Association of 10 Polymorphisms in PLA2R1 and HLA DQA1 Genes with Primary Membranous Nephropathy Risk: A Meta-Analysis and a Meta-Regression

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ABSTRACT

BACKGROUND: Although, several studies have assessed the association of the phospholipase A2 receptor (PLA2R) and HLA-DQA1 SNPs with primary membranous nephropathy (PMN), results were inconsistent and between-studies heterogeneity needs to be investigated.

OBJECTIVES: The aim of this review was to summarize existing data on the contribution of 10 SNPs in the PLA2R and HLA-DQA1 genes to PMN susceptibility and to investigate the between-studies heterogeneity by subgroup analyses and meta-regressions.

DESIGN: This study was performed according to the PRISMA guidelines for systematic reviews and meta-analyses.

DATA SOURCES AND METHODS: An electronic literature search for eligible studies among all papers published prior to January 10, 2024, was conducted through PubMed, EMBASE, Web of science and Scopus databases. Meta-analyses together with subgroup analyses and meta-regressions were performed for the 10 following SNPs: rs4664308, rs3749117, rs3749119, rs35771982, rs3828323, rs16844715, rs1511223, rs6757188, rs2715918, and rs2187668.

RESULTS: Combined analyses revealed a significant increase in PMN risk conferred by the following alleles: rs4664308*A, rs3749117*T, rs3749119*C, rs35771982*G, rs3828323*C, rs16844715*C, rs1511223*A, rs2715918*A, and rs2187668*A, all *P*-values < .001. Moreover, the PLA2R-rs4664308/HLA-DQA1-rs2187668 interaction was significantly associated with an increased PMN risk, *P* < .001. However, there was a substantial between-studies heterogeneity for some SNPs. Subgroup analyses by ethnicity for the 9 PLA2R SNPs did not show any cross-ethnic disparity. Inversely, the risk conferred by the HLA-DQA1 rs2187668*A allele was significantly higher in Caucasians (OR [95% CI] = 3.929 [3.251–4.748]) than in Asians (OR [95% CI] = 2.537 [1.94–3.318], *P* = .007. Besides, meta-regressions revealed for the majority of investigated SNPs significant correlations of the effect size with albumin, 24-hours proteinuria, serum creatinine, and eGFR levels. Hence, the influence on PMN risk conferred by the PLA2R and HLA-DQA1 SNPs was rather noted in patients with a severe disease.

CONCLUSION: This meta-analysis showed that 9 out of the 10 investigated SNPs in PLA2R and HLA-DQA1 genes were associated with increased PMN risk. Heterogeneity could be due to disparate patient groups in terms of disease presentation for almost all SNPs, and ethnicity for the HLA-DQA1 rs2187668 SNP.

REGISTRATION: This review has been registered on PROSPERO: CRD42024506729. Available from: https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42024506729

PLAIN LANGUAGE SUMMARY
Genetic factors in primary membranous nephritis

Why was the study done? Primary membranous nephritis (PMN) is the most common etiology of adult-onset nephrotic syndrome. Understanding risk factors, particularly genetic ones, would provide a better understanding of its pathophysiological mechanisms in order to prevent and treat patients more effectively. What did the researchers do? The research team summarized published data on genetic factors associated with PMN including phospholipase A2 receptor (PLA2R) and HLA-DQA1 genes. What did the researchers find? The total number of included studies was 27. Nine out of ten genetic factors were found to be associated with PMN risk. Moreover, we noted significant interaction between PLA2R and HLA-DQA1 in potentializing PMN risk. Nevertheless, there was a significant between-studies heterogeneity which was found to be explained in part by disease severity. What do the findings mean? This study has identified some important some genetic factors associated with PMN together with confounding factors that could influence the aforementioned association.

KEYWORDS: Idiopathic membranous nephropathy, phospholipase A2 m-type receptor, HLA-DQA1, polymorphism, meta-analysis, meta-regression

Introduction

Membranous nephropathy (MN) is the most common etiology of adult-onset nephrotic syndrome.[1] Approximately 20% of MN are secondary membranous nephropathy (SMN) which can be related to an autoimmune disease such as systemic lupus erythematosus or due to infections, drugs, or cancers.[2] The remaining 80% of MN are referred to as idiopathic or primary membranous nephropathy (PMN).[2]

Since the discovery in 2009 of the phospholipase A2 receptor (PLA2R) as a target autoantigen in PMN by Beck et al[3] several proteomic and genomic studies focusing on PLA2R have been carried out to better understand its pathogenic mechanisms. In addition, some authors have hypothesized that renal overexpression of PLA2R may trigger an autoimmune response, resulting in the production of PLA2R antibody (Ab).[4] Indeed, PLA2R expression study by direct immunofluorescence and immunochemistry revealed a significant increase in PLA2R deposit in case of PMN comparatively to SMN or tubular nephropathies.[4,5] Besides, the PLA2R Ab production by autoreactive B cells requires the presentation of PLA2R epitopes to T cells by class II human leukocyte antigens (HLA) including HLA-DR, -DP, and -DQ. Hence, numerous studies investigated HLA class II polymorphisms in PMN risk.

Three previous meta-analyses assessing PMN genetic risk; 2 for the PLA2R rs4664308 polymorphism (SNP) in 2020[6,7]; and 1 for the HLA-DQA1 rs2187668 SNP in 2018[8]; have been already published. Nevertheless, these aforementioned meta-analyses included only a few studies (7–11) and revealed a high level of between-study heterogeneity with inconsistent results.[6,8] Moreover, subgroup analyses by ethnicity failed to explain the heterogeneity and the between-studies inconsistency.[6,8] Furthermore, these previous meta-analyses did not perform extensive meta-regressions in order to investigate the aforementioned between-studies heterogeneity. Therefore, including more studies and a deeper investigation of the sources of heterogeneity are needed. In addition, the risk conferred by the PLA2R rs4664308 SNP (OR = 2.27)[6,7] and the HLA-DQA1 rs218668 SNP (OR = 3.34)[8] represents only a small part of the genetic component predisposing to PMN. Indeed, other SNPs could contribute to PMN susceptibility, and thus require further investigation to better characterize the predisposing genetic landscape.

The aim of this review was to summarize existing data on the contribution of 10 SNPs in the PLA2R and HLA-DQA1 genes to PMN susceptibility and to investigate the between-studies heterogeneity by subgroup analyses and meta-regressions.

Materials and Methods

Search strategy

This study was performed according to the PRISMA guidelines for systematic reviews and meta-analyses.[9] An electronic literature search for eligible studies among all papers published prior to January 10, 2024, was conducted through PubMed, EMBASE, Web of science, and Scopus databases. The following search string was used: (([PLA2R] OR [phospholipase A2 receptor] OR [HLA-DQA]) AND ([Polymorphism] OR [SNP] OR [Variant] OR [Mutation] OR [Allele] OR [Allelic] OR [Genotype] OR [Genotypic] OR [rs4664308] OR [rs3749117] OR [rs3749119] OR [rs35771982] OR [rs3828323] OR [rs16844715] OR [rs1511223] OR [rs6757188] OR [rs2715918] OR [rs2187668]) AND ([membranous nephropathy] OR [membranous nephritis])). The literature search was carried out without any language restriction.

Selection criteria

All studies were independently assessed and evaluated by 2 reviewers (Tarak Dhaouadi, T.D. and Imen Sfar, I.S.) for the inclusion and the exclusion. The following selection criteria were adopted:

Inclusion criteria:

- Studies of case-control (retrospective) or cohort (prospective) design.
- Studies assessing the association between PLA2R and/or HLA-DQA1 polymorphisms and PMN risk.
- Studies providing precise results with genotypes or alleles frequencies.

Exclusion criteria:

- Genotype frequencies are not consistent with the Hardy-Weinberg Equilibrium (HWE) as recommended by Trikalinos et al.[10]
- Studies carried out in patients with SMN.
- Case series of subjects, narrative or systematic review, comments, or meta-analysis.
- If many studies have been carried out using duplicate cases, only the study with complete data and the largest sample size was included.

Definition of primary membranous nephropathy

Primary membranous nephropathy was defined as a biopsy-proven MN with negative antinuclear antibody and serology of hepatitis B, C, and HIV, and the absence of neoplasm evidence.

Data extraction

Data were extracted using a predeveloped form and entered in an Excel datasheet. Two investigators (T.D. and I.S.) independently extracted the following information: first author, year of publication, study design, country, ethnicity, mean or median

age, gender ratio (M/F), number of patients, number of controls, mean or median albumin (g/L), mean or median urinary protein (g/24 hours), mean or median serum creatinine, mean or median eGFR, PLA2R, and HLA-DQA1 SNPs genotyping method, the frequencies of genotypes and alleles for each of the following SNPs: rs4664308, rs3749117, rs3749119 rs35771982, rs3828323, rs16844715, rs1511223, rs6757188, rs2715918, and rs2187668 (Table 1). A third investigator (Awatef Riahi) compared the results of the extracted data for potential discrepancies.

Quality assessment

The quality of eligible studies was assessed independently by 2 reviewers (T.D. and I.S.) using the Newcastle-Ottawa Scale (NOS)[11] which is based on the following 3 general categories: selection (4 points), Comparability of the study groups (2 points), and ascertainment of outcome (3 points). Studies with a score ≥7 were classified as high-quality reports. Additionally, risk of bias was assessed for each included study through a generic form (Excel spreadsheet) and visualized via the Cochrane ROBVIS online tool (https://mcguinlu.shinyapps.io/robvis/). Two additional independent reviewers (Taïeb Ben Abdallah and Yousr Gorgi) examined the quality-assessment results.

Study endpoints

The primary endpoint of this meta-analysis was to estimate the strength of the association between PLA2R and HLA-DQA1 SNPs and PMN risk. The secondary endpoint was to evaluate potential confounding factors that might influence the impact of the aforementioned SNPs on PMN risk in order to identify the sources of heterogeneity.

Statistical analysis

Statistical analysis was carried out using the Cochrane Review Manager 5.4 software, the OpenMeta-Analyst software, and the online available software MetaGenyo (https://metagenyo.genyo.es/). The associations of PLA2R and HLA-DQA1 SNPs with PMN risk were assessed using pooled Odds Ratios (ORs) with the 95% confidence interval (95% CI). The statistical significance of pooled ORs was tested by Z-test with a threshold of significance set at .05. Random effects models (DerSimonian-Laird) were used as recommended by Borenstein et al.[12] Indeed, as long as the eligible studies were carried out in genetically diverse populations, the random-effects model applies.[12] Forest-plots were generated to display the distribution of effect size (OR) across included studies. Sensitivity analyses were carried out to test the results stability by omitting sequentially each individual study. The heterogeneity of between-studies was tested by Q test (significance threshold: 0.1), quantified via I^2 calculation (proportion of true

effects variance) and analyzed through the determination of 95% prediction intervals (PI). PI were obtained through the CMA Prediction Intervals free software. The calculation of the 95% PI was based on the following 4 items: OR, upper bound of 95% CI, Tau[2], and number of included studies. Subsequently, the heterogeneity was explored for potential sources by subgroup analyses and meta-regressions. Briefly, studies were stratified by continent and type of treatment. Meta-regressions were performed using age, gender ratio (male/female), the control/patient ratio, the minor allele frequency (MAF) in control group, albumin, urinary protein, serum creatinine, and eGFR as independent variables. Both univariate and multivariate models of meta-regression were generated in order to assess the presence of potential confounding factors. Publication bias were assessed by Egger's test and visualized through the generation of funnel plots. HWE was examined for each study and for every SNP by assessing both individual-univariate and multivariate-adjusted *P*-values. HWE adjusted *P*-values in control groups are available for each SNP in Table 2. In this study, the codominant genetic model (allele contrast) was applied. Additionally, results of recessive and dominant genetic models were displayed.

A Supplemental File 1 with additional tables and figures is available with the full-manuscript. A PRISMA checklist is available as a Supplemental File 2.

Systematic review registration

This review has been registered on PROSPERO: CRD42024506729, Available from: https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42024506729.

Results

Search results and study characteristics

A PRISMA flow diagram was generated to depict the study selection process (Figure 1). Overall, 27 studies with a total of 5353 PMN cases and 10,777 controls were included in the present study.[13–35] The characteristics of included studies are summarized in Table 1. Genotyping data of cases and controls are depicted in Supplemental Tables S1–S10. Biological features of included studies patients are illustrated in Supplemental Table S11. Eighteen studies were included for the rs4664308 SNP,[13–17,20,22,23,25,27,28,30,32–34] 13 for the rs3749117 SNP,[16,18,22,23,25–27,30,33] 10 for the rs3749119 SNP,[13,18,20,25,29–33] 16 for the rs35771982 SNP,[16,18–23,25,26,29–31,33,35] 12 for the rs3828323 SNP,[13,18,19,25–27,30,31,35] 5 for the rs16844715 SNP,[13,20,22,29,31] 7 for the rs1511223 SNP,[13,26,27,29] 6 for the rs6757188 SNP,[13,21,26,31,32] 4 studies for the rs2715918 SNP,[18,22,31,32] and 17 for the rs2187668 SNP.[13,15,16,18,20,22–27,32–34] The NOS quality score results for each included study are shown in Table 1. Included SNPs characteristics are described in Supplemental Table S12. Risks of bias are summarized in Figure 2.

Table 1. Characteristics of included studies.

REF.	FIRST AUTHOR	YEAR	COUNTRY	ETHNICITY	STUDY DESIGN	PATIENT AGE (Y)	GENDER RATIO (MALE/FEMALE)	SAMPLE SIZE PATIENTS	CONTROLS	GENOTYPING METHOD	QUALITY SCORE
13	Berchtold	2021	Europe + America	Caucasian	Retrospective	50.4 ± 12.8	4.3	105	192	NGS	7
14	Bobyleva	2019	Russia	Caucasian	Retrospective	47.4	1.3	70	100	PCR-RFLP	6
15	Bullich	2014	Spain	Caucasian	Retrospective	46.6 ± 14.4	2.3	89	286	Real-time PCR	8
16	Cui	2017	China	Asian	Retrospective	48.8 ± 15.6	1.3	261	599	Sequencing	6
17	Dhaouadi	2020	Tunisia	North African	Prospective	44 ± 15.7	1.7	68	60	Real-time PCR	7
18	Kaga	2018	Japan	Asian	Retrospective	61.3 ± 12.9	NS	58	50	Sequencing	6
19	Kim	2011	South Korea	Asian	Retrospective	50.4 ± 15.5	1.9	199	356	Sequencing	7
20	Latt	2018	Japan	Asian	Prospective	NS	NS	222	701	NGS	6
21	Liu	2010	Taiwan	Asian	Retrospective	57.37	1.5	129	106	PCR-RFLP	7
22	Liu	2021	China	Asian	Prospective	49 ± 13.21	1.6	86	90	Sequencing	7
23	Lv	2013	China	Asian	Retrospective	49 ± 13	1.6	1112	1020	Sequencing	8
24	Qin	2017	China	Asian	Prospective	48.1 ± 14.1	1.9	509	601	MALDI-TOF	7
25	Ramachandran	2016	India	Asian	Prospective	41.4 ± 13.3	1.4	114	95	Real-time PCR	8
26	Saeed (AA)	2014	USA	African American	Unclear	53.6 ± 17	2.1	115	218	Real-time PCR	7
	Saeed (Cau)	2014	USA	Caucasian	Unclear	53.6 ± 18	2.1	280	337	Real-time PCR	7
27	Stanescu (British)	2011	UK	Caucasian	Retrospective	52.5 ± 13.3	2.2	335	349	NGS	6
	Stanescu (Dutch)	2011	Netherlands	Caucasian	Retrospective	51.8 ± 14.2	2.9	146	1832	NGS	6
	Stanescu (French)	2011	France	Caucasian	Retrospective	49.8 ± 15.3	3.4	75	157	NGS	6
28	Sun	2019	China	Asian	Retrospective	46.9 ± 12.4	2.2	131	113	NS	7
29	Thiri	2016	Japan	Asian	Retrospective	61 ± 15	1.3	183	805	Real-time PCR	6
30	Tian (Group A)	2020	China	Asian	Prospective	49.1 ± 15.5	1.7	166	144	Real-time PCR	8
	Tian (Group B)	2020	China	Asian	Prospective	49.4 ± 9.6	2.1	212	162	Real-time PCR	8
31	Tian	2023	China	Asian	Prospective	55.5 ± 10.1	4	35	25	Sequencing	6
32	Wang	2019	China	Asian	Retrospective	49.2 ± 13.6	1.1	314	354	Real-time PCR	7
33	Wang	2021	China	Asian	Retrospective	50.2	1.8	97	138	Real-time PCR	6
34	Wunnenburger	2017	Germany	Caucasian	Prospective	60 ± 12	NS	147	1655	NGS	7
35	Zhou	2013	China	Asian	Retrospective	52.2 ± 7.2	1.6	95	232	PCR-RFLP	6

Abbreviation: NS, not specified.

Table 2. HWE adjusted *P*-values in control groups.

| REF.	FIRST AUTHOR	YEAR	RS4664308	RS3749117	RS3749119	RS35771982	RS3828323	RS16844715	RS1511223	RS6557188	RS2715918	RS2187668
13	Berchtold	2021	.1014	–	.112	–	.2613	.1314	.1036	.0571	–	.2952
14	Bobyleva	2019	.5606	–	–	–	–	–	–	–	–	–
15	Bullich	2014	.2989	–	–	–	–	–	–	–	–	.6782
16	Cui	2017	.9611	.198	–	.4498	–	–	–	–	–	.0608
17	Dhaouadi	2020	.8681	–	–	–	–	–	–	–	–	–
18	Kaga	2018	–	.156	.1597	.253	.2438	–	–	–	.8269	.9431
19	Kim	2011	–	–	–	.9411	.0728	–	–	–	–	–
20	Latt	2018	.9624	–	.1996	.0687	–	.0805	–	–	–	.1389
21	Liu	2010	–	–	–	.7629	–	–	–	.0611	–	–
22	Liu	2021	.8019	.868	–	.868	–	.9268	–	–	.1865	.2952
23	Lv	2013	.9762	.0861	–	.0749	–	–	–	–	–	.5534
24	Qin	2017	–	–	–	–	–	–	–	–	–	.1322
25	Ramachandran	2016	.3177	.2509	.2241	.3351	.2111	–	–	–	–	.1795
26	Saeed (AA)	2014	–	.172	–	.8399	.1033	–	.4778	.0509	–	.9269
	Saeed (Cau)	2014	–	.06	–	.893	.0689	–	.0716	.1734	–	.9127
27	Stanescu (British)	2011	.9572	.112	–	–	–	–	.1512	–	–	.9734
	Stanescu (Dutch)	2011	.9823	.091	–	–	.0782	–	.0709	–	–	.9518
	Stanescu (French)	2011	.916	.239	–	–	.1364	–	.1046	–	–	.5293
28	Sun	2019	.2811	–	–	–	–	–	–	–	–	–
29	Thiri	2016	–	–	.0686	.0826	–	.0602	.1207	–	–	–
30	Tian (Group A)	2020	.6261	.1164	.7077	.8101	.1144	–	–	–	–	–
	Tian (Group B)	2020	.9316	.1914	.6407	.2368	.1008	–	–	–	–	–
31	Tian	2023	–	–	.0633	.1147	.0922	.1195	–	.4732	.2724	–
32	Wang	2019	.3307	–	.4431	–	–	–	–	–	.077	.3391
33	Wang	2021	.5202	.6115	.0718	.6115	–	–	–	.1512	–	.0553
34	Wunnenburger	2017	.6552	–	–	–	–	–	–	–	–	.8562
35	Zhou	2013	–	–	–	.1645	.5337	–	–	–	–	–

Abbreviation: NS, not specified.
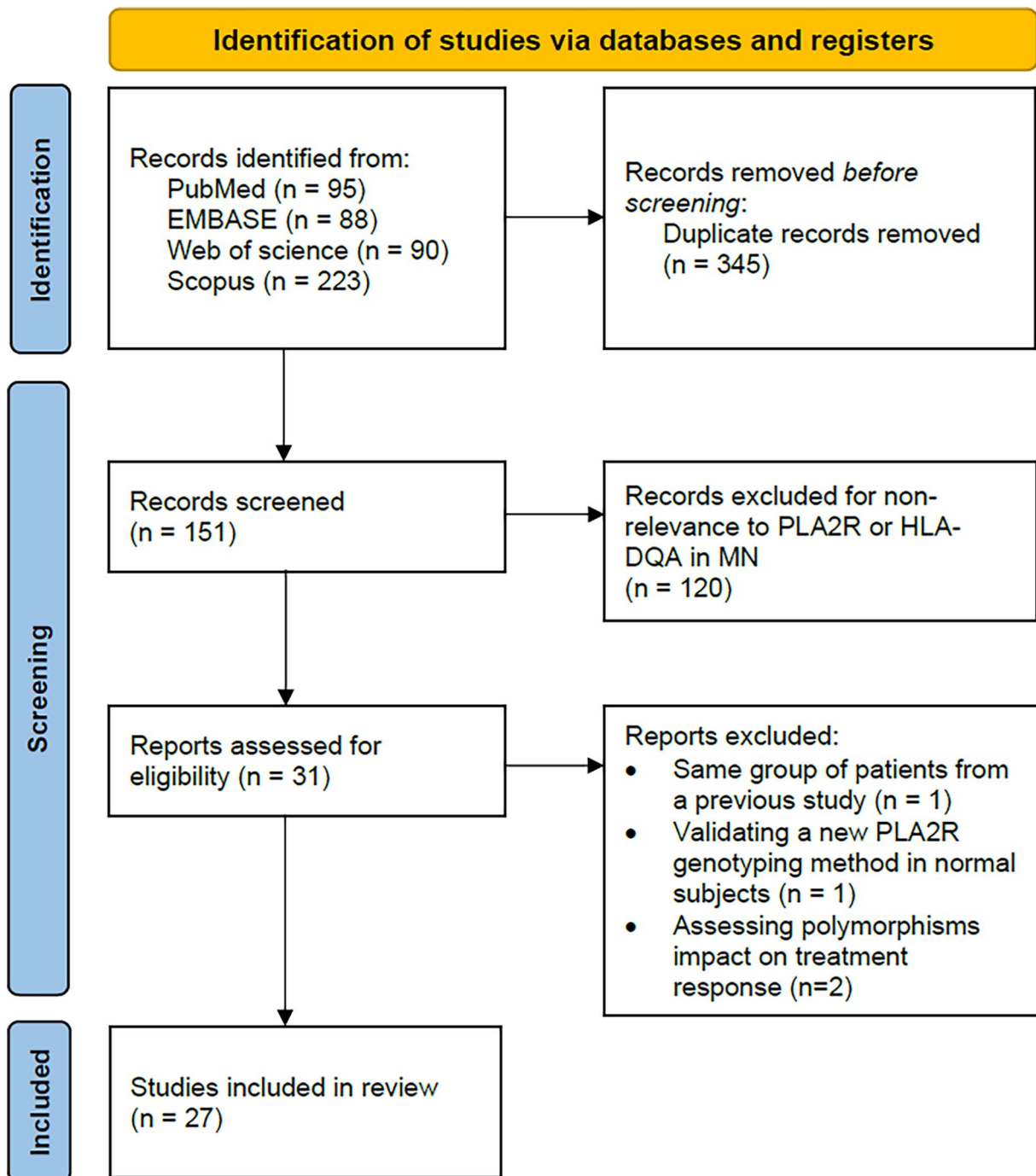
Figure 1. PRISMA flow diagram for study selection.

PLA2R rs4664308 SNP meta-analysis

The rs4664308*A wild (major) allele was significantly associated with increased PMN risk, OR (95% CI) = 2.32 (2.13–2.53), $P < 1E–10$ (Figure 3). This association noted with the codominant model was also statistically significant when recessive and dominant models were applied (Table 3). Besides, there was a low level of between-studies heterogeneity, $I^2 = 17\%$, $Tau^2 = 0.0057$, 95% PI = (1.93–2.8), and $P = .25$. Subgroup analysis by ethnicity revealed that the increase of PMN risk conferred by the A allele remained significant in all ethnic groups,

Caucasian, Asian, and North-African (Supplemental Figure S1). Subsequent meta-regression revealed significant negative correlation of the effect size with both gender ratio (M/F) and albumin level, $P = .045$ and $P = .015$, respectively (Supplemental Table S13 and Supplemental Figure S2).

PLA2R rs3749117 SNP meta-analysis

The integrated analysis revealed a significant association between the rs3749117*T wild allele and susceptibility to PMN, OR (95% CI) = 2.13 (1.79–2.52), $P < 1E–10$ (Figure 4).

Figure 2. Summary of study risk of bias.

Likewise, recessive and dominant genetic models exhibited significant association with PMN risk (Table 3). Nevertheless, the heterogeneity between included studies was substantial, $I^2 = 73\%$, $Tau^2 = 0.0642$, 95% PI = (1.18–3.83), $P < 1E–10$. Subgroup analysis by ethnicity showed that both the association with PMN risk and the between-studies heterogeneity remained statistically significant in all ethnic groups (Supplemental Figure S3). Meta-regression revealed significant positive correlations of the effect size with age and urinary protein level and a negative correlation with albumin level, $P = .012$, $P = .035$, and $P = .013$, respectively (Supplemental Table S14).

PLA2R rs3749119 SNP meta-analysis

The rs3749119*C wild allele was significantly associated with PMN susceptibility, OR (95% CI) = 2.22 (1.78–2.77), $P < 1E–10$ (Figure 5). This significant association was also noted in recessive and dominant genetic models (Table 3). However, there was a significant amount of between-studies

heterogeneity, $I^2 = 71\%$, $Tau^2 = 0.0821$, 95% PI = (1.09–4.51), $P < 1E–10$. There was only 1 included study performed in a Caucasian population while all the other studies were carried out in Asian groups. Hence, subgroup analysis did not bring further explanation of the observed heterogeneity (Supplemental Figure S4). Meta-regression revealed a significant negative correlation between the effect size and albumin level, $P = .024$ (Supplemental Table S15).

PLA2R rs35771982 SNP meta-analysis

Combined analysis showed a significant association of the rs35771982*G major allele with increased PMN risk, OR (95% CI) = 2.44 (2.17–2.74), $P < 1E–10$ (Figure 6). Both recessive and dominant genetic models confirmed this significant association with PMN (Table 3). Nonetheless, there was a significant between-studies heterogeneity, $I^2 = 41\%$, $Tau^2 = 0.0199$, 95% PI = (1.76–3.39), $P = .04$. As the mainstream of included studies were performed in Asian populations, the subgroup analysis did not provide the present study with information on potential sources of heterogeneity (Supplemental Figure S5). Inversely, the meta-regression model showed significant negative correlations of the effect size with albumin level and eGFR and a positive correlation with urinary protein level, $P = .013$, $P = .001$, and $P = .045$ (Supplemental Table S16 and Supplemental Figure S6). Therefore, the association of the rs35771982*G allele with PMN risk is rather observed in case of severe disease with lower albumin and eGFR levels and higher 24-hours proteinuria.

PLA2R rs3828323 SNP meta-analysis

The rs3828323*C wild allele was significantly associated with PMN susceptibility, OR (95% CI) = 1.65 (1.44–1.89), $P < 1E–10$ (Figure 7). Likewise, recessive and dominant genetic models confirmed the significant association between the rs382832 SNP and PMN risk (Table 3). Besides there was a moderate between-studies heterogeneity, $I^2 = 35\%$, $Tau^2 = 0.0188$, 95% PI = (1.17–2.32), $P = .11$. Subgroup analysis by ethnicity revealed that the association remained significant in all ethnic groups (Supplemental Figure S7). Conversely, meta-regression revealed a significant negative correlation of the effect size with albumin level ($P = .002$), and a positive correlation with 24-hours proteinuria level ($P = .027$) (Supplemental Table S17 and Supplemental Figure S8).

PLA2R rs16844715 SNP meta-analysis

Only 5 studies were included in the rs16844715 SNP meta-analysis. The C major allele was significantly associated with increased PMN risk, OR (95% CI) = 2.32 (2.02–2.67), $P < 1E–10$ (Figure 8). This association was also significant when recessive and dominant genetic models were applied (Table 3). In addition, there was no evidence of heterogeneity
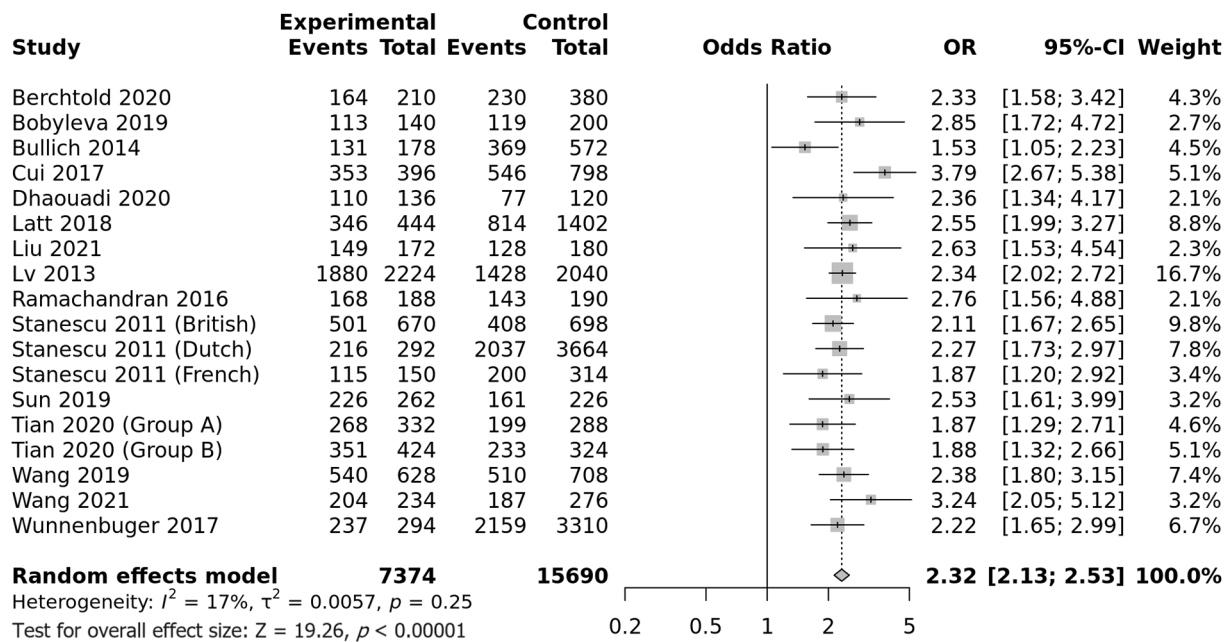
Study	Experimental Events	Total	Control Events	Total	Odds Ratio	OR	95%-CI	Weight
Berchtold 2020	164	210	230	380		2.33	[1.58; 3.42]	4.3%
Bobyleva 2019	113	140	119	200		2.85	[1.72; 4.72]	2.7%
Bullich 2014	131	178	369	572		1.53	[1.05; 2.23]	4.5%
Cui 2017	353	396	546	798		3.79	[2.67; 5.38]	5.1%
Dhaouadi 2020	110	136	77	120		2.36	[1.34; 4.17]	2.1%
Latt 2018	346	444	814	1402		2.55	[1.99; 3.27]	8.8%
Liu 2021	149	172	128	180		2.63	[1.53; 4.54]	2.3%
Lv 2013	1880	2224	1428	2040		2.34	[2.02; 2.72]	16.7%
Ramachandran 2016	168	188	143	190		2.76	[1.56; 4.88]	2.1%
Stanescu 2011 (British)	501	670	408	698		2.11	[1.67; 2.65]	9.8%
Stanescu 2011 (Dutch)	216	292	2037	3664		2.27	[1.73; 2.97]	7.8%
Stanescu 2011 (French)	115	150	200	314		1.87	[1.20; 2.92]	3.4%
Sun 2019	226	262	161	226		2.53	[1.61; 3.99]	3.2%
Tian 2020 (Group A)	268	332	199	288		1.87	[1.29; 2.71]	4.6%
Tian 2020 (Group B)	351	424	233	324		1.88	[1.32; 2.66]	5.1%
Wang 2019	540	628	510	708		2.38	[1.80; 3.15]	7.4%
Wang 2021	204	234	187	276		3.24	[2.05; 5.12]	3.2%
Wunnenburger 2017	237	294	2159	3310		2.22	[1.65; 2.99]	6.7%
Random effects model		**7374**		**15690**		**2.32**	**[2.13; 2.53]**	**100.0%**

Heterogeneity: $I^2 = 17\%$, $\tau^2 = 0.0057$, $p = 0.25$
Test for overall effect size: $Z = 19.26$, $p < 0.00001$

Figure 3. Forest plot for the association between the PLA2R rs4664308 SNP and PMN risk: Significant increase in PMN risk in case of rs4664308*A allele with a low between-studies inconsistency.

between included studies, $I^2 = 0\%$, Tau$^2 = 0$, 95% PI = (2.02–2.67), $P = .87$.

PLA2R rs1511223 SNP meta-analysis

Seven studies were involved in the rs1511223 SNP meta-analysis. The combined analysis showed a significant association of the A wild allele with susceptibility to PMN, OR (95% CI) = 1.62 (1.35–1.94), $P < 1E$–10 (Figure 9). Both recessive and dominant models confirmed this significant association (Table 3). However, the was a significant between-studies heterogeneity, $I^2 = 57\%$, Tau$^2 = 0.0311$, 95% PI = (0.97–2.7) $P = .03$. Subgroup analysis by ethnicity was not informative as the majority of included studies were performed in Caucasian groups (Supplemental Figure S9). Subsequent meta-regression revealed a significant negative correlation between the effect size and the rs1511223*C minor allele frequency in control groups, $P = .015$ (Supplemental Table S18 and Supplemental Figure S10).

PLA2R rs6757188 SNP meta-analysis

Six studies were included to assess the impact of the rs6757188 on predisposition to PMN. Combined analysis did not show any association between the T major allele and PMN risk, OR (95% CI) = 1.16 (0.88–1.54), $P = .3$ (Figure 10). Similarly, no association observed when recessive and dominant genetic models were applied (Table 3). Of note, there was a important amount of between-studies heterogeneity, $I^2 = 72\%$, Tau$^2 = 0.0827$, 95% PI = (0.48–2.84), $P = .0027$. Subgroup analysis by ethnicity did not unveil any useful information on heterogeneity as the association between the rs6757188 SNP and PMN risk remained non-significant in all ethnic groups

(Supplemental Figure S11). Inversely, meta-regression showed significant positive correlations of the effect size with the control/patient ratio and the C minor allele frequency in control groups, $P = .002$ and $P < .0001$, respectively (Supplemental Table S19 and Supplemental Figure S12).

PLA2R rs2715918 SNP meta-analysis

Only 4 studies investigated the influence of the rs2715918 on susceptibility to PMN. All included studies were performed in Asian populations. The rs2715918*A minor allele was significantly associated with increased PMN risk, OR (95% CI) = 2.63 (1.5–4.62), $P = .0007$ (Figure 11). Both recessive and dominant genetic models confirmed the significant association with predisposition to PMN (Table 3). Nevertheless, there was a significant heterogeneity between included studies, $I^2 = 65\%$, Tau$^2 = 0.1945$, 95% PI = (0.27–5.32), $P = .04$. Subsequent meta-regression revealed significant negative correlations of the effect size with the gender ratio ($P = .021$) and the A allele frequency in control groups ($P = .005$) and a positive correlation with the 24-hours proteinuria level ($P = .011$) (Supplemental Table S20 and Supplemental Figure S13).

HLA-DQA1 rs2187668 SNP meta-analysis

Combined analysis showed a significant association of the rs2187668*A minor allele with increased PMN risk, OR (95% CI) = 3.11 (1.5–4.62) $P < 1E$–10 (Figure 12). This association with increased PMN risk remained significant when recessive and dominant models were applied (Table 3). However, there was a considerable amount of between-studies heterogeneity, $I^2 = 73\%$, Tau$^2 = 0.1$, 95% PI = (1.54–6.29), $P < 1E$–10. Subgroup analysis by ethnicity revealed that if heterogeneity was

Table 3. Main results of PLA2R1 and HLA-DQA1 SNPs in PMN risk.

SNP	GENETIC MODEL	CONTRAST	PMN RISK		HETEROGENEITY	
			OR (95% CI)	*P*-VALUE	*I²*	*P*-VALUE
rs4664308	Codominant	A vs G	2.3245 (2.1333–2.5328)	<1E–10	17%	.2451
	Recessive	AA vs AG + GG	2.6996 (2.4660–2.9553)	<1E–10	0	.4846
	Dominant	AA + AG vs GG	3.1187 (2.3926–4.0651)	<1E–10	30%	.1112
rs3749117	Codominant	T vs C	2.1252 (1.7920–2.5203)	<1E–10	73%	<1E–10
	Recessive	TT vs TC + CC	2.1708 (1.7257–2.7309)	<1E–10	75%	<1E–10
	Dominant	TT + TC vs CC	2.9387 (2.3850–3.6208)	<1E–10	17%	.2707
rs3749119	Codominant	C vs T	2.2199 (1.7811–2.7669)	<1E–10	71%	.0003
	Recessive	CC vs CT + TT	2.2317 (1.8187–2.7386)	<1E–10	48%	.0446
	Dominant	CC + CT vs TT	3.7148 (2.2711–6.0763)	1.7E–7	63%	.0039
rs35771982	Codominant	G vs C	2.4407 (2.1740–2.7401)	<1E–10	41%	.0441
	Recessive	GG vs GC + CC	2.5956 (2.2144–3.0424)	<1E–10	53%	.0071
	Dominant	GG + GC vs CC	3.7221 (2.5799–5.3700)	<1E–10	63%	.0003
rs3828323	Codominant	C vs T	1.6500 (1.4404–1.8900)	<1E–10	35%	.108
	Recessive	CC vs CT + TT	1.8047 (1.4829–2.1963)	3.8E–9	47%	.0354
	Dominant	CC + CT vs TT	1.7528 (1.4423–2.1301)	1.7E–8	0	.8771
rs16844715	Codominant	C vs T	2.3189 (2.0158–2.6674)	<1E–10	0	.8743
	Recessive	CC vs CT + TT	2.4229 (1.8685–3.1419)	<1E–10	37%	.1772
	Dominant	CC + CT vs TT	2.9975 (2.1867–4.1090)	<1E–10	18%	.2983
rs1511223	Codominant	A vs C	1.6207 (1.3545–1.9394)	1.3E–7	57%	.0306
	Recessive	AA vs AC + CC	1.6444 (1.3835–1.9545)	1.7E–8	25%	.2353
	Dominant	AA + AC vs CC	2.0016 (1.2816–3.1260)	.0022	69%	.0032
rs6557188	Codominant	T vs C	1.1614 (0.8762–1.5393)	.298045	72%	.0027
	Recessive	TT vs TC + CC	1.1659 (0.9129–1.4891)	.218766	43%	.1219
	Dominant	TT + TC vs CC	1.2144 (0.6290–2.3448)	.562745	72%	.003
rs2715918	Codominant	G vs A	2.6338 (1.5015–4.6200)	.000731	65%	.0374
	Recessive	GG vs GA + AA	5.1840 (2.1950–12.2431)	.000174	0	.9512
	Dominant	GG + GA vs AA	2.7731 (1.5010–5.1230)	.001126	63%	.0438
rs2187668	Codominant	A vs G	3.1082 (2.5765–3.7498)	<1E–10	73%	<1E–10
	Recessive	AA vs AG + GG	4.8323 (3.2408–7.2054)	<1E–10	45%	.0324
	Dominant	AA + AG vs GG	3.5328 (2.8325–4.4062)	<1E–10	73%	<1E–10

significant in both Caucasian and Asian populations, the risk conferred by the A allele was significantly higher in Caucasians (OR [95% CI] = 3.929 [3.251–4.748]) than in Asians (OR [95% CI] = 2.537 [1.94–3.318], *P* = .007) (Figure 13). Moreover, meta-regression showed significant negative correlation between the effect size and both albumin and eGFR levels and
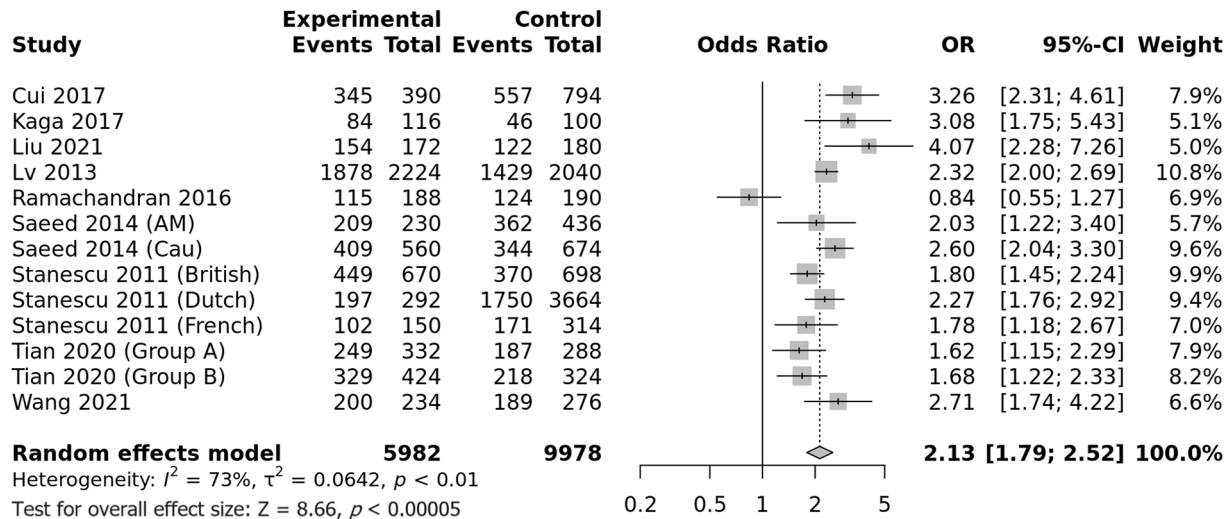
Study	Experimental Events Total		Control Events Total		Odds Ratio	OR	95%-CI	Weight
Cui 2017	345	390	557	794		3.26	[2.31; 4.61]	7.9%
Kaga 2017	84	116	46	100		3.08	[1.75; 5.43]	5.1%
Liu 2021	154	172	122	180		4.07	[2.28; 7.26]	5.0%
Lv 2013	1878	2224	1429	2040		2.32	[2.00; 2.69]	10.8%
Ramachandran 2016	115	188	124	190		0.84	[0.55; 1.27]	6.9%
Saeed 2014 (AM)	209	230	362	436		2.03	[1.22; 3.40]	5.7%
Saeed 2014 (Cau)	409	560	344	674		2.60	[2.04; 3.30]	9.6%
Stanescu 2011 (British)	449	670	370	698		1.80	[1.45; 2.24]	9.9%
Stanescu 2011 (Dutch)	197	292	1750	3664		2.27	[1.76; 2.92]	9.4%
Stanescu 2011 (French)	102	150	171	314		1.78	[1.18; 2.67]	7.0%
Tian 2020 (Group A)	249	332	187	288		1.62	[1.15; 2.29]	7.9%
Tian 2020 (Group B)	329	424	218	324		1.68	[1.22; 2.33]	8.2%
Wang 2021	200	234	189	276		2.71	[1.74; 4.22]	6.6%
Random effects model		**5982**		**9978**		**2.13**	**[1.79; 2.52]**	**100.0%**

Heterogeneity: $I^2 = 73\%$, $\tau^2 = 0.0642$, $p < 0.01$
Test for overall effect size: $Z = 8.66$, $p < 0.00005$

(Axis: 0.2 0.5 1 2 5)

Figure 4. Forest plot for the association between the PLA2R rs3749117 SNP and PMN risk: Significant increase in PMN risk in case of rs3749117*T allele with a significant between-studies inconsistency.
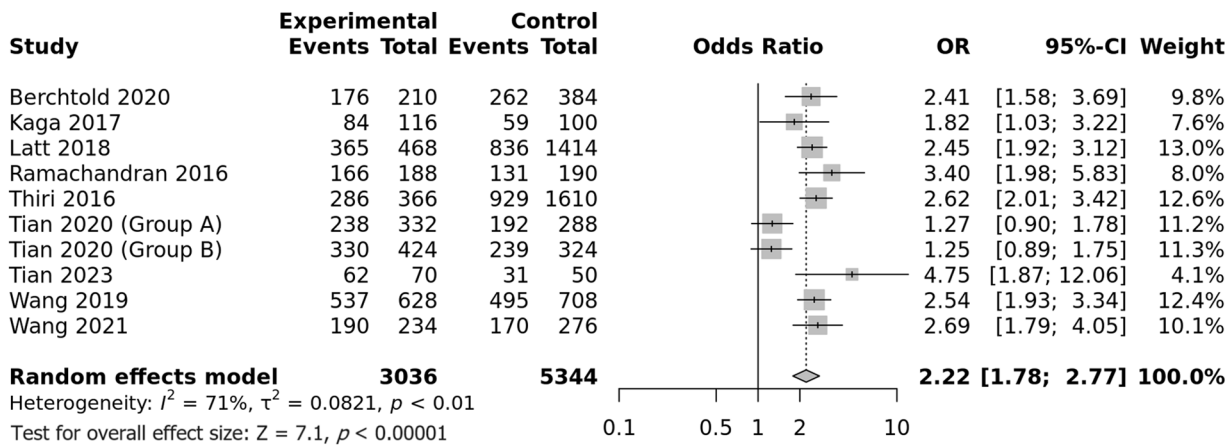
Study	Experimental Events Total		Control Events Total		Odds Ratio	OR	95%-CI	Weight
Berchtold 2020	176	210	262	384		2.41	[1.58; 3.69]	9.8%
Kaga 2017	84	116	59	100		1.82	[1.03; 3.22]	7.6%
Latt 2018	365	468	836	1414		2.45	[1.92; 3.12]	13.0%
Ramachandran 2016	166	188	131	190		3.40	[1.98; 5.83]	8.0%
Thiri 2016	286	366	929	1610		2.62	[2.01; 3.42]	12.6%
Tian 2020 (Group A)	238	332	192	288		1.27	[0.90; 1.78]	11.2%
Tian 2020 (Group B)	330	424	239	324		1.25	[0.89; 1.75]	11.3%
Tian 2023	62	70	31	50		4.75	[1.87; 12.06]	4.1%
Wang 2019	537	628	495	708		2.54	[1.93; 3.34]	12.4%
Wang 2021	190	234	170	276		2.69	[1.79; 4.05]	10.1%
Random effects model		**3036**		**5344**		**2.22**	**[1.78; 2.77]**	**100.0%**

Heterogeneity: $I^2 = 71\%$, $\tau^2 = 0.0821$, $p < 0.01$
Test for overall effect size: $Z = 7.1$, $p < 0.00001$

(Axis: 0.1 0.5 1 2 10)

Figure 5. Forest plot for the association between the PLA2R rs3749119 SNP and PMN risk: Significant increase in PMN risk in case of rs3749119*C allele with a significant between-studies inconsistency.
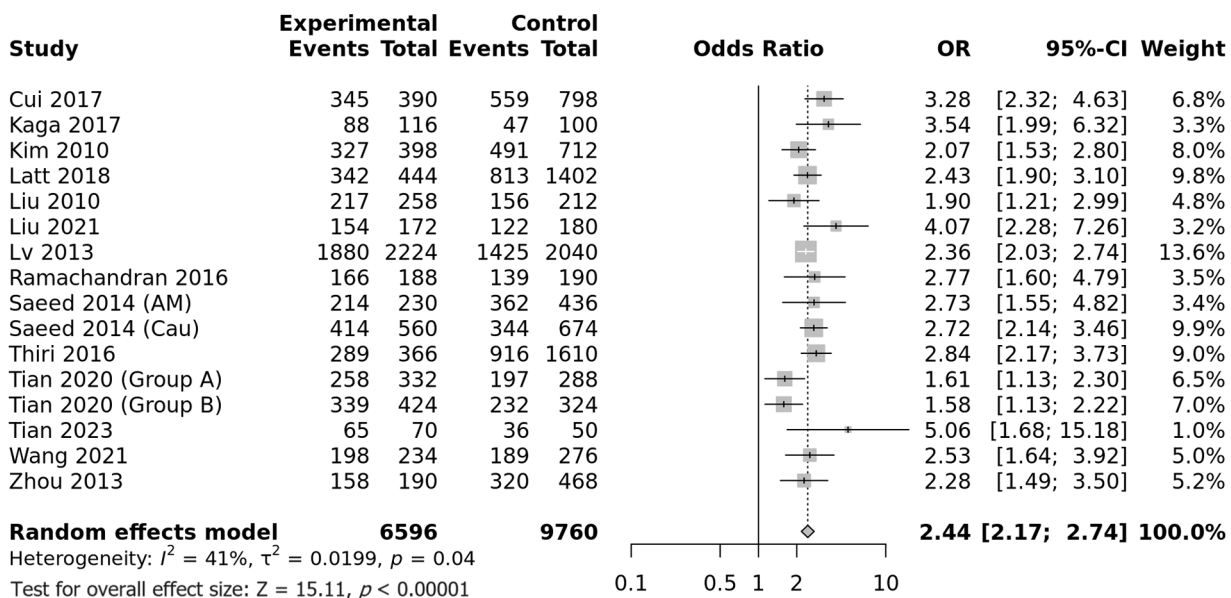
Study	Experimental Events Total		Control Events Total		Odds Ratio	OR	95%-CI	Weight
Cui 2017	345	390	559	798		3.28	[2.32; 4.63]	6.8%
Kaga 2017	88	116	47	100		3.54	[1.99; 6.32]	3.3%
Kim 2010	327	398	491	712		2.07	[1.53; 2.80]	8.0%
Latt 2018	342	444	813	1402		2.43	[1.90; 3.10]	9.8%
Liu 2010	217	258	156	212		1.90	[1.21; 2.99]	4.8%
Liu 2021	154	172	122	180		4.07	[2.28; 7.26]	3.2%
Lv 2013	1880	2224	1425	2040		2.36	[2.03; 2.74]	13.6%
Ramachandran 2016	166	188	139	190		2.77	[1.60; 4.79]	3.5%
Saeed 2014 (AM)	214	230	362	436		2.73	[1.55; 4.82]	3.4%
Saeed 2014 (Cau)	414	560	344	674		2.72	[2.14; 3.46]	9.9%
Thiri 2016	289	366	916	1610		2.84	[2.17; 3.73]	9.0%
Tian 2020 (Group A)	258	332	197	288		1.61	[1.13; 2.30]	6.5%
Tian 2020 (Group B)	339	424	232	324		1.58	[1.13; 2.22]	7.0%
Tian 2023	65	70	36	50		5.06	[1.68; 15.18]	1.0%
Wang 2021	198	234	189	276		2.53	[1.64; 3.92]	5.0%
Zhou 2013	158	190	320	468		2.28	[1.49; 3.50]	5.2%
Random effects model		**6596**		**9760**		**2.44**	**[2.17; 2.74]**	**100.0%**

Heterogeneity: $I^2 = 41\%$, $\tau^2 = 0.0199$, $p = 0.04$
Test for overall effect size: $Z = 15.11$, $p < 0.00001$

(Axis: 0.1 0.5 1 2 10)

Figure 6. Forest plot for the association between the PLA2R rs35771982 SNP and PMN risk: Significant increase in PMN risk in case of rs35771982*G allele with a significant between-studies inconsistency.
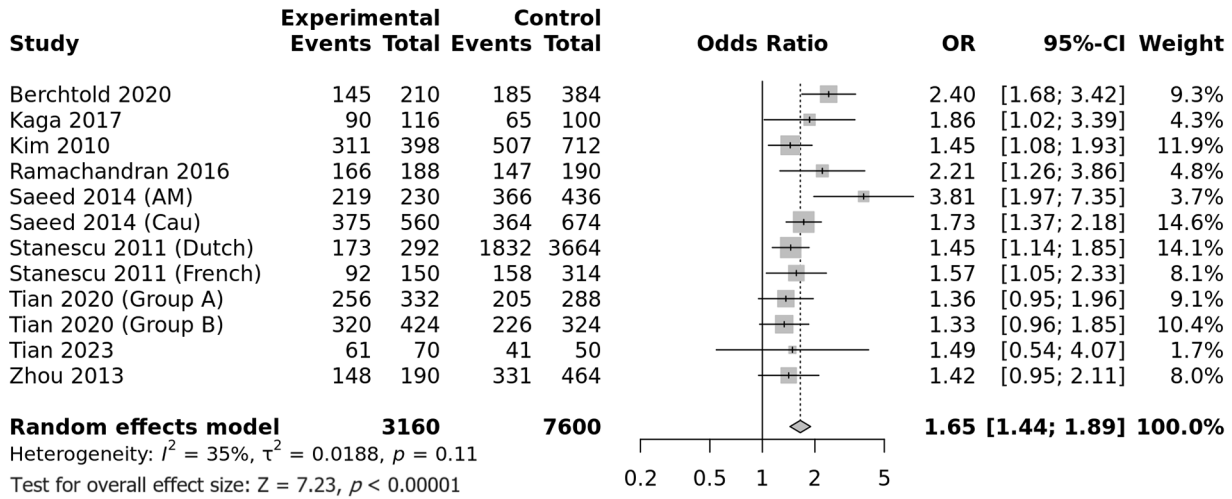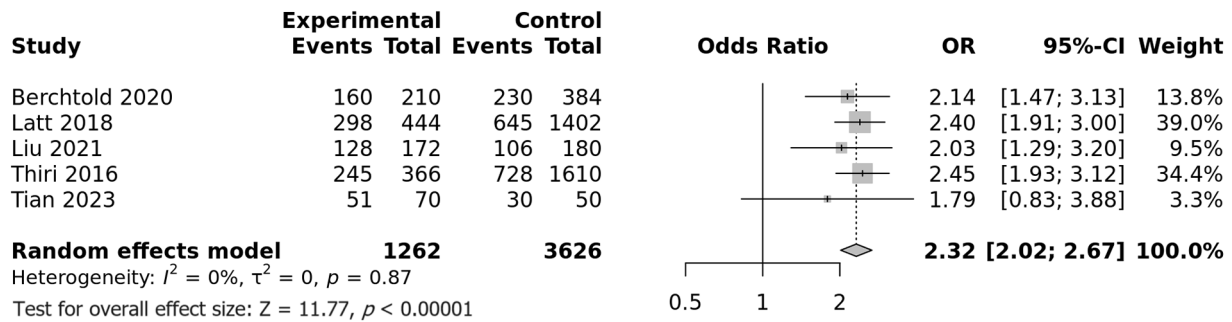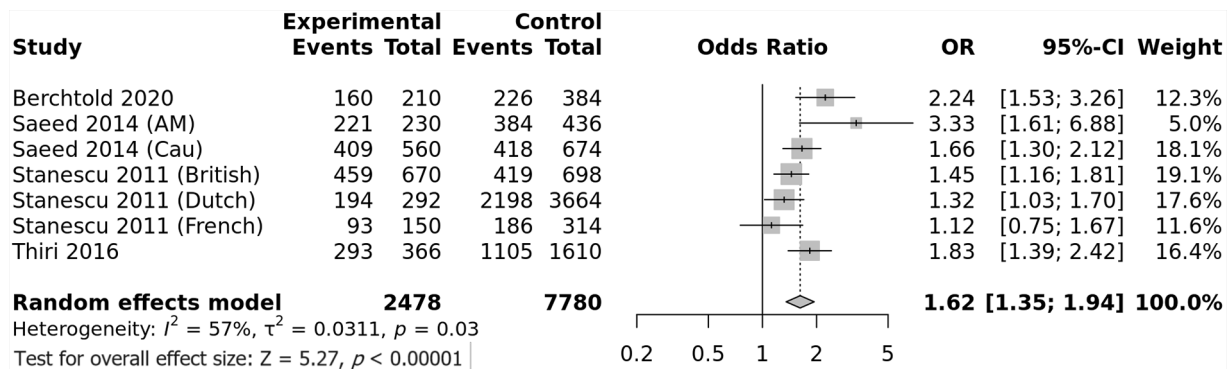
Study	Experimental Events	Total	Control Events	Total	Odds Ratio	OR	95%-CI	Weight
Berchtold 2020	145	210	185	384		2.40	[1.68; 3.42]	9.3%
Kaga 2017	90	116	65	100		1.86	[1.02; 3.39]	4.3%
Kim 2010	311	398	507	712		1.45	[1.08; 1.93]	11.9%
Ramachandran 2016	166	188	147	190		2.21	[1.26; 3.86]	4.8%
Saeed 2014 (AM)	219	230	366	436		3.81	[1.97; 7.35]	3.7%
Saeed 2014 (Cau)	375	560	364	674		1.73	[1.37; 2.18]	14.6%
Stanescu 2011 (Dutch)	173	292	1832	3664		1.45	[1.14; 1.85]	14.1%
Stanescu 2011 (French)	92	150	158	314		1.57	[1.05; 2.33]	8.1%
Tian 2020 (Group A)	256	332	205	288		1.36	[0.95; 1.96]	9.1%
Tian 2020 (Group B)	320	424	226	324		1.33	[0.96; 1.85]	10.4%
Tian 2023	61	70	41	50		1.49	[0.54; 4.07]	1.7%
Zhou 2013	148	190	331	464		1.42	[0.95; 2.11]	8.0%
Random effects model		**3160**		**7600**		**1.65**	**[1.44; 1.89]**	**100.0%**

Heterogeneity: $I^2 = 35\%$, $\tau^2 = 0.0188$, $p = 0.11$
Test for overall effect size: $Z = 7.23$, $p < 0.00001$

0.2 0.5 1 2 5

Figure 7. Forest plot for the association between the PLA2R rs3828323 SNP and PMN risk: Significant increase in PMN risk in case of rs3828323*C allele with a moderate between-studies inconsistency.

Study	Experimental Events	Total	Control Events	Total	Odds Ratio	OR	95%-CI	Weight
Berchtold 2020	160	210	230	384		2.14	[1.47; 3.13]	13.8%
Latt 2018	298	444	645	1402		2.40	[1.91; 3.00]	39.0%
Liu 2021	128	172	106	180		2.03	[1.29; 3.20]	9.5%
Thiri 2016	245	366	728	1610		2.45	[1.93; 3.12]	34.4%
Tian 2023	51	70	30	50		1.79	[0.83; 3.88]	3.3%
Random effects model		**1262**		**3626**		**2.32**	**[2.02; 2.67]**	**100.0%**

Heterogeneity: $I^2 = 0\%$, $\tau^2 = 0$, $p = 0.87$
Test for overall effect size: $Z = 11.77$, $p < 0.00001$

0.5 1 2

Figure 8. Forest plot for the association between the PLA2R rs16844715 SNP and PMN risk: Significant increase in PMN risk in case of rs16844715*C allele without any between-studies inconsistency.

Study	Experimental Events	Total	Control Events	Total	Odds Ratio	OR	95%-CI	Weight
Berchtold 2020	160	210	226	384		2.24	[1.53; 3.26]	12.3%
Saeed 2014 (AM)	221	230	384	436		3.33	[1.61; 6.88]	5.0%
Saeed 2014 (Cau)	409	560	418	674		1.66	[1.30; 2.12]	18.1%
Stanescu 2011 (British)	459	670	419	698		1.45	[1.16; 1.81]	19.1%
Stanescu 2011 (Dutch)	194	292	2198	3664		1.32	[1.03; 1.70]	17.6%
Stanescu 2011 (French)	93	150	186	314		1.12	[0.75; 1.67]	11.6%
Thiri 2016	293	366	1105	1610		1.83	[1.39; 2.42]	16.4%
Random effects model		**2478**		**7780**		**1.62**	**[1.35; 1.94]**	**100.0%**

Heterogeneity: $I^2 = 57\%$, $\tau^2 = 0.0311$, $p = 0.03$
Test for overall effect size: $Z = 5.27$, $p < 0.00001$

0.2 0.5 1 2 5

Figure 9. Forest plot for the association between the PLA2R rs1511223 SNP and PMN risk: Significant increase in PMN risk in case of rs1511223*A allele with a significant between-studies inconsistency.

positive correlations with 24-hours proteinuria and serum creatinine levels, all *P*-values < .0001 (Supplemental Table S21 and Supplemental Figure S15).

Interaction between PLA2R rs4664308 and HLA-DQA1 rs2187668 SNPs

Only 3 studies[15,25,27] provided the rs4664308/rs2187668 genotype combinations in PMN patients and controls. The high-risk combination was defined as the rs4664308*A/A genotype with rs2187668*G/A or A/A genotypes whereas the low-risk combination was defined as the rs4664308*G/G with the rs2187668*G/G genotypes. Comparatively to all other combinations, the high-risk combination conferred a significant increase in PMN risk, OR (95% CI) = 6.54 (4.38–9.71), *P* < 1E-10 (Figure 14A). Furthermore, when compared to low-risk combination, the high-risk combination was associated with an even higher risk of PMN, OR (95% CI) = 15.1 (4.94–46.19),
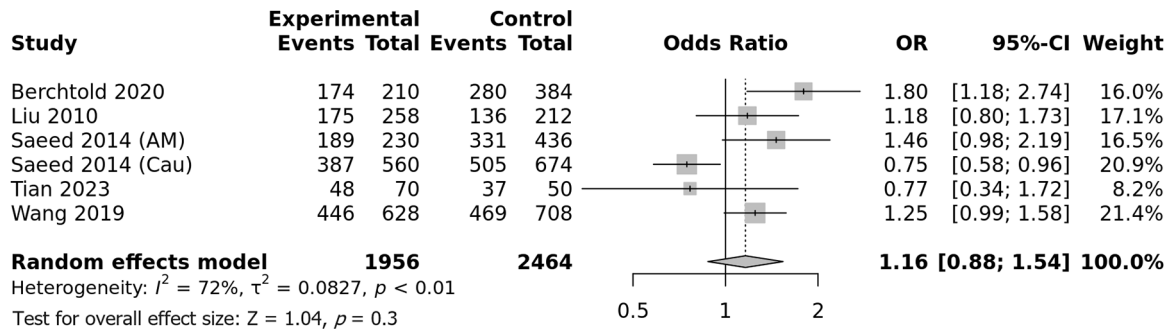
Figure 10. Forest plot for the association between the PLA2R rs6757188 SNP and PMN risk: No association between the rs6757188 SNP and PMN risk with a significant between-studies inconsistency.
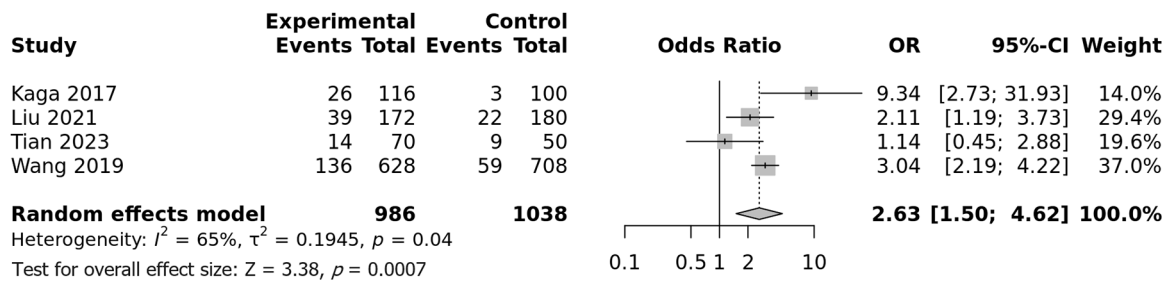

Figure 11. Forest plot for the association between the PLA2R rs2715918 SNP and PMN risk: Significant increase in PMN risk in case of rs2715918*A allele with a significant between-studies inconsistency.
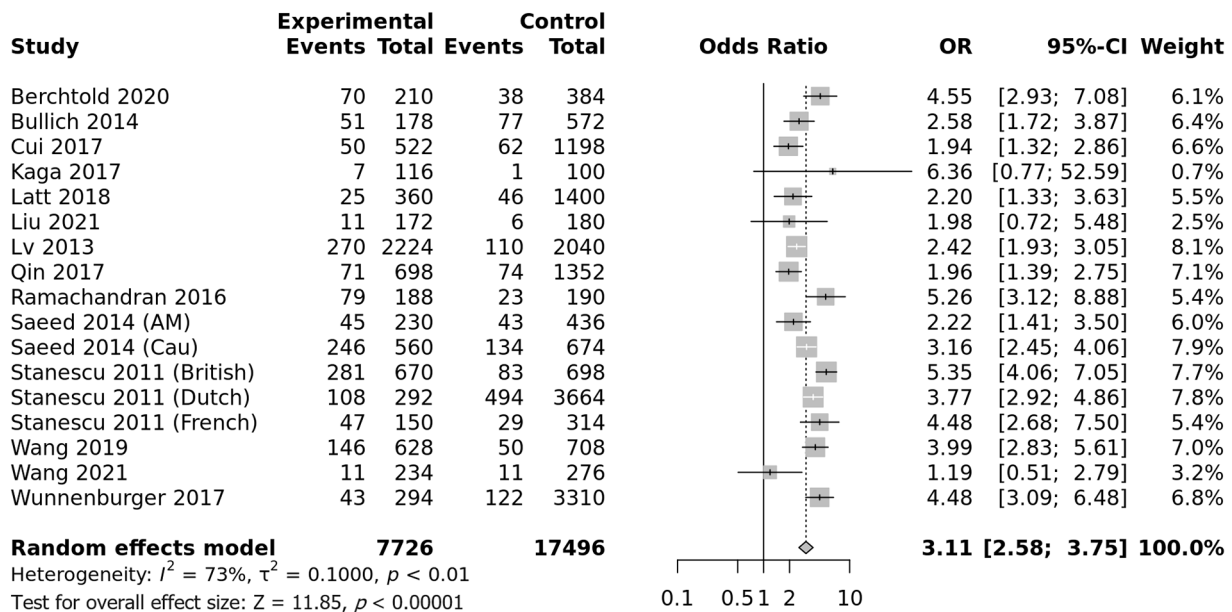

Figure 12. Forest plot for the association between the HLA-DQA1 rs2187668 SNP and PMN risk: Significant increase in PMN risk in case of rs2187668*A allele with a significant between-studies inconsistency.

$P < 1E-10$ (Figure 14B). However, both comparisons suffered from between-studies heterogeneity as the small number of included studies did not allow to suitably estimate Tau².

Sensitivity analysis

The sensitivity analysis revealed that association results were stable for all performed meta-analyses (Supplemental Figures S15–S24), suggesting a high level of integrity with reliable results.

Publication bias

Generated funnel plots (Figure 15) were found to be overall symmetrical and Egger's tests confirmed these findings with non-significant *P*-values for the 10 investigated SNPs (0.6949,
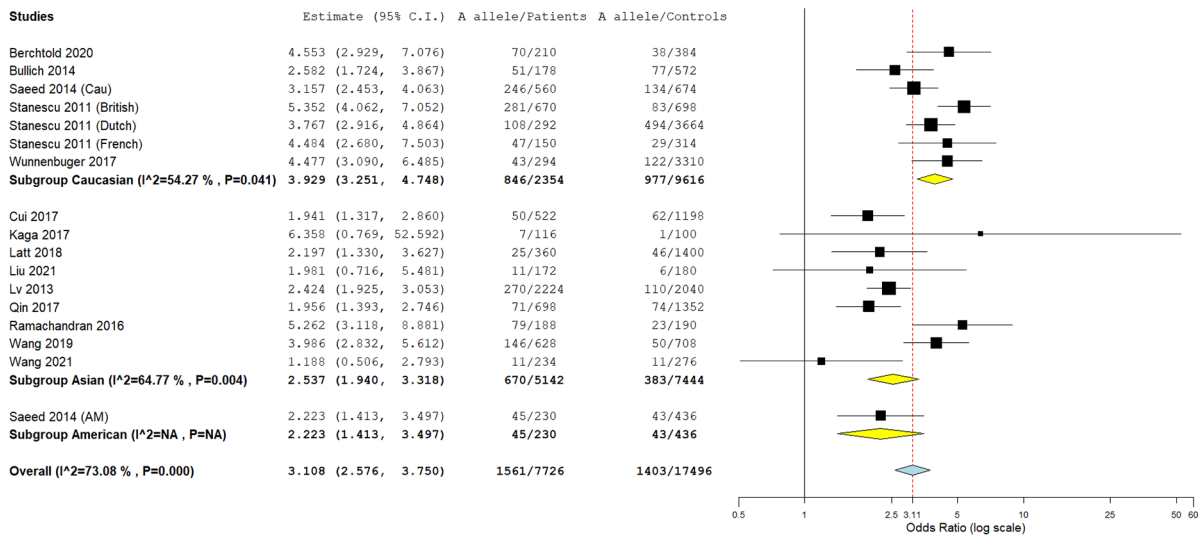
Figure 13. Subgroup analysis by ethnicity for the HLA-DQA1 rs2187668 SNP: Significant higher PMN risk conferred by the rs2187668*A allele in Caucasians comparatively to Asians and Americans.
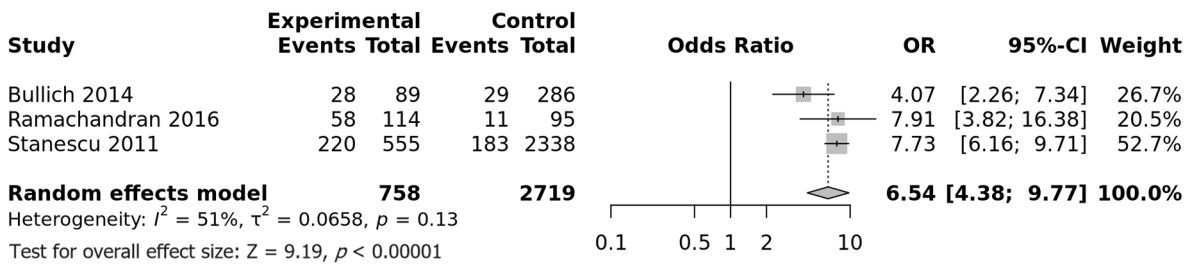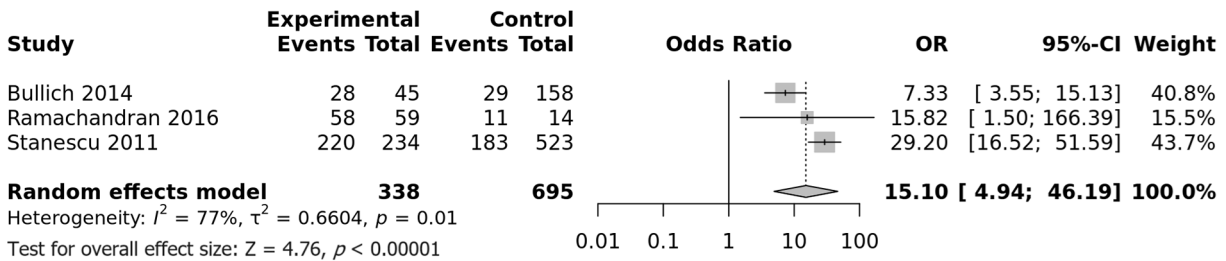
Figure 14. Forest plot for the association of the PLA2R rs4664308/HLA-DQA1-rs2187668 interaction with PMN risk: Significant interaction between rs4664308 and rs2187668 in increased PMN risk.

0.8552, 07506, 0.3805, 0.2065, 0.086, 0.2552, 0.6636, 0.948, and 0.6886), which indicated that results were not weakened by publication biases.

Overall, apart from the rs6757188 SNP, all other SNPs in the PLA2R and HLA-DQA1 genes were found to be associated with PMN risk. For most SNPs, the association with PMN risk was influenced by serum albumin, eGFR, and 24-hours proteinuria levels. Moreover, combined results revealed a significant interaction between PLA2R and HLA-DQA1 genes in the increased risk of PMN.

Discussion

PMN is an antibody-mediated glomerular disease caused by the formation of a large number of IgG4 immune-complexes in the subepithelial space of the glomerular basement membrane.[3] In the majority of PMN patients, these IgG4 colocalize with the PLA2R[3] which have been reported to be overexpressed on podocytes.[5] In addition, to their diagnostic specificity for PMN, anti-PLA2R Abs have also been reported to be of a prognostic significance and could predict a non-remission outcome.[17,25]

Like other autoimmune diseases, PMN etiology is complex including multigenetic background and environmental factors.[17] Among genetic factors, PLA2R and HLA genes were the most associated with PMN risk. In fact, over the past decade several studies focused on PLA2R and HLA-DQA1 gene polymorphisms, though with inconsistent findings. Although, previous meta-analyses were published in 2018 for the
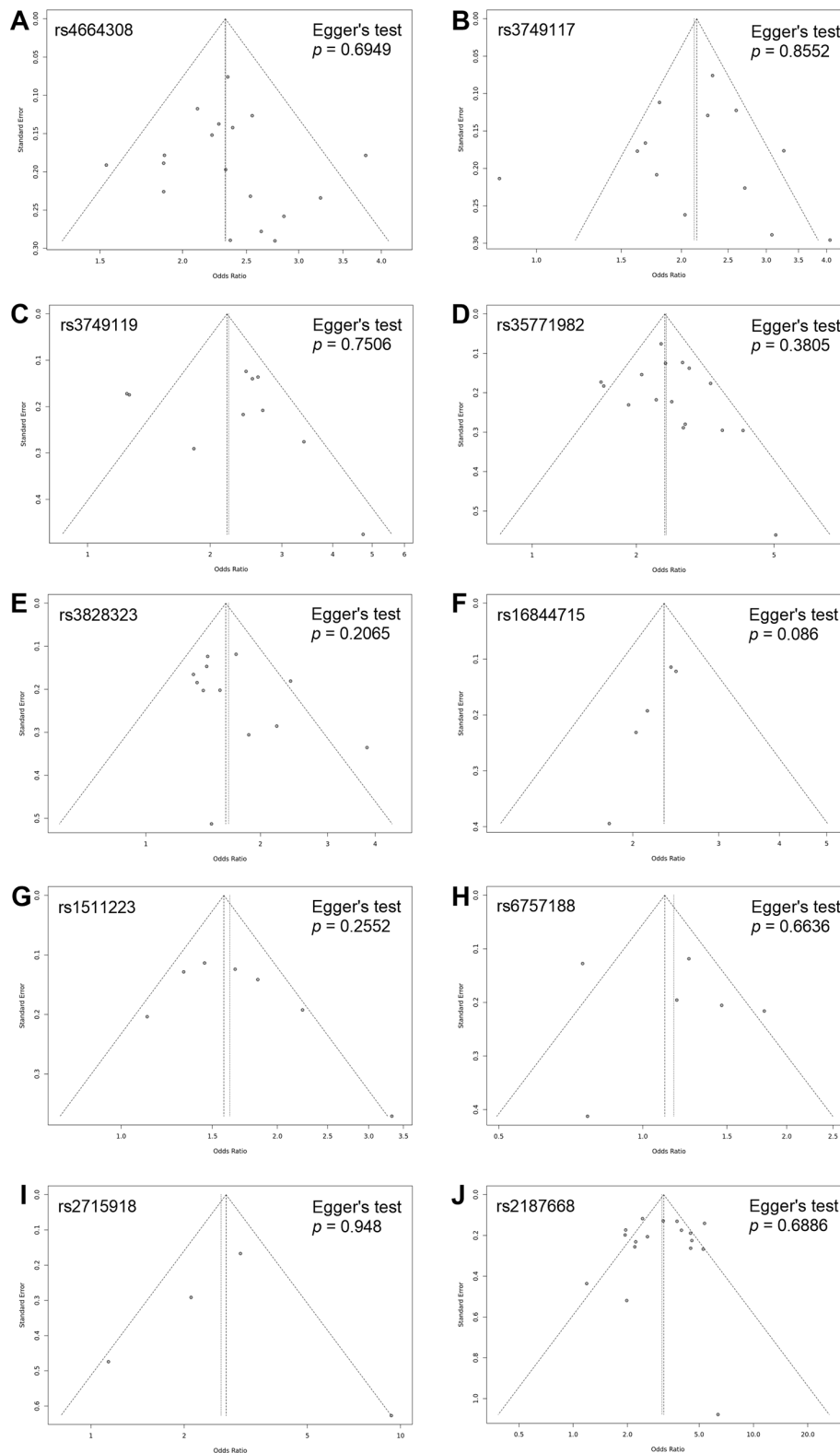
Figure 15. Funnel plots assessing publication bias: Symmetrical funnel plots with no evidence of publication biases. A: rs4664308 SNP. B: rs3749117 SNP. C: rs3749119 SNP. D: rs35771982 SNP. E: rs3828323 SNP. F: rs16844715 SNP. G: rs1511223 SNP. H: rs6757188 SNP. I: rs2715918 SNP. J: rs2187668 SNP.

HLA-DQA1 rs2187668 SNP,[8] and in 2020 for the PLA2R rs4664308 SNP,[6,7] the literature search cut-off time in our study was fresher. Besides, to the best of our knowledge, this study was the first to summarize published results on PMN risk conferred by the following PLA2R SNPs, rs3749117, rs3749119, rs35771982, rs3828323, rs16844715, rs1511223,

rs6757188, and rs2715918. In addition, while previous meta-analyses involved 7 to 11 studies, our meta-analysis included 27 reports which could provide a more comprehensive and accurate estimation of PLA2R and HLA-DQA1 SNPs influence on PMN risk. Moreover, we have integrated the results of 8 other SNPs which were not previously meta-analyzed, together with a meta-analysis of the PLA2R-rs4664308/ HLA-DQA1-rs2187668 SNPs interaction effect on PMN risk. Furthermore, we assessed the between-studies heterogeneity by building univariate and multivariate models of meta-regression.

The present study revealed that the PLA2R rs4664308*A allele was associated with approximately 132% increase in PMN risk with a low level of between-studies heterogeneity. In addition, if the subgroup analysis displayed a stable association among all ethnic groups, meta-regression showed a negative correlation with albumin level in PMN patients. This peculiar finding indicates that the association with PMN risk is greater in patients with a deeper hypoalbuminemia. Hence, the rs4664308 SNP could as well influence the disease presentation and prognosis. Nevertheless, more studies are needed to accurately assess its impact on treatment response and PMN outcome.

Besides, the PLA2R rs3749117*T allele was associated with just over 2-fold increased risk of PMN. Nonetheless, there was a considerable amount of heterogeneity which could not be explained by ethnicity-subgroup analysis. Interestingly, meta-regression revealed an inverse correlation of the association strength with albumin level on the one hand, and a positive correlation with 24-hours proteinuria level on the other hand. Hence, the association between the rs3749117*T allele and PMN risk was rather noted in case of a more severe disease with lower levels of albumin and greater proteinuria. However, prognostic studies are needed to verify this particular result.

This study showed that the PLA2R rs3749119*C was associated with roughly 122% increased PMN risk. Nevertheless, there was a high between-studies heterogeneity and the predicted risk might fluctuate between 9% and 351% increase in PMN risk. Almost all included studies were performed in Asians, thus, subgroup analysis by ethnicity could not be useful. Conversely, meta regression revealed that the effect size was negatively correlated with albumin level. This finding indicated that the association of the rs3749119*C allele with PMN risk was greater in patients with lower albumin level.

In the current study, the PLA2R rs35771982*G allele was associated with approximately 2.44-fold higher PMN risk. The heterogeneity was moderate and the true effect in 95% of comparable populations could vary from 1.76 to 3.39 times. Subgroup analysis by ethnicity was of no help as most include studies were carried out in Asian populations. Inversely, meta-regression showed negative correlations of the association strength with albumin and eGFR levels and a positive correlation with 24-hours proteinuria amount. Therefore, the association of the rs35771982*G allele with PMN risk was rather

observed in case of severe disease with lower albumin and eGFR levels and higher 24-hours proteinuria. However, the rs35771982 impact on disease presentation and outcome has to be assessed in future studies.

Besides, the combined analysis revealed a 65% increase in PMN risk conferred by the PLA2R rs3828323*C allele. Between-studies heterogeneity was moderate and true effect could fall between 44% and 89% increased PMN risk. While subgroup analysis did not show any cross-ethnic disparity, meta-regression unveiled a negative correlation of the association strength with albumin level and a positive correlation with 24-hours proteinuria extent. This finding indicated that the rs3828323*C allele effect on PMN risk could be greater in case of severe disease with lower albumin level and higher 24-hours proteinuria level.

In this study, the PLA2R rs16844715*C allele was associated with approximately 2.32 times increased PMN risk and no between-studies heterogeneity was noted. Of note only 5 studies were included in the rs16844715 SNP meta-analysis. Hence, further studies examining the influence of this SNP on PMN risk are needed.

Integrated analysis showed a 62% increase in PMN risk conferred by the PLA2R rs1511223*A allele, though with a significant amount of heterogeneity. Thus, the true effect in 95% of comparable populations could vary between a 3% decrease and 170% increase in PMN risk. As most of included studies were performed in Caucasian populations, subgroup analysis by ethnicity was not informative. Interestingly, the protective C allele frequency in control groups was negatively correlated with the effect size. Hence, the association between the rs1511223 and PMN risk seemed to be rather noted in populations with lower frequency of the protective C allele.

In the present study, the PLA2R rs6757188 SNP was not found to be associated with PMN risk, with though an important amount of between-studies heterogeneity. Therefore, the true effect could fall between 52% decrease and 54% increase in PMN risk. Subsequent subgroup analysis by ethnicity did not provide the analysis with useful information about heterogeneity sources. Conversely, the protective C allele frequency in control groups was positively correlated with the strength of the association with susceptibility to PMN. Of note, only 6 studies were included in the rs6557188 SNP meta-analysis. Thus, studies on independent and large cohorts are required to better evaluate the influence of the rs6557188 on PMN risk.

In this study, only 4 reports were included in the PLA2R rs2715918 SNP meta-analysis. The A allele was associated with 163% increased PMN risk. Nonetheless, a substantial amount of between-studies heterogeneity was noted, and the true effect could fall between 73% decrease and 432% increase in PMN risk. As all included studies were carried out in Asian populations subgroup analysis by ethnicity could not be informative on heterogeneity sources. Inversely, meta-regression revealed significant correlation of the effect size with gender ratio, the A minor allele frequency in control groups, and

the 24-hours proteinuria level. Hence, and like for other studied SNPs, the strength of the association with PMN risk could be influenced by disease severity in the selected patient groups as well as other clinical and biological features.

Besides, the combined analysis revealed that the HLA-DQA1 rs2187668*A allele conferred 3.11-fold higher PMN risk. However, the between-studies heterogeneity was important, and the true effect in 95% of comparable populations could vary between 1.5- and 6.29-fold higher PMN risk. Subgroup analysis revealed the PMN risk conferred by the A allele was significantly higher in Caucasians ($\approx$4) comparatively to Asians (2.537). In addition, meta-regression showed significant correlations of the effect size with albumin, 24-hours proteinuria, serum creatinine, and eGFR levels. Thus, the risk of PMN conferred by the rs2187668*A allele was greater in patients with a more severe disease.

In the current study, we sought to summarize the PLA2R-rs4664308/HLA-DQA1-rs2187668 interaction influence on PMN risk. Unfortunately, only 3 studies[15,25,27] reported genotype combinations in both patients and controls. The high-risk combination, rs4664308*A/A genotype associated with rs2187668*G/A or A/A, conferred 6.54-fold higher PMN risk when compared to all other combinations. Moreover, and when compared to the low-risk combination, the high-risk combination was associated with a 15.1-fold increase in PMN risk. Nevertheless, between-studies heterogeneity was significant. Hence, more studies are needed to better estimate the PLA2R/HLA-DQA1-interaction impact on susceptibility to PMN, and to more accurately investigate the sources of heterogeneity.

In summary, the present meta-analysis revealed that PLA2R and HLA-DQA1 SNPS could play a significant role in susceptibility to PMN. However, it is noteworthy to admit the substantial between studies heterogeneity for some studied SNPs. To the best of our knowledge, this study was the first to perform several meta-regressions in order to explore the aforementioned heterogeneity. Meta-regression provided the study with some interesting findings such as significant correlations with disease severity. However, there are some limitations that need to be acknowledged. Firstly, like other autoimmune disease, PMN risk depends on several factors such as environmental, infectious, psychological, and genetic factors, and as the present study analyses derived from pooling PLA2R and HLA-DQA1 SNPs aggregate findings without any access to raw data, there was a lack of further adjustment for baseline characteristics. Secondly, the majority of included studies were performed in Caucasians and Asians. Hence, the present meta-analysis results cannot be generalized to sub-Saharan-African and South-American populations. Thirdly, some of investigated SNPs included only a few numbers of studies and consequently require replication in independent large cohorts. Fourthly, only 3 study investigated the PLA2R-rs4664308/HLA-DQA1-rs2187668 interaction influence on PMN risk

which prevented us from accurately analyzing the between-studies heterogeneity. Moreover, other gene-gene interactions could influence susceptibility to PMN which need further investigation in future studies.

Conclusion

This study demonstrated that several PLA2R and HLA-DQA1 SNPs as well as the PLA2R-rs4664308/HLA-DQA1-rs2187668 interaction could influence the susceptibility to PMN. However, more studies in independent cohorts are needed, mainly in Sub-Saharan African and South-American populations to further validate the results of the present meta-analysis.

Abbreviations

Ab: antibody
eGFR: estimated glomerular filtration rate
HIV: human immunodeficiency virus
HLA: human leukocyte Antigen
MAF: minor allele frequency
NOS: Newcastle-Ottawa Scale
PI: prediction interval
PLA2R: phospholipase A2 receptor
PMN: primary membranous nephropathy
SMN: secondary membranous nephropathy

Declarations

Ethics Approval and Consent to Participate

Not applicable.

Consent for Publication

N/A.

Author Contributions

TD: conceptualization, investigation, writing—original draft, methodology, validation, visualization, writing—review & editing, software, formal analysis, data curation, supervision. AR: conceptualization, investigation, funding acquisition, validation, visualization, supervision. TBA: conceptualization, funding acquisition, visualization, validation, project administration, resources. YG: conceptualization, funding acquisition, validation, visualization, project administration, resources. IS: conceptualization, funding acquisition, validation, visualization, formal analysis, project administration, resources, investigation, methodology, data curation, supervision.

Availability of Data and Materials

The authors confirm that the data supporting the findings of this study are available within the article [and/or] its Supplemental Materials.

Supplemental Material

Supplemental material for this article is available online.

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