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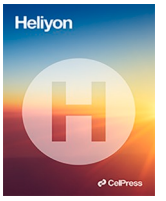

Research article

Integrative genomic analysis of RNA-modification-single nucleotide polymorphisms associated with kidney function

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A R T I C L E I N F O

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A B S T R A C T

Introduction: Increasing evidence suggests that RNA modification plays a significant role in the kidney and may be an ideal target for the treatment of kidney diseases. However, the specific mechanisms underlying RNA modifications in the pathogenesis of kidney disease remain unclear. Genome-wide association studies (GWAS) have identified numerous genetic loci involved in kidney function and RNA modifications. The identification and exploration of RNA modification-related single-nucleotide polymorphisms (RNAm-SNPs) associated with kidney function can help us to comprehensively understand the underlying mechanism of kidney disease and identify potential therapeutic targets.

Methods: First, we examined the association of RNAm-SNPs with eGFR. Second, we performed expression quantitative trait locus (eQTL) and protein quantitative trait locus (pQTL) analyses to explore the functions of the identified RNAm-SNPs. Finally, we evaluated the causality between RNAm-SNP-associated gene expression and circulating proteins and kidney function using a Mendelian randomization (MR) analysis.

Results: A total of 252 RNA m-SNPs related to m^6A, m^1A, A-to-I, m^5C, m^7G, and m^5U were identified. All these factors were significantly associated with the eGFR. A total of 119(47.22 %) RNAm-SNPs showed cis-eQTL effects in blood cells, whereas 72 (28.57 %) RNAm-SNPs showed cis-pQTL effects in plasma. 47 (18.65 %) RNAm-SNPs exhibited cis-eQTL and cis-pQTL effects. In addition, we demonstrated a causal association between RNAm-SNP-associated gene expression, circulating protein levels, and eGFR decline. Some of the identified genes and proteins have been reported to be associated with kidney diseases, such as CDK10 and SDCCAG8.

Conclusions: This study reveals an association between RNAm-SNPs and kidney function. These SNPs regulate gene expression and protein levels through RNA modifications, eventually leading to kidney dysfunction. Our study provides novel insights that connect the genetic risk of kidney disease to RNA modification and suggests potential therapeutic targets for the prevention and treatment of kidney disease.

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1. Introduction

Kidney diseases are highly complex. Diabetes and hypertension are the primary causes of kidney disease are diabetes and hypertension [1]. The prevalence of kidney disease is high and is increasing globally owing to the increasing prevalence of diabetes and hypertension. The glomerular filtration rate (GFR) is currently the best indicator of kidney function [2]. Although kidney diseases often progress slowly, patients with kidney disease have a lower quality of life than the general population. As GFR declines, patients experience nephron loss and develop chronic kidney disease (CKD). CKD is a major cause of death [3]. Genetic predispositions have been well documented in patients with renal diseases. The heritability of renal disease ranged 25–44 % in a large analysis of medical records [3]. Genome-wide association studies (GWAS) have rapidly become a major focus for studying the genetics of diseases. In recent years, GWAS of renal disease and kidney function-related traits have resulted in striking gains [4].

RNA modification, a new frontier of epigenetic changes, has attracted considerable attention. Emerging evidence indicates that RNA modifications are strongly associated with critical biological functions and disease pathways [5]. To date, more than 170 different types of RNA modifications have been characterized in living organisms [6]. N^6-methyladenosine (m^6A) methylation has received considerable attention. m^6A modifications have been recently widely reported to play important roles in kidney diseases, particularly diabetic kidney disease [7]. There is growing evidence that other types of RNA modifications play important roles in disease pathogenesis, which may imply the potential for repurposing RNA modifications as targets for the prevention and treatment of kidney disease. However, the association between kidney function and RNA modifications has not been systematically evaluated because of the complexity of RNA modifications.

Variants affecting RNA modifications can influence RNA modifications by substituting the nucleotide at the modified position or by changing the nucleotide sequence in the proximal flanking region [8]. RNA modification-related single-nucleotide polymorphisms (SNPs; RNAm-SNPs) play a critical role in several diseases. Focusing on the interplay between the genetics of kidney function and RNA modifications will provide a novel perspective for identifying novel therapeutic targets for kidney diseases. The goal of this study was to assess the effects of RNA mSNPs on kidney function. In addition, expression quantitative trait loci (eQTL), quantitative trait loci (pQTL), and Mendelian randomization (MR) analyses were performed to identify putative causal genes and circulating proteins.

2. Methods

2.1. Data sources and determination of RNAm-SNPs for kidney function

In this study, we used summary-level data from a large-scale multiracial study to identify potential functionally related RNAm-SNPs. Stanzick et al. performed a meta-analysis on kidney function (glomerular filtration rate estimated by serum creatinine (eGFRcrea)), including 1,201,909 individuals obtained from the CKDGen and Pan-UK Biobank. They identified several independent loci, most of which were validated using eGFR based on cystatin C levels (eGFRcys) and/or blood urea nitrogen (BUN). The data are publicly available online at the CKDGen [9] (https://ckdgen.imbi.uni-freiburg.de, accessed on 04/01/2023).

RNAm-SNP data are available in the online database at http://rmvar.renlab.org (accessed 12/01/2023) [10]. The RMVar database of functional variants involved in RNA modifications can continually update RMVar and provide important RM-associated variant information. Currently (accessed on 12/01/2023), RMVar contains 1,678,126 RNA modification-associated variants. It contains several common RNA modifications: N^6-methyladenosine (m^6A), N^6-dimethyladenosine (m^6Am), N^1-methyladenosine (m^1A), pseudouridine (ψ), 5-methylcytosine (m^5C), ribose methylations (2'-O-Me), 7-methylguanosine (m^7G), 5-methyluridine (m^5U), and adenosine-to-inosine (A-to-I). These RNAm SNPs were divided into low-, median-, and high-confidence levels according to the different levels of RNA modification site confidence.

To select the RNAm-SNPs associated with kidney function, we annotated the GWAS-tested SNPs with RNA modifications in the GWAS summary dataset using the "merge" function of the R program. Next, the data containing the eGFR-associated SNPs were combined with the RNAm-SNP annotation files based on the "rsID" and the RNAm-SNPs associated with kidney function were screened out ($p < 5.0 \times 10^{-8}$).

SNP positions and their genomic region information were annotated in the human genome build GRCh37 (hg19) using ANNOVAR [11]. ANNOVAR is freely available at http://www.openbioinformatics.org/annovar/(accessed 12/01/2023).

2.2. eQTL analysis for kidney function-associated RNAm-SNPs

RNA modifications have recently emerged to play a critical role as post-transcriptional regulators of gene expression. Therefore, we hypothesized that eGFR-associated RNAm-SNPs can influence kidney function by affecting gene expression.

To confirm the association between the eGFR-associated RNAm-SNPs and mRNA expression levels in several types of tissues, we performed gene expression quantitative loci (eQTL) analysis ($p < 5 \times 10^{-8}$). eQTL are genetic variants that have genotype-specific effects on gene expression levels and can be divided into two categories: cis-eQTL and trans-eQTL. Only cis-eQTLs (within 1 Mb of their target gene) were used in this study to minimize the possibility of pleiotropy, while maximizing the possibility of causality. We searched for eQTL signals from the GTEx project (v8 release) (https://www.gtexportal.org/home/datasets, accessed 12/01/2023) and Westra eQTL summarized data (http://www.genenetwork.nl/bloodeqtlbrowser, 12/01/2023) [12,13]. Next, we evaluated the association between gene expression levels in eight relevant tissues from the GTEx project: whole blood ($n = 670$). The Westra eQTL contains eQTL information from the peripheral blood of 5311 European healthy individuals and 2775 European individuals in replication. We used the summary-data-based Mendelian randomization (SMR) method, which can integrate summary-level data from

kidney function GWAS with data from eQTL studies, to identify genes whose expression levels are associated with the kidney function phenotype due to pleiotropy [14,15].

2.2.1. SMR and heterogeneity in dependent instruments (HEIDI) analysis

Three primary assumptions were made in the SMR and HEIDI analyses. First, the cis-eQTLs used as IVs were significantly correlated with gene expression and reached the genome-wide significance threshold (Assumption 1). Second, the IVs were independent of the confounding factors (Assumption 2). Third, IVs should only affect kidney function through gene expression, and other pathways or routes are unavailable (Assumption 3). We used two eQTL datasets, Westra eQTL and GTEx v8 (whole blood) eQTL data. The eQTL summary data in SMR binary format can be downloaded from http://cnsgenomics.com/software/smr/download.html (accessed on 12/01/2023).

We conducted SMR analysis using SMR software (https://yanglab.westlake.edu.cn/software/smr/#Download). Detailed information regarding the SMR method can be found in a previous publication: cis-regions with a window of 2000 Kb [14]. We used the genome-wide significance level for SMR ($p < 5 \times 10^{-6}$) and HEIDI (heterogeneity in dependent instruments) to test for heterogeneity. Genes without heterogeneity (PHEIDI>0.05) were considered statistically significant.

2.3. Differential expression analysis

Three gene expression datasets (GSE37171) were obtained from the Gene Expression Omnibus (GEO) database. GSE37171 includes peripheral blood mononuclear cell (PBMC) expression data from uremic patients and 20 healthy controls [16]. We used t-tests to assess differential expression of eGFR-related genes.

2.4. pQTL analysis for kidney function-associated RNAm-SNPs

pQTL analysis was used to determine the functional potential of the identified eGFR-associated RNAm-SNPs to identify their functional potentials. Only $p < 5 \times 10^{-6}$ of cis-pQTL signals were considered in this study. Summary of cis-pQTL data were obtained from two proteomic GWAS. The discovery cohort was the pQTL GWAS by Zhang et al., who identified 2004 proteins in 7213 European American populations using modified aptamers (SOMAmer reagents) [17]. Summary statistics for this study are available from the ARIC Consortium (http://nilanjanchatterjeelab.org/pwas/; accessed 04/01/2023). To replicate our findings, we used the largest pQTL GWAS from a DeCODE study to date. Ferkingstad et al. [18]. measured the plasma protein abundance using 4907 aptamers in 35,559 individuals using the SomaScan assay and 4719 plasma proteins were measured. Summary statistics for this study are available on the deCODE genetics website (https://www.decode.com/, accessed 04/01/2023).

2.5. Two-sample Mendelian randomization (TSMR)

We estimated the effect of kidney function-associated RNAm-SNPs on circulating protein levels identified by pQTL analysis using two-sample MR on a proteome-wide scale. First, SNPs associated with exposure at the genome-wide significance level ($p < 5 \times 10^{-6}$) were selected as IVs. We then excluded SNPs in linkage disequilibrium (LD) ($r^2 < 0.01$ within a 10,000 kb distance) to ensure that the instruments used for exposure were independent of each other. Finally, we performed inverse-variance weighting (IVW), MR-Egger, weighted median, and weighted modes. The IVW was used as the primary method for causal estimation. In addition, the MR-Egger regression method was used to identify and adjust for pleiotropy. The intercept representation of the MR-Egger regression was used to estimate horizontal pleiotropy. The presence of horizontal pleiotropy is indicated by $p > 0.05$ [19]. Heterogeneity between the estimates for each SNP was evaluated using the Cochran's Q test. If significant heterogeneity existed among the selected IVs, the IVW with multiplicative random effects (IVW-MRE) was used [20]. We used R 4.2.2 to perform our statistical analysis and TSMR analysis was performed using the "TwoSampleMR" R package.

Table 1
Number of each type of RNAm-SNPs examined in this study.

RNA modification types	Number of SNPs found in eGFR GWAS	Number of SNPs with $p < 0.05$	Number of SNPs with $p < 5 \times 10\text{-}8$
m6A	13898	2427	218
m6Am	13	1	0
m1A	728	190	15
m5C	73	22	5
m5U	5	1	1
m7G	210	41	5
2′-O-Me	4	1	0
A-to-I	491	102	11
Pseudouridine	3	3	0

RNAm-SNP: RNA modification-associated single-nucleotide polymorphism; GWAS: genome-wide association study; eGFR: estimated glomerular filtration rate.

3. Results

3.1. Kidney function-associated RNAm-SNPs

We identified 252 RNAm-SNPs that were significantly associated with eGFR (eGFR-RNAm-SNPs) at $p < 5.0 \times 10^{-8}$, containing six types of RNA modifications, including 218 m^6A, 15 m^1A, 11 A-to-I, 5 m^5C, 5 m^7G, and 1 m^5U (Table 1, Supplementary Table S1, Figs. 1 and 2). We noticed that the three eGFR-RNAm-SNPs were related to two types of RNA modification. rs897172 ($p = 1.285 \times 10^{-27}$) in ORC4 was associated with both m^6A and m^1A. rs2229503 ($p = 1.738 \times 10^{-10}$) in SPTBN1 was related to both m^6A and m5C. rs34317102 ($p = 1.855 \times 10^{-9}$) in LACTB was associated with both m^6A and m^1A. Among the 252 SNP identified, 107, 85, and 62 belonged to high-, medium-, and low-confidence regions, respectively. Based on the annotation information of the SNP (hg19), 227 (90.08 %) eGFR-RNAm-SNPs mapped to 199 protein-coding genes, and 23 (9.13 %) mapped to lncRNAs or pseudogenes. The 227 identified SNPs located in the protein-coding genes were primarily located in introns (97, 42.73 %), UTR (75, 33.04 %), CDS (36, 15.86 %), exons (20, 8.81 %), and upstream (7, 3.08 %) and downstream (5, 2.2 %) regions. The top 20 most significant eGFR-RNAm-SNPs located in protein-coding genes are listed in Table 2. Among these 20 SNPs, 17 were related to m^6A, one to A-to-I, one to m^1A, and one to m^7G. We noticed that rs7210488 and rs10491129 showed strong linkage disequilibrium in FBXL20 ($r^2 = 0.928$).

m^6A-SNPs are the important genetic functional variants, we found that the number of eGFR-associated SNPs related to m^6A ($n = 218$, 86.51 %) was the largest (Supplementary Table S1). These m^6A-SNPs were of three confidence levels: 80 (36.7 %) had high confidence, 53(24.31 %) had medium confidence, and 85(38.99 %) had low confidence. Most of the eGFR-m^6A-SNPs (190; 87.16 %) were located in protein-coding genes. Among these, 92 (48.42 %) were introns, 39 (20.53 %) were in the UTR, 30 (15.79 %) were in the CDS, and 29 (15.26 %) were exons. Notably, the m^6A-SNP rs9895661 in the intron of BCAS3 had the strongest association signal ($p = 2.51 \times 10^{-114}$). This SNP is a functionally loss m^6A SNP and belongs to the low confidence type. Notably, some significant m^6A-SNPs in key inherited kidney disease (IKD) susceptibility genes were identified [21], including rs10193972 ($p = 4.899 \times 10^{-74}$) and rs142800387 ($p = 2.152 \times 10^{-43}$) in ALMS1, rs9572787 ($p = 5.5 \times 10^{-23}$) in DACH1, rs7210 ($p = 1.753 \times 10^{-41}$) in TPRKB, rs56076827 ($p = 3.57 \times 10^{-20}$) in IFT172 and rs2275155 ($p = 5.569 \times 10^{-19}$) in SDCCAG8.

For the m^1A-SNPs, we identified 15 SNPs that were significantly associated with eGFR, all of which were functionally lost (Supplementary Table S1). Ten m^1A-SNPs belonged to the high-confidence group and five m^1A-SNPs belonged to the medium confidence group. We observed that rs2952151 in PGAP3 ($p = 7.35 \times 10^{-34}$) had the highest signal among all m^1A-SNPs. For m^7G modification, five functional loss m^7G-SNPs with medium confidence were identified. In addition, rs7262393 in LAMA5 is susceptible to IKD. Eleven A-to-I-SNPs, five m^5C-SNPs, and one m^5U-SNPs were significantly associated with eGFR, and all were associated with functional loss (Supplementary Table S1).

3.2. Gene expression analysis

We investigated whether RNA mSNPs are associated with gene expression. eQTL analysis ($p < 5.0 \times 10^{-8}$) was performed for the identified RNAm-SNPs associated with eGFR. In total, 271 cis-eQTL signals were identified for the 119 eGFR-RNAm-SNPs (Supplementary Table S2). Forty-one SNPs were associated with host gene expression. Among the 119 RNAm-SNPs with cis-eQTL effects, 101 (84.87 %) were related to m^6A, nine (7.56 %) to m^1A, seven (5.88 %) to A-to-I, two (1.68 %) to m^7G, and two (1.68 %) to m^5C. A total of 100 RNAm-SNPs showed cis-eQTL effects in whole blood and 56 in peripheral blood. rs526897 in CNOT9 showed the largest number of cis-eQTL signals in blood ($n = 9$). Twenty-nine RNAm-SNPs showed the same cis-eQTL effects in whole and peripheral blood, and rs2295443 in PIGU showed significant cis-eQTL signals in the peripheral blood, which could affect MAP1LC3A ($P_{eQTL} = 2.019 \times 10^{-200}$).

After SMR testing, 178 gene associations with eGFR were identified in whole blood (Bonferroni correction, $p < 7.48 \times 10^{-6}$, 0.05/6682), and 124 did not pass the HEIDI test. Of the remaining 54 genes, 46 were protein-coding genes, five were lncRNA genes, and three were pseudogenes. A total of 210 gene associations with eGFR were identified in the peripheral blood (Bonferroni correction, $p < 8.42 \times 10^{-6}$, 0.05/5939); 146 did not pass the HEIDI test, and the remaining 64 genes were coding genes. Finally, we identified 14 genes that were significantly associated with the 21 RNAm-SNPs were identified were detected (Supplementary Table S3). These RNAm-SNPs may be causal variants that affect both gene expression and eGFR in the blood. The most common signal-associated

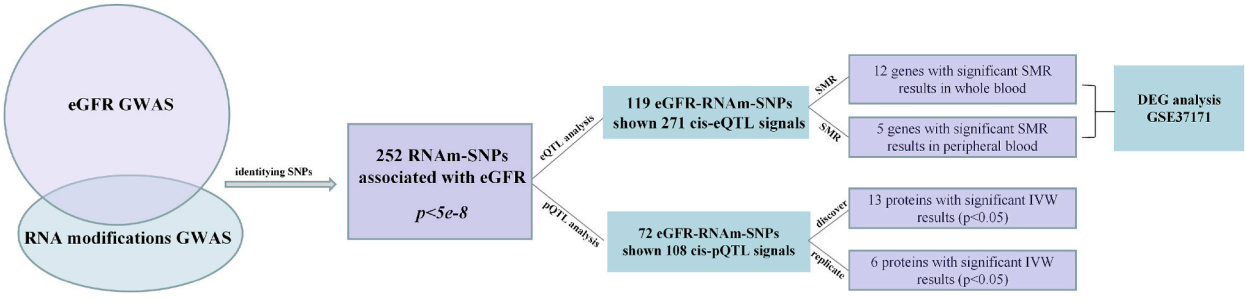

Fig. 1. Flow chart of study design and the main results.

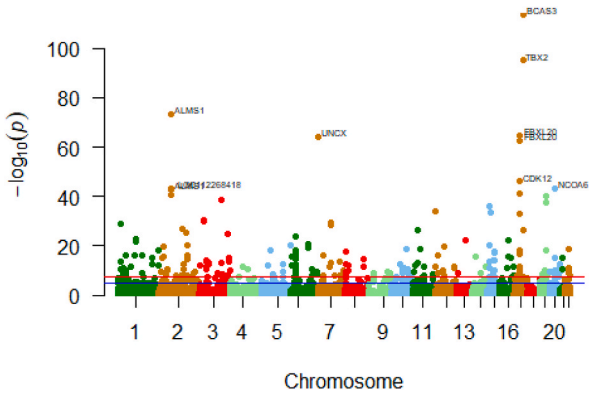

Fig. 2. Genome-wide identified RNAm-SNPs associated with eGFR.
This Manhattan plot shows the associations between RNAm-SNPs and kidney function of 15425 variants. The x-axis indicates chromosome positions. The y-axis indicates -$\log_{10}$ p-values of the association. The solid black and red line as suggestive threshold indicates the genome-wide significance level of 5.0×10^{-5} and 5.0×10^{-8}, respectively. Genes containing the top 10 most significant RNAm-SNPs were annotated. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

protein-coding gene was LACTB ($P_{SMR} = 9 \times 10^{-12}$). We also noticed that the m^{6}A-SNP rs2275155 in SDCCAG8 was the top cis-eQTL.

Three signals were replicated using the following datasets: CDC14A, LACTB, and C8orf58. The m^{6}A-SNP rs529224 in CDC14A was associated with CDC14A mRNA levels ($P = 4.40 \times 10^{-18}$ in whole blood and $P = 4.55 \times 10^{-78}$ in peripheral blood) (Fig. 3A–D). The A-to-I-SNP rs2729823 in LACTB was associated with LACTB mRNA levels ($P = 2.36 \times 10^{-11}$ in whole blood and $P = 9.79 \times 10^{-155}$ in peripheral blood) (Fig. 4A–D). The m^{6}A-SNP rs3735894 in PDLIM2 and the m^{6}A-SNP rs1869 in CCAR2 were associated with C8orf58 mRNA levels (in whole blood, $P = 8.5 \times 10^{-10}$ and $P = 1.25 \times 10^{-9}$, respectively; in peripheral blood, $P = 4.36 \times 10^{-13}$ and $P = 1.09 \times 10^{-9}$, respectively) (Fig. 5A–F). For the three genes identified in the SMR analysis according to the two blood eQTL datasets, we compared their expression levels in the peripheral blood of patients with uremia and controls in the GSE37171 dataset. The expression of CDC14A and LACTB was significantly decreased in the PBMCs of uremic patients compared to that in healthy controls (NC). ($p < 0.001$ and $p < 0.001$, respectively) (Fig. 6).

3.3. pQTL analysis and TSMR

In the discovery cohort, we identified 108 pQTL signals from 72 RNAm-SNPs involving 60 proteins (Supplementary Table S4). A total of 91 signals were detected for m^{6}A, ten for m^{1}A, three for A-to-I, three for m^{5}C, and one for m^{7}G. The association between m^{6}A-SNP rs4687552 and ITIH1 ($p = 8.954 \times 10^{-301}$) was followed by that between m^{6}A-SNP rs3755806 and ITIH3 ($p = 3.382 \times 10^{-276}$). We tested whether these 60 proteins were genetically associated with the eGFR using TSMR. IVW analysis showed that 13 proteins were causally associated with kidney function (Table 3).

Furthermore, we performed a TSMR analysis for cross-validation using the deCODE study data. First, 13 proteins were selected from the discovery cohort. Second, 14 proteins, including BIN3, C8orf58, CCHCR1, CDC14A, CDK10, DNAJC8, LACTB, MAST2, MBD5, MUSTN1, SLC25A21, SLC7A6, DHX36, and SDCCAG8, were also considered because the expression of these protein-coding candidate genes was associated with eGFR in our SMR analysis. We assessed whether these 27 proteins were genetically associated with the eGFR using several MR methods (weighted median, IVW, MR-Egger, weighted mode, and IVW-MRE). In the IVW analyses, we found a causal relationship exists between 6 proteins and eGFR six proteins, including BTN3A3, COL2A1, INHBC, ITPA, PFKM, and PRSS3 (Table 4 and Fig. 7). The associations between circulating protein levels of COL2A1, INHBC, PFKM, and PRSS3 were significant in the analyses using all four methods. Indeed, five RNAm-SNPs that were significantly associated with eGFR were also significantly associated with circulating levels of COL2A1, INHBC, PFKM, and PRSS3. The m^{6}A-SNP rs2732484 is associated with plasma levels of COL2A1. The A-to-I-SNP rs540730 was associated with plasma levels of INHBC. The m^{6}A-SNP rs2732484 is associated with plasma levels of PFKM. The m^{6}A-SNP rs2005617 and m^{1}A-SNP rs307658 were associated with plasma levels of PRSS3.

4. Discussion

The present study examined the association between RNA modifications and eGFR by conducting a joint association study using two large-scale GWAS. The eGFR-associated RNAm-SNPs identified by the GWAS were related to several RNA modification types, including m^{6}A, m^{1}A, A-to-I, m^{5}C, m^{7}G, and m^{5}U. Our findings indicated that RNA modifications may play a role in kidney function. Notably, some eGFR-associated RNAm-SNPs showed eQTL effects in blood or kidney tissues, or pQTL effects in blood tissues, which can regulate gene or protein expression. We used eQTL-based SMR, eQTL-based coloc, and pQTL-based TSMR to assess causal relationships. Our findings provide noteworthy evidence for the existence of a causal relationship between RNA modification of genes or proteins and kidney function. To the best of our knowledge, this is the first MR study to evaluate the potential causal relationship between eGFR and RNA modification of genes or proteins. A particularly interesting and novel finding was the identification of risk

Table 2
Top 20 RNAm-SNPs associated with eGFR.

SNP	P_eGFRcrea GWAS	Chromosome	Position	Modification type	Gene	Gene region	Mutation	Confidence level	Function
rs9895661	2.51E-114	17	59456589	m^6A	BCAS3	intron		Prediction:(Low)	Functional Loss
rs8074151	1.03E-95	17	59485017	m^6A	TBX2	intron		Prediction:(Low)	Functional Gain
rs10193972	4.889E-74	2	73717656	m^6A	ALMS1	CDS	missense	MeRIP-seq:(Medium)	Functional Loss
rs7210488	2.402E-65	17	37539819	m^6A	FBXL20	intron		m6A-Label-seq:(High)	Functional Loss
rs10491129	2.048E-63	17	37461643	m^6A	FBXL20	intron		m6A-Label-seq:(High)	Functional Loss
rs8069074	1.025E-46	17	37685401	m^6A	CDK12	intron		MeRIP-seq:(Medium)	Functional Loss
rs2295354	6.124E-44	20	33356541	m^6A	NCOA6	intron		m6A-Label-seq:(High)	Functional Loss
rs142800387	2.152E-43	2	73693466	m^6A	ALMS1	intron		MeRIP-seq:(Medium)	Functional Loss
rs1877031	5.684E-42	17	37814080	m^7G	STARD3	exon	missense	MeRIP-seq:(Medium)	Functional Loss
rs7210	1.753E-41	2	73957124	m^6A	TPRKB	intron	synonymous	Prediction:(Low)	Functional Gain
rs55743020	1.228E-40	19	38188233	m^6A	ZNF607	intron		MeRIP-seq&m6A-Seal-seq:(Medium)	Functional Loss
rs9942	1.805E-39	3	141663752	m^6A	TFDP2	3'UTR		Prediction:(Low)	Functional Gain
rs8111790	1.614E-38	19	38056763	m^6A	ZNF571	intron	missense	MeRIP-seq:(Medium)	Functional Loss
rs632887	1.756E-34	12	3392351	m^6A	TSPAN9	3'UTR		MeRIP-seq:(Medium)	Functional Loss
rs11070458	1.963E-34	15	45771751	m^6A	SLC30A4	intron		Prediction:(Low)	Functional Loss
rs2952151	7.35E-34	17	37828496	m^1A	PGAP3	3'UTR		m1A-quant-seq:(High)	Functional Loss
rs7372545	2.917E-31	3	38507570	m^6A	ACVR2B	intron		m6A-Label-seq:(High)	Functional Loss
rs4147025	8.688E-31	3	38456531	m^6A	XYLB	intron		MeRIP-seq:(Medium)	Functional Loss
rs10441228	5.783E-30	7	77370662	A-to-I	RSBN1L	intron		RNA-Seq:(High)	Functional Loss
rs12741552	1.542E-29	1	15853276	m^6A	DNAJC16	upstream		m6A-Label-seq:(High)	Functional Loss

RNAm-SNP: RNA modification-associated single-nucleotide polymorphism; eGFR: estimated glomerular filtration rate.

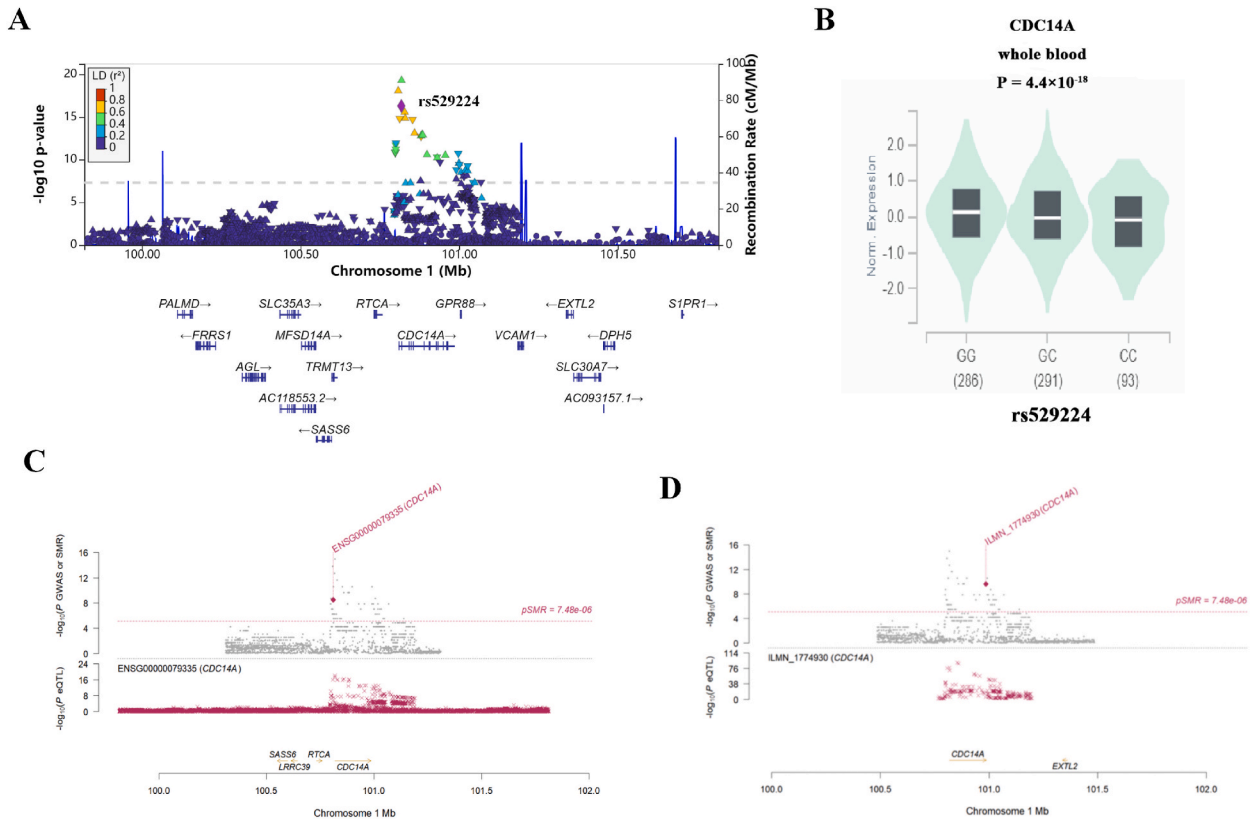

Fig. 3. Association between the CDC14A gene and eGFR.
A. The m^{6}A-SNP rs529224 in the CDC14A gene was associated with eGFR.
B. The C allele carriers of rs529224 had lower mRNA expression levels of CDC14A in whole blood.
C-D. The expression levels of the CDC14A gene in whole blood (C) and peripheral blood (D) were associated with eGFR.

genes and circulating proteins related to RNA modification in CKD. Our study provides new evidence regarding the potential pathogenesis of kidney diseases.

In recent years, there has been an increase in the discovery and application of GWAS. These results have made key contribute to a deeper biological understanding of kidney diseases. Liu et al. reported the results of the largest meta-analysis of kidney function, comprising 1.5 million samples. Although numerous kidney disease variants have been identified, only a few seem to have been seriously accounted for. RNA modifications are involved in diverse cellular and biological processes and can affect various steps including transcription, splicing, stability, transport, and translation. These modifications can affect the processing, stability, and function of RNA molecules, ultimately leading to changes in gene and protein expression levels, which may be key regulatory mechanisms in kidney disease [5,22]. The annotation of genetic variants for their functional consequences on RNA modifications is necessary to further investigate the potential pathogenesis of RNA modifications. The m^{6}A modification is the most abundant modification in eukaryotic mRNA and has been studied in several diseases, including renal diseases [23–25]. In recent years, other types of RNA modifications have been widely investigated in cancers; however, the role of RNA modifications in renal diseases has not been extensively considered. In this study, we integrated kidney function GWAS data with information from the RMVar database and identified 370 RNAm-SNPs associated with kidney function. Six types of RNA modifications were involved in these RNAm-SNPs: m^{6}A, m^{1}A, A-to-I, m^{5}C, m^{7}G, and m^{5}U.

We also observed that most of the identified SNPs were located in non-coding regions. Although most genetic studies have focused on protein-coding regions, non-coding regions, including regulatory and intergenic regions, also play important role [26–28]. A deeper understanding of the functions of the non-coding regions could significantly contribute to our understanding of the underlying mechanisms of diseases [29,30]. There is growing evidence that SNPs located in non-coding regions can influence gene expression by regulating regulatory sequences or elements of genes. Therefore, we explored the potential functions of all the identified RNAm-SNPs using public databases and determined the causal relationship between these variants and eGFR. In eQTL analysis, 223 eGFR-RNAm-SNPs related to m^{6}A, m^{1}A, A-to-I, m^{7}G, m^{5}C, or m^{5}U showed cis-eQTL signals. This confirms that some RNA mSNPs could affect gene expression. Further SMR analysis confirmed the presence of multiple putative causal genes in blood.

We also identified 14 genes that were potentially causally associated with eGFR. CDK10 and SDCCAG8 are known candidate causal genes for kidney disease. CDK10 mutations cause multicystic dysplastic kidneys, whereas SDCCAG8 mutations cause retinal-renal ciliopathy [31,32]. However, other genes have not been confirmed to be involved in kidney disease in this population. The

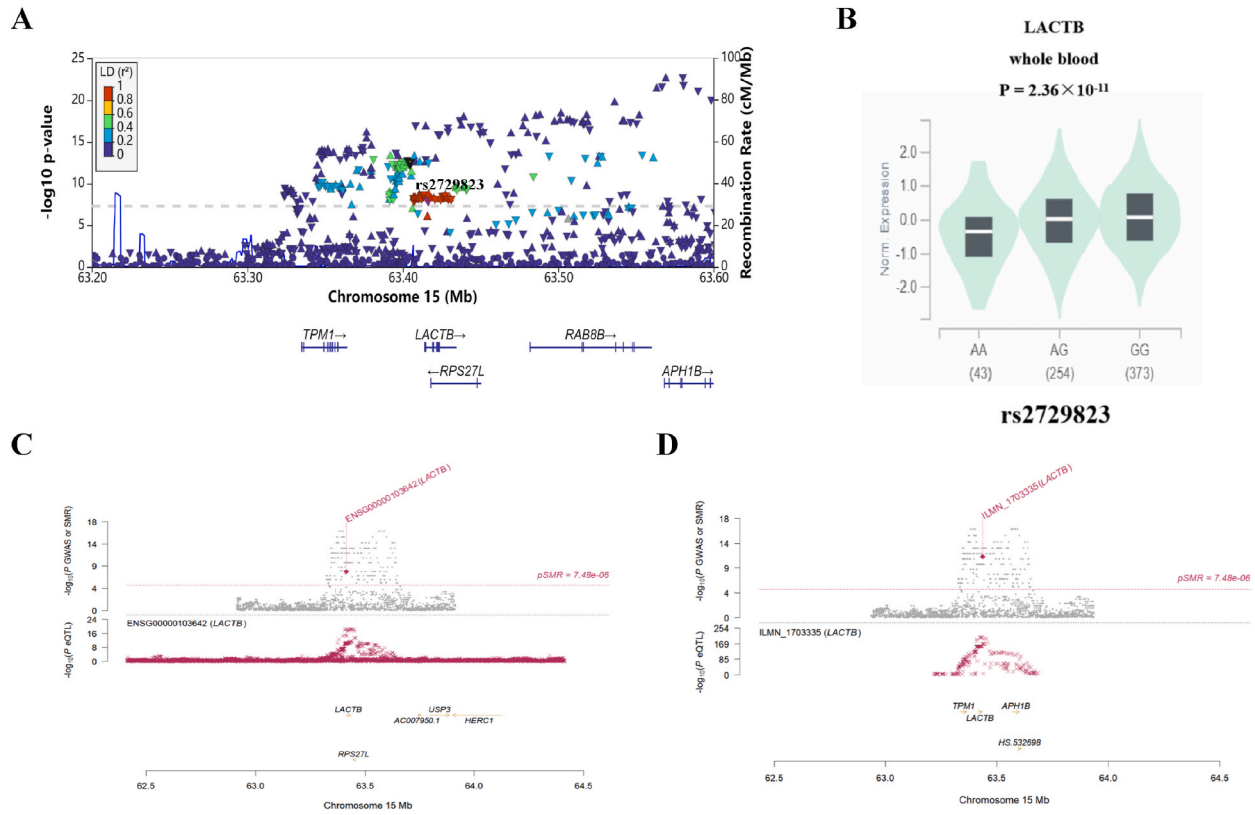

Fig. 4. Association between the LACTB gene and eGFR.
A. The A-to-I-SNP rs2729823 in the LACTB gene was associated with eGFR.
B. The C allele carriers of rs2729823 had lower mRNA expression levels of CDC14A in whole blood.
C-D. The expression levels of the LACTB gene in whole blood (C) and peripheral blood (D) were associated with eGFR.

expression of these genes is affected by RNA mSNPs and may play an important role in kidney disease.

In addition, considering that RNAm-SNPs can affect protein expression levels [33,34], 72 eGFR-RNAm-SNPs related to m^6A, m^1A, A-to-I, m^7G, and m^5C showed pQTL signals. We conducted a TSMR analysis between the pQTL datasets as exposures and eGFR as the outcome. Genetically determined eGFR values were associated with 13 proteins in the ARIC consortium. The association between these six proteins was successfully replicated in an independent replication cohort. Thus, these causal blood protein candidates may be suitable drug targets for the treatment of kidney diseases. Several studies have shown that HSPB1, PCK1, and GPX1 are involved in the progression and deterioration of kidney disease [35,36]. Therefore, protein expression affected by RNAm-SNPs plays an important role in kidney diseases. This evidence suggests that the identified RNAm-SNPs may affect kidney function by altering RNA modification. Thus, the detailed mechanisms underlying the relationship between these RNAm-SNPs and kidney function require further investigation.

This study has some potential limitations. First, the results from the bioinformatics approaches could not provide direct evidence, and we did not experimentally test whether the identified RNAm-SNPs functionally affected RNA modifications. The roles of these RNAm-SNPs, RNA modifications, and genes in the kidney have not yet been clearly identified and further experiments are needed. Second, m^6A, one of the most important RNA modifications, has the largest m^6A-SNP set; however, data on other types of RNA modifications are rare. Third, data are currently publicly limited; therefore, only the potential impact of plasma pQTL was explored in this study.

5. Conclusion

To our knowledge, this is the first study to evaluate the association between RNAm-SNPs and kidney function, and some eGFR-associated RNAm-SNPs have been identified. Our study suggests that RNAm-SNPs may affect kidney function by altering gene and protein expression levels. We also identified a potential causal relationship between the eGFR and RNA modification genes or proteins. These findings indicate that RNA modification may play a role in kidney disease and may explain the underlying mechanisms of RNA modification in kidney function. Our study provides novel insights into potential therapeutic targets for the treatment of kidney diseases.

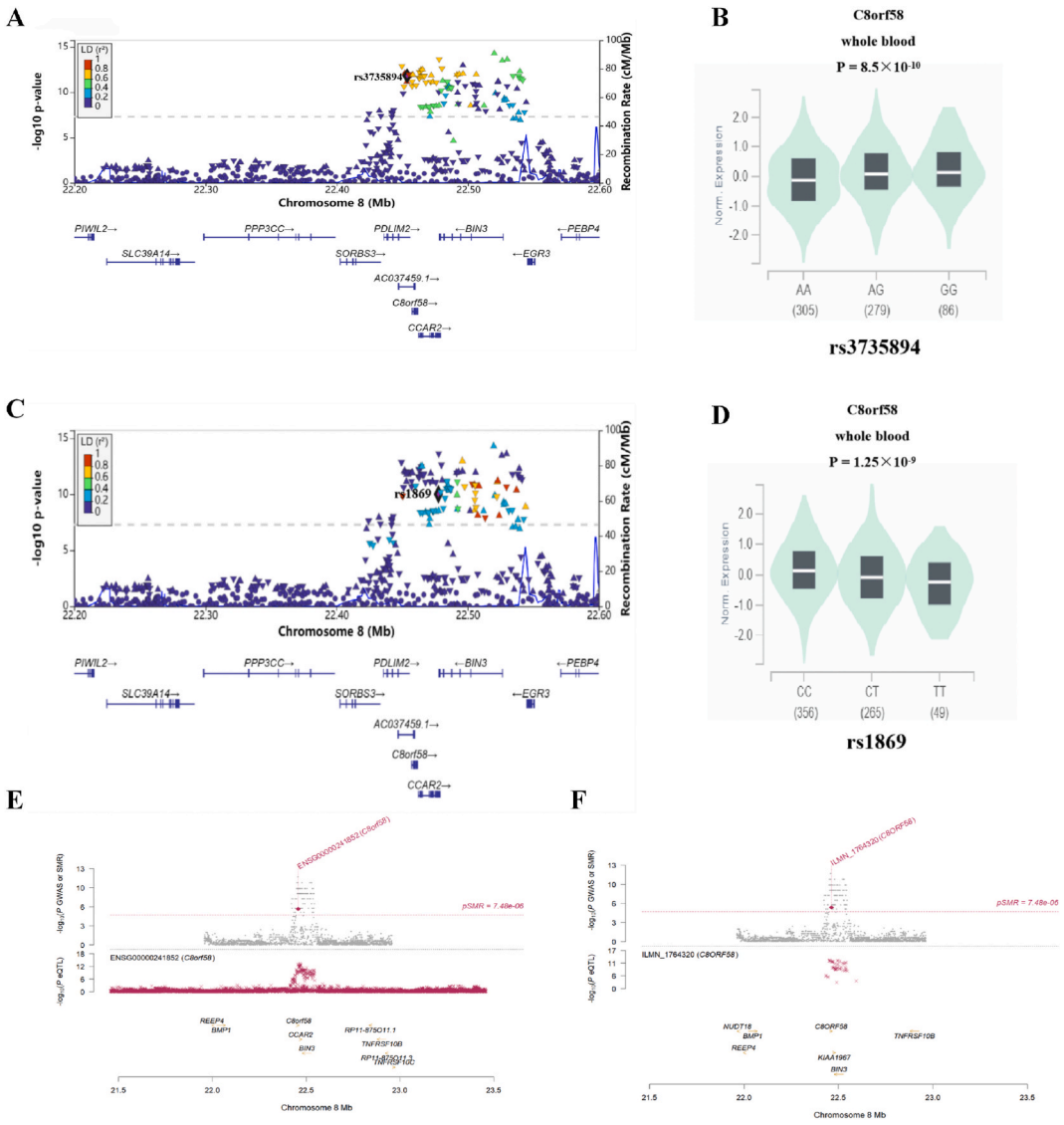

Fig. 5. Association between the C8orf58 gene and eGFR.
A. The m6A-SNP rs3735894 in the C8orf58 gene was associated with eGFR.
B. The A allele carriers of rs3735894 had lower mRNA expression levels of C8orf58 in whole blood.
C. The m6A-SNP rs1869 in the C8orf58 gene was associated with eGFR.
D. The C allele carriers of rs1869 had higher mRNA expression levels of C8orf58 in whole blood.
E-F. The expression levels of the C8orf58 gene in whole blood (E) and peripheral blood (F) were associated with eGFR.

Availability of data and materials

All data used in this study were obtained from openly available databases and consortiums, which have been mentioned in the data sources in this study. We express our sincere appreciation to them.

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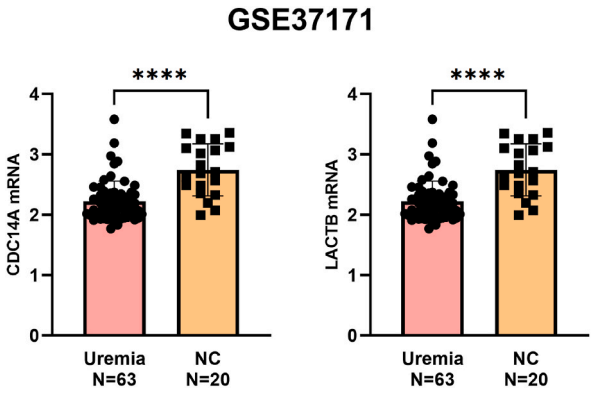

Fig. 6. Patients with uremia have significantly lower mRNA expression levels of CDC14A and LACTB in peripheral blood mononuclear cells (PBMCs) compared to healthy controls.

Table 3
Mendelian randomization estimates for circulating protein levels and eGFR in discovery cohort.

Protein	EA-ID	SNP	Modification type	No. of IVs	Method	β_TSMR	SE_TSMR	P_TSMR
HSPB1	SeqId_11103_24	rs1637045	m^6A	6	IVW	0.004	0.002	2.36E-02
APOF	SeqId_12370_30	rs1043011	m^6A	1	IVW	0.003	0.001	2.70E-03
PRXL2A	SeqId_13423_94	rs10887869	m^6A	1	IVW	−0.019	0.003	1.97E-09
GUSB	SeqId_15562_24	rs9530	m^6A	1	IVW	−0.01	0.002	1.97E-09
GPX1	SeqId_15591_28	rs13068038	m^6A	1	IVW	−0.014	0.002	1.24E-15
INHBC	SeqId_15686_49	rs540730	A-to-I	7	IVW	−0.002	0.001	7.19E-03
PFKM	SeqId_17384_110	rs2732484	m^6A	1	IVW	0.004	0.002	4.55E-02
BTN3A3	SeqId_17692_2	rs2073531	m^6A	5	IVW	0.001	0.001	1.37E-02
BTN3A3	SeqId_17692_2	rs3757138	m^6A	5	IVW	0.001	0.001	1.37E-02
PCK1	SeqId_18182_24	rs4811872	m^6A	2	IVW	−0.005	0.001	3.54E-06
PRSS3	SeqId_18864_7	rs2005617	m^6A	8	IVW	0.003	0.001	9.16E-07
PRSS3	SeqId_18864_7	rs307658	m^6A	8	IVW	0.003	0.001	9.16E-07
COL2A1	SeqId_18875_125	rs2732484	m^6A	4	IVW	−0.004	0.001	2.00E-03
DNAJB4	SeqId_18884_22	rs644359	m^6A	5	IVW	−0.004	0.002	2.40E-02
ITPA	SeqId_18916_25	rs73077077	m^6A	6	IVW	−0.002	0.001	1.86E-03

Ethics approval consent to participate

Not applicable.

CRediT authorship contribution statement

Xinran Liu: Writing – original draft, Data curation. **Sai Zhu:** Data curation. **Xueqi Liu:** Data curation. **Xiaomei Luo:** Data curation. **Chaoyi Chen:** Data curation. **Ling Jiang:** Writing – review & editing, Supervision, Funding acquisition. **Yonggui Wu:** Writing – review & editing, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:Yonggui Wu reports financial support was provided by the National Science Foundation of China. Ling Jiang reports financial support was provided by the National Science Foundation of China, Anhui Provincial Department of Education University research project, Anhui Province translational medicine project cultivation project. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Abbreviations

A-to-I adenosine-to-inosine
BUN blood urea nitrogen
CKD chronic renal disease
eGFRcrea glomerular filtration rate estimated by serum creatinine
eGFRcys eGFR based on cystatin C level

Table 4
Mendelian randomization estimates for circulating protein levels and eGFR in replication cohort.

Protein	ID	No. of IVs	MR results				MR-Egger regression		Heterogeneity analyses		
			Method	beta	SE	P	Intercept	p_intercept	Method	Q	p_Q
BTN3A3	SeqId_17692_2	247	MR Egger	4.81E-04	3.24E-04	1.388E-01	−2.00E-05	6.810E-01	MR Egger	211.58	9.399E-01
		247	Weighted median	7.30E-04	3.05E-04	1.664E-02			IVW	211.75	9.443E-01
		247	IVW	3.73E-04	1.88E-04	4.763E-02					
		247	Weighted mode	8.18E-04	3.48E-04	1.962E-02					
COL2A1	SeqId_18875_125	244	MR Egger	−1.11E-03	3.94E-04	5.145E-03	7.13E-05	1.753E-01	MR Egger	272.04	8.967E-02
		244	Weighted median	−7.48E-04	3.67E-04	4.150E-02			IVW	274.12	8.297E-02
		244	IVW	−6.79E-04	2.32E-04	3.409E-03					
		244	Weighted mode	−7.80E-04	3.68E-04	3.510E-02					
DNJB4	SeqId_18884_22	26	MR Egger	−1.40E-03	2.14E-03	5.178E-01	5.41E-05	8.004E-01	MR Egger	35.93	5.580E-02
		26	Weighted median	−5.41E-04	1.27E-03	6.701E-01			IVW	36.02	7.123E-02
		26	IVW	−9.20E-04	9.71E-04	3.436E-01					
		26	Weighted mode	−3.31E-04	1.50E-03	8.267E-01					
PRXL2A	SeqId_13423_94	13	MR Egger	4.42E-03	4.92E-03	3.871E-01	−9.95E-05	8.385E-01		15.24	1.719E-01
		13	Weighted median	0.00E+00	1.71E-03	1.000E+00			IVW	15.30	2.255E-01
		13	IVW	−5.64E-05	1.28E-03	9.648E-01					
		13	Weighted mode	3.24E-04	2.29E-03	8.894E-01					
GUSB	SeqId_15562_24	79	MR Egger	1.67E-03	1.15E-03	1.499E-01	−9.54E-05	4.617E-01	MR Egger	158.53	1.359E-07
		79	Weighted median	1.59E-03	7.74E-04	4.013E-02			IVW	159.66	1.466E-07
		79	Weighted mode	1.21E-03	5.38E-04	2.409E-02					
		79	IVW-MRE	2.79E-03	1.59E-03	8.463E-02					
HSPB1	SeqId_11103_24	74	MR Egger	4.42E-04	9.84E-04	6.549E-01	−3.20E-04	3.332E-03	MR Egger	108.56	3.483E-03
		74	Weighted median	−8.83E-04	7.63E-04	2.473E-01			IVW	122.47	2.575E-04
		74	Weighted mode	−9.80E-04	1.16E-03	4.002E-01					
		74	IVW-MRE	−5.54E-04	4.12E-04	1.779E-01					
INHBC	SeqId_15686_49	454	MR Egger	−1.32E-03	2.43E-04	9.951E-08	6.67E-05	9.306E-02	MR Egger	395.78	9.733E-01
		454	Weighted median	−1.53E-03	2.45E-04	3.575E-10			IVW	398.61	9.687E-01
		454	IVW	−9.84E-04	1.42E-04	4.461E-12					
		454	Weighted mode	−1.88E-03	3.38E-04	4.421E-08					
ITPA	SeqId_18916_25	176	MR Egger	−4.50E-04	3.32E-04	1.768E-01	3.62E-06	9.462E-01	MR Egger	201.14	7.770E-02
		176	Weighted median	−4.15E-04	3.66E-04	2.568E-01			IVW	201.14	8.556E-02
		176	IVW	−4.33E-04	2.15E-04	4.389E-02					
		176	Weighted mode	−5.06E-04	4.08E-04	2.168E-01					
PCK1	SeqId_18182_24	43	MR Egger	1.22E-04	1.20E-03	9.190E-01	−4.26E-05	7.473E-01	MR Egger	47.23	2.329E-01
		43	Weighted median	0.00E+00	8.99E-04	1.000E+00			IVW	47.36	2.633E-01
		43	IVW	−2.16E-04	5.76E-04	7.072E-01					
		43	Weighted mode	−8.24E-06	1.01E-03	9.936E-01					
PFKM	SeqId_17384_110	15	MR Egger	8.56E-03	3.44E-03	2.722E-02	−3.58E-04	2.540E-01	MR Egger	26.09	1.655E-02
		15	Weighted median	6.03E-03	1.59E-03	1.517E-04			IVW	28.95	1.063E-02
		15	Weighted mode	6.50E-03	1.68E-03	1.692E-03					
		15	IVW-MRE	4.93E-03	1.63E-03	2.518E-03					
PRSS3	SeqId_18864_7	125	MR Egger	1.92E-03	7.42E-04	1.080E-02	−1.39E-04	9.992E-02	MR Egger	189.56	1.095E-04
		125	Weighted median	1.18E-03	5.82E-04	4.230E-02			IVW	193.80	6.176E-05
		125	Weighted mode	1.58E-03	6.46E-04	1.583E-02					
		125	IVW-MRE	9.03E-04	4.20E-04	3.152E-02					
APOF	SeqId_12370_30	15	MR Egger	−7.14E-03	3.85E-03	8.678E-02	7.16E-04	6.102E-02	MR Egger	20.81	7.687E-02
		15	Weighted median	−3.20E-04	1.97E-03	8.709E-01			IVW	27.54	1.639E-02
		15	Weighted mode	−6.55E-04	2.88E-03	8.231E-01					
		15	IVW-MRE	3.30E-05	1.79E-03	9.853E-01					
GPX1	SeqId_15591_28	12	MR Egger	2.72E-03	6.00E-03	6.598E-01	−3.06E-04	4.996E-01	MR Egger	28.89	1.299E-03
		12	Weighted median	−8.10E-04	2.77E-03	7.699E-01			IVW	30.31	1.418E-03
		12	Weighted mode	−3.53E-04	4.72E-03	9.418E-01					
		12	IVW-MRE	−8.81E-04	3.02E-03	7.704E-01					

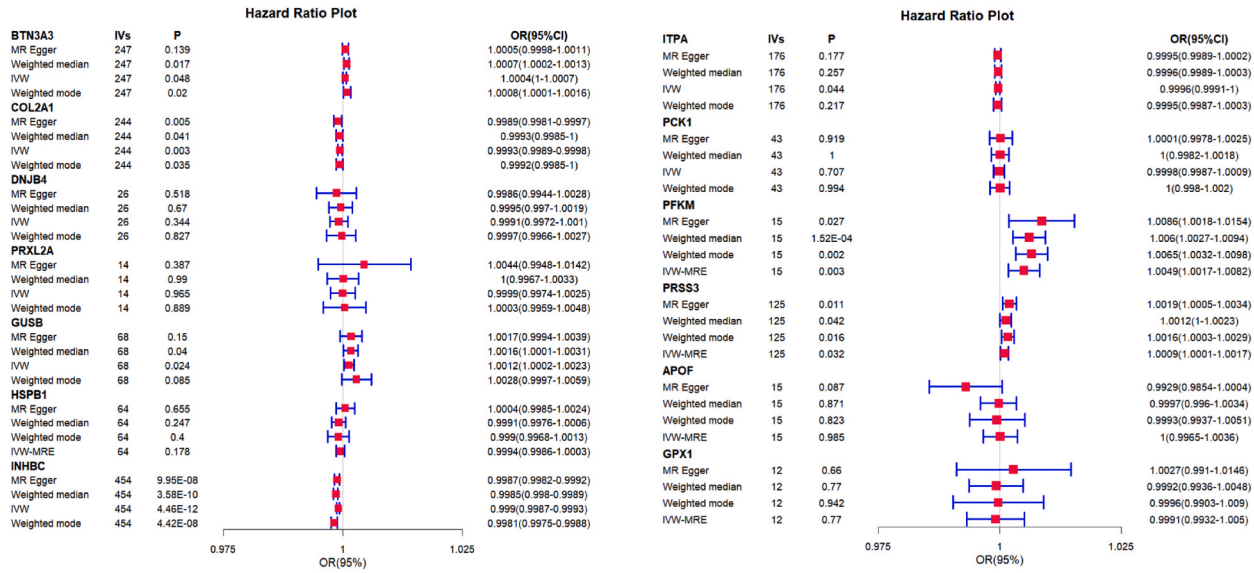

Fig. 7. Forest plot for estimated causal effects between circulating protein levels and eGFR by using different Mendelian randomization (MR) analysis methods, $p < 0.05$ were considered significant.

eQTL	expression quantitative trait loci
GFR	glomerular filtration rate
GWAS	genome-wide association studies
HEIDI	heterogeneity in dependent instruments
IVW	inverse-variance weighted
MR	Mendelian randomization
m1A	N1-methyladenosine
m^5C	5-methylcytosine
m5U	5-methyluridine
m^6A	N6-methyladenosine
m^6Am	N6-dimethyladenosine
m^7G	7-methylguanosine
ψ	pseudouridine
PBMC	peripheral blood mononuclear cells
pQTL	protein quantitative trait loci
RNAm-SNPs	RNA modification-related single nucleotide polymorphisms
SMR	summary-data-based Mendelian randomization
SNPs	single nucleotide polymorphisms
TSMR	two-sample Mendelian randomization
2'-O-Me	ribose methylations

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e38815.

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