CETP Gene may be Associated with Advanced Age-Related Macular Degeneration in the Chinese Population

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ABSTRACT

Objectives: This study aims to investigate whether variations in LIPC, CETP, ABCA1 and LPL, which are involved in high-density lipoprotein (HDL) metabolism, are associated with advanced age-related macular degeneration (AMD) in the Chinese population.

Design and Methods: A total of 119 Chinese patients with advanced AMD and 99 control individuals were recruited. Genomic DNA was extracted from peripheral blood leukocytes. Genotypes of seven single nucleotide polymorphisms (SNPs) including rs1061170 and rs1410996 in CFH, rs10490924 in HTRA1, rs10468017 in LIPC, rs3764261 in CETP, rs1883025 in ABCA1 and rs12678919 near LPL were determined by polymerase chain reaction (PCR) followed by allele-specific restriction enzyme digestion or SNaPshot. Unconditional logistic regression analyses were performed to generate a risk predictive model.

Results: We observed the frequency of allele A of rs3764261 in CETP to be significantly lower in advanced AMD after Bonferroni correction (15.5% in patients with AMD and 20.7% in controls; OR = 0.49, 95% CI: 0.29–0.85; p = 0.011). Furthermore, we found that it was also associated with reduced risk of both unilateral AMD (OR = 0.52, 95% CI: 0.28–0.98; p = 0.043) and bilateral AMD (OR = 0.45, 95% CI: 0.22–0.91; p = 0.026). Rs10468017 in LIPC, rs12678919 near LPL and rs1883025 in ABCA1 were not found to be associated with advanced AMD (all p > 0.05).

Conclusion: Our data suggested that the allele A in rs3764261 in CETP gene may be associated with a decreased risk of advanced AMD in Chinese population.

Keywords: Age-related macular degeneration, CETP, genetic polymorphism, HDL

INTRODUCTION

Age-related macular degeneration (AMD) is a progressive neurodegenerative disease and a common cause of blindness in the elderly population, particularly in developed countries.¹ AMD is also a leading cause of blindness in the aging Chinese population.² The disease affects the macular region of the retina, which is necessary for sharp central vision.

There are two forms of advanced AMD. “Dry” AMD, or geographic atrophy, is characterized by confluent areas of retinal pigment epithelium and photoreceptor cell death, while “wet” AMD, or choroidal neovascularization, is characterized by an abnormal growth of blood vessels under the macula. AMD is a complex disease with genetic and environmental factors contributing to its pathogenesis by various proportions. Numerous previous studies have repeatedly shown...
significant associations between AMD and polymorphisms in landmark genes including complement factor H (CFH)\(^3\)–7 and high temperature requirement factor A-1 (HTRA1)\(^8\)–10. Variants in genes that play roles in the alternative complement pathway, including CFB/C2,\(^5,11\) C3\(^12\)–13 and CFH,\(^14\) also contribute to the risk of AMD. Recently, a well-powered, genome-wide association study (GWAS) reported that a variant in the non-complement gene LIPC, which encodes for a hepatic lipase that regulates triglyceride hydrolysis and plasma high density lipoprotein cholesterol (HDL-C) levels,\(^15\) was associated with a decreased risk of advanced AMD.\(^15\)–18 Since lipid metabolism and regulation are complex and involve multiple genes, there may also be associations between AMD and other variants in genes that are heavily involved in the high-density lipoprotein (HDL) metabolic pathway, including cholesteryl ester transfer protein (CETP) gene,\(^15\)–18 lipoprotein lipase (LPL) gene,\(^15,16\) and ATP-binding cassette transporter A1 (ABCA1) gene.\(^15\)–18 However, there are few studies targeting the association of polymorphisms in these genes in Chinese AMD patients. The purpose of our study was to investigate whether LIPC, CETP, ABCA1 and LPL polymorphisms were related to the risk of advanced AMD in a Chinese cohort.

MATERIALS AND METHODS

Participants

The use of human tissue and the protocol in this study adhered strictly to the principles of the Declaration of Helsinki and were approved by the Ethical Committee of the West China Hospital, Sichuan University. Written informed consent was obtained from all subjects before their participation in the study. A total of 119 Chinese patients with advanced AMD and 99 healthy controls were recruited from the West China Hospital. All patients and control subjects were unrelated native Chinese. As described previously,\(^19\) participants underwent a standard ophthalmologic examination, which included visual acuity measurements, applanation tonometry, dilated slit lamp biomicroscopy, and indirect ophthalmoscopy. Imaging studies including stereoscopic color fundus photography, optical coherence tomography and fluorescein fundus angiography were performed as needed. Diagnosis of advanced AMD was based on the presence of geographic atrophy (GA) or choroidal neovascularization (CNV). Determination of unilateral or bilateral AMD was made at the final visit. Control subjects were defined as being >60 years old, having fewer than five small drusen of which none exceeded 63 \(\mu\)m in diameter, and not exhibiting retinal pigment epithelium (RPE) abnormalities.

Genotyping

Blood samples were collected and stored at \(-80^\circ\)C before DNA extraction. Genomic DNA was extracted from peripheral blood leukocytes according to established protocols. Seven single nucleotide polymorphisms (SNPs) in six genes associated with AMD were selected according to the literature (Table 1), which were rs1061170 and rs1410996 in CFH;\(^3\)–5 rs10490924 in HTRA1;\(^8\)–10 rs1068017 in LIPC;\(^15\)–18 rs3764261 in CETP;\(^15\)–18 rs12678919 near LPL;\(^15\)–18 and rs1883025 in ABCA1.\(^15\)–18

Genotyping was performed with polymerase chain reaction (PCR) followed by allele-specific restriction enzyme digestion or SNaPshot. Briefly, DNA fragments containing SNPs were amplified by PCR. Primers used for sequence amplification and the size of PCR products were given in Table 1. Rs10468017 was genotyped by allele-specific restriction enzyme (SspI) digestion method according to the manufacturer’s recommendations (New England Biolabs Inc, Ipswich, Massachusetts). With a mutation from C to T, the PCR product can be digested by the restriction enzyme SspI. All other SNPs were genotyped using the SNaPshot method as described previously.\(^20\) Briefly, the SNP was amplified by PCR, and the PCR product was purified by Exo I and shrimp alkaline phosphatase, the product was analyzed on an ABI 3130xl genetic analyzer (Applied Biosystems Inc., Foster City, California). After an additional purification step using shrimp alkaline phosphatase, the product was analyzed on an ABI 3130xI genetic analyzer (Applied Biosystems Inc.). The purified PCR product and the SNaPshot primer (Supplementary Table 1 – online only) were then used to perform a single base-pair extension with the SNaPshot multiplex mix (Applied Biosystems Inc., Foster City, California).

Table 1. Characteristics of the study cohorts with or without advanced AMD.

| Characteristics | AMD (n = 119) | Controls (n = 99) | p Value |
|-----------------|--------------|-----------------|--------|
| Age (years)     | 65.8 ± 8.5   | 66.2 ± 6.9      | 0.69   |
| Gender, Male n (%) | 38 (31.9%) | 41 (41.4%) | 0.147  |

The data are presented as mean ± SEM (standard error of the mean). The p values are calculated either with the t-test or the Chi-square test.
multivariate unconditional logistic regression with adjusting for potential confounders such as age. \textit{CFH} and \textit{HTRA1} were also included as potential confounders due to their prominent genetic contribution to AMD that has been well established. Since these empirical \( p \) values adjust for the three SNPs including \textit{rs1061170} and \textit{rs1410996} in \textit{CFH} and \textit{rs10490924} in \textit{HTRA1}, we have carried out 3 tests on each SNP of the data. A stringent multiple testing correction for this would be to use the Bonferroni correction, which would require testing at the 0.05/3 = 0.017 level. The gender was not included as the covariate since it has been established that gender is not related to AMD.\(^4,21\) The power of a statistical test is calculated to avoid making a false negative decision, which is over 0.95 for each SNP. Furthermore, we tested the association of each SNP in control, unilateral and bilateral AMD using multinomial logistic regression. All analyses were performed using Statistical Analysis System software version 9.1 (SAS Institute Inc., Cary, NC, USA).

**RESULTS**

A total of 218 unrelated Chinese individuals including 119 cases with advanced AMD and 99 controls were enrolled in the study. The mean ages of patients and controls were 65.8 and 66.2, respectively. There were no significant differences in age and gender between cases and controls (\( p = 0.69 \) and 0.147, respectively) (Table 1).

Table 2 summarizes the association between advanced AMD and the seven SNPs. All genotype distributions of the SNPs were in Hardy-Weinberg equilibrium in patients and control subjects. The genotypes of \textit{rs1061170 (CFH)}, \textit{rs1410996 (CFH)} and \textit{rs10490924 (HTRA1)} were distributed significantly differently between patients and controls (\( p < 0.05 \)), while other SNPs were not. In addition, \textit{CFH} variant \textit{rs1061170} and \textit{HTRA1} variant \textit{rs10490924} exhibited significant associations with AMD on allelic \( p \) values under the multiplicative model (\( p < 0.05 \)). The odds ratios (ORs) of the risk alleles of \textit{rs1061170} (\( \text{OR} = 0.49, 95\% \text{CI}: 0.29–0.85; p = 0.011 \)) and \( 2.48 (95\% \text{CI}: 1.14–5.41, p = 0.022) \) and 2.11 (95\% CI: 1.37–3.24, \( p = 0.001 \)) were roughly 2.48 (95\% CI: 1.14–5.41, \( p = 0.022 \)) and 2.11 (95\% CI: 1.37–3.24, \( p = 0.001 \)), respectively. Interestingly, we observed that the allele A at \textit{rs3764261} in \textit{CETP} was associated with a reduced AMD risk (\( \text{OR} = 0.49, 95\% \text{CI}: 0.29–0.85; p = 0.011 \)). The allelic of \textit{rs1061170} at \textit{LIPC}, \textit{rs12678919} near \textit{LPL}, \textit{rs1833025} at \textit{ABCA1} and \textit{rs1410996} at \textit{CFH} were not significantly associated with advanced AMD.

Among the 119 cases with AMD, 71 (60\%) had unilateral and 48 (40\%) had bilateral advanced AMD. We compared the distributions of SNPs between unilateral and bilateral advanced AMD. As shown in Supplementary Table 2 (online only), only the genotypes of \textit{rs10468017} (\textit{LIPC}) were distributed significantly differently between unilateral and bilateral advanced AMD (\( p = 0.039 \)). However, it did not exhibit an association with AMD on allelic \( p \) values

### TABLE 2. Distributions of SNPs in patients with advanced AMD and controls.

| Genotypes | Number (freq.%) | \( p \) | Adjusted OR* |
|-----------|-----------------|--------|--------------|
| Genotypes | Number (freq.%) | \( p \) | (95% CI) |
| rs1061170 (CFH) | AMD 86 (72.3) 33 (27.7) | 0.001 | T 205 (86.1) 33 (13.9) 0.022 2.48 |
| Controls 89 (89.9) 10 (10.1) | | | C 188 (95.0) 10 (5.0) [1.14–5.41] |
| rs1410996 (CFH) | AMD 12 (10.1) 52 (43.7) 55 (46.2) | 0.022 | T 76 (31.9) 162 (68.1) 1.36 |
| Controls 16 (16.2) 55 (55.6) 28 (28.3) | | | C 87 (43.9) 111 (56.1) [0.88–2.11] |
| rs10490924 (HTRA1) | AMD 9 (7.6) 47 (39.5) 63 (52.9) | <0.001 | G 65 (27.3) 173 (72.7) 0.001 2.11 |
| Controls 18 (18.2) 55 (55.6) 26 (26.3) | | | T 91 (46.0) 107 (54.0) [1.37–3.24] |
| rs10468017 (LIPC) | AMD 93 (78.2) 24 (20.1) 2 (1.7) | 0.912 | C 210 (87.4) 28 (12.6) 0.709 0.88 |
| Controls 78 (78.8) 20 (20.2) 1 (1.0) | | | T 176 (88.9) 22 (11.1) [0.46–1.68] |
| rs3764261 (CETP) | AMD 85 (71.4) 31 (26.1) 3 (2.5) | 0.377 | C 201 (84.5) 37 (15.5) 0.011 0.49 |
| Controls 63 (49.5) 31 (41.4) 5 (9.1) | | | A 157 (79.3) 41 (20.7) [0.29–0.85] |
| rs12678919 (LPL) | AMD 101 (85.6) 17 (13.6) 1 (0.8) | 0.616 | A 219 (92.0) 19 (8.0) 0.066 1.93 |
| Controls 79 (79.8) 19 (19.2) 1 (1.0) | | | G 177 (89.4) 21 (10.6) [0.96–3.91] |
| rs1833025 (ABCA1) | AMD 71 (59.7) 43 (36.1) 5 (4.2) | 0.282 | C 185 (77.7) 53 (22.3) 0.227 0.72 |
| Controls 69 (69.7) 26 (26.3) 4 (4.0) | | | T 164 (82.8) 34 (17.2) [0.42–1.23] |

*Adjusted OR is calculated by using multivariate unconditional logistic regression analysis with covariates of age, \textit{CFH} and \textit{HTRA1}. Bold values signify that \( p < 0.05 \) was considered statistically significant.
under the multiplicative model \((p = 0.714)\). The distributions of all other SNPs in either genotype or allele were not statistically significant.

By multinomial logistic regression, as shown in Table 3, when compared with control subjects, HTRA1 variant rs10490924 exhibited significant association with increased risk of both unilateral AMD (OR = 2.02, 95% CI: 1.24–3.31; \(p = 0.005\)) and bilateral AMD (OR = 2.41, 95% CI: 1.35–4.30; \(p = 0.003\)), while CFH rs1061170 only confers significantly an increased risk of unilateral AMD (OR = 2.67, 95% CI: 1.14–6.25; \(p = 0.024\)). Notably, CETP variant rs3764261 was found to be associated with reduced risk of both unilateral AMD (OR = 0.52, 95% CI: 0.28–0.98; \(p = 0.043\)) and bilateral AMD (OR = 0.45, 95% CI: 0.22–0.91; \(p = 0.026\)). Rs10468017 in LIPC, rs12678919 near LPL and rs1883025 in ABCA1 were not associated with advanced AMD.

### DISCUSSION

In this study, we described significant associations between advanced AMD and rs1061170 in CFH, rs10490924 in HTRA1 and rs3764261 in CETP in a Chinese cohort. We did not detect a significant association between advanced AMD and genetic variants of LIPC, ABCA1 and LPL genes.

The most severe visual loss due to AMD occurs when the disease progresses to one of the two advanced forms: geographic atrophy (GA) or choroidal neovascularization (CNV).\(^{23}\) Although GA and CNV are pathophysiologically and clinically distinct,\(^ {23}\) they have one common early hallmark, which is drusen between the RPE and Bruch’s membrane.\(^ {24}\) Drusen are extracellular deposits composed mainly of lipids and proteins, including esterified and unesterified cholesterol.\(^ {25–28}\) The mechanism of drusen initiation is unclear, though it has been hypothesized that age-related lipid accumulation in the Bruch’s membrane may induce early physiologic changes and lesions in the RPE.\(^ {29}\) These age-related lipid accumulations may then interact with other lipids, local ligands, or additional secreted self-aggregating proteins, such as those in the complement complex, to manifest as clinically detectable drusen.\(^ {24,29}\)

The CETP gene encodes cholesterylster transfer protein (CETP), which shuttles cholesterol esters from high-density lipoprotein particles (HDL) to low-density lipoproteins (LDL)\(^ {30}\). As HDL supports reverse cholesterol transport to the liver, patients with rare genetic defects in CETP present with numerous lipid abnormalities.\(^ {31}\) Rs3764261 is located in the CETP gene on chromosome 16. In this study, we observed that the A allele of rs3764261 in CETP was associated with a decrease in the risk of AMD (OR = 0.49, 95% CI: 0.29–0.85; \(p = 0.011\)), a finding that is constant with some reports in Caucasian populations.\(^ {32}\) Furthermore, we found that it was also associated with decreased risk of both unilateral and bilateral AMD in our cohort. As abnormal lipid metabolism and accumulation, especially under oxidative stress conditions in the eye as well as systemically, is thought to contribute to the risk of AMD, the role that CETP plays in the pathogenesis of AMD most likely has to do with its transport of cholesterol from the retina to the liver. Interestingly, several other variants of the CETP gene were not observed to be associated with the risk of AMD in a recent study,\(^ {33}\) which did not include the SNP rs3764261. Our study is the first to examine the association between rs3764261 and advanced AMD in Chinese population.

LIPC (hepatic lipase), LPL (lipoprotein lipase) and ABCA1 (ATP-binding cassette transporter A1) regulate triglyceride hydrolysis and plasma high density lipoprotein cholesterol (HDL-c) levels.\(^ {15,34,35}\) Rs10468017 is located in the LIPC gene on chromosome 15, while rs12678919 is near LPL on chromosome 8, and rs1883025 is located in the ABCA1 gene on chromosome 9. Our study showed that the three SNPs of LPL, LIPC, and ABCA1 were not associated significantly with AMD in the Chinese population, although they were associated with AMD in previous genome wide association studies (GWAS) or candidate studies in Caucasian populations.\(^ {15–18}\)

Genetic variants at CFH and HTRA1 were previously reported to be strongly and consistently associated with AMD in Caucasian and Chinese cohorts.\(^ {3–10,34,36}\) Our findings are consistent with those of published reports. For HTRA1 rs10490924, the risk allele T confers a 2.11-fold increased risk of

## TABLE 3. Risk of SNPs for unilateral and bilateral advanced AMD.

| SNP (Gene) | Adjusted OR* | 95% CI     | p Value |
|------------|--------------|------------|---------|
| Unilateral AMD versus Controls |              |            |         |
| rs1061170(CFH) | 2.67         | 1.14–6.25  | 0.024   |
| rs1410996(CFH) | 0.45         | 0.74–1.98  | 1.209   |
| rs10490924(HTRA1) | 2.02        | 1.24–3.31  | 0.005   |
| rs10468017(LIPC) | 0.63         | 0.28–1.38  | 0.245   |
| rs3764261(CETP) | 0.52         | 0.28–0.98  | 0.043   |
| rs12678919(LPL) | 2.38         | 0.99–5.64  | 0.051   |
| rs1883025(ABCA1) | 0.70         | 0.38–1.28  | 0.242   |
| Bilateral AMD versus Controls |              |            |         |
| rs1061170(CFH) | 2.22         | 0.90–5.48  | 0.083   |
| rs1410996(CFH) | 1.76         | 0.98–3.15  | 0.057   |
| rs10490924(HTRA1) | 2.41        | 1.35–4.30  | 0.003   |
| rs10468017(LIPC) | 1.29         | 0.61–2.75  | 0.498   |
| rs3764261(CETP) | 0.45         | 0.22–0.91  | 0.026   |
| rs12678919(LPL) | 1.54         | 0.66–3.58  | 0.317   |
| rs1883025(ABCA1) | 0.75         | 0.39–1.44  | 0.385   |

*Adjusted OR is calculated by using multivariate unconditional logistic regression analysis with covariates of age, CFH and HTRA1.

Bold values signify that \(p < 0.05\) was considered statistically significant.
advanced AMD. For CFH rs1061170, the risk allele T had a 2.48-fold increased risk of advanced AMD. Interestingly, compared with wild genotypes, HTRA1 rs10490924 was significantly associated with both uni- and bilateral AMD, while CFH variant rs1061170 was found to be significantly associated with unilateral but not bilateral AMD.

In conclusion, our data suggest that CETP variant rs3764261 is significantly associated with reduced risk of both uni- and bilateral advanced AMD. The variants rs10468017 in LIPC, rs12678919 near LPL, and rs1883025 in ABCA1 were not associated with advanced AMD in this Chinese cohort. Further studies with a larger sample size would be required to confirm this observation.

DECLARATION OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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