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ORIGINAL ARTICLE

Analysis of *PLA2R1* and *HLA-DQA1* sequence variants in Japanese patients with idiopathic and secondary membranous nephropathy

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Abstract
Background Several recent studies in patients with idiopathic membranous nephropathy (iMN) from Western and Asian counties showed that some single nucleotide polymorphisms (SNPs) within the *PLA2R1* and *HLA-DQA1* genes are significantly associated with iMN. However, there is only 1 report on analysis of *PLA2R1* and *HLA* regions in Japanese patients with iMN.
Methods A total of 58 patients with iMN, 26 patients with secondary MN (sMN), and 50 patients with other diseases were enrolled. All patients were Japanese. We selected 6 SNPs within *PLA2R1* and 1 SNP within *HLA-DQA1*, which were significantly associated with iMN in reported white European cohorts, and sequenced these exons using genomic DNA prepared from peripheral mononuclear cells from each patient. We then analyzed differences in *PLA2R1* and *HLA-DQA1* sequence variants among the 3 groups.
Results Genotypic and allelic frequency distributions for 3 out of 6 SNPs within *PLA2R1*, rs3749117, rs35771982, and rs2715918 were significantly different between the iMN and control groups. Allelic frequency distributions for SNP rs2187668 within *HLA-DQA1* were significantly different between the iMN and control groups. There were no correlations between *PLA2R1* and *HLA-DQA1* sequence variants and clinical parameters in patients with iMN. There were no significant differences in genotypic or allelic frequency distributions for examined SNPs between the sMN and control groups.
Conclusions There are some differences in *PLA2R1* SNP distributions between previously reported cohorts from other countries and our Japanese cohort of patients with iMN, while there is a significant association between SNP rs35771982 and iMN in most of reported cohorts.

Keywords Bucillamine · Japanese cohort · Membranous nephropathy · M-type phospholipase A2 receptor · Single nucleotide polymorphism

Introduction

Membranous nephropathy (MN), characterized by the subepithelial deposition or in situ formation of immune complexes, is an autoimmune disease usually associated with a nephrotic syndrome [1]. MN can be classified as either idiopathic (iMN) or secondary MN (sMN) depending on its etiology. Although the pathogenesis of iMN has been not fully understood, Beck et al. [2] demonstrated that the major target antigen of autoantibodies in iMN is an M-type phospholipase A2 receptor (PLA2R) in 2009. They also showed that the anti-PLA2R antibody was found in 70% of patients with iMN, but was not found in patients with sMN, disease controls, or healthy controls [2].

The prevalence of anti-PLA2R antibodies in iMN patients with nephrotic syndrome from western countries, China, and Korea is approximately 70–80% (for reviews, see 3 and 4, and references therein). On the contrary, recent studies in 2 cohorts of Japanese patients with iMN (47–54% of patients had a nephrotic syndrome) showed

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that the prevalence of anti-PLA2R antibodies is 50–53% [5, 6]. Akiyama et al. [5] speculated some reasons of the low positivity of anti-PLA2R antibodies in Japanese patients with iMN, including genetic differences.

Several recent studies in patients with iMN and sMN from Western and Asian countries have provided further validation that the *PLA2R1* gene has a role in iMN [7–15]. The association of *HLA-DQA1* with iMN has also been shown in several cohorts [8, 11–14]. However, there is only 1 report on analysis of the genetic background of *PLA2R1* and *HLA* regions in Japanese patients with iMN [15]. In the present study, we analyzed 6 single nucleotide polymorphisms (SNPs) within *PLA2R1* and 1 SNP within *HLA-DQA1*, which were shown to be significantly associated with iMN in white European cohorts [8, 10], in our Japanese cohort of patients with iMN and sMN.

Methods

Patients

We enrolled 58 patients with iMN, 26 patients with sMN, and 50 patients with other diseases as a control in this study. All of patients were Japanese, and admitted to our hospital and affiliated hospitals between 1990 and 2013. iMN and sMN were diagnosed based on renal biopsy findings and screening for causes of sMN, including malignancy, hepatitis B virus infection, lupus nephritis, and drug side effects [1, 3, 4]. Among 26 patients in the sMN group, 24 patients were regarded to be bucillamine (a disease modified anti-rheumatic drug)-related [16], and 2 patients were diagnosed with lupus nephritis (ISN/RPS 2003 classification class V [17]). The diagnosis of bucillamine-related sMN was clinically made in patients with rheumatoid arthritis treated with bucillamine. The control group included the following cases: 13 cases of minimal change nephrotic syndrome, 11 cases of IgA nephropathy, 3 cases of diabetic nephropathy, 3 cases of tubulointerstitial nephritis, 3 cases of Gitelman's syndrome, 3 cases of systemic lupus erythematosus without lupus nephritis, 3 cases of glomerulosclerosis, 3 cases of malignancy without MN, 2 cases of focal segmental glomerulosclerosis, 1 case of membranoproliferative glomerulonephritis, 1 case of antineutrophil cytoplasmic antibodies-associated vasculitis, 1 case of immunoglobulin deposition disease, 1 case of renal amyloidosis, 1 case of familial Mediterranean fever, and 1 case of drug-induced hypersensitivity syndrome.

Clinical data were collected from medical records for age, sex, urinary protein, serum total protein, albumin, and creatinine (Cre) at the time of renal biopsy.

Sequencing

Genomic DNA was prepared from peripheral blood mononuclear cells from each patient, using a DNA isolation kit (QIAamp DNA Blood Mini Kit; Qiagen, Hilden, Germany). Previous studies in white European cohorts of patients with iMN showed 6 SNPs within *PLA2R1* (rs3747119, rs4665143, rs3749117, rs35771982, rs2715918, and rs3828323) and 1 SNP within *HLA-DQA1* (rs2187668) were significantly associated with iMN [8, 10]. In the Coenen's cohort [10], all 30 exons of *PLA2R1*, including essential splice sites, were sequenced. They identified 18 sequence variants, and confirmed significant associations between the above-mentioned 6 SNPs and iMN. In our study, these SNPs were selected for analysis. Polymerase chain reaction (PCR) primers used in this study are shown in Table 1. PCR was performed using Taq PCR Core kit (Qiagen). These PCR products were sequenced directly using a DNA sequencing kit (BigDye Terminator Cycle Sequencing Kit; Applied Biosystems Japan, Tokyo, Japan) on an automated DNA sequencer (ABI 3130 genetic analyzer; Applied Biosystems, Foster City, CA, USA). Sequence analysis and mutation identification were performed using the Sequencing Analysis software (DNASIS for Mac; Hitachi, Tokyo, Japan).

Statistical analysis

Data are presented as the mean ± standard deviation, median with a range, or counts and percentages. Differences between groups were evaluated using Kruskal–Wallis test, Welch's t test, Chi-square test, or Fisher's exact test. All analyses were performed using the SPSS Statistics Version 21 software (IBM Japan, Tokyo, Japan). P values <0.05 were considered statistically significant in all analyses. The Bonferroni correction (with a correction factor derived from the number of SNPs tested) was used to adjust for multiple testing, and P values for truly significant results were calculated as 0.05/n.

Results

Patients

Clinical characteristics of patients in the iMN, sMN, and control groups are shown in Table 2. Most of patients diagnosed with bucillamine-related sMN achieved remission of proteinuria after steroid therapy with or without immunosuppressive agents, in addition to discontinuation of bucillamine treatment. The mean ages of patients with iMN, sMN, and other diseases were 61.3, 55.8, and

Table 1 Polymerase chain reaction primers for genomic DNA amplification of *PLA2R1* and *HLA-DQA1*

SNPs		Forward primer	Reverse primer	Annealing temperature (°C)
PLA2R1	rs3749119	AGTGGGTCTCCAGGGCTC	AGGACTGCTGACACACGAAC	64
	rs4665143	AGATCTGTGGGAAATGGCTG	GGTCCAGGCTTCGTGTACTTC	57
	rs3749117 rs35771982	TGAGCCTGCTCTGTTTCTCA	TGGCTTACACCCAAATCCTC	57
	rs2715918	GCCTCTCTGGAGCTTCCTCT	AAAAGGTCGGGGATAGGTACA	64
	rs3828323	CAACATGGATCATAGTGACAAGG	AGGCTGTTCATAAGGTTGGTC	64
HLA-DQA1	rs2187668	AGGCATGAGCCACCTTGTCT	GCCTGGAGGTCTCTATGTCGT	60

SNPs single nucleotide polymorphisms

All primer sequences given are from 5′ to 3′

Table 2 Characteristics of patients with membranous nephropathy and control subjects

	iMN ($n = 58$)	sMN ($n = 26$)	Control ($n = 50$)	P value*
Age (years)	61.3 ± 12.9	55.8 ± 11.1	51.1 ± 19.5	0.048
UP (g/gCre)	5.01 ± 3.68	7.92 ± 7.40	2.81 ± 4.75	0.179
Serum TP (g/dL)	5.30 ± 0.88	5.57 ± 1.00	6.28 ± 1.25	<0.001
Serum Alb (g/dL)	2.68 ± 0.85	2.80 ± 0.76	2.91 ± 1.39	<0.001
Serum Cre (mg/dL)	0.82 ± 0.32	0.75 ± 0.31	1.33 ± 1.41	<0.001

Alb albumin, *Cre* creatinine, *iMN* idiopathic membranous nephropathy, *sMN* secondary membranous nephropathy, *TP* total protein, *UP* urinary protein

* Kruskal–Wallis test

51.1 years, respectively ($P = 0.048$). The mean levels of urinary protein were 5.01, 7.92, and 2.81 g/gCre, respectively ($P = 0.179$). The mean levels of serum total protein were 5.30, 5.57, and 6.28 g/dL, respectively ($P < 0.001$). The mean levels of serum albumin were 2.68, 2.80, and 2.91 g/dL, respectively ($P < 0.001$). The mean levels of serum Cre were 0.82, 0.75, and 1.33 mg/dL, respectively ($P < 0.001$).

Sequencing

Table 3 summarizes genotypic and allelic frequency distributions of 6 SNPs within *PLA2R1* and 1 SNP within *HLA-DQA1* in the iMN, sMN, and control groups (the Bonferroni corrected significant level: $P < 0.0024$). Table 3 also indicates minor allele frequency (MAF) from the Japanese in Tokyo (JPT) population in the HapMap database. Among 7 examined SNPs, each MAF value in our cohort was similar to that in HapMap JPT population except for 1 SNP (rs3749117).

Genotypic frequency distributions of 3 SNPs within *PLA2R1* (rs3749117, rs35771982, and rs2715918) were significantly different between the iMN and control groups ($P = 0.0008$, $P = 0.0006$, and $P = 0.0002$, respectively). There were no significant differences in genotypic frequency distributions of 7 SNPs within *PLA2R1* and *HLA-DQA1* between the sMN and control groups. Allelic frequency distributions of 3 SNPs within *PLA2R1* (rs3749117, rs35771982, and rs2715918) and SNP within *HLA-DQA1* (rs2187668) were significantly difference between the iMN and control groups ($P = 0.0001$, $P < 0.0001$, $P < 0.0001$, and $P < 0.0001$, respectively). There were no significant differences in allelic frequency distributions of 7 SNPs within *PLA2R1* and *HLA-DQA1* between the sMN and control groups. Between the iMN and sMN groups, there were no significant differences in genotypic or allelic frequency distributions of SNPs within *PLA2R1* and *HLA-DQA1*.

Table 4 shows clinical characteristics in patients with iMN according to SNP genotypes of *PLA2R1* and *HLA-DQA1*. There were no significant differences between *PLA2R1* and *HLA-DQA1* sequence variants and clinical parameters.

Discussion

Table 5 summarizes previously reported data and our data on the association between SNPs within *PLA2R1* and *HLA-DQA1* and MN. In the present study, we examined 6 SNPs within *PLA2R1* and 1 SNP within *HLA-DQA1*, which were shown to be significantly associated with iMN in white European cohorts [8, 10]. Among 6 SNPs within *PLA2R1*, 3 SNPs (rs3749117, rs35771982, and rs2715918) were also

Table 3 *PLA2R1* and *HLA-DQA1* polymorphisms in patients with membranous nephropathy

Gene variation	Genotype	IMN $N = 58$ (%)	SMN $N = 26$ (%)	Control $N = 50$ (%)	Allele	IMN $N = 5$ 8 (%)	SMN $N = 26$ (%)	Control $N = 50$ (%)	HapMap JPT[#] MAF
PLA2R1									
rs3749119	TT	3 (5.2)	1 (3.8)	6 (12.0)	C	84 (72.4)	36 (69.2)	59 (59.0)	
	TC	26 (44.8)	14 (53.8)	29 (58.0)	T	32 (27.6)	16 (20.8)	41 (41.0)	0.41 (T)
	CC	29 (50.0)	11 (42.3)	15 (30.0)					
P value*			0.7423*[a]	0.0799*[b]			0.6729*[,a]	0.0377*[,b]	
P value*				0.3599*[c]				0.2164*[,c]	
rs4665143	GG	17 (29.3)	9 (34.6)	18 (36.0)	A	54 (46.5)	22 (42.3)	40 (40.0)	0.35 (A)
	GA	28 (48.3)	12 (46.2)	24 (48.0)	G	62 (53.5)	30 (57.7)	60 (60.0)	
	AA	13 (22.4)	5 (19.2)	8 (16.0)					
P value*			0.8759*[,a]	0.6252*[,b]			0.6094*[,a]	0.3328*[,b]	
P value*				0.9400*[,c]				0.7836*[,c]	
rs3749117	CC	5 (8.6)	3 (11.5)	19 (38.0)	T	84 (72.4)	32 (60.9)	46 (46.0)	
	CT	22 (37.9)	14 (53.8)	16 (32.0)	C	32 (27.6)	20 (39.1)	54 (54.0)	0.42 (C)
	TT	31 (53.4)	9 (34.6)	15 (30.0)					
P value*			0.2782*[,a]	0.0008*[,b]			0.1586*[,a]	0.0001*[,b]	
P value*				0.0424*[,c]				0.0690*[,c]	
rs35771982	CC	5 (8.6)	3 (11.5)	19 (38.0)	G	88 (75.9)	34 (65.4)	47 (47.0)	0.42 (G)
	CG	18 (31.0)	12 (46.2)	15 (30.0)	C	28 (23.1)	18 (34.6)	53 (53.0)	
	GG	35 (60.3)	11 (42.3)	16 (32.0)					
P value*			0.3048*[,a]	0.0006*[,b]			0.1591*[,a]	<0.0001*[,b]	
P value*				0.0522*[,c]				0.0311*[,c]	
rs2715918	GG	35 (60.3)	20 (76.9)	47 (94.0)	A	26 (22.4)	6 (11.5)	3 (3.0)	0.16 (A)
	GA	20 (34.5)	6 (23.1)	3 (6.0)	G	90 (77.6)	46 (88.5)	97 (97.0)	
	AA	3 (5.2)	0	0					
P value*			0.2402*[,a]	0.0002*[,b]			0.0707**[,a]	<0.0001**[,b]	
P value*				0.0289*[,c]				0.0430**[,c]	
rs3828323	TT	4 (6.9)	2 (7.7)	8 (16.0)	C	88 (75.9)	37 (71.1)	65 (65.0)	
	TC	18 (31.0)	10 (38.5)	19 (38.0)	T	28 (24.1)	15 (28.9)	35 (35.0)	0.36 (T)
	CC	36 (62.1)	14 (53.8)	23 (46.0)					
P value*			0.7722*[,a]	0.1610*[,b]			0.5179*[,a]	0.0799*[,b]	
P value*				0.5728*[,c]				0.4436*[,c]	
HLA-DQA1									
rs2187668	GG	51 (87.9)	25 (96.2)	49 (98.0)	G	109 (94.0)	51 (98.1)	99 (99.0)	
	GA	7 (12.1)	1 (3.8)	1 (2.0)	A	7 (6.0)	1 (1.9)	1 (1.0)	0.03 (A)
	AA	0	0	0					
P value*			0.2352*[,a]	0.0463*[,b]			0.2303**[,a]	<0.0001**[,b]	
P value*				0.6333*[,c]				0.5687**[,c]	

Underlined *P* values: not significant after the Bonferroni correction

iMN idiopathic membranous nephropathy, *sMN* secondary membranous nephropathy, *JPT* Japanese in Tokyo, *MAF* minor allele frequency

[a] iMN vs. sMN

[b] iMN vs. control

[c] sMN vs. control

* Chi square for independence test

** Fisher's exact test

[#] http://www.ncbi.nlm.nih.gov/projects/snp/

Table 4 Clinical characteristics in patients with idiopathic membranous nephropathy according to SNP genotypes of *PLA2R1* and *HLA-DQA1*

PLA2R1	*n*	Age (years)	Sex (F/M)	UP (g/gCre)	Serum Alb (g/dL)	Serum Cre (mg/dL)
rs3749119						
TT	4	58 ± 21.9	2/2	5.15 ± 2.12	1.97 ± 1.40	1.02 ± 0.60
TC	25	62.3 ± 1.2.5	9/16	5.92 ± 3.94	2.62 ± 1.02	0.88 ± 0.33
CC	29	60.9 ± 12.2	16/13	4.07 ± 3.43	2.71 ± 0.63	0.73 ± 0.24
P value*		0.797	0.479	0.534	0.793	0.398
rs4665143						
GG	17	60.8 ± 14.3	9/8	4.11 ± 3.52	2.77 ± 1.07	0.81 ± 0.42
GA	28	62.8 ± 11.0	14/14	5.83 ± 3.52	2.60 ± 0.85	0.82 ± 0.29
AA	13	58.7 ± 15.2	4/9	4.73 ± 4.54	2.62 ± 0.51	0.83 ± 0.25
P value*		0.535	0.533	0.178	0.944	0.553
rs3749117						
CC	6	55.8 ± 22.6	1/5	4.57 ± 3.01	2.65 ± 1.28	1.10 ± 0.55
CT	21	62.6 ± 10.5	8/13	5.85 ± 4.12	2.51 ± 0.89	0.86 ± 0.30
TT	31	61.4 ± 12.2	18/13	4.56 ± 3.49	2.75 ± 0.75	0.73 ± 0.24
P value*		0.906	0.198	0.962	0.737	0.278
rs35771982						
CC	6	56.3 ± 22.7	2/4	3.17 ± 2.88	2.73 ± 1.29	0.94 ± 0.49
CG	17	62.6 ± 11.8	7/10	6.04 ± 3.67	2.71 ± 0.84	0.83 ± 0.32
GG	35	61.5 ± 11.5	18/17	4.91 ± 3.77	2.62 ± 0.80	0.79 ± 0.29
P value*		0.97	0.705	0.588	0.917	0.922
rs2715918						
GG	29	63.3 ± 13.5	19/18	4.91 ± 3.56	2.61 ± 0.96	0.82 ± 0.33
GA	18	58.9 ± 10.1	7/12	4.73 ± 3.27	2.82 ± 0.65	0.83 ± 0.33
AA	3	50.7 ± 15.3	1/2	14.6	2.23 ± 0.21	0.68 ± 0.06
P value*		0.078	0.699	0.338	0.463	0.478
rs3828323						
TT	6	56.0 ± 23.0	4/2	3.50 ± 2.78	2.93 ± 1.33	0.69 ± 0.21
TC	16	60.2 ± 11.8	6/10	6.79 ± 4.46	2.60 ± 0.81	0.88 ± 0.38
CC	36	62.7 ± 11.3	17/19	4.66 ± 3.32	2.63 ± 0.79	0.77 ± 0.23
P value*		0.808	0.575	0.057	0.994	0.581
HLA-DQA1	*n*	Age (yr)	Sex (F/M)	UP (g/gCre)	Serum Alb (g/dL)	Serum Cre (mg/dL)
rs3828323						
GG	51	61.3 ± 13.3	23/28	5.07 ± 3.78	2.62 ± 0.82	0.80 ± 0.28
GA	7	61.4 ± 9.5	4/3	5.06 ± 3.26	2.90 ± 1.10	0.94 ± 0.55
AA	0					
P value**		0.485	0.2944	0.501	0.273	0.259

Alb albumin, *Cre* creatinine, *F* female, *M* male, *SNP* single nucleotide polymorphism, *UP* urinary protein

* Kruskal–Wallis test

** Welch's *t* test

significantly associated with iMN in our Japanese cohort even after the Bonferroni correction, while 3 SNPs (rs3749119, rs4665143, and rs382832) were not. Regarding 1 SNP within *HLA-DQA1* (rs2187668), this SNP was also significantly associated with iMN in our cohort even after the Bonferroni correction.

SNP rs3749119 within *PLA2R1* is located in the 5′-untranslated region of exon 1 [10]. The association between rs3749119 and iMN was examined in 3 previous reports. SNP rs3747119 was significantly associated with iMN in cohorts of white European [10], Indian [14], and Japanese [15]. Our results were not consistent with these data.

Synonymous SNP rs4665143 within *PLA2R1* (p.Ser87Ser in the cysteine-rich domain) is located in a coding region of exon 2 [10]. The association between rs4665143 and iMN was examined in 1 previous report. SNP rs4665143 was significantly associated with iMN in white European cohort [10], while this SNP was not associated with iMN in our Japanese cohort.

Table 5 Summary of reports on the association between SNPs within *PLA2R1* and *HLA-DQA1* and membranous nephropathy

References (year)	Ethnicity of patients	MN	Anti-PLA2R Abs	*PLA2R1*										*HLA-DQA1*
				Rs3749119 5′-UTR	Rs4664308 Intron	Rs4665143 Ser87= Synonymous	Rs3749117 Met292Val Missense	Rs35771982 His300Asp Missense	Rs17830307 Intron	Rs2715918 Intron	Rs3828323 Gly1106Ser Missense	Rs1511223 Intron	Rs1511223 3′-UTR	Rs2187668 Intron
[7] (2010)	Taiwanese–Chinese	I						Yes						
[8] (2011)	French/Dutch/British	I			Yes									Yes
[9] (2011)	Korean	I						Yes						
		S						No						
[10] (2013)	French/Dutch	I		Yes		Yes	Yes	Yes		Yes	Yes			
[11] (2013)	Chinese	I			Yes		Yes	Yes						Yes
[12] (2014)	Spanish	I			Yes									Yes
[13] (2014)	Caucasian	I	+				Yes	Yes	Yes		Yes	Yes	Yes	Yes
	African American	I	+				Yes	No	No		No	No	No	Yes
[14] (2016)	Indian	I		Yes	Yes		No	Yes			Yes			Yes
[15] (2016)	Japanese	I		Yes				Yes					Yes	
Present study	Japanese	I		No		No	Yes*,#	Yes*,#		Yes*#	No			Yes#
		S		No		No	No	No		No	No			No

Abs antibodies, *I* idiopathic, *MN* membranous nephropathy, *PLA2R* phospholipase A2 receptor, *S* Secondary, *UTR* untranslated region

* Genotypic frequency distribution

\# Allelic frequency distribution

Nonsynonymous SNP 3749117 within *PLA2R1* (p.Met292Val in the 1st C-type lectin-like domain) is located in a coding region of exon 5 [10]. The association between rs3749117 and iMN was examined in 4 previous reports. This SNP was significantly associated with iMN in cohorts of white Europeans [10], Chinese [11], and anti-PLA2R antibody-positive Caucasians and African Americans [13]. Our results were consistent with these data. On the contrary, Ramachandran et al. [14] reported that there was no association between rs3749117 and iMN in an Indian cohort. There was no association between rs3749117 and bucillamine-related sMN in our cohort.

Nonsynonymous SNP rs35771982 within *PLA2R1* (p.His300Asp in the 1st C-type lectin-like domain) is located in a coding region of exon 5 [10]. The association between rs35771982 and iMN was examined in 7 previous reports. This SNP was significantly associated with iMN in cohorts of Taiwanese-Chinese [7], Koreans [9], white Europeans [10], Chinese [11], anti-PLA2R antibody-positive Caucasians [13], Indians [14], and Japanese [15]. Our results were consistent with these data. On the contrary, Saeed et al. [13] reported that there was no association between rs35771982 and iMN in a cohort of anti-PLA2R antibody positive African Americans. Regarding the association between rs35771982 and sMN, there was no association in a Korean cohort [9], as well as in our Japanese cohort.

SNP rs2715918 within *PLA2R1* is located in a noncoding region of exon 16 [10]. The association between rs2715918 and iMN was examined in 1 previous report. This SNP was significantly associated with iMN in a cohort of white Europeans [10], as well as in our Japanese cohort. There was no association between rs2715918 and sMN in our cohort.

Nonsynonymous SNP rs3828323 within *PLA2R1* (p.Gly1106Ser in a linker region between the 6th and 7th C-type lectin-like domains) is located in a coding region of exon 24 [10]. The association between rs3828323 and iMN was examined in 3 previous reports. Although SNP rs3828323 was significantly associated with iMN in cohorts of white Europeans [10], anti-PLA2R antibody-positive Caucasians [13], and Indians [14], this SNP was not associated with iMN in our Japanese cohort.

Intronic SNP rs2187668 within *HLA-DQA1* was significantly associated with iMN in European cohorts [8, 12], Chinese cohort [11], Caucasian and African American cohorts positive for anti-PLA2R antibodies [13], and Indian cohort [14]. In our Japanese cohort, there was also an association between rs2187668 and iMN.

Anti-PLA2R antibodies have emerged as an excellent biomarker of disease activity in iMN. At presentation, approximately 70–80% of patients with iMN will test positive for serum anti-PLA2R antibodies [3, 4]. Recent studies in 2 Japanese cohorts demonstrated that Japanese patients with iMN have a lower percentage of anti-PLA2R positivity (approximately 50%) [5, 6]. Francis et al. [4] suggested that this variability in sensitivity has more to do with the biology of the disease and perhaps ethnicity rather than characteristics of the assays used. Coenen et al. [10] showed that 3 nonsynonymous SNPs within *PLA2R1* (rs3749117 [p.Met292Val], rs35771982 [p.His300Asp], and rs2828323 [p.Gly1106Ser]) were significantly associated with iMN in their white European cohort, and suggested that these variants are responsible for important amino acid substitutions that may change the antigenicity of PLA2R. In our Japanese cohort, rs3749117 [p.Met292Val] and rs35771982 [p.His300Asp] were significantly associated with iMN, while rs2828323 [p.Gly1106Ser] was not. It is possible that this difference may be related with the low prevalence of anti-PLA2R antibodies in Japanese patients.

There are several limitations in our study. We studied patients only in northern Japan. The control group included patients with several diseases. Comparison analyses between subgroups according to SNPs within *PLA2R1* and *HLA-DQA1* may be limited by small sample size. Some difference in MAF of examined SNPs between HapMap JPT population and our Japanese cohort could be attributed to these limitations. Nationwide large-scale studies including healthy individuals are needed to solve these problems.

In conclusion, our study suggests that there are some differences in the genetic background of *PLA2R1* between Japanese patients with iMN and patients with iMN from other counties. These differences may be partly related to the low prevalence of anti-PLA2R antibodies in Japanese patients with iMN. In addition, our literature review suggests the strong genetic association between nonsynonymous SNP rs35771982 (p.His300Asp) within *PLA2R1* and iMN except for African Americans.

Compliance with ethical standards

Conflict of interest All the authors have declared no competing interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee at which the studies were conducted (IRB approval number 1026) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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