Title: *PLA2R1 and HLA-DQA1* Interaction Confers Anti-PLA2R Antibodies and Membranous Nephropathy

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Supplemental **Table1. Characteristic of patients in IMN patients and heal controls**

Characteristic	IMN patients	Health controls	P value
No. of individual	1112	1020	
Gender			
Male	620	527	
Female	492	493	
Gender ratio	1.26:1	1.07:1	0.06
Age at diagnosis	49±13	35±10	<0.001

Supplemental **Table 2. PLA2R1&HLA-DQA1 genotype distribution in patients with IMN and healthy controls in Chinese population**

Gene	Variants	Position		Control (n=1020)	IMN (n=1112)	OR(95%CI) [a,b]	*P* value	Adjusted *p* value [c]
PLA2R1	rs35771982	Exon 5	CC	81 (7.9)	33(3.0)	1	$2.34x10^{-18}$	$1.04x10^{-17}$
			CG	453(44.4)	278(25.0)	1.42(0.88-2.31)		
			GG	486(47.6)	801(72.0)	3.58(2.23-5.74)		
PLA2R1	rs3749117	Exon 5	CC	80(7.8)	35(3.1)	1	$4.75x10^{-18}$	$2.85x10^{-17}$
			CT	451(44.2)	276(24.8)	1.33(0.82-2.14)		
			TT	489(47.9)	801(72.0)	3.35(2.10-5.33)		
PLA2R1	rs4664308	Intron	AA	489(47.9)	803(72.2)	3.57(2.22-5.75)	$5.37x10^{-18}$	$3.22x10^{-17}$
			GA	449(44.0)	274(24.6)	1.44(0.88-2.35)		
			GG	82 (8.0)	35(3.1)	1		
HLA-DQA1	rs2187668	Intron	AA	2(0.2)	11(1.0)	5.08(1.00-25.76)	$2.36x10^{-11}$	$1.42x10^{-10}$
			GA	106(10.4)	248(22.3)	2.74(2.05-3.67)		
			GG	912(89.4)	853(76.6)	1		
HLA-DOB	rs11244	3'Prime UTR variant	CC	563 (55.2)	607(54.6)	1	0.899	____
			CT	380(37.3)	427(38.4)	1.04(0.87-1.25)		
			TT	77 (7.5)	78 (7.0)	0.94(0.67-1.34)		
HLA-DQB2	rs2301271	Intron	CC	727(71.3)	771(69.3)	1	0.618	____
			CT	265(26.0)	309(27.8)	1.10(0.91-1.33)		
			TT	28(2.7)	32(3.0)	1.08(0.64-1.81)		

[a]. OR ,odds ratio modeled for minor allele.
[b]. The odds ratio and the adjacent p value is performed by binary logistic regression while gender and age was included as co-variance.
[c]. Bonferroni correction was used when adjusted p value was reported.

***Supplemental* Table 3.** Logistic regression analysis of 3 SNPs in PLA2R1

	$P_{v.s.con}$	OR	95%CI
rs35771982	0.594	1.388	0.415-4.640
rs37491117	0.691	1.272	0.389-4.165
rs4664308	***0.007***	1.644	1.143-2.364
gender	0.479	0.927	0.752-1.143
age	<0.001	1.110	1.099-1.121

p values for each SNP were controlled for other two SNPs ,gender and age.

***Supplemental* Table 4 .Results of genetic model of 4 SNPs associated with IMN**

SNP	P value by different model		
	Recessive	Additive	Dominant
rs35771982	$4.62x10^{-19}$	$2.34x10^{-18}$	$1.02x10^{-4}$
rs3749117	$7.28x10^{-19}$	$4.75x10^{-18}$	$2.41x10^{-4}$
rs4664308	$1.11x10^{-18}$	$5.37x10^{-18}$	$9.50x10^{-5}$
rs2187668	0.076	$2.36x10^{-11}$	$2.85x10^{-12}$

P values for each SNP under the recessive, additive, or dominant model were calculated by logistic regression analysis controlled for the 4 SNPs, gender and age.

Interaction analysis by logistic regression and Chi-square test

Logistic regression analysis detected that rs4664308 could best explain the signal of the region in PLA2R1(Supplemental Table 3). Thus, a possible genetic interaction was performed between rs4664308 in PLA2R1 and rs2187668 in HLA-DQA1.

Multiplicative interactive effect of the SNPs was estimated by a multiple logistic regression model. For each individual key variables were defined as (1) a binary variable indicating case-control status, (2) 2 SNPs variables ranging from 0-2 indicating the number of risk alleles that the individual has. For each SNP pair, a logistic regression model was built to predict case-control status based on the indicator variables (gender, age) and the two SNP variables (a total of 4 variables and an intercept). We tested whether the log-likelihood of the model was significantly improved by adding an additional multiplicative pairwise interaction term for those two SNPs. Results showed that a multiplicative interaction could be observed between HLA-DQA1 and PLA2R1($p=5.85x10^{-3}$).

To test possible additive interactions, Chi-square test were used .According to the analysis of genetic model in Supplemental Table 4,we explore the possible additive interaction under the two conditions.(1)rs4664308 recessive interact with rs2187668 dominant (Supplemental Table 5). (2)rs4664308 additive interact with rs 2187668 dominant(Supplemental Table 6.2)

***Supplemental* Table 5. Additive interaction analysis of PLA2R1 and HLA-DQA1 in genotype combinations by chi-square test .**

Combination	Number (%)		$p_{vs.\ con}$	OR (95% CI)
	Controls	IMN		
rs4664308/*rs2187668*-0/0	464	235		1.00
rs4664308/*rs2187668*-0/1	67	74	$2.26X10^{-5}$	2.18(1.51-3.14)
rs4664308/*rs2187668*-1/0	448	618	$1.33X10^{-23}$	2.72(2.23-3.32)
rs4664308/*rs2187668*-1/1	41	185	$9.64X10^{-37}$	8.91(6.14-12.94)
		AP = 0.562	***S=2.722***	***RERI= 5.003***

Genotype combinations were conducted under the dominant model for rs2187668 and recessive for rs4664308 based on Supplemental Table 4. The overall significance for the difference in risk genotype counts between patients and controls in all groups was high. And the risk genotype combination contributed the most to the overall interaction. **RERI**, relative excess risk due to interaction; **AP**, attributable proportion due to interaction; **S**, synergy index

Supplemental* Table 6.1. Analysis of gene-gene interaction: Odds Ratios for IMN, according to Single-Nucleotide Polymorphism (SNP)and Genotype Combinations.

HLA-DQA1(rs2187668)	PLA2R1 (rs4664308)		
	GG	GA	AA
GG No.of case/control OR(95%CI)	 30 / 74 1.00	 205 / 390 1.30(0.821-2.047)	 618 / 448 3.40(2.19-5.29)
GA No.of case/control OR(95%CI)	 3 / 8 0.93(0.23-3.73)	 64 / 58 2.72(1.57-4.73)	 181 / 40 11.16 (6.47-19.25)
AA No.of case/control OR(95%CI)	 2 / 0 0.29(0.21-0.39)	 5 / 1 12.33(1.38-110.04)	 4 / 1 9.87(1.06-91.94)

* Nine combination of genotype analysis for gene-gene interaction**.** Persons who were homozygous for the low-risk allele (GG within rs2187668 and GG within rs4664308) constituted the reference category.

Supplemental* Table 6.2. Analysis of gene-gene interaction: Odds Ratios for IMN, according to Single-Nucleotide Polymorphism (SNP)and Genotype Combinations.

HLA-DQA1(rs2187668)	PLA2R1 (rs4664308)		
	GG	GA	AA
GG			
No.of case/control	30 / 74	205 / 390	618 /448
OR(95%CI)	1.00	1.30(0.82-2.05)	3.40(2.19-5.29)
AA+GA			
No.of case/control	5 / 8	69 / 59	185 / 41
OR(95%CI)	1.54(0.47-5.09)	2.89(1.67-4.99)	11.13(6.47-19.15)

*Persons who were homozygous for the low-risk allele (GG within rs 2187668 and GG within rs4664308) constituted the reference category.

***Supplemental Table7.* Clinical and pathological characteristics of IMN in subgroup by genotype of PLA2R1 and HLA-DQA1**

Clinical parameters	**rs35771982(PLA2R1)**				**rs3749117(HLA-DQA1)**			
Genotype	GG	CG	CC	P value	CC	CT	TT	*p* value
No. of patient	801	278	33	----	35	276	801	----
Gender(M/F)	482 / 319	125 / 153	13 / 20	***<0.001***	14 / 21	123/ 153	483 / 318	***<0.001***
Age(years)	50±13	48±13	45±11	0.044	45±11	48±13	50±13	0.043
SBP(mmHg)	129±18	127±19	126±20	0.327	125±20	127±19	129±18	0.287
DBP(mmHg)	81±11	81±12	79±11	0.573	79±11	81±12	81±12	0.480
Proteinuria(g/24h)[a]	4.55 (2.80-7.07)	4.60 (3.10-6.77)	4.58 (1.93-8.08)	0.829	4.58 (2.00-7.86)	4.60 (3.10-6.78)	4.55 (2.80-7.07)	0.789
Serum albumin (g/L)	26.25±6.92	25.82±7.39	27.28±7.91	0.464	27.34±7.75	25.87±7.40	26.23±6.92	0.485
Serum creatinine (umol/L)[b]	72.00 (61.00-88.00)	68.00 (57.50-80.85)	69.10 (58.10-69.10)	0.031	68.55 (57.15-86.60)	68.00 (57.25-80.92)	72.00 (61.00-88.00)	0.037
TG (mmol/L)	2.98±2.86	2.52±1.90	2.85±2.25	0.064	2.90±2.26	2.50±1.86	2.99±2.86	0.044
Tchol (mmol/L)	7.83±2.64	8.09±3.13	8.34±4.41	0.331	8.23±4.29	8.06±3.07	7.84±2.67	0.477
eGFR ($ml/min/1.73m^2$)	104.66±38.41	109.47±37.37	106.37±46.72	0.219	107.33±46.35	109.34±37.39	104.66±38.41	0.233
Pathology [c]								
I-MN	341(42.6)	143(51.4)	26(78.8)	***<0.001***	340(42.4)	142(51.4)	28(80)	***<0.001***
II-MN	347(43.3)	106(38.2)	6(18.2)		348(43.4)	105(38.1)	6(17.1)	
III-MN	113(14.1)	29(10.4)	1(3.0)		113(14.1)	275(10.5)	1(2.9)	

SBP : systolic pressure; DBP: diastolic pressure;DUP: Urinary protein of One day; Scr : Serum creatinin; TG:Total Triglyceride;
Tchol:Total cholesterol;eGFR: estimated GFR-MDRD for Chinese population (published in J Am Soc Nephrol 17: 2937–2944, 2006)

[a,b.] Neither Proteinuria or Serum creatinine conform to normal distribution,so we perform a Nonparamatric test . Other parameters using One-Way ANOVA or T -test(Continuous variable) or X^2 Test (Dichotomous variable)

[c.] A multivariable analysis ,adjusted for age,gender,duration of disease,blood pressure(SBP and DBP)and treatment, was used to evaluate the association of PLA2R and HLA-DQA1 risk alleles with stage of pathology