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# Geneticpolymorphism of SERPING1 rs2511989 and age-related macular degeneration: a Meta-analysis

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## SERPING1 基因 rs2511989 多态性与年龄相关性黄斑变性相关性研究的 Meta 分析

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**摘要**

**目的**:探讨经典通路 SERPING1 基因 rs2511989 基因多态性与年龄相关性黄斑变性(age-related macular degeneration, AMD)的相关性。

**方法**:检索中国学术期刊网(CNKI)、PubMed、Cochrane、Embase 以及 Web of Science 数据库,使用随机效应模型,使用 *OR* 值及其 95% 可信区间评价 SERPING1 rs2511989 基因多态性与 AMD 易感性的关联程度,同时对入选文献异质性,敏感性以及发表偏倚等进行评估。

**结果**:共纳入 15 项病例对照研究,收集 8657 例 AMD 患者,对照组 5393 例。各个遗传模型中均未发现 SERPING1 基因多态性与 AMD 发病具有相关性。(显性模型:*OR*=0.960,95% *CI*:0.918~1.003,*P*=0.009;隐性模型:*OR*=0.898,95% *CI*:0.791~1.019,*P*=0.035;共显性纯合模型:*OR*=0.881,95% *CI*:0.770~1.008,*P*=0.003;共显性杂合模型:*OR*=0.962,95% *CI*:0.917~1.010,*P*=0.050)。但进一步研究发现 SERPING1 基因多态性与新生血管型 AMD 显著相关。(显性模型:*OR*=0.691,95% *CI*:0.547~0.872;共显性纯合模型:*OR*=0.661,95% *CI*:0.450~0.971;共显性杂合模型:*OR*=0.754,95% *CI*:0.589~0.964)。亚组分析未发现种族与国家对 rs2511989 基因多态性与 AMD 有影响。

**结论**:通常情况下 SERPING1 rs2511989 基因多态性与 AMD 无相关性,但在新生血管类型 AMD 可能与其存在相关性。期待更多研究来证实该假说。

**关键词**:年龄相关性黄斑变性;SERPING1;基因多态性;Meta 分析

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## Abstract

• AIM: To explore the association between the polymorphism rs2511989 in the classical pathway geneSERPING1 (C1 inhibitor) and age-related macular degeneration (AMD).

• METHODS: A random-effect Meta-analysis was performed. An electronic search was done in CNKI, PubMed, the Cochrane Collaboration's Database, Embase, and the ISI Web of Knowledge. Odds ratios (OR) and their 95% confidence interval (CI) were calculated to assess the strength of association between SERPING1 rs2511989 polymorphism and AMD susceptibility. Heterogeneity, sensitivity analysis and publication bias were also tested.

• RESULTS: A total of 15 case-control studies with 8657 cases and 5393 controls were finally included in this Meta-

analysis. There was no significant association between SERPING1 and AMD in all genetic models. (Dominant model: OR = 0.960, 95% CI: 0.918-1.003, $P$ = 0.009; recessive model: OR = 0.898, 95% CI: 0.791-1.019, $P$ = 0.035; homozygote model: OR = 0.881, 95% CI: 0.770-1.008, $P$=0.003; heterozygote model: OR=0.962, 95% CI: 0.917-1.010, $P$ = 0.050). However, the associations between SERPING1 and neovascular AMD were significant in three models (dominant model: OR = 0.691, 95% CI: 0.547-0.872; homozygote model: OR = 0.661, 95% CI: 0.450-0.971; heterozygote model: OR = 0.754, 95% CI: 0.589-0.964). Subgroup analysis by ethnicity and country did not find significant association between rs2511989 polymorphism and AMD susceptibility.

• CONCLUSION: SERPING1 rs2511989 does not associates with AMD generally but may associate with neovascular AMD. More studies are required to verify the hypothesis.

• KEYWORDS: age-related macular degeneration; SERPING1; polymorphism; Meta-analysis
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## INTRODUCTION

Age-related macular degeneration (AMD) is the leading cause of legal blindness in aged population in the industrialized world, and also the third most frequent cause of visual impairment globally[1]. The genetic factors, as a prevailing view, might have an important role on AMD etiology[2]. One of the most prominent discoveries about the cause of AMD is the identification of the complement factor H (CFH) and LOC387715 genes[3]. In addition, several genes encoding proteins involved in the innate immune response were considered established risk factors, such as complement factor B (CFB) and complement component 2 (C2), C3, and apolipoprotein E (APOE)[4-6].

Recently, the serpin peptidase inhibitor, clade G, member 1 (SERPING1) gene which encodes complement component 1 inhibitor (C1 inhibitor), was reported to have a protective effect against development of AMD[7]. SERPING1 gene contains eight exons, and is located on chromosome 11$q$12.1[8]. As an important regulator of the classical complement pathway, SERPING1 is a plausible candidate gene for AMD. Recent studies have evaluated the association of SERPING1 polymorphisms with AMD susceptibility[9-11].

Ennis[8] first explored the association between SERPING1 and AMD susceptibility. They found the mRNA expression of SERPING1 in the retina-RPE-choroid complex, and reported a protective effect on AMD for the minor allele of a SNP (rs2511989) within intron 6 of the SERPING1 gene. Later, Lee *et al*[12] reported that SERPING1 SNPs rs2511989 conferring a protective effect significantly. However, some recent studies, such as Park *et al*[13] and Lu *et al*[14], have drawn conflicting conclusions that they were unable to replicate the reported association between SERPING1 and AMD. Thus the best way to solve this discrepancy is to perform a Meta-analysis. But no meta-analyses on this topic, to our knowledge, have been published. Therefore, we systematically reviewed all available studies between SERPING1 and AMD and pooled their results to ascertain the possible association between SERPING1 gene polymorphism and AMD susceptibility.

## METERIALS AND METHODS

**Search Strategy** We searched CNKI, PubMed, the Cochrane Collaboration's Database, Embase, and the ISI Web of Knowledge (up to July 2014). The following terms were used: ("age-related macular degeneration" OR "AMD") AND ("SERPING1" OR "serpin peptidase inhibitor, clade G, member 1") AND ("polymorphism" OR "mutation" OR "variation").

**Inclusion and Exclusion Criteria** Studies were included when they met all of the following three criteria: 1) the association of the SERPING1 polymorphisms with AMD should be clearly explored; 2) only the unrelated case-control studies were considered; 3) the genotype frequency in both AMD cases and controls should also be offered. Accordingly, the exclusion criteria were: 1) review, editorial, or comment; 2) laboratory molecular or animal studies; and 3) The genotype distribution in control group does not meet Hardy-Weinberg equilibrium (HWE). All eligible and relevant studies, as well as their references were manually retrieved to identify additional relevant studies. For studies based on the same data, the more detailed one was included. When a study reported the results on different subpopulations, we considered them as independent studies.

**Quality Score Assessment and Data Extraction** The quality of genetic association studies were assessed by two authors (Qin Y and Pan CS) independently using the Newcastle-Ottawa Scale[15]. The scores ranged from 0 (worst) to 10 (best). The following information was extracted: the first author's name, year of publication, countries of participants, ethnicity, number of cases and controls, distribution of genotypes, genotype frequencies in both case and control groups, genotype detecting method, source of samples and AMD diagnostic criteria. Data were extracted using standard guidelines published by the Cochrane Collaboration[16]. Any disagreement was adjudicated by another experienced author (Zhao JY). When genotype frequency was not reported, we contacted the corresponding author by e-mail to obtain the original data.

**Statistical Analysis** ORs and 95% CIs were used to estimate the strength of the associations between SERPING1 rs2511989 polymorphisms and AMD. Heterogeneity between eligible studies was tested using $\chi^2$ tests (A $P$<0.1 was considered to be statistically significant) and $I^2$ tests ($I^2$>50%: significant heterogeneity; $I^2$<25%: insignificant heterogeneity), fixed-effect or random-effect models were used appropriately according to the criteria[17]. Hardy-Weinberg equilibrium (HWE) of genotype was tested by the $\chi^2$ test, and $P$<0.05

**Table 1 Main characteristics of eligible studies included in the Meta-analysis**

| First author(a) | *n* | Country | Ethnicity | Sample Size | | GG (genotype) | | GA (genotype) | | AA (genotype) | | Genotyping methods |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Cases | Controls | Case | Controls | Case | Controls | Case | Controls | |
| Ennis 2008 | 953 | UK | C | 476 | 477 | 191 | 132 | 215 | 236 | 70 | 109 | Illumina |
| Ennis 2008 | 500 | America | C | 248 | 252 | 100 | 79 | 122 | 124 | 26 | 49 | |
| Park 2009 | 780 | America | C | 470 | 310 | 179 | 103 | 211 | 157 | 80 | 50 | |
| Park 2009 | 1516 | America | C | 1221 | 295 | 436 | 115 | 563 | 127 | 222 | 53 | |
| Allikmets 2009 | 1367 | Columbia | C | 1004 | 363 | 449 | 151 | 431 | 171 | 124 | 41 | |
| Allikmets 2009 | 483 | America | C | 368 | 115 | 116 | 37 | 178 | 59 | 74 | 19 | |
| Allikmets 2009 | 595 | Holland | C | 338 | 257 | 107 | 84 | 184 | 131 | 47 | 42 | |
| Allikmets 2009 | 1859 | Holland | C | 1017 | 842 | 328 | 285 | 518 | 407 | 171 | 150 | TaqMan assay |
| Allikmets 2009 | 1068 | Australia | C | 741 | 327 | 251 | 105 | 367 | 157 | 123 | 65 | |
| Allikmets 2009 | 1723 | Germany | C | 998 | 725 | 377 | 284 | 485 | 341 | 136 | 100 | |
| Lee 2010 | 812 | America | C | 556 | 256 | 213 | 74 | 273 | 135 | 70 | 47 | |
| Lu 2010 | 475 | China | A | 194 | 281 | 147 | 215 | 42 | 63 | 5 | 3 | |
| Nakata 2011 | 735 | Japan | A | 401 | 334 | 293 | 248 | 102 | 76 | 6 | 10 | |
| James 2011 | 189 | America | C | 94 | 95 | 38 | 29 | 39 | 52 | 17 | 14 | |
| Tian 2012 | 995 | China | A | 531 | 464 | 422 | 371 | 96 | 86 | 13 | 7 | |

C: Caucasian; A: Asian. All study types are case control.

was considered as significantly disequilibrium[18]. Sensitivity analyses were performed by repeating the statistical analysis after omitting studies to reflect the influence of the individual study on the pooled OR. Publication bias was assessed by Begg's funnel plot[19] and Egger's linear regression tests[20]. A $P<0.05$ was considered significant. Visual inspection of asymmetry in funnel plot was conducted to estimate the potential publication bias[21]. Subgroup analyses were performed by ethnicity and country. For the studies concerning neovascular AMD, an additional analysis was performed. All statistical analysis was conducted using STATA version 11.0.

## RESULTS

**Studies and Data Included in the Meta-analysis** Twenty articles relevant to the role of SERPING1 rs2511989 polymorphisms on AMD susceptibility were identified from electronic search. During data extraction, two were excluded after abstract review[22,23], five were laboratory molecular studies[7,24-27], four did not provide genotyping frequency data[28-31], one cohort study was excluded[11] and one case-control study was excluded for being not consistent with HWE. No eligible studies were found by manual search of references. If one study report cases from different countries, we divided them into several sub-studies[8,13,32]. Finally we selected 8 relevant papers, including 15 independent case-control studies that involved 8657 AMD cases and 5393 matched controls in our Meta-analysis[8-10,12-14,32,33] (Figure 1). Of all the 8 eligible papers, 5 were conducted in Caucasians and 3 were in Asians for SERPING1 rs2511989 polymorphism; 2 case-control studies were population-based and the other 13 were hospital-based. Meanwhile, 3 studies were included with neovascular AMD patients[10,12,14]. The basic information of the 15 case-control studies is summarized in Table 1.

**Analysis of SERPING1 rs2511989 Gene Polymorphism and Age-related Macular Degeneration Risk** When all

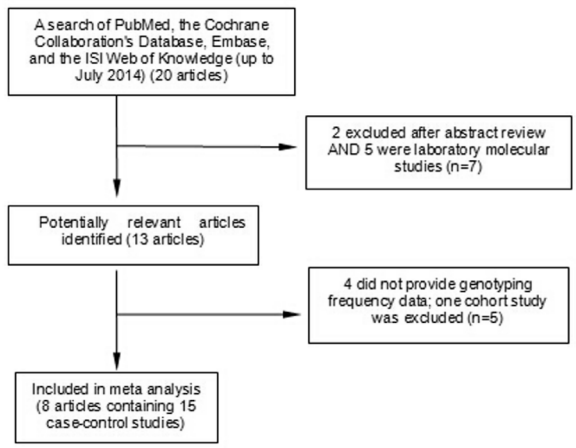


**Figure 1 The flow diagram of selection of studies and specific reasons for exclusion from the Meta-analysis.**

the 15 studies were pooled into the Meta-analysis, statistical significant increased AMD risk was not observed in all genetic models: in the dominant model (GA + AA *vs* GG; OR = 0.960, 95% CI: 0.918-1.003, $P=0.009$; $I^2=52.8\%$, Figure 2); in the recessive model (AA *vs* GA+GG; OR = 0.898, 95% CI: 0.791-1.019, $P=0.035$; $I^2=43.9\%$); in the homozygote model (AA *vs* GG; OR=0.881, 95% CI: 0.770-1.008, $P=0.003$; $I^2=58.0\%$); in the heterozygote model (GA *vs* GG OR=0.962, 95% CI: 0.917-1.010, $P=0.050$; $I^2=40.8\%$). Subgroup analysis by ethnicity did not detect any significant association between SERPING1 rs2511989 and AMD risk in either Asians or Caucasians. Subgroup analysis by country found that there was not significant association between SERPING1 rs2511989 and AMD risk except in UK. The result of analysis with neovascular AMD showed SERPING1 rs2511989 was associated with neovascular AMD in homozygote, heterozygote and dominant model. The main results of our Meta-analysis about SERPING1 rs2511989 and the heterogeneity test were listed in Table 2.

**Table 2 Main results of the Meta-analysis of SERPING1 rs2511989 polymorphism on AMD risk**

| Analysis | Homozygote | | Heterozygote | | Dominant model | | Recessive model | |
|---|---|---|---|---|---|---|---|---|
| | OR (95% CI) | $P^{het}/I^2$(%) | OR (95% CI) | $P^{het}/I^2$(%) | OR (95% CI) | $P^{het}/I^2$(%) | OR (95% CI) | $P^{het}/I^2$(%) |
| Total | 0.881 (0.770-1.008) | 0.003/58.0 | 0.962 (0.917-1.010) | 0.050/40.8 | 0.960 (0.918-1.003) | 0.009/52.8 | 0.898 (0.791-1.019) | 0.035/43.9 |
| Ethnicity | | | | | | | | |
| Caucasian | 0.815 (0.668-0.995) | 0.002/62.8 | 0.900 (0.792-1.022) | 0.020/51.3 | 0.874 (0.763-1.002) | 0.002/62.0 | 0.869 (0.748-1.009) | 0.040/46.2 |
| Asian | 1.184 (0.477-2.937) | 0.134/50.2 | 1.034 (0.841-1.273) | 0.795/0.0 | 1.045 (0.855-1.276) | 0.991/0.0 | 1.177 (0.463-2.994) | 0.119/53.1 |
| Country | | | | | | | | |
| America | 0.792 (0.557-1.126) | 0.015/64.5 | 0.839 (0.686-1.025) | 0.133/40.8 | 0.825 (0.665-1.023) | 0.056/53.7 | 0.884 (0.661-1.182) | 0.037/57.7 |
| Columbia | 1.017 (0.683-1.515) | - | 0.848 (0.656-1.095) | - | 0.88 (0.691-1.123) | - | 1.107 (0.760-1.611) | - |
| Holland | 0.964 (0.760-1.224) | 0.681/0.00 | 1.105 (0.924-1.321) | 0.989/0.00 | 1.068 (0.902-1.266) | 0.902/0.00 | 0.908 (0.734-1.123) | 0.645/0.00 |
| Australia | 0.792 (0.543-1.154) | - | 0.978 (0.728-1.313) | - | 0.923 (0.700-1.219) | - | 0.802 (0.575-1.120) | - |
| Germany | 1.025 (0.758-1.384) | - | 1.071 (0.871-1.318) | - | 1.061 (0.872-1.291) | - | 0.986 (0.747-1.302) | - |
| UK | 0.444 (0.306-0.645) | - | 0.063 (0.472-0.840) | - | 0.571 (0.435-0.749) | - | 0.582 (0.418-0.811) | - |
| China | 1.836 (0.840-4.012) | 0.648/0.00 | 0.979 (0.754-1.271) | 0.982/0.00 | 1.034 (0.805-1.329) | 0.968/0.00 | 1.843 (0.845-4.022) | 0.645/0.00 |
| Japan | 0.508 (0.182-1.417) | - | 1.136 (0.807-1.599) | - | 1.063 (0.764-1.478) | - | 0.492 (0.177-1.369) | - |
| Neovascular AMD | 0.661 (0.450-0.971) | 0.089/58.7 | 0.754 (0.589-0.964) | 0.336/8.3 | 0.691 (0.547-0.872) | 0.115/53.8 | 0.806 (0.570-1.140) | 0.087/59.1 |

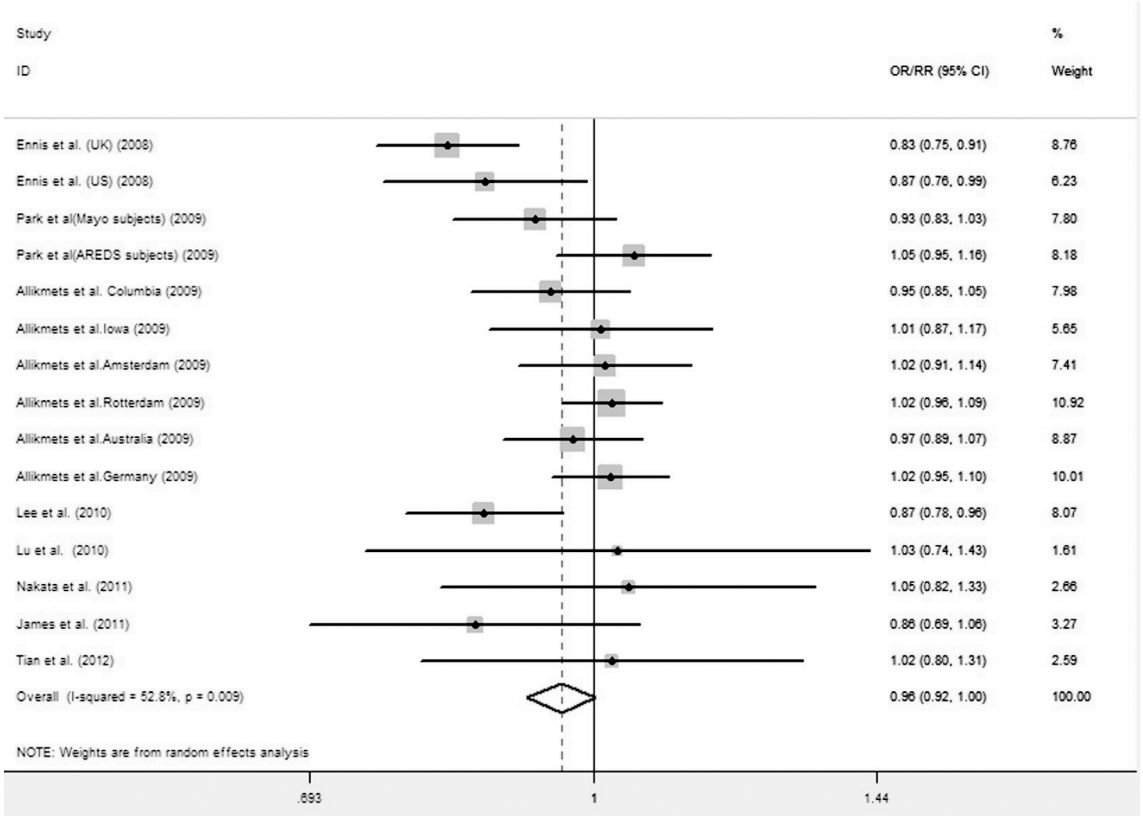


**Figure 2 Meta-analysis for the overall association between the SERPING1 rs2511989 gene polymorphism and AMD (dominant model).**

**Heterogeneity Test** Significant heterogeneity among studies was observed between eligible studies (GA+AA *vs* GG, $P=0.009$; AA *vs* GA+GG, $P=0.035$; AA *vs* GG, $P=0.003$). Therefore, random effects model was applied to synthesize the data. Moreover, subgroup analyses by ethnicity showed that the heterogeneity was still significant in Caucasian populations. However, the heterogeneity almost disappeared when we stratify the results by country. Meanwhile, the heterogeneity no longer existed for the SERPING1 rs2511989 polymorphism when the study used an Illumina genotype method was excluded[8] (dominant model, $P=0.167$; $I^2=26.8\%$, recessive model, $P=0.137$; $I^2=30.0\%$, homozygote model, $P=0.063$; $I^2=39.6\%$, and heterozygote model, $P=0.063$; $I^2=18.9\%$). The findings revealed that the study used an Illumina genotype method might be the main cause of heterogeneity.

**Sensitivity Analysis** The significance of pooled ORs in total population and subgroup analyses was not influenced by omitting any single study, indicating that our results were stable.

**Publication Bias** Begg's funnel plot and Egger's test were performed to detect the publication bias of the studies. There seems no publication bias by examining funnel plots visually (Figure 3). All the *P* values of Egger's tests were more than 0.05 ($P=0.923$ for AA *vs* GG; $P=0.406$ for GA *vs* GG; $P=0.634$ for dominant model GA+AA *vs* GG; and $P=0.629$ for recessive model AA *vs* GA+GG), suggesting that there was no publication bias in this Meta-analysis.

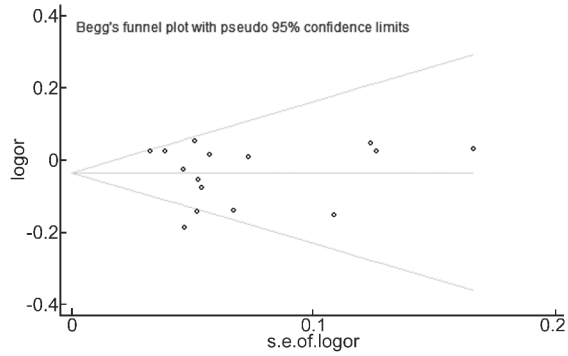


**Figure 3 Funnel plots for publication bias of SERPING1 rs2511989 polymorphism and AMD risk in the overall populations (dominant model). Each point represents a separate study for the indicated association. Funnel plot for dominant model GA + AA *vs* GG in overall analysis ($P=0.634$).**

## DISSCUSSION

This paper summarizes the evidence to date regarding the association between SERPING1 rs2511989 polymorphism and AMD. Although SERPING1 cannot influence the incidence of AMD, it may be a protective factor for neovascular AMD.

SERPING1 is expressed in the neural retina (RPE), andchoroid of human is likely to be involved in regulating the complement system in the eye. However, SERPING1 G allele could not contribute to the protection of AMD in our paper. Subgroup analysis by ethnicity did not detect any significant association between SERPING1 rs2511989 and AMD either in Asians or Caucasians. Nor did subgroup analysis by country. However the heterogeneity reduced significantly during the analysis, the declined heterogeneity could be explained by two reasons: first, stratifications may affect the heterogeneity. Second, as raises by Allikmets *et al*[32], demographic differences in different country can lead to significant heterogeneity. For example, the minor allele frequency (MAF) for the rs2511989 varied between studies from 0.35 to 0.45. Moreover, Allikmets also mentioned that other possible bias, such as non-random sample, may lead to the heterogeneity. Unfortunately, this did not work in our paper. Genotyping errors may be another factor that affects the pooled ORs. Ennis *et al*[8] used Illumina genotype methods in UK subjects while others was genotyped with a TaqMan genotyping assay. The heterogeneity sharply diminished when subjects from the UK[8] were excluded. Subgroup analysis by ethnicity did not detect any significant association either in Asians or Caucasians. Sensitivity analysis indicated that any individual study did not affect the pooled analysis and that our results were reliable. This Meta-analysis is unlikely to suffer publication bias since funnel plots are generally symmetrical and *P* value of Egger's test is larger than 0.05.

Another important finding in our study is the associationbetween SERPING1 rs2511989 and neovascular AMD. It conflicts with previous studies on AMD patients. One reasonable explanation is that pathophysiology may differ between different subtypes of AMD, so it is possible that SERPING1 rs2511989 is only associated with the neovascular form. Note that this is an unverified hypothesis that based on merely three studies with insufficient sample. Therefore the possibility of false association (type I error) cannot be neglected. Above all, SERPING1 rs2511989 may has a modest effect on neovascular AMD, and more studies with larger sample size are needed to verify the hypothesis.

However, although we have put considerable effortsand resources into testing the possible association between SERPING1 rs2511989 polymorphism and AMD risk, there are still some limitations inherited from the published studies. First, our results were based on single-factor estimations without adjustment for other risk factors including alcohol usage, environmental factors and other lifestyles. Second, we only included the studies published in English and Chinese. Some eligible studies published in other languages might be missed in our Meta-analysis. Third, the controls were not uniformly defined. Some studies used a healthy population as the reference group, whereas others selected hospital patients without AMD as the reference group. Therefore misclassification bias is possible because these studies may have included the control groups who have different risks of developing AMD.

In conclusion, this Meta-analysis indicates that SERPING1 rs2511989 polymorphism is not associated with AMD risk but may have an association with neovascular AMD. Further studies with better design and larger sample size are required to validate this conclusion.

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