Supplemental Material

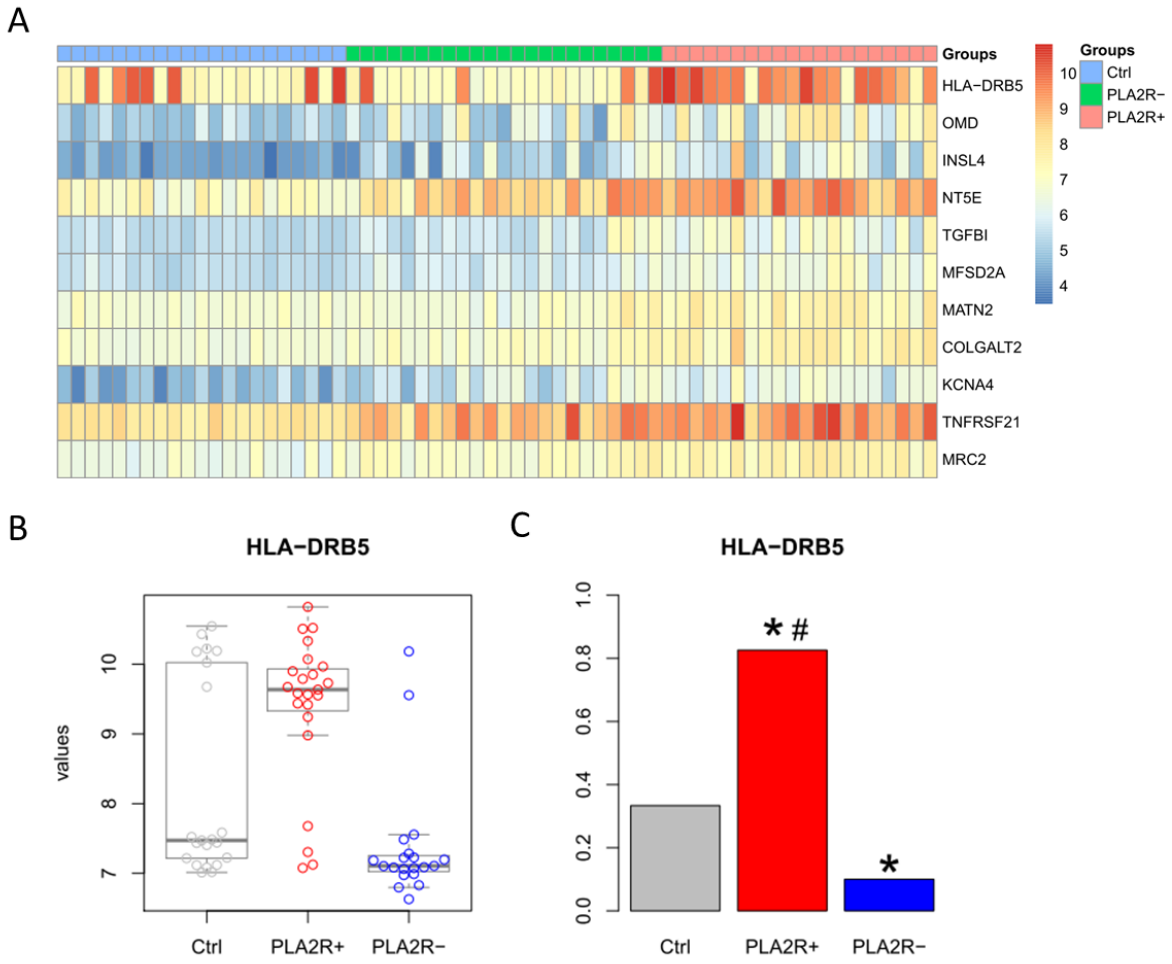


Figure S1, Differentially expressed genes (DEGs) in the micro-dissected glomeruli between the PLA2R-related MN and PLA2R-unrelated MN groups (fold change >1.5, FDR<0.05). A microarray analysis of the micro-dissected glomeruli from 44 biopsied renal tissues in patients with MN (23 with PLA2R-related MN and 20 with PLA2R-unrelated MN) as well as from 21 paracancerous renal tissues as normal control from patients without proteinuria and who underwent a nephrectomy for renal cancer. After either renal biopsy or nephrectomy, the renal tissue was submerged in RNase inhibitor and stored at -80 degrees at the Biobank. Total RNA was isolated from the micro-dissected glomeruli tissue. The Affymetrix Human Transcriptome Array 2.0 (HTA 2.0) was used in this study. Panel A shows the heat

map of the11 DEGs. Panel B shows the all-or-none expression pattern of the HLA-DRB5 gene, because the three additional distinct DRB genes DRB3, DRB4, and DRB5, are not ubiquitous throughout the population.. Panel C shows the frequency of HLA-DRB5 was 33.3%, 82.6% and 10% in the control group, PLA2R-related MN group, and the PLA2R-unrelated MN group, respectively.

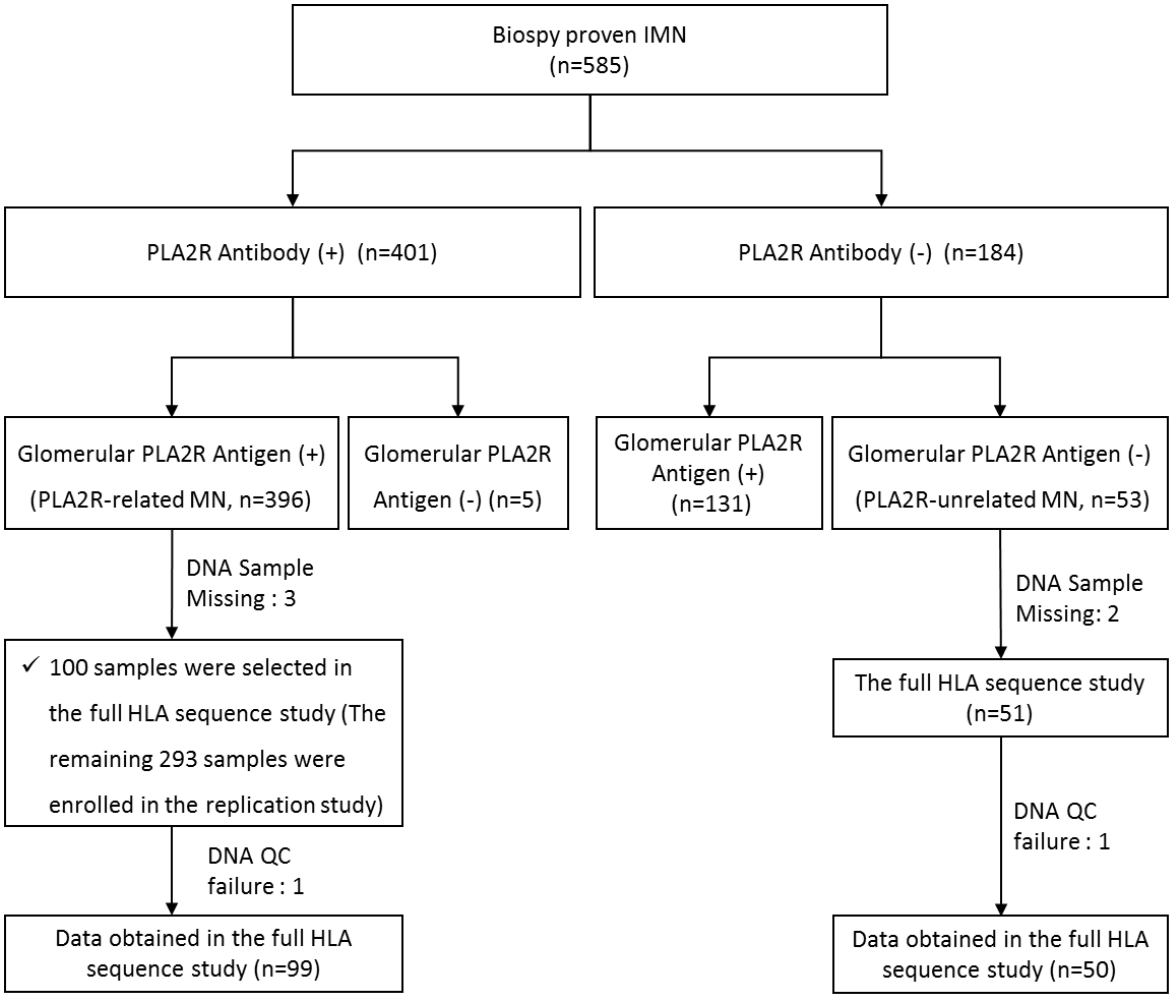


Figure S2. Flow chart of patient screening for inclusion in this study.

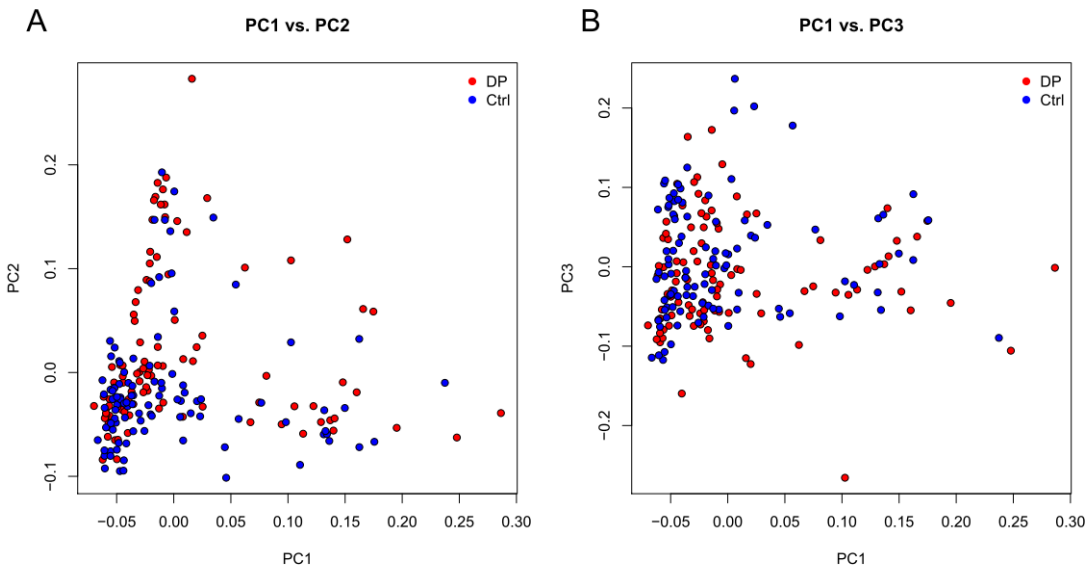


Figure S3. Plots of first three principal components. Panels A and B show plots for the first three principal components from the principal components analysis of the PLA2R-related MN samples and clinical controls.

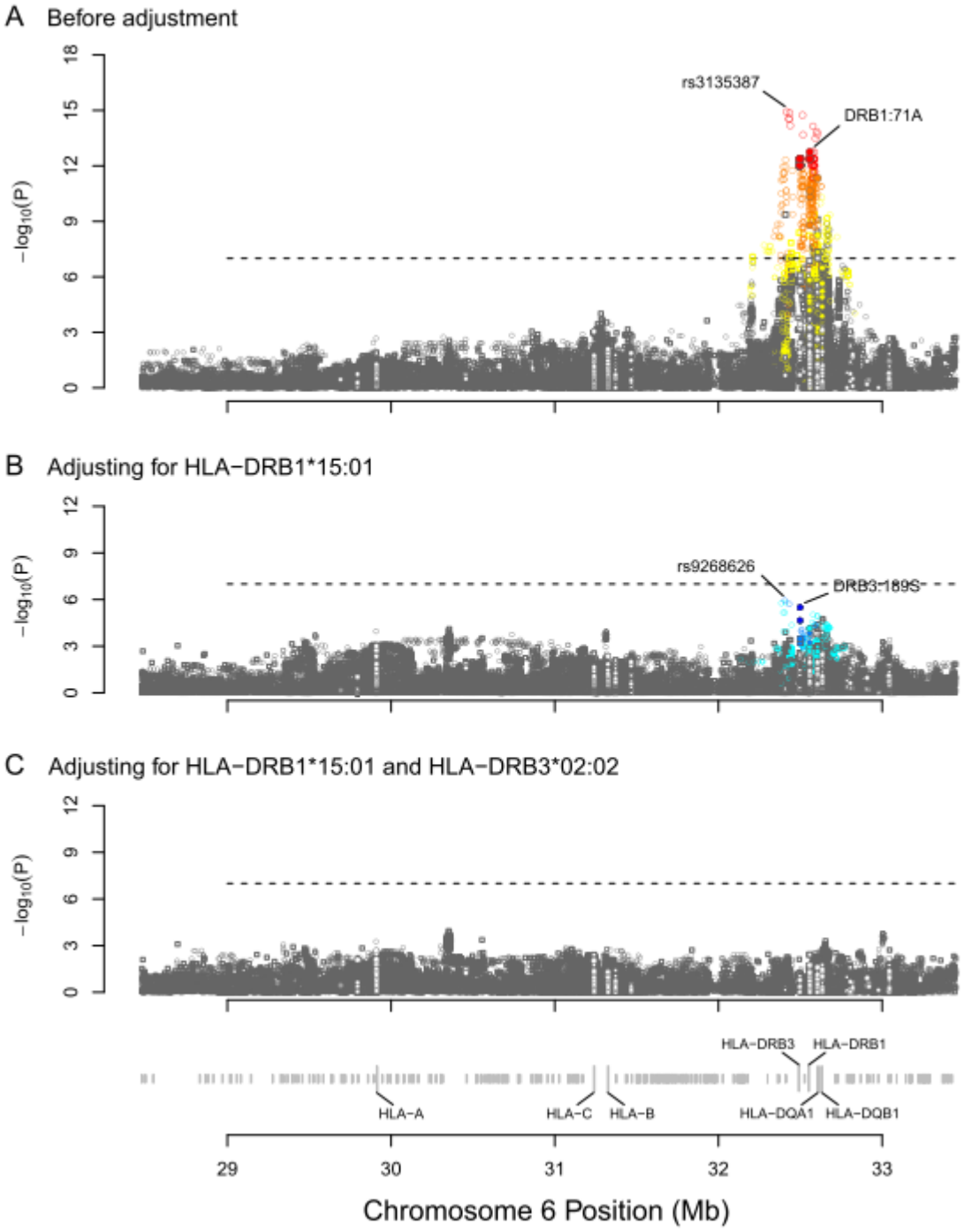


Figure S4. Association tests for SNPs and amino acid variants within the entire HLA region to PLA2R-related MN for the discovery data set (dominant model). Circles represent SNPs with an ORs > 1, whereas squares represent those with an OR ≤ 1. Colors denote the strength of the LD of the SNPs or amino acid variants to HLA-DRB1*15:01. Red denotes an R^2 value of 0.8 or more, orange denotes 0.5 to less than 0.8, yellow denotes 0.25 to less than 0.5, and white denotes less than 0.25. Panel A shows that, of the 36,576 tested SNPs or amino acid variants, rs3135387 had the strongest association with

PLA2R-related MN (OR = 18.0, $p = 1.7\times10^{-15}$). HLA-DRB1*15:01 and rs3135387 had a high level of LD ($R^2 = 0.89$, D' = 0.96). Panel B shows that, after adjusting for HLA-DRB1*15:01, the strongest signal was that of rs9268626 (OR = 13.2, $p = 1.1\times10^{-6}$), which was linked with HLA-DRB3*02:02 ($R^2 = 0.59$, D' = 0.92). Colors denote the strength of the LD of the alleles to HLA-DRB3*02:02. Blue denotes an R^2 value of 0.8 or more, azure denotes 0.5 to less than 0.8, cyan denotes 0.25 to less than 0.5, and white denotes less than 0.25. Panel C shows that adjusting for both HLA-DRB1*15:01 and HLA-DRB3*02:02 eliminated the effects of all SNPs and amino acid variants.

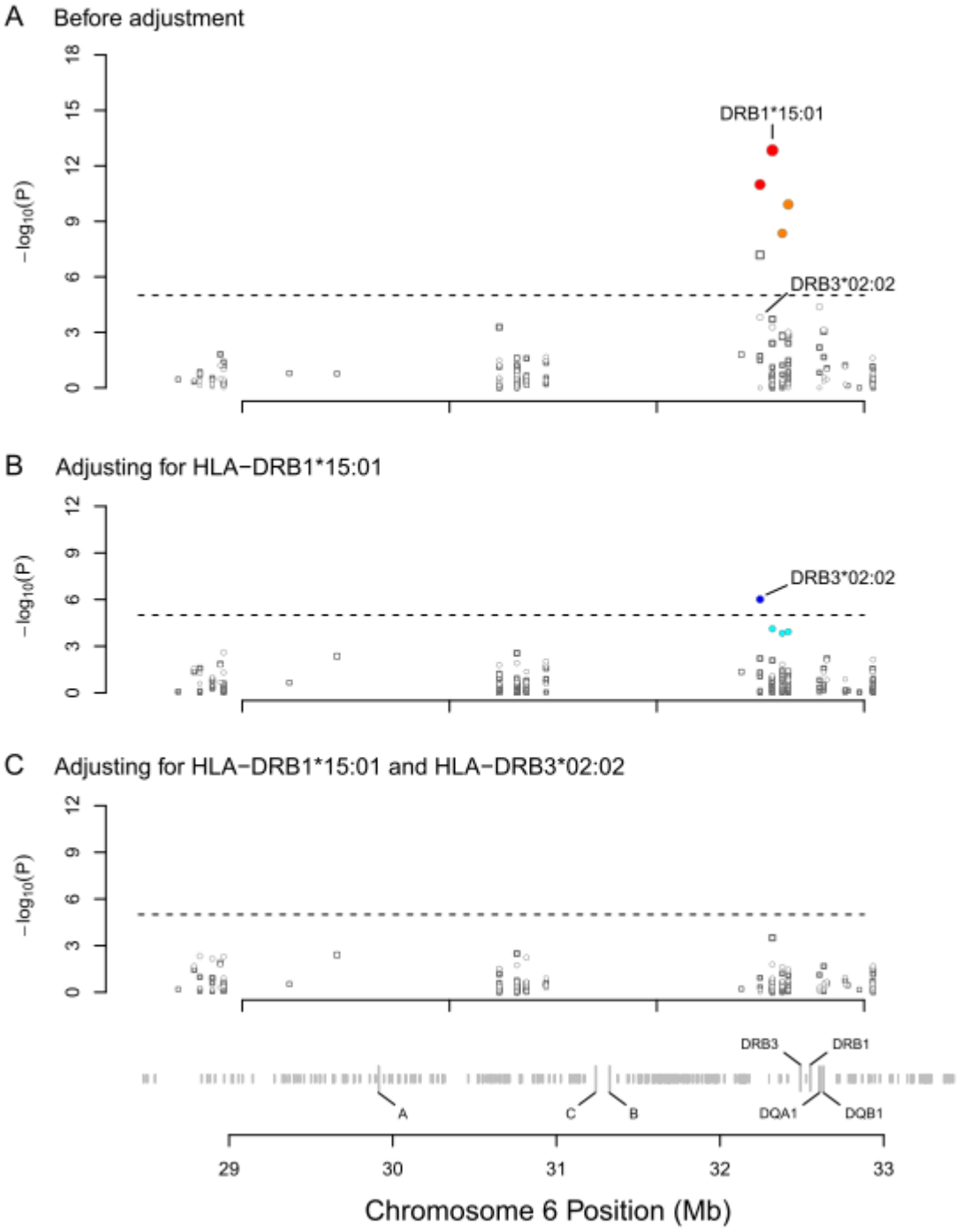


Figure S5. Association tests for all HLA variants to PLA2R-related MN in a logistic regression analysis for the discovery data set (multiplicative model).

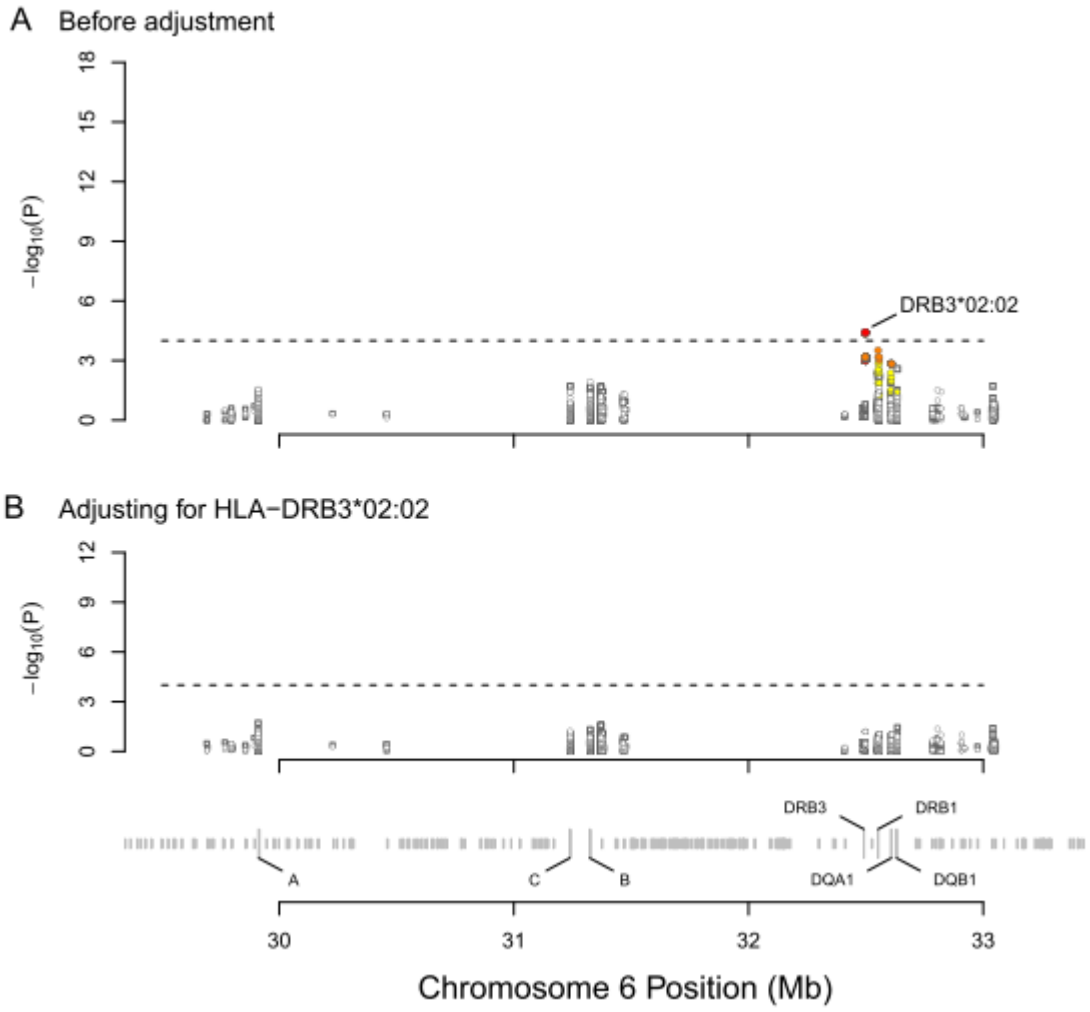


Figure S6. Association tests between all HLA alleles and PLA2R-unrelated MN in the discovery data set. Circles denote alleles with ORs > 1, whereas squares denote alleles with ORs ≤ 1. Panel A shows that HLA-DRB3*02:02 (OR = 4.6, $p = 3.9\times10^{-5}$) had the strongest signal. Because of the limited sample size, other candidate risk alleles cannot be identified. Colors denote the strength of the LD of the alleles to HLA-DRB3*02:02. Red denotes an R^2 value of 0.8 or more, orange denotes 0.5 to less than 0.8, yellow denotes 0.25 to less than 0.5 and white denotes less than 0.25. Panel B shows the association plot after adjusting for HLA-DRB3*02:02.

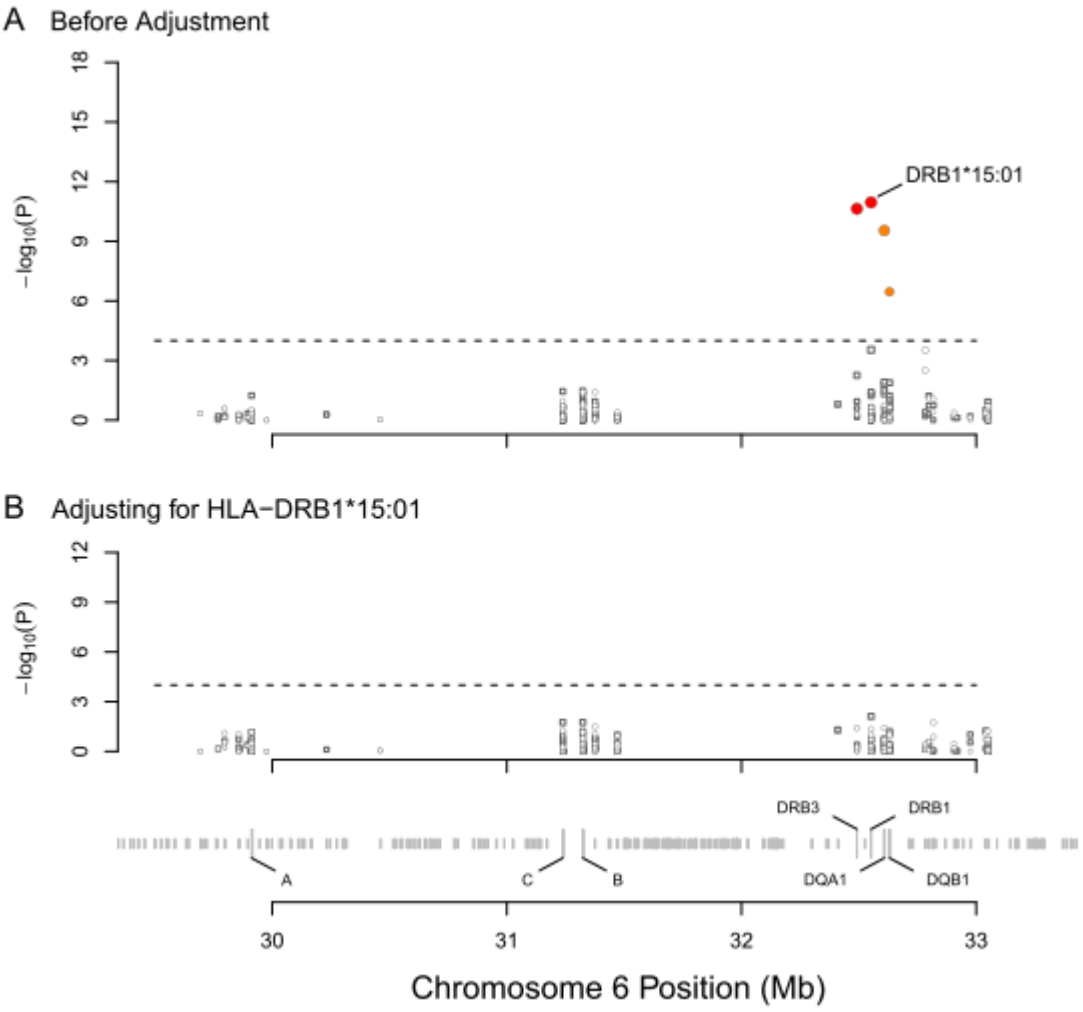
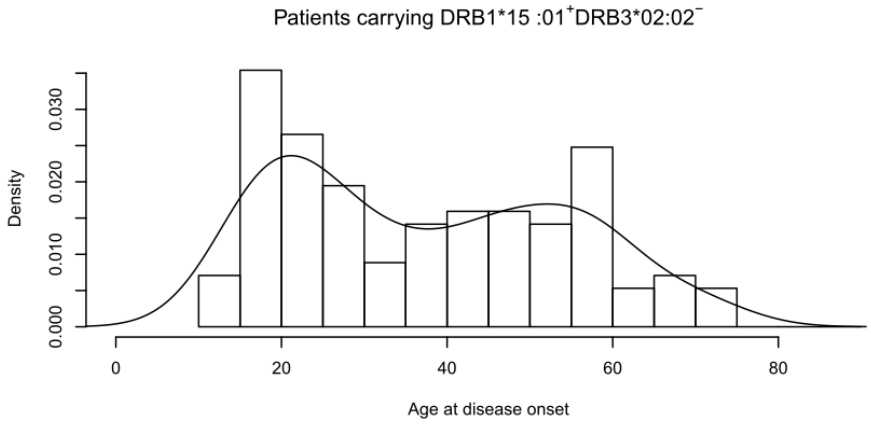
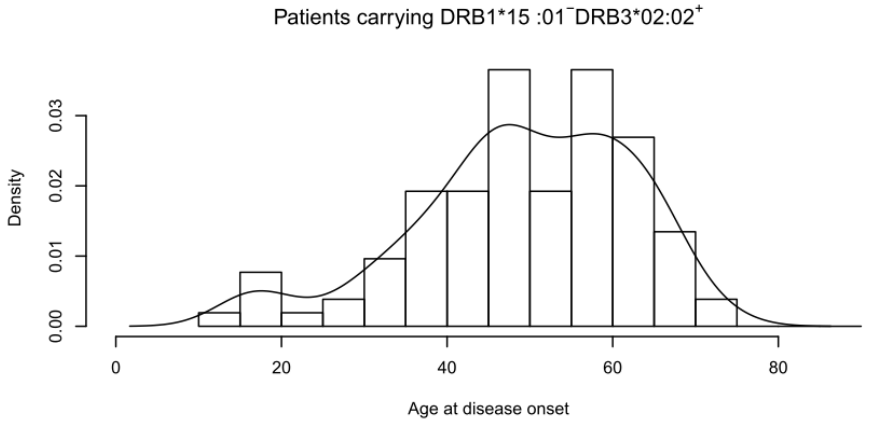
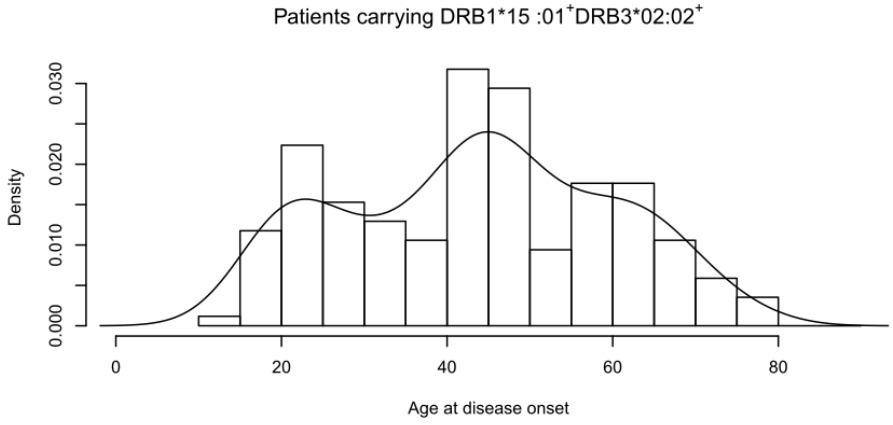


Figure S7. The different HLA distributions between PLA2R-related and PLA2R-unrelated MN. Association tests were performed for all HLA alleles for PLA2R-related MN in contrast to PLA2R-unrelated MN.


Patients carrying DRB1*15 :01+DRB3*02:02−
Density
0.000
0.010
0.020
0.030
0
20
40
60
80
Age at disease onset


Patients carrying DRB1*15 :01−DRB3*02:02+
Density
0.00
0.01
0.02
0.03
0
20
40
60
80
Age at disease onset


Patients carrying DRB1*15 :01+DRB3*02:02+
Density
0.000
0.010
0.020
0.030
0
20
40
60
80
Age at disease onset

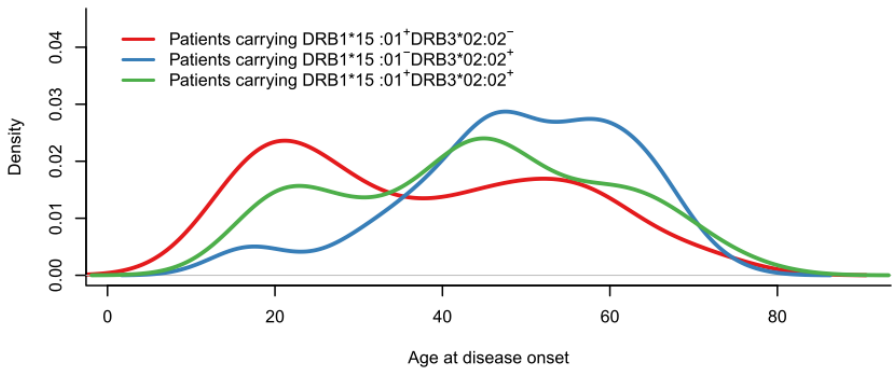

Patients carrying DRB1*15 :01+DRB3*02:02−
Patients carrying DRB1*15 :01−DRB3*02:02+
Patients carrying DRB1*15 :01+DRB3*02:02+
Density
0.00
0.01
0.02
0.03
0.04
0
20
40
60
80
Age at disease onset

Figure S8. Histogram and probability distribution plots of the age at disease onset of PLA2R-related MN in patients carrying various HLA risk alleles. There are two obvious peaks of the age distribution for patients with DRB1*15:01^{+}DRB3*02:02^{-} (approximately 20 and 50 years old, respectively), in which the former peak at approximately 20 years of age was even higher than the second peak at approximately 50 years of age. In conversely, there is approximately only one peak of onset age distribution in patients with DRB1*15:01^{-}DRB3*02:02^{+} (approximately 50 years old). Patients with DRB1*15:01^{+}DRB3*02:02^{+} which also presents two peaks of the age distribution and likely represents a mixture of these two subgroups of patients.

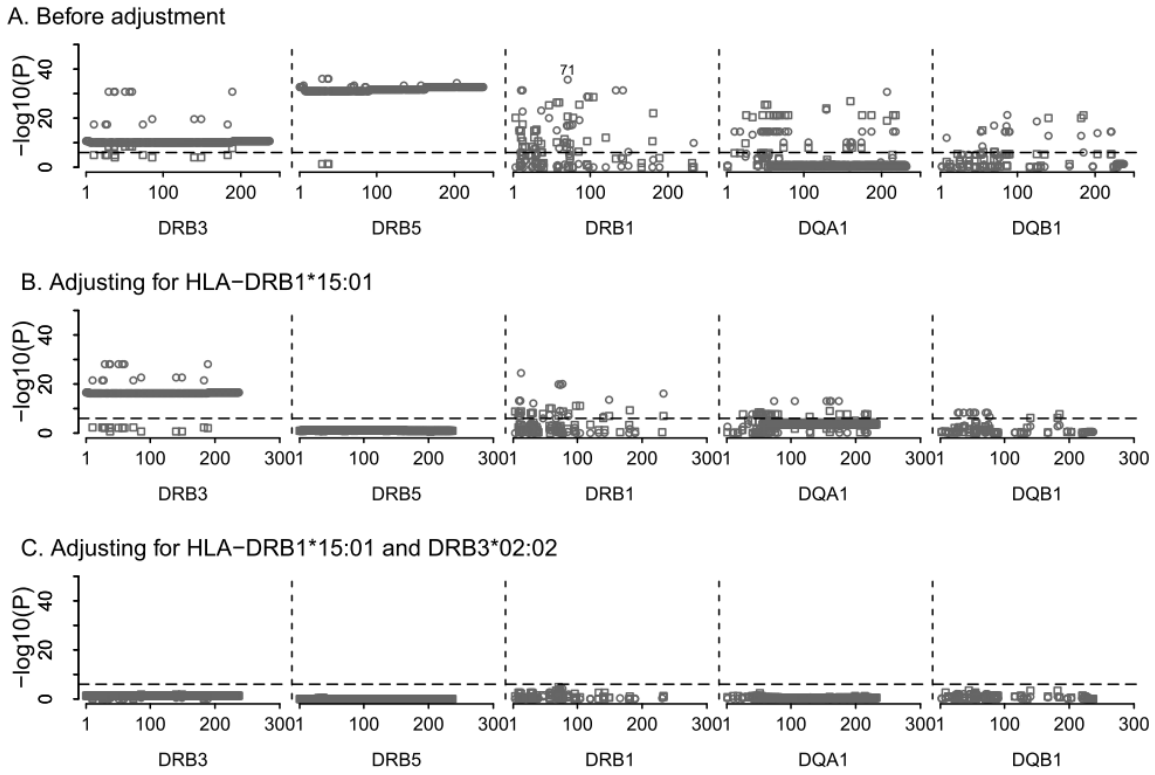


Figure S9. Association results for the amino acid variants in DRβ3, DRβ5, DRβ1, DQα1 and DQβ1 in the combined data set. (A) The strongest signals were mapped to positions 30Asp, 37Asp and 38Leu in DRβ5, all of which correspond to the DRB5*01:01 allele (all ORs = 7.9; $p = 8.6 \times 10^{-37}$) and to position 71Ala in DRβ1, which corresponds to the DRB1*15:01 allele (OR = 7.8, and $p = 1.9 \times 10^{-36}$). It is not surprising that the most significant amino acid variants was mapping to DRβ5 because the DRB1*15:01 allele is almost completely linked to the DRB5*01:01 allele, and there are significantly fewer polymorphisms in the HLA-DRB5 gene than in the HLA-DRB1 gene. (b) Subsequent analyses that adjusted for DRB1*15:01 showed that all effects of the amino acid variants in DRβ5 were eliminated, whereas the effects of the amino acid variants in DRβ3 that corresponded to DRB3*02:02 remained significant (OR 17.3; $P = 8.0 \times 10^{-29}$). (C) After adjusting for both DRB1*15:01 and DRB3*02:02, we found that all effects of the amino acid variants were eliminated.

Table S1. The clinical characteristics for patients enrolled in the micro-dissected glomerular gene expression study.

	Control	PLA2R-related MN	PLA2R-unrelated MN
Number of Patients	21	23	21
Han Chinese Ethnicity (%)	100%	100%	100%
Age	50.0 ± 11.5	37.7 ± 14.6	45.1 ± 14.3
Female (%)	33.3	26.1	57.1
Proteinuria (g/24h)	-	4.6 (3.3 – 6.2)	3.8 (2.3 – 5.3)
Serum albumin (g/L)	44.2 ± 3.8	27.2 ± 6.1	27.2 ± 6.5
Estimated GFR*	107 ± 15	112 ± 23	100 ± 23

*Estimated GFR was calculated using the equation of CKD-EPI formula.

Table S2. Characteristics of patients and healthy control subjects in the HLA typing study.

Characteristic	Discovery study			Replication study	
	PLA2R-related MN	PLA2R-unrelated MN	Health controls	PLA2R-related MN	Health controls
No. of patients	99	50	100	293	285
Male/ Female	54/45	21/29	50/50	188/105	156/129
Age at DNA sample collection (yr)	37 ± 14	43 ± 13	30 ± 7	47 ± 16	39 ± 12
Age at disease onset (yr)	36 ± 14	42 ± 13	-	46 ± 16	-
Ethnicity (Chinese Han)	99 (100%)	50 (100%)	100 (100%)	293 (100%)	285 (100%)
Serum anti-PLA2R levels	70.6 (38.5 – 129.6)	-	-	83.1 (39.4 – 178.2)	-
Proteinuria (g/24 h)	3.9 (2.3 – 5.8)	3.6 (2.6 – 4.6)	-	3.9 (2.3 – 6.2)	-
Serum albumin (g/L)	27.9 (25.3 – 31.5)	29.6 (24.9 – 34.1)	-	27.9 (24.3 – 31.9)	-
Serum creatinine (mg/dl)	0.76 ± 0.22	0.79 ± 0.34		0.81 ± 0.36	-
Estimated GFR*	112 ± 22	105 ± 25	-	99 ± 26	-
Total cholesterol	8.4 ± 2.7	8.5 ± 2.7		8.2 ± 2.3	
Total triglyceride	2.6 ± 1.4	2.7 ± 1.5		2.7 ± 1.8	
Positive glomerular PLA2R staining	99 (100%)	0 (0%)	-	100%	-
Global glomeruli sclerosis (%)	0 (0 – 4.1)	3.0 (0 – 11.1)		3.6 (0 – 9.1%)	-
Interstitial fibrosis					
None or trace, <10%	84.7%	78.3%		68.8%	

Mild, 10%-25%	14.1%	17.4%	24.1%
Moderate, 26%-50%	1.2%	4.3%	6.4%
Severe, >50%	0%	0%	0.71%

*eGFR was calculated using the equation of CKD-EPI formula.

Table S3. Summary of sequencing data quality in the discovery samples.

	Case	Control	Total
No. of individuals	99	100	199
Raw data(Gb)	1.04(±0.28)	1.04(±0.18)	1.04(±0.24)
Mapped bases(Gb)	0.67(±0.17)	0.67(±0.11)	0.67(±0.14)
Mapped bases on MHC region(Gb)	0.45(±0.11)	0.46(±0.08)	0.46(±0.10)
Capture specificity(%)	67.82(±0.87)	69.60(±2.71)	68.71(±2.20)
Average sequencing depth(fold)	98.19(±24.93)	100.68(±17.68)	99.44(±21.59)
Fraction of target covered>=1X(%)	97.57(±0.39)	97.40(±0.38)	97.48(±0.40)
Fraction of target covered>=4X(%)	96.65(±0.45)	96.41(±0.43)	96.53(±0.45)
Fraction of target covered>=10X(%)	95.54(±0.68)	95.35(±0.51)	95.44(±0.61)
Fraction of target covered>=20X(%)	93.33(±1.83)	93.61(±0.78)	93.47(±1.41)

Table S4. The top 25 HLA alleles with significant associations with PLA2R-related MN in the discovery data set.

Allele	OR	SE	L95	U95	P
DRB1*15:01	16.93	0.36	8.39	34.15	2.75E-15
DRB5*01:01	12.40	0.35	6.28	24.50	4.17E-13
DQA1*01:02	10.50	0.34	5.39	20.44	4.57E-12
DQB1*06:02	12.29	0.37	5.94	25.44	1.41E-11
DRB4*01:03	0.22	0.30	0.12	0.41	8.55E-07
DRB3*02:02	3.96	0.30	2.18	7.17	5.73E-06
DOB*01:02	4.57	0.37	2.21	9.45	4.16E-05
DRB1*09:01	0.19	0.45	0.08	0.45	0.0001867
DRB1*03:01	4.19	0.41	1.86	9.42	0.0005296
TAP2*01:01	3.00	0.33	1.57	5.74	0.0009035
TAP2*01:04	0.33	0.33	0.17	0.64	0.0009035
DQB1*02:01	3.73	0.40	1.70	8.16	0.0009972
C*01:02	0.32	0.35	0.16	0.63	0.001019
DQA1*05:01	3.55	0.40	1.62	7.79	0.001589
DQA1*03:03	0.04	1.04	0.01	0.29	0.001628
DQA1*01:03	0.27	0.42	0.12	0.61	0.001661
DQB1*03:03	0.27	0.42	0.12	0.61	0.001661
DQA1*03:02	0.24	0.46	0.10	0.59	0.00183
DQB1*04:01	0.05	1.04	0.01	0.38	0.003918
DRB1*04:05	0.05	1.04	0.01	0.38	0.003918
DRB1*08:03	0.19	0.57	0.06	0.59	0.003952
DPB1*05:01	0.47	0.30	0.27	0.85	0.01195
A*02:07	0.38	0.39	0.17	0.81	0.0129
K*01:02	0.49	0.29	0.28	0.86	0.01349
TAP1*02:01	2.15	0.31	1.17	3.95	0.01405

Table S5. The top 25 HLA alleles with significant associations with PLA2R-related MN in the replication data set.

Allele	OR	SE	L95	U95	P
DRB1*15:01	8.32	0.19	5.71	12.14	3.44E-28
DRB3*02:02	7.72	0.19	5.34	11.18	2.29E-27
DRB5*01:01	7.06	0.19	4.89	10.20	1.93E-25
DQA1*01:02	5.57	0.18	3.90	7.96	3.81E-21
DQB1*06:02	4.94	0.20	3.33	7.34	2.11E-15
DRB1*03:01	8.41	0.27	4.93	14.33	5.17E-15
DQB1*02:01	7.94	0.27	4.70	13.39	8.21E-15
DQA1*05:01	7.70	0.27	4.57	13.00	2.07E-14
DRB1*09:01	0.12	0.29	0.07	0.22	1.70E-12
DQA1*03:02	0.16	0.28	0.09	0.27	1.80E-11
DQB1*03:03	0.20	0.25	0.12	0.32	5.77E-11
DQA1*02:01	0.28	0.27	0.16	0.47	1.77E-06
DRB1*07:01	0.28	0.27	0.16	0.47	1.77E-06
DQB1*02:02	0.24	0.31	0.13	0.43	2.35E-06
DQB1*03:02	0.29	0.32	0.15	0.54	0.0001158
DRB3*01:01	0.21	0.40	0.10	0.47	0.0001349
DQA1*03:01	0.28	0.33	0.15	0.54	0.0001454
DRB1*12:01	0.19	0.46	0.08	0.47	0.0003127
DRB1*04:05	0.28	0.36	0.14	0.56	0.0003364
DQA1*03:03	0.35	0.31	0.19	0.65	0.0007579
DQB1*04:01	0.31	0.36	0.15	0.62	0.001087
DRB3*03:01	0.45	0.25	0.28	0.74	0.00147
DRB1*14:05	3.66	0.41	1.64	8.17	0.001559
DQA1*06:01	0.42	0.28	0.24	0.73	0.002082
DRB1*08:03	0.37	0.33	0.19	0.71	0.002561

Table S6. The top 25 HLA alleles with significant associations with PLA2R-related MN in the combined data set.

Allele	OR	SE	L95	U95	P
DRB1*15:01	9.74	0.17	7.01	13.55	1.14E-41
DRB5*01:01	7.91	0.16	5.74	10.89	8.61E-37
DRB3*02:02	6.44	0.16	4.71	8.81	1.71E-31
DQA1*01:02	6.42	0.16	4.70	8.78	1.97E-31
DQB1*06:02	6.18	0.18	4.38	8.74	5.22E-25
DRB1*03:01	6.97	0.23	4.47	10.85	9.20E-18
DQB1*02:01	6.45	0.22	4.19	9.93	2.78E-17
DQA1*05:01	6.23	0.22	4.04	9.61	1.13E-16
DRB1*09:01	0.14	0.25	0.09	0.23	1.23E-15
DQA1*03:02	0.17	0.24	0.11	0.28	1.37E-13
DQB1*03:03	0.21	0.21	0.14	0.32	4.10E-13
DQA1*03:03	0.24	0.28	0.14	0.42	5.62E-07
DRB1*04:05	0.20	0.33	0.11	0.39	1.18E-06
DQB1*04:01	0.22	0.33	0.11	0.41	3.71E-06
DQB1*02:02	0.34	0.24	0.21	0.54	6.28E-06
DQA1*02:01	0.38	0.22	0.25	0.58	8.50E-06
DRB3*01:01	0.19	0.37	0.09	0.40	1.06E-05
DRB1*07:01	0.39	0.22	0.26	0.60	1.39E-05
DQB1*03:02	0.32	0.27	0.19	0.54	1.72E-05
DRB1*08:03	0.31	0.28	0.18	0.54	3.34E-05
DQA1*03:01	0.35	0.27	0.21	0.59	7.85E-05
DRB3*03:01	0.42	0.22	0.27	0.65	9.62E-05
DRB1*12:01	0.22	0.40	0.10	0.47	0.0001213
DQA1*06:01	0.42	0.25	0.26	0.67	0.0003597
DRB1*12:02	0.43	0.25	0.27	0.70	0.0007164

Table S7. Demographic and clinical data according to the presence of the HLA- DRB3*02:02 allele in patients with PLA2R-unrelated MN

	DRB3*02:02^{+}	DRB3*02:02^{-}	*p* value
No. of Cases	32	18	
Gender (Male, %)	11 (34.4%)	10 (55.6%)	0.25
Age at disease onset	42 (32 - 49)	42 (34 - 57)	0.54
Estimated GFR	105.2 ± 32.0	100.4 ± 17.4	0.55
Serum albumin (g/L)	29.4 ± 5.4	29.2 ± 7.0	0.90
24-h proteinuria	3.4 (2.6 – 4.6)	3.6 (2.7 – 4.9)	0.81
Total cholesterol	7.8 ± 2.7	8.2 ± 2.6	0.69
Total triglyceride	2.8 ± 1.6	2.5 ± 1.1	0.43
Global glomeruli sclerosis (%)	3.7 (0 – 10.8)	1.0 (0 – 11.9)	0.12
Interstitial fibrosis (%)			
None or trace, <10%	75.0%	83.3%	0.18
Mild, 10%-25%	25.0%	5.6%	
Moderate, 26%-50%	0%	11.1%	
Severe, >50%	0%	0%	

Table S8. The distribution of HLA risk alleles between the PLA2R-related group and the PLA2R-unrelated group

	PLA2R-related MN	PLA2R-unrelated MN	Healthy Controls
No. of patients	392	50	385
DRB1*15:01^{+}	283 (72.2%)	8 (16.0%)	81 (21.0%)
DRB3*02:02^{+}	274 (69.9%)	32 (64.0%)	102 (26.5%)

Table S9. ORs for PLA2R-related MN according to the SNP rs35771982 within PLA2R1.

	Patients	Control subjects	OR (95% CI)	P
rs35771982				
CC	2 (0.5%)	33 (8.6%)	1.0	
CG	72 (18.4%)	162 (42.1%)	7.3 (2.1 – 46.0)	7.2×10^{-3}
GG	318 (81.1%)	190 (49.4%)	27.6 (8.3 – 171.5)	6.1×10^{-6}

Table S10. Frequency of the SNP rs35771982 and the HLA risk alleles (DRB1*15:01 and/or HLA-DRB3*02:02) in the combined data set.

Risk	Patients	Healthy Controls
No HLA Risk Alleles		
rs35771982 CC	0	20
rs35771982 GC	0	98
rs35771982 GG	5	98
Any HLA Risk Allele		
rs35771982 CC	2[#]	13
rs35771982 GC	72	64
rs35771982 GG	313	92

Both patients with the rs35771982 genotype CC were positive for the DRB3*02:02 allele and negative for the DRB1*15:01 allele.

Table S11, Demographic and clinical data based on the SNP rs35771982 genotype status in patients with PLA2R-related MN

Items	GG	GC	*p* value
No. of Patients	318	72	
Gender (Male, %)	63%	54%	0.18
Age at disease onset (years)	45 (32 - 56)	43 (22 - 55)	0.13
Serum Anti-PLA2R (RU/ml)	77.7 (38.2 – 170.0)	83.3 (51.0 – 147.2)	0.63
Estimated GFR	101 ± 26	105 ± 27	0.30
Serum uric acid (μmmol/L)	369 ± 95	338 ± 84	0.03
Serum albumin (g/L)	28.2 ± 5.3	29.2 ± 5.3	0.17
24-h proteinuria (g/24 h)	3.9 (2.4 – 5.9)	3.6 (1.9 – 6.2)	0.74
Global glomeruli sclerosis (%)	2.9 (0 – 7.7)	2.9 (0 – 6.0)	0.74
Interstitial fibrosis			
None or trace, <10%	72.8%	73.0%	
Mild, 10%-25%	21.2%	22.2%	
Moderate, 26%-50%	5.3%	4.8%	0.98
Severe, >50%	0.67%	0%	
HLA risk alleles			
DRB1*15:01^{+}DRB3*02:02^{-}	29.2%	27.8%	
DRB1*15:01^{-}DRB3*02:02^{+}	24.5%	33.3%	0.40
DRB1*15:01^{+}DRB3*02:02^{+}	44.7%	38.9%	
DRB1*15:01^{-}RB3*02:02^{-}	1.6%	0%	