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Gut microbiota can relieve proteinuria: A two-sample Mendelian analysis and genetic database mining

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Abstract: *Despite a mounting body of evidence suggesting a role for the gut microbiota in kidney diseases, the precise mechanisms underpinning this relationship remain unclear. In this context, we utilized a two-sample Mendelian randomization (MR) analysis and extensive genetic database mining to delineate the potential causal link between the gut microbiota and proteinuria. By utilizing data from European cohorts and genome-wide association studies (GWAS), we identified 14 significant instrumental variables associated with gut microbiome practices. Through rigorous statistical analyses, including MR Egger regression and Inverse Variance Weighted (IVW) method, we demonstrated a significant causal relationship between gut microbiota and proteinuria (P = 0.0004).Sensitivity analysis further corroborated the robustness of our findings. Moreover, by leveraging the Gene Expression Omnibus (GEO) database, we carried out a comprehensive analysis of differentially expressed genes in patients with membranous nephropathy, with a particular focus on those associated with proteinuria. Our findings uncovered key pathways, such as taste transduction and transporter activity, that are modulated by the gut microbiota. Furthermore, we identified specific genes, including znf784 and tmem125, whose expression is regulated by the intestinal flora and is implicated in kidney function.*

Keywords: *Gut microbiota, proteinuria, kidney diseases, two-sample Mendelian analysis*

1. Introduction

The gut microbiome plays a pivotal role in human health and affects the onset of chronic diseases, spanning from metabolic disorders to gastrointestinal issues and colorectal cancer. These conditions, which are increasingly prevalent in Western societies, impose a significant burden on healthcare systems[1].In recent years, a considerable body of evidence has emerged highlighting the significant regulatory role of gut microbiota in kidney diseases. [2-6]The underlying mechanisms and the correlations involved are currently subjects of extensive discussion and research.

Proteinuria arises from two distinct mechanisms. The first is characterized by an abnormal transglomerular transit of proteins, which is a consequence of enhanced permeability of the glomerular capillary wall. The second involves the compromised reabsorption of these proteins by the epithelial cells within the proximal tubules.[7]Proteinuria is a key biomarker in nephrology. This sentence states that proteinuria is crucial in the process of diagnosing diseases and assessing risks, and it is also the main focus of numerous significant therapeutic interventions. Etiologies resulting in pathological proteinuria include congenital and acquired disorders, as well as both glomerular (immune/non-immune mediated) and tubular defects[8].

Mendelian randomization (MR) is a statistical method that infers a causal relationship from an exposure to an outcome by utilizing genetic variants linked to the exposure as a proxy for the exposure, thereby assessing the association between the proxy and the outcome[9].Gene Expression Omnibus (GEO), NCBI's publicly available genomics database, which collects submitted high throughput gene expression data.10To further explore the causal relationship between gut microbiota and kidney disease, we will conduct a two-sample Mendelian randomization analysis of gut microbiota and proteinuria, and perform gene mining based on the GEO database.

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2. Methods

2.1 MR Verification Ideas

To identify suitable instrumental variables in a two-sample Mendelian Randomization (MR) study, this research formulates three pivotal hypotheses(Figure 1):

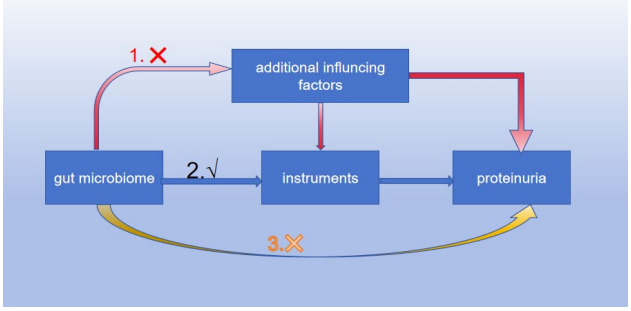

Figure 1: Mendelian Three Verification Ideas

2.2 MR Data Sources

The genetic data pertaining to the gut microbiome, which is based on probiotics, is obtained from the European Bioinformatics Institute (EBI-a-GCST90027857). This dataset includes a European cohort consisting of 555,907 individuals and encompasses 35,115,690 variants. The data concerning proteinuria are obtained from the Pan-UKB team (ukb-e-30500 AFR), which includes a sample size of 3,036 subjects and a total of 15,523,141 SNPs identified through genome-wide association studies (GWAS).The use of publicly available databases did not require additional ethical approval.

2.3 Selection of MR Instrumental Variables

In this study, instrumental variables significantly associated with the gut microbiome were pre-selected using a threshold of $p < 1 \times 10^{-5}$. A parameter setup with an r^2 threshold of 0.1 and a kilobase pair (kb) limit of 500 was implemented to reduce interference from linkage disequilibrium (LD). As a result, statistically significant SNPs associated with the gut microbiome were identified.

2.4 Statistical Analysis

This investigation primarily utilized Version 4.1.2 of the R programming language to execute a Two-Sample Mendelian Randomization (MR) analysis, employing five regression models: MR-Egger regression, Weighted median, Inverse variance weighted (IVW) regression, MR-Egger regression with a radial IVW approach, and a Simple mode regression.

2.5 Sensitivity Analysis

Sensitivity analysis primarily encompassed heterogeneity testing and the leave-one-out approach.

2.6 Differential Gene Analysis

Differential Gene Analysis (DEG) encompasses the statistical examination of gene expression data obtained from treatments in comparison to a reference, or control, group. The objective is to pinpoint gene sets that exhibit substantial alterations in expression levels, which are subsequently referred to as differentially expressed gene sets. This investigation performed a Differential Gene Analysis (DEG) on patients with membranous nephropathy (with proteinuria as the index) and control groups, utilizing the data from the GEO database. The aim was to identify key genes linked to the onset of membranous nephropathy in patients (with proteinuria as the index) and to explore their correlation with the gut microbiome. Microarray experiments using a single-color labeling technique were conducted on peripheral blood mononuclear cells (PBMCs) from a total of eight patients diagnosed with membranous nephropathy, as well as from a pool of healthy subjects, by Nagasawa Y et al. (GSE73953).

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3. Results

3.1 Instrumental Variables

Subsequent to comparing the data with confounding variables such as age, gender, and family history of hereditary diseases, and computing their respective F-values ($F = 0.736$, $p > 0.05$), it was determined that these factors did not contribute Significantly to the model. In the present study, a total of 14 significant instrumental variables were ultimately identified. The causal effect of exposure on the outcome is estimated via the Wald ratio for each SNP individually, and these estimates are depicted in a forest plot. Additionally, the MR estimates derived from all SNPs utilizing the MR Egger and IVW methods are presented. A regression model intercept test was carried out, resulting in a regression intercept coefficient of $b = -0.012$ and a P-value of 0.813 for gut microbiome SNPs(Table 1), suggesting no horizontal pleiotropy ($P > 0.05$). Consequently, the selected SNPs do not exhibit genetic pleiotropy, making Mendelian Randomization an effective approach for causal inference in this study. (Refer to Table 2 for details.)

Table 1: Intercept Test of Regression Model

Method	Variable	Intercept	Standard Error	P
MR-Egger	gut microbiome	-0.012	0.049	0.813

3.2 Mendelian Randomization Analysis Results

The effects of SNPs on the outcome are depicted in relation to their effects on the exposure (only SNPs with negative effects on the exposure are shown, with the sign of the effect on the outcome reversed). The slope of the line signifies the causal relationship, and each method corresponds to a distinct line. The Egger estimate is the only line that does not pass through the origin automatically. Utilizing the IVW method, instrumental variables were identified as significantly associated with proteinuria. Following multiple-method correction, as presented in Table 2, the directions of the Betas were found to be consistent. Consequently, the results from the IVW method should be considered definitive. The data indicates a significant causal correlation, with a p-value of 0.0004 ($P < 0.05$). The outcomes of the multiple regression analysis are represented in Figure 2.

Table 2: Mendelian Randomization Analysis Results

Method	SNP Quantity	b	se	Pval
MR Egger	14	-0.05226	0.3091	0.8685
Weighted median	14	-0.1117	0.08887	0.2088
Inverse variance weighted	14	-0.1249	0.06964	0.07287
IVW radial	14	-0.125	0.03579	0.0004
Simple mode	14	-0.1152	0.1413	0.4294

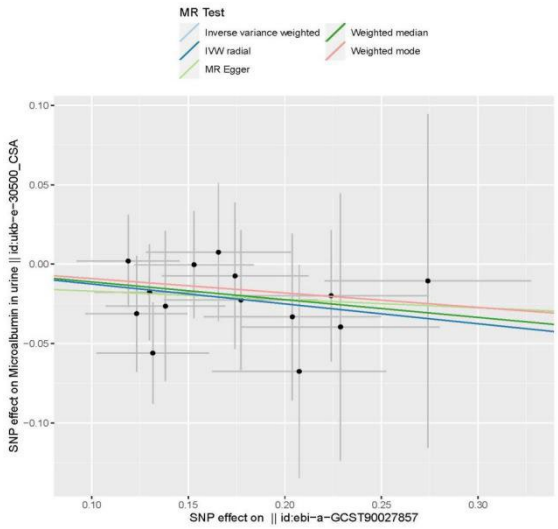

Figure 2: Regression Analysis Graph under Different Statistical Methods

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3.3 Sensitivity Analysis

Sensitivity analysis was performed using the leave-one-out approach (see Figure 3). When the gut microbiome was treated as the exposure factor, the results remained consistently below the zero line and aligned with the null hypothesis upon exclusion of each SNP individually. This demonstrates the robustness of the findings. The funnel plot in Figure 4revealed no notable outliers, providing additional evidence for the stability of the results.

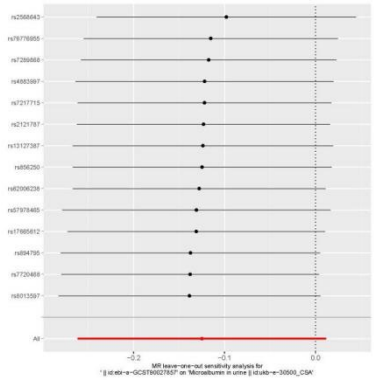

Figure 3: Leave-One-Out Method

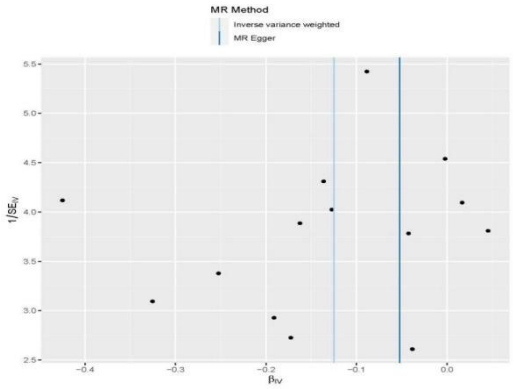

Figure 4: MR Funnel Plot

3.4 Differential Gene Expression Results

This study performed a screening of differentially expressed genes between membranous nephropathy patients and control groups, utilizing the GEO database as a resource(Figure 6). A bubble chart is employed to visualize the analysis outcomes. The findings are depicted in Figure 5, where larger bubble sizes and darker colors signify higher levels of differential expression. Key significant pathways, responsive to stimuli, and other principal discoveries are summarized in Table 3,4.

Table 3: Main factors about gene expression

Name	up/down	Associations with gut microbiome	References
Taste transduction	up	Metabolite Production: (e.g.SCFAs)	[10-12]
		Modulation of Taste Receptors	
		Impact on Satiety and Appetite	
		Neurotransmitter Production(like serotonin)	
Symporter activity	up/down	nutrient competition and syntrophy	[13-16]
		Short-Chain Fatty Acids and Bile Acids:host symporter activity	
		Quorum Sensing and Bacterial Metabolites:like indole	
		Immune Response and Barrier Function:the activity,localization	

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		Enzymatic Activity	
Secondary active transmembrance transporter activity	Up/down	ditto	
Salmonella infection	down	Microbial Competition	[17-18]
		Maintaining Epithelial Integrity	
		Blocking Adhesion Sites	
Amytrophic lateral sclerosis	down	Gut-Brain Axis	[19-20]
		Short-Chain Fatty Acids (SCFAs)	
		Neurotoxin Production	
		Energy Homeostasis	

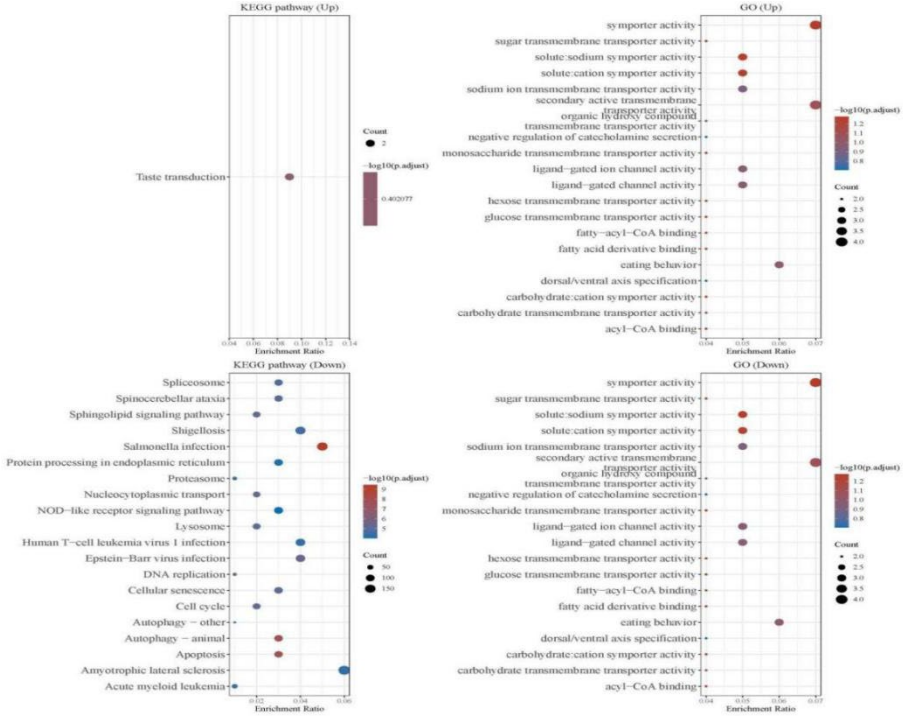

Figure 5: KEGG analysis

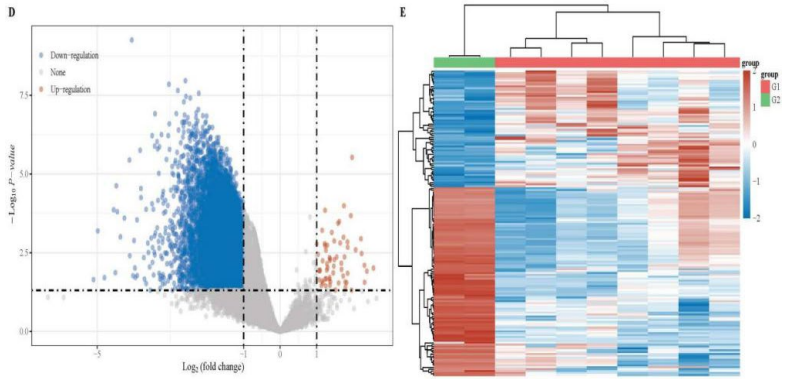

Figure 6: Volcano Plot of Differentially Expressed Genes

Table 4: Differential Gene Expression

Genes	up/down	Function	Reference
znf784	up	belongs to the family of Krüppel-like zinc finger proteins,nonfunctional	[21]
tmem125	up	cell signaling, membrane trafficking, or ion transport,regulated by intestinal flora	[22]
ccdc42	up	localize various cellular compartments, including the cytoplasm and possibly the cell membrane	[23]

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six5	up	a transcription factor involved in embryonic development and muscle function	[24]
asah2	up	responsible for encoding acid ceramidase, and its expression and activity are subject to regulation in different tissues	[25]
mstn	down	Myostatin is a protein that inhibits muscle growth	[26]
ccm2l	down	Mutations in the CCM2L gene have been associated with familial forms of cerebral cavernous malformations	[27]
fam168b	down	play a role in cell proliferation, differentiation, and apoptosis (programmed cell death)	[28]
rad51ap1	down	plays a critical role in the homologous recombination (HR) pathway	[29]
C8b	down	known as complement component 8 beta chain,Participate in immune process	[30]

4. Conclusions

Through Mendelian randomization experiments, we have discovered that, using European samples as an example, the gut microbiome functions as a direct mitigating factor for proteinuria. The gut microbiome, free from the influence of other confounding factors, acts as a direct causal factor in preventing proteinuria, exhibiting a high level of significance.Simultaneously, at the genetic and pathway levels, it also demonstrates regulatory potential. However, this study, which relies on genetic samples from European populations, still carries certain limitations, as indicated by statistical analysis revealing a highly significant causal relationship.

Disruption of barrier function can alter the transcellular and paracellular transport of molecules, thereby impacting the expression and activity of transport proteins. The modulation of host signaling pathways involves the activation of signaling cascades by gut microbiota-derived molecules, such as microbe-associated molecular patterns (MAMPs) and metabolites. These signaling molecules interact with nuclear receptors and transcription factors, which, in response, regulate the expression of transport proteins crucial for nutrient uptake, xenobiotic transport, and maintenance of ion homeostasis. Recently, a growing body of evidence has indicated that the gut microbiota may mitigate the progression of Amyotrophic Lateral Sclerosis (ALS), a finding that starkly contrasts with the etiology of proteinuria. The pivotal functions attributed to the gut microbiota are intimately associated with the expression of key genes identified through screening. These genes are subject to modulation and regulation by the gut microbiota in various critical domains, including apoptosis, mutation in gene expression, immune response, ion channel function, and the onset of cancer. We confidently predict that the alleviation of proteinuria by the gut microbiota is intricately linked to the activation and modulation of these critical genes. However, additional research and empirical evidence from experimental studies are essential to validate and expand upon this hypothesis.

This investigation indicates that probiotics can potently mitigate proteinuria; however, the accuracy and efficacy of the mediation by related genes and pathways remain a significant area for future exploration.

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