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Clinical phenotypes associated with the Complement Factor H Y402H variant in age-related macular degeneration

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Abstract

Purpose—To determine whether the complement factor H (CFH) Y402H variant is associated with specific age-related macular degeneration (AMD) clinical phenotypes.

Design—Retrospective, case-control study.

Methods—188 Caucasian subjects with AMD and 189 control subjects were genotyped for the T-to-C polymorphism in exon-9 of the CFH gene by restriction-fragment length analysis and DNA sequencing using genomic DNA from mouthwash samples. AMD phenotypes were characterized by clinical examination, fundus photography, and fluorescein angiography.

Results—Heterozygosity for the at-risk genotype (TC) increased the likelihood for AMD 2.1-fold (95% CI 1.3–3.3) while homozygosity for the genotype (CC) increased the likelihood for AMD 6.5-fold (95% CI 3.4–12.5) in our population. The C allele was significantly associated with predominantly classic choroidal neovascularization (OR 2.01, 95% CI 1.34–3.30). Neovascular lesion size was similar among the three genotypes ($p=0.67$).

Conclusions—The Y402H CFH variant carried a significantly increased risk for developing AMD in our population. Genotype/phenotype correlations regarding choroidal neovascular lesion type were observed

Introduction

Age-related macular degeneration (AMD) is the most common irreversible cause of severe vision loss throughout the world in individuals over the age of 50. Studies over the past decade pointed to a link between AMD and inflammation,^{1–4} a relationship that was confirmed recently when a coding variation (Y402H) in the complement factor H (CFH) gene on chromosome 1q32 was determined to be strongly associated with AMD.^{5–8} The predicted tyrosine-to-histidine substitution at amino acid position 402 in the CFH protein is the result of a T-to-C transition at nucleotide position 1277 in exon-9 of the gene. In these initial studies, the presence of a single C at this position (genotype TC) led to a significantly increased risk of AMD (with odds ratios ranging from 2.5 to 4.6) while homozygosity (genotype CC) resulted in an even higher risk (odds ratios ranging from 3.3 to 7.4). Additional reports have confirmed

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the association of the CFH Y402H variant with AMD in numerous populations throughout the world,^{9–19} although this relationship does not appear to be present in the Japanese population.^{20,21} A meta-analysis of eight reports calculated the population attributable risk for the combined genotypes CC and TC to be 58.9%,²² implying that the CFH-Y402H polymorphism contributes to over half of all AMD. Recent work has discovered additional CFH polymorphisms that are also associated with AMD,^{23,24} and the role of complement in AMD pathogenesis is further supported by the discovery that variations in complement factor B and complement component 2 genes are also linked to AMD.²⁵

The CFH Y402H polymorphism has been associated with both exudative and advanced atrophic AMD,^{10,17} but few data are available evaluating AMD clinical presentations with regard to CFH genotype.²⁶ The purpose of this study was to determine if there is an association between AMD clinical phenotypes and CFH genotype.

Methods

Patients and clinical examination

This study was approved by the Washington University Human Research Protection Office and the Barnes Retina Institute Study Center and was conducted in accordance with HIPAA regulations. All participants were enrolled from the clinical offices of the Barnes Retina Institute and signed written informed consent prior to participation.

Mouthwash samples were collected from 203 Caucasian subjects with AMD (Age Related Eye Disease Study²⁷ category 3 or 4 in at least one eye). AMD phenotypes were characterized by clinical examination including dilated fundus exam, fundus photography, and fluorescein angiography. Fluorescein angiograms obtained upon initial presentation of active choroidal neovascular lesions were classified as either predominantly classic (>50% classic: a definable vascular complex appearing early in the angiogram followed by late leakage), minimally classic (<50% classic), or occult (leakage appearing only late in the angiogram with no classic component). Disciform scars were not included in the lesion type analysis. Angiograms were read independently by two retina specialists (MAB and RSA) masked to CFH genotype. Any discrepancies in lesion classification were openly adjudicated. The majority of the angiograms (57%) were obtained digitally with a Zeiss fundus camera and imaging software (OIS, Sacramento, CA); the remainder were obtained using film with a Zeiss FF4 fundus camera. Snellen visual acuity was recorded for all AMD subjects at initial presentation. Control subjects (n=205) were Caucasian patients from the Barnes Retina Institute over the age of 55 with diagnoses other than AMD. Exclusions for enrollment included history of ocular infection, trauma, or tumor.

DNA preparation and genotyping

Participants provided buccal tissue samples by expectorating into 50 ml conical tubes (Falcon) after vigorously rinsing for 30 seconds with 20 ml Scope mouthwash (Procter & Gamble). Genomic DNA was prepared from buccal cells using the Puregene kit (Gentra Systems) and quantified by absorbance at 260nm (GeneQuant, Pharmacia). Exon 9 of the CFH gene was PCR-amplified using AmpliTaq Gold Universal PCR Master Mix, (ABI) and gene-specific primers located in intron-8 (5'-cttggtagtaacttagtctgtcttcag) and intron-9 (5'-acaaggtgacataaacatttggcc). For restriction fragment length analysis, amplicons (~445 bp) were digested with Hsp92 II (Promega) and sized on 2.5% agarose-gels stained with SYBR Gold (Molecular Probes). For sequence analysis, amplicons were treated with ExoSAP-IT (USB), then cycle-sequenced using the Big Dye Terminator Ready Reaction Mix (ABI) and nested primers in the forward (5'-tcattgttatggctctag) and reverse (5'-catgtaactgtgtgtctgcgc) directions,

and analyzed by capillary-electrophoresis on a 3130xl Genetic Analyzer running SeqScape software (ABI).

Data Analysis

Descriptive statistics for all demographic and clinical variables were calculated. Comparisons were made between cases and controls using chi-square tests for the nominal variables and t-test for continuous variables. The association between genotype (or other potential risk factors) and AMD status was assessed using logistic regression. Univariate analysis was conducted and any risk factor with an association at the $p \leq 0.10$ level was included in the final multivariate model along with risk factors known to be associated with risk of AMD as a result of previous epidemiological research. Odds ratios with 95% confidence intervals are reported.

Results

A total of 203 patients with AMD (AMD group) and 205 age-matched patients without AMD (control group) were recruited over a six-week period. Of 408 mouthwash samples collected, 377 (92.4%) yielded DNA of ample quantity (~20 kb) and concentration (50–100 µg) for successful genotyping by restriction fragment length analysis and DNA sequencing.

Genotyping for the CFH-Y402H polymorphism was performed for 188 AMD cases and 189 controls (Table 1). The mean age of the AMD patients was 78.8 (SD=8.0) years and the control group was 69.5 (SD=9.5) years ($p<0.001$). The percentage of female patients was 63% in the AMD group and 61% in the control group. The CFH-Y402H genotypes in our study are summarized in Table 1. The at-risk CC genotype was seen in 30.3% of AMD patients and 9.5% of control subjects. The overall frequency of the C allele was 55% in the AMD group compared to 34% in the control group ($p<0.001$). Individuals with at least one copy of the C allele were 2.1 times more likely to have AMD than those without a C allele (95% CI 1.3–3.3). Homozygous CC individuals were 6.5 times more likely to have AMD than homozygous TT and heterozygous TC individuals (95% CI 3.4–12.5). Adjustment for age and gender did not affect these associations.

Clinical phenotypes were examined in 184 AMD subjects with complete follow-up data to determine if there were associations of clinical findings and the different CFH genotypes (Table 2). Of these subjects, 153 (83.2%) had exudative AMD, defined by the presence of an active choroidal neovascular complex or fibrovascular scar in one or both eyes. The homozygous genotype (CC) had the highest percentage of exudative AMD (89.3%), followed by TC group (84.4%) and the TT group (76.3%) ($p=0.24$).

Fluorescein angiograms obtained upon initial presentation of active choroidal neovascular lesions were classified as predominantly classic, minimally classic, or occult. These angiograms were graded independently by two retina specialists (intergrader correlation $\kappa=0.76$) masked to patient genotype. In eyes with gradable lesions ($n=185$), the CC genotype had the greatest percentage of predominantly classic lesions (47.0%), compared to 36.9% for the TC genotype and only 17.1% for the TT genotype. This difference was statistically significant. Specifically, for each additional C allele, there was a significantly increased likelihood of having predominantly classic lesion (OR 2.01, 95% CI 1.34–3.00) (Table 2).

To compare choroidal neovascular lesion size among the CFH genotypes, we measured the greatest linear dimension (GLD) of the lesions on the angiogram obtained at the time of initial presentation of active choroidal neovascularization. The mean GLD for lesions in patients with the CC genotype was 2816 µm, compared to 2816 µm for the TC genotype, and 3064 µm for the TT genotype. These values were not significantly different ($p=0.67$).

Discussion

The purpose of this study was to examine the relationship between the CFH genotypes and AMD clinical phenotypes to give clinicians a better understanding of the clinical significance of the Y402H variant. We collected mouthwash samples for DNA analysis from more than 400 subjects over a six-week period, demonstrating this method of sample collection to be efficient and to have a negligible impact on the flow of a busy clinical practice. The odds ratios linking CFH to AMD, 2.1 (95% CI 1.3–3.3) for the TC genotype and 6.5 (95% CI 3.4–12.5) for the CC genotype, are consistent with findings reported by other groups.

There have been few previous reports examining AMD clinical phenotypes and their relationship to CFH. We first looked at whether there was an association of the CFH-Y402H genotype with exudative AMD. In our study population, for each additional C allele present, there was an increased likelihood of having exudative disease, but this difference was not statistically significant. Because AMD patients were recruited from a primarily referral-based retinal subspecialty practice, it is not surprising that a large majority of the patients (82.3%) had exudative AMD. This high prevalence of exudative disease likely limited our ability to detect a significant difference in risk of exudative AMD based on CFH genotype, but it is nevertheless interesting that the percentage of patients with exudative AMD increased with each additional C allele.

We wished to know if there was any relationship between the CFH-Y402H genotype and the type of neovascular lesion that formed, as this may have implications in a lesion's response to treatment. We identified a significant association of the C allele with predominantly classic lesions. Predominantly classic lesions are felt to have the poorest natural history,²⁸ and these data interestingly suggest an association of the (at-risk) CC genotype with the more aggressive phenotype of exudative AMD. Previous studies have reported various rates of predominantly classic lesions,^{29,30} but a widely quoted figure is that predominantly classic lesions make up about 25% of all choroidal neovascular complexes secondary to AMD.³⁰ In our patients with the CC genotype (n=66), the percentage of predominantly classic lesions was close to twice that (47.0%). In fact, compared to the TT group, we observed predominantly classic lesions at a rate 2.2 times greater for the TC group and 2.7 times greater for the CC group, suggesting an important relationship between the presence of the C allele and the predominantly classic lesion subtype. Therefore, while our study did not identify an association of the CFH-Y402H variant with the *presence* of neovascularization in our patients with AMD, we did see an association with the *type* of neovascular lesion in these patients. This suggests that the development of neovascular tissue may be more dependent on other factors (e.g. other genes and environmental factors such as smoking), but that the CFH variant may have a role in determining retinal or choroidal architecture such that it affects the type of lesion that forms.

This study is limited by our use of selected samples from a case-control study to make genotype-phenotype correlations, and by the sample sizes. However, we believe this study has value as an investigation of AMD genotype-phenotype correlations, and that these data can serve as a starting point for prospective trials evaluating such associations. In summary, we have utilized a rapid, efficient method for collecting samples suitable for genotypic analysis in AMD patients and described relationships between CFH genotypes and choroidal neovascularization lesion type.

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Biography

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Table 1
Demographics and CFH genotype for AMD patients and controls

Characteristic	AMD	Controls	Odds Ratio (95% CI) for AMD
Total number	188	189	
Age, mean (SD) yr	79.8 (8.0)	69.5 (9.5)	
Female, ratio	0.63	0.61	
Frequency of C allele	55%	34%	
TT genotype (%)	38 (20.2)	78 (41.3)	
TC genotype (%)	93 (49.5)	93 (49.2)	2.1 (1.3–3.3)
CC genotype (%)	57 (30.3)	18 (9.5)	6.5 (3.4–12.5)

CFH = complement factor H; AMD = age-related macular degeneration

Table 2

Clinical phenotypes of AMD patients by CFH genotype.

Characteristic	CC	TC	TT	p value/Odds Ratio
Total number of patients	56	90	38	
AMD patients with exudative AMD (%)	50 (89.3)	76 (84.4)	29 (76.3)	0.24
Eyes with predominantly classic lesions (% of gradable lesions)	31/66 (47.0)	31/84 (36.9)	6/35 (17.1)	OR=2.01 (CI 1.34–3.30) [*]
GLD of gradable lesions (μm)	2816	2816	3064	0.67

CFH = complement factor H; AMD = age-related macular degeneration; GLD = greatest linear dimension;

*
For each additional C allele.