Association between *PLA2R* gene polymorphism and idiopathic membranous nephropathy in Heilongjiang Chinese

Tian Tian, Jiaxin Zheng, Yefan Li, Qiang Guo, Yihong Jiang

Department of Nephrology, The Second Affiliated Hospital of Heilongjiang University of Chinese Medicine, Harbin, China

Contributions: (I) Conception and design: J Zheng; (II) Administrative support: None; (III) Provision of study materials or patients: Q Guo; (IV) Collection and assembly of data: T Tian; (V) Data analysis and interpretation: Y Li, Y Jiang; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

Correspondence to: Jiaxin Zheng. Department of Nephrology, The Second Affiliated Hospital of Heilongjiang University of Chinese Medicine, Harbin, China. Email: zjxemail@163.com.

Background: The aim of this study was to investigate the correlation between the phospholipase A2 receptor (*PLA2R*) gene polymorphism and idiopathic membranous nephropathy (IMN) in Heilongjiang Chinese.

Methods: Thirty-five patients with IMN confirmed by renal biopsy attending the Heilongjiang Hospital of Traditional Chinese Medicine between June 2021 and December of 2021 were selected as the IMN group, and a group of 25 healthy participants from the Physical Examination Center of Heilongjiang Hospital of Traditional Chinese Medicine were enrolled as healthy controls. Polymerase chain reaction (PCR) was used to identify and genotype 8 single-nucleotide polymorphism (SNP) loci (rs16844715, rs2715918, rs2715928, rs35771982, rs3749119, rs3828323, rs4665143, and rs6757188) of *PLA2R* and to analyze the *PLA2R* gene polymorphisms that correlated with IMN. SPSS 26.0 statistical software was used for data analysis, and the chi-squared (χ^2) goodness-of-fit test was used to determine whether each SNP genotype and allele in the *PLA2R* gene complied with the Hardy-Weinberg equilibrium. The qualitative data were analyzed via χ^2 or Fisher exact probability method. Logistic regression was used to analyze risk factors, and the odds ratios (ORs) values and 95% confidence intervals (CIs) were calculated. α=0.05 was taken as the test level, and P<0.05 was considered statistically significant.

Results: Statistically significant differences were found in the genotype and allele frequencies of rs35771982 and rs3749119 between the IMN and control groups (P<0.05). Logistic regression analysis showed that the genotypes rs35771982 GG and rs3749119 CC were associated with IMN susceptibility. Statistically significant differences in uric acid level were found between the rs35771982 GG and CG + CC genotypes (P<0.05), while statistically significant differences in serum albumin were detected between rs3749119 CC and the CT + TT genotypes (P<0.05). Multivariate logistic regression analysis showed that gender, age, and triglyceride levels affected the occurrence of IMN (P<0.05).

Conclusions: The *PLA2R* gene polymorphisms rs35771982 and rs3749119 in Heilongjiang Chinese may be related to IMN susceptibility and correlated with clinical indicators of IMN. Gender, age, and triglyceride levels may influence the occurrence of IMN.

Keywords: Idiopathic membranous nephropathy (IMN); M type phospholipase A2 receptor; gene polymorphism

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Introduction

Membranous nephropathy (MN) is currently one of the most common causes of adult nephrotic syndrome, accounting for 20% to 30% of glomerular disease, with an annual incidence of approximately 1.2 in 100,000 individuals (1,2). Based on the etiology, MN can be further categorized into secondary membranous nephropathy (SMN) and idiopathic membranous nephropathy (IMN). IMN accounts for 75% of MN and is common in the middle-aged and older adult population, with a male-to-female prevalence of approximately 2:1 (3). Approximately 25% of patients achieve complete spontaneous remission within 5 years, while 20% develop end stage renal disease (ESRD) within 10 years (4).

The pathogenesis of IMN is still unclear. In 2009, Beck *et al.* (5) found PLA2R antibody in the serum samples of 37 patients with IMN. PLA2R is mainly expressed in glomerular podocytes and is the main target antigen of IMN, which is of milestone significance for the study of adult IMN. PLA2R is a receptor for PLA2 and belongs to the sPLA2 subtype, which is composed of nerve (N) and muscle (M) types. M-type PLA2R is a glycoprotein complex purified from glomerular extract, which can be expressed in lung and liver, but mainly in kidney (6). Dong *et al.* (7) proved that there are two configurations of PLA2R: flexural conformation and extended conformation, and the change of PH can make the two conformations interconvert. Due to the amplification of PLA2R structure under alkaline PH conditions, the main epitopes located in CysR, CTLD1 and CTLD7 regions may be used to produce different antibodies, and the conformational changes will lead to the exposure of internal domains, thus causing different autoimmune reactions (8,9). Stanescu *et al.* (10) performed a genomewide association analysis and identified the encoding gene *PLA2R* on chromosome 2q24 [single-nucleotide polymorphism (SNP) rs4664308] and *HLA-DQA1* encoding the human leukocyte antigen (HLA) class II alpha chain on chromosome 6p21 (SNP rs2187668) as the IMN susceptibility genes in caucasian. Inspired by this pioneering study, Chinese researchers investigated the susceptibility loci for MN in Sichuan, Chengde, and Xinjiang in China (11-13), but the results of different regions and different races are different. In addition, there are few research data on the alpine region of Heilongjiang, so we selected 8 corresponding gene loci for research, hoping to find the risk gene of idiopathic membranous nephropathy in Heilongjiang and then carry out the next research. We present the following article in accordance with the MDAR reporting checklist (available at https://atm.amegroups.com/article/view/10.21037/atm-22-6648/rc).

Highlight box

Key findings

- Through research, we found that the genetic polymorphisms of the phospholipase A2 receptor (*PLA2R*) SNP rs35771982 and rs3749119 loci in patients with IMN in Heilongjiang were related to the susceptibility to IMN, and the GG genotype of the rs35771982 locus and the CC genotype of the rs3749119 locus were the risk genotypes.

What is known and what is new?

- There is a close association between idiopathic membranous nephropathy and M-type phospholipase A2 receptor gene polymorphism.
- In this study, 8 SNP sites on *PLA2R* that are associated with IMN were selected to explore the association between IMN and *PLA2R* gene polymorphism in Heilongjiang.

What is the implication, and what should change now?

- Differences in study results in different regions are attributable to many factors, including race, geographical environment, eating habits, and living environment. How these factors are related to gene polymorphism should be investigated further in future research.

Methods

Case information

Between June 2021 and December 2021, 35 cases of IMN diagnosed via renal biopsy in the First Department of Nephrology, Heilongjiang Hospital of Traditional Chinese Medicine, were selected. The pathology was based on the diagnostic criteria for Ehrenreich-Churg MN (14). Concurrently, 25 healthy patients in the Heilongjiang Hospital of Traditional Chinese Medicine Physical Examination Center were selected as the research participants. Both groups were unrelated to Han Chinese. The IMN group included 28 males and 7 females aged between 25 and 73 (55.48±10.09) years. The healthy control group included 5 males and 20 females, with an age ranging from 18 to 75 (33.32±11.62) years. Clinical demographics were collected, including gender, age, systolic blood pressure, diastolic blood pressure, total cholesterol, triglyceride levels, serum albumin, urea nitrogen, serum creatinine, uric acid, and 24-hour urinary protein. The study was conducted in accordance with the Helsinki Declaration (revised in 2013). The study was approved by

the Ethics Committee of Heilongjiang Provincial Hospital of Traditional Chinese Medicine (No. 2021-023-01) and the informed consent of all patients was obtained.

Exclusion criteria

IMN group

The exclusion criteria for the IMN group were the following: (I) patients with SMN; (II) patients with other kidney diseases; (III) use of hormones or immunosuppressants to treat patients; (IV) patients with severe infection; and (V) patients with serious primary diseases of the brain, lung, heart, or liver system. Meanwhile, the exclusion criteria for the healthy control group were the following: (I) abnormal results on physical examination report; (II) previous chronic medical history of hypertension, hyperlipidemia, or diabetes; and (III) pregnant or lactating women.

Reagents and instruments

The Magnetic Beads Whole Blood Genome Extraction Kit (item No. BGI-LH-303-02) and a polymerase chain reaction (PCR) amplification kit (item No. BGI-LH-001) were purchased from Beijing Liuhe Huada Gene Technology Co. (Wuhan, China). The PCR product purification was achieved with magnetic beads (item No. PMSi200001) purchased from Xiamen PuriMag. Biotechnology Co. Agarose (item No. BGI-LH-007) was ordered from Sunma Biotech Corp. (Xiamen, China), the centrifuge (model 5417R) from Eppendorf, the PCR instrument (model 9700) from Applied Biosystems (Shanghai, China), the electrophoresis instrument (model DYY-8C) from Beijing Liuyi Instrument Factory, the water bath (model DK-24) from Shanghai Jinghong Experimental Equipment Co. (Shanghai, China), and the sequencer (model 3730XL) from Applied Biosystems.

Clinical data collection

Clinical demographics, including gender, age, systolic blood pressure, diastolic blood pressure, total cholesterol, triglycerides, serum albumin, urea nitrogen, blood creatinine, uric acid, and 24-hour urinary protein levels, were collected from the IMN group and the healthy controls.

DNA extraction

A 5-mL aliquot of fasting venous blood was collected from the participants in the morning, transferred to an ethylenediaminetetraacetic acid (EDTA) anticoagulant tube, and stored in a refrigerator at –80 ℃. DNA was extracted according to the experimental procedure described in the whole blood genome extraction kit (Beijing Liuhe Huada Gene Technology Co.) using magnetic beads after specimen collection. DNA purity and concentration were determined using the UV spectrophotometer. The absorbance at a 260 nm/280 nm wavelength was measured to determine DNA purity above 1.80. The DNA was stored in a refrigerator at –80 ℃.

Gene sequencing

PCR resequencing was used to identify the genotypes and alleles of the *PLA2R* genes rs16844715, rs2715918, rs2715928, rs35771982, rs3749119, rs3828323, rs4665143, and rs6757188 loci. Primer Premier 5.0 software was used to design primers (listed in *Table 1*) for amplification by Beijing Liuhe Huada Gene Technology Co., Ltd. PCR amplification was performed using a 25-μL amplification reaction system, containing Primer F (1 μL), Primer R (1 μL), and DNA template (1 ng/μL) in ddH_2O (9.5 μL) to obtain a total volume of 12.5 μL. PCR amplification conditions were as follows: predenaturation at 96 ℃ for 5 min, denaturation at 96 ℃ for 20 s with 35 cycles, annealing at 52 ℃ for 30 s with 35 cycles, 72℃ extension 30 s, 35 cycles; extended at 72 ℃ for 10 min and stored at 4 ℃. The target gene amplification product was digested with restriction endonuclease Bbs I at 37 ℃ for 4 h and terminated at 65 ℃ for 30 min. The digested product was subjected to 1.0% agarose gel electrophoresis, and the band distribution was observed in a gel imaging system. PCR product purification was performed strictly according to the experimental criteria indicated in the PCR product purification kit with the magnetic bead method. The purified PCR products were sequenced. PCR amplification was conducted by Beijing Liuhe Huada Gene Technology Co.

Statistical analysis

SPSS 26.0 statistical software was used for data analysis. The chi-squared (χ^2) goodness-of-fit test was used to determine whether the genotypes and alleles of each group at the SNP locus of the *PLA2R* gene conformed to the Hardy-Weinberg law of equilibrium. The measurement data conforming to a normal distribution are expressed as mean ± standard deviation ($\bar{x}\pm s$), and an independent samples *t*-test

Table 1 PCR primer sequences

Genetic loci	Direction	Primer sequences (5'-3')	Primer length (bp)
rs16844715	F	AGGTTCTTTCCACTCCTGCTC	400
	R	CCTGTGGAAAATTTGTCTTCTGT	
rs2715918	F	GATTTGGTCACTCAACATT	694
	R	ATGAGTAAGTGGAAACACC	
rs2715928	F	TCAGCAGTACCCAATTCACT	346
	R	ACTTTCCTCTGTAGCTCCCT	
rs35771982	F	GCTTACACCCAAATCCTCCT	680
	R	ACACCTCTTCCTCCACCCTA	
rs3749119	F	CGACAGCAGCATCGCTAACCACT	453
	R	TCAAATCGCTCACCCACAACTCC	
rs3828323	F	CGGTTGAGGACAACAGTGAG	542
	R	AAGCCAAGTGTAGTTAGTGGAGTG	
rs4665143	F	CACAAATGTCTCCACCACCT	560
	R	ATGCTTCCCAAGAAACTCCT	
rs6757188	F	GATTTGGTCACTCAACATT	374
	R	ATGAGTAAGTGGAAACACC	

PCR, polymerase chain reaction; F, forward; R reverse.

was used to compare the 2 groups. The data that did not conform to a normal distribution ae expressed as medians and interquartile range. Mann-Whitney nonparametric test was used for comparison between groups, and the χ^2 test or Fisher exact probability method was used to compare the qualitative data of the groups. Logistic regression was used for risk factor analysis. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated. α=0.05 was used as the test level, and P<0.05 was considered to indicate a statistically significant difference.

Results

Clinical and demographic characteristics

Age, systolic blood pressure, diastolic blood pressure, total cholesterol, triglycerides, urea nitrogen, creatinine, and uric acid levels were all higher in the IMN group than in the healthy control group, while serum albumin levels were lower in the IMN group than in the healthy controls, with statistically significant differences (P<0.05). In the healthy control group, 24-hour urine protein was not quantified because no abnormalities were found in routine urine examination (*Table 2*).

Genotypic analysis of the PLA2R locus based on PCR resequencing

Chromas software was used to analyze the peaks and revealed CC, CT, and TT genotypes at locus rs16844715; AA, AG, and GG genotypes at locus rs2715918; and AA, AG, and GG genotypes at locus rs2715928. The rs35771982 locus contained CC, CG, and GG genotypes; each of the loci rs3749119, rs3828323, and rs6757188 carried CC, CT, and TT genotypes; and the rs4665143 locus carried AA, AG, and GG genotypes (*Figures 1-8*).

Hardy-Weinberg equilibrium

The PLA2R SNP loci rs16844715, rs2715918, rs2715928, rs35771982, rs3749119, rs3828323, rs4665143, and rs6757188 were tested for the Hardy-Weinberg genetic equilibrium. All loci tested for genotype number in both the IMN and healthy groups were in Hardy-Weinberg equilibrium (P>0.05), as shown in *Table 3*.

Table 2 Comparison of the clinical demographics of the IMN group and healthy group

Characteristic	IMN (n=35)	HC (n=25)	χ^2/t/z	P
Gender			21.212	0.001
Male	28	5		
Female	7	20		
Age, years	55.48±10.91	33.32±11.62	−5.601	0.001
SBP, mmHg	135.00 (120.00, 150.00)	112.00 (107.50, 120.00)	−4.606	0.001
DBP, mmHg	80.00 (80.00, 90.00)	75.00 (72.50, 80.00)	−3.466	0.001
CHOL, mmol/L	7.10 (6.53, 8.78)	3.99 (3.40, 4.72)	−6.110	0.001
TG, mmol/L	2.62 (1.59, 3.67)	0.80 (0.58, 1.17)	−6.440	0.001
ALB, g/L	25.90±7.25	45.65±2.34	−15.060	0.001
BUN, mmol/L	6.32 (4.84, 7.71)	5.26 (4.23, 5.61)	−2.437	0.015
SCR, μmol/L	75.00 (57.4, 90.20)	50.40 (46.85, 56.55)	−4.791	0.001
UA, μmol/L	341.80 (291.20, 409.50)	300.70 (247.95, 344.40)	−2.714	0.007
UPRO, g/d	3.69 (2.23, 6.79)	–	–	–

Data are presented as number, mean ± SD, median (range). IMN, idiopathic membranous nephropathy; HC, healthy controls; SBP, systolic pressure; DBP, diastolic pressure; CHOL, total cholesterol; TG, triglyceride; ALB, albumin; BUN, urea nitrogen; SCR, serum creatinine; UA, uric acid; UPRO, 24-hour urine protein quantification.

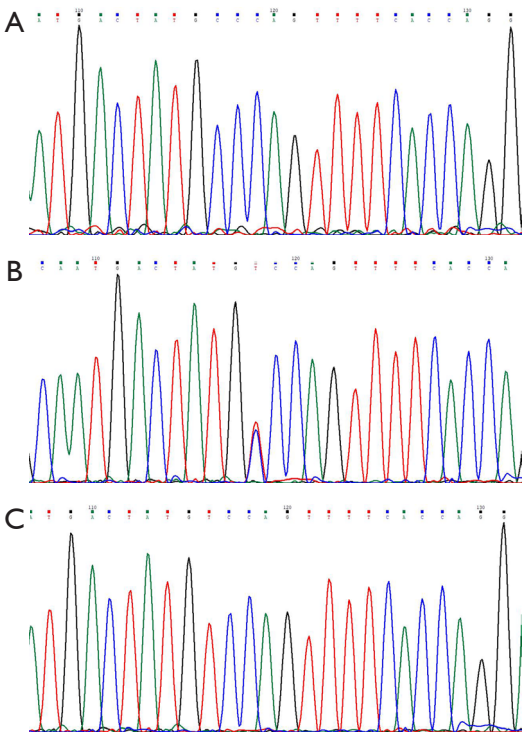

Figure 1 Genotype of the rs16844715 locus. (A) CC type; (B) CT type; (C) TT type. C, allele c; T, allele t; A, allele a; G, allele g.

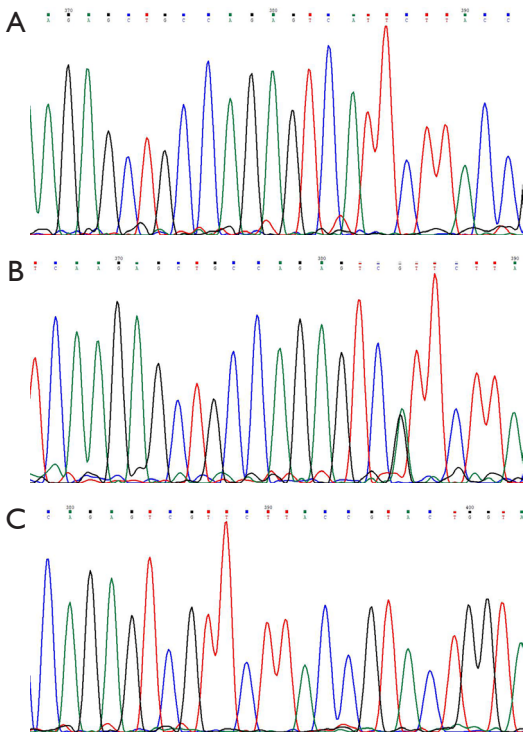

Figure 2 Genotype of the rs2715918 locus. (A) AA type; (B) AG type; (C) GG type. C, allele c; T, allele t; A, allele a; G, allele g.

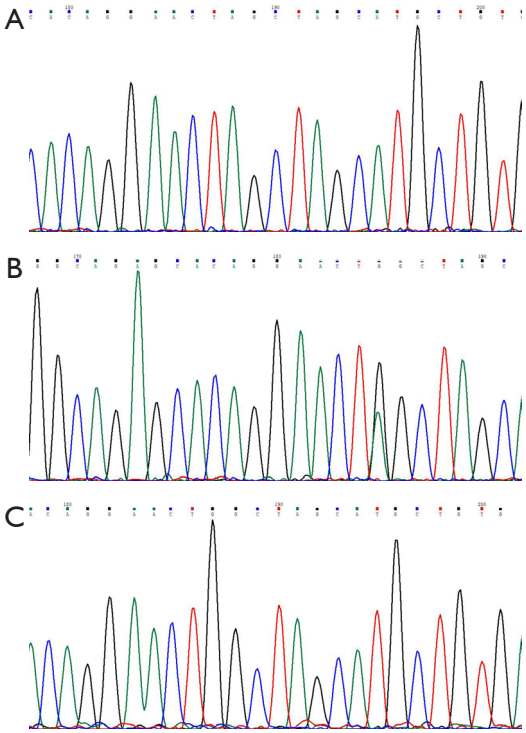

Figure 3 Genotype of the rs2715928 locus. (A) AA type; (B) AG type; (C) GG type. C, allele c; T, allele t; A, allele a; G, allele g.

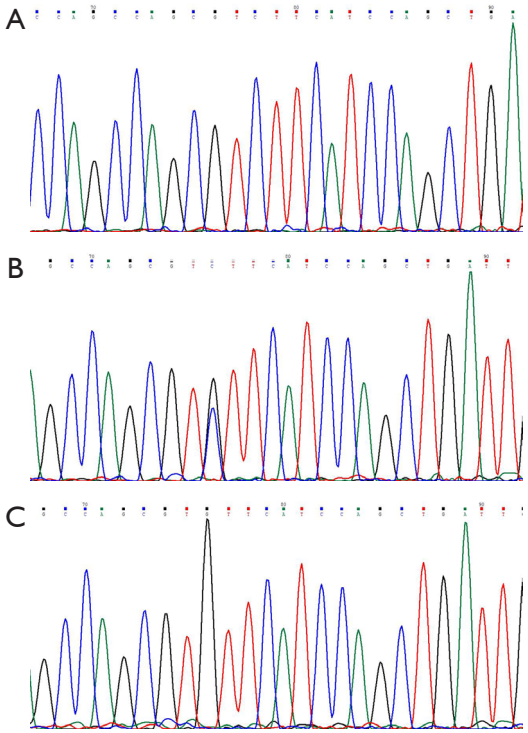

Figure 4 Genotype of the rs35771982 locus. (A) CC type; (B) CG type; (C) GG type. C, allele c; T, allele t; A, allele a; G, allele g.

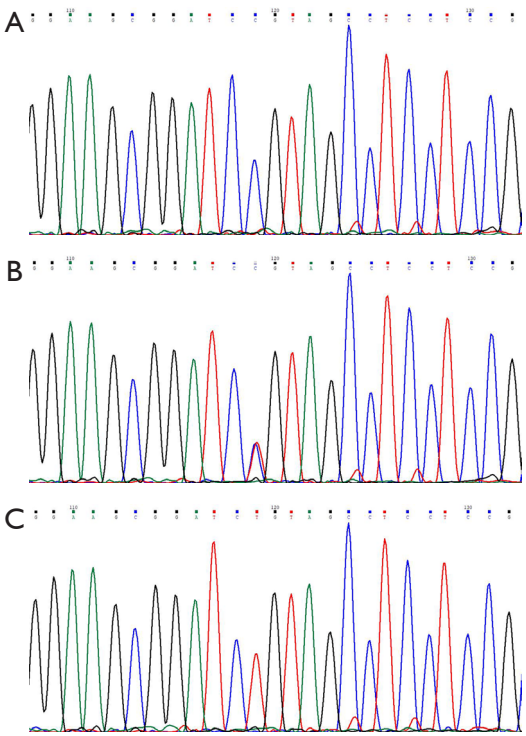

Figure 5 Genotype of the rs3749119 locus. (A) CC type; (B) CT type; (C) TT type. C, allele c; T, allele t; A, allele a; G, allele g.

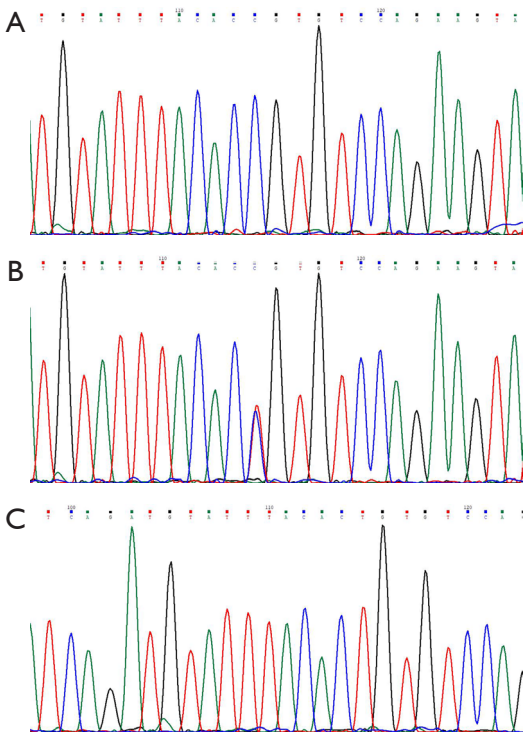

Figure 6 Genotype of the rs3828323 locus. (A) CC type; (B) CT type; (C) TT type. C, allele c; T, allele t; A, allele a; G, allele g.

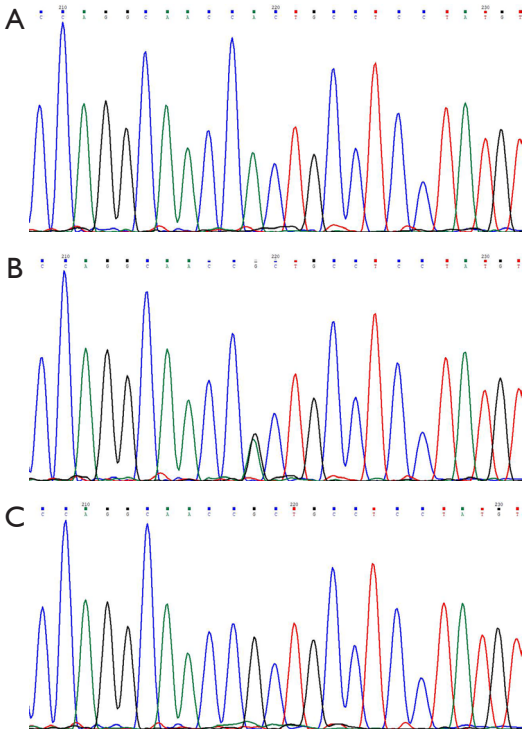

Figure 7 Genotype of the rs4665143 locus. (A) AA type; (B) AG type; (C) GG type. C, allele c; T, allele t; A, allele a; G, allele g.

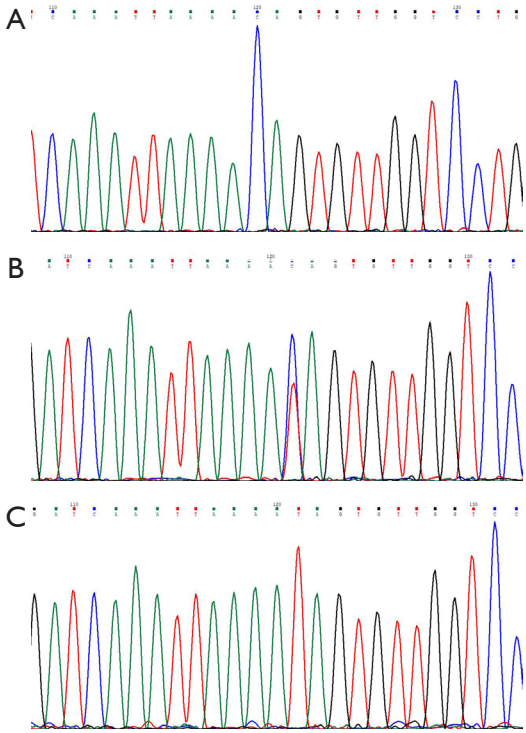

Figure 8 Genotype of the rs6757188 locus. (A) CC type; (B) CT type; (C) TT type. C, allele c; T, allele t; A, allele a; G, allele g.

Distribution of the different SNP genotypes and alleles in the IMN and healthy groups

The χ^2 test revealed statistically significant differences in genotype and allele frequencies of the *PLA2R* SNP loci rs35771982 and rs3749119 (P<0.05) of the 2 groups. When the IMN and healthy groups were compared, the more frequent of the GG genotype and allele G for rs35771982 and that of the CC genotype and allele C for rs3749119 were more common those of the control group. In contrast, the distribution of individual genotypes and allele frequencies of *PLA2R* SNPs rs16844715, rs2715918, rsrs2715928, rs3828323, rs4665143, and rs6757188 was not statistically different (P>0.05) between the 2 groups (*Table 4*).

Analysis of genetic risk factors for IMN

The rs35771982 and rs3749119 loci of the *PLA2R* SNP were analyzed. The risk of rs35771982 carrying the G allele was 5.056-fold higher than the risk associated with carrying the C the allele in the additive model (G *vs.* C). In the dominant model (GG *vs.* CG + CC), the risk of the GG genotype was 4-fold higher than that of the CG + CC genotype. The risk of rs3749119 carrying the C allele in the additive model (C *vs.* T) was 4.75-fold higher than that of the T allele, and in the dominant model (CC *vs.* CT + TT), the risk of the CC genotype was 4.333-fold higher than that of the CT + TT genotype. In the recessive model (CC + CT *vs.* TT), the risk of the CC + CT genotype was 10.737-fold higher than that of the TT genotype (*Tables 5*,*6*).

Correlations of the IMN risk genotypes with clinical indicators

A statistically significant difference in uric acid (P<0.05) level existed between the groups carrying the rs35771982 locus GG and those carrying the CC + CG genotypes. No statistically significant difference (P>0.05) was found for sex, age, systolic blood pressure, diastolic blood pressure, total cholesterol, triglycerides, albumin, urea nitrogen, creatinine, or 24-hour urine protein quantification. The rs3749119 locus CC and the CC + TT genotypes showed a statistically significant difference in albumin (P<0.05) with respect to the CT + TT genotype group, but no statistically significant difference was found for sex, age, systolic blood pressure, diastolic blood pressure, total cholesterol,

Table 3 Results of the Hardy-Weinberg equilibrium test

Genetic locus	Genotype	IMN	HC	P1	P2
rs16844715	CC	17	11	0.555	0.613
	CT	17	8		
	TT	1	6		
rs2715918	AA	1	0	1.000	0.762
	AG	12	9		
	GG	22	16		
rs2715928	AA	25	12	0.836	1.000
	AG	8	10		
	GG	2	3		
rs35771982	CC	0	4	1.000	0.400
	CG	5	6		
	GG	30	15		
rs3749119	CC	28	12	1.000	0.479
	CT	6	7		
	TT	1	6		
rs3828323	CC	26	19	1.000	0.282
	CT	9	3		
	TT	0	3		
rs4665143	AA	8	8	0.687	0.628
	AG	21	8		
	GG	6	9		
rs6757188	CC	3	1	1.000	1.000
	CT	16	11		
	TT	16	13		

P1, P value for the IMN group; P2, P value for the healthy group. IMN, idiopathic membranous nephropathy; HC, healthy controls; C, allele c; T, allele t; A, allele a; G, allele g.

triglycerides, urea nitrogen, creatinine, uric acid, or 24-hour urine protein levels (P>0.05; *Tables 7*,*8*).

Analysis of factors associated with IMN

Univariate logistic regression analysis revealed a correlation between IMN incidence (dependent variable) and gender, age, systolic blood pressure, diastolic blood pressure, total cholesterol, triglyceride, serum albumin, urea nitrogen, serum creatinine, and uric acid (independent variables). Multivariate logistic regression analysis of gender, age, systolic blood pressure, diastolic blood pressure, total cholesterol, triglyceride, serum albumin, urea nitrogen, serum creatinine, and uric acid showed that gender, age, and total cholesterol were associated with the occurrence of IMN (P<0.05), as shown in *Table 9*.

Discussion

IMN currently has no equivalent term in traditional Chinese medicine (TCM). Based on clinical signs and symptoms, most physicians describe the disease as “edema”,

Table 4 Distribution of the different genotypes and alleles of SNPs in the IMN and healthy groups

Genetic locus	IMN		HC		χ^2	P
	Number of samples	Frequency (%)	Number of samples	Frequency (%)		
rs16844715						
Genotypes					6.283	0.046
CC	17	48.57	11	44.00		
CT	17	48.57	8	32.00		
TT	1	2.86	6	24.00		
Alleles					2.198	1.138
C	51	72.86	30	60.00		
T	19	27.14	20	40.00		
rs2715918						
Genotypes					0.710	1.000
AA	1	2.86	0	0		
AG	12	34.29	9	36.00		
GG	22	62.85	16	64.00		
Alleles					0.075	0.784
A	14	20.00	9	18.00		
G	56	80.00	41	82.00		
rs2715928						
Genotypes					3.456	0.215
AA	25	71.43	12	48.00		
AG	8	22.86	10	40.00		
GG	2	5.71	3	12.00		
Alleles					3.599	0.058
A	58	82.86	34	68.00		
G	12	17.14	16	32.00		
rs35771982						
Genotypes					7.229	0.016
CC	0	0	4	16.00		
CG	5	14.29	6	24.00		
GG	30	85.71	15	60.00		
Alleles					9.521	0.002
C	5	7.14	14	28.00		
G	65	92.86	36	72.00		

Table 4 (*continued*)

Table 4 (*continued*)

Genetic locus	IMN		HC		χ^2	P
	Number of samples	Frequency (%)	Number of samples	Frequency (%)		
rs3749119						
Genotypes					8.350	0.015
CC	28	80.00	12	48.00		
CT	6	17.14	7	28.00		
TT	1	2.86	6	24.00		
Alleles					11.810	0.001
C	62	88.57	31	62.00		
T	8	11.43	19	38.00		
rs3828323						
Genotypes					5.002	0.055
CC	26	74.29	19	76.00		
CT	9	25.71	3	12.00		
TT	0	0	3	12.00		
Alleles					0.605	0.437
C	61	87.14	41	82.00		
T	9	12.86	9	18.00		
rs4665143						
Genotypes					4.897	0.086
AA	8	22.86	8	32.00		
AG	21	60.00	8	32.00		
GG	6	17.14	9	36.00		
Alleles					0.275	0.600
A	37	52.86	24	48.00		
G	33	47.14	26	52.00		
rs6757188						
Genotypes					0.570	0.783
CC	3	8.58	1	4.00		
CT	16	45.71	11	44.00		
TT	16	45.71	13	52.00		
Alleles					0.416	0.529
C	22	31.43	13	26.00		
T	48	68.57	37	74.00		

SNP, single-nucleotide polymorphism; IMN, idiopathic membranous nephropathy; HC, healthy controls; C, allele c; T, allele t; A, allele a; G, allele g.

Table 5 Correlation of the IMN prevalence risk with genotype at the rs35771982 locus

Model	Groups	IMN	HC	χ^2	P	OR	95% CI
Additive model	G	65	36	9.521	0.002	5.056	1.684–15.177
	C	5	14				
Explicit model	GG	30	15	5.143	0.023	4.000	1.158–13.817
	CG + CC	5	10				

IMN, idiopathic membranous nephropathy; HC, healthy controls; OR, odds ratio; CI, confidence interval; G, the g allele of rs35771982; C, the c allele of rs35771982.

Table 6 Correlation between IMN prevalence risk and the genotype at the rs3749119 locus

Model	Group	IMN	HC	χ^2	P	OR	95% CI
Additive model	C	62	31	11.810	0.001	4.750	1.871–12.061
	T	8	19				
Explicit model	CC	28	12	6.720	0.010	4.333	1.385–13.561
	CT + TT	7	13				
Implicit model	CC + CT	34	19	4.441	0.035	10.737	1.201–95.953
	TT	1	6				

IMN, idiopathic membranous nephropathy; HC, healthy controls; OR, odds ratio; CI, confidence interval; C, the c allele of rs3749119; T, the t allele of rs3749119.

Table 7 Comparison between the rs35771982 genotype and clinical indicators

Characteristics	GG genotype	CG + CC genotype	χ^2/t/z	P
Gender			0.352	0.687
Male	24	4		
Female	6	1		
Age, years	55.50±9.85	51.40±6.84	–0.890	0.380
SBP, mmHg	137.67±24.87	130.00±10.00	–0.673	0.505
DBP, mmHg	82.50 (80.00, 90.00)	80.00 (75.00, 85.00)	–0.947	0.344
CHOL, mmol/L	7.06 (6.47, 8.74)	7.98 (6.60, 12.72)	–0.754	0.451
TG, mmol/L	2.85±1.25	2.78±2.00	–0.117	0.907
ALB, g/L	26.59±7.38	21.76±5.12	–1.400	0.171
BUN, mmol/L	6.35 (4.65, 7.98)	5.61 (4.10, 7.11)	–0.424	0.671
SCR, μmol/L	76.70 (57.63, 92.48)	67.90 (56.30, 107.60)	–0.707	0.480
UA, μmol/L	348.30 (308.18, 413.13)	291.20 (273.00, 314.70)	–2.168	0.030
UPRO, g/d	3.68 (2.23, 5.95)	5.16 (2.43, 8.87)	–0.849	0.396

Data are presented as number, mean ± SD, median (range). GG, the gg genotype of rs35771982; CG, the cg genotype of rs35771982; CC, the cc genotype of rs35771982; SBP, systolic pressure; DBP, diastolic pressure; CHOL, total cholesterol; TG, triglyceride; ALB, albumin; BUN, urea nitrogen; SCR, serum creatinine; UA, uric acid; UPRO, 24-hour urine protein quantification.

Table 8 Comparison of the rs3749119 genotype and clinical indicators

Characteristic	CC genotype	CT + TT genotype	χ^2/t/z	P
Gender			0.011	0.916
Male	23	5		
Female	5	2		
Age, years	54.82±10.41	55.29±4.92	–0.114	0.910
SBP, mmHg	130 (120.00, 145.00)	140 (130.00, 160.00)	–1.312	0.189
DBP, mmHg	80 (80.00, 90.00)	90 (80.00, 90.00)	–0.637	0.524
CHOL, mmol/L	7.02 (6.34, 8.62)	7.98 (6.85, 16.17)	–1.608	0.108
TG, mmol/L	2.72±1.12	3.32±2.07	–0.738	0.485
ALB, g/L	26.67±7.86	22.84±2.49	2.176	0.037
BUN, mmol/L	6.47 (4.46, 7.99)	6.20 (5.15, 6.87)	–0.330	0.741
SCR, μmol/L	76.70 (58.45, 97.03)	69.10 (55.50, 83.00)	–0.949	0.343
UA, μmol/L	348.30 (304.73, 411.30)	305.60 (276.30, 343.70)	–1.361	0.174
UPRO, g/d	3.72 (2.27, 7.17)	3.69 (1.28, 5.16)	–0.722	0.470

Data are presented as number, mean ± SD, median (range). CC, the cc genotype of rs3749119; CT, the ct genotype of rs3749119; TT, the tt genotype of rs3749119; SBP, systolic pressure; DBP, diastolic pressure; CHOL, total cholesterol; TG, triglyceride; ALB, albumin; BUN, urea nitrogen; SCR, serum creatinine; UA, uric acid; UPRO, 24-hour urine protein quantification.

Table 9 Multifactor logistic regression analysis

Characteristic	P	OR	95% CI
Gender	0.001	1.440	0.010–0.193
Age	0.026	1.145	1.017–1.223
SBP	0.251	1.100	0.935–1.295
DBP	0.585	0.938	0.746–1.179
CHOL	0.009	5.602	1.542–20.351
TG	0.769	0.969	0.788–1.193
ALB	0.052	0.523	0.273–1.004
BUN	0.922	0.974	0.576–1.647
SCR	0.401	1.080	0.902–1.294
UA	0.162	1.009	0.996–1.022

OR, odds ratio; CI, confidence interval; SBP, systolic pressure; DBP, diastolic pressure; CHOL, total cholesterol; TG, triglyceride; ALB, albumin; BUN, urea nitrogen; SCR, serum creatinine; UA, uric acid.

"urine turbidity", or "asthenia", among other terms. The etiology and pathogenesis of IMN is mostly attributed to disorders of the lung, and especially a defective spleen and kidney, along with a deficiency of qi and yin, resulting in dampness, damp heat, and blood stasis. Wang and Chen (15) proposed a dialectical method of "microscopic syndrome differentiation" and believed that "pathological changes of glomerular basement membrane in MN are equivalent to the accumulation of dampness and heat into stasis in TCM". According to modern medicine, the occurrence of IMN is mainly related to immune dysfunction, target antigen exposure, and genetic and environmental factors.

IMN is one of the most common types of adult nephrotic syndrome, with diffuse thickening of the glomerular basement membrane and massive deposition of epithelial-side immune complex as the main pathological changes (16). In 2009, Beck *et al.* (5) identified M-type PLA2R antibodies in up to 70% of patients with IMN, indicating that M-type PLA2R is an important target antigen of IMN. M-type PLA2R represents a type I transmembrane glycoprotein, a member of the mannitol receptor family, with a molecular weight of 180 KDa and a coding sequence located on chromosome 2q23-24. It is a type II fibronectin-like protein composed of 1465 amino acids, carrying a cysteine-rich extracellular region from amino- to carboxyl terminals, 8 repeated tandem C-type lectin domains, a transmembrane domain, and a short cytoplasmic inner tail (17). rs35771982 is located on the fifth exon of *PLA2R*. In our study, we

found a higher frequency of the GG genotype and G alleles of rs35771982 in the IMN group, suggesting that the GG genotype was associated with susceptibility to IMN, with the G allele being the susceptible allele. This result is consistent with findings in the French (18), Chinese/Taiwanese (19), and Sichuan populations (11). Similar studies performed by Kim *et al.* (20) in Korea, Zhou *et al.* (21) in Northeast China, and Guo (13) in Xinjiang China indicated that the CC genotype of rs35771982 is associated with IMN susceptibility. rs3749119 is located in the *PLA2R* region, which is transcribed to the 5' untranslated region (5'UTR) of *PLA2R* messenger RNA (mRNA). PLA2R 5'UTR plays an important role in gene regulation. In the IMN group in our study, the CC genotype and C allele frequencies of rs3749119 locus were higher, suggesting that the CC genotype is associated with susceptibility to IMN, with the C allele being the susceptible allele. This result was consistent with studies involving French (18) and Japanese (22) populations. A meta-analysis of studies investigating the correlation between the *PLA2R* gene loci rs3749117 and rs3749119 and IMN susceptibility (23) concluded that the 2 loci were related to Asian IMN, with the C allele of rs3749119 being the susceptible allele. The susceptibility of IMN is related to *PLA2R* rs2715928 and rs16844715. The risk genes of IMN are the A allele of rs2715928, the C allele of rs16844715, the G allele of rs35771982, the T allele of rs3749117, and the A allele of rs4664308. The combination of the AA genotype of rs2715928 and the GG genotype of rs35771982 is the biggest risk factor for the disease. Haploid has certain influence on the susceptibility of IMN, but has no obvious effect on its clinical symptoms (24). One study (25) discovered a linkage of familial MN to chromosome Xp11.3-11.22. Family members affected with MN have a significantly lower genetic risk score than do individuals with anti-PLA2R-associated MN, suggesting that X-linked familial MN represents a separate etiologic entity.

Environmental pollution may also be one of the factors contributing to MN. Studies show that in the area with PM2.5 >70 mg/m^3, the incidence of MN increases by 14% for every 10 mg/m^3 increase in PM2.5 concentration (2). $PM_{2.5}$ induces the expression of *PLA2R* in inflammatory cells of the lung. Anti-PLA2R antibodies are generated against *PLA2R* presented by antigen-presenting cells. Both $PM_{2.5}$ and anti-PLA2R antibodies enter glomerular capillaries, and anti-PLA2R antibodies cross endothelial cells and glomerular basement membrane and bind with PLA2R on podocytes to form immune complexes, resulting in the onset of MN (26).

We established different genetic models of rs35771982 and rs3749119, and logistic regression analysis showed that the rs35771982 GG and rs3749119 CC genotypes were associated with susceptibility to IMN. The results showed statistically significant differences in uric acid levels ($P<0.05$) between the rs35771982 GG and CG + CC genotypes, and between the rs3749119 CC and CT + TT genotypes. Serum albumin levels showed statistical significance ($P<0.05$). Multiple logistic regression analysis showed that gender, age, and triglyceride levels affected the occurrence of IMN ($P<0.05$). We believe that a variety of factors contribute to the differences in results derived from different populations. First, the studies included different ethnic groups with different genetic backgrounds. Second, geography, living environment, and dietary habits may also be key factors, and further studies are needed to elucidate their role in gene polymorphism. At the same time, there are some shortcomings in this experiment. The sample size is too small. The collection of sample size should be expanded in the future.

At present, studies have shown that the PLA2R gene polymorphism site is associated with IMN, but there are still many problems to be solved. Because the podocytes of animals do not express PLA2R, many studies have become difficult. Regional differences make the PLA2R gene polymorphism site different susceptibility to IMN, and the reasons for the differences are not clear. To study IMN from the perspective of genetics, a large amount of data is still needed to verify the relationship between polymorphic sites of genes and IMN in the future. Therefore, it is not necessary to detect PLA2R gene polymorphism in IMN diagnosis at present.

Conclusions

In conclusion, the rs35771982 and rs3749119 polymorphisms were associated with IMN susceptibility in Heilongjiang Chinese and were related to the susceptibility genotypes GG and CC, respectively. The GG genotype was positively correlated with uric acid, while the CC genotype was negatively correlated with albumin. Gender, age, and triglyceride levels may influence the occurrence of IMN. It is hoped that this study will provide some theoretical basis for finding gene targets for the prevention and treatment of IMN and exploring the pathogenesis of membranous nephropathy.

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Footnote

Reporting Checklist: The authors have completed the MDAR reporting checklist. Available at https://atm.amegroups.com/article/view/10.21037/atm-22-6648/rc

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://atm.amegroups.com/article/view/10.21037/atm-22-6648/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Helsinki Declaration (revised in 2013). The study was approved by the Ethics Committee of Heilongjiang Provincial Hospital of Traditional Chinese Medicine (No. 2021-023-01) and the informed consent of all patients was obtained.

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