RENAL FAILURE
2025, VOL. 47, NO. 1, 2476053
https://doi.org/10.1080/0886022X.2025.2476053

Taylor & Francis
Taylor & Francis Group

CLINICAL STUDY

Deciphering the causal link between gut microbiota and membranous nephropathy: insights into potential inflammatory mechanisms

Jianbo Qing[a,b,c], Changqun Li[a] and Nan Jiao[b,c,d,e]

[a]Department of Nephrology, Sir Run Run Shaw Hospital, Zhejiang University School of Medicine, Hangzhou, China; [b]Department of Nephrology, Shanxi Provincial People's Hospital, Shanxi Medical University, Taiyuan, China; [c]Department of Nephrology, The Fifth Clinical Medical College, Shanxi Medical University, Taiyuan, China; [d]Big Data Center of Kidney Disease, Shanxi Provincial People's Hospital, Taiyuan, China; [e]Shanxi Provincial Key Laboratory of Kidney Disease, Taiyuan, China

ABSTRACT

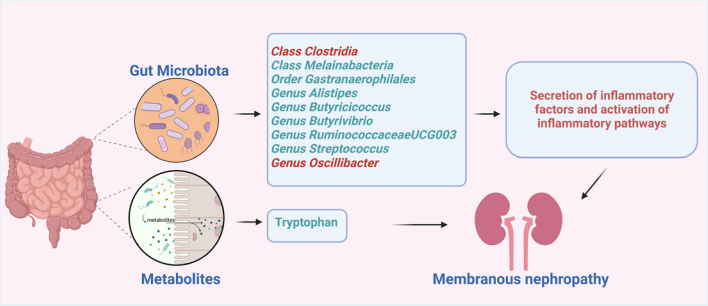
Background: Membranous nephropathy (MN), a leading cause of adult nephrotic syndrome and renal failure, has been linked to gut microbiota (GM) and their metabolites. However, direct causal relationships and therapeutic implications remain unclear.

Methods: We utilized a comprehensive GWAS dataset that encompasses GM, metabolites, and MN through two-sample Mendelian randomization (MR) analyses, bidirectional MR evaluations, and detailed sensitivity tests.

Results: We identified strong causal associations between nine specific types of GM, including class *Clostridia* (OR = 1.816, 95%CI: 1.021–3.236, p = .042), class *Melainabacteria* (OR = 0.661, 95%CI: 0.439–0.996, p = .048), order *Gastranaerophilales* (OR = 0.689, 95%CI: 0.480–0.996, p = .044), genus *Alistipes* (OR = 0.480, 95%CI: 0.223–0.998, p = .049), genus *Butyricicoccus* (OR = 0.464, 95%CI: 0.216–0.995, p = .048), genus *Butyrivibrio* (OR = 0.799, 95%CI: 0.639–0.998, p = .048), genus *Ruminococcaceae UCG003* (OR = 0.563, 95%CI: 0.362–0.877, p = .011), genus *Streptococcus* (OR = 0.619, 95%CI: 0.393–0.973, p = .038), and genus *Oscillibacter* (OR = 1.90, 95%CI: 1.06–3.40, p = .031). Additionally, the metabolite tryptophan also exhibited a significant causal influence on MN (OR = 0.852, 95%CI: 0.754–0.963, p = .010). Sensitivity and reverse MR analyses confirmed the robustness of these findings. Further exploration using gutMGene database suggests that GM may influence MN by affecting the release of inflammatory factors and modulating inflammatory pathways.

Conclusion: This study offers a comprehensive understanding of the causal links between GM, their metabolites, and MN, which highlight potential pathways for developing new preventive and therapeutic strategies for this condition.

GRAPHICAL ABSTRACT



ARTICLE HISTORY

Received 29 December 2024
Revised 23 February 2025
Accepted 24 February 2025

KEYWORDS

Membranous nephropathy; Gut microbiota; Metabolites; Mendelian randomization; Inflammation

1. Introduction

Membranous nephropathy (MN) represents a leading autoimmune glomerulonephritis in adults, marked by autoreactive antibodies and immune complex accumulation. This pathology incites complement activation, culminating in pronounced renal injury and proteinuria [1]. While insights into its pathogenesis and rituximab use have ushered in therapeutic advancements [2], corticosteroids and agents such as cyclophosphamide remain pivotal in disease control [3]. At the

CONTACT Nan Jiao stefaniej0913@163.com Department of Nephrology, Shanxi Provincial People's Hospital, Shanxi Medical University, Taiyuan 030001, China

Supplemental data for this article can be accessed online at https://doi.org/10.1080/0886022X.2025.2476053.

same time, the complications of immunosuppression-centered treatment principle impose a heavy physical and mental burden on patients, and elapse or disease progression is not uncommon [4], underscoring the urgency for novel treatment strategies rooted in a deeper mechanistic understanding.

The gut microbiome (GM) is pivotal for immune equilibrium and has emerged as a potential player in autoimmune maladies [5]. Alterations in microbial communities and their metabolic byproducts can compromise the mucosal barrier, potentially instigating the genesis of pathogenic IgG [6]. Pertinently, MN patients exhibit distinct microbiome profiles compared to both healthy subjects and those with other renal pathologies [7]. Importantly, GM modulation attenuates proteinuria in MN, endorsing its potential as an adjunctive therapeutic avenue [8].

However, extant studies, constrained by limited cohort sizes, leans heavily on correlation analysis, sidestepping a conclusive causal link between the GM, its metabolites, and MN. While expansive prospective studies are constrained by financial and methodological challenges, the deployment of Mendelian randomization (MR) in the analysis of GWAS datasets from extensive cohorts offers a means to bypass confounders typical of observational research [9]. Such an approach promises a refined understanding of the intricate nexus between the GM, its metabolites, and MN, pinpointing high-fidelity therapeutic targets. The main workflow of this study is shown in Figure 1.

2. Methods

2.1. Data source

Every piece of GWAS data leveraged in this study was meticulously sourced from publicly accessible databases. We systematically downloaded the most up-to-date summary-level data pertaining to GM from the MiBioGen consortium, a comprehensive amalgamation of 24 cohorts incorporating a total of 18,340 participants [10]. This rich dataset spans 211 microbial taxa meticulously classified from phyla to genera. For MN, we sourced data comprising 5829 controls and 2450 idiopathic MN patients from the IEU Open GWAS Project [11]. In conjunction, we referred to a seminal study by Rhee et al. for data on 12 prevalent metabolites, which encompassed a sample of 2076 participants [12].

Each cohort involved in our study comprised individuals of exclusive European descent and had been subjected to rigorous ethical approval protocols at the time of their respective data collection processes. To ensure transparency and facilitate scholarly pursuit, we have provided exhaustive details pertaining to data access, including precise URLs and specific timeframes, in Table S1, allowing for thorough examination and validation by peers in the field.

2.2. Instrumental variables

To meticulously select instrumental variables (IVs), we adopted rigorous selection parameters. First and foremost, we implemented a stringent p value cutoff for SNPs, anchoring it at 5×10^{-8}. While this threshold is stringent, it acknowledges the potential of sidelining some pertinent results. In scenarios where IVs are scant at this p value, we then resort to an adjusted threshold of $p < 1 \times 10^{-5}$, a benchmark that has gained in prior scholarly endeavors [13]. To ascertain the autonomy of IVs for analogous exposures, we employed a linkage disequilibrium cutoff, setting r^2 at less than 0.001 and a clumping radius of 10,000 kb. Palindromic SNPs, known to impede allele congruence for both exposure and resultant outcomes, were categorically excluded from our analysis [14].

In our bid to curtail the perturbations from potential confounding factors, we leveraged the capabilities of Phenoscanner, systematically excising SNPs with affiliations to confounders like hypertension and other autoimmune maladies [15]. Furthermore, to ensure the potency of our selected IVs, we subjected them to an F-statistics assessment, disqualifying any with F-values $((R^2/(R^2 - 1)) \times ((N - K - 1)/K))$ less than 10 [16]. Only the SNPs which rigorously adhered to this comprehensive criterion matrix were christened as valid IVs for our study.

2.3. Mendelian randomization

For assessing the association between exposures and outcomes, we employed four diversified MR techniques. These techniques include the inverse variance weighted (IVW), weighted median, weighted mode, and the MR Egger regression methods. Each technique offers its intricacies and merits, and our intent is to synergize insights derived from this multi-method analytical repertoire to distill a concrete causal nexus.

The IVW method enjoys a laudable reputation for its robustness, grounding its computation on the weighted effects and variances of the IVs. However, a caveat is its presupposition of a singular causal trajectory, potentially sidelining the phenomenon of horizontal pleiotropy [17]. To counteract this limitation, the MR Egger is used to accommodate multiple causal pathways and pleiotropic effects. This technique permits non-zero intercept terms, thereby ameliorating directional pleiotropy and refining IVW-derived outcomes [18]. Additionally, during encounters with compromised IVs, the weighted Median exemplifies resilience, but with the prerequisite that bona fide IVs contribute over half of the total. Both the weighted median and weighted mode techniques serve as arbitrators, especially when reconciling divergent findings between the IVW and MR Egger methodologies [19]. For all our analyses, we anchored our significance threshold at a p value below .05. As this research is inherently exploratory, we chose not to implement the Bonferroni correction to mitigate the risk of type II errors potentially overlooking genuine effects.

2.4. Sensitivity analysis

Causal inference in MR is susceptible to confounding and bias. To mitigate these, we adopted a systematic approach. We began with MR Egger to evaluate horizontal pleiotropy. A p value below .05 indicates its presence; otherwise, it is

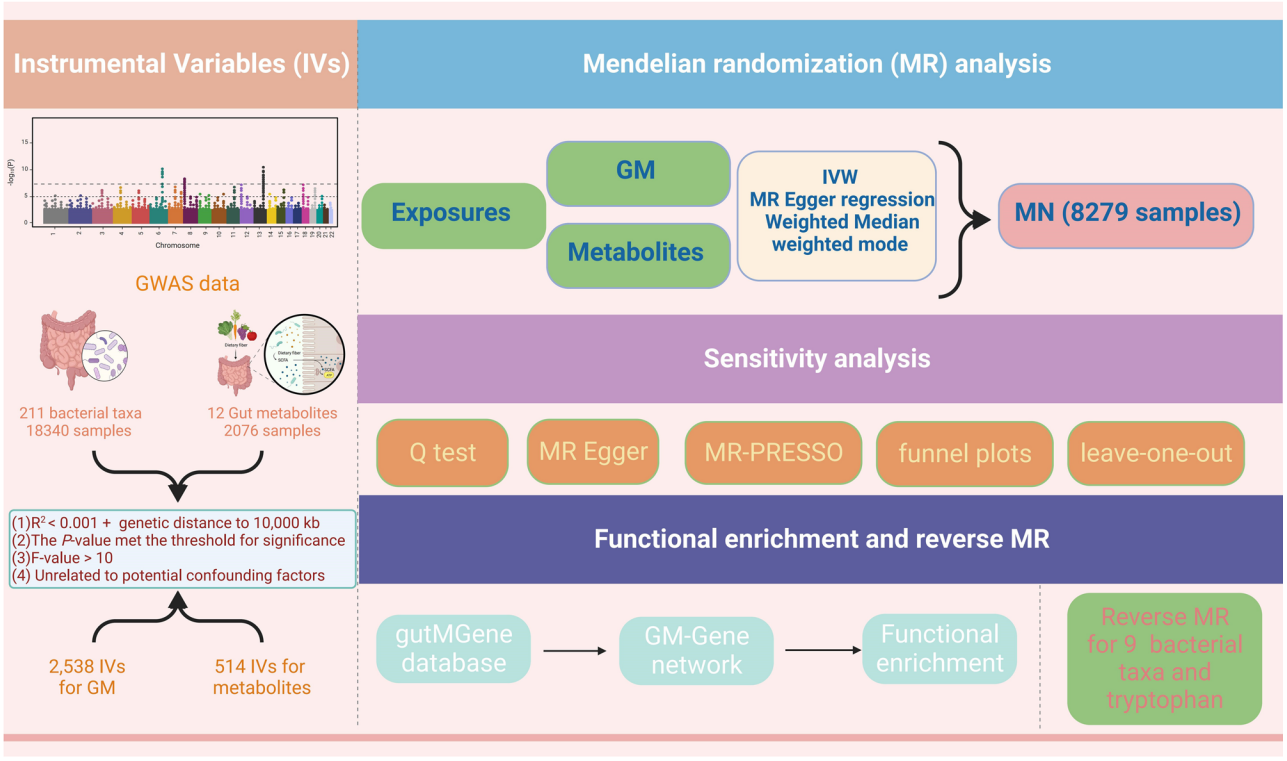


Figure 1. An overview of this study. By sourcing GWAS data for GM and metabolites from public repositories, rigorously selecting instrumental variables (IVs), conducting two-sample bidirectional MR to validate their causal relationships on MN, and exploring potential mechanisms.

deemed absent. For enhanced precision and identification of potential outliers, we turned to MR-PRESSO tests, which are adept at pinpointing and excising these deviations [20]. Moreover, to investigate heterogeneity among IVs, Cochran's *Q* tests were performed. A *Q*-value below 0.05 denotes significant heterogeneity, warranting the adoption of a random-effects (REs) model [21]. This model caters to heterogeneity, yielding a precise weighted average of causal effect estimates. We also incorporated funnel plots to further evaluate potential bias and heterogeneity [22]. Lastly, the 'leave-one-out' criterion was implemented to discern the influence of individual SNPs on the derived outcomes [23].

2.5. Bidirectional Mendelian randomization

We initiated our analysis by designating GM or metabolites as the exposure variable, with MN as the outcome in the MR framework. Next, IVW method was used to ascertain the presence of a consequential causal link between specific GM or metabolites and MN. After this affirmation, a reverse causal inference was undertaken to investigate if MN induces alterations in these GM or metabolites. The analytical strategies and methodologies utilized aligned with the previously detailed descriptions.

2.6. Functional enrichment

To further investigate the potential mechanistic pathways through which GM might influence MN, we extracted relevant GM data from the gutMGene database. This database consolidates substantial relationships between GM and target genes, based on results from both *in vivo* and *in vitro* studies. By entering the names of GM into the database, the associated genes can be retrieved [24]. Utilizing Cytoscape (Version 3.8.2), we then built a network to depict the interplay between GM and their target genes, resulting in a comprehensive GM-Gene network. In the subsequent phase, we conducted functional enrichment analyses on the genes regulated by GM. For this, we employed the 'ClusterProfiler' package (Version 4.2.2), cross-referencing with both KEGG and GO databases for a holistic understanding.

2.7. Analysis platform and visualization

All analyses were performed in the R platform (Version 4.3.1) (R Foundation for Statistical Computing, Vienna, Austria), MR was performed by 'TwoSampleMR' package (Version 0.5.6), the forest map was drawn using the 'forestploter' package (1.1.0), and the enrichment analysis map was beautied using 'ggplot2' package (Version 3.4.2).

3. Results

3.1. IVs for MR

Adhering to the strict threshold of $p < 5 \times 10^{-8}$, our efforts to identify an adequate number of IVs for GM proved unfruitful. This necessitated a threshold adjustment to $p < 1 \times 10^{-5}$. Subsequent to this change, we eliminated SNPs in linkage disequilibrium and discarded those correlating with potential

confounding factors. This process culminated in the identification of 2538 IVs spread across 196 GM variables. Using analogous criteria but for the 12 metabolites, we pinpointed 514 IVs, with the notable exception of setting the *p* value threshold at 5×10^{-5} (Tables S2 and S3).

3.2. GM and MN

Upon executing the MR, significant causal associations emerged between MN and nine GM variables. These spanned across two classes, one order, and six genera. In the class category, divergent associations with MN risk were observed: a heightened abundance of *Clostridia* correlated with an increased MN risk (OR = 1.816, 95%CI: 1.021–3.236, p = .042), whereas elevated *Melainabacteria* levels suggested a diminished MN risk (OR = 0.661, 95%CI: 0.439–0.996, p = .048). At the order tier, a surge in *Gastranaerophilales* was significantly inversely associated with MN risk (OR = 0.689, 95%CI: 0.480–0.996, p = .044). On the genus front, the prevalences of *Alistipes* (OR = 0.480, 95%CI: 0.223–0.998, p = .049), *Butyrivibrio* (OR = 0.799, 95%CI: 0.639–0.998, p = .048), *Butyricicoccus* (OR = 0.464, 95%CI: 0.216–0.995, p = .048), *Ruminococcaceae UCG003* (OR = 0.563, 95%CI: 0.362–0.877, p = .011), and *Streptococcus* (OR = 0.619, 95%CI: 0.393–0.973, p = .038) were all substantially inversely linked to MN risk. In contrast, *Oscillibacter*'s abundance presented a positive tie to MN risk (OR = 1.90, 95%CI: 1.06–3.40, p = .031). However, sensitivity evaluations flagged significant heterogeneity in the IVs for *Oscillibacter*, denoted by a *Q*-value of 0.01. Fortunately, following adjustment using the IVW-RF model, the causal association between *Oscillibacter* and MN persisted even after accounting for heterogeneity ($P_{IVW\text{-}RE} < .0001$) (Figure 2 and Table S4).

Further exhaustive analyses on other GM, comprising MR-PRESSO tests, MR-Egger tests, Cochran's *Q* tests (Table 1), funnel plots (Figure S1), and unearthed neither multicollinearity nor outliers, affirming our study's precision. Notably, the lack of prominent outliers in the leave-one-out assessment bolsters the resilience of our results (Figure S2). Additionally, due to an insufficient number of SNPs, the funnel and leave-one-out plots for genus *Alistipes* are missing.

3.3. GM-Gene network and functional enrichment

The intricate regulatory influence of GM on target gene expression is a compelling subject of investigation. Leveraging insights from the gutMgene database, we discerned specific downstream genes modulated by *Ruminococcaceae UCG-003* (inclusive of *TNF*, *IL6*, *NFKB1*, *IL1B*, *TJP*1), *Streptococcus* (encompassing *CCL20*, *CXCL6*), and *Oscillibacter* (comprising *LBP*, *CD163*, *LCN2*, *CD44*, *IL1B*, *CD14*, *IL6*, *IL12A*, *SMN1*, *TGFBR1*, *TNF*, and *SMURF2*). These associations are visually depicted in Figure 3(A). Given their inverse correlation with MN risk, *Ruminococcaceae UCG-003* and *Streptococcus* were postulated as protective agents. Our enrichment analysis elucidated that barring *TJP1*, the protective factors suppress the expression of six genes, predominantly engaged in adaptive immune responses. These encompass pivotal pathways such as IL-17 and TNF signaling and mechanisms that augment inflammatory processes.

Conversely, *Oscillibacter*, based on its positive correlation with MN, was earmarked as a risk modulator. This microbe amplifies the expression of a constellation of 11 genes, with *SMURF2* being the sole exception. Intriguingly, these genes orchestrate KEGG pathways and biological processes that overlap considerably with those steered by the protective agents. Notable pathways include Toll-like receptor signaling and the upregulation of IL-8 synthesis. Of paramount interest is the observation that both protective and risk GM share

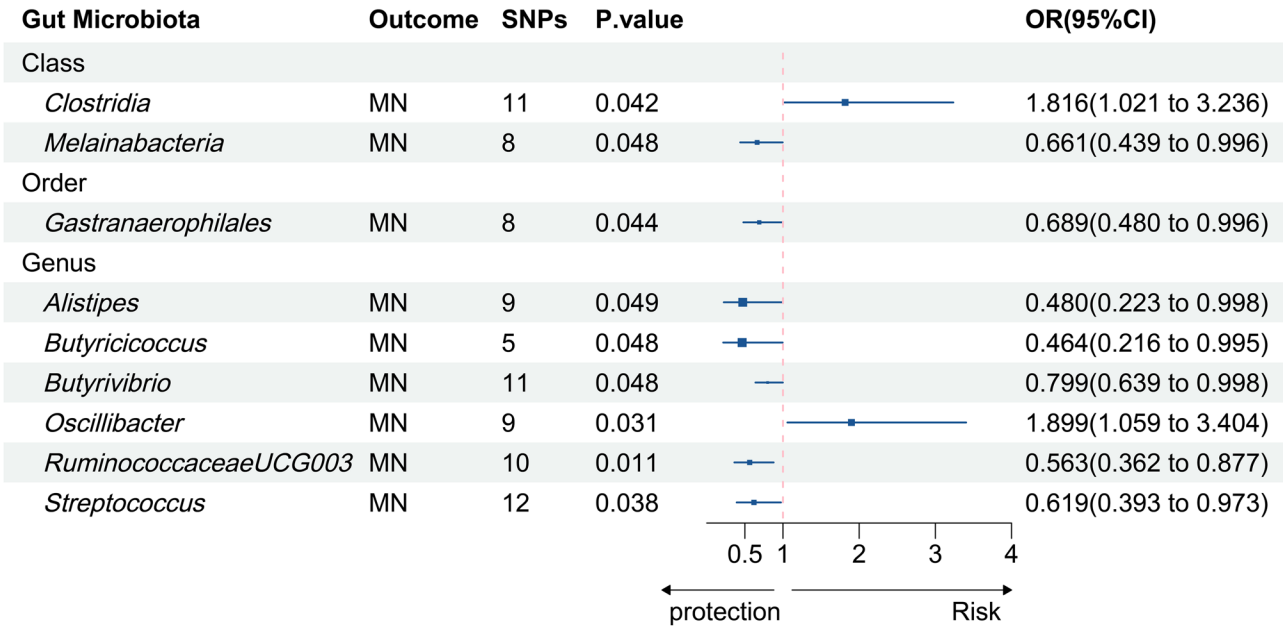


Figure 2. The forest plot of MR between GM and MN. CI: confidence interval; Int pval: intercept *p* value; MN: membranous nephropathy; OR: odds radio.

Table 1. MR results for the effect of GM on MN.

Gut microbiota	Outcome	SNPs	Methods	β	OR (95%CI)	p Value	Q value	Int pval
Class *Clostridia*	MN	11	IVW	0.597	1.023–3.236	.042	0.266	.631
			MR Egger	−0.108	0.052–15.406	.942	0.216	
Class *Melainabacteria*	MN	8	IVW	−0.414	0.439–0.996	.048	0.120	.255
			MR Egger	0.305	0.414–4.449	.633	0.170	
Order *Gastranaerophilales*	MN	8	IVW	−0.37	0.48–0.99	.044	0.252	.342
			MR Egger	0.18	0.40–3.60	.764	0.264	
Genus *Alistipes*	MN	9	IVW	−0.751	0.223–0.998	.049	0.073	.693
			MR Egger	−0.004	0.026–38.351	.998	0.051	
Genus *Butyricicoccus*	MN	5	IVW	−0.768	0.216–0.995	.048	0.913	.771
			MR Egger	−1.535	0.002–25.706	.574	0.831	
Genus *Butyrivibrio*	MN	11	IVW	−0.225	0.639–0.998	.048	0.572	.917
			MR Egger	−0.291	0.217–2.570	.655	0.478	
Genus *Ruminococcaceae UCG003*	MN	10	IVW	−0.574	0.362–0.877	.011	0.676	.788
			MR Egger	−0.381	0.164–2.857	.616	0.586	
Genus *Streptococcus*	MN	12	IVW	−0.480	0.393–0.973	.038	0.427	.348
			MR Egger	−1.244	0.060–1.409	.155	0.431	
Genus *Oscillibacter*	MN	9	IVW	0.641	1.060–3.404	.031	0.012	.243
			MR Egger	−0.905	0.035–4.650	.491	0.025	
			IVM-RE	0.641	1.060–3.404	<.0001		

CI: confidence interval; Int pval: intercept *p* value; IVW: inverse variance weighted; MN: membranous nephropathy; OR: odds radio; RE: random effect.

several target genes, namely *IL6*, *TNF*, and *IL1B*. These microbial taxa seem to wield opposing regulatory sway on multiple inflammation-centric pathways. Such an intricate interplay could very well serve as a fulcrum determining the predisposition and manifestation of MN (Figure 3(B–D)).

3.4. Metabolites and MN

Upon meticulous evaluation, a salient causal linkage emerged between one out of the 12 scrutinized metabolites and MN. Precisely, a marked inverse association was observed between tryptophan concentrations and MN susceptibility (OR = 0.852, 95%CI: 0.754–0.963, p = .010). Rigorous sensitivity analyses buttressed the validity of this association, revealing no discernible anomalies or outliers (Table 2, Figure S3, and Table S5).

3.5. Reverse causal inference

To substantiate the causative relationships of the pinpointed GM and metabolites with MN, we embarked on a reverse causation verification. Notably, MN failed to manifest discernible causal impacts on any of the examined factors. Data derived from our principal analytical approach, IVW, is elucidated in Table 3.

4. Discussion

While our comprehension of MN's pathogenesis has deepened, therapeutic avenues remain constrained. A significant proportion of MN patients find themselves tethered to immunosuppressants, grappling with the dual challenges of adverse drug ramifications and looming recurrence risks. This situation precipitates notable economic and psychological tolls of patients [25]. A pivotal nexus exists between the GM, their metabolites, and the human immune system, playing an instrumental role in disease onset and progression. Earlier research focusing on MN patients has underscored significant shifts in GM composition, with these alterations bearing a potent correlation to disease severity [26]. Intriguingly, fecal microbial transplantation (FMT) has emerged as a beacon of therapeutic hope, demonstrating efficacy in symptom alleviation with a diminished adverse effect profile [27]. Yet, the landscape of prior observational research is marred by limitations, chiefly an over-reliance on correlation analyses and constrained sample sizes. Such impediments obfuscate a holistic grasp of the intricacies binding MN to GM and thwart advancements in FMT optimization.

This present inquiry offers a comprehensive elucidation of the causal interplay between GM, their affiliated metabolites, and MN. We anchor our research in an expansive, population-centric GWAS dataset, harnessing the precision of MR analysis. A cornerstone of our approach lies in the immutable nature of genetic markers, rendering them impervious to external influences, thus sidestepping the pitfalls of confounding and bias endemic to conventional cohort explorations. Utilizing statistical measures, specifically the p value and F value, we meticulously evaluated independent variables exhibiting notable correlation with exposure, excluding any latent confounding factors. Subsequent to this, a rigorous two-sample MR analysis complemented by sensitivity analysis was undertaken to reinforce the robustness of our results. Conclusively, our investigations discerned nine GM and a singular metabolite with pronounced causal implications on MN risk.

At the class level, a marked positive relationship exists between the prevalence of *Clostridia* and MN risk. *Clostridia* are frequently implicated in intestinal infections, often leading to intestinal inflammation and heightened permeability [28]. This bacterium can also stimulate B cell proliferation, enhance IgG antibody production, and, in severe instances, possibly introduce toxic substances into the bloodstream, potentially instigating kidney damage [29,30]. The intriguing aspect is that it also exhibits a pronounced inverse correlation with serum albumin levels [31]. Conversely, an augmented abundance of *Melainabacteria* is associated with a

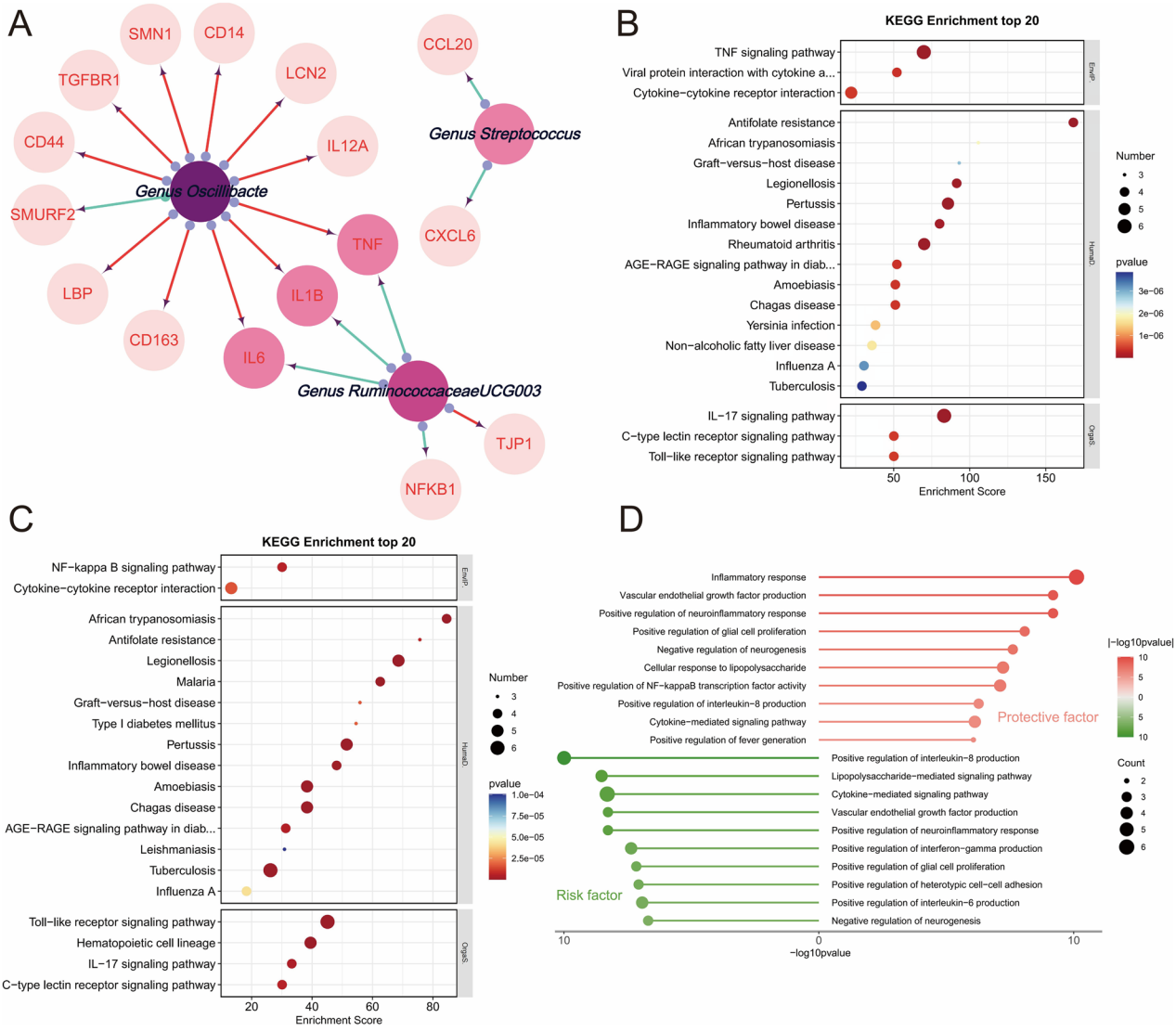


Figure 3. The GM-Gene network and functional enrichment. (A) The GM-Gene network diagram, where arrow direction indicates the direction of influence, with red arrows signifying promotion of target gene expression, and blue arrows indicating a reduction in target gene expression. (B) The KEGG enrichment analysis for target genes of protective factors, excluding *TJP1*. (C) The KEGG enrichment analysis for target genes of risk factor, excluding *SMURF2*. (D) GO biological process analysis for target genes of both protective and risk factors, with gene *TJP1* and *SMURF2* excluded. Red represents risk factor, while green signifies protective factors.

diminished IMN risk. While the precise role of *Melainabacteria* in the human physiological framework remains partially understood, it plays a pivotal role in preserving GM diversity and equilibrium. This stability potentially translates into host benefits *via* the amplified expression of vitamin synthetic genes [32]. The order *Gastranaerophilales*, a member of the class *Melainabacteria*, not only mirrors the same functionalities but also stands as a prominent producer of indoles in the intestine [33]. Remarkably, these indoles can curtail oxidative stress and suppress pro-inflammatory factor production, effectively modulating immunity and stalling MN's progression [34].

Excitingly, we delineated a robust causal nexus between six distinct genera and MN. Notably, barring *Oscillibacter*, these genera appear to convey a protective effect against the occurrence of MN. *Oscillibacter*, under normophysiological conditions, remains a minor constituent of the human GM. However, under pathophysiological perturbations, its relative abundance escalates, thereby influencing both intestinal permeability and pyruvate metabolism. Such perturbations may instigate an enhanced biosynthesis of uremic toxins and orchestrate a coalescent inflammatory milieu with other bacterial constituents, culminating in progression of MN and renal functional perturbations [35]. Moreover, the functional attributes of *Alistipes* within the human physiological milieu oscillate between pro-inflammatory and anti-inflammatory paradigms. However, an accumulating body of evidence underscores its prodigious capability to biosynthesize short-chain fatty acids (SCFAs) [36]. Such SCFA production could serve as a counteractive measure against inflammatory cascades, thereby affording a renoprotective shield [37]. In tandem with this, an ascendant presence of *Alistipes* has

Table 2. MR results for the effect of metabolites on MN.

Metabolites	Outcome	Methods	SNPs	β	OR (95%CI)	p Value	Q value	Int pval
Tryptophan	MN	IVW	22	−0.160	0.754–0.963	.010	0.091	.114
Tryptophan		MR Egger		−0.332	0.566–0.908	.012	0.151	
Tryptophan		Weighted median		−0.169	0.726–0.981	.027		
Tryptophan		Weighted mode		−0.242	0.652–0.944	.018		

CI: confidence interval; Int pval: intercept p value; IVW: inverse variance weighted; MN: membranous nephropathy; OR: odds radio.

Table 3. Reverse MR results for the effect of MN on specific GM and metabolites.

Exposure	Outcome	Method	p Value
MN	Class *Clostridia*	IVW	.085
	Class *Melainabacteria*		.335
	Order *Gastranaerophilales*		.344
	Genus *Alistipes*		.314
	Genus *Butyricicoccus*		.788
	Genus *Butyrivibrio*		.886
	Genus *Ruminococcaceae UCG003*		.143
	Genus *Streptococcus*		.275
	Genus *Oscillibacter*		.069
	Tryptophan		.391

been empirically associated with attenuated renal inflammatory cell infiltration, inhibition of renal fibrotic pathways, and a deceleration of vascular calcification trajectories in nephropathic phenotypes [38,39]. Furthermore, in patients manifesting advanced chronic kidney disease (CKD), an empirical decrement in *Butyricicoccus* abundance has been observed across multiple investigative studies [40]. Butyrate, a metabolic end product orchestrated by *Butyricicoccus*, not only fuels the intestinal epithelium but also ameliorates intestinal inflammatory perturbations, fortifies mucosal barrier function, and orchestrates the immune balance, particularly within the Treg/Th17 cellular milieu in CKD cohorts [41]. *Butyrivibrio*, sharing metabolic parallels with *Butyricicoccus*, emerges as a linchpin in maintaining intestinal integrity and a systemic immunological equipoise, often through harmonious interactions within the broader GM consortium [42,43]. Additionally, *Ruminococcaceae UCG-003*, yet another pivotal player, wields its influence by modulating mucosal immunity, primarily through the synthesis of SCFA. A decline in its relative abundance could potentially usher in a milieu conducive to inflammatory disorders and dysregulated immune cell functionality [44]. Intriguingly, *Streptococcus*, traditionally anchored within the pathogenic spectrum, especially in the context of acute glomerulonephritis, paradoxically demonstrates an inverse relationship with MN risk. It is imperative to acknowledge the taxonomic diversity within the genus *Streptococcus*, where specific species like *Streptococcus salivarius* and *Streptococcus thermophilus* are reported to confer mucosal health benefits [45–47].

Subsequent dissection of the GM-genes nexus furnishes a nuanced understanding of their causal interplay. Genera including *Ruminococcaceae UCG-003*, *Streptococcus*, and *Oscillibacter* modulate the transcriptional landscape of a myriad of genes underpinning inflammatory cascades. This modulation orchestrates perturbations in cytokine and chemokine equilibria, serving as the fulcrum for inflammation initiation and perpetuation. Notably, *Ruminococcaceae UCG-003* attenuates the transcription of pivotal cytokines like *IL6*, *IL1B*, and *TNF*. In stark contrast, *Oscillibacter* amplifies their transcriptional profiles, thereby positioning itself as a key pro-inflammatory protagonist. IL6, IL1B, and TNF could emerge as central to anti-PLA2R biosynthesis, playing instrumental roles in mediating renal lesions in MN [48–50]. Their significance is further underscored by their potential as viable therapeutic targets in MN therapeutic strategies [51]. Positioning *Oscillibacter* as a harbinger of risk contrasts starkly with *Streptococcus* and *Ruminococcaceae UCG-003*, which are delineated as protective factor. Notably, the enrichment analyses of the target genes for both protective and risk factors exhibit marked congruence, primarily participating in the biosynthesis of inflammatory mediators and the activation of inflammatory pathways. Microbial dysbiosis can lead to abnormal inflammatory mediator release and pathway activation, precipitating the onset and swift progression of MN. Ensuring a balanced interplay between these elements might hold the key to optimizing MN outcomes and minimizing recurrence rates [52]. Beyond the realm of microbial influence, the concentration of tryptophan exhibits an antithetical relationship with MN risk. As tryptophan undergoes metabolic catabolism, it spawns indole derivatives. These metabolites, intriguingly, attenuate the aryl hydrocarbon receptor signaling axis, providing a potential mechanism for MN mitigation [53].

Presently, considerable progress has been made in discerning the pathogenic genes and pathways linked to MN. Yet, interventions centered on these elements largely hinge on immunosuppression, often ushering in undesirable side effects. Shifting focus, the modulation of GM to maintain intestinal mucosal immune equilibrium will serve as a compelling adjunctive therapy for MN. This strategy not only lessens dependence on immunosuppressants but also assures therapeutic efficacy and curtails recurrence, paving the way for improved diagnostic and therapeutic avenues for patients.

5. Limitations

This study presents several limitations. First, the population under investigation is exclusively of European ancestry, which lacks representation from other ethnic backgrounds. Second, all patients in the MN cohort are diagnosed with primary MN; hence, the causal relationships between GM, its metabolites, and secondary MN remain undetermined. Furthermore, although we have preliminarily identified potential relationships between the GM, metabolites, and MN using statistical methods in this study, these findings still require further validation through more rigorous experimental approaches.

Future work will need to involve the collection of a large number of biological samples to validate these results and obtain more precise conclusions.

6. Conclusion

Utilizing genetic tools, we have comprehensively uncovered the causal relationships between GM, its metabolites, and MN. We successfully identified nine types of GM and tryptophan as having significant causal links with MN and also elucidated potential mechanisms of action. This sheds further light on the pathogenic mechanisms underlying MN and provides more robust support for the development of expanded therapeutic strategies.

Acknowledgements

We extend our gratitude to the volunteers who selflessly contributed human tissue samples for the advancement of scientific research. We also express our appreciation to those individuals who have generously offered us invaluable assistance.

Author contributions

J.Q.: conceptualization, formal analysis, investigation, methodology, software, writing-original draft, resources, investigation, validation, and visualization. C.L.: writing-review and editing. N.J.: funding acquisition. All authors read and approved the final manuscript.

Ethical approval

The studies that contributed the data had been approved by ethical standards committees; no additional ethical approval is required for this study.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This research was funded by the Shanxi Provincial Administration of Traditional Chinese Medicine Scientific Research Program (2023ZYYC041), Natural Science Research Project of Basic Research Programme in Shanxi Province (202203021221268), Young Scientists Fund of Natural Science Research Project of Basic Research Programme in Shanxi Province (202303021222361), and Young Scientists Fund of National Natural Science foundation of China (82305030).

Data availability statement

The data utilized in this study are all sourced from public repositories. Specific access links and key data generated in this research are provided in the supplementary files. For more detailed data and the complete set of analysis codes, please reach out to the corresponding author via email.

References

[1] Hoxha E, Reinhard L, Stahl RAK. Membranous nephropathy: new pathogenic mechanisms and their clinical implications. Nat Rev Nephrol. 2022;18(7):466–478. doi: 10.1038/s41581-022-00564-1.

[2] Teisseyre M, Cremoni M, Boyer-Suavet S, et al. Advances in the management of primary membranous nephropathy and rituximab-refractory membranous nephropathy. Front Immunol. 2022;13:859419. doi: 10.3389/fimmu.2022.859419.

[3] Fernández-Juárez G, Rojas-Rivera J, Logt AV, et al. The STARMEN trial indicates that alternating treatment with corticosteroids and cyclophosphamide is superior to sequential treatment with tacrolimus and rituximab in primary membranous nephropathy. Kidney Int. 2021;99(4):986–998. doi: 10.1016/j.kint.2020.10.014.

[4] Safar-Boueri L, Piya A, Beck LHJr., et al. Membranous nephropathy: diagnosis, treatment, and monitoring in the post-PLA2R era. Pediatr Nephrol. 2021;36(1):19–30. doi: 10.1007/s00467-019-04425-1.

[5] Miyauchi E, Shimokawa C, Steimle A, et al. The impact of the gut microbiome on extra-intestinal autoimmune diseases. Nat Rev Immunol. 2023;23(1):9–23. doi: 10.1038/s41577-022-00727-y.

[6] Sanidad KZ, Amir M, Ananthanarayanan A, et al. Maternal gut microbiome-induced IgG regulates neonatal gut microbiome and immunity. Sci Immunol. 2022;7(72):eabh3816. doi: 10.1126/sciimmunol.abh3816.

[7] Shang J, Zhang Y, Guo R, et al. Gut microbiome analysis can be used as a noninvasive diagnostic tool and plays an essential role in the onset of membranous nephropathy. Adv Sci. 2022;9(28):e2201581. doi: 10.1002/advs.202201581.

[8] Li M, Wei L, Sun J, et al. Association of gut microbiota with idiopathic membranous nephropathy. BMC Nephrol. 2022;23(1):164. doi: 10.1186/s12882-022-02797-5.

[9] Birney E. Mendelian randomization. Cold Spring Harb Perspect Med. 2022;12(4):a041302. doi: 10.1101/cshperspect.a041302.

[10] Wang J, Kurilshikov A, Radjabzadeh D, et al. Meta-analysis of human genome–microbiome association studies: the MiBioGen consortium initiative. Microbiome. 2018;6(1):101. doi: 10.1186/s40168-018-0479-3.

[11] Xie J, Liu L, Mladkova N, et al. The genetic architecture of membranous nephropathy and its potential to improve non-invasive diagnosis. Nat Commun. 2020;11(1):1600. doi: 10.1038/s41467-020-15383-w.

[12] Rhee EP, Ho JE, Chen MH, et al. A genome-wide association study of the human metabolome in a community-based cohort. Cell Metab. 2013;18(1):130–143. doi: 10.1016/j.cmet.2013.06.013.

[13] Bowden J, Holmes MV. Meta-analysis and Mendelian randomization: a review. Res Synth Methods. 2019;10(4):486–496. doi: 10.1002/jrsm.1346.

[14] Haycock PC, Burgess S, Nounu A, et al. Association between telomere length and risk of cancer and non-neoplastic diseases: a Mendelian randomization study.

JAMA Oncol. 2017;3(5):636–651. doi: 10.1001/jamaoncol.2016.5945.
[15] Kamat MA, Blackshaw JA, Young R, et al. PhenoScanner V2: an expanded tool for searching human genotype–phenotype associations. Bioinformatics. 2019;35(22):4851–4853. doi: 10.1093/bioinformatics/btz469.
[16] Borges MC, Haycock PC, Zheng J, et al. Role of circulating polyunsaturated fatty acids on cardiovascular diseases risk: analysis using Mendelian randomization and fatty acid genetic association data from over 114,000 UK Biobank participants. BMC Med. 2022;20(1):210. doi: 10.1186/s12916-022-02399-w.
[17] Cao Z, Wu Y, Li Q, et al. A causal relationship between childhood obesity and risk of osteoarthritis: results from a two-sample Mendelian randomization analysis. Ann Med. 2022;54(1):1636–1645. doi: 10.1080/07853890.2022.2085883.
[18] Burgess S, Thompson SG. Interpreting findings from Mendelian randomization using the MR-Egger method. Eur J Epidemiol. 2017;32(5):377–389. doi: 10.1007/s10654-017-0255-x.
[19] Li P, Wang H, Guo L, et al. Association between gut microbiota and preeclampsia–eclampsia: a two-sample Mendelian randomization study. BMC Med. 2022;20(1):443. doi: 10.1186/s12916-022-02657-x.
[20] Verbanck M, Chen CY, Neale B, et al. Detection of widespread horizontal pleiotropy in causal relationships inferred from Mendelian randomization between complex traits and diseases. Nat Genet. 2018;50(5):693–698. doi: 10.1038/s41588-018-0099-7.
[21] Tabrizi R, Saneei P, Lankarani KB, et al. The effects of caffeine intake on weight loss: a systematic review and dose–response meta-analysis of randomized controlled trials. Crit Rev Food Sci Nutr. 2019;59(16):2688–2696. doi: 10.1080/10408398.2018.1507996.
[22] van der Willik EM, van Zwet EW, Hoekstra T, et al. Funnel plots of patient-reported outcomes to evaluate health-care quality: basic principles, pitfalls and considerations. Nephrology. 2021;26(2):95–104. doi: 10.1111/nep.13761.
[23] Guan M, Yan L, Li R, et al. Integration of leave-one-out method and real-time live cell reporter array system to assess the toxicity of mixtures. Environ Res. 2022;214(Pt 3):114110. doi: 10.1016/j.envres.2022.114110.
[24] Cheng L, Qi C, Yang H, et al. gutMGene: a comprehensive database for target genes of gut microbes and microbial metabolites. Nucleic Acids Res. 2022;50(D1):D795–D800. doi: 10.1093/nar/gkab786.
[25] Wu L, Lai J, Ling Y, et al. A review of the current practice of diagnosis and treatment of idiopathic membranous nephropathy in China. Med Sci Monit. 2021;27:e930097. doi: 10.12659/msm.930097.
[26] Yu W, Shang J, Guo R, et al. The gut microbiome in differential diagnosis of diabetic kidney disease and membranous nephropathy. Ren Fail. 2020;42(1):1100–1110. doi: 10.1080/0886022x.2020.1837869.
[27] Zhou G, Zeng J, Peng L, et al. Fecal microbiota transplantation for membranous nephropathy. CEN Case Rep. 2021;10(2):261–264. doi: 10.1007/s13730-020-00560-z.
[28] Harris RA, Anniballi F, Austin JW. Adult intestinal toxemia botulism. Toxins. 2020;12(2):81. doi: 10.3390/toxins12020081.
[29] Lohia S, Vlahou A, Zoidakis J. Microbiome in chronic kidney disease (CKD): an omics perspective. Toxins. 2022;14(3):176. doi: 10.3390/toxins14030176.
[30] Cimolai N. Are *Clostridium difficile* toxins nephrotoxic? Med Hypotheses. 2019;126:4–8. doi: 10.1016/j.mehy.2019.03.002.
[31] Dong R, Bai M, Zhao J, et al. A comparative study of the gut microbiota associated with immunoglobulin a nephropathy and membranous nephropathy. Front Cell Infect Microbiol. 2020;10:557368. doi: 10.3389/fcimb.2020.557368.
[32] Hu C, Rzymski P. Non-photosynthetic *Melainabacteria* (cyanobacteria) in human gut: characteristics and association with health. Life. 2022;12(4):476. doi: 10.3390/life12040476.
[33] Rosario D, Bidkhori G, Lee S, et al. Systematic analysis of gut microbiome reveals the role of bacterial folate and homocysteine metabolism in Parkinson's disease. Cell Rep. 2021;34(9):108807. doi: 10.1016/j.celrep.2021.108807.
[34] Konopelski P, Mogilnicka I. Biological effects of indole-3-propionic acid, a gut microbiota-derived metabolite, and its precursor tryptophan in mammals' health and disease. Int J Mol Sci. 2022;23(3):1222. doi: 10.3390/ijms23031222.
[35] Kim JE, Kim HE, Park JI, et al. The association between gut microbiota and uremia of chronic kidney disease. Microorganisms. 2020;8(6):907. doi: 10.3390/microorganisms8060907.
[36] Parker BJ, Wearsch PA, Veloo ACM, et al. The genus *Alistipes*: gut bacteria with emerging implications to inflammation, cancer, and mental health. Front Immunol. 2020;11:906. doi: 10.3389/fimmu.2020.00906.
[37] Zhu H, Cao C, Wu Z, et al. The probiotic *L. casei* Zhang slows the progression of acute and chronic kidney disease. Cell Metab. 2021;33(10):1926–1942.e8. doi: 10.1016/j.cmet.2021.06.014.
[38] Gharaie S, Lee K, Newman-Rivera AM, et al. Microbiome modulation after severe acute kidney injury accelerates functional recovery and decreases kidney fibrosis. Kidney Int. 2023;104(3):470–491. doi: 10.1016/j.kint.2023.03.024.
[39] Bao WH, Yang WL, Su CY, et al. Relationship between gut microbiota and vascular calcification in hemodialysis patients. Ren Fail. 2023;45(1):2148538. doi: 10.1080/0886022x.2022.2148538.
[40] Gryp T, Faust K, Van Biesen W, et al. Gut microbiome profiling uncovers a lower abundance of *Butyricicoccus* in advanced stages of chronic kidney disease. J Pers Med. 2021;11(11):1118. doi: 10.3390/jpm11111118.
[41] Serrano M, Srivastava A, Buck G, et al. Dietary protein and fiber affect gut microbiome and Treg/Th17 commitment in chronic kidney disease mice. Am J Nephrol. 2022;53(8–9):646–651. doi: 10.1159/000526957.
[42] Pidcock SE, Skvortsov T, Santos FG, et al. Phylogenetic systematics of *Butyrivibrio* and *Pseudobutyrivibrio* genomes illustrate vast taxonomic diversity, open genomes and an abundance of carbohydrate-active enzyme family isoforms. Microb Genom. 2021;7(10):000638. doi: 10.1099/mgen.0.000638.
[43] Cai H, Su S, Li Y, et al. Danshen can interact with intestinal bacteria from normal and chronic renal failure rats. Biomed Pharmacother. 2019;109:1758–1771. doi: 10.1016/j.biopha.2018.11.047.
[44] Kaushal A, Noor R. Association of gut microbiota with inflammatory bowel disease and COVID-19 severity: a possible outcome of the altered immune response. Curr Microbiol. 2022;79(6):184. doi: 10.1007/s00284-022-02877-7.
[45] Satoskar AA, Parikh SV, Nadasdy T. Epidemiology, pathogenesis, treatment and outcomes of infection-associated

glomerulonephritis. Nat Rev Nephrol. 2020;16(1):32–50. doi: 10.1038/s41581-019-0178-8.

[46] MacDonald KW, Chanyi RM, Macklaim JM, et al. *Streptococcus salivarius* inhibits immune activation by periodontal disease pathogens. BMC Oral Health. 2021;21(1):245. doi: 10.1186/s12903-021-01606-z.

[47] Roux E, Nicolas A, Valence F, et al. The genomic basis of the *Streptococcus thermophilus* health-promoting properties. BMC Genomics. 2022;23(1):210. doi: 10.1186/s12864-022-08459-y.

[48] Zhang XD, Lin CX, Cui Z, et al. Mapping the T cell epitopes of the M-type transmembrane phospholipase A2 receptor in primary membranous nephropathy. Kidney Int. 2023;103(3):580–592. doi: 10.1016/j.kint.2022.11.021.

[49] Liu B, Lu R, Li H, et al. Zhen-wu-tang ameliorates membranous nephropathy rats through inhibiting NF-κB pathway and NLRP3 inflammasome. Phytomedicine. 2019;59:152913. doi: 10.1016/j.phymed.2019.152913.

[50] Santoro D, Postorino A, Costantino G, et al. Anti-TNF-α therapy in membranous glomerulonephritis. Clin Kidney J. 2012;5(5):487–488. doi: 10.1093/ckj/sfs105.

[51] Alsharhan L, Beck LHJr. Membranous nephropathy: core curriculum 2021. Am J Kidney Dis. 2021;77(3):440–453. doi: 10.1053/j.ajkd.2020.10.009.

[52] Rojas-Rivera JE, Ortiz A, Fervenza FC. Novel treatments paradigms: membranous nephropathy. Kidney Int Rep. 2023;8(3):419–431. doi: 10.1016/j.ekir.2022.12.011.

[53] Miao H, Wang YN, Yu XY, et al. *Lactobacillus* species ameliorate membranous nephropathy through inhibiting the aryl hydrocarbon receptor pathway via tryptophan-produced indole metabolites. Br J Pharmacol. 2024;181(1):162–179. doi: 10.1111/bph.16219.