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Nonsynonymous single nucleotide polymorphisms in the complement component 3 gene are associated with risk of age-related macular degeneration: A meta-analysis

Yu Qian-Qian, Yao Yong *, Zhu Jing, Bao Xin, Xie Tian-Hua, Sun Chao, Cao Jia

Department of Ophthalmology, Affiliated Wuxi People's Hospital of Nanjing Medical University, Wuxi, Jiangsu, China

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ABSTRACT

Nonsynonymous single nucleotide polymorphisms (SNPs) in complement component 3 (CC3) are associated with the risk of age-related macular degeneration (AMD), however, this association is not consistent among studies. To thoroughly address this issue, we performed an updated meta-analysis to evaluate the association between nine SNPs in the CC3 gene and AMD risk. A search was conducted of the PubMed database through 3rd Aug, 2014. Odds ratios (ORs) and 95% confidence intervals (CIs) were used to assess the strength of associations. Based on the search criteria for manuscripts reporting AMD susceptibility related to CC3 in nine SNPs, 57 case–control studies from 22 different articles were retrieved. Significantly positive associations were found for the rs2230199 C/G SNP and AMD in the Caucasian population, as well as for the rs1047286 C/T SNP. Moreover, a relationship between the rs11569536 G/A SNP and AMD was detected. By contrast, a negative association was observed between rs2250656 A/G SNP and AMD risk. The present meta-analysis suggests that these four SNPs in the CC3 gene are potentially associated with the risk of AMD development. Further studies using larger sample sizes and accounting for gene–environment interactions should be conducted to elucidate the role of CC3 gene polymorphisms in AMD risk.

1. Introduction

Age-related macular degeneration (AMD) is the major cause of legal blindness in older people in industrialized countries (Jager et al., 2008). The early stages, characterized by subretinal deposits (drusen) on the Bruch membrane and the extracellular matrix separating the choriocapillaris from the retinal pigment epithelium (RPE), affect 15.4% of those aged more than 65 years; the late stages, including abnormal blood vessels growing from the choriocapillaris through the Bruch membrane (wet AMD) and the degeneration of photoreceptors and RPE cells resulting in geographic atrophy (dry AMD), occur in 3.3% of those individuals (Augood, 2006). Genetic and environmental factors, including family history, race, smoking, and dietary factors, are considered important risk factors in AMD etiology (Priya et al., 2012; Swaroop et al., 2009).

The complement system is an important mediator of natural and acquired immunity in humans (Reis, 2006). A dysfunctional complement pathway has been proposed to increase retinal cell damage via increased formation of drusen deposits, atrophy, and cell degeneration and progression to choroidal neovascularization (CNV) (Hageman et al., 2005; Anderson et al., 2002). The central element of the complement cascade, complement component 3 (CC3), is a plausible candidate since its cleavage product, C3a, was found in drusen. Recent studies suggest that genetic variants in the CC3 gene may alter the risk of AMD (Hageman et al., 2001; Johnson et al., 2001).

The human C3 gene is located on chromosome 19 and exhibits nine common genetic SNPs (rs2230199 C/G-R102G, rs1047286 C/T-P314L, rs2241394 G/C, rs2250656 A/G-I90V, rs344542 A/G, rs2230205 A/G, rs339392 T/G, rs3745565 C/G, and rs11569536 G/A-Q1270H). Considering the possibility that AMD results from nonsynonymous mutations of the CC3 gene, a number of studies have explored the association between these polymorphisms and AMD. However, individual studies have yielded inconsistent or conflicting findings, possibly caused by the limitations associated with individual studies. Positive findings were detected in a previously published meta-analysis (Thakkinstian et al., 2011), but this study was not sufficiently large for a comprehensive analysis. Moreover, a number of novel reports (larger individuals and novel SNPs) have been published in the last three years. It is necessary to re-analyze associations.

Abbreviations: CC3, complement component 3; AMD, age-related macular degeneration; OR, odds ratio; SNP, single nucleotide polymorphism; CI, confidence interval; RPE, retinal pigment epithelium; CNV, choroidal neovascularization; PB, population-based; HB, hospital-based; PCR-RFLP, polymerase chain reaction-restrictive fragment length polymorphism; MALDI-TOF MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; GWAS, genome-wide association studies.

* Corresponding author at: Department of Ophthalmology, Affiliated Wuxi People's Hospital of Nanjing Medical University, 214023 Wuxi, Jiangsu, China.
E-mail address: yaoyongmeta@sina.com (Y. Yong).

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To shed light on these contradictory results and to more precisely evaluate the relationships between CC3 gene polymorphisms and AMD risk, we performed an updated meta-analysis of 57 published case–control studies.

2. Materials and methods

2.1. Search strategy

We conducted searches of the PubMed database through 3rd Aug, 2014, using the keywords "age-related macular degeneration," "AMD," "polymorphism," and "complement component 3." With these terms, a total of 101 different articles were retrieved, 22 of which coincided with our inclusion criteria. We also screened references of the retrieved articles and reviewed all articles via a hand search.

2.2. Inclusion and exclusion criteria

For inclusion, studies were required to (i) assess the correlation between AMD risk and CC3 gene polymorphisms; (ii) be case-controlled, and (iii) contain sufficient numbers of genotypes (WW + WM + MM, where W indicates the wild-type allele and M indicates the mutant allele: GG + GC + CC for rs2230199 C/G, TT + TC + CC for rs1047286 C/T, CC + CG + GG for rs2241394 G/C, GG + GA + AA for rs2250656 A/G, GG + GA + AA for rs344542 A/G, GG + GA + AA for rs2230205 A/G, GG + GT + TT for rs339392 T/G, GG + GC + CC for rs3745565 C/G, and AA + AG + GG for rs11569536 G/A) for cases and controls. Studies were excluded if they (i) included no control population; (ii) did not report genotype frequency data, and (iii) were duplicated publications.

2.3. Quality score assessment

The Newcastle-Ottawa Scale (Wells et al.) was selected to assess the quality of each study. This measure assesses aspects of methodology in observational studies related to study quality, including selection of cases, comparability of populations and ascertainment of exposure to risks. The NOS ranges from zero (worst) to nine stars (best). Studies with a score of seven stars or greater were considered as high quality.

2.4. Data extraction

Two authors (Yu QQ and Yao Y) independently extracted all data that complied with the selection criteria. These data included the first author's last name, year of publication, country of origin, ethnicity of the study population, polymorphism site and genotyping method. Ethnicity was categorized as Caucasian or Asian. The control subgroups were population-based (PB) and hospital-based (HB).

2.5. Statistical analysis

Based on the genotype frequencies for cases and controls, crude odds ratios (ORs) with 95% confidence intervals (CI) were used to measure the strengths of associations between the nine SNPs in the CC3 gene and AMD risk. The statistical significance of the OR was determined with the *Z* test. The heterogeneity assumption among studies was evaluated using a χ^2-square-based *Q* test. A *P*-value of >0.10 for the *Q* test indicated a lack of heterogeneity among studies. If significant heterogeneity was detected, the DerSimonian–Laird random-effects model or the Mantel–Haenszel fixed-effects model was chosen (Mantel and Haenszel, 1959; DerSimonian and Laird, 1986). We investigated the relationship between genetic variants of the CC3 gene and AMD risk by testing the allelic contrast (M versus W), heterozygote comparison (MW versus WW), and dominant genetic model (MM + MW versus WW). A sensitivity analysis was performed by omitting studies, one after another, to assess the stability of results. The departure of frequencies of the CC3 polymorphisms from expectation under HWE was assessed by the Pearson's χ^2 test, $P < 0.05$ was considered significant. Publication bias was diagnosed using Egger's linear regression method and funnel plots. A *P*-value <0.05 in Egger's linear regression indicated the presence of a potential publication bias (Hayashino et al., 2005). Bioinformatics tools such as Polyphen and SIFT technology are used to predict the impact of CC3 SNPs at protein level. For Polyphen (http://genetics.bwh.harvard.edu/pph2/), the score ranges from 0.0 (benign) to 1.0 (probably damaging). For SIFT (http://sift.jcvi.org/), if the score <0.05, this SNP may influence the protein function. All statistical tests for this meta-analysis were performed using version 10.0 Stata software (StataCorp LP, College Station, TX, USA).

3. Results

3.1. Study characteristics

Using various combinations of key terms, a total of 101 article titles were garnered by a literature search using the PubMed database. As shown in Supplementary Fig. 1, 50 articles were excluded after screening the Abstract sections of the manuscripts. The full texts were then evaluated, and 29 additional articles were excluded due to duplication (one), meta-analysis (one), polymorphism for Alzheimer's disease (one), review (seven), other SNPs in CC3 (seven), no case–control study (five), lack of SNP data (three), and no data available for cases and controls (four). Finally, 22 different articles (Buentello-Volante et al., 2012; Bergeron-Sawitzke et al., 2009; Edwards et al., 2008; Scholl et al., 2008, 2009; Wu et al., 2013; Cui et al., 2010; Park et al., 2009; Tian et al., 2012; Puche et al., 2013; Havvas et al., 2014; Kim et al., 2012; Smailhodzic et al., 2012; Francis et al., 2009; Despriet et al., 2009; Pei et al., 2009; Gu et al., 2009; McKay et al., 2010; Zerbib et al., 2010; Yates et al., 2007; Reynolds et al., 2009; Seitsonen et al., 2008) were included in our meta-analysis, including 27 studies about rs2230199 C/G, 10 studies about rs1047286 C/T, 2 studies about rs2241394 G/C, 3 studies about rs2250656 A/G, 4 studies about rs344542 A/G, 3 studies about rs2230205 A/G, 2 studies about rs339392 T/G, 3 studies about rs3745565 C/G, and 3 studies about rs11569536 G/A (Table 1). For example, for the rs2230199 C/G site, there were 10,531 cases and 12,051 controls. The frequency of the G allele was found to be significantly higher in control individuals of Caucasian ethnicity than in those of Asian ethnicity (19.6% vs. 0.6%, $P < 0.001$). A similar trend was found for the G allele among Asian and Caucasian individuals in AMD group (26.6% vs. 0.6%, $P < 0.001$) (Supplementary Figs. 2, 3). For the rs1047286 C/T site, there were 4956 cases and 7747 controls. The frequency of the T allele was higher in Caucasians than in Asians in both the case and control groups (Supplementary Figs. 4, 5). The genotyping methods included polymerase chain reaction-restrictive fragment length polymorphism (PCR-FLIP) and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), the MassARRAY Compact System, DNA sequencing, ldSelect, deCODE Genetics, multiplex PCR, primer extension methodology, and the genotyping module of the BeadStudio 3 software. The NOS results showed that the average score was 7.12, which indicated that the methodological quality was generally good (data not shown).

3.2. Quantitative synthesis

3.2.1. Rs2230199 C/G

Results of the overall meta-analysis were suggestive of positive associations between this polymorphism and AMD susceptibility in all three genetic models (dominant model, OR = 1.53, 95% CI = 1.38–1.70, $P_{\text{heterogeneity}} = 0.001$; heterozygote comparison, OR = 1.45, 95% CI = 1.32–1.60, $P_{\text{heterogeneity}} = 0.028$; allelic comparison, OR = 1.46, 95% CI = 1.32–1.60, $P_{\text{heterogeneity}} = 0.000$) (Table 2). Analysis of the ethnicity subgroups indicated a marginally statistically significant association