Association between Polymorphisms of Complement Pathway Genes and Age-Related Macular Degeneration in a Chinese Population

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Purpose. We assessed the association between complement pathway genes and age-related macular degeneration (AMD) in a Chinese population.

Methods. In a case-control study, 165 AMD patients and 216 unrelated controls were recruited from two hospitals in central China. We selected and genotyped six single nucleotide polymorphisms (SNPs) of four complement pathway genes, including rs800292 and rs1410996 of complement H (*CFH*), rs9332739 of complement 2 (*C2*), rs4151667 of complement factor B (*CFB*), and rs2241394 and rs2230199 of complement 3 (*C3*). The associations between SNPs and AMD, adjusted by age and sex, were assessed by using logistic regression models and haplotype association analysis.

Results. In our study, two SNPs of *CFH* and their haplotypes were associated significantly with AMD, and the adjusted odd ratios (ORs) were 2.45 (95% confidence interval [CI] 1.25–4.79) for rs800292 (genotype GG versus AA), 2.49 (95% CI 1.24–5.00) for rs1410996 (genotype TT versus CC), and 4.45 (95% CI 2.32–8.55) for haplotype block of rs800292–rs1410996 (haplotype G–C versus A–C), respectively. The haplotype of *C2/CFB* also was associated significantly with AMD, and the adjusted OR was 8.86 (95% CI 1.88–41.69) for the haplotype block of rs9332739–rs4151667 (haplotype G–A versus G–T), though no relationship was found in genotype association analysis of the two SNPs of *C2/CFB*. With the sample size of our study, no relationship was found for AMD and the two SNPs of *C3*.

Conclusions. Gene variants in *CFH* and *C2/CFB* contribute to AMD in the Chinese population. (*Invest Ophthalmol Vis Sci.* 2013;54:170–174) DOI:10.1167/iovs.12-10453

Age-related macular degeneration (AMD), which causes damage to the retina and results in a loss of vision in the center of the visual field, is the leading cause of irreversible visual decreases and vision impairment in people over age 50 in developed countries.[1–4] AMD is a complex disease derived from inherited and environmental exposures, and the United States twin study of AMD quantified the proportions of variance in this disease due to genetic and environmental factors as 46% to 71% and 19% to 37%, respectively.[5] The complement pathway has been implicated in the pathogenesis of many diseases, and recently variants in several genes, such as complement H (*CFH*),[6–9] complement factor B (*CFB*), complement 2 (*C2*),[10–12] complement 3 (*C3*),[13,14] and complement factor I (*CFI*)[15,16] encoding complement pathway proteins, have been identified associated with AMD. However, the associations between these candidate genes and AMD were varied due to genetic variation within a population (different ethics) in a country and across different populations (different ethics or races) worldwide.[17] Our study aimed to assess the association between genetic variants of the complement pathway genes (*CFH*, *CFB*, *C2*, and *C3*) with AMD in a Chinese population.

MATERIALS AND METHODS

Participants

In our case-control study, 165 AMD patients and 216 health controls were recruited from ophthalmic clinics of Wuhan Union Hospital and Wuhan General Hospital of Guangzhou Military Region. All participants were asked to take comprehensive eye examinations and to take an epidemiologic survey by using a structural questionnaire, which included age, sex, ethnicity, diseases history, and common risk factors of AMD, such as smoking, diet, and health-related lifestyles. AMD patients were diagnosed according to the Age-Related Eye Disease Study (AREDS).[15] Controls had no family relationship with the AMD patients. Our study was conducted in accordance with the tenets of the Declaration of Helsinki. Informed written consents had been obtained from all participants before the study. Blood samples were collected for DNA extraction and single nucleotide polymorphism (SNP) genotyping.

Genotyping

Peripheral venous blood (2 mL) was collected from each participant and stored at −80°C before DNA extraction. A total of 250 μL venous blood was used to isolate genomic DNA by using an AxyPrep Blood DNA Purification Kit (Aisijin Biotech, Hangzhou, China) according to the manufacturer's instructions. We genotyped 5 SNPs in the 356 subjects with the Taqman SNP genotyping assay (Applied Biosystems, Foster City, CA). Master mix for 5 μL reaction was as follows: 2.5 μL 2 ×

Universal PCR Master mix, 0.125 μL 40 × Probe/primer mix, 1.375 μL deionized water, and 1 μL template DNA. The reaction mix was spotted onto six 384-well plates, respectively. Samples were denaturing at 95°C for 10 minutes followed by 40 cycles according to the following parameters: denaturation at 95°C for 15 seconds, and anneal and extension at 60°C for 1 minute. After PCR amplification, allelic discrimination was done with the ABI Prism 7900HT-based quantitative PCR instrument. Every run was recorded and analyzed on ABI software (SDS2.3; ABI, Foster City, CA).

Statistical Analysis

We used the Wald χ^2 test to test the Hardy-Weinberg equilibrium (HWE) of genotype distribution of selected markers in AMD patients and controls, and a probability value $P > 0.05$ was considered a test distribution followed HWE. The association between AMD and the genotype of a given SNP, adjusted by age and sex, was evaluated by using a logistic regression model. The association between AMD and the haplotypes of SNPs on an identical chromosome, adjusted by age and sex, was estimated by using an open source R package hapassoc.[18-20] The hypotheses tests were considered statistically significant if $P < 0.05$ or an adjusted P value $< (0.05/n)$ according to Bonferroni correction for "n times" of multiple comparisons. For the hypotheses test with $P > 0.05$, the statistical power (Power) was calculated to estimate the probability that a negative result ($P > 0.05$) was true.

RESULTS

We enrolled in our study a total of 165 (88 men and 77 women) AMD patients (wet AMD 60 cases and dry AMD 105 cases), aged (mean ± SD) 69.36 ± 10.15 years (range 50-91 years), and 216 unrelated health controls (120 men and 96 women), aged 64.45 ± 8.01 years (range 52-85 years). All study subjects were of Chinese Han ethnicity.

The proportions of female subjects in the AMD and control groups were 46.67% (77/165) and 44.44% (96/216), respectively. The AMD group had a higher proportion of female subjects, though the difference in sex distribution between the AMD group and controls was not statistically significant ($\chi^2 = 0.1863$, $P = 0.6660$). The proportion of persons aged 65 and above in the AMD and control groups was 75.52% (118/165) and 45.37% (98/216), respectively. The AMD group had a higher proportion of elder persons, and the difference in age distribution between the AMD group and controls was statistically significant ($\chi^2 = 26.0432$, $P < 0.0001$). Therefore, we needed to adjust age and sex as a common confounder in the analysis of the association between SNPs and AMD.

We selected six SNPs from four complement pathway genes, including rs800292 (*CFH*), rs1410996 (*CFH*), rs9332739 (*C2*), rs4151667 (*CFB*), rs2241394 (*C3*), and rs2230199 (*C3*), to test the SNPs' association with AMD. All genotype frequencies of all tested SNPs, among controls and AMD patients, followed the Hardy-Weinberg equilibrium ($P > 0.05$).

In the analysis of the association between *CFH* variant and AMD, two SNPs, rs800292 (alleles G/A, G > A) and rs1410996 (alleles T/C, T > C), and their haplotypes were associated significantly with AMD. In allele association and genotype association analysis, the age- and sex-adjusted odd ratios (ORs) of rs800292 were 1.53 (95% confidence interval [CI] 1.12-2.08) for allele G versus A, and 2.45 (95% CI 1.25-4.79) for genotype GG versus AA, respectively (Table 1). Similarly, the adjusted ORs of rs1410996 were 1.42 (95% CI 1.01-1.84) for allele T versus C, 2.49 (95% CI 1.24-5.00) for genotype TT versus CC, and 2.44 (95% CI 1.25-4.73) for genotype TC versus CC, respectively (Table 1). In the haplotype association analysis, the adjusted ORs of block rs800292-rs1410996 were

4.45 (95% CI 2.32-8.55) for haplotypes G-C versus A-C, 4.18 (95% CI 1.99-8.75) for haplotypes A-T versus A-C, and 1.70 (95% CI 1.20-2.41) for haplotypes G-T versus A-C, respectively (Table 2). The results showed not only a strong evidence of association between *CFH* variant and AMD, but also implied strong joint effects of the two SNPs of *CFH*, since the OR of haplotypes G-C of block rs800292-rs1410996 was higher compared to that of genotype association results (point estimation 4.45 vs. 2.45 or 2.49).

In the analysis of the association between *C2/CFB* variant and AMD, the haplotypes of two SNPs, rs9332739 (G/C, G > C) and rs4151667 (T/A, T > A), also were associated significantly with AMD, and the adjusted OR was 8.86 (95% CI 1.88-41.69) for the haplotype block of rs9332739-rs4151667 (haplotypes G-A versus G-T, Table 2), though no relationship was found in genotype association analysis of these two SNPs (Table 1). The results showed not only a very strong evidence of association between *C2/CFB* variants and AMD, but also implied strong joint effects between these two SNPs, since the OR of haplotype G-A of block rs9332739-rs4151667 was higher compared to that of genotype association results (point estimation 8.86 vs. 1.07 or 2.01).

With the sample size of our study, no relationship was found for AMD and the two SNPs of *C3*, rs2241394 (G/C, G > C) and rs2230199 (C/G, C > G), while the results had less statistical power (Power < 0.80, Table 1). The lower statistical powers mainly were due to the very low frequencies of rare alleles in the SNPs of *C3*. For example, the frequencies of rare allele (G) of rs2230199 were 0.6% in AMD patients and 0.9% in controls, respectively (Table 1). There was no association between haplotypes of the block of rs2241394-rs2230199 (Table 2). The results showed no strong evidence of association between *C3* variants and AMD, though they have not enough statistical power (<0.80).

DISCUSSION

In a sample set of AMD patients and controls in a Chinese population, we observed associations between haplotypes in complement pathway genes (*CFH* and *C2/CFB*) and AMD. The results showed that genetic variants at chromosomes 1q32 and 6p21 involved complement pathway are associated strongly with susceptibility to AMD in the Chinese population.

The complement system consists of over 40 proteins and regulators found in the systemic circulation, which has a key role in host defense against pathogens, the elimination of immune complexes and apoptotic cells, and adaptive immune responses.[21,22] Growing evidence suggests that *CFH*, a key regulator of the complement system of innate immunity, has a key role in the development of AMD. The variant in *CFH* on chromosome 1q32 was the first major AMD susceptibility allele.[6] Many independent studies showed that the *CFH* gene has been identified as a causal polymorphism in western and Chinese populations.[6-9,23,24] Our results showed strong evidence that the genotypes and the haplotypes of two SNPs (rs800292 and rs1410996) of the *CFH* variant associate with AMD, and the joint effects might be strong for these two SNPs of *CFH*, but this finding must be replicated in future studies.

In the European-American population, a study showed that combined analysis of the *CFB* and *C2* haplotypes, and *CFH* variants revealed that variation in the two loci can predict the clinical outcome in 74% of the affected individuals and 56% of the controls.[10] *CFB* and *C2* have nearly identical modular structures, and both regulate the production of *C3*. The genetic effects of *CFB* and *C2* polymorphisms are known to be important for AMD in the Caucasian population.[11] However, the associations between *C2/CFB* and AMD were not

TABLE 1. Allele Association and Genotype Association between Polymorphisms of Complement Pathway Genes and AMD

SNP ID (Allele)	Designation (Location)	Allele	Allele Association					Genotype	Genotype Association			
			AMD n (%)	Control n (%)	OR* (95% CI)	P	Power	Genotype	AMD n (%)	Control n (%)	OR* (95% CI)	P
rs800292 (G/A, G > A)	*CFH*, 162V Exon 2 (1q32)	A	105 (31.8)	179 (41.4)	Ref			GG†	77 (46.7)	75 (34.7)	2.45 (1.25, 4.79)‡	0.0093**
		G	225 (68.2)	253 (58.6)	1.53 (1.12, 2.08)	0.0069	–	GA	77 (44.0)	103 (47.7)	1.37 (0.87, 2.17)§	0.1779
								AA	17 (10.3)	38 (17.6)	1.65 (0.85, 3.21)¶	0.1391
rs1410996 (T/C, T > C)	*CFH* Intron 14 (1q32)	C	117 (34.5)	183 (42.4)	Ref			TT†	64 (38.8)	74 (34.3)	2.49 (1.24, 5.00)‡	0.0101**
		T	213 (65.5)	249 (57.6)	1.42 (1.01, 1.84)	0.0473	–	TC	85 (51.5)	101 (46.8)	0.92 (0.58, 1.46)§	0.7376
								CC	16 (9.7)	41 (18.9)	2.44 (1.25, 4.73)¶	0.0086**
rs9332739 (G/C, G > C)	*C2*, E318D Exon 7 (6p21.3)	G	311 (97.8)	422 (97.7)	Ref			CC†	0 (0)	0 (0)	NA	
		C	7 (2.2)	10 (2.3)	1.07 (0.41, 2.81)	0.8888	0.0502	GC	8 (4.9)	10 (4.6)	NA	
								GG	157 (95.1)	206 (95.4)	1.07 (0.40, 2.85)¶	0.8874
rs4151667 (T/A, T > A)	*CFB*, L9H Exon 1 (6p21.3)	T	311 (96.1)	422 (97.7)	Ref			AA†	0 (0)	0 (0)	NA	
		A	13 (3.9)	10 (2.3)	1.97 (0.84, 4.63)	0.1121	0.2563	TA	13 (7.9)	10 (4.6)	NA	
								TT	152 (92.1)	206 (95.4)	NA	
rs2241394 (G/C, G > C)	*C3* Intron (19p13.2)	G	277 (91.7)	399 (92.4)	Ref			GG†	128 (84.8)	183 (84.7)	2.01 (0.84, 4.82)¶	0.1159
		C	25 (8.3)	33 (7.6)	1.03 (0.59, 1.79)	0.9235	0.0655	GC	21 (13.9)	33 (15.3)	1.26 (0.68, 2.31)‡	0.4671
								CC	2 (1.3)	0 (0)	NA	
rs2230199 (C/G, C > G)	*C3*, R102G Exon 3 (19p13.2)	G	2 (0.6)	4 (0.9)	Ref			CC†	163 (98.8)	212 (98.6)	1.16 (0.20, 6.67)‡	0.8679
		C	328 (99.4)	428 (99.1)	1.16 (0.20, 6.60)	0.8686	0.0692	CG	2 (2.1)	4 (1.4)	NA	
								GG	0 (0)	0 (0)	NA	

NA, the OR was not available where the number of individuals with two copies of the risk allele was zero; Ref, the reference group in groups' comparisons.

* ORs were adjusted by age and sex.

† Homozygous for the risk allele.

‡ Comparing the likelihood of AMD among individuals with two copies of risk allele versus individuals with no copies of risk allele.

§ Comparing the likelihood of AMD among individuals with two copies of risk allele versus one copy of risk allele.

¶ Comparing the likelihood of AMD among individuals with one copy of risk allele versus no copy of risk allele.

** ORs represent significance at α (0.05/3) = 0.0167 (Bonferroni correction for 3 times of multiple comparisons).

TABLE 2. Age and Sex Adjusted Haplotype Association Analysis in AMD Patients and Controls

Haplotype*	% Frequencies (95% CI)†	OR (95% CI)‡	*P*
Block 1 (1q32), *CFH*: rs800292-rs1410996			
A–C	37.71 (34.1, 41.32)	Ref	
G–C	3.63 (2.53, 4.73)	4.45 (2.32, 8.55)	0.0000§
A–T	4.69 (3.49, 5.89)	4.18 (1.99, 8.75)	0.0002§
G–T	53.97 (50.21, 57.74)	1.70 (1.20, 2.41)	0.0029§
Block 2 (6p21.3), *C2/CFB*: rs9332739-rs4151667			
G–T	97.32 (96.13, 98.51)	Ref	
G–A	0.41 (0.19, 0.63)	8.86 (1.88, 41.69)	0.0058§
C–T	0.50 (0.15, 0.84)	3.84 (0.74, 20.02)	0.1102
C–A	1.78 (0.67, 2.89)	0.37 (0.07, 2.05)	0.2546
Block 3 (19p13.2), *C3*: rs2241394-rs2230199			
G–C	91.1 (88.95, 93.26)	Ref	
G–G	8.07 (6.05, 10.09)	0.87 (0.49, 1.55)	0.6445
C–G	0.60 (0.01, 1.19)	0.95 (0.16, 5.50)¶	0.9514
C–C	0.23 (0.00, 0.62)		

* The risk haplotypes are noted in roman font, and the protective haplotypes are noted in italic font.
† The frequencies of haplotypes, from subsequent iterations of the EM algorithm in hapassoc (an open sources R package), were estimated based on the genotype data from all subjects combined.
‡ The ORs, listed in descending order, were age- and sex-adjusted in generalized linear models (GLM) by using the hapassoc package.
§ ORs with $P < 0.05$.
¶ Two rare haplotypes of block 3 (rs2241394–rs2230199), C–G and C–C, were pooled together to prevent estimation algorithm failed to converge due to very low frequency of rare alleles.

consistent in the Asian population, partly due to low risk allele frequency of these SNPs.[23,25] In our study, we found for the first time to our knowledge a strong evidence of association with haplotype block rs9332739–rs41516677 (*C2/CFB*). The strong joint effects still imply some potential interaction effects of these two SNPs of *C2/CFB*, but this finding also must be replicated in future studies

C3 is a key complement protein, and its cleavage into C3a and *C3b* is central to complement pathways.[21,26] The association between *C3* and AMD has been confirmed in many studies on the subjects of western populations,[13,14,23,26–28] but the evidences were not strong in the studies on the subjects from Asian populations.[24,25]. With the sample size of our study, no relationship was found for AMD and two SNPs, rs2241394 and rs2230199, of *C3* gene, which was consistent with the negative findings of a few previous studies in Asian populations.[24,25,29] However, the statistical powers of the negative findings were below 0.80 due to very rare risk allele, which might lead to false negative findings. Furthermore, we also cannot exclude the possibility that other variants of *C3* may have a role in the pathogenesis of AMD in a Chinese population. Therefore, the relationship between *C3* and AMD must be tested in future lager sample size studies, in which more variants of *C3* should be tested.

Our study has two limitations. Firstly, the sample size was not large enough to detect rare allele association in variants of *C3*. Secondly, the haplotypes, estimated from population data, could introduce uncertainty for rare alleles.[30] However, those limitations might have minor effects on SNPs of *CFH* variants and *C2/CFB* variants, given the facts that most of our genotype analysis results were consistent with the results reported in an Asian population.

In summary, increasing evidence shows that genetic effects of complement pathway genes have key roles in development of AMD in various ethnic groups worldwide. Our study not only confirmed some reported findings of the association between *CFH* variants and AMD, but also, for the first time to our knowledge, found a significant association between a haplotype of *C2/CFB* and AMD in the Chinese population.

Based on these findings, we concluded that the gene variants in *CFH* and *C2/CFB* contribute to AMD in the Chinese population.

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