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Complement factor B polymorphism (rs641153) and susceptibility to age-related macular degeneration: evidence from published studies

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Received: 2013-07-10 Accepted: 2013-09-22

Abstract

• **AIM: To determine whether single nucleotide polymorphism (SNP) rs641153 is associated with the risk of age-related macular degeneration (AMD), we performed a systematic meta-analysis of 15 eligible studies. SNP in the complement factor B (CFB) gene is considered to have significant association with AMD susceptibility, but there is great discrepancy in these results.**

• **METHODS: The eligible studies were identified by searching the databases of PubMed, EMBASE, and Web of Science. Odds ratios (ORs) with 95% confidence intervals (CIs) were used to assess the association. All data were analyzed using Stata software.**

• **RESULTS: The association between rs641153 and AMD risk was statistically significant under the homozygous model (AA *vs* GG: OR=0.26, 95%CI=0.15-0.45, R_h=0.973, I^2=0.0%, fixed effects), dominant model (AA+GA *vs* GG: OR=0.49, 95%CI=0.40-0.59, R_h=0.004, I^2=56.4%, random effects) and recessive model (AA *vs* GA+GG: OR=0.30, 95%CI=0.17-0.51, R_h=0.983, I^2=0.0%, fixed effects). The same results were also observed in the stratified analyses by ethnicity, source of control and sample size.**

• **CONCLUSION: Our meta-analysis suggests that rs641153 in the CFB gene may play a protective role in AMD susceptibility, the late AMD in particular, both in Caucasians and in Asians.**

• **KEYWORDS:** complement factor B; rs641153; age-related macular degeneration; meta-analysis

DOI:10.3980/j.issn.2222-3959.2013.06.21

Wang X, Zhang Y, Zhang MN. Complement factor B polymorphism (rs641153) and susceptibility to age-related macular degeneration: evidence from published studies. *Int J Ophthalmol* 2013;6(6):861-867

INTRODUCTION

Age-related macular degeneration (AMD), also known as age-related maculopathy (ARM), is the leading cause of irreversible blindness and a major public health threat in the elderly of western countries[1]. The prevalence of the disease increases significantly with age, accounting for approximately 4% among the population over 60 years and more than 10% of individuals older than 75 [1,2]. Environmental factors, such as smoking and exposure to chronic infection are involved in the onset of AMD [3]. Genetic susceptibility, however, plays a predominant role in the disease progression and aetiology [4,5]. The strong genetic association with AMD directs widespread attention to the mechanism underlying the pathogenesis of this aggressive disease.

Common variants in a wide range of genes have been identified to have genetic contributions to AMD susceptibility [6]. The variants in complement factor H (CFH) gene as well as the single nucleotide polymorphisms (SNPs) in HTRA1 and ARMS2 genes are strongly related to AMD [7-10]. The discovery of CFH variants and HTRA1 and ARMS2 SNPs in the etiology of AMD has promoted many following investigations on other genes of the complement cascade, including C2,C3 and complement factor B(CFB) [11-13]. CFB, located downstream on human chromosome 6p21, contains a common SNP rs641153 that is closely correlated with AMD susceptibility [14-20]. Nevertheless, no significant association with the risk of AMD is simultaneously shown in accumulated investigations [21-25]. Whether the rs641153 in CFH gene is truly involved in AMD remains to be elucidated. Hence in the present study, we hypothesized that rs641153 might modify the risk of AMD. To test this hypothesis, we performed a meta-analysis of 15 case-control studies.

SUBJECTS AND METHODS

Identification and Eligibility of Relevant Studies We systematically identified articles pertaining to rs641153 and AMD in PubMed, EMBASE, and Web of Science databases, using the keywords "CFB", "R32Q" or "rs641153", "polymorphism" or "polymorphisms", and "age-related macular degeneration" or "AMD" (the last search was

updated on February 19, 2013). The search was restricted to English language. Additional relevant articles were identified through screening the reference lists of the retrieved studies and journals that are known to publish related articles. When several publications included the same population, only the most recent or the largest study was used in this meta-analysis. We selected the studies based on the following criteria: 1) evaluation of rs641153 and AMD risk; 2) a case-control study; 3) the genotype distribution of controls is in accordance with Hardy-Weinberg equilibrium (HWE); and 4) contains sufficient genotype data to calculate the odds ratios (ORs) with 95% confidence intervals (CIs).

Data Extraction Data extraction was performed by two investigators independently and consensus was reached on all items. The following information was recorded from each article: first author, year of publication, country of study, ethnicity (Caucasian or Asian), source of control (population- or hospital-based controls), allele and genotype frequencies in cases and controls and numbers of cases and controls. For studies including populations of different ethnicities, we extracted the data separately and categorized them into Caucasians or Asians.

Statistical Analysis The numbers of allele and genotype frequencies in cases and controls were extracted from each study to estimate the risk of AMD development by ORs and 95% CIs. We further performed subgroup analyses by ethnicity, source or controls, stage of AMD (early/late AMD) and sample size (<500 or >500). HWE was detected for control subjects of each study, using the goodness-of-fit χ^2-test (P<0.10 was considered representative of departure from HWE). ORs with 95% CIs were used to assess the strength of association between rs641153 and the risk of AMD. The pooled ORs were calculated under homozygous model (AA *vs* GG), dominant model (AA+GA *vs* GG) and recessive model (AA *vs* GA+GG).

The Chi-square-based Q-test was performed to assess heterogeneity across studies and P<0.10 suggested presence of significant heterogeneity [26]. Between-study heterogeneity was also quantified with the I^2 statistic, which takes values between 0% and 100% with higher values denoting greater degree of heterogeneity (I^2=0-25%: no heterogeneity; I^2=25%-50%: moderate heterogeneity; I^2=50%-75%: large heterogeneity; I^2=75%-100%: extreme heterogeneity) [27]. Both fixed effects model and random effects model were applied for the pooled ORs. The fixed-effects model (Mantel-Haenszel method) was used if P>0.10, which assumes the same homogeneity of effect size across all studies [28]; otherwise, the random-effects model (DerSimonian and Laird method) was more appropriate, which tends to provide wider 95% CIs when differences occur in the results of the constituent studies[29].

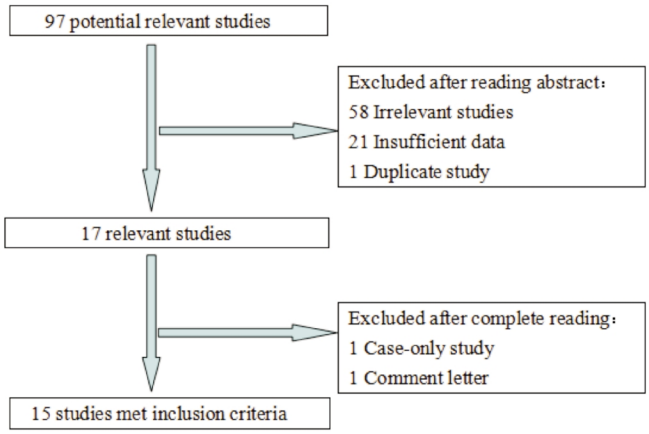

Figure 1 The flow diagram of included/excluded studies.

To determine the influence of independent studies on the overall AMD risk, sensitivity analysis was performed by meta-analyses of omitting each study, one at a time and recalculating the ORs and 95%CIs. Potential publication bias was assessed by Begg's funnel plot and Egger's test, P<0.10 was considered significant[30].

All statistical data were done using Stata software (version 12.0, Stata Corp LP, College Station, TX, USA). A significant P value was defined at 0.10.

RESULTS

Characteristics of Studies A total of 97 potentially relevant articles were extracted by initial search in PubMed, EMBASE, and Web of Science. Among them, 23 were subjected to further examination. After reading the full text, eight studies were ultimately excluded. Three of them did not provide available genotype data [11,31-34]; one contained duplicate information that had been included in the present meta-analysis [35]; one was a case-only study [13]; one was a comment letter [36]. The flow chart for study selection is presented in full detail in Figure 1.

In total, there were 15 eligible studies [14-25,37-39] for rs641153 with 6 712 cases and 4 669 controls in this meta-analysis. The main characteristics of all included studies are listed in Table 1, including first author, publication date, ethnicity, source of controls, and allele and HWE. No derivation from HWE was indicated in the controls of each study included in the current meta-analysis.

Quantitative Synthesis When pooling all eligible studies into one dataset for the meta-analysis, we found statistical evidence for an association between rs641153 and overall reduced risk of AMD under the homozygous model (AA *vs* GG: OR=0.26, 95%CI=0.15-0.45, P_h=0.973, I^2=0.0%, fixed effects), dominant model (AA+GA *vs* GG: OR=0.49, 95% CI=0.40-0.59, P_h=0.004, I^2=56.4%, random effects) and recessive model (AA *vs* GA+GG: OR=0.30, 95% CI= 0.17-0.51, P_h=0.983, I^2=0.0%, fixed effects). Significant decreased risk was also showed under all of the analyzed comparisons in the analysis restrained to late AMD studies (Table 2).

Table 1 Characteristics of all studies included in meta-analysis

First author	Year	Ethnicity	Control source	Case						Control						HWE
				Total	AA	GA	GG	A	G	Total	AA	GA	GG	A	G	
Maller[37]	2006	Caucasian	PCC	1238	3	106	1129	112	2364	934	10	171	753	191	1677	0.933
Gold[16]	2006	Caucasian	PCC	551	2	52	497	56	1046	269	3	53	213	59	479	0.883
Spencer[17]	2007	Caucasian	HCC	698	2	66	630	70	1326	282	3	50	229	56	508	0.883
Scholl[14]	2008	Caucasian	HCC	112	0	6	106	6	218	67	0	10	57	10	124	0.509
Chu[21]	2008	Asian	HCC	144	1	30	113	32	256	126	4	32	90	40	212	0.582
Farwick[22]	2009	Caucasian	PCC	776	0	26	750	26	1526	119	0	26	93	26	212	0.181
Reynolds[38]	2009	Caucasian	ND	103	0	6	97	6	200	57	0	11	46	11	103	0.420
Richardson[15]	2009	Caucasian	PCC	529	2	54	473	58	1000	199	3	41	155	47	351	0.878
Seddon[23]	2009	Caucasian	ND	279	0	23	256	23	535	1167	6	138	1023	150	2184	0.566
McKay[39]	2009	Caucasian	PCC	271	2	25	244	29	513	235	4	50	181	58	412	0.799
Pei[18]	2009	Asian	HCC	123	0	18	105	18	228	130	0	18	112	18	242	0.396
Kaur[19]	2010	Asian	HCC	162	2	18	142	22	302	158	10	53	95	73	243	0.483
Liu[24]	2010	Asian	HCC	238	0	17	221	17	459	220	1	25	194	27	413	0.841
Chen[20]	2011	Caucasian	HCC	1335	3	128	1204	134	2536	509	4	83	422	91	927	0.971
Kim[25]	2012	Asian	PCC	153	2	16	135	20	286	197	2	30	165	34	360	0.630

PCC: Population-based case-control study; HCC: Hospital-based case-control study; ND: Not defined; HWE: Hardy-Weinberg equilibrium.

Table 2 Meta-analyses of SNP rs641153 and risk of AMD in each subgroup

Variables	*n* (case/control)	AA *vs* GG			AA+GA *vs* GG			AA *vs* GA+GG		
		OR (95%CI)	P_h	I^2	OR (95%CI)	P_h	I^2	OR (95%CI)	P_h	I^2
Ethnicity										
Caucasian	5892/3838	0.26 (0.13, 0.49)	0.999	0.0%	0.45 (0.36, 0.55)	0.018	54.9%	0.29 (0.15, 0.54)	0.999	0.0%
Asian	820/831	0.27 (0.11, 0.71)	0.406	0.0%	0.63 (0.42, 0.94)	0.057	56.3%	0.32 (0.12, 0.84)	0.488	0.0%
Source of controls										
PCC	3518/1953	0.32 (0.15, 0.65)	0.653	0.0%	0.42 (0.31, 0.57)	0.008	68.2%	0.35 (0.17, 0.73)	0.679	0.0%
HCC	2812/1492	0.21 (0.09, 0.47)	0.984	0.0%	0.56 (0.44, 0.72)	0.135	38.5%	0.24 (0.10, 0.54)	0.997	0.0%
ND	382/1224	0.31 (0.02, 5.50)	NA	NA	0.52 (0.25, 1.07)	0.173	46.2%	0.32 (0.02, 5.72)	NA	NA
Sample size										
<500	1585/2357	0.23 (0.12, 0.48)	0.998	0.0%	0.43 (0.33, 0.57)	0.005	69.9%	0.26 (0.13, 0.53)	0.998	0.0%
>500	5127/1314	0.30 (0.13, 0.66)	0.705	0.0%	0.56 (0.43, 0.73)	0.106	39.3%	0.34 (0.15, 0.76)	0.778	0.0%
Stage										
Late	3646/3355	0.31 (0.15, 0.62)	0.778	0.0%	0.55 (0.48, 0.64)	0.158	33.9%	0.34 (0.17, 0.68)	0.789	0.0%
Total	6712/4669	0.26 (0.15, 0.45)	0.973	0.0%	0.49 (0.40, 0.59)	0.004	56.4%	0.30 (0.17, 0.51)	0.983	0.0%

PCC: Population-based case-control study; HCC: Hospital-based case-control study; ND: Not defined; NA: Not available; P_h: *P* value of heterogeneity test; CI: Confidence interval; OR: Odds ratio.

In the stratified analysis according to ethnicity, the risks for the AA genotype, compared with the GG genotype, were 0.26 (95%CI=0.13-0.49, P_h=0.999, I^2=0.0%, fixed effects) in Caucasians and 0.27 (95%CI=0.11-0.71, P_h=0.406, I^2=0.0%, fixed effects) among Asians. A significantly decrease in AMD risk between rs641153 and the analyzed subgroups was also seen in dominant model and recessive model (Table 2).

Stratifying by control source and sample size indicated that the risk of AMD was decreased both in population-based case-control studies and in hospital-based case-control studies, and that for rs641153, the sample sizes was not a modifier of the reduced risk observed in the above analyses (Table 2).

Heterogeneity and Sensitivity Analysis No substantial heterogeneity was detected in the three genetic models except for the dominant model (P_h=0.004, I^2=56.4%) and the random-effects model was selected for the pooled OR. Subsequently, the leave-one-out sensitivity analysis was performed to identify the heterogeneity source. The results indicated two datasets may constitute the main contribution to the obvious heterogeneity across the studies [19,22]. The exclusion of these two articles increased homogeneity among the remaining studies (P_h=0.438, I^2=0.8%). The general

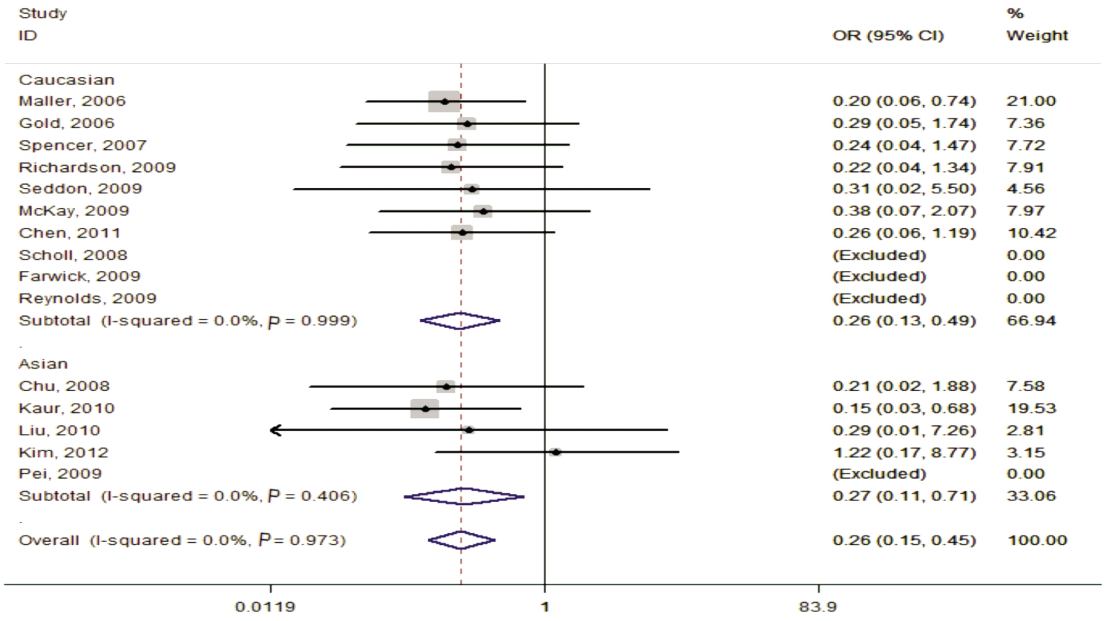

Figure 2 Forest plot of AMD risk associated with SNP rs641153 (AA *vs* GG) in the stratified analyses by ethnicity The squares and horizontal lines correspond to the study-specific OR and 95%CI. The area of the squares reflects the weight (inverse of the variance). The diamond represents the summary OR (0.26) and 95%CI (0.15, 0.45).

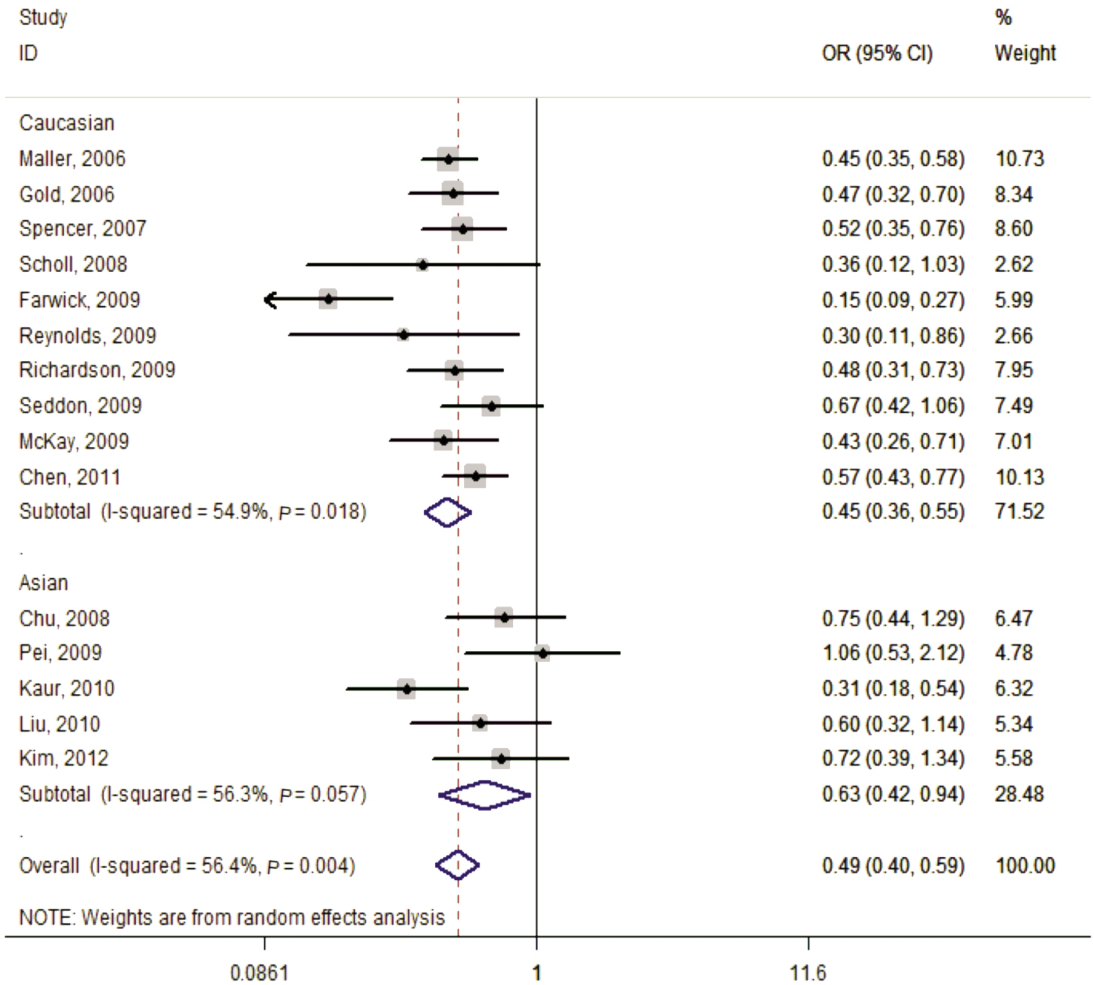

Figure 3 Forest plot of AMD risk associated with SNP rs641153 (AA+GA *vs* GG) in the stratified analyses by ethnicity The squares and horizontal lines correspond to the study-specific OR and 95%CI. The area of the squares reflects the weight (inverse of the variance). The diamond represents the summary OR (0.49) and 95%CI (0.40, 0.59).

result, however, was not significantly changed with or without them (OR=0.53, 95%CI=0.47-0.60)(Figures 2-4).

Publication Bias Begg's funnel plot and Egger's test were performed to determine the publication bias of included

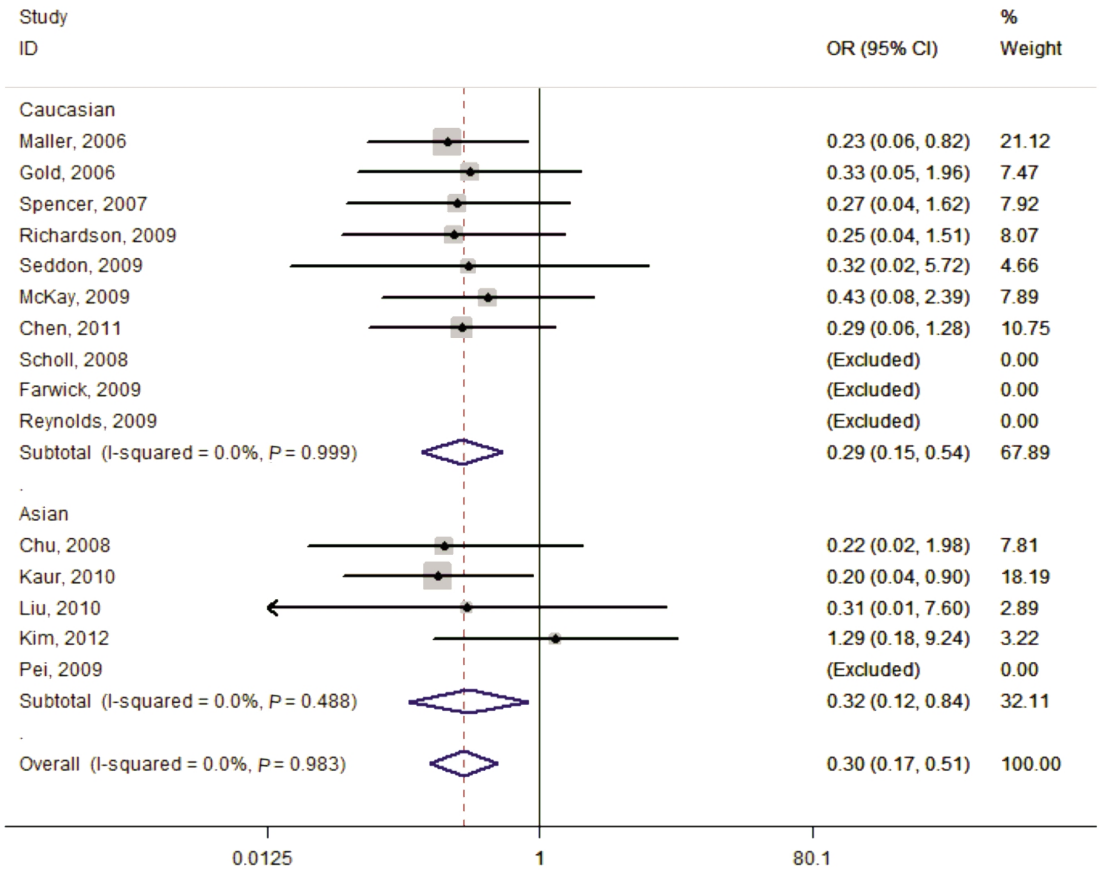

Figure 4 Forest plot of AMD risk associated with SNP rs641153 (AA *vs* GA+GG) in the stratified analyses by ethnicity The squares and horizontal lines correspond to the study-specific OR and 95%CI. The area of the squares reflects the weight (inverse of the variance). The diamond represents the summary OR (0.30) and 95%CI (0.17, 0.51).

studies. Statistical evidence of publication bias was showed neither in the funnel plot nor in the Egger's test (t =0.45, P= 0.666 for AA *vs* GG) (Figure 5)

DISCUSSION

We performed a systematic meta-analysis of 15 eligible studies involving 6 712 cases and 4 669 controls. We found a significant decrease of AMD risk in the general analysis (26% under the homozygous model, 49% under the dominant model and 32% under the recessive model). Due to the incomplete data from three studies for analyses of the AA *vs* GG and the AA *vs* GA+GG genetic models (for example, in the AA *vs* GG genetic model, AA genotype frequency for all of the studies are 0 in both cases and controls), they were automatically excluded when performing meta-analysis and the results were summarized in pooling data consisting of 12 publications [14,22,38]. The protective effects remained stable when analysis was restrained to the studies focusing on late AMD. Several reports have indicated that inflammatory processes may play a central role in the development of AMD by inducing the formation of drusen, which is an important characteristic of early AMD[40,41]. Therefore, we can conclude that rs641153 in the CFB gene may act as an inhibitor against AMD progression, the late AMD in particular by suppressing inflammation.

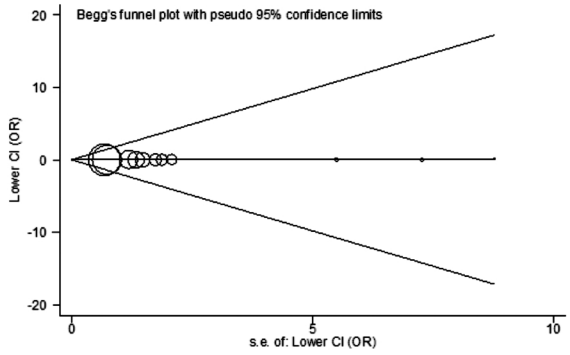

Figure 5 Begg´s funnel plot for publication bias test (AA *vs* GG) Each point represents a separate study for the indicated association. Log [OR]: Natural logarithm of the odds ratio; Horizontal line: Mean effect size.

Meta-analysis is deemed as an important tool with strong statistical power in defining the association of selected genetic polymorphisms with the risk of disease and identifying potential between-study heterogeneity. A previously published meta-analysis based on 8 case-control studies, showed a tendency for strong protective effects on AMD risk, which was especially exhibited in Caucasians[42]. Following this study, a larger assessment including 14 case-control publications provided a robust estimate of the protective association of rs641153 with AMD, with an

absolute lowering risk among Caucasian populations [43]. However, in our meta-analysis, we found strongly protective impact on the risk of AMD in Caucasians, as well as in Asians. Since rs641153 is common among Caucasians and Asians, and there is no obvious ethnic difference in the associations with AMD risk, the biological significance of this SNP may be comparable in different ethnic groups.

While most studies in current meta-analysis have already been included in the meta-analysis of the two published papers and no obvious new information is provided, there are some differences. First, compared to the reference 43 (the larger study of the two published papers), new data have been included in our meta-analysis, which helps to enlarge the sample size of the general and subgroup analysis, thus deriving a more estimate of the true association [25]. Second, for a meta-analysis involving a large number of subjects, accuracy of data is critically important. By carefully checking the published papers, especially the reference 43, some data included in the meta-analysis are not matching those provided in the original article (for example, reference 39), which may increase the chance of false positives.

Consistent with the protective effects indicated in the general analysis, stratification analyses according to source of control and sample size suggested that there was significant association between rs641153 and susceptibility to AMD. It is especially important to use typically representative populations and large sample sizes for genetic association studies, which could contribute to a more precise estimation. There was no apparent difference in the results in the subgroups of control source and sample size, implying that rs641153 itself may have a strong protective role in the development and progression of AMD.

Although our meta-analysis is based on all eligible case-control studies to date and indicated no significant publication bias facilitating the accuracy and credibility of the results, some limitations should be highlighted. First, lack of the original data from the included studies limited further assessment of potential interactions between gene-to-gene and gene-to-environment, because such interactions may modify AMD risk. Second, significant heterogeneity detected in the dominant model may have influenced the results, although no statistical evidence was indicated in the meta-analysis. Third, the included case-control studies were carried out among Caucasians or Asians, thus the results from this meta-analysis may not be applicable to other ethnic groups.

Despite these limitations, our meta-analysis provided evidence of significant association between rs641153 and AMD risk, supporting the hypothesis that this SNP confers protection to the susceptibility to AMD, especially the late AMD. To confirm our findings, further well-designed studies including gene-to-gene and gene-to-environment interactions in diverse ethnic populations are necessary.

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