

## PLA2R1 and HLA-DQA1 gene variations in idiopathic membranous nephropathy in South China

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### ABSTRACT

**Introduction:** Associations of variations in PLA2R1 and HLA-DQA1 genes with susceptibility to idiopathic membranous nephropathy (IMN) have been well documented. Association with spontaneous remission, however, is poorly defined in the Chinese Han population.

**Methods:** A Chinese cohort of 117 IMN patients and 138 healthy controls were recruited between July 2009 and November 2019. Case-control studies for single-nucleotide polymorphisms (SNPs) within HLA-DQA1 (rs2187668) and PLA2R1 (rs35771982, rs4664308, rs3749117, rs3749119) genes were performed. The contributions of these polymorphisms to predict susceptibility, titre of autoantibodies against the M-type phospholipase A<sub>2</sub> receptor (anti-PLA2R1), glomerular PLA2R1 expression, and spontaneous remission were analysed.

**Results:** We found that variations in PLA2R1 (SNPs rs35771982, rs4664308, rs3749117) were strongly associated with IMN susceptibility, while SNP (rs2187668) within HLA-DQA1 did not increase the risk of IMN. All SNPs in PLA2R1 and HLA-DQA1 were not statistically associated with anti-PLA2R1 titre, glomerular PLA2R1 expression and spontaneous remission after Bonferroni correction ( $P > 0.0167$ ). Clinical and pathological parameters such as lower levels of serum albumin, higher levels of anti-PLA2R1 and glomerular PLA2R1 expression were independent risk factors for non-spontaneous remission.

**Conclusion:** This study confirms that variations in PLA2R1 (SNPs rs35771982, rs4664308, rs3749117) are risk factors for IMN. We found excellent association of serum albumin level, anti-PLA2R1 titre and glomerular PLA2R1 positivity with non-spontaneous remission in IMN.

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**Keywords:** HLA-DQA1, idiopathic membranous nephropathy, PLA2R1, susceptibility, spontaneous remission

### INTRODUCTION

Membranous nephropathy is an organ-specific autoimmune disease and is the most common cause of adult-onset nephrotic syndrome.<sup>1</sup> The diagnosis of membranous nephropathy mainly depends on pathological characteristics observed through various techniques such as diffuse thickening of the glomerular basement membrane and spike formation by light microscopy, granular deposition of immunoglobulin G (IgG) and complement 3, along with the glomerular capillary loops by immunofluorescence, and subepithelial electron-dense deposits by electron microscopy.<sup>2</sup> In recent years, the discovery of M-type phospholipase A<sub>2</sub> receptor (PLA2R1) and demonstration of its function in idiopathic membranous nephropathy

(IMN) have played an important role in distinguishing IMN from secondary membranous nephropathy and predicting the treatment efficacy and kidney outcome in IMN patients as well.<sup>3-6</sup>

Genome-wide association studies in white ancestry populations have demonstrated the association of single-nucleotide polymorphisms (SNPs) in PLA2R1 and major histocompatibility complex, class II, DQ alpha 1 (HLA-DQA1) with IMN susceptibility.<sup>7</sup> Subsequent studies conducted in Asian and Western populations achieved consistent results.<sup>8-15</sup> However, only a few studies analysed the genetic background of PLA2R1 and HLA-DQA1 in Chinese patients with primary membranous nephropathy. Moreover, their results are inconsistent with each other.<sup>9,14,15</sup> Lv et al.

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found that 3 SNPs (rs35771982, rs3749117, rs4664308) within PLA2R1 were strongly associated with IMN in a northern Chinese cohort.<sup>9</sup> Another study in Taiwan found that only SNP rs35771982 within PLA2R1 was significantly associated with IMN susceptibility.<sup>14</sup> Wang et al. found that SNP rs2187668 within HLA-DQA1 and SNPs rs2715918 and rs4665143 within PLA2R1 increased the risk of IMN.<sup>15</sup> The variable clinical course of IMN and treatment strategies make treatment decisions challenging.<sup>16–18</sup> About two-thirds of IMN patients experience non-spontaneous remission (NSR), and a significant number of patients have an inadequate response to non-immunosuppressive therapy and progress to end-stage renal disease.<sup>19–23</sup> Older age at onset, female sex, baseline proteinuria >8g/dL, urinary excretion of  $\beta_2$ -microglobulin or IgG and preserved renal function at presentation, the level of autoantibodies against PLA2R1 (anti-PLA2R1) are predictors of NSR.<sup>24–27</sup> Nevertheless, few studies have investigated whether genetic factors affect spontaneous remission (SR) in IMN patients. Some studies have found that SNPs (rs2187668, HLA-DQA1\*05:01, HLA-DQB1\*02:01, HLA-DRB1\*15:02, HLA-DRB1\*03:01) within HLA were associated with anti-PLA2R1 levels, and SNP within PLA2R1 such as rs35771982 and SNPs (rs2187668, HLA-DRB1\*15:01, HLA-DRB3\*02:02) within HLA were related to PLA2R1-positive staining in IMN.<sup>9,10</sup> Moreover, Wang et al. found that HLA-DRB1\*15:02 was associated with significantly lower estimated glomerular filtration rates at baseline and a significantly worse renal outcome in a Chinese cohort.<sup>15</sup> However, studies in Taiwan and Japan demonstrated that SNP rs35771982 and HLA-DRB1\*15:01 did not relate to the kidney outcome in IMN patients.<sup>12–14</sup> The goals of this study were: (1) to validate the association of PLA2R1 and HLA-DQA1 risk alleles with IMN susceptibility in a Chinese population; (2) to evaluate the relationship of risk alleles in PLA2R1 and HLA-DQA1 genes with anti-PLA2R1 titre and PLA2R1 deposits; and (3) to assess the use of these genetic variants, clinical parameters, pathological immunofluorescence variables in predicting SR in patients with IMN.

## METHODS

### Study population

This prospective study was carried out at the Department of Nephrology, Huashan Hospital, Fudan University, Shanghai, China, between July 2009 and November 2019. Chinese Han patients aged 18–80 years who have been diagnosed with IMN by biopsy and using non-immunosuppressive therapy were enrolled. Patients

with secondary membranous nephropathy were excluded. The control group consisted of 138 Chinese adults without nephropathy. The study was approved by the ethics committees of Huashan Hospital, Fudan University. Spontaneous remission was defined as achieving either partial or complete remission. Partial remission was defined as proteinuria <3.5g/dL and a 50% or more significant reduction from peak values, accompanied by an improvement of the serum albumin concentration and a stable serum creatinine level. Complete remission was defined as proteinuria <0.3g/dL, accompanied by normal serum albumin concentration and normal serum creatinine level in the absence of immunosuppressive therapy. Patients with PLA2R1 staining positive in the glomeruli were defined as PLA2R1-related IMN. Written informed consent was obtained from all participants.

### HLA-DQA1 and PLA2R1 SNP genotyping

Genomic DNA was extracted from peripheral blood samples anticoagulated with K2-EDTA (TIANamp Blood DNA Kit, Beijing, China) according to the manufacturer's instructions. We genotyped SNPs in HLA-DQA1 (rs2187668) and PLA2R1 (rs35771982, rs4664308, rs3749117, rs3749119), which have been confirmed to have a relationship with susceptibility in IMN patients both in Asians and other ancestries. Amplification reactions were performed on an ABI 3730XL real-time polymerase chain reaction system (Applied Biosciences, Hamburg, Germany) according to the manufacturer's standard. The genotyping efficiency of each variant exceeded 95%.

### Kidney biopsy and detection of circulating anti-PLA2R1

A kidney biopsy was performed at the time of diagnosis in all patients. The fluorescence intensities of PLA2R1 were determined using a semi-quantitative scale of 0 to 4: 0=negative, 1=weak, 2=moderate, 3=intense and 4=glaring staining. Circulating anti-PLA2R1 were detected using commercial enzyme-linked immunosorbent assay kits (EUROIMMUN AG, Lübeck, Germany) according to the standard instructions.

### Statistical analyses

Data are expressed as number, percentage, mean and standard deviation (SD), median and interquartile range (IQR). Normally distributed variates are expressed as mean (SD), and continuous variates of non-normal distribution as median with IQR. Categorical variables are expressed as absolute values and percentages. Student's t-test and Mann-Whitney U test were performed in order to compare the clinical characteristics

of patients with PLA2R1-related versus PLA2R1-unrelated IMN. Unadjusted and adjusted logistic regression analyses were performed to evaluate the relationship between disease risk, clinical parameters, SR and genetic variables. A Kaplan-Meier curve was used to analyse the risk factors for SR. Predictors of NSR were analysed using the Cox regression model. Results are expressed as odds ratio (OR) or hazard ratio (HR) with 95% confidence intervals. Statistical analyses were 2-tailed. All statistical calculations were performed using SPSS Statistics software version 26.0 (IBM Corp, Armonk, US) and SNPAssoc R software package. The control group was tested for Hardy-Weinberg equilibrium using Fisher's Exact test. Analyses of allelic frequencies found that the genotype of the controls at SNP rs3749119 was not in Hardy-Weinberg equilibrium ( $P=0.0001$ ), so the SNP was excluded for analysis. Analyses were performed for 5 different inheritance models: dominant, co-dominant, recessive, over-dominant and additive.  $P$  values were modified with the Bonferroni method to correct for multiple test comparisons. The adjusted level for statistical significance was established at  $P<0.0167$ .

## RESULTS

### Association of HLA-DQA1 and PLA2R1 SNPs with IMN susceptibility

Single-nucleotide polymorphisms within HLA-DQA1 (rs2187668) and PLA2R1 (rs35771982, rs4664308, rs3749117, rs3749119) genes were successfully genotyped in 117 IMN patients and 138 controls. The proportion of men was 55.8% in the control group and was 65.81% in IMN patients. The average age was 45.4 years in the control group and 49.8 years in IMN patients. Since the age and sex of the control group did not match the IMN populations ( $P=0.02706$  and  $P=0.01459$ , respectively), the  $P$  value of disease risk was adjusted by the 2 parameters. Allelic frequencies of the following SNPs in healthy controls were determined: rs2187668 (CC:  $n=131$ ; CT:  $n=3$ ; TT:  $n=4$ ); rs35771982 (CC:  $n=15$ ; GC:  $n=57$ ; GG:  $n=66$ ); rs4664308 (AA:  $n=65$ ; AG:  $n=57$ ; GG:  $n=16$ ); rs3749117 (CC:  $n=15$ ; CT:  $n=57$ ; TT:  $n=66$ ); and rs3749119 (CC:  $n=63$ ; CT:  $n=44$ ; TT:  $n=31$ ). Allelic frequencies of the following SNPs in IMN patients were also analysed: rs2187668 (CC:  $n=110$ ; CT:  $n=3$ ; TT:  $n=4$ ); rs35771982 (CC:  $n=3$ ; GC:  $n=30$ ; GG:  $n=84$ ); rs4664308 (AA:  $n=88$ ; AG:  $n=28$ ; GG:  $n=1$ ); rs3749117 (CC:  $n=2$ ; CT:  $n=30$ ; TT:  $n=85$ ); and rs3749119 (CC:  $n=76$ ; CT:  $n=38$ ; TT:  $n=3$ ). Our results indicated that A allele at rs4664308 was significantly

associated with IMN under the 5 different inheritance models (codominant: OR 2.73, OR 19.82,  $P=3.58\times 10^{-6}$ ; dominant: OR 3.33,  $P=1.08\times 10^{-5}$ ; recessive: OR 13.85,  $P=3.68\times 10^{-4}$ ; over-dominant: OR 2.22,  $P=4.56\times 10^{-3}$ ; additive: OR 3.12,  $P=8.61\times 10^{-7}$ ). G allele at rs35771982 was significantly associated with IMN under all the inheritance models (codominant: OR 2.39, OR 5.96,  $P=3.50\times 10^{-4}$ ; dominant: OR 2.73,  $P=1.97\times 10^{-4}$ ; recessive: OR 4.37,  $P=1.12\times 10^{-2}$ ; over-dominant: OR 2.03,  $P=1.10\times 10^{-2}$ ; additive: OR 2.41,  $P=6.64\times 10^{-5}$ ). T allele at rs3749117 was also associated with IMN under the 5 different inheritance models (codominant: OR 2.42, OR 8.84,  $P=1.09\times 10^{-4}$ ; dominant: OR 2.84,  $P=1.16\times 10^{-4}$ ; recessive: OR 6.45,  $P=3.60\times 10^{-3}$ ; over-dominant: OR 2.03,  $P=1.09\times 10^{-2}$ ; additive: OR 2.60,  $P=2.18\times 10^{-5}$ ; Table 1).

### Association of HLA-DQA1 and PLA2R1 SNPs with PLA2R1 titre, glomerular PLA2R1 expression

For all genotype-phenotype correlation studies, patients who received immunosuppressive therapy before or after biopsy immediately ( $n=9$ ), patients with no clinical information ( $n=4$ ), and patients followed up for <6 months ( $n=7$ ) were excluded (Fig. 1). Baseline characteristics and follow-up data of the remaining 97 patients were obtained from medical records until an endpoint (remission) was reached or until November 2019 (Fig. 1). Patients with a minimum follow-up of 6 months were classified according to their clinical outcome into SR or NSR patients, and the latter group was separated into 2 subgroups: receiving immunosuppressive therapies ( $n=45$ ) or non-immunosuppressive therapies ( $n=9$ ) (Fig. 1).

The IMN cohort was further divided into PLA2R1-related subgroup and PLA2R1-unrelated subgroup according to glomerular PLA2R1 expression (Table 2). Comparison of the clinical and biochemical parameters showed no differences between the PLA2R1-related and PLA2R1-unrelated patients, except for circulating anti-PLA2R1 positivity and titre ( $P=0.030$ ,  $P=0.003$ ; Table 2). All SNPs in PLA2R1 and HLA-DQA1 that we genotyped were not statistically associated with positivity of anti-PLA2R1 and glomerular PLA2R1 expression after Bonferroni correction ( $P>0.0167$ ) (Table 3).

### Association of genetic variants and clinical parameters with SR

We tested whether SNPs within HLA-DQA1 (rs2187668) and PLA2R1 (rs35771982, rs4664308, rs3749117) were associated with SR in IMN in a group comprising

Table 1. Relationship between HLA-DQA1 and PLA2R1 single-nucleotide polymorphisms (SNPs) and disease risk<sup>a</sup>

Model		OR	95% CI	P value
<b>SNP rs2187668</b>				
Codominant	CC/CT/TT	0.72	0.13–3.89	0.93
		0.94	0.22–3.95	
Dominant	CC/CT+TT	0.84	0.28–2.55	0.76
Recessive	CC+CT/TT	0.95	0.23–3.97	0.94
Over-dominant	CC+CT/TT	0.72	0.13–3.89	0.70
Additive	0, 1, 2	0.93	0.48–1.18	0.83
<b>SNP rs4664308</b>				
Codominant	AA/AG/GG	2.73	1.54–4.82	3.58×10 <sup>-6</sup>
		19.82	2.54–154.78	
Dominant	AA/AG+GG	3.33	1.92–5.79	1.08×10 <sup>-5</sup>
Recessive	AA/AG+GG	13.85	1.79–107.13	3.68×10 <sup>-4</sup>
Over-dominant	AA+GG/AG	2.22	1.27–3.87	4.56×10 <sup>-3</sup>
Additive	0, 1, 2	3.12	1.91–5.09	8.61×10 <sup>-7</sup>
<b>SNP rs35771982</b>				
Codominant	GG/GC/CC	2.39	1.36–4.20	3.50×10 <sup>-4</sup>
		5.96	1.63–21.81	
Dominant	GG/GC+CC	2.73	1.59–4.67	1.97×10 <sup>-4</sup>
Recessive	GG+GC/CC	4.37	1.21–15.78	1.12×10 <sup>-2</sup>
Over-dominant	GG+CC/GC	2.03	1.17–3.52	1.10×10 <sup>-2</sup>
Additive	0, 1, 2	2.41	1.53–3.80	6.64×10 <sup>-5</sup>
<b>SNP rs3749117</b>				
Codominant	TT/TC/CC	2.42	1.38–4.25	1.09×10 <sup>-4</sup>
		8.84	1.92–40.61	
Dominant	TT/TC+CC	2.84	1.65–4.88	1.16×10 <sup>-4</sup>
Recessive	TT+TC/CC	6.45	1.42–29.32	3.60×10 <sup>-3</sup>
Over-dominant	TT+CC/TC	2.03	1.17–3.53	1.09×10 <sup>-2</sup>
Additive	0, 1, 2	2.60	1.63–4.16	2.18×10 <sup>-5</sup>

CI: confidence interval; HLA-DQA1: major histocompatibility complex; class II, DQ alpha 1 gene; OR: odds ratio; PLA2R1: M-type phospholipase A2 receptor; SNP: single-nucleotide polymorphisms

<sup>a</sup> P values have been adjusted by sex and age. Multiple test correction cut-off is set to  $P < 0.0167$ .

43 patients with SR and 54 with NSR. No significant association was found for any of these variants (Table 3). Univariate Cox regression analyses showed that urinary  $\beta_2$ -microglobulin (HR 1.194, 95% CI 1.031–1.383,  $P=0.018$ ), level of anti-PLA2R1 (HR 1.001, 95% CI 1.000–1.003,  $P=0.034$ ), and glomerular PLA2R1 positivity (HR 6.523, 95% CI 1.846–23.051,

$P=0.004$ ) were risk factors for NSR in IMN patients. The higher level of serum albumin at baseline on biopsy (HR 0.892, 95% CI 0.811–0.980,  $P=0.018$ ) was a protective factor for NSR. Multivariate analyses identified that anti-PLA2R1 level (HR 1.001, 95% CI 1.000–1.002,  $P=0.041$ ) and glomerular PLA2R1 positivity (HR 3.432, 95% CI 1.237–9.519,  $P=0.018$ )

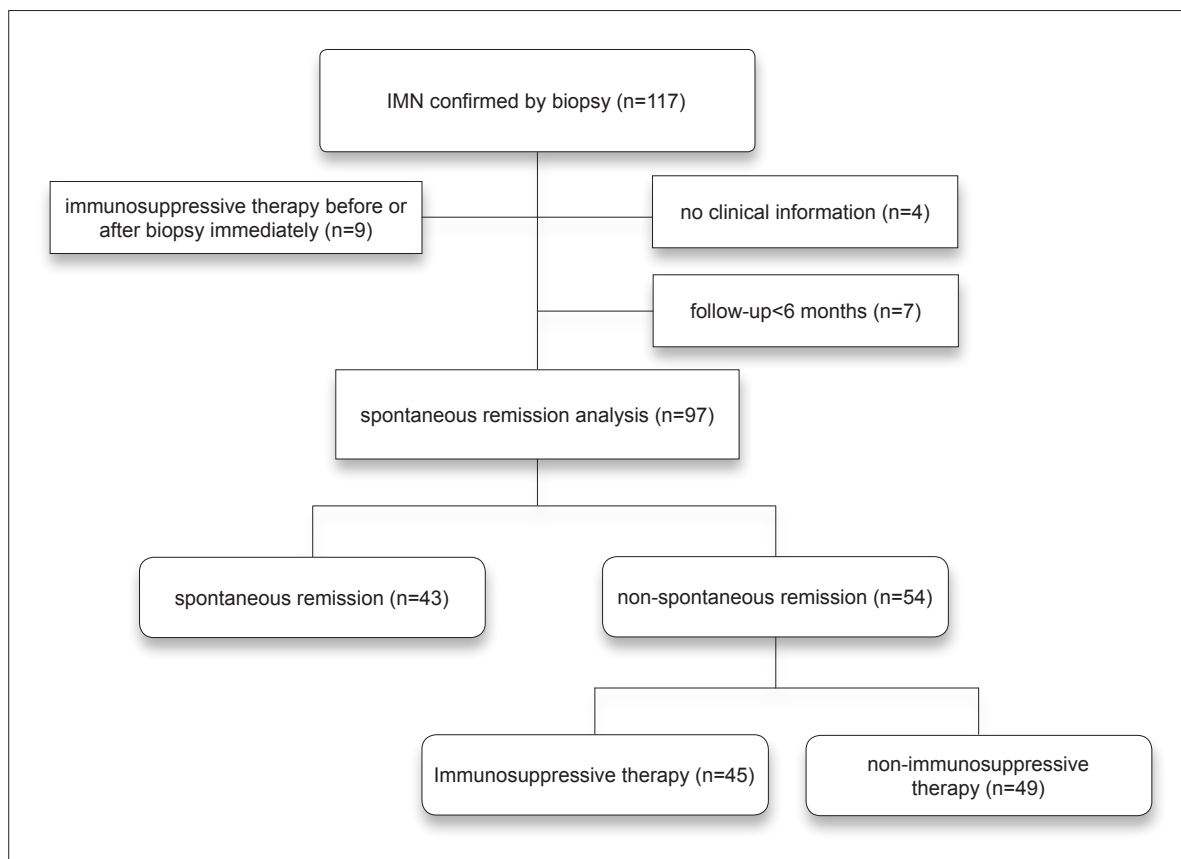


Fig. 1. Flowchart for the classification of idiopathic membranous nephropathy (IMN) patients included in the genotype–phenotype correlation studies.

Table 2. Comparison of clinical and serology parameters of PLA2R1-related and PLA2R1-unrelated cases of idiopathic membranous nephropathy<sup>a</sup>

	PLA2R1-related IMN (n=79)	PLA2R1-unrelated IMN (n=18)	<i>P</i> value
Male, n (%)	51 (64.56)	11 (61.11)	0.998
Age, years	51.2±14.8	45.9±18.6	0.274
<b>Serology parameters</b>			
Serum creatinine, mmol/L	70.46±16.15	69.89±20.34	0.898
Albumin, g/L	26.32±5.61	26.25±7.67	0.966
Proteinuria, g/24 h	4.19 (2.27, 8.06)	2.93 (2.37, 6.19)	0.328
Cholesterol, mmol/L	7.01±2.16	7.79±3.97	0.254
Triglycerides, mmol/L	2.99±2.75	2.10±0.93	0.220
Urinary β <sub>2</sub> -microglobulin, mg/L	0.90±1.90	0.46±0.64	0.378
Anti-PLA2R1 positivity, n (%)	62 (78.48)	9 (50)	0.030
Anti-PLA2R1 level, RU/mL	104.96 (26.14, 263.31)	38.15 (1.82, 109.72)	0.003
Remission, n (%)	31 (39.24)	12 (66.67)	0.064
Follow-up duration, month	34 (16.50, 47.00)	34 (26.75, 41.75)	0.846

Anti-PLA2R1: autoantibodies against M-type phospholipase A2 receptor; PLA2R1: M-type phospholipase A2 receptor

<sup>a</sup> Values are expressed as mean ± standard variation or median (interquartile range) unless otherwise indicated.

Table 3. Relationship between HLA-DQA1 and PLA2R1 single-nucleotide polymorphisms and anti-PLA2R1 level, anti-PLA2R1 positivity or PLA2R1 positivity in the glomeruli in a group comprising 43 patients with SR and 54 with NSR

	Codominant	Dominant	Recessive	Over-dominant	Additive
<b>SNP rs2187668</b>					
Anti-PLA2R1 level	0.962	0.990	0.835	0.849	0.919
Anti-PLA2R1 positivity	0.808	0.544	0.562	0.800	0.808
Glomerular PLA2R1 positivity	0.169	0.371	1.000	0.062	0.169
Spontaneous remission	0.524	0.256	0.429	0.429	0.281
<b>SNP rs4664308</b>					
Anti-PLA2R1 level	0.579	0.367	0.490	0.454	0.319
Anti-PLA2R1 positivity	0.035	0.045	0.268	0.099	0.035
Glomerular PLA2R1 positivity	0.192	0.869	0.186	0.488	0.192
Spontaneous remission	0.794	0.700	1.000	0.544	0.794
<b>SNP rs35771982</b>					
Anti-PLA2R1 level	0.162	0.059	0.798	0.063	0.093
Anti-PLA2R1 positivity	0.495	0.237	0.800	0.260	0.280
Glomerular PLA2R1 positivity	0.341	0.310	0.535	0.167	0.534
Spontaneous remission	0.244	0.669	0.252	0.274	0.244
<b>SNP rs3749117</b>					
Anti-PLA2R1 level	0.165	0.059	0.475	0.090	0.060
Anti-PLA2R1 positivity	0.455	0.237	0.481	0.331	0.210
Glomerular PLA2R1 positivity	0.222	0.310	0.309	0.137	0.583
Spontaneous remission	0.455	0.669	0.501	0.387	0.455

Anti-PLA2R1: autoantibodies against M-type phospholipase A2 receptor; CI: confidence interval; HLA-DQA1: major histocompatibility complex, class II, DQ alpha 1 gene; OR: odds ratio; PLA2R1: M-type phospholipase A2 receptor; SNP: single-nucleotide polymorphisms

Table 4. Risk factors for non-spontaneous remission in patients with idiopathic membranous nephropathy

Predictor	Univariate Cox regression			Multivariate Cox regression		
	HR	95% CI	P value	HR	95% CI	P value
Male	1.267	0.342–4.693	0.723			
Age, years	1.009	0.986–1.034	0.438			
Serum creatinine, mmol/L	0.981	0.947–1.016	0.273			
Albumin, g/L	0.892	0.811–0.980	0.018	0.893	0.828–0.964	0.004
Proteinuria, g/24 h	0.979	0.895–1.071	0.638			
Cholesterol, mmol/L	1.016	0.842–1.225	0.868			
Triglycerides, mmol/L	1.073	0.927–1.242	0.344			
Urinary $\beta_2$ -microglobulin, mg/L	1.194	1.031–1.383	0.018			
Anti-PLA2R1, U/mL	1.001	1.000–1.003	0.034	1.001	1.000–1.002	0.041
Serum anti-PLA2R1 positivity	1.357	0.452–4.069	0.586			
Glomerular PLA2R1 positivity	6.523	1.846–23.051	0.004	3.432	1.237–9.519	0.018

Anti-PLA2R1: autoantibodies against M-type phospholipase A2 receptor; CI: confidence interval; HR: hazard ratio; PLA2R1: M-type phospholipase A2 receptor



were 2 independent risk factors for NSR, and serum albumin level at baseline on biopsy (HR 0.893, 95% CI 0.828–0.964,  $P=0.004$ ) was an independent protective factor for NSR (Table 4). Kaplan-Meier curve analysis also indicated that patients with glomerular PLA2R1 positivity showed no difference in SR compared with patients without it ( $P=0.051$ ) (Fig. 2).

## DISCUSSION

Our study aims to evaluate the association of HLA-DQA1 and PLA2R1 risk alleles with IMN susceptibility and SR in Chinese subjects with IMN, and to assess the use of these genetic variants and clinical parameters in predicting SR in patients with IMN. We found that variations in PLA2R1 (SNPs rs35771982, rs4664308, rs3749117) were strongly associated with IMN susceptibility, especially SNP rs4664308, while the SNP (rs2187668) within HLA-DQA1 did not increase the risk of IMN. In a genome-wide association study, Stanescu et al. reported the association between SNP rs2187668 in HLA-DQA1 and SNP rs4664308 in PLA2R1 with IMN in the white population.<sup>7</sup> The risk association was stronger for HLA-DQA1 than for PLA2R1 gene. Lv et al. investigated the association of IMN with variations at 3 loci each in the HLA-DQA1 and PLA2R1 genes in a Chinese population.<sup>9</sup> The strongest associations of IMN were with SNPs in the PLA2R1 gene (rs3749117:

OR 2.32, 95% CI 2.00–2.69; rs4664308: OR 2.35, 95% CI 2.02–2.73).<sup>9</sup> In our study, the strongest association was with variation at SNP rs4664308 in the PLA2R1 gene. Previous studies have shown evidence of gene–gene interaction between HLA-DQA1 (SNP rs2187668) and PLA2R1 (SNP rs4664308) as determinants of IMN risk.<sup>7,9</sup> Homozygosity for the rare variants at both loci conferred a 78.5-fold (range 34.55–178.17) higher risk of developing IMN in the white population.<sup>7</sup> In contrast, the risk was higher in the Chinese IMN patients bearing AA at the rs4664308 locus in HLA-DQA1 and GA at the rs2187668 locus in PLA2R1 (OR 12.33, 95% CI 1.38–110.04).<sup>9</sup> The variable results of different studies may be due to the small sample size, different races and variable study designs.

In our study, none of the SNPs within HLA-DQA1 and PLA2R1 that we genotyped were related to anti-PLA2R1 titre and glomerular PLA2R1 expression, which was inconsistent with previous studies. In 2013, Lv et al. found that variations in HLA-DQA1 (SNP rs2187668) and PLA2R1 (SNPs rs2715918, rs4665143) were associated with anti-PLA2R1 positivity.<sup>9</sup> Another study conducted in South Asia found that only HLA-DQA1 (SNP rs2187668) was related to anti-PLA2R1 positivity.<sup>10</sup> Wang et al. demonstrated that DRB1\*0301 within HLA-DQA1 was associated with a significantly higher level of anti-PLA2R1 (OR 1.58, 95%

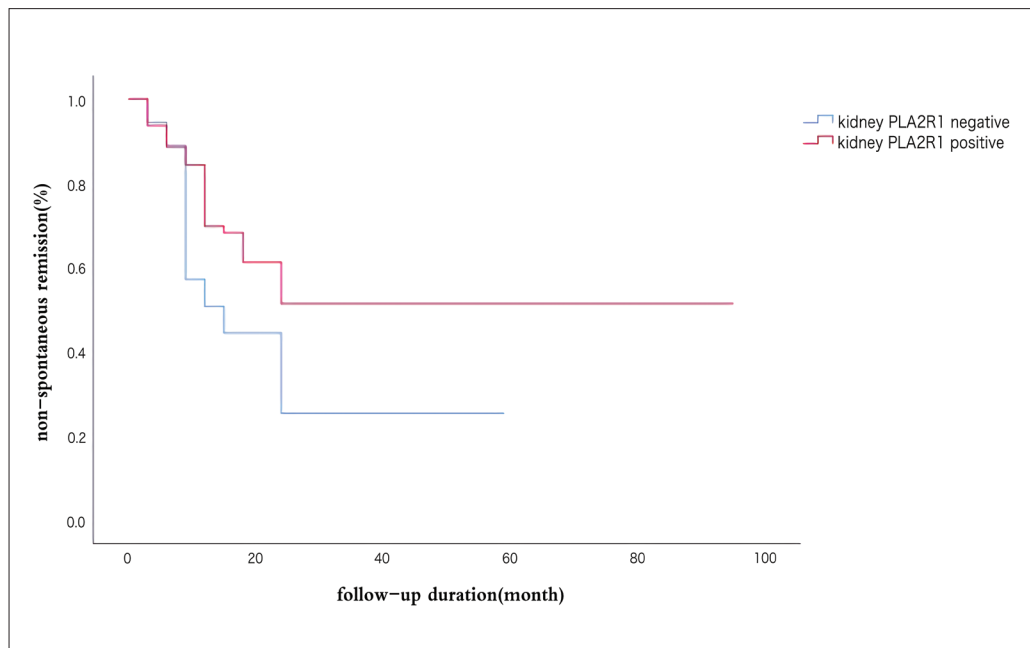


Fig. 2. Kaplan-Meier curve analysis for spontaneous remission in patients with idiopathic membranous nephropathy: a comparison between the patients with and without glomerular PLA2R1 expression. The 2 groups showed no difference in spontaneous remission during follow-up.

CI 1.13–2.22) in 258 Chinese patients.<sup>28</sup> The difference in these observations may be due to racial differences, small sample size and variable study designs.

We also investigated whether the 4 SNPs that we genotyped contributed to predicting IMN prognosis. We found no relationship between the polymorphisms of HLA-DQA1 (rs2187668) or PLA2R1 (rs35771982, rs4664308, rs3749117), and SR in our cohort. Bullich et al. showed that the risk SNPs (rs2187668, rs4664308) for IMN development also predicted response to immunosuppressive therapy and protection to renal function decline.<sup>8</sup> A study published in 2018 reported that DRB1\*1502 in HLA-DQA1 was associated with a significantly worse renal and higher risk of end-stage renal disease.<sup>28</sup> The authors assumed that HLA genes might control anti-PLA2R1 production and primary membranous nephropathy severity and outcome.<sup>28</sup> The clinical course of IMN is incredibly variable, so searching for prognostic markers of clinical outcome is vital. Age at onset, female sex, baseline proteinuria >8g/dL, urinary excretion of  $\beta_2$ -microglobulin or IgG and preserved renal function at presentation, and the level of anti-PLA2R1 are predictors of SR.<sup>24–27</sup> The genetic variants analysed in this study showed no significant association with SR. Still, clinical and pathological parameters such as a lower level of serum albumin, higher level of anti-PLA2R1 and glomerular PLA2R1 expression were independent risk factors for NSR. The clinical complicity of the disease suggests that a combination of prognostic markers would be the best option for the prediction of clinical outcomes.

Our study verifies 3 facts: (1) the strong association of PLA2R1 (SNPs rs35771982, rs4664308, rs3749117) risk alleles with IMN susceptibility; (2) no association of the genetic variants with anti-PLA2R1 level or with glomerular PLA2R1 expression in IMN patients; and (3) no relationship between SNPs in HLA-DQA1 and PLA2R1 and SR, and the excellent association of a lower level of serum albumin, higher level of anti-PLA2R1 and glomerular PLA2R1 positivity with NSR in IMN populations. Our study provides further support for the genetic variants for IMN susceptibility and SR. It suggests the importance of a combination of clinical and pathological markers when assessing the possibility of SR.

Our study has a few limitations. We studied only those patients who received non-immunosuppressive therapy; we are thus unable to comment on the relationship of genetic variants and clinical parameters in patients

who received immunotherapy. Besides, the duration of follow-up was limited, and the sample size for the genetic analysis was small. Moreover, we only tested serum anti-PLA2R1 at biopsy, and the absence of more frequent antibody testing hindered us from assessing the relationship between the decrease in anti-PLA2R1 concentration and SR. Finally, we tested only candidate SNPs in HLA-DQA1 and PLA2R1 confirmed in Asians and Caucasians.

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