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Original Article

ORIGINAL ARTICLE

Uromodulin and progression of IgA nephropathy

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ABSTRACT

Background. This study investigates the link between genetic variants associated with kidney function and immunoglobulin A (IgA) nephropathy (IgAN) progression.

Methods. We recruited 961 biopsy-proven IgAN patients and 651 non-IgAN end-stage renal disease (ESRD) patients from Ruijin Hospital. Clinical and renal pathological data were collected. The primary outcome was the time to ESRD. A healthy population was defined as estimated glomerular filtration rate >60 mL/min/1.73 m^2 without albuminuria or hematuria. Fifteen single-nucleotide polymorphisms (SNPs) were selected from a genome-wide association study of kidney function and genotyped by the SNaPshot. Immunohistochemistry in renal tissue and ELISA in urine samples were performed to explore the potential functions of genetic variations.

Results. The rs77924615-G was independently associated with an increased risk for ESRD in IgAN patients after adjustments for clinical and pathologic indices, and treatment (adjusted hazard ratio 2.10; 95% confidence interval 1.14–3.88). No significant differences in ESRD-free survival time were found among different genotypes in non-IgAN ESRD patients (log-rank, $P = .480$). Moreover, rs77924615 exhibited allele-specific enhancer activity by dual-luciferase reporter assay. Accordingly, the urinary uromodulin–creatinine ratio (uUCR) was significantly higher in healthy individuals with rs77924615 AG or GG than in individuals with AA. Furthermore, uromodulin expression in tubular epithelial cells was higher in patients with rs77924615 AG or GG. Finally, we confirmed that an increased uUCR ($P = .009$) was associated with faster IgAN progression.

Conclusion. The SNP rs77924615, which modulates the enhancer activity of the *UMOD* gene, is associated with renal function deterioration in IgAN patients by increasing uromodulin levels in both the renal tubular epithelium and urine.

Keywords: ESRD, IgAN, renal function progression, rs77924615, uromodulin

INTRODUCTION

Immunoglobulin A (IgA) nephropathy (IgAN) is the most common glomerulonephritis with IgA deposition in mesangium of glomeruli and remains a leading cause of end-stage renal disease (ESRD) in young people [1]. It is now well recognized that IgAN has significant heterogeneity in its clinical presentation,

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KEY LEARNING POINTS

What was known:

- Immunoglobulin A (IgA) nephropathy (IgAN) has significant heterogeneity in its clinical presentation, pathological features and disease progression.
- It is challenging for clinicians to predict kidney outcomes at the time of biopsy accurately.
- As IgAN has clear genetic susceptibility in previous studies, more genetic contributors to IgAN severity and progression need to be identified.
- This can help us better understand the mechanisms of IgAN progression and stratify the risk of IgAN patients.

This study adds:

- Our study demonstrated that rs77924615, which modulates the enhancer activity of the *UMOD* gene, is independently associated with renal function deterioration in IgAN patients by increasing uromodulin levels in both the renal tubular epithelium and urine.
- We provide novel insight into tubular-interstitial genetic variants for IgAN progression.

Potential impact:

- Some additional genetic factors may be involved in the progression of IgAN.
- In the future, the risk stratification system for IgAN prognosis also needs to consider common genetic variations in the renal tubulointerstitium.

pathological features, disease progression and long-term outcome across different ethnic populations. Hence, it is challenging for clinicians to accurately predict kidney outcomes at the time of biopsy.

A few studies have focused on the genetic contributors to IgAN progression. The HLA-DQ/DR region was found to be associated with IgAN progression according to Wang's analysis of 315 Chinese IgAN patients [2]. Genetic risk scores (GRS) have been established based on susceptibility loci identified in genome-wide association studies (GWAS) of IgAN. The GRS was associated with IgAN progression independent of clinical and pathologic risk factors [3, 4]. More genetic contributors to IgAN severity and progression need to be identified, as they may help us predict the progression of IgAN more accurately. In addition, identifying new genetic predictors has potential value for increasing our understanding of the pathogenic mechanisms underlying IgAN and new biomarkers.

A trans-ancestry meta-analysis of a GWAS published in 2019, including more than 1 million individuals, reported a catalog of genetic loci associated with kidney function. We hypothesized that genetic variations related to kidney function might contribute to IgAN progression. Our study aims to validate these genetic variations based on our extended multicenter IgAN cohort to explore the mechanism underlying candidate genetic predictors.

MATERIALS AND METHODS

Study design and participants

We studied the link between kidney function genetic variations and ESRD in a large IgAN cohort from Ruijin Hospital affiliated with Shanghai Jiao Tong University School of Medicine. We then validated our findings in the non-IgAN ESRD cohort of Ruijin Hospital. Further analyses were done to explore the function of the single-nucleotide polymorphism (SNP) using the public expression quantitative trait loci (eQTL) database, a dual-luciferase reporter assay and protein expression in IgAN patients and a healthy population.

We enrolled biopsy-proven primary IgAN participants at Ruijin Hospital affiliated with Shanghai Jiao Tong University School of Medicine from 1985 to 2018. The inclusion criteria were age at biopsy ≥18 years old and at least one follow-up visit. The exclusion criteria were an estimated glomerular filtration rate (eGFR) <15 mL/min/1.73 m^2 at the time of biopsy, IgAN secondary to Henoch-Schönlein purpura, systemic lupus erythematosus, liver disease or other systemic diseases. The time of study entry was defined as the time of renal biopsy. The clinical variables at the time of admission were recorded, including demographic variables, blood pressure and laboratory tests. Kidney histological injury was scored according to the Oxford classification. The primary outcome was ESRD after biopsy, defined as eGFR <15 mL/min/1.73 m^2 or needing maintenance dialysis or renal transplantation. Patients were censored at the time of loss to follow-up or death. The flowchart of IgAN patient recruitment is shown in Fig. 1.

The ESRD patients were recruited from patients with maintenance dialysis treatment (hemodialysis or peritoneal dialysis) in Ruijin Hospital from 1986 to 2020. ESRD patients due to IgAN were excluded. The healthy population, defined as people with normal kidney function (eGFR >60 mL/min/1.73 m^2) and without microalbuminuria or hematuria, was enrolled at the Physical Examination Center of Ruijin Hospital.

This study was performed by the Helsinki Declaration and approved by the Ethics Research Committee of Ruijin Hospital affiliated to Shanghai Jiao Tong University School of Medicine [2021(350)]. Written informed consent was obtained from all the participants before inclusion in the study.

SNP selection and genotyping

Seventeen SNPs associated with chronic kidney disease (CKD; defined as an eGFR <60 mL/min/1.73 m^2) at the genome-wide significance level of 5×10^{-8} from the 264 replicated eGFR-associated variants in the CKDGen trans-ethnic meta-analysis were selected [5]. All the SNPs were likely relevant to kidney function based on the association with eGFR/blood urea nitrogen in the opposite direction. Among all SNPs, two SNPs (rs187355703 and rs10254101) were excluded due to their low frequency (minor allele frequency <0.05) in East Asian. Four SNPs (rs8096658, rs28817415, rs62435145, rs2411192) that failed to pass the preliminary experiment were replaced by four proxy SNPs, rs549752 ($r^2 = 0.93$), rs4859682 ($r^2 = 0.99$), rs10277115 ($r^2 = 0.99$)

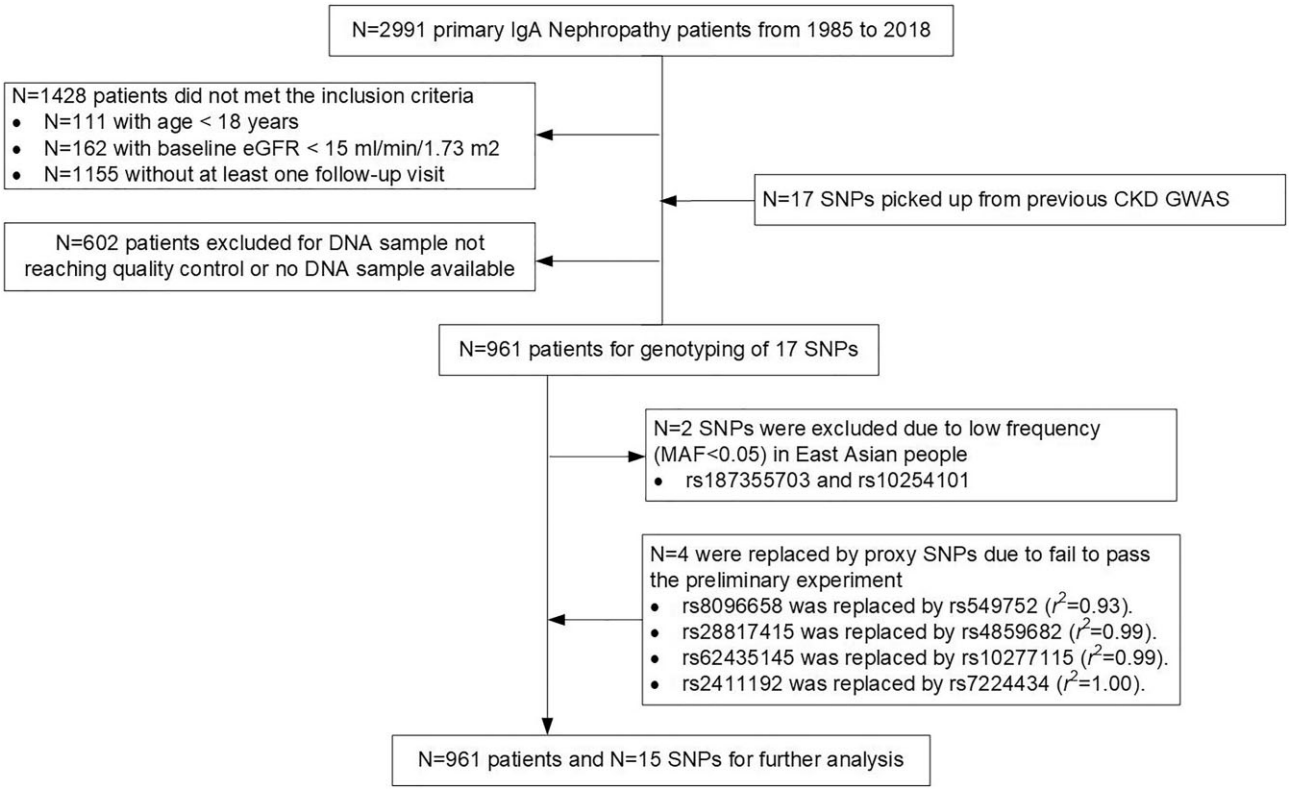

Figure 1: The flowchart of our study.

and rs7224434 ($r^2 = 1.00$). Finally, 15 SNPs were genotyped in our study (Supplementary data, Table S1). All SNPs were genotyped using SNaPshot technology (ABI 3730xl DNA Analyzer) developed by Applied Biosystems (ABI) in the IgAN cohort, the ESRD cohort and the healthy population. The results were analyzed using GeneMapper 4.0 software. Both the SNP call rate and the individual call rate were 100%.

Gene expression in *cis*-eQTL

We used a public database of *cis*-eQTL of the glomerular and tubulointerstitial tissues from the kidneys of 187 participants from the Nephrotic Syndrome Study Network (NEPTUNE) cohort [6]. We searched SNP numbers in the NephVS eQTL Browser (nephqtl.org) and then summarized the adjusted expression level *P*-value.

Dual-luciferase reporter assay

A 500-bp putative enhancer fragment containing rs77924615, a 2000-bp promoter fragment surrounding the uromodulin (UMOD) promotor start site, was amplified from genomic DNA and cloned into a pGL3-Basic vector. The primers are listed in Supplementary data, Table S2. The constructed plasmids were transfected into HEK293 cells by using the HG Transfection Reagent (Genomeditech, Shanghai, China). The phRL plasmid containing Renilla luciferase was simultaneously transfected into the cells as an internal control. After 48 h of transfection, the luciferase activity with the Renilla luciferase vector phRL as the internal reference luciferase signal was detected using the Dual-Luciferase Reporter Assay System (Genomeditech).

Urinary UMOD

Fresh morning urine samples from 251 healthy populations and 185 IgAN patients were collected. The urinary UMOD (uUMOD) concentration was measured by ELISA (BioVendor, Czech Republic, Cat No. RD191163200R) (for details, see Supplementary data). Uromodulin was represented as uUMOD and the urinary uromodulin–creatinine ratio (uUCR).

Immunohistochemistry

Twenty-nine IgAN patients were selected for immunohistochemistry through simple cluster sampling (9 with the AA genotype, 10 with the AG genotype and 10 with the GG genotype). The selection process of IgA patients for UMOD immunohistochemistry and the procedure for immunodetection are shown in the Supplementary data. Quantification of immunohistochemistry images was using ImageJ.

Statistical analysis

Demographic and clinical characteristics were analyzed as frequencies and means/medians. *t*-Tests were used for comparisons, with chi-squared tests for categorical variables. SNPs were tested for linkage disequilibrium before analysis. We used statistical analyses like log-rank tests and Kaplan–Meier curves to examine variables and outcomes. We identified significant SNPs through false-discovery rate (FDR) correction and conducted Cox proportional hazards models adjusted for the International Risk-Predicted score [7] and CLIN-PATH equations plus treatment [8]. Subgroup analysis of the associations between risk alleles and ESRD was performed through unadjusted

Table 1: Clinical and pathological characteristics of IgAN patients in overall and by rs77924615 genotype.

	Overall	rs77924615		
		AA	AG	GG
	(*n* = 961)	(*n* = 27)	(*n* = 271)	(*n* = 663)
Male, *n* (%)	482 (50.2)	15 (55.6)	145 (53.5)	322 (48.6)
Onset age, years	35.3 ± 12.4	35.4 ± 14.8	36.2 ± 12.3	34.9 ± 12.2
Age at biopsy, years	37.2 ± 12.4	37 ± 14	38 ± 13	37 ± 12
MAP, mmHg	96.7 ± 13.0	93.8 ± 14.1	97.2 ± 13.0	96.7 ± 12.9
eGFR at biopsy, mL/min/1.73 m^2	70.8(45.9,99.3)	90 (63–116)	69 (45–100)[b]	70 (45–97)[b]
Urine protein, g/24 h	1.26(0.62–2.48)	0.9 (0.4–1.7)	1.2 (0.6–2.4)[b]	1.3 (0.6–2.5)[b]
Albumin, g/L	34.9 ± 6.5	34.2 ± 8.0	34.7 ± 6.3	35.0 ± 6.5
Hemoglobulin, g/L	127.9 ± 20.6	132.4 ± 14.9	128.0 ± 20.4	127.6 ± 20.9
Uric acid, μmol/L	387.5 ± 102.9	373.4 ± 91.3	389.2 ± 101.7	387.4 ± 103.9
Use of RASi, *n* (%)	626 (71.6)	17 (68.0)	181 (74.5)	428 (70.6)
Use of glucocorticosteroids, *n* (%)	532 (60.9)	19 (76.0)	156 (64.2)	357 (58.9)
Oxford classification[a], *n* (%)				
M1	303(40.2)	6 (27)	92 (43)	205 (40)
E1	259 (34.4)	8 (36)	77 (36)	174 (34)
S1	636 (84.5)	15 (68)	178 (84)	443 (86)
T1	185 (24.6)	4 (18.2)	52 (24.4)	129 (24.9)
T2	104 (13.8)	0 (0)	31 (14.6)	73 (14.1)
C1	286 (39.6)	8 (40.0)	92 (44.7)	186 (37.4)
C2	44 (6.1)	2 (10.0)	14 (6.8)	28 (5.6)
Follow-up, months	36.7 (11.6–51.2)	40.9 (16.3–114.6)	27.8 (13.3–49.1)	25.6 (11.1–51.2)
ESRD, *n* (%)	93 (9.7)	1 (3.7)	19 (7.0)	73 (11.0)

[a]Oxford M, E, S and T scores were available for 753 patients, while Oxford C scores were available in the 723 patients.
[b]$P < .05$, compared with the AA genotype.
RASi, renin-angiotensin system inhibitor.

Cox proportional hazards models. Subgroups analysis included sex (female or male), onset age (≤35 or >35 years old), age at biopsy (≤35 or >35 years old), eGFR (≤60 or >60 mL/min/1.73 m^2), proteinuria (≤1 or >1 g/day) and serum albumin (≤35 or >35 g/L). Due to the smaller sample size of the rs77924615 AA genotype, a lifespan study was conducted in non-IgAN ESRD patients to analyze the association between genetic variations (AA or AG vs GG) and ESRD initiation in the ESRD cohort. Missing data were not imputed. The eGFR was calculated using the four-variable abbreviated Chronic Kidney Disease Epidemiology Collaboration equation based on age, race, sex and serum creatinine [9]. The mean arterial pressure (MAP) was calculated using MAP = SBP + 2 × DBP)/3. We analyzed the association between the urinary UMOD and renal function progression using the annual eGFR decline rate instead of ESRD because of the low rate of ESRD. All statistical analyses were performed using STATA version 15.0, and a *P*-value <.05 was considered indicative of statistical significance.

RESULTS

Study cohort and SNP genotyping

Fifteen susceptibility loci were genotyped in 961 patients with IgAN. Of these patients, 482 were male. The mean onset age was 35.3±12.4 years old, the mean age at biopsy was 37.2 ± 12.4 years old, and the median eGFR was 70.8 [interquartile range (IQR) 45.9–99.3] mL/min/1.73 m^2. Ninety-three (9.7%) patients reached ESRD during a median follow-up time of 36.7 (IQR 11.6–51.2) months (Table 1). All 15 SNPs were in accordance with Hardy–Weinberg equilibrium, and no inter-SNP pairwise linkage disequilibrium was observed (Supplementary data, Table S3). The CKD susceptibility SNPs were tested for associations with clinical features at the time of renal biopsy (Supplementary data, Tables S4–S6).

SNPs and kidney function progression in IgAN patients

We found that rs77924615 and rs2490391 were significantly associated with ESRD through Cox proportional hazards models, even after FDR correction [hazard ratio (HR) 1.81; 95% confidence interval (CI) 1.17–2.79, adjusted $P = .013$ for rs77924615; HR 1.65; 95% CI 1.15–2.36, adjusted $P = .007$ for rs2490391, Table 2]. However, only rs77924615-G was stably associated with a higher risk of ESRD after adjusting for the CLIN-PATH equations plus treatment (HR 2.10; 95% CI 1.14–3.88) (Table 2) and International Risk-Predicted model (adjusted HR 2.30; 95% CI 1.24–4.30). Kaplan–Meier curves showed that compared with patients with the AG and GG genotypes, patients with the AA genotype of rs77924615 had a longer ESRD-free survival time (AA vs AG, log-rank $P = .085$; AA vs GG, log-rank $P = .027$; AG vs GG, log-rank $P = .082$) (Fig. 2A). The results for the associations between risk alleles and ESRD showed the same trend in different subgroups (Fig. 2B). The clinical characteristics of patients by rs77924615 genotypes are shown in Table 1. IgAN patients with AG and GG genotypes had a significantly lower level of eGFR (AG vs AA, $P = .013$; GG vs AA, $P = .008$) and notably higher 24-h proteinuria (AG vs AA, $P = .033$; GG vs AA, $P = .043$) than patients with the AA genotype. No significant differences of follow-up time are observed between different genotypes (AG vs AA, $P = .183$; GG vs AA, $P = .097$).

Lifespan survival analysis of rs77924615 in IgAN and non-IgAN ESRD cohort

Sixty hundred and fifty-one non-IgAN ESRD patients were enrolled, 59.8% of whom were male. The dialysis initiation age

Table 2: Univariate Cox regression analysis of SNPs and the risk of ESRD in the discovery cohort.

			Univariate Cox model					Multivariate Cox model[b]		
No.	RS number	Effect allele	HR	95% CI	*P*-value	Rank	Q value[a]	HR	95% CI	*P*-value
1	rs2490391	C	1.65	1.15–2.36	**.006**	1	0.007	1.34	0.83–2.17	.226
2	rs11123169	T	0.79	0.56–1.12	.191	3	0.020			
3	rs4859682	C	0.85	0.61–1.17	.317	7	0.047			
4	rs12509595	T	1.06	0.79–1.43	.689	14	0.093			
5	rs1362800	C	1.31	0.70–2.45	.398	9	0.060			
6	rs3812036	C	0.91	0.64–1.29	.586	12	0.080			
7	rs10277115	A	1.08	0.80–1.44	.619	13	0.087			
8	rs80282103	A	1.30	0.79–2.15	.298	6	0.040			
9	rs963837	T	1.20	0.89–1.60	.226	4	0.027			
10	rs690428	C	0.89	0.67–1.18	.426	10	0.067			
11	rs77924615	G	1.81	1.17–2.79	**.007**	2	0.013	2.10	1.14–3.88	**.017**
12	rs7224434	T	0.99	0.73–1.35	.958	15	0.100			
13	rs16942751	C	1.20	0.86–1.68	.274	5	0.033			
14	rs549752	G	1.16	0.85–1.58	.341	8	0.053			
15	rs2823139	G	1.17	0.80–1.71	.430	11	0.073			

[a]Multiple significance testing was performed by the Benjamini–Hochberg procedure with an FDR of 0.1. The SNP was considered significant if the raw *P*-value was smaller than the Benjamini–Hochberg-adjusted *P*-value.
[b]The Cox proportional hazard model was adjusted by CLIN-PATH equations plus treatment [8].

was 52.7 ± 15.7 years old. Overall, 14.0% (91 out of 651) of patients were ESRD with diabetes. rs77924615-G was associated with ESRD onset in IgAN patients (HR 1.92; 95% CI 1.17–3.15, $P = .010$). No significant differences in ESRD-free survival time and rs77924615 in non-IgAN ESRD patients (HR 1.06; 95% CI 0.90–1.26, $P = 480$), ESRD patients with diabetes (HR 1.55; 95% CI 0.99–2.43, $P = .058$) or without diabetes (HR 0.97; 95% CI 0.81–1.17, $P = .779$, Supplementary data, Table S7).

eQTL analysis and geographic variation of rs77924615

rs77924615 is located upstream of the *UMOD* gene and was found to be associated with the mRNA expression of *UMOD*. According to the NephVS *eQTL* Databank, rs77924615 was associated with the mRNA expression of *UMOD* in the renal tubulointerstitium ($P = .001$) (Fig. 3A). In addition, there are noticeable regional differences in genetic variation (Fig. 3B). The mean frequency of allele G was higher in East Asia (mean frequency 0.83) than in the USA (mean frequency 0.77) and Europe (mean frequency 0.80) (Fig. 3C, Supplementary data, Table S8).

rs77924615 acts as an allele-specific enhancer of the UMOD gene

To experimentally validate the allelic regulation between rs77924615 and the UMOD promoter, we compared the regulatory activity of genomic fragments containing different genotypes of rs77924615 by using dual-luciferase reporter assays in HEK293 cells. The rs77924615-G allele could reinforce the expression of the pGL-3 basic promoter ($P < .001$) and UMOD promotor ($P < .001$, Fig. 3D).

rs77924615 and uUMOD levels

The uUMOD level was 5.67 (IQR 4.07–7.91) μg/mL in 251 healthy people, while the uUCR was 2.86 (IQR 1.96–4.71) mg/gCr. The uUMOD of the AA genotype was 3.73 (IQR 1.58–5.01) while that of the AG and GG genotypes were 5.77 (IQR 4.35–8.44) and 5.68 (IQR 4.18–7.78). For uUCR, the levels of AA, AG and GG genotype were 2.34 (IQR 1.69–2.44), 3.08 (IQR 2.04–4.94) and 2.93 (IQR 1.96–4.72). Individuals with the AA genotype had significantly lower uUMOD (AA vs AG, $P < .001$; AA vs GG, $P < .001$) and uUCR (AA vs AG, $P = .037$; AA vs GG, $P = .044$, Table 3).

rs77924615 and UMOD expression in kidney tissue

Diffuse cytoplasmic staining of UMOD in tubular epithelial cells was observed in 29 IgAN patients, while there was no irregular pattern of UMOD aggregation in tubular epithelial cells (patients' information is shown in Supplementary data, Table S9). The maximal intensity on apical membranes was observed in IgAN patients with AG and GG genotypes but not in patients with the AA genotype. Accordingly, the mean density of UMOD staining in patients with GG and AG genotypes was significantly higher than that in patients with the AA genotype (AA vs AG, $P = .001$; AA vs GG, $P = .002$, Fig. 4, Supplementary data, Table S10).

uUMOD and IgAN progression

The median uUMOD level of 185 IgAN patients was 4.05 (IQR 2.88–6.35) μg/mL, and the median uUCR was 3.10 (IQR 2.00–8.84) mg/gCR. The associations between urinary UMOD and clinical data are shown in Supplementary data, Table S11.

Both increased uUMOD ($\beta = 4.14$, $P = .011$) and uUCR($\beta = 2.66$, $P = .009$) levels were significantly associated with a faster annual decrease in eGFR (Fig. 5). The level of uUMOD ($\beta = 3.58$, $P = .037$) and uUCR ($\beta = 2.50$, $P = .014$) were steadily associated with the decrease in eGFR after adjustment by CLIN-PATH equations plus treatment (Table 4).

The uUMOD and uUCR levels of patients with stable renal function (defined as annual eGFR decline rate <2 mL/min/1.73 m^2/year) were lower than those of patients with progressive renal function (defined as annual eGFR decline rate ≥5 mL/min/1.73 m^2/year) (Supplementary data, Table S12). Furthermore, patients in the highest tertile group had a higher annual eGFR decline rate (uUMOD grouping: tertile 3 vs tertile 1, 3.36 ± 11.34 vs −3.68 ± 14.07 mL/min/1.73 m^2/year, $P = .003$; uUCR grouping: tertile 3 vs tertile 1, 2.88 ± 1.66 vs −1.73 ± 17.32 mL/min/1.73 m^2/year, $P = .078$) (Fig. 5).

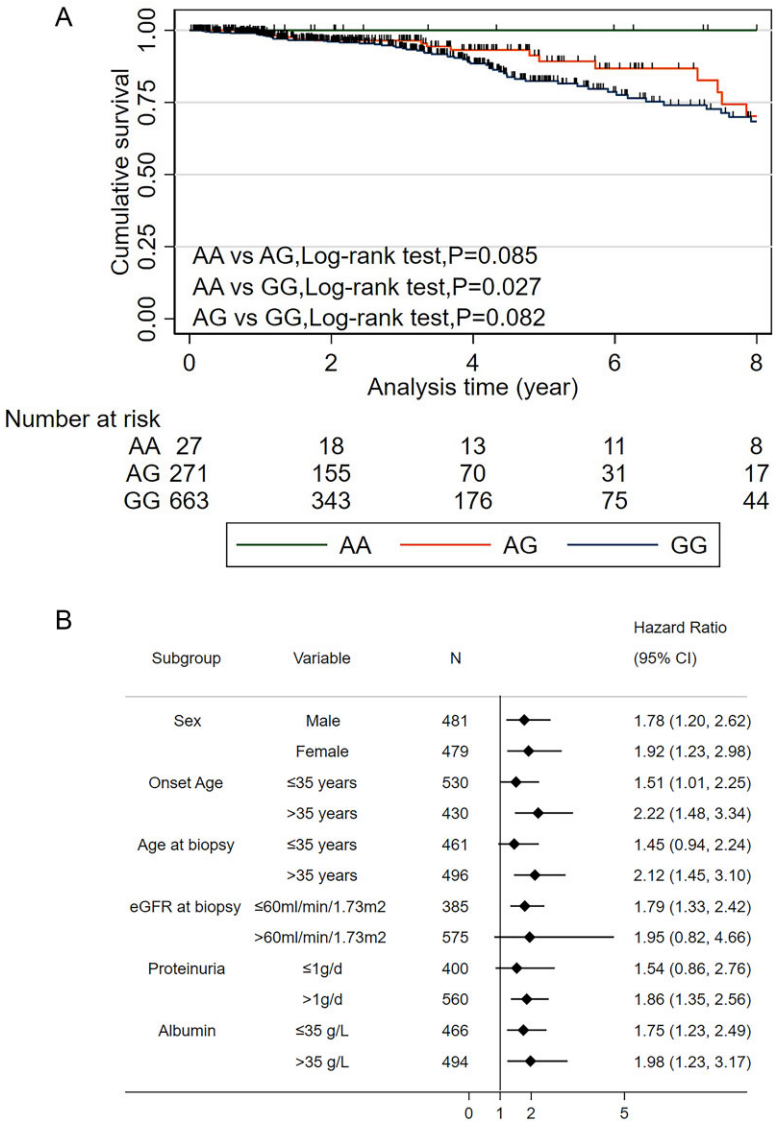

Figure 2: The association of rs77924615 with ESRD in IgAN cohorts. (**A**) Kaplan–Meier survival curves by rs77924615 genotype. The median follow-up times of patients with the AA, AG and GG genotypes were 3.4, 2.3 and 2.1 years, respectively. (**B**) A forest plot of subgroup analysis for the HR and 95% CIs of the associations between risk alleles (G) and ESRD. All of the models were unadjusted models.

DISCUSSION

It has been widely recognized that genetic factors play an essential role in the development and progression of IgAN [10–13]; however, the exact genetic mechanisms have not been fully elucidated. In this study, we included genetic variants of kidney function identified from an extended multiethnic meta-analysis and validated their associations with IgAN progression based on our large IgAN cohorts [5]. We found that rs77924615 was independently associated with IgAN progression among all genetic variants related to kidney function in a large IgAN cohort. Then, experiments showed that rs77924615 could enhance the expression of UMOD. We determined UMOD expression in tubular epithelial cells, and urinary UMOD levels were correlated with genotypes of rs77924615. Furthermore, we confirmed that an elevated urinary UMOD level was independently associated with renal function deterioration. Our study demonstrated that rs77924615, which modulates the enhancer activity of the *UMOD* gene, is independently associated with renal function deterioration in IgAN patients by increasing UMOD levels in both the renal tubular epithelium and urine.

Approximately 20%–40% of IgAN patients will progress to ESRD. Baseline kidney function and interstitial fibrosis/tubular atrophy were considered the most important risk factors for renal function decline [14, 15]. On the other hand, studies showed that an increased risk of ESRD was observed in familial IgAN patients compared with sporadic IgAN patients [16, 17]. Additionally, new rare coding variants were also uncovered in IgAN pathogenesis [18–20]. These findings suggested that the genetic mechanisms associated with IgAN progression are worth investigating, however genetic susceptibility of tubulointerstitial injury has not been well studied. We observed that rs77924615-AA had milder tubulointerstitial injury which indicates a protective effect. This is consistent with higher ESRD risk among population with a lower frequency of the allele A. IgAN patients from

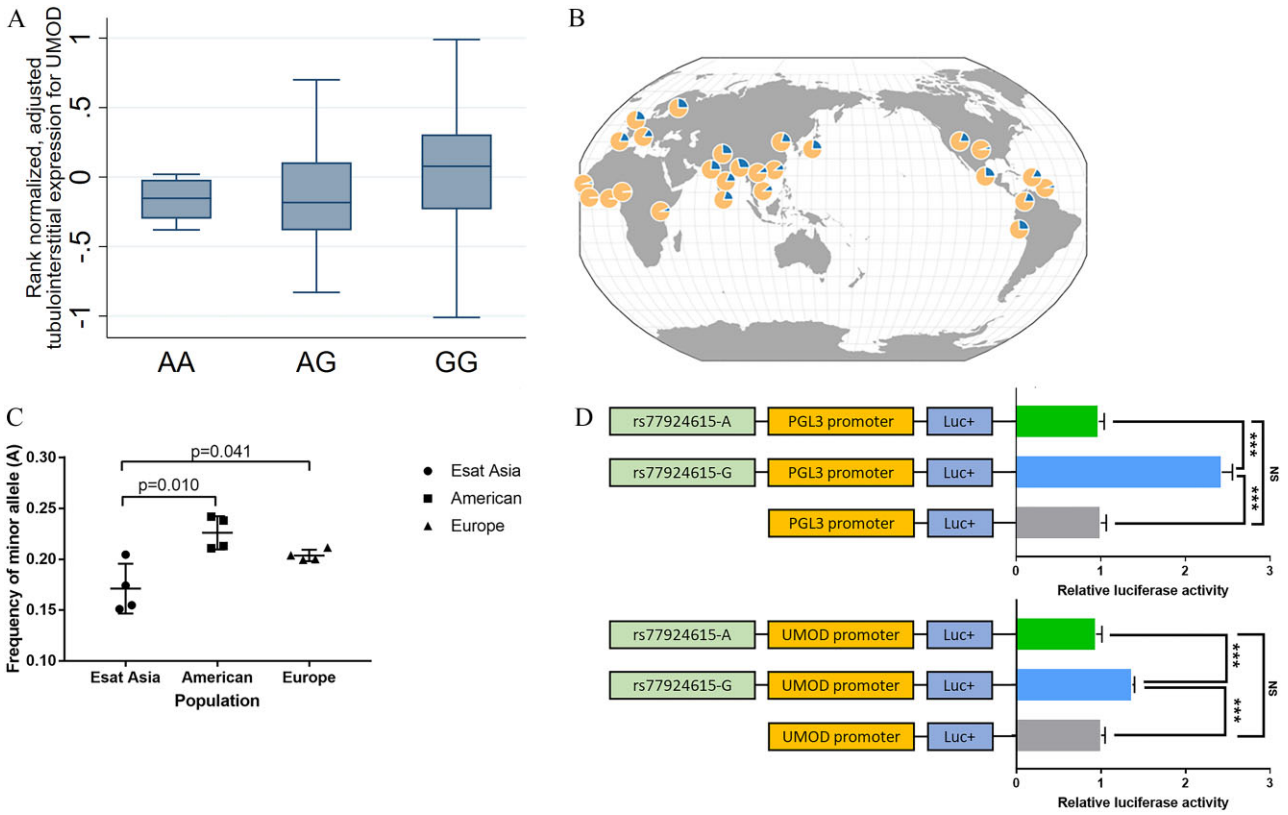

Figure 3: Uromodulin mRNA expression of kidney, geographic variations by rs77924615 genotype, and the dual-luciferase assay for the pGL3 and *UMOD* promoter. (**A**) The associated signals for *UMOD* mRNA expression in the tubulointerstitial. Association plots show association –log10(*P*-value) (y-axis) plotted against rs77924615 genotype (x-axis). The AA genotype had the lowest level of *UMOD* mRNA expression (A as effect allele; β, the estimated regression coefficient from MatrixEQTL, –0.23; t-statistic, –3.27; $P = .00138$). (**B**) The geography of the rs77924615 variant. The blue part of each pie chart represents the minor allele frequency (frequency scale = proportion out of 1) (from https://popgen.uchicago.edu/dev-integrated/welcome). (**C**) The frequency of a minor allele in East Asia was significantly lower than that in the USA and Europe. (**D**) The dual-luciferase assay for the pGL3 and the *UMOD* promoter containing the region surrounding rs77924615-A or rs77924615-G. Luciferase signals were normalized to Renilla signals. $^{***}P < .001$.

Table 3: uUMOD levels by the genotype of rs77924615 in healthy controls.

		Genotype of rs77924615		
Variable	Total	AA	AG	GG
N	251	10	81	160
Male, *n* (%)	159 (63)	7 (70)	48 (59)	111 (65)
Age, years	42.7 ± 11.6	40.6 ± 8.2	42.6 ± 11.2	42.9 ± 12.0
SCr, μmol/L	79.0 ± 14.3	79.0 ± 16.7	79.5 ± 14.6	78.8 ± 14.1
Uric acid, μmol/L	345 ± 76	322 ± 84	341 ± 71	349 ± 77
eGFR[c], mL/min/1.73 m^2	96.4 ± 13.5	99.8 ± 12.2	95.1 ± 13.0	96.9 ± 13.9
UMOD, μg/mL	5.67 (4.07, 7.91)	3.73 (1.58, 5.01)[a,b]	5.77 (4.35, 8.44)	5.68 (4.18, 7.78)
uUCR, mg/gCr	2.86 (1.96, 4.71)	2.34 (1.69, 2.44)[a,b]	3.08 (2.04, 4.94)	2.93 (1.96, 4.72)

[a]$P < 0.05$, compared with the AG genotype.
[b]$P < 0.05$, compared with the GG genotype.
[c]eGFR, eGFR calculated by Chronic Kidney Disease Epidemiology Collaboration formula.
SCr, serum creatine.

East Asia had a rapid kidney function progression with a lower frequency of the allele A.

Interestingly, the association between rs77944615 and ESRD was not observed in non-IgAN ESRD patients. The results indicated that this correlation might only exist in IgAN patients. However, the reasons for this need further investigation. Our investigation implies that additional genetic factors may be involved in the progression of IgAN, underscoring the importance of our results. In the future, it may be necessary to consider integrating common genetic variations into the risk stratification system for IgAN prognosis.

Uromodulin, also known as Tamm-Horsfall protein, encoded by *UMOD*, is located on chromosome 16p23. It is generated in the thick ascending loop of Henle and distal convoluted tubule, and

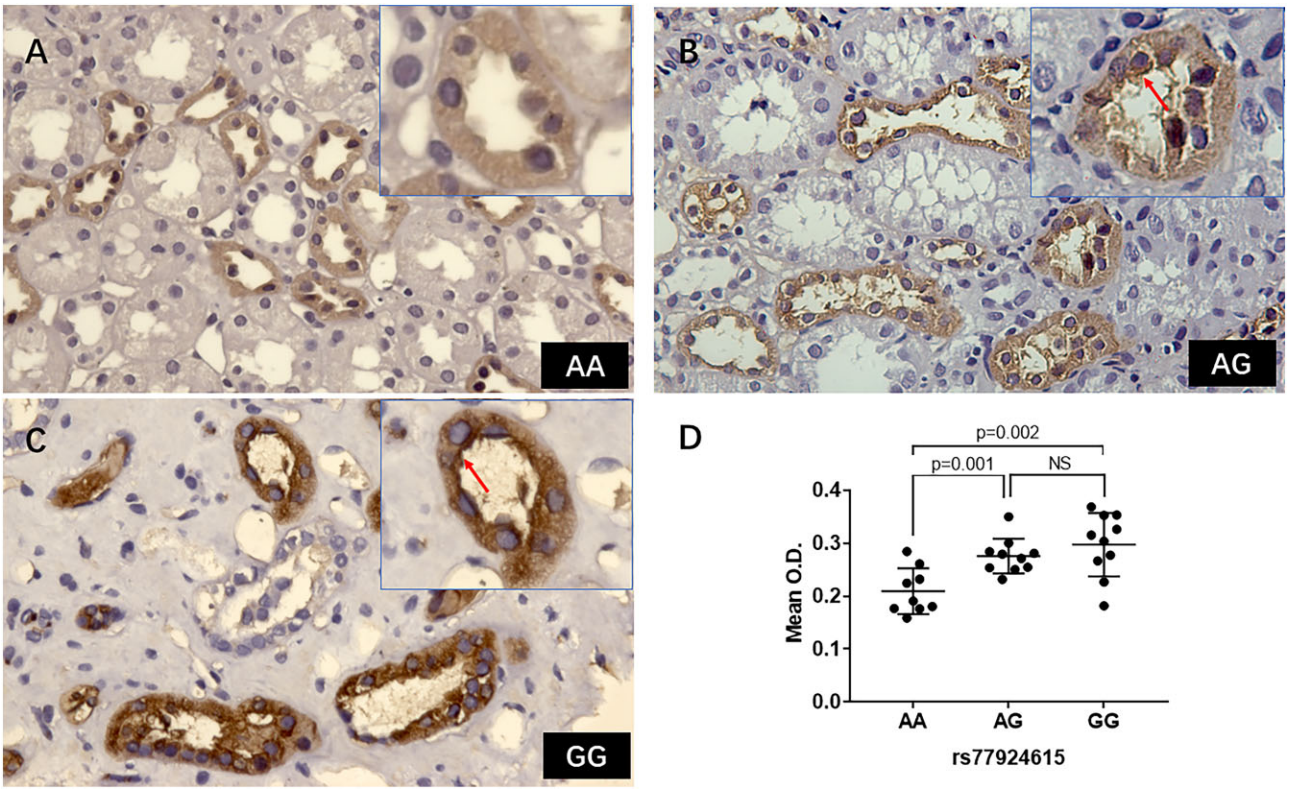

Figure 4: Renal tubular UMOD expression in IgAN patients with different rs77924615 genotypes. Diffuse cytoplasmic staining of UMOD in tubular epithelial cells was observed in all patients with AA(A), AG(B) and GG genotype(C). The maximal intensity on apical membranes was observed in the AG and GG genotypes [arrow in (**B**) and (**C**)]. The mean optical density of UMOD in kidney sections was significantly lower in the AA genotype than in the AG and GG genotypes (**D**).

it is released into the tubular lumen and blood [21, 22]. Rare variants of *UMOD* are among the main causes of autosomal dominant tubulointerstitial kidney disease (ADTKD). In patients with *UMOD*-related ADTKD, decreased uUMOD concentrations could be found, as well as an accumulation of mutated UMOD in tubular epithelial cells [23, 24]. In contrast, common variants in *UMOD* were associated with decreased eGFR in the general population, but these patients had elevated urinary UMOD concentrations, which were different from rare variants, suggesting that common and rare variants have different mechanisms for causing kidney damage [23, 24]. It has been speculated that overexpression of UMOD, decreased membrane-anchoring efficiency, faster protein sorting to the apical membrane or increased proteolytic release are the main mechanisms for common variants of *UMOD* [25]. Studies in transgenic mice showed that the 3.0–3.7 kb 5′-flanking sequence from the promoter region of the *UMOD* gene could drive the thick ascending limb–specific expression and affect urinary excretion [26, 27]. The SNP rs77924615, located close to the promoter region of *UMOD*, may modulate the promotor activity through a Cicero co-accessibility mechanism [23].

Previous studies have produced contradictory results on the relationship between uUMOD concentrations and kidney disease progression. Consistent with our findings, lower uUMOD levels have been reported to correlate with a reduced risk of kidney function progression in CKD patients and a lower risk of incident CKD in the community population [5, 28, 29]. In contrast, lower uUMOD levels were reported to be correlated with faster kidney function decline and higher mortality independent of eGFR and other risk factors in an elderly population [30] and IgAN patients [31]. Due to the inconsistent causality between uUMOD levels and kidney function progression, a recent population-based study reported that higher uUMOD levels had a direct effect on faster eGFR decline by using Mendelian randomization analysis [32]. Consistent with this study, we found that patients with AG and GG have a higher risk of progression to ESRD, which is related to the higher levels of UMOD in the urine and tubular epithelial cell expression. For the possible mechanism, the accumulation of UMOD in renal tubular epithelial cells could lead to defects in protein folding, and misfolded immature UMOD could be trapped in the endoplasmic reticulum and cause cell damage and apoptosis by endoplasmic reticulum stress. In a transgenic mouse model of *UMOD* overexpression, focal lesions and increased expression of damage markers (lipocalin-2 and Kim-1) were observed in the kidneys with aging [33]. This was also supported by significant interactions between *UMOD* variants, and aging and comorbidities shown in population studies [34]. In our study, the protective effect of the minor allele of 77 924 615 is more significant in patients older than 35 years old.

Our study has multiple strengths compared with previous work in this area. First, our study was based on extended multicenter IgAN cohorts and used the hard endpoint of ESRD as the study endpoint. Second, our study integrated data from the genome, urine and kidney tissue, allowing us to validate genetic findings at the mRNA and protein levels. This study also has several limitations. First, due to the retrospective nature of this study, some patients were excluded due to lack of follow-up data or DNA samples, which may lead to potential bias. Therefore, prospective studies are needed to validate our findings before the results of this study can be applied to the clinic practice. Second, we did not validate our findings in other independent

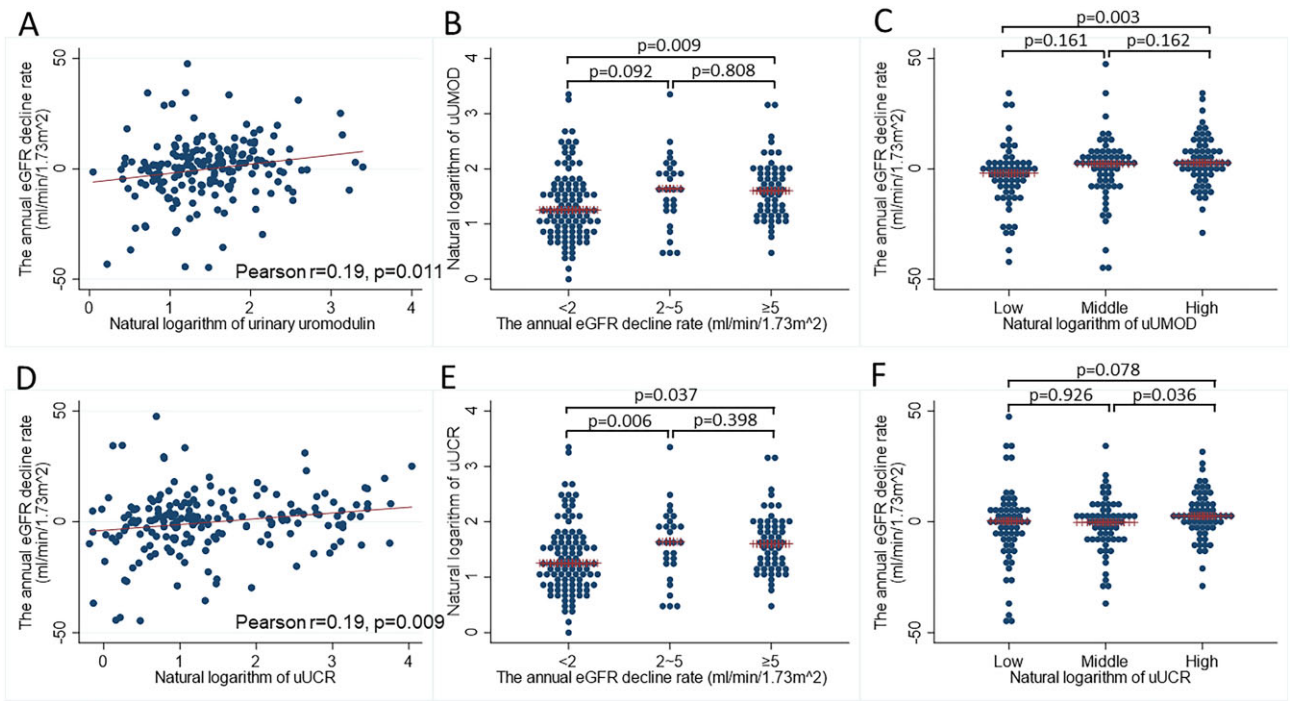

Figure 5: The association of urinary UMOD with the decrease in the eGFR from the baseline eGFR in IgAN patients ($n = 158$). (**A**) A scatter plot for the natural logarithm of uUMOD and the annual eGFR decline rate. (**B**) The uUMOD level for the annual eGFR decline rate group. (**C**) The annual eGFR decline rate of the tertile of uUMOD. (**D**) A scatter plot for the natural logarithm of uUCR and the annual eGFR decline rate. (**E**) The uUCR level for the annual eGFR decline rate group. (**F**) The annual eGFR decline rate of the tertile of uUCR.

Table 4: The association of uUMOD and the annual decrease in eGFR from the baseline eGFR ($n = 185$).

Variable	Coefficient	95% CI	*P*-value
Natural logarithm of uUMOD (μg/mL)			
Unadjusted	4.14	0.96–7.31	.011
Model 1	4.08	0.86–7.29	.013
Model 2	3.91	0.35–7.46	.031
Model 3	3.58	0.22–6.93	.037
Natural logarithm of uUCR (mg/gCr)			
Unadjusted	2.66	0.65–4.56	.009
Model 1	2.82	0.68–4.84	.010
Model 2	2.89	0.73–5.94	.009
Model 3	2.50	0.49–4.41	.014

Model 1 was adjusted by biopsy age and sex.
Model 2 was adjusted by the International Risk-Predicted score [7].
Model 3 was adjusted by CLIN-PATH equations plus treatment [8].

IgAN cohorts. Third, our findings do not exclude the possibility that rs77924615 is in linkage disequilibrium with another functional variant. Fourth, only one case out of 27 patients with the AA genotype has progressed to ESRD, therefore further validation studies are needed. Fifth, because of the sample size of our study, we did not have enough samples to analyze SNPs with a small effect size. Finally, our study restricted the genetic analysis of 17 SNPs that have previously been associated with kidney function. Other potential genetic factors have not been well investigated.

In conclusion, our study demonstrated that rs77924615 is associated with renal function deterioration in IgAN patients by increasing UMOD levels in both the renal tubular epithelium and urine. Our results provide novel insight into tubular-interstitial genetic variants for IgAN progression. The underlying mechanism of UMOD in kidney damage should be further investigated.

SUPPLEMENTARY DATA

Supplementary data are available at *Clinical Kidney Journal* online.

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AUTHORS' CONTRIBUTIONS

J.X., X.J.Z. and Z.C. conceptualized the study; Z.C., L.L.X., Y.O., Z.F. and J.L. were responsible for data curation; Z.C., L.L.X. and W.D. were responsible for formal analysis; J.X., X.J.Z. and Z.C. were responsible for investigation; X.Y., X.G. and L.X. were responsible for methodology; J.X., X.J.Z., N.C. and H.Z. provided supervision; Z.C., L.L.X. and W.D. wrote the original draft; J.X., X.J.Z., Y.J., J.M., Z.W., X.P., W.Z., H.R., W.W. and X.C. reviewed and edited the manuscript. Each author contributed important intellectual content during manuscript drafting or revision and accepts accountability for the overall work by ensuring that questions about the accuracy or integrity of any portion of the work are appropriately investigated and resolved. All authors read and approved the final manuscript.

DATA AVAILABILITY STATEMENT

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

CONFLICT OF INTEREST STATEMENT

All the authors declared no competing interests.

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