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3′UTR variants of *TNS3*, *PHLDB1*, *NTN4*, and *GNG2* genes are associated with IgA nephropathy risk in Chinese Han population

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ABSTRACT

Background: Immunoglobulin A nephropathy (IgAN) is the most common primary glomerulonephritis and is characterized by mesangial cell proliferation and agglomeration of the mesangial matrix.
Methods: In this study, we aimed to explore the role of *TNS3*, *PHLDB1*, *NTN4*, and *GNG2* 3′untranslated region (3′UTR) polymorphisms with the risk of IgAN in a Chinese Han cohort. A logistic recession model was used to calculate the effects of candidate single nucleotide polymorphism (SNP) on IgAN risk after adjusting age and gender difference. In silico prediction was conducted to identify potential functions of SNPs.
Results: The analysis revealed a significant relationship between the homozygotic genotype for *NTN4* rs1362970 A/A and higher risk of IgAN ($p = 0.003$). Statistically significant associations were found when the sample was stratified by gender and Lee's grade. As a result, *NTN4* rs1362970 A/A and *GNG2* rs3204008 G/G genotypes were associated with enhanced IgAN risk in males ($p = 0.006$, $p = 0.023$, respectively), and the association between the *PHLDB1* rs7389 G/T genotype and higher IgAN risk was found in females ($p = 0.008$). In the Lee's grade III–V subgroup, the rs1369270 in *NTN4* was significantly correlated with the risk of IgAN ($p = 0.004$). Bioinformatics prediction suggested that rs1362970 within *NTN4* 3′UTR was located in the potential target sequence of miR-483-5p.
Conclusions: Our research confirmed that *NTN4*, *GNG2*, and *PHLDB1* gene polymorphisms were implicated in IgAN susceptibility in Chinese Han population. Further research should be conducted to investigate and validate the mechanism by which the above-mentioned polymorphisms affect IgAN.

1. Introduction

Immunoglobulin A nephropathy (IgAN) is the most common dominant form of primary glomerulonephritis worldwide. IgAN is characterized by diffuse glomerular mesangial IgA-containing immune compound deposition in the glomerular mesangium along with the characteristic of mesangial cell proliferation and agglomeration of the mesangial matrix [1,2]. The incidence of IgAN is more prevalent among areas of relative socioeconomic deprivation, especially in Asian populations [3]. It usually occurs in young or middle-aged adults and is frequent in men [4]. The majority of patients are difficult to observe. Previously, the diagnosis has been based on clinical manifestations, but currently, diagnosis can be confirmed by obtaining an invasive and somewhat dangerous renal biopsy [5]. Therefore, there is an urgent need to identify the issues of IgAN causation and seek effective and noninvasive biomarkers to improve early detection and individual treatment.

TNS3 (Tensin 3) encoding an intracellular protein, TNS3, may play an important role in actin remodeling and the dissociation of the integrin-tensin-actin complex [6]. *PHLDB1* (Pleckstrin Homology-Like Domain Family B Member 1) facilitates insulin-dependent Akt phosphorylation in adipocytes and also plays a crucial role in laminin-dependent microtubule anchoring at the epithelial cell basal cortex [7]. *NTN4* (Netrin-4), which encodes Ntn4, a member of the netrin family, is a candidate released from vascular endothelial cells, and its restricted spatial expression suggests that it might play a role in neural, kidney,

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stabilization of mature blood vessels, and perhaps vascular remodeling [8,9]. *GNG2* (G protein subunit gamma 2) encodes one of the gamma subunits of a G-protein, and among its related pathways, PI3 kinase/AKT molecules, c-SRC, FAK and translation regulation through Alpha-1 adrenergic receptors are activated [10]. A recent study showed that *TNS3*, *PHLDB1*, *NTN4*, and *GNG2* gene polymorphisms were associated with various diseases [6,7,11,12]. However, less is known about the association between locus variations among these genes and the risk of IgAN.

Genetic polymorphisms at 3′UTR may modify regulatory elements affecting the interaction of the UTRs with proteins and microRNAs, which may lead to altered IgAN susceptibility. In the present study, we explored the 3′UTR region polymorphisms of predicted genes *TNS3*, *PHLDB1*, *NTN4*, and *GNG2* on IgAN risk in a Chinese Han population, which could potentially extend our understanding regarding the pathogenesis of IgAN and serve as non-invasive biomarkers for this disease.

2. Materials and methods

2.1. Study participants

Briefly, 357 IgAN patients and 384 unrelated healthy controls in a large cohort of Chinese Han population were recruited to investigate whether the 3′UTR region variations of genes in our study have influence on IgAN. IgAN patients were diagnosed according to the Kidney Disease: Improving Global Outcomes (KDIGO) clinical practice guideline for glomerulonephritis in the First Affiliated Hospital of Xi'an Jiaotong University [13]. Control subjects, without any clinical symptoms or medical history of any type of nephropathy, were also selected from the same hospital during the same period. The protocol of the present study was approved by the clinical investigative ethical committee of the same hospital (the ethical approval number is XJTUG-80), and all procedures were performed in compliance with the ethical standards of the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Blood samples from each individual were harvested at the time of initial diagnosis. The design of the case-control study is presented in Supplementary Fig. 1.

2.2. SNP selection and genotyping

Four genes (*TNS3*, *PHLDB1*, *NTN4*, and *GNG2*) were selected to evaluate the effect of 3′UTR region polymorphisms on IgAN susceptibility. We researched the databases of the 1000 Genomes Project (http://www.1000genomes.org/) and dbSNP (https://www.ncbi.nlm.nih.gov/projects/SNP/) to select the SNPs with minor allele frequency (MAF) > 5%. Ultimately, this method yields 6 SNPs (rs3750163, rs17748, rs7389, rs1362970, rs3825596, rs3204008) involved in this study. Following the manufacturer's guidelines, GoldMag whole blood genomic DNA purification kit (GoldMag Co. Ltd., Xi'an, China) was adopted to extract genomic DNA from peripheral blood samples of participants. DNA concentration was measured by NanoDrop 2000C spectrophotometer (Thermo Scientific, Waltham, MA, USA) [14]. The Agena Bioscience Assay Design Suite V2.0 software (Agena Bioscience, San Diego, CA, USA, https://agenacx.com/online-tools/) was performed to design primers for the amplification reactions [15]. The MassARRAY iPLEX platform and Agena Bioscience TYPER version 4.0 software were used for SNP genotyping and data analysis, respectively.

2.3. In silico analysis

HaploReg v4.1 (https://pubs.broadinstitute.org/mammals/haploreg/haploreg.php) and SNPinfo Web Server (https://snpinfo.niehs.nih.gov/) were used to predict the potential functions of SNPs and the putative miRNAs whose targeting sites contain the 3′UTR variants.

2.4. Statistical analysis

The SPSS 16.0 (SPSS, Chicago, IL, USA) software was used for statistical analysis in our study [16,17], and two-sided $p < 0.05$ was confirmed to be statistically significant. Hardy-Weinberg equilibrium (HWE) p values were obtained from exact tests [18]. The correlation between individual alleles and genotype and IgAN susceptibility was estimated with the values of odds ratios (ORs) and 95% confidence intervals (CIs) based on logistic regression model analysis [19]. Furthermore, the stratified analyses were also performed to access the relationship between each SNP and the risk of IgAN in different subgroups. Genetic model analyses were performed using SNPstats online tools software (http://bioinfo.iconcologia.net/snpstats/start.htm). The one-way analysis of variance (ANOVA) tests were carried out to analyze clinical characteristics among different genotypes.

3. Results

3.1. Participant characteristics

We recruited 357 IgAN patients (mean age 32.44 ± 11.80) consisting of 246 males and 111 females and 384 unrelated healthy individuals (mean age 51.16 ± 11.49) containing of 265 healthy males and 119 females in this study. The basic characteristics of them were shown in Table 1.

3.2. Association between NTN4 and GNG2 variations and IgAN risk

In our study, the basic information on SNPs is listed in Table 2. All candidate SNPs were in accordance with HWE (p value ≥0.05), indicating good sample selection. The minor allele “A” of rs1362970 in the *NTN4* gene and rs3204008 in the *GNG2* gene was significantly correlated with enhanced risk of IgAN (OR = 1.37, 95% CI = 1.09–1.73, $p = 0.007$; OR = 1.24, 95% CI = 1.00–1.53, $p = 0.045$, respectively). Five genetic models, including the codominant model, the dominant model, the recessive model, the overdominant model, and the Log-additive model were carried out to analyze the relationship between SNPs genotypes and IgAN risk. The

Table 1
Baseline characteristics of samples.

Variables	Case (%)	Control (%)
	$N = 357$	$N = 384$
Gender		
Male	246 (65.3%)	265 (72.4%)
Female	111 (34.7%)	119 (27.6%)
Age, year (mean ± SD)	32.44 ± 11.80	51.16 ± 11.49
Urine RBC (n/μL)	250.12 ± 28.82	
Proteinuria (g/24 h)	2.71 ± 0.34	
ALB (g/L)	33.99 ± 0.43	
CHO (mmol/L)	6.13 ± 1.01	
IgA (g/L)	2.75 ± 0.07	
C3 (g/L)	1.03 ± 0.01	
C4 (g/L)	0.02 ± 0.01	
BUN (mmol/L)	8.20 ± 0.32	
Scr (μmol/L)	145.75 ± 8.55	
UA (μmol/L)	380.87 ± 11.41	
Serum β macroglobulin (mg/L)	2992.78 ± 155.35	
Urine β macroglobulin (mg/L)	639.78 ± 62.42	
HB (g/dL)	127.27 ± 1.29	
FIB (g/L)	17.76 ± 13.97	
Lee's grade		
I–II	120 (33.61%)	
III–V	215 (60.22%)	

ALB, serum albumin; BUN, blood urea nitrogen; CHO, serum cholesterol; C3, complement 3; C4, complement 4; FIB, fibrinogen; HB, hemoglobin; IgA, serum immunoglobulin A; Scr, serum creatinine; UA, serum uric acid.

Table 2
Basic characteristics and allele frequencies among SNPs.

SNP	Genes	Chr	Function	Position	Allele	Minor allele frequency		HWE *p* value	OR (95% CI)	*p*[a]
						Case	Control			
rs3750163	*TNS3*	7	3′UTR	47,317,510	A/G	0.098	0.079	1.000	1.26(0.88–1.80)	0.207
rs17748	*PHLDB1*	11	3′UTR	118,528,424	G/T	0.248	0.260	0.895	0.94(0.74–1.18)	0.581
rs7389	*PHLDB1*	11	3′UTR	118,528,466	T/C	0.476	0.430	0.603	1.21(0.98–1.48)	0.072
rs1362970	*NTN4*	12	3′UTR	96,052,526	A/C	0.293	0.231	0.250	1.37(1.09–1.73)	0.007*
rs3825596	*GNG2*	14	3′UTR	52,434,883	G/T	0.174	0.176	0.861	0.99(0.75–1.29)	0.917
rs3204008	*GNG2*	14	3′UTR	52,435,059	A/G	0.409	0.358	0.824	1.24(1.00–1.53)	0.045*

CI, confidence interval; HWE, Hardy-Weinberg equilibrium; OR, odds ratio; SNP, single nucleotide polymorphism.
* $p < 0.05$ indicates statistical significance.
[a] *p* Values calculated with two-sided $\chi 2$.

results were presented in Table 3. The significant positive association was found between *NTN4* rs1362970 A/A genotypes and IgAN susceptibility in the recessive model (OR = 3.06, 95% CI = 1.43–6.52, $p = 0.003$) after adjustment for gender and age. However, we observed no significant correlation between any genotypes among other SNPs and IgAN risk. Furthermore, the clinical parameters among patients were analyzed, and the remarkable SNPs are listed in Supplementary Table 1.

According to gender parameters, stratified analysis regarding the effects of SNPs on IgAN risk is summarized in Table 4. In males, *NTN4* rs1362970 A/A and *GNG2* rs3204008 G/G genotypes were correlated with improved risk of IgAN in the recessive model (OR = 3.17, 95% CI = 1.35–7.45, $p = 0.006$; OR = 2.16, 95% CI = 1.10–4.23, $p = 0.023$, respectively). In females, genotype "G/T" of rs7389 in *PHLDB1* was significantly correlated with an enhanced IgAN risk in the codominant model (OR = 3.69, 95% CI = 1.55–8.78, $p = 0.008$) and the overdominant model (OR = 2.48, 95% CI = 1.18–5.18, $p = 0.014$). The genotype "G/T + G/G" of rs7389 was associated with an increased IgAN risk in the dominant model (OR = 3.22, 95% CI = 1.46–7.12, $p = 0.003$). We also found that rs7389 increased IgAN risk in the Log-additive model (OR = 1.69, 95% CI = 1.03–2.75, $p = 0.034$). The Lee's grading system was also performed. As shown in Table 5, the results revealed that the A/A genotype of rs1362970 in the *NTN4* gene was related to enhanced IgAN risk in the codominant model, the recessive model, and the Log-additive model (OR = 3.54, 95% CI = 1.51–8.30, $p = 0.004$; OR = 3.50, 95% CI = 1.52–8.04, $p = 0.003$ and OR = 1.44, 95% CI = 1.02–2.03, $p = 0.037$, respectively) in the Lee's grade III–V subgroup. No significant association was found between rs1369270 genotypes and IgAN risk were observed in the Lee's grade I–II subgroup.

3.3. In silico analysis

In silico analysis, HaploReg v4.1 and SNPinfo Web Server were conducted to assess the function of the selected SNPs in the 3′UTR among these genes. The integration of 2 online tools for function annotation suggested that rs7389, rs1362970, and rs3204008 were associated with enhancer histone marks, DNase and selected eQTL hits. Furthermore, our results revealed that *PHLDB1* rs7389 was located in the seed region of 3 miRNA binding sites (miR-298, miR-639, and miR-802) and that *TNT4* rs1362970 and *GNG2* rs3204008 were located within the potential target sequence of miR-483-5p and miR-601 respectively. It may influence the banding of miRNAs with the target genes and resulted in decrease or increase in the target mRNA translation, which is correlated with IgAN susceptibility. All putative results are displayed in Supplementary Table 2.

4. Discussion

The 3′UTR variants have been recognized as major contributors to the pathogenesis and development of most diseases. In this study, we aimed to clarify the correlation between 3′UTR polymorphisms of *TNS3*, *PHLDB1*, *NTN4*, and *GNG2* genes and IgAN susceptibility in a Chinese Han population. The results suggested that the minor allele "A" of rs1362970 in the *NTN4* gene and rs3204008 in the *GNG2* gene was correlated with enhanced IgAN risk. We also found that the *NTN4* rs1362970 homozygotic genotype A/A was a contributor to higher risk of IgAN. After stratifying the sample by gender and Lee's grading system, associations were found between higher risk and *NTN4* rs1362970 A/A as well as *GNG2* rs3204008 G/G genotypes in males. The *PHLDB1* rs7389 "G/T" and "G/T + G/G" genotypes were related to higher IgAN risk in females. Similarly, the *NTN4* rs1362970 A/A genotype significantly increased IgAN risk in the Lee's grade III–V subgroup.

The definite pathogenesis of IgAN remains unclear, but several studies have demonstrated correlations between multiple gene variations and IgA nephropathy, usually using a candidate gene approach [20], which show candidate gene encoding protein association with proteinuria, glycosylation of IgAN [21], mesangial matrix expansion [22], or immunity against pathogen diversity [2]. Our study extends these observations by investigating the predicted genes *TNS3*, *PHLDB1*, *NTN4*, and *GNG2*, which are known to be involved in various biological activities, such as cell invasion, differentiation, angiogenesis, and proliferation [7,8,10,11], but whose association with IgAN has not been previously assessed. Our present results provide evidence for the new functional roles of these genes in IgAN. The reasons for the polymorphism within these genes affecting the susceptibility are may be that the variants modify relevant molecular pathways and cellular processes, which may lead to the disease processes. Several studies have been conducted recently in which Ntn4 restricted spatial expression might play a role in neural [23], kidney [8], stabilization of mature blood vessels and perhaps vascular remodeling through encoding a member of laminin-related proteins [12].

Recently, an increasing number studies has demonstrated that SNPs located in the miRNA binding sites affected the binding of miRNAs with the target genes, resulting in an increase in the target mRNA translation, and thus being related to the risk of kidney disease [12,22,24,25]. Computational analysis revealed that the rs7389 SNP was identified within the 3′UTR of *PHLDB1* and target binding site of 3 miRNAs, *TNT4* rs1362970 within the potential target sequence of miR-483-5p, and *GNG2* rs3204008 within the potential target sequence of miR-601. It is biologically plausible that the 3′-UTR variation perhaps changes the biological activities by affecting the translation of *PHLDB1*, *NTN4*, and *GNG2* gene, thus inducing the higher risk of IgAN.

Another possible explanation for this is that *NTN4* rs1362970 AA, *GNG2* rs3204008 G/G, and *PHLDB1* rs7389 G/T + G/G might play pivotal roles in the proliferation of the mesangial cells and the renin-angiotensin system via affecting the miRNA effect on IgAN. Recently, sufficient studies have elucidated the connections between miRNAs and several diseases, including kidney disease [26,27]. Especially, miRNA let-7 family members appeared to have similar functions in the

Table 3
Association between SNP genotypes and the risk of IgA nephropathy based on unconditional logistic regression model analysis.

SNP	Genotype	Control	Case	Adjustment analysis		Crude analysis	
		N (%)	*N* (%)	OR (95% CI)[a]	*p*[a]	OR (95% CI)[b]	*p*[b]
rs3750163							
	G/G	325 (84.6%)	290 (81.2%)	1.00		1.00	
Codominant	G/A	57 (14.8%)	64 (17.9%)	1.23 (0.74–2.04)	0.180	1.26 (0.85–1.86)	0.450
	A/A	2 (0.5%)	3 (0.8%)	7.06 (0.80–62.12)		1.68 (0.28–10.13)	
Dominant	G/G	325 (84.6%)	290 (81.2%)	1.00		1.00	
	G/A + A/A	59 (15.4%)	67 (18.8%)	1.32 (0.80–2.17)	0.280	1.27 (0.87–1.87)	0.220
Recessive	G/G + G/A	382 (99.5%)	354 (99.2%)	1.00		1.00	
	A/A	2 (0.5%)	3 (0.8%)	6.82 (0.78–59.97)	0.093	1.62 (0.27–9.74)	0.590
Overdominant	G/G + A/A	327 (85.2%)	293 (82.1%)	1.00		1.00	
	G/A	57 (14.8%)	64 (17.9%)	1.21 (0.73–2.00)	0.470	1.25 (0.85–1.85)	0.260
Log-additive	–	–	–	1.39 (0.86–2.23)	0.170	1.26 (0.88–1.82)	0.200
rs17748							
	C/C	209 (54.4%)	199 (56.4%)	1.00		1.00	
Codominant	T/C	150 (39.1%)	133 (37.7%)	0.95 (0.64–1.41)	0.910	0.93 (0.69–1.26)	0.860
	T/T	25 (6.5%)	21 (6.0%)	0.84 (0.36–1.97)		0.88 (0.48–1.63)	
Dominant	C/C	209 (54.4%)	199 (56.4%)	1.00		1.00	
	T/C + T/T	175 (45.6%)	154 (43.6%)	0.93 (0.64–1.37)	0.720	0.92 (0.69–1.24)	0.600
Recessive	C/C + T/C	359 (93.5%)	332 (94.0%)	1.00		1.00	
	T/T	25 (6.5%)	21 (6.0%)	0.86 (0.37–1.98)	0.720	0.91 (0.50–1.65)	0.750
Overdominant	C/C + T/T	234 (60.9%)	220 (62.3%)	1.00		1.00	
	T/C	150 (39.1%)	133 (37.7%)	0.96 (0.65–1.42)	0.840	0.94 (0.70–1.27)	0.700
Log-additive	–	–	–	0.93 (0.68–1.28)	0.670	0.94 (0.74–1.18)	0.580
rs7389							
	T/T	122 (31.8%)	96 (27.0%)	1.00		1.00	
Codominant	G/T	194 (50.5%)	181 (50.8%)	1.13 (0.73–1.76)	0.850	1.19 (0.85–1.66)	0.190
	G/G	68 (17.7%)	79 (22.2%)	1.11 (0.64–1.92)		1.48 (0.97–2.25)	
Dominant	T/T	122 (31.8%)	96 (27.0%)	1.00		1.00	
	G/T + G/G	262 (68.2%)	260 (73.0%)	1.13 (0.74–1.71)	0.580	1.26 (0.92–1.73)	0.150
Recessive	T/T + G/T	316 (82.3%)	277 (77.8%)	1.00		1.00	
	G/G	68 (17.7%)	79 (22.2%)	1.02 (0.64–1.65)	0.920	1.33 (0.92–1.90)	0.130
Overdominant	T/T + G/G	190 (49.5%)	175 (49.2%)	1.00		1.00	
	G/T	194 (50.5%)	181 (50.8%)	1.09 (0.74–1.59)	0.670	1.01 (0.76–1.35)	0.930
Log-additive	–	–	–	1.06 (0.81–1.39)	0.670	1.21 (0.98–1.49)	0.069
rs1362970							
	C/C	222 (57.8%)	181 (51.0%)	1.00		1.00	
Codominant	C/A	146 (38.0%)	140 (39.4%)	0.98 (0.65–1.46)	0.012	1.18 (0.87–1.59)	0.008*
	A/A	16 (4.20%)	34 (9.6%)	3.03 (1.40–6.57)		2.61 (1.39–4.87)	
Dominant	C/C	222 (57.8%)	181 (51.0%)	1.00		1.00	
	C/A + A/A	162 (42.2%)	174 (49.0%)	1.18(0.80–1.72)	0.400	1.32 (0.99–1.76)	0.062
Recessive	C/C + C/A	368 (95.8%)	321 (90.4%)	1.00		1.00	
	A/A	16 (4.2%)	34 (9.6%)	3.06 (1.43–6.52)	0.003*	2.44 (1.32–4.50)	0.003*
Overdominant	C/C + A/A	238 (62.0%)	215 (60.6%)	1.00		1.00	
	C/A	146 (38.0%)	140 (39.4%)	0.86 (0.58–1.28)	0.460	1.06 (0.79–1.43)	0.690
Log-additive	–	–	–	1.34 (0.99–1.80)	0.059	1.38 (1.09–1.74)	0.007*
rs3825596							
	G/G	260 (67.7%)	244 (68.9%)	1.00		1.00	
Codominant	A/G	113 (29.4%)	97 (27.4%)	0.92 (0.60–1.40)	0.930	0.91 (0.66–1.26)	0.710
	A/A	11 (2.9%)	13 (3.7%)	1.00 (0.33–3.06)		1.26 (0.55–2.86)	
Dominant	G/G	260 (67.7%)	244 (68.9%)	1.00		1.00	
	A/G + A/A	124 (32.3%)	110 (31.1%)	0.93(0.62–1.39)	0.710	0.95(0.69–1.29)	0.720
Recessive	G/G + A/G	373 (97.1%)	341 (96.3%)	1.00		1.00	
	A/A	11 (2.9%)	13 (3.7%)	1.02 (0.34–3.12)	0.970	1.29 (0.57–2.92)	0.540
Overdominant	G/G + A/A	271 (70.6%)	257 (72.6%)	1.00		1.00	
	A/G	113 (29.4%)	97 (27.4%)	0.92 (0.61–1.40)	0.690	0.91 (0.66–1.25)	0.540
Log-additive	–	–	–	0.95 (0.66–1.35)	0.760	0.99 (0.75–1.29)	0.920
rs3204008							
	C/C	159 (41.5%)	124 (35.4%)	1.00		1.00	
Codominant	G/C	174 (45.4%)	166 (47.4%)	1.20 (0.79–1.81)	0.210	1.22 (0.89–1.68)	0.140
	G/G	50 (13.1%)	60 (17.1%)	1.71 (0.94–3.11)		1.54 (0.99–2.40)	
Dominant	C/C	159 (41.5%)	124 (35.4%)	1.00		1.00	
	G/C + G/G	224 (58.5%)	226 (64.6%)	1.30(0.88–1.92)	0.190	1.29(0.96–1.74)	0.091
Recessive	C/C + G/C	333 (87.0%)	290 (82.9%)	1.00		1.00	
	G/G	50 (13.1%)	60 (17.1%)	1.55 (0.89–2.70)	0.120	1.38 (0.92–2.07)	0.120
Overdominant	C/C + G/G	209 (54.6%)	184 (52.6%)	1.00		1.00	
	G/C	174 (45.4%)	166 (47.4%)	1.05 (0.71–1.53)	0.820	1.08 (0.81–1.45)	0.590
Log-additive	–	–	–	1.28 (0.97–1.69)	0.084	1.24 (1.00–1.53)	0.047*

CI, confidence interval; OR, odds ratio; SNPs, Single nucleotide polymorphisms.
* $p < 0.05$ indicates statistical significance.
[a] *p* Values calculated with unconditional logistic regression adjusted by gender and age.
[b] *p* Values calculated with unconditional logistic regression without adjustment by gender and age.

Table 4
Association between SNPs and the risk of IgA nephropathy after stratification analysis subgroups of gender.

SNP	Model	Genotype	Male		Female	
			OR (95% CI)	p^a	OR (95% CI)	p^a
rs7389						
	Codominant	T/T	1.00		1.00	
		G/T	0.70(0.41–1.19)	0.410	3.69(1.55–8.78)	0.008*
		G/G	0.75(0.38–1.48)		2.53(0.94–6.79)	
	Dominant	T/T	1.00		1.00	
		G/T + G/G	0.71 (0.43–1.18)	0.190	3.22 (1.46–7.12)	0.003*
	Recessive	T/T + G/T	1.00		1.00	
		G/G	0.95(0.54–1.70)	0.870	1.19(0.51–2.74)	0.690
	Overdominant	T/T + G/G	1.00		1.00	
		G/T	0.78(0.50–1.23)	0.290	2.48(1.18–5.18)	0.014*
	Log-additive	–	0.85(0.61–1.18)	0.330	1.69(1.03–2.75)	0.034*
rs1362970						
	Codominant	C/C	1.00		1.00	
		C/A	0.91(0.56–1.48)	0.023	1.14(0.54–2.38)	0.460
		A/A	3.06(1.28–7.35)		2.80(0.54–14.68)	
	Dominant	C/C	1.00		1.00	
		C/A + A/A	1.14 (0.73–1.79)	0.570	1.27 (0.62–2.58)	0.510
	Recessive	C/C + C/A	1.00		1.00	
		A/A	3.17(1.35–7.45)	0.006*	2.67(0.52-13.67)	0.230
	Overdominant	C/C + A/A	1.00		1.00	
		C/A	0.79(0.50–1.27)	0.330	1.05(0.51–2.16)	0.900
	Log-additive	–	1.33(0.94–1.89)	0.110	1.35(0.75–2.43)	0.320
rs3204008						
	Codominant	C/C	1.00		1.00	
		G/C	1.08(0.66–1.77)	0.073	1.50(0.69–3.26)	0.480
		G/G	2.25(1.10–4.62)		0.92(0.30–2.77)	
	Dominant	C/C	1.00		1.00	
		G/C + G/G	1.28 (0.81–2.03)	0.290	1.35 (0.65–2.81)	0.420
	Recessive	C/C + G/C	1.00		1.00	
		G/G	2.16(1.10–4.23)	0.023*	0.72(0.26-1.98)	0.530
	Overdominant	C/C + G/G	1.00		1.00	
		G/C	0.89(0.56–1.40)	0.610	1.54(0.76–3.12)	0.230
	Log-additive	–	1.37(0.99–1.92)	0.059	1.07(0.63–1.80)	0.810

* $p < 0.05$ indicates statistical significance.
[a] p Values calculated with unconditional logistic regression adjusted by age.

glycosylation process of IgA1 molecules [25] and human renal fibrosis via participating pathways including p38MAPK or NF-κB signal [28], and this finding is similar to that of Xing LN et al. [29] and Min QH et al. [30], in that another miRNA family miR-29 is suggested to be capable of affecting IgAN via regulating inflammation during IgAN pathogenesis, cell apoptosis or proliferation in renal cell carcinoma. The specific markers of miRNAs in IgAN, as described above, remain to be investigated.

Significantly, previous research revealed that the distribution of IgAN risk score along with relevant risk alleles was more correlated with geography and showed an even greater difference among ethnic groups [31,32]. It is therefore important to investigate the allele distribution in the Chinese Han population, the most populous nationality in China. While the study focus and the methods are valid given our project's aims, the design of the current research may still be limited by the hospital-based case-control study and the small number of samples, which may limit the statistical power. There was also lack of information on the renal outcome, despite our efforts to obtain this information. We used bioinformatics software to predict the potential functions of the SNPs in the 3′UTR region of the aim gene. However, further functional experimentation is necessary to confirm this hypothesis. Overall, the present study shed novel light on *GNG2*, *NTN4*, and *PHLDB1* as potential contributors for IgAN susceptibility in the Chinese Han population, which provides new insights into the

Table 5
Association between SNPs and the risk of IgA nephropathy after stratification analysis subgroups of Lee's grading system.

SNP	Model	Genotype	Lee's grading system			
			I–II		III–V	
			OR (95% CI)	p^a	OR (95%CI)	p^a
rs1362970	Codominant	C/C	1.00		1.00	
		C/A	0.94 (0.53–1.67)	0.842	1.03 (0.65–1.64)	0.895
		A/A	1.75 (0.52–5.83)	0.366	3.54 (1.51–8.30)	0.004*
	Dominant	C/C	1.00		1.00	
		C/A + A/A	1.01 (0.59–1.75)	0.964	1.27 (0.82–1.96)	0.283
	Recessive	C/C + C/A	1.00		1.00	
		A/A	1.79 (0.55–5.83)	0.336	3.50 (1.52–8.04)	0.003*
	Log-additive	–	1.10 (0.69–1.74)	0.697	1.44 (1.02–2.03)	0.037*

* $p < 0.05$ indicates statistical significance.
[a] p Values calculated with unconditional logistic regression adjusted by age.

pathogenesis of this disease. Further study with more focus on the interaction between the above-mentioned genes and specific miRNA involved in IgAN, as well as signaling mechanisms based on mesangial cell culture in vitro or knockdown of targeted genes in mice are therefore suggested to verify these findings and provide a new theoretical basis that *GNG2*, *TNT4*, and *PHLDB1* may be used as alternatively promising markers for better screening and early diagnosis of IgAN.

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Declaration of interest

The authors have declared that they have no conflicts of interest. The authors alone are responsible for the content of this manuscript.

Author contributions

Conceived and designed the experiments: JiaLi Wei and Chunyang Ma. Performed the experiments: Yan Su, Ziyang Jing, Xiaohong Yang. Analyzed the data: Daofa Zhang, Maowei Xie, Wenning Li. Wrote the paper: Yuan Feng.

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