Association of Two Polymorphisms, rs1061170 and rs1410996, in Complement Factor H with Age-Related Macular Degeneration in an Asian Population: A Meta-Analysis

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Key Words
Age-related macular degeneration · Complement factor H polymorphism · Meta-analysis

Abstract
Background: With the increasing number of studies indicating that two single-nucleotide polymorphisms (SNPs), rs1061170 and rs1410996, in complement factor H (CFH) might be associated with the susceptibility to age-related macular degeneration (AMD), the exact association still remains uncertain. Thus, we conducted a meta-analysis to systematically summarize and clarify the association between the two SNPs and the AMD risk particularly in an Asian population. Methods: A systematic search of studies on the association of two SNPs with the susceptibility to AMD was conducted in PubMed, Embase and Web of Science. Summary odds ratios (ORs) and 95% confidence intervals (CIs) of allele contrast and genotype contrast were estimated using the random or fixed effects model. The Q statistic test was used to identify heterogeneity, and the funnel plot was adopted to evaluate publication bias. A total of 19 case-control studies on rs1061170 and 8 studies on rs1410996 were included. Results: Clearly a significantly increased trend of AMD was observed with the rs1061170 (T vs. C: OR = 1.91, 95% CI = 1.71–2.13, p_H = 0.029; TC vs. CC: OR = 2.11, 95% CI = 1.30–3.42, p_H = 0.792; TT vs. CC: OR = 3.90, 95% CI = 2.45–6.22, p_H = 0.774). Similarly, the rs1410996 polymorphism also showed a rising AMD tendency (T vs. C: OR = 1.48, 95% CI = 1.17–1.87, p_H < 0.001; TC vs. CC: OR = 1.52, 95% CI = 1.13–2.04, p_H = 0.002; TT vs. CC: OR = 2.10, 95% CI = 1.27–3.49, p_H < 0.001). What is more, subgroup analysis revealed that both polymorphisms indicated a high risk of nAMD (neovascular AMD) in Asian populations. Conclusions: This meta-analysis suggested that CFH rs1061170 and rs1410996 polymorphisms were associated with AMD risk, both of which demonstrated a higher susceptibility to AMD, especially to nAMD. However, the results of rs1410996 should be interpreted with caution due to the limited sample and heterogeneity. Large-scale and well-designed studies are needed to validate our findings.

Introduction

Age-related macular degeneration (AMD) is the leading cause of irreversible blindness for elderly populations in developed countries. A variety of previous studies indicate it is a multifactorial and heterogeneous disease,
which may generally be divided into two types: one is nonexudative (dry or atrophic) AMD, and the other is exudative (wet or neovascular) AMD, distinguished by the presence of choroidal neovascularization beneath the fovea. For the latter type, the neovascular AMD (nAMD) is commonly associated with aging, multiple genetic factors and environmental influences such as smoking status and serum cholesterol levels. Meanwhile, as a kind of progressive disease, the early stage usually indicates pigmentary irregularities of the retinal pigment epithelium, including hyperpigmentation or depigmentation and formation of hard and soft drusen, and the late (or advanced) stage is characterized by either a geographic atrophy of the retinal pigment epithelium and the underlying choriocapillaris (dry type) or subretinal and intraretinal exudation caused by a subretinal choroidal neovascularization (wet type). Soft drusen is a hallmark risk factor for developing AMD [1]. Recently much progress has been made on the pathology and epidemiology of AMD, especially in the genetic fields. Some genes have been identified to be related to AMD susceptibility, of which the complement factor H (CFH) gene is one of the most important genes [2–4]. Besides, more and more scientists tend to provide insights into the molecular basis of AMD.

The Y402H coding variant (rs1061170) in the CFH gene located in the heparin and C-reactive protein-binding domain may cause complement dysregulation and lead to the pathogenesis of AMD [5]. Multiple independent studies have been performed to assess the association between Y402H and AMD in Asian populations [6–24]; meanwhile, rs1410996 is another polymorphism in the CFH gene, having been substantially reported to be related with AMD risk in Asian populations as well [10, 13, 17, 21, 23–26], but the results are still contradictory and inclusive.

In addition, as for the polymorphism rs1061170, only one meta-analysis-based study [27] investigating the impact of Y402H on AMD for Asian populations was published to date, incorporating only 13 raw studies on the deadline of 2010, after which 9 new records with diverse and meaningful results emerged; thus, it is extremely necessary to perform another meta-analysis dealing with more related studies. Moreover, this is the first meta-analysis to clarify the relation between rs1410996 and AMD. With the objective of fulfilling the requirements above, we carried out a meta-analysis based on totally 21 case-control studies to better determine the role of the two single-nucleotide polymorphisms (SNPs) in the susceptibility to AMD.

### Methods

#### Identification of Eligible Studies

In order to collect the eligible literature, we performed a comprehensive search of PubMed, Embase and Web of Science databases by using the following search terms: 'complement factor H or CFH', 'polymorphism or polymorphisms' and 'age-related macular degeneration or AMD'. The retrieval was restricted to the English literature, and the last search was updated in December 2014. The reference lists of retrieved studies and recent reviews were also manually searched for further relevant studies.

#### Inclusion and Exclusion Criteria

Studies in this meta-analysis must meet the following inclusion criteria: (1) the original major objective was to explore the relationship between rs1061170 and rs1410996 polymorphisms and AMD; (2) the studies were designed on the basis of unrelated case-control studies with available data of allele and genotype distributions and sufficient data for estimating odds ratios (ORs) with 95% confidence intervals (CIs); (3) as for the duplicated articles, the latest or the largest one was selected; (4) the papers were written in English and published in peer-reviewed journals. Exclusion criteria were: (1) duplication of previous publications; (2) comment, review and editorial; (3) family-based studies of pedigrees; (4) study with no detailed genotype data. When there were multiple publications from the same population, only the largest study was included. Study selection was achieved by two investigators independently, according to the inclusion and exclusion criteria by screening the title, abstract and full text. Any dispute was solved by discussion.

#### Data Extraction

The data of the eligible studies were extracted in duplicate by two investigators independently (Wu and Guo). The following contents were collected: name of first author, year of publication, the largest one was selected; (4) the papers were written in English and published in peer-reviewed journals. Exclusion criteria were: (1) the original major objective was to explore the relation-ship between rs1061170 and rs1410996 polymorphisms and AMD; (2) the studies were designed on the basis of unrelated case-control studies with available data of allele and genotype distributions and sufficient data for estimating odds ratios (ORs) with 95% confidence intervals (CIs); (3) as for the duplicated articles, the latest or the largest one was selected; (4) the papers were written in English and published in peer-reviewed journals. Exclusion criteria were: (1) duplication of previous publications; (2) comment, review and editorial; (3) family-based studies of pedigrees; (4) study with no detailed genotype data. When there were multiple publications from the same population, only the largest study was included. Study selection was achieved by two investigators independently, according to the inclusion and exclusion criteria by screening the title, abstract and full text. Any dispute was solved by discussion.

#### Methodological Quality Assessment

The quality of studies was independently evaluated by two reviewers according to the Newcastle-Ottawa Scale criteria [28], which were adjusted from a previous publication [29]. In this scale, three aspects were carefully checked: (1) subject selection, 0–4; (2) comparability of subjects, 0–2, and (3) clinical outcome, 0–3. Total scores ranged from 0 (worst) to 9 (best) with a score ≥7 indicating good quality. Any disagreement was adjudicated by a third author.

#### Statistical Analysis

We conducted our meta-analysis according to the PRISMA checklists and followed the guideline [30]. Hardy-Weinberg equilibrium was evaluated for each study by the $\chi^2$ test in control groups, and $p < 0.05$ was considered a significant departure from Hardy-Weinberg equilibrium. ORs and 95% CIs were calculated to evaluate the strength of the association between rs1061170 and...
rs1410996 SNPs and susceptibility to AMD. Pooled ORs were performed for allelic comparison (rs1061170/rs1410996: T vs. C), heterozygote model (rs1061170/rs1410996: TC vs. CC), homozygote model (rs1061170/rs1410996: TT vs. CC), dominant model (rs1061170/rs1410996: TC + TT vs. CC) and recessive model (rs1061170/rs1410996: TT vs. TC + CC), respectively. The statistically significant level was determined by the Z test with the p value <0.05. Heterogeneity was evaluated by the Q statistic (significance level of p < 0.1) and I² statistic (>50% as evidence of significant inconsistency) [31]. Either the fixed-effects or random-effects model was used to pool the effect sizes according to the heterogeneity [32]. Sensitivity analysis was also performed to evaluate the effect of each study on the combined ORs by omitting each study in each turn. In the meantime, a cumulative meta-analysis was conducted to inspect the stability of accumulative data over time. Besides, meta-regression analysis was taken to detect the feature of studies included and explore the source of heterogeneity with a significant level of p < 0.1 [33]. The following variables were studied: mean age of study subjects, percentage of male participants, phenotype of cases (nAMD vs. other), country (Chinese vs. Japanese) and sample size. Potential publication bias was checked by Begg’s funnel plots [34] and Egger’s regression test [35]. An asymmetric plot and the p value of Egger’s test <0.05 was considered a significant publication bias. All statistical analyses were performed with Stata 12.0 software (Stata Corp., College Station, Tex., USA). A two-tailed p < 0.05 was considered significant except for specified conditions, where a certain p value was declared necessary.

**Results**

**Characteristics of Studies**

A total of 1,558 studies were acquired from PubMed, Embase and Web of Science databases (PubMed: 490, Embase: 589, Web of Science: 479). The literature selection process and characteristics of each study are shown in figure 1 and table 1. In 6 studies [10, 13, 17, 21, 23, 24], genotype frequencies of the two SNPs were presented separately, thus each of them were treated as separate studies.

**Association between rs1061170 Polymorphism and AMD Susceptibility**

We first analyzed the association between the CFH rs1061170 polymorphism and the susceptibility to AMD. No significant heterogeneity was identified by the Q test and I² statistic in the heterozygote model, homozygote model, genetic models, dominant model and allelic comparison; therefore a fixed-effects model was used. A random-effects model was used in the recessive model due to the presence of heterogeneity. The significant association was identified in all of the genetic models (T vs. C: OR = 1.91, 95% CI = 1.71–2.13, pH  = 0.029; TC vs. CC: OR =
Association between rs1410996 Polymorphism and AMD Susceptibility

The association between the CFH rs1410996 polymorphism and the risk of AMD was analyzed in 8 independent studies. For the limited studies and heterogeneity,
this result should be treated with caution. The random-effects model was used in all of the 5 models for the presence of heterogeneity. A significantly increased risk of AMD was also observed in each genetic model (T vs. C: OR = 1.48, 95% CI = 1.17–1.87, \( p_H < 0.001 \); TC vs. CC: OR = 1.52, 95% CI = 1.13–2.04, \( p_H = 0.002 \); TT vs. CC: OR = 2.10, 95% CI = 1.27–3.49, \( p_H < 0.001 \); TT vs. TC/CC: OR = 1.37, 95% CI = 0.85–2.21, \( p_H < 0.001 \); TT vs. TC/CC: OR = 1.68, 95% CI = 1.21–2.33, \( p_H = 0.003 \); fig. 3).

Besides, subgroup analysis of 4 records for the nAMD was assessed. A significant statistical association was identified in all the genetic models, with the random-effects model only being used for the heterozygote model (T vs. C: OR = 1.58, 95% CI = 1.15–2.18, \( p_H = 0.004 \); TC vs. CC: OR = 1.70, 95% CI = 1.37–2.11, \( p_H = 0.113 \); TT vs. CC: OR = 2.49, 95% CI = 1.28–4.84, \( p_H = 0.007 \); TT/TT vs. CC: OR = 1.28, 95% CI = 0.57–2.89, \( p_H < 0.001 \); TT vs. TC/CC/CC: OR = 1.89, 95% CI = 1.16–3.08, \( p_H = 0.039 \); table 2).

Sensitivity Analysis and Cumulative Meta-Analysis
Sensitivity analysis was performed to examine the influence set by the individual study on the pooled ORs for CFH rs1061170 and rs1410996 by deleting each study
once in every genetic model. The summary OR remained stable, indicating that our results were not driven by any single study. Besides, cumulative meta-analysis of rs1061170 revealed that the summary ORs were stable and that the 95% CIs narrowed with accumulation of data over time (fig. 4, T vs. C). Nevertheless, the results for rs1410996 showed an irregularity on account of the limited research (fig. 5, T vs. C).

**Meta-Regression**

To explore the effect of study characteristics on the estimate of effect size, we performed a univariate meta-regression analysis for the two polymorphisms. No statistically significant effect was observed for both rs1061170 and rs1410996 on the summary estimate by the 5 variables studied, including mean age of study subjects (p = 0.188, 0.238), percentage of male participants (p = 0.65, 0.323), phenotype of cases (nAMD vs. other; p = 0.698, 0.602), country (Chinese vs. Japanese; p = 0.058, 0.453) and sample size (p = 0.612, 0.930).

**Publication Bias**

No publication bias for the association between the two polymorphisms and AMD susceptibility was identified by Begg’s funnel plot or Egger’s regression test. Symmetrical funnel plots for rs1061170 were obtained in all the genetic models (fig. 6, T vs. C). For rs1410996, the imperfect funnel plots may be owed to the limited number of included studies (fig. 7, T vs. C).

**Discussion**

The reliable assessment of the association for CFH rs1061170 and rs1410996 with AMD has been hindered by the low frequency of variant alleles and small sample sizes in studies. Thus, we conducted a systematic meta-analysis to further identify the correlation between them, representing a pooled total of 19 case-control studies involving 7,716 subjects for rs1061170 and 8 case-control studies including 3,625 subjects for rs1410996. Consistently with a previous meta-analysis focused on the association between rs1061170 and AMD [27], an obviously increased effect has been found between rs1061170 and AMD susceptibility in any genetic model. What is more, this rising trend became more noticeable in the subgroup analysis by the type nAMD. Entirely similar results were found for the association between rs1410996 and AMD susceptibility, a positive relationship between them was also identified in all of these observed models, with a more significant result for nAMD as well. However, this finding should be interpreted with caution due to the limited sample heterogeneity.
With the increased knowledge about the genetic determinants for AMD, researchers have tried to find genetic evidence for the pathogenesis of AMD. Previous studies have revealed that inflammation plays a role in the initiation and progression of AMD. Complement dysregulation has emerged as an important pathogenetic factor in AMD. As a key regulator of the complement system of innate immunity, CFH consists of 20 complement control protein modules, and the Y402H polymorphism (rs1061170) is located within a binding site for heparin.
and C-reactive protein, which was reported to play a role in AMD pathogenesis [36]. Therefore, changes in this region of the protein may result in a malfunctioning CFH that is not able to inhibit the complement cascade properly. The association between the CFH polymorphisms and AMD has been replicated in many well-established AMD cohorts in different populations. It has been thought that the magnitude for the association between CFH Y402H (rs1061170) and AMD appears to become lower when studies move from the West to the East, with an OR in patients homozygous for the Y402H variant to be only 2.45 in a Russian study and even no evidence of Y402H association with AMD in Asian populations, whereas ORs higher than 3 have been reported in Western populations. To some extent, this is consistent with the low minor allele frequency of rs1061170 with only 6.7 in Han populations and 28.2 in those of European descent reported in the study of Yang et al. [17]. However, our meta-analysis reported a summary allele OR of 1.91 (p < 0.001) for AMD and 2.02 (p < 0.001) for nAMD, which strongly supported the notion that the Y402H variant is a risk factor for AMD in Asian populations. For the other polymorphism we observed, the rs1410996, it is located in the noncoding loci of the CFH gene, with a comparatively higher minor allele frequency in the Han population than in individuals of European descent (43.3 and 40.8, respectively) [17]. Thus, more and more attention has been paid to it, especially by these Asian scientists. This analysis shows a higher risk of AMD with the C allele of rs1410996 in the Asian population. Compared with individuals with the wild-type TT genotype of rs1410996, individuals with the homozygous CC genotype had a 2.10-fold increased risk of AMD. However, the mechanism of the relationship between AMD and the noncoding variant of the CFH gene is unclear. One intriguing hypothesis is that the associated noncoding variant modulates the risk of AMD by regulating the expression of CFH, rather than disrupting the CFH protein function [37].

A significant heterogeneity was found for the association of CFH rs1410996 in all 5 genetic models. However, when we restricted the type to nAMD, the heterogeneity became smaller, suggesting that the type of AMD, to some extent, contributed to the source of heterogeneity. Though heterogeneity existed, our results remained stable, and the results became more significant for nAMD. Additionally, no significant heterogeneity was identified for CFH rs1061170. The results from our subgroup and sensitivity analyses were consistent and robust. During the subgroup analysis, we found that the type had a prominent effect on the association for both rs1061170 and rs1410996 with AMD in any of the genetic models, suggesting the two SNPs might be more relevant for the formation of choroidal neovascularization, but the related mechanism still remained to be seen. Meanwhile, the cumulative meta-analysis for both polymorphisms indicates that far more stabilizing experimental results had been presented and adopted. Furthermore, meta-regression analysis revealed no significant effect of demographics of study participants, phenotype of cases, type of AMD, country and sample size on the summary effect size.

Our meta-analysis has several strengths. First of all, this is the first meta-analysis focused on the association between CFH rs1410996 polymorphism and the susceptibility to AMD in the Asian population, and the positive
Conclusion drawn will greatly help explore the etiology of AMD. Additionally, for the polymorphism rs1061170, compared with the former meta-analysis, another 9 studies were included, and supplementary analysis such as subgroup analysis was performed. In addition, all the included studies had high qualities according to the methodological quality assessment. Moreover, no publication bias was identified by either Begg’s funnel plot or Egger’s regression test. Finally, no limitation was made in the literature search; thus, the selection bias was well controlled.

In spite of the considerable efforts to explore the possible relationship between the two SNPs and AMD risk, some limitations should be considered. Firstly, the number of included studies for the rs1410996 polymorphism limited further analysis. Results might be treated with more caution due to the existing high heterogeneity; even homogeneity was achieved after excluding the 2 studies in the sensitivity analysis, and the conclusion still remained stable. Secondly, for the rs1061170, there was a study which did not conform to Hardy-Weinberg equilibrium expectations, but when restricted to those who were in Hardy-Weinberg equilibrium, the pooled estimate of the association between the rs1061170 polymorphism and susceptibility to AMD remained significant. Thirdly, heterogeneity was detected in rs1061170 only in the allelic comparison but in all genetic models of 1410996. The country of origin of the subjects and the type of AMD might contribute to the heterogeneity.

In conclusion, our results suggested that both CFH rs1061170 and rs1410996 were significantly associated with an increased risk of AMD in Asian populations, especially for nAMD. However, there was insufficient data to fully confirm the association of AMD and rs1410996, and the results should be interpreted with caution. Well-designed studies with a larger sample size and more ethnic groups are required to validate the risk identified in the current meta-analysis.

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Disclosure Statement

The authors declare that there is no conflicts of interest regarding the publication of this paper.

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