Meta-Analysis of Observational Studies in Epidemiology

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Association between *PLA2R1* rs4664308 and susceptibility to idiopathic membranous nephropathy

Protocol for a systematic review and meta-analysis of case-control studies

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Abstract

Previous studies have evaluated the association between the phospholipase A2 m-type receptor (*PLA2R1)* rs4664308 polymorphism and the risk of idiopathic membranous nephropathy (IMN), but the results need to be integrated. We hypothesized that the *PLA2R1* rs4664308 polymorphism is associated with IMN risk in different ethnicities and assessed this hypothesis by using meta-analysis and case-control studies.

A literature searches on *PLA2R1* rs4664308 and IMN risk was conducted using the EMBASE, PubMed, Cochrane Library, and Chinese Medical Databases. The relationship between *PLA2R1* rs4664308 and IMN risk was evaluated in 5 genetic models, namely, allelic (AG), recessive (RG), dominant (DG), homozygous (HMG), and heterozygous (HTG) models. Subgroup analysis was conducted by ethnicity on Asian and non-Asian populations.

Eight sets of data from 6 articles met study objectives were selected and 6797 subjects (IMN: 2324 Controls: 4,473) were included. Heterogeneity was found in the DG, HMG, and HTG models but not in the AG or RG models. The minor allele(G) of *PLA2R1* rs4664308 showed a significant negative correlation with IMN risk in all genetic random models: odds ratio of AG: 0.44(0.37-0.51), RG: 0.35(0.29-0.42), DG: 0.38(0.31-0.48), HMG: 0.26(0.19-0.37), and HTG: 0.61(0.48-0.77; $P < .00001$), and Asians and non-Asians showed the same effect of *PLA2R1* rs4664308 on IMN risk. Analysis of Asians and non-Asians revealed no publication bias in any of the 5 genetic models.

The minor allele of *PLA2R1* rs4664308 has a protective activity against IMN in Asians and non-Asians. It provided new insights into potential curative and preventative treatments for IMN.

Abbreviations: AG = allelic genetic model, Cis = confidence intervals, DG = dominant genetic model, *HLA* = human leukocyte antigen, *HLA-DQA1* = HLA alpha chain-1, HMG = homozygous genetic model, HTG = heterozygous genetic model, HWE = Hardy-Weinberg Equilibrium, IMN = idiopathic membranous nephropathy, ORs = odds ratio, *PLA2R1* = phospholipase A2 m-type receptor, RG = recessive genetic model, SNPs = single nucleotide polymorphisms, STREGA = strengthening the reporting of genetic association.

Keywords: phospholipase A2 m-type receptor, rs4664308, idiopathic membranous nephropathy, Asians, risk of bias

1. Introduction

Idiopathic membranous nephropathy (IMN) is an autoimmune disease caused by a variety of drugs and infections.[1] IMN is the most common cause of non-diabetic adult kidney disease and usually occurs in the sixth decade of life.[2] IMN is mainly caused by an immune complex attack, which destroys glomerular podocytes and damages the glomerular filtration barrier, thereby producing proteinuria, which, in turn, increases the long-term risk of kidney failure.[3] Renal failure affects appetite (anorexia), the nervous system (lassitude), the cardiovascular system (hypertension), and blood (anemia), and thus, seriously affects the quality of life.[4]

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The prevalence of IMN is ethnicity- and age-dependent but is not influenced by sex. The 70% of IMN in the United States is primary membranous nephropathy, whereas it is 50%, 22.8%, and 12.3% in Japan, China, and South Korea, respectively.[5–7] In contrast, its prevalence in Bangladesh and Saudi Arabia is low, at 7.37% and 9.90%, respectively.[8,9] IMN has been also reported to have a greater prevalence in Caucasians than in Asians.[10] IMN accounts for 20% to 30% of nephrotic syndrome cases in Caucasian adults, and this proportion is also increasing in Asian adults. The disease is considered to have a genetic basis, though environmental factors are known to contribute.[11]

Despite ongoing research on the topic, the pathogenesis and causes of IMN remain unclear. Nevertheless, the most crucial identified factors that confer susceptibility to IMN are antibodies of specific genes such as the human leukocyte antigen (*HLA*) gene and phospholipase A2 m-type receptor (*PLA2R1*).[12,13] It has been well established that ~70% of patients from various ethnic groups with active disease have antibodies against *PLA2R1*. Furthermore, the interaction between *PLA2R1* and *HLA* is known to induce IMN. Bao et al[14] have concluded that the HLA alpha chain-1 (*HLA-DQA1*) gene has a greater prevalence in Asians, Europeans, and Caucasians with IMN than in normal controls in a meta-analysis on the relation between *HLA-DQA1* and IMN. Genome-wide association studies have found that *PLA2R1* and *HLA-DQA1* single nucleotide polymorphisms (SNPs) are high-risk to IMN, and the SNPs are interacted to induce IMN. However, IMN has not been associated with the low-affinity immunoglobulin γFc region receptor III genes.[15] These 2 studies have also confirmed the presence of risk alleles (rs4664308, rs2187668) at 2 genomic loci containing *PLA2R1* and *HLA-DQA1*, which both contribute to the development of IMN. The latest published article has shown that the prevalence of *PLA2R1* rs4664308 is greater in IMN in the northwestern region of China using the European population as a control group.[16] In 2019, the latest publication on the relationship between IMN and multiple SNPs in the *PLA2R1* gene in people in Hebei, China. The results indicate that PLA2R1 rs4664308 is related to IMN.[17] Furthermore, Li et al[18] have confirmed that in the Chinese population, the *PLA2R1* gene is related to other autoimmune diseases like systemic lupus erythematosus and lupus nephritis.

The association between IMN and *PLA2R1* rs4664308 has been studied in India, [19] China, [17,20–22] Spain, France, the United Kingdom,[15] and the Netherlands.[23] These studies have revealed that the prevalence of IMN is dependent on the *PLA2R1* polymorphism, geographic location, and ethnicities. The reasons for these differences have not been identified, although it has been established that different ethnicities exhibit different sensitivities to *PLA2R1* rs4664308. Furthermore, it should be noted that the qualities of the studies affect their results. For example, the studies have often conducted in small cohorts, and the insufficient power of sample size results in an incorrect conclusion. We hypothesized that *PLA2R1* rs4664308 differently influenced the prevalence of IMN in different ethnicities, and we examined this relation by meta-analysis using previous case-control studies conducted in Asians and non-Asians.

2. Materials and methods

2.1. Publication search and inclusion criteria

The electronic literature was searched using and keywords "IMN," "idiopathic membranous nephropathy," "membranous nephropathy," "*PLA2R1*," and "rs4664308," and the following search engines: NCBI (Pubmed), Wiley, Web of Science, EMBASE, Cochrane Library, Springer Link, Science Direct, Chinese electronic literature search sites (China National Knowledge Infrastructure, Wan Fang Data, and China Journal Database), Korean electronic database and dissertations. Articles published in English, Korean, and Chinese were searched. The search was conducted from 1973 until August 31, 2020. Because this study is a meta-analysis to combine the existing research, it did not require ethical approval from an ethics review committee.

2.2. Eligibility criteria for studies

The following criteria were used to select articles for the meta-analysis:

1) Evaluation of the relationship between the *PLA2R1 rs4664308* polymorphism and IMN,
2) Sufficient data for each allele,
3) A *P*-value >.05 for Hardy-Weinberg Equilibrium (HWE), and
4) Subjected to peer review.

2.3. Data extraction

WXG and ZT were responsible for searching for appropriate documents in databases. QJY was responsible for analyzing data and confirming that articles met the study criteria. A third reviewer (SP) reconciled conflicts between the literature search and the data analysis. Duplicated articles were deleted. Authors' names, publication times, study location, numbers of cases and controls, numbers of genotypes, diagnostic criteria, and author's conclusions were recorded.

2.4. Eligibility criteria for studies used in this review

All clinical studies to investigate the association of *PLA2R1 rs4664308* polymorphism and IMN were included in this systematic review and meta-analysis. Exclusion criteria included *in vitro* studies, *in vivo* studies in non-human species, studies that were only published in abstract form or included insufficient data to properly evaluate the outcomes, studies with different genetic variants of *PLA2R1*, and duplicated studies. A flow diagram of the article selection process is shown in Figure 1. Although no language barriers were imposed, all studies included in this review were written in English. Dissertations about randomized clinical studies were also included.

2.5. Quality assessment and publication bias of selected studies

The qualities of selected papers were determined using 12 quality checklists provided by the statement of Strengthening the Reporting of Genetic Association Studies (STREGA).[24] The 12 checklist items included in STREGA are; HWE, genotyping errors, modeling haplotype variation, replication, selection of participants, treatment effects when studying quantitative traits, the rationale for the choice of genes and variants subjected to study, statistical methods, reporting of descriptive and outcome data, and data volume. Each of the 12 checklist items was evaluated by awarding a score of 0 or 1 point. The quality of the study was presented by the summation of scores of the 12 checklists.

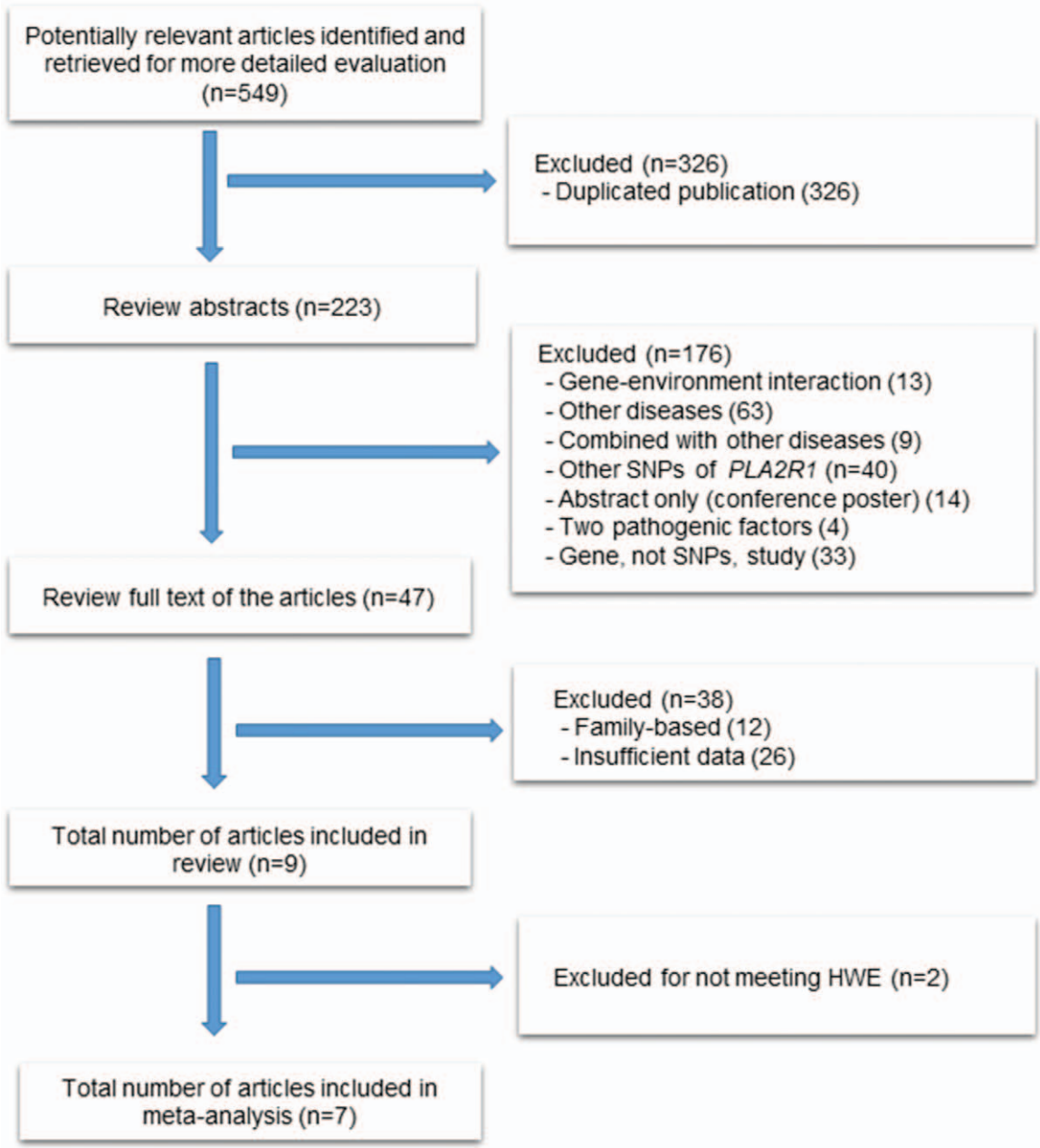


Figure 1. Flow chart of the study selection process. HWE = Hardy-Weinberg equilibrium, SNP = single nucleotide polymorphism, *PLA2R1* = phospholipase A2 m-type receptor.

2.6. Statistical analyses

Statistical analyses were performed using Comprehensive Meta-Analysis software (CMA, Biostat, Englewood, NJ) and Review manager 5.3 (Cochrane Community, London). The relationship between *PLA2R1* rs4664308 and IMN was analyzed using 5 genetic models using the A allele (the major allele of *PLA2R1* rs4664308) and the G allele (the minor allele). The number of the assigned alleles in each of the 5 genetic models was analyzed using the following equations:

(1) the allelic genetic model (AG; major allele x 2 + heterozygous allele VS heterozygous allele + minor allele x 2),
(2) recessive genetic model (RG; major allele VS minor allele + heterozygous allele),
(3) dominant genetic model (DG; heterozygous allele + major allele VS minor allele),
(4) homozygous genetic model (HMG; major allele VS minor allele), and
(5) heterozygous genetic model (HTG; heterozygous allele VS minor allele).

Because studies were conducted in different countries, they were categorized by subject ethnicity as Asian or non-Asian.[2,5]

Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated by pooling the results of selected studies to determine the risks of IMN by *PLA2R1* rs4664308 genotype. ORs and CIs were also calculated for Asians and non-Asians. Heterogeneities of data sets were analyzed using I^2 statistics. The random model

was used when $I^2 \geq 30\%$ and the fixed model when $I^2 < 30\%$. Funnel plots were applied to estimate potential publication bias and Funnel plot symmetry was taken to indicate a lack of publication bias. Publication bias was also evaluated using 2-tailed Egger regression using Comprehensive Meta-Analysis software.[26] Statistical significance was accepted for P values $<.05$.

3. Results

3.1. Literature search and summary of included studies

The process used to select articles for meta-analysis is presented in Figure 1. A total of 549 articles were initially identified. However, 326 articles were deleted for duplication after screening titles, 223 articles have remained. By reviewing their abstracts, 176 articles addressed other diseases, mixed diseases, different SNPs, contained no genetic variants, or concerned gene-environmental interactions were removed. Finally, by 47 reading the articles, 12 family-inherited studies, and 26 articles with no or insufficient data were eliminated. The HWE of the remaining nine articles was calculated, and 2 of them did not meet the requirements. Therefore, 7 articles were included in the meta-analysis.[15,17,19–23]

These seven studies[15,17,19–23] were conducted in the United Kingdom, the Netherlands, Spain, France, China, and India (Table 1). One study included subjects with 3 different ethnicities, and these were treated as 3 separate study groups. Thus, a total of seven articles including nine studies were subjected to meta-analysis. The number of subjects with major, heterozygous, and minor alleles was determined for the IMN (case) and healthy (control) groups (Table 1).

A total of 6797 subjects (IMN: 2324 Control: 4473) were included in the 9 studies.[15,17,19–23] All studies were conducted using a case-control design and included 95 to 1767 subjects. Subjects' ages ranged from 40 to 65 years, and average ages in the IMN and control groups were 41 to 52 years and 35 to 45 years, respectively. Five studies were conducted on Asians[17,19–22] and 4 on Europeans.[15,23] STREGA scores for the 6 articles ranged from 6 to 11, and thus, article qualities were sufficient for inclusion in the meta-analysis (Table 2).

HWE of each of the 6 articles satisfied the criterion $P > .05$. The author's conclusion of each study was summarized in Table 3 and most studies concluded that *PLA2R1* rs4664308 was associated with IMN (Table 3).

3.2. Heterogeneity analysis during meta-analysis

After pooling all nine studies, the AG, DG, RG, HMG, and HTG models provided heterogeneity indices (I^2) of 55%, 5%, 49%, 33%, and 0%, respectively (Table 4). AG and HMG models were used as random models and the other 3 genetic models were used as fixed models due to the heterogeneity scores.

Since I^2 for all 5 genetic models was <30% for non-Asians, genetic analysis was conducted using the fixed model. I^2 was $\geq$ 30% by the AG, RG, and HMG models, and thus, the random model was used, but $I^2 < 30\%$ by the DG and HTG models and the fixed model was applied. Because Review manager 5.3 was used to analyze the overall effect of *PLA2R1* rs4664308 on IMN in total and subgroup analysis in 1 analysis, a random model was conservatively chosen to analyze the overall effect in a forest plot. Therefore, 2 of the 5 genetic models (AG and RGM) were adopted as fixed models and 3 (DG, HMG, and HTGM) as random models. When non-Asian studies were analyzed separately using the fixed genetic model, the results of statistical analysis were the same as using the random genetic model.

3.3. Association between the PLA2R1 rs4664308 genotype and IMN risk with ethnic subgroups

The ORs and CIs for IMN risk by *PLA2R1* rs4664308 genotype are summarized in Table 4 and Figure 2:

(1) AGM: 0.44 and 0.37-0.51 ($P < .00001$),
(2) RGM: 0.35 and 0.29-0.42 ($P < .0001$),
(3) DGM: 0.38 and 0.31-0.48 ($P < .0001$),
(4) HMGM: 0.26 and 0.19-0.37 ($P < .0001$), and
(5) HTGM: 0.61 and 0.48-0.77 ($P < .0001$).

Genetic model results after pooling the 9 studies showed that the minor (G) allele protected against IMN development.

To examine the genetic impact of *PLA2R1* rs4664308 in Asian and non-Asians, we conducted subgroup analysis. Data on Asians[17,19–22] came from China and India and data for non-Asians[15,23] from France, Britain, the Netherlands, and Spain. ORs and CIs of these subgroups are shown in Figure 2,

(1) AGM (Fig. 2A): 0.39 (0.30-0.49) in Asians ($P < .00001$) and 0.49 (0.41-0.59) in non-Asians ($P < .00001$),
(2) RGM (Fig. 2B): 0.31 (0.26-0.47) in Asians ($P < .00001$) and 0.39 (0.31-0.50) in non-Asians ($P < .00001$);
(3) DMG (Fig. 2C): 0.34 (0.25-0.47) in Asians ($P < .00001$) and 0.42 (0.31-0.58) in non-Asians ($P < 0.00001$);

Table 1

Genotype distribution of the *PLA2R1* rs4664308 polymorphisms in idiopathic membranous nephropathy (IMN) patients and controls.

			Case (IMN) genotype			Control genotype			IMN		Control	
Author	Year	Country	Major (AA)	Hetero (AG)	Minor (GG)	Major (AA)	Hetero (AG)	Minor (GG)	A[a]	G	A	G
Stanescu et al/1[22]	2011	French	97	79	14	562	865	340	273	107	1989	1545
Stanescu et al/2[22]	2011	Dutch	178	94	23	170	269	92	450	140	609	453
Stanescu et al/3[22]	2011	British	42	23	5	13	18	6	107	33	44	30
Lv et al[19]	2013	China	803	274	35	489	449	82	1880	344	1427	613
Bullich et al[14]	2014	Spanish	51	29	9	115	139	32	131	47	369	203
Ramachandran et al[18]	2015	India	75	18	1	52	39	4	168	0	143	47
Dong[20]	2016	China	0	25	120	3	18	205	25	265	24	428
Cui et al[21]	2016	China	159	35	4	187	171	40	353	43	545	251
Sun[16]	2019	China	96	34	1	55	51	7	226	36	161	65

PLA2R1 = phospholipase A2 m-type receptor.
[a] Calculate the frequency of the A and G alleles by the formula of AA × 2 + AG and GG × 2 + AG.

(4)

Table 2

Summaries of studies to determine the association between *PLA2R1* rs4664308 and idiopathic membranous nephropathy (IMN).

Author	Year	Country	Diagnostic criteria of IMN	Sample size			Participant Age (yr)	Gender (number)	Study design	Total quality scores[a]
				Total	IMN	Control				
Stanescu et al[22]	2011	French	Renal biopsy	1957	190	1767	IMN: 49.8±15.3 Control: ND	IMN: Male 58 Female 17 Control: ND	Case-control	9
Stanescu et al[22]	2011	Dutch	Renal biopsy	826	295	531	IMN: 51.8±14.2 Control: ND	IMN: Male 109 Female 37 Control: ND	Case-control	9
Stanescu et al[22]	2011	British	Renal biopsy	222	118	104	IMN: 52.5±13.3 Control: ND	IMN: Male 231 Female 104 Control: ND	Case-control	9
Lv et al[19]	2013	China	Kidney biopsy	2132	1112	1020	IMN: 49±13 Control: 35±10	IMN: Male 620 Female 492 Control: Male 527 Female 493	Case-control	11
Bullich et al[14]	2014	Spanish	Renal biopsy	375	89	286	IMN: 46.6±14.4 Control: ND	IMN: Male 58 Female 25 Control: ND	Case-control	10
Ramachandran et al[18]	2015	India	Renal biopsy	189	94	95	IMN: 41.7±12.8 Control:40.0±15.8	IMN: Male 56 Female 38 Control: Male 11 Female 09	Case-control	11
Dong[20]	2016	China	Kidney biopsy	371	145	226	IMN:48.8±13.9 Control:45.3±5.3	IMN: Male 90 Female 55 Control: Male 146 Female 80	Case-control	10
Cui et al[21]	2016	China	Kidney biopsy	596	198	398	IMN:48.74±15.61 Control: ND	ND	Case-control	9
Sun[16]	2019	China	Kidney biopsy	244	131	113	IMN:46.92±12.38 Control:46.93±10.7	IMN: Male 83 Female 38 Control: Male 69 Female 44	Case-control	10

ND = no data given in the article. *PLA2R1* = phospholipase A2 m-type receptor.

[a] Total quality scores were added according to each of the scoring criteria provided in each of the reporting of genetic association studies. Total score 12 (12 listed include genotyping errors, population stratification, modelling haplotype variation, HWE, replication, selection of participants, rationale for choice of genes and variants selected, treatment effects in studying quantitative traits, statistical methods, reporting of descriptive and outcome data, and volume of data.).

HMGM (Fig. 2D): 0.21 (0.10-0.45) in Asians ($P < .00001$) and 0.30 (0.19-0.46) in non-Asians ($P < .00001$);

(5) HTGM (Fig. 2E): 0.57 (0.41-0.79) in Asians ($P < .00001$) and 0.65 (0.47-0.91) in non-Asians ($P < .00001$).

These analyses showed that *PLA2R1* rs4664308 was significantly associated with IMN risk in Asians and non-Asians by all 5 genetic models, and that the minor G allele of *PLA2R1* rs4664308 reduced the risk of IMN.

Table 3

***PLA2R1* rs4664308 study author's conclusion and HWE value.**

Author (reference)	Year	*P* value of HWE	Authors' conclusion
Stanescu et al[22]	2011	.619	- *PLA2R1* rs4664308 had a significant association with IMN.
Stanescu et al[22]	2011	.195	- *PLA2R1* rs4664308 was significantly associated with IMN.
Stanescu et al[22]	2011	.421	- *PLA2R1* rs4664308 exhibited a significant association with IMN.
Lv et al[19]	2013	.232	- The interaction between *PLA2R1* and *HLA-DQA1* risk alleles associates with the development of IMN in the Chinese population.
Bullich et al[14]	2014	.877	- The *HLA-DQA1* and *PLA2R1* SNPs had association with IMN in a Spanish cohort and the risk increased when combining both risk genotypes.
Ramachandran et al[18]	2015	.640	- The SNPs rs3749119, rs3749117, and rs4664308 in *PLA2R1* were significantly associated with IMN.
Dong[20]	2016	.245	- The polymorphisms of *PLA2R1* and *HLA-DQA1* were closely associated with the susceptibility of IMN. AA genotype at PLA2R1 and AA/AG genotype at *HLA-DQA1* were risk factors for IMN.
Cui et al[21]	2016	.088	- The rs2187668 in *HLA-DQA1* shows a greater risk effect than the rs4664308 in *PLA2R1*. But the *PLA2R1* rs4664308 is more closely related to the Asian IMN.
Sun[16]	2019	.339	- *PLA2R* rs4664308 and rs35771982 are related to the susceptibility of IMN.

HWE = Hardy-Weinberg equilibrium; IMN = Idiopathic membranous nephropathy; *PLA2R1* = phospholipase A2 m-type receptor; *HLA-DQA1* = human leukocyte antigen alpha chain-1.

Table 4

Meta-subgroup analysis of the relationship between *PLA2R1* rs4664308 and idiopathic membranous nephropathy.

	Overall effects				Heterogeneity		Publication bias[b]	
Genetic model1[a]	OR (95% CI)	*Z* value	*P* value	Model	I^2%	*P* value	*Z* value	*P* value
Allelic genetic model								
Asian subgroup	0.39 (0.30-0.49)	7.89	<.00001	Random	50%	.09	0.361	.742
Non-Asian subgroup	0.49 (0.41-0.59)	7.68	<.00001	Random	29%	.24	0.567	.628
Total	0.44 (0.37-0.51)	10.62	<.00001	Random	49%	.05	0.086	.934
Recessive genetic model								
Asian subgroup	0.31 (0.23-0.42)	7.57	<.00001	Random	49%	.10	0.220	.839
Non-Asian subgroup	0.39 (0.31-0.50)	7.71	<.00001	Random	29%	.24	0.322	.778
Total	0.35 (0.29-0.42)	11.20	<.00001	Random	42%	.09	0.582	.579
Dominant genetic model								
Asian subgroup	0.34 (0.25-0.47)	6.69	<.00001	Fixed	0%	0.42	1.563	.216
Non-Asian subgroup	0.42 (0.31-0.58)	5.34	<.00001	Fixed	30%	0.23	0.583	.619
Total	0.38 (0.31-0.48)	8.43	<.00001	Fixed	8%	0.37	0.727	.491
Homozygous genetic model								
Asian subgroup	0.23 (0.16-0.33)	8.07	<.00001	Fixed	38%	0.17	0.163	.881
Non-Asian subgroup	0.28 (0.20-0.39)	7.54	<.00001	Fixed	35%	0.20	0.670	.572
Total	0.26 (0.20-0.33)	10.96	<.00001	Fixed	31%	0.17	0.107	.918
Heterozygous genetic model								
Asian subgroup	0.57 (0.41-0.79)	3.35	<.00001	Fixed	0%	0.62	1.495	.232
Non-Asian subgroup	0.65 (0.47-0.91)	2.49	<.00001	Fixed	34%	0.21	0.491	.672
Total	0.61 (0.48-0.77)	4.12	<.00001	Fixed	0%	0.48	0.579	.581

OR = odds ratio; CI = confidence intervals; HWE = Hardy-Weinberg equilibrium.

[a] Genetic models of the allelic (AA x 2 + AG VS GG x 2 + AG), recessive (AA VS GG + AG), dominant (AA + AG VS GG), homozygous (AA VS GG), heterozygous(AG VS GG).

[b] Tested by regression analysis of 2-tailed Egger test.

3.4. Sensitivity analysis

To confirm that overall analysis results were not unduly affected by a single study, the analysis was conducted using each model after sequentially removing 1 study. This sensitivity analysis showed no single study affected overall findings or alter the risks of IMN conferred by *PLA2R1* rs4664308 (Table 5).

3.5. Publication bias

The publication bias was examined for all 5 genetic models. No publication bias was observed in Asians or non-Asians for any genetic models, as determined by the Funnel plot or Egger regression test ($P > .05$, Table 4, Fig. 2).

4. Discussion

A meta-analysis of the 9 study datasets confirms that the minor G allele of *PLA2R1* rs4664308 potentially protects against IMN development in Asians and non-Asians (IMN: 2324 Controls: 4473) in the 5 genetic models using random effects. Meta-analysis can effectively reduce random probabilities, provide detailed analysis of aggregated data, and eliminate false positive and negative results that might occur in single studies. Furthermore, it allows subgroup analysis to be conducted in different countries and the determination of the impacts of racial differences, and thus, increases the reliabilities of research results. Thus, the meta-analysis conducted in the present study provides a conclusion regarding the effect of *PLA2R1* rs4664308 on IMN risk. To the best of our knowledge, the present study is the first to investigate the associated between *PLA2R1* rs4664308 and IMN risk using a meta-analysis based approach.

IMN is an autoimmune disease that targets glomerular podocyte antigens in middle-aged adults.[27] On the other hand, *PLA2R1* may prevent pathogens from producing immunoglobulins that can cause kidney damage and increase the risk of IMN.[28]*PLA2R1* is a transmembrane protein. Its N terminus contains a 20-amino acid signal peptide, whereas its C terminus contains the extracellular domain of an endocytic motif, a transmembrane domain, and a short intracellular domain. The cysteine-rich domain, the type II fibronectin domain, and eight different C-type lectin domains of PLA2R1 bind to the anti-PLA2R1 antibodies to interfere with the action of PLA2R1. Furthermore, these epitopes can also be used to produce anti-PLA2R1 antibodies.[29] Autoantibodies are produced in major histocompatibility complex class II molecules, and some epitopes in PLA2R1 interact with major histocompatibility complex class II molecules, although the mechanisms involved have not been elucidated.[30] B cells are also known to play a crucial role in immune response and dysregulated B cell response has been suggested to influence the pathogeneses of various autoimmune diseases, including that of IMN. PLA2R1 has been also shown to modulate the receptor response of B cells, and it acts as a major factor in IMN pathogenesis.[31]

It has been reported that the HLA class II gene interacts with the *PLA2R1* gene to promote the development of IMN. The *HLA-DQA1* allele on chromosome 6p21 is closely related to IMN, mainly in Caucasian populations, and this allele may promote autoimmune responses to targets, which include *PLA2R1* variants.[32]*HLA-DQA1* rs2187668 is known to be associated with IMN, and carriers of the major allele of *HLA-DQA1* rs2187668 have a higher probability of harboring *PLA2R1* rs4664308 major allele to increase the IMN risk. However, studies have shown Asians are less likely to exhibit *PLA2R1* genetic variations to increase IMN risk.[14] In 2014, a meta-analysis showed the presence of the PLA2R1 antibody could be used to diagnose IMN and suggested a serum PLA2R1 antibody test might be useful for detecting IMN.[33] In the same year, a new type of assay was developed to determine the presence

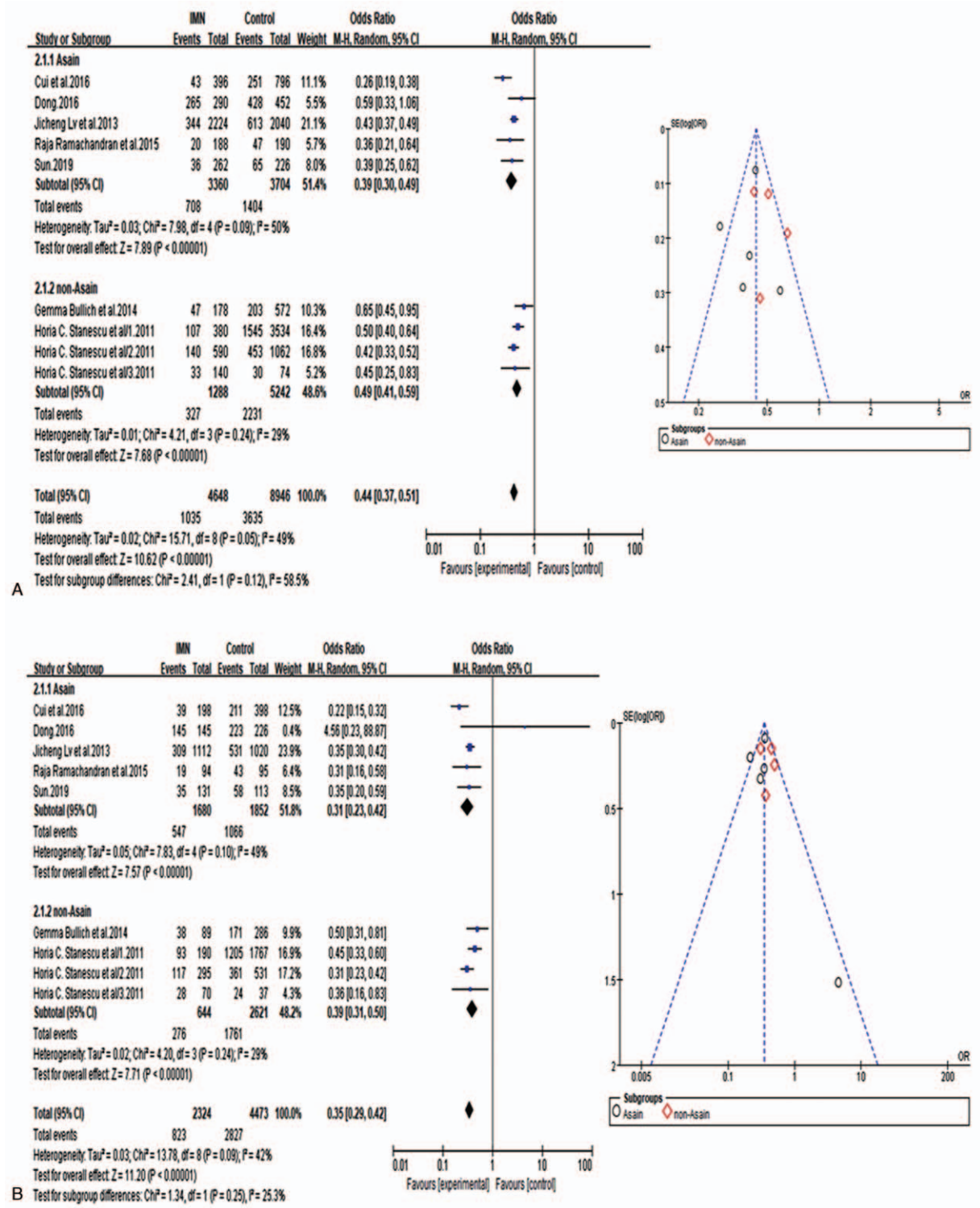


Figure 2. Forest and funnel plots of the association between the *PLA2R1* rs4664308 polymorphism and the risk of idiopathic membranous nephropathy. A. = allele genetic model, B. = recessive genetic model, C. = dominant genetic model, D. = homozygous genetic model, E. = heterozygous genetic model, CI = confidence intervals.

of the PLA2R1 antibody in IMN.[34] Furthermore, multiple SNPs in *PLA2R1* (in addition to rs4664308), that is, rs35771982,[35] rs3828323,[36] and rs3749117,[37] have reported to be associated with IMN. Therefore, *PLA2R1* is reported to play a crucial role in IMN pathogenesis, and it may have a different impact on IMN risk in Non-Asians and Asians.

The pathogenesis of IMN has yet to be elucidated. The research also shows the susceptibility to IMN is associated with *PLA2R1*

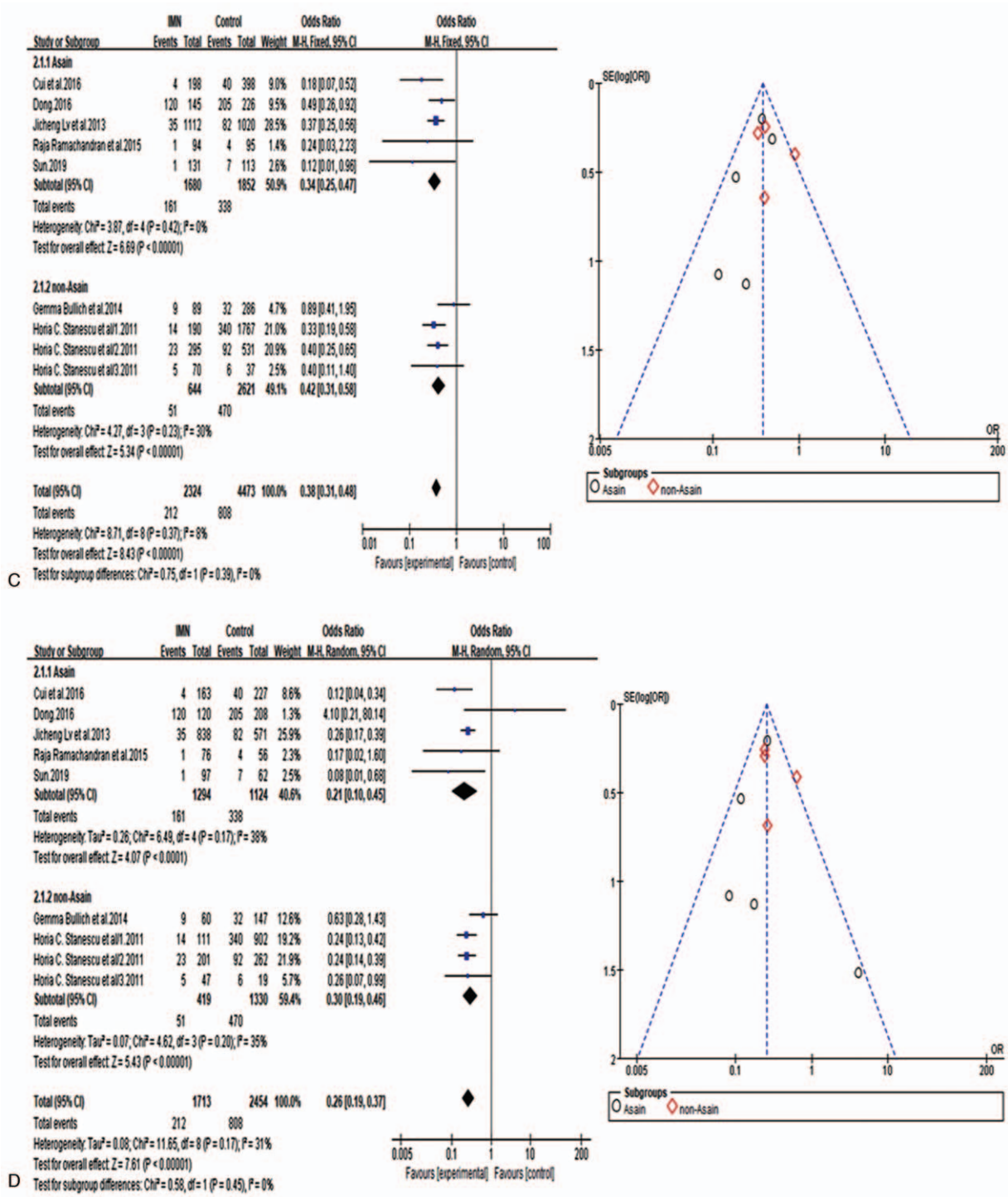
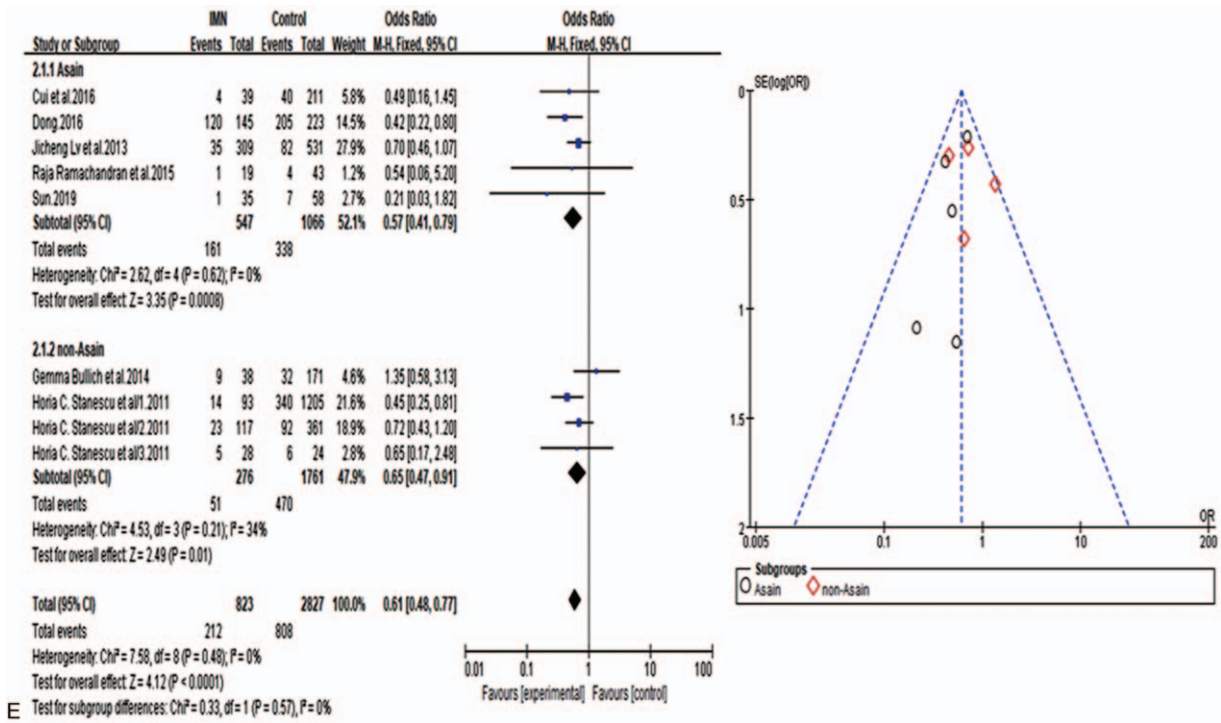


Figure 2. Continued.

rs4664308 in Asians and non-Asians. Other studies have shown *PLA2R1* rs4664308 is more closely associated with IMN in Europeans, and it should be added that few studies on this relationship have been conducted on Asian populations.[38] Nonetheless, our findings show *PLA2R1* rs4664308 is also closely associated with IMN in Asians. However, the present study only provides an overview of the distribution of *PLA2R1* rs4664308 and does not give details of the effects of environmental, lifestyle, dietary intake, and other factors.

The present study has some limitations that warrant consideration. First, the causes of autoimmune diseases include environmental and psychological factors as well as genetic variants, and further research is needed to characterize environmental/genetic interactions. However, the data used in



E

Figure 2. Continued.

this meta-analysis did not include data on environmental factors. Although the total subjects included in the meta-analysis were 6797 subjects including 2324 IMN and 4473 control, each study was not big enough to have statistical power. No publication bias was shown in the statistical analysis, but no significant results might be unpublished. Second, this meta-analysis was conducted with the case-control studies, and they were not applied to the cause and effect relationship. The rigorous longitudinal studies are warranted, but the present meta-analysis can overcome the limitation of individual research with a small number of subjects. Third, this study was conducted by combining the published data of each article selected, and it contained a possible bias like publication bias. We analyzed the publication bias by Egger test and funnel plot. These results suggested the statistical publication bias, but some studies have not been published when there are no positive results. Finally, a sufficient effect size was limited in both ethnicities. Some studies with small samples may have not been published since they had the insufficient statistical power to demonstrate the positive associations, and the studies with negative results are less likely to be published.

5. Conclusion

This meta-analysis shows the *PLA2R1* rs4664308 polymorphism was associated with IMN susceptibility in Asians, Europeans, and Caucasians. We recommend that additional studies be conducted to determine the influences of racial and environmental factors of the relation between *PLA2R1* rs4664308 and the risk of IMN and to expand data on the prognosis and optimal treatment of IMN in children and adolescents. Importantly, the present study also shows the minor G allele of *PLA2R1* rs4664308 protects against IMN development in Asians and non-Asians. It is necessary to conduct in-depth longitudinal research on these limitations in the future.

Table 5

Sensitivity analysis of allelic genetic model.

Studies included	Overall effects			Model	Heterogeneity		Publication bias[a]	
	OR (95% CI)	*Z* value	*P* value		I^2, %	*P* value	*Z* value	*P* value
[16], [18–22]	0.42 (0.38-0.46)	17.46	<.00001	Fixed	39%	.140	0.321	.759
[14], [18–22]	0.44 (0.37-0.52)	9.610	<.00001	Random	55%	.003	0.179	.864
[14], [16], [19–22]	0.44 (0.37-0.52)	9.830	<.00001	Random	54%	.003	0.271	.795
[14], [16], [18], [20–22]	0.44 (0.36-0.53)	8.090	<.00001	Random	55%	.003	0.074	.943
[14], [16], [18,19], [21,22]	0.43 (0.36-0.50)	10.48	<.00001	Random	52%	.004	0.229	.827
[14], [16], [18–20], [22]	0.45 (0.41-0.50)	15.85	<.00001	Fixed	5%	.390	0.714	.502
[14], [16], [18–21]	0.42 (0.33-0.54)	6.730	<.00001	Random	64%	.002	0.070	.947

Sensitivity analysis was conducted by removing one study and overall meta-analysis was performed in allelic genetic model.

[a] Tested by regression analysis of 2-tailed Egger test.

These results suggested that carriers with the minor allele (G) of *PLA2R1* rs4664308 had a protective activity in IMN risk in Asians and non-Asians with some limitations.

Author contributions

JYQ and SP designed the experiment of this study. SP supervised the findings of this work. JYQ, XGW, and TZ analyzed the data. SP and JYQ wrote the first draft of the manuscript. All authors reviewed and edited the manuscript and approved the final version of it. The datasets analyzed during the current study are available from the corresponding author on reasonable request.
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Writing – original draft: Jing Yi Qiu, Sunmin Park.
Writing – review & editing: Sunmin Park.

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