Molecular Vision 2014; 20:1434-1442 <http://www.molvis.org/molvis/v20/1434>
Received 21 December 2013 | Accepted 15 October 2014 | Published 17 October 2014

Relationship between *SERPING1* rs2511989 polymorphism and age-related macular degeneration risk: A meta-analysis

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Purpose: We conducted a meta-analysis aiming to evaluate the relationship between a common polymorphism (rs2511989 G>A) in the *SERPING1* gene and the risk of age-related macular degeneration (AMD).
Methods: The PubMed, CISCOM, CINAHL, Web of Science, Google Scholar, EBSCO, Cochrane Library, and CBM databases were searched for relevant articles published before November 1, 2013, without any language restrictions. A meta-analysis was conducted using STATA 12.0 software. We calculated a crude odds ratio (OR) with a 95% confidence interval (95% CI) to evaluate the relationships under five genetic models.
Results: Seven case-control studies with a total of 7,159 patients with AMD and 5,797 healthy subjects met the inclusion criteria. The results of our meta-analysis showed that the *SERPING1* rs2511989 polymorphism might be correlated with an increased risk of AMD (G allele versus A allele: OR = 1.09, 95% CI = 1.03–1.15, p = 0.020; GG + GA versus AA: OR = 1.14, 95% CI = 1.03–1.26, p = 0.014; GG versus GA+AA: OR = 1.10, 95% CI = 1.02–1.19, p = 0.012; GG versus AA: OR = 1.20, 95% CI = 1.07–1.34, p = 0.002; respectively). Results of subgroup analysis by ethnicity revealed positive correlations between the *SERPING1* rs2511989 polymorphism and risk of AMD among Caucasians under five genetic models (all p<0.05), but not among Asians (all p>0.05).
Conclusions: The current meta-analysis shows that the *SERPING1* rs2511989 polymorphism may have a positive effect on the risk of AMD, especially among Caucasians.

Age-related macular degeneration (AMD) refers to the medical condition that usually occurs in older people with loss of vision in the center of the vision field resulting from damage to the retina [1]. AMD is the leading cause of blindness worldwide, and with aging populations in many countries, more than 20% might have the disorder [2]. Generally, AMD is a complex disease influenced by several risk factors, such as cigarette smoking, nutritional factors, and cardiovascular diseases [2]. Furthermore, genetic factors have also been demonstrated to play significant roles in the development of AMD [2,3]. Previous evidence has suggested that complement activation may contribute to the pathogenesis of AMD [4,5]. Currently, several candidate genes that can encode proteins associated with complement activation have been implicated in the risk of AMD [4,6].

Serpin peptidase inhibitor, clade G (C1 inhibitor), member 1, encoded by the *SERPING1* gene (OMIM 606860), is a protease inhibitor in the serine proteinase inhibitor super-family, which mainly functions as an inhibitor of the complement system to prevent spontaneous activation [7]. The complement component 1 (C1) inhibitor, expressed in the neural retina, retina pigment epithelium, and choroidal tissues, has a crucial role in inhibiting C1 and might implicate the classic pathway of complement activation in AMD [6,8]. The human *SERPING1* gene, composed of eight exons and seven introns, is located on chromosome 11q11–q13.1 [9]. Although the specific etiology of AMD is still not well elaborated, it has been hypothesized that single nucleotide polymorphisms in the *SERPING1* gene may have an impact on production and function, leading to complement activation, and thus conducive to an individual's susceptibility to AMD [10]. Additionally, numerous studies have investigated the potential associations between common polymorphisms in the *SERPING1* rs2511989 polymorphism and risk of AMD [6,11]. The rs2511989 polymorphism as the haplotype-tagging single nucleotide polymorphism (SNP) has been reported to be positively associated with the risk of AMD in Caucasians [5]. Recent studies have focused on the relationship between a common polymorphism (rs2511989 G>A) in the *SERPING1* gene and the risk of AMD, but the results were inconclusive [5,12]. The purpose of the current meta-analysis was to evaluate whether the *SERPING1* rs2511989 polymorphism contributes to susceptibility to AMD.

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METHODS

Search strategy: The PubMed, CISCOM, CINAHL, Web of Science, Google Scholar, EBSCO, Cochrane Library, and CBM databases were searched for relevant articles published before November 1, 2013, without any language restrictions. The following keywords and MeSH terms were used: ["SNP" or "mutation" or "genetic polymorphism" or "variation" or "polymorphism" or "single nucleotide polymorphism" or "variant"] and ["age-related macular degenerations" or "AMD" or "age-related maculopathy" or "senile macular degeneration"] and ["pigment epithelium-derived factor" or "PEDF" or "SERPIN-F1" or "serpin peptidase inhibitor, clade G, member 1" or "C1 inhibitor" or "SERPING1"]. We also performed a manual search of the reference lists from the relevant articles to find other potential articles.

Selection criteria: The studies must meet all four of the following criteria: (1) The study design must be a case-control study that focused on the relationship between the *SERPING1* rs2511989 polymorphism and susceptibility to AMD; (2) all patients met diagnostic criteria for AMD; (3) the genotype frequencies of the healthy controls should follow the Hardy–Weinberg equilibrium (HWE); and (4) the study must provide sufficient information about the genotype frequencies. If the study did not meet the inclusion criteria, it was excluded. The most recent or the largest sample size publication was included when the authors published several studies using the same subjects.

Data extraction: Relevant data were systematically extracted from all included studies by two observers using a standardized form. The researchers collected the following data: language of publication, publication year of article, the first author's surname, geographic location, design of study, sample size, the source of the subjects, genotype frequencies, source of samples, genotyping method, evidence of HWE, etc.

Quality assessment: Methodological quality was evaluated separately by two observers using the Newcastle-Ottawa Scale (NOS) criteria [13]. The NOS criteria included three aspects: (1) subject selection: 0–4, (2) comparability of subjects: 0–2, and (3) clinical outcome: 0–3. NOS scores ranged from 0 to 9 with a score ≥7 indicating good quality.

Statistical analysis: STATA version 12.0 (Stata Corp, College Station, TX) software was used for meta-analysis. We calculated crude odds ratio (OR) with a 95% confidence interval (95% CI) to evaluate the relationships under five genetic models. Genotype frequencies of healthy controls were tested for HWE using the χ^2 test. The statistical significance of pooled ORs was assessed with the *Z* test. Cochran's *Q*-statistic and the I^2 test were used to evaluate potential heterogeneity between studies [14]. If the *Q* test showed a $p<0.05$ or the I^2 test exhibited >50%, which indicates significant heterogeneity, the random effects model was conducted, or else the fixed-effects model was used. We also performed subgroup and meta-regression analyses to investigate potential sources of heterogeneity. A sensitivity analysis was conducted to assess the influence of single studies on the overall ORs. Beggar's funnel plots and Egger's linear regression test were used to investigate publication bias [15].

RESULTS

Baseline characteristics of included studies: Initially, the searched keywords identified 36 articles. We reviewed the titles and abstracts of all articles and excluded 14 articles; full texts were also reviewed, and 15 articles were further excluded. Finally, seven case-control studies with a total of 7,159 patients with AMD and 5,797 healthy subjects met our inclusion criteria for qualitative data analysis [4-6,11,12,16,17]. Figure 1 shows the selection process for the eligible articles. The distribution of the number of topic-related works in electronic databases during the last decade is shown in Figure 2. Overall, five studies were conducted among Caucasians and two studies among Asians. The TaqMan assay method was conducted in six studies, and only one study used MassARRAY. None of the studies deviated from the HWE (all $p>0.05$). The NOS scores of all included studies were ≥5. We summarize the study characteristics and methodological quality in Table 1.

Quantitative data synthesis: Meta-analysis findings for the relationship between the *SERPING1* rs2511989 polymorphism and the risk of AMD are shown in Table 2. The random-effects model was conducted because obvious heterogeneity existed between studies. Our meta-analysis indicated that the *SERPING1* rs2511989 polymorphism might be correlated with an increased risk of AMD under four genetic models (G allele versus A allele: OR = 1.09, 95% CI = 1.03–1.15, p = 0.020; GG + GA versus AA: OR = 1.14, 95% CI = 1.03–1.26, p = 0.014; GG versus GA+AA: OR = 1.10, 95% CI = 1.02–1.19, p = 0.012; GG versus AA: OR = 1.20, 95% CI = 1.07–1.34, p = 0.002; respectively).

Subgroup analyses were conducted to investigate the impact of potential factors on an individual's risk of AMD. Results of subgroup analysis by ethnicity revealed positive correlations between the *SERPING1* rs2511989 polymorphism and AMD risk among Caucasians under five genetic models (all $p<0.05$), but not among Asians (all $p>0.05$; Figure 3). In the stratified analysis based on country, the results suggested that the *SERPING1* rs2511989 polymorphism might increase

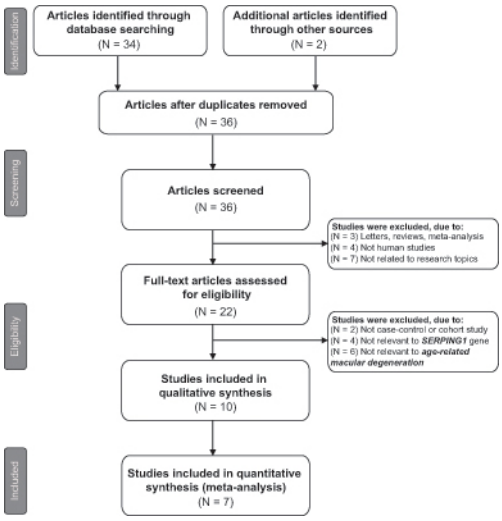

Figure 1. Flowchart of the literature search and study selection. Seven case-control studies were included in the meta-analysis.

the risk of AMD in the populations of the United Kingdom and the United States, but not in the populations of Japan and China (all p>0.05; Figure 4). We also performed subgroup analyses with the source of controls and sample size. These results also indicated significant associations between the *SERPING1* rs2511989 polymorphism and an increased risk of AMD in the population-based and large sample-size subgroups; however, no positive correlations were observed in the hospital-based and small sample-size subgroups (as shown in Table 2).

Meta-regression analysis confirmed that none of the factors may be the dominant sources of heterogeneity (as shown in Table 3). The results of the sensitivity analysis indicated that the overall pooled ORs were not affected by a single study (Figure 5). No evidence for asymmetry was observed in the Beggar funnel plots (Figure 6). Egger's test also failed to reveal any evidence of publication bias (all p>0.05).

DISCUSSION

The present meta-analysis indicated that the *SERPING1* rs2511989 polymorphism was associated with an increased risk of AMD, suggesting that this polymorphism may be a causative factor for the pathogenesis of AMD. The results could be explained by the fact that the C1 inhibitor, encoded by the *SERPING1* gene, expressed in the neural retina, retina pigment epithelium, and choroidal tissues, is an important complement regulator of the classical complement pathway by inhibiting proteolytic activity [8,18]. The *SERPING1* rs2511989 polymorphism may give rise to a dysfunctional protein or influence the expression levels of SERPING1, leading to hereditary angioedema, which may increase the risk of developing AMD [6,10,19].

Considering the possibility of obvious heterogeneity, which may have a negative influence on the results of relevant studies, we carefully performed stratified analysis based on ethnicity, genotyping method, and sample size. The results of subgroup analysis by ethnicity revealed positive correlations between the *SERPING1* rs2511989 polymorphism and the pathogenesis of AMD among Caucasians, but not among Asians. The *SERPING1* rs2511989 polymorphism may result in dysfunctional protein and subnormal concentrations of the SERPING1 protein that have been shown to affect the complement system, a powerful component of innate immunity recognizing and facilitating the elimination of pathogens and unwanted host material, thus contributing to the pathogenesis of AMD [6]. Subgroup analyses based on the source of the controls and sample size indicated significant associations between the *SERPING1* rs2511989 polymorphism and an increased risk of AMD in population-based and large sample-size subgroups. Our results were in accordance with a previous study that demonstrated genetic variation in

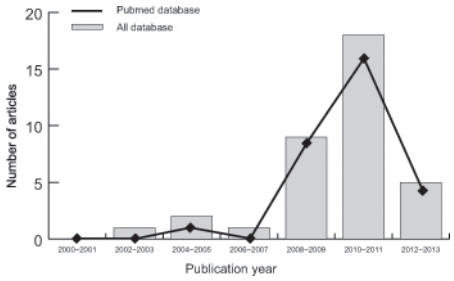

Figure 2. Distribution of the number of topic-related works in electronic databases during the last decade.

Table 1. Baseline characteristics and methodological quality of all included studies.

First author	Year	Country	Ethnicity	Source	Sample size		Gender (M/F)		Age (years)		Genotyping method	HWE test (p value)	NOS score
					Case	Control	Case	Control	Case	Control			
Nakata et al. [5]	2011	Japan	Asian	HB	401	336	287/114	142/194	77.4±8.4	74.2±8.4	TaqMan assay	0.59	8
				PB	401	1194	287/114	493/701	77.4±8.4	50.3±15.9			
Carter et al. [4]	2011	UK	Caucasian	PB	94	95	26/68	32/63	51~94	55~89	TaqMan assay	0.23	7
Lu et al. [11]	2010	China	Asian	PB	272	285	126/146	132/153	68.2±9.8	68.4±7.2	TaqMan assay	0.49	8
Lee et al. [16]	2010	USA	Caucasian	PB	556	256	177/379	116/140	79	70	MassArray	0.29	8
Park et al. [17]	2009	USA	Caucasian	HB	476	310	169/307	141/169	76.9±9.6	69.5±8.2	TaqMan assay	0.44	8
Allikmets et al. [15]	2009	UK	Caucasian	PB	4881	2842	-	-	-	-	TaqMan assay	0.55	7
Ennis et al. [6]	2008	UK	Caucasian	PB	479	479	181/298	232/247	77.9±8.8	70.6±9.4	TaqMan assay	0.85	8

M=male; F=female; SNP=single nucleotide polymorphism; HWE=Hardy–Weinberg equilibrium; NOS=Newcastle-Ottawa Scale; HB=hospital-based; PB=population-based.

Table 2. Meta-analysis of the association between *SERPING1* gene and age-related macular degeneration.

Subgroup analysis	G allele versus A (Allele model)			GG + GA versus AA (Dominant model)			GG versus GA + AA (Recessive model)			GG versus AA (Homozygous model)			GG versus GA (Heterozygous model)		
	OR	95%CI	*P*	OR	95%CI	*P*	OR	95%CI	*P*	OR	95%CI	*P*	OR	95%CI	*P*
Overall	1.09	1.03–1.15	0.002	1.14	1.03–1.26	0.014	1.10	1.02–1.19	0.012	1.20	1.07–1.34	0.002	1.08	1.00–1.17	0.063
Ethnicity															
Caucasians	1.09	1.03–1.15	0.001	1.14	1.02–1.26	0.017	1.12	1.03–1.21	0.008	1.19	1.06–1.34	0.003	1.09	1.00–1.19	0.046
Asians	1.03	0.85–1.24	0.755	1.25	0.61–2.53	0.541	1.02	0.82–1.25	0.874	1.25	0.61–2.54	0.543	1.00	0.81–1.24	0.983
Country															
USA	1.21	1.05–1.41	0.010	1.19	0.90–1.57	0.223	1.37	1.10–1.70	0.005	1.42	1.04–1.93	0.028	1.35	1.07–1.71	0.010
UK	1.07	1.01–1.14	0.021	1.13	1.01–1.26	0.037	1.08	0.99–1.18	0.086	1.16	1.03–1.32	0.018	1.05	0.96–1.16	0.267
China	0.95	0.66–1.36	0.763	0.55	0.13–2.33	0.417	0.98	0.66–1.46	0.922	0.55	0.13–2.34	0.421	1.02	0.68–1.53	0.932
Japan	1.06	0.85–1.33	0.584	1.63	0.68–3.90	0.269	1.03	0.81–1.32	0.804	1.63	0.68–3.90	0.271	1.00	0.77–1.28	0.978
Source of control															
Population-based	1.09	1.03–1.15	0.004	1.15	1.03–1.28	0.012	1.10	1.01–1.19	0.022	1.20	1.06–1.35	0.003	1.07	0.98–1.17	0.108
Hospital-based	1.08	0.92–1.25	0.345	1.04	0.74–1.47	0.822	1.11	0.92–1.34	0.285	1.19	0.81–1.74	0.373	1.10	0.90–1.35	0.335
Sample size															
Small (n<300)	1.06	0.93–1.21	0.356	0.96	0.71–1.31	0.818	1.11	0.94–1.31	0.201	1.12	0.80–1.57	0.503	1.12	0.95–1.34	0.182
Large (n≥300)	1.09	1.03–1.16	0.003	1.16	1.04–1.30	0.007	1.10	1.01–1.20	0.029	1.21	1.07–1.36	0.002	1.07	0.97–1.17	0.162

OR=odds ratios; 95%CI=95% confidence interval.

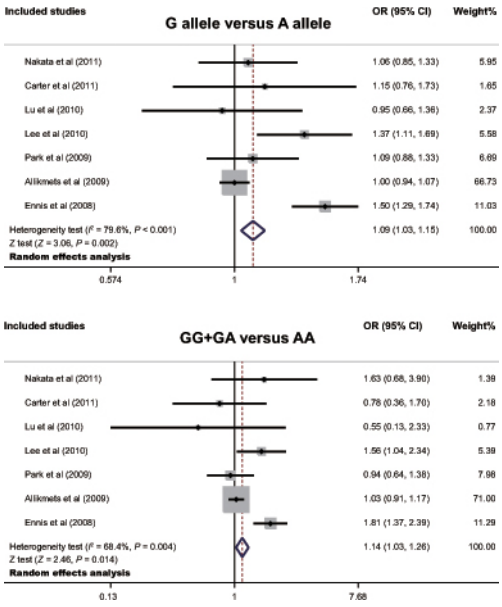

Figure 3. Forest plots of the relationship between the *SERPING1* rs2511989 polymorphism and the risk of age-related macular degeneration (AMD) under the allele and dominant models.

SERPING1 might cause hereditary angioedema implications and inhibit the classic pathway of complement activation in AMD [16]. In short, our findings were consistent with previous studies that *SERPING1* genetic variations may be implicated in the pathogenesis of AMD, suggesting that this polymorphism may be a helpful biomarker for early diagnosis of AMD.

The current meta-analysis also had several limitations that should be acknowledged. First, our results lacked sufficient statistical power to assess the correlations between the *SERPING1* rs2511989 polymorphism and the occurrence of AMD. Second, meta-analysis is a retrospective study that may lead to subject selection bias, and thus affect the reliability of our results. Third, our meta-analysis failed to obtain original data from the studies included, which may limit further evaluation of the potential role of the*SERPING1* rs2511989 polymorphism in the development of AMD. Although our study has many limitations, this is the first meta-analysis focusing on the relationships between the *SERPING1* rs2511989 polymorphism and the pathogenesis of AMD. Furthermore, we performed a highly sensitive literature search strategy for electronic databases. A manual search of the reference lists from the relevant articles was also conducted to find other potential articles. The selection process for eligible articles was based on strict inclusion and exclusion criteria. Importantly, rigorous statistical analysis of SNP data provided a basis for pooling information from individual studies.

In conclusion, our findings provide empirical evidence that the *SERPING1* rs2511989 polymorphism may conduce susceptibility to AMD. Thus, the *SERPING1* rs2511989 polymorphism could be used as a helpful biomarker for early diagnosis of AMD. However, due to study limitations, more

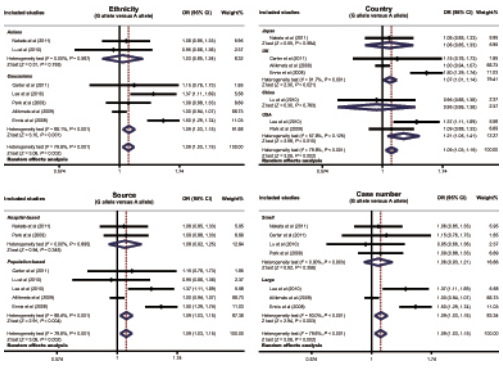

Figure 4. Subgroup analyses of the relationship between the *SERPING1* rs2511989 polymorphism and the risk of age-related macular degeneration under the allele model.

Table 3. Univariate and multivariate meta-regression analyses of potential source of heterogeneity.

Heterogeneity factors	Coefficient	SEM	*Z*	*P*	95%CI	
					LL	UL
Publication year						
Univariate	−0.063	0.070	−0.89	0.371	−0.200	0.075
Multivariate	−0.043	0.198	−0.22	0.828	−0.432	0.346
Ethnicity						
Univariate	−0.169	0.171	−0.99	0.323	−0.505	0.166
Multivariate	−0.427	0.948	−0.45	0.653	−2.285	1.431
Country						
Univariate	−0.052	0.070	−0.74	0.461	−0.189	0.086
Multivariate	0.180	0.482	0.37	0.708	−0.764	1.124
Source of controls						
Univariate	−0.104	0.162	−0.64	0.521	−0.421	0.214
Multivariate	−0.167	0.639	−0.26	0.794	−1.421	1.086
Sample size						
Univariate	0.163	0.143	1.14	0.255	−0.118	0.444
Multivariate	0.042	0.491	0.09	0.931	−0.920	1.005

SE=standard error; 95%CI=95% confidence interval; UL=upper limit; LL=lower limit.

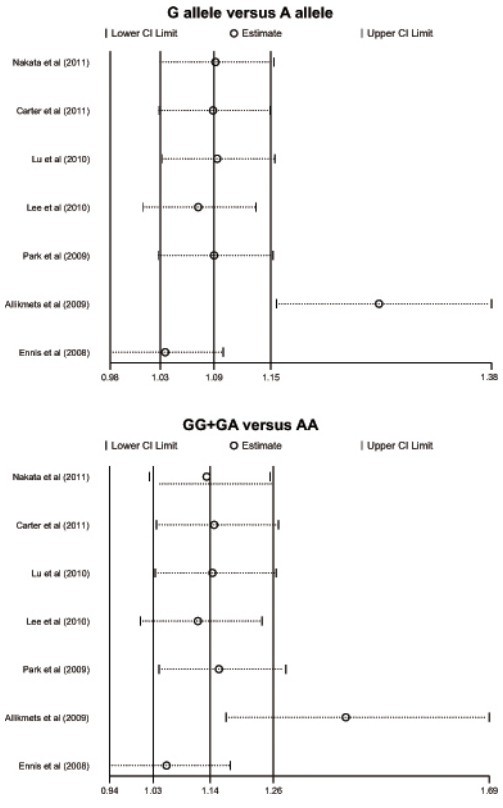

Figure 5. Sensitivity analysis of the summary odds ratio coefficients on the relationship between the *SERPING1* rs2511989 polymorphism and the risk of age-related macular degeneration under the allele and dominant models.

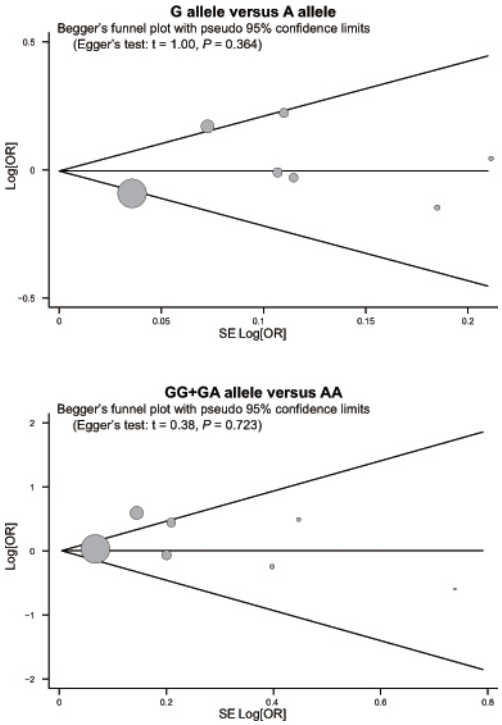

Figure 6. Beggar's funnel plot of publication biases for the relationship between the *SERPING1* rs2511989 polymorphism and the risk of age-related macular degeneration under the allele and dominant models.

studies with larger sample sizes are needed to provide a more representative statistical analysis.

ACKNOWLEDGMENTS

We thank the reviewers for their helpful comments.

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Articles are provided courtesy of Emory University and the Zhongshan Ophthalmic Center, Sun Yat-sen University, P.R. China. The print version of this article was created on 17 October 2014. This reflects all typographical corrections and errata to the article through that date. Details of any changes may be found in the online version of the article.