Complement Factor H Variant Y402H Is a Major Risk Determinant for Geographic Atrophy and Choroidal Neovascularization in Smokers and Nonsmokers


PURPOSE. The complement factor H (CFH) gene polymorphism Y402H (1277T–C) has been associated with susceptibility to age-related macular degeneration (AMD). The purpose of this study was to confirm this association in a U.K. population, to determine whether the association holds for both geographic atrophy (GA) and choroidal neovascularization (CNV), and to investigate interactions with smoking.

METHODS. A case–control study was undertaken in 443 cases of AMD, with 262 spouses as control subjects. All subjects completed a health and lifestyle questionnaire, had an ophthalmic assessment with fundus photography, and were genotyped.

RESULTS. The frequencies of the C allele and CC genotype were significantly higher in cases than in controls. In comparison to the TT genotype, the odds ratios for AMD associated with the CT and CC genotypes were 3.1 (CI 2.0–4.6) and 6.5 (CI 3.8–10.4), respectively. The results were similar in subgroup analyses confined to cases with GA or CNV. The findings were also similar for subgroup analyses restricted to subjects who had never smoked, moderate smokers, or heavier smokers (>20 pack years of smoking). Heavier smokers with the CC genotype may be particularly at risk. The frequency of the CC genotype did not differ significantly between cases with and without a family history of AMD. There was no evidence that genotype had any influence on age at onset of disease.

CONCLUSIONS. The CFH Y402H variant is strongly associated with both GA and CNV in the U.K. population. This association is similar in smokers and nonsmokers. Heavier smokers with the CC genotype may be at particular risk. (Invest Ophthalmol Vis Sci. 2006;47:536–540) DOI:10.1167/iovs.05-1143

Age-related macular degeneration (AMD) is the leading cause of visual impairment in the elderly and the commonest cause of blindness in Western populations.1 As such, it represents a major public health problem, and improvements in life expectancy are increasing the number of persons at risk. The condition is characterized in the early stages by the deposition of macular drusen. Later, the disease manifests as extensive atrophy of the retinal pigment epithelium and overlying photoreceptor cells (geographic atrophy, GA) or aberrant choroidal angiogenesis (choroidal neovascularization, CNV) leading to loss of central vision. The pathogenesis of AMD is incompletely understood. In common with other late-onset chronic diseases, susceptibility is influenced by age, ethnicity, and a combination of environmental and genetic risk factors. The adverse effects of smoking are well established,1 and family history is an important risk factor.2

Efforts have been made to identify susceptibility genes for AMD, because this would contribute to understanding the pathogenesis of the disease and could lead to novel strategies for therapy or prevention. Recent reports have shown that a common polymorphism Y402H in the complement factor H (CFH) gene is a major predictor of risk for AMD.3–7 The single nucleotide change 1277T→C present in approximately a third of copies of the gene and resulting in the substitution of histidine for tyrosine at codon 402 of the CFH protein is associated with a more than twofold increase in risk of AMD in CT heterozygotes and a three- to sixfold increase in risk in CC homozygotes compared with the TT genotype.3–7 It seems likely from the genetic data that this amino acid substitution itself is responsible for influencing the risk of AMD rather than being a marker for a causal variant elsewhere in the gene.3–5 CFH is a key regulator of the alternative complement pathway—preventing uncontrolled complement activation. The demonstration that the CFH gene is implicated in AMD, together with the finding that drusen contain proteins associated with inflammation and immune-mediated processes,9 supports the hypothesis that inflammation and complement activation play a role in the formation of drusen and the pathogenesis of AMD.

The first purpose of this study was to confirm the association between Y402H and AMD in the U.K. population and explore the relationship with the two end-stage manifestations of AMD—geographic atrophy and choroidal neovascularization—which have not been addressed in detail in published reports. Because more than half the population carry at least one copy of the C allele but the majority never have AMD, there must be further genetic and environmental factors that influence risk. An important question is whether Y402H and smoking are independent risk factors, and investigating this was the second objective of the study.

METHODS

Subjects

A case–control study was undertaken in white subjects. Unrelated cases with end-stage AMD were compared with spouse control sub-
jects. Most of the patients were ascertained from hospital ophthalmic clinics in the East Anglia region of England and the remainder from general practices, optometrists, and charitable societies for people with visual impairment. Patients with a spouse willing to act as a control subject were prioritized for recruitment, but cases without a spouse were also accepted. Informed consent was obtained from all subjects after a full explanation of the purpose and nature of the study. The research protocol was in keeping with the Declaration of Helsinki, and Multicenter Research Ethics Committee and Local Research Ethics Committee approvals were obtained.

All study subjects were examined by an ophthalmologist and completed a health and lifestyle questionnaire modified from that used for the European Prospective Investigation of Cancer (EPIC) study. This included family history and a detailed smoking history. All subjects had color stereoscopic fundus photography of the macular region (field 2 of the modified Airlie House classification), and photographs were graded according to the International Classification of Age-Related Maculopathy and Macular Degeneration. Patients were accepted as cases if they were confirmed to have geographic atrophy or choroidal neovascularization in one or both eyes. Spouses with a normal macula or early changes of age-related maculopathy were accepted as control subjects. The control group therefore included subjects with non-ex- tensive small or intermediate drusen and minimal hypo- or hyperpig- mentation and matched the criteria for groups 1 and 2 of the AREDS trials. Spouses found to have AMD were reclassified as cases. All subjects were selected to be more than 50 years of age and were excluded if they had greater than 6 D of myopic refractive error. If pseudophakic or aphakic, they were included only if a preoperative refraction was available. Cases and control subjects were excluded if they had evidence of inflammatory or retinovascular disease, such as retinal vessel occlusion, diabetic retinopathy, or choriororetinitis, that could contribute to the development or confound the diagnosis of maculopathy. Fundus photographs were graded at the Reading Centre, Moorfields Eye Hospital and were subjected to both a preliminary and final grading process by investigators who had no knowledge of their provisional status as cases or controls. Discrepancies between prelimi- nary and final grading were adjudicated by an experienced ophthalmologist (ACB). Discrepancies between photographic grading and clinical examination were decided by reference to the medical records and any previous color photographs or fluorescein angiography.

Pack years of cigarette smoking was taken as the best measure of smoking behavior, because we have shown that this variable is the one most strongly associated with risk of development of AMD (data reported elsewhere). The number of cigarettes smoked daily in each decade of life was obtained from the questionnaire. To calculate pack years of smoking, the average number of cigarettes smoked per day was divided by 20, to give packs per day, and multiplied by the total number of years of smoking. Age at onset of disease was taken as the age at which vision was first noted to be significantly impaired and AMD was subsequently found to be the cause, or if vision impairment was not initially noted (such as in unilateral AMD) the age at which a diagnosis of AMD was first made by an ophthalmologist.

Genotyping

Genomic DNA was extracted from peripheral blood leukocytes by using a standard protocol. The region of CFH exon 9 spanning the Y402H polymorphism was amplified by PCR. Genotyping was performed on a genetic analyzer according to the manufacturer’s instructions (Prism SNAPSHOT dNTP Primer Extension Kit and a 3100 Genetic Analyser; Applied Biosystems, Inc., [ABI], Foster City, CA).

Statistical Analysis

The χ2 test was used for comparisons of categorical variables and allele and genotype frequencies and to check for Hardy-Weinberg equilib- rium. The Mann-Whitney test was used to compare age and pack years of smoking in cases and controls and to investigate age at onset of disease as a function of genotype. Logistic regression analysis was used to investigate interactions between CFH Y402H genotype and other variables and to estimate odds ratios and 95% confidence intervals. The covariables age, family history of AMD, and pack years of cigarette smoking were included in the model when appropriate and if significant in prior univariate analysis. Odds ratios for categorical variables were estimated in relation to a reference category. Data were analyzed on computer (SPSS ver.11.0; SPSS Inc., Chicago, IL).

RESULTS

The study comprised 443 cases with end-stage AMD and 262 spouse controls. In 265 cases, CNV was the only manifestation of AMD; in 106, only GA; and in 72, a mixed phenotype of both CNV and GA. There was no significant difference in gender between cases and controls, with 243 (54.9%) and 153 (58.4%) being women, respectively. The mean age of cases was 80.3 ± 6.9 (SD) years and of controls 75.8 ± 7.8 years, a significant difference (P < 0.0005). A family history of AMD was reported by 81 (18.3%) cases and 5 (1.9%) controls (P < 0.0005). The mean number of pack years of cigarette smoking was 15.4 ± 18.8 in the cases and 10.7 ± 14.5 in the controls (P = 0.01).

Allele and genotype data for the Y402H polymorphism in the complement factor H gene are given in Table 1. The genotype frequencies in cases and controls were in Hardy-Weinberg equilibrium. Compared with controls the frequency of the C allele was significantly higher in the cases, and this difference was present in subgroups with GA only, CNV only, and a mixed phenotype. The frequency of the CC genotype was significantly higher in the cases and in all three phenotypic subgroups compared with the controls. The results were similar when the data were analyzed taking into account smoking history (Table 2), with the CC genotype being significantly higher in cases than in controls in subjects who had never smoked, moderate smokers (0.1–20 pack years of cigarettes), and heavier smokers (>20 pack years of smoking).

The results of logistic regression analysis are given in Table 3, with all comparisons made to the TT genotype. The odds ratio for AMD associated with the CC genotype was 6.3 (CI 3.8–10.4), twice that associated with the genotype CT. The results were similar if the analysis was confined to cases with GA only or CNV only. The CC genotype was associated with a substantially increased risk of AMD in smokers and in subjects who had never smoked. The data suggested a particularly high risk for CC homozygotes who were heavier smokers, but it should be noted that this was not simply the result of a higher frequency of the CC genotype among such cases but was partly attributable to a lower frequency of this genotype among controls who smoked, particularly in those who were heavier smokers (Table 2).

In cases with a family history of AMD, the frequencies of the CC, CT, and TT genotypes were 45.7% (n = 37), 43.2% (n = 35), and 11.1% (n = 9) respectively. In cases without a family history of AMD the corresponding frequencies were 35.9% (n = 130), 46.7% (n = 169), and 17.4% (n = 65) but the differences in genotype frequencies were not statistically signifi- cant (P = 0.18). In the five controls with a family history of AMD, two were CC homozygotes, one was heterozygous for CT, and two were homozygous for TT. There was no evidence that genotype had any influence on age at onset of disease, with mean age at onset in cases with TT, CT, and CC genotypes being 73.7 ± 10.1, 73.6 ± 8.5, and 73.5 ± 7.8 years, respec- tively.

DISCUSSION

We have shown a strong association between age-related macular degeneration and the C allele of the Y402H polymorphism
in the complement factor H gene in whites in a U.K. population. In comparison with the TT genotype, the odds ratios for AMD associated with the CT and CC genotypes were 3.1 (CI 2.0–4.6) and 6.3 (CI 3.8–10.4), respectively. These results are similar to the published findings for the North American population.3–7

There is likely to be much overlap in the disease processes leading to GA and CNV; and, not uncommonly, these occur together in the same or fellow eyes. However, there is a moderate degree of concordance between eyes and also between affected relatives in familial cases of AMD which raises the possibility of environmental or genetic factors favoring one outcome over the other. The published association studies of Y402H in AMD include cases with both GA and CNV and sometimes early AMD as well, but with one exception, these investigators did not report separate odds ratios for AMD subtypes. Haines et al.4 reported that when their analysis was restricted to CNV, the odds ratios were higher, being 3.4 and 5.6 for the CT and CC genotypes, respectively. Zareparsi et al.7 noted that the frequency of the CC genotype was increased in both GA and CNV and sometimes early AMD as well, but with one exception, these investigators did not report separate odds ratios for AMD subtypes. Haines et al.4 reported that when their analysis was restricted to CNV, the odds ratios were higher, being 3.4 and 5.6 for the CT and CC genotypes, respectively. Zareparsi et al.7 noted that the frequency of the CC genotype was increased in both GA and CNV.7 Hageman et al.6 reported that associations with markers spanning the CHF locus were particularly strong in cases with early AMD and CNV but less so in cases with GA. In our data, the odds ratios for AMD, GA, and CNV are very similar, providing good evidence that the Y402H variant is similar, providing good evidence that the Y402H variant is influencing the risk of AMD rather than being a marker for a causal variant elsewhere in the gene. It had previously been suggested that this variant may have functional implications,15 and this marker showed the strongest association with AMD in the genetic analysis.3–5 CFH is a key regulator of the alternative complement pathway preventing uncontrolled complement activation which would

### Table 1. Allele and Genotype Frequencies for the Y402H Polymorphism in the Complement Factor H Gene in Cases and Controls

<table>
<thead>
<tr>
<th></th>
<th>Controls n = 262</th>
<th>GA Only n = 106</th>
<th>CNV Only n = 265</th>
<th>Mixed Cases n = 72</th>
<th>All AMD n = 443</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Alleles</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T</td>
<td>354 (63.7)</td>
<td>80 (37.7)</td>
<td>222 (41.9)</td>
<td>46 (31.9)</td>
<td>348 (39.3)</td>
</tr>
<tr>
<td>C</td>
<td>190 (36.3)</td>
<td>132 (62.3)</td>
<td>308 (58.1)</td>
<td>98 (68.1)</td>
<td>538 (60.7)</td>
</tr>
<tr>
<td><strong>Significance</strong></td>
<td><em>P &lt; 0.0005</em></td>
<td><em>P &lt; 0.0005</em></td>
<td><em>P &lt; 0.0005</em></td>
<td><em>P &lt; 0.0005</em></td>
<td><em>P &lt; 0.0005</em></td>
</tr>
<tr>
<td><strong>Genotypes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>112 (42.7)</td>
<td>18 (37.0)</td>
<td>48 (18.1)</td>
<td>6 (8.3)</td>
<td>72 (16.3)</td>
</tr>
<tr>
<td>CT</td>
<td>110 (42.0)</td>
<td>44 (41.5)</td>
<td>126 (47.5)</td>
<td>34 (47.2)</td>
<td>204 (46.0)</td>
</tr>
<tr>
<td>CC</td>
<td>40 (15.3)</td>
<td>44 (41.5)</td>
<td>91 (34.3)</td>
<td>32 (44.4)</td>
<td>167 (37.7)</td>
</tr>
<tr>
<td><strong>Significance</strong></td>
<td><em>P &lt; 0.0005</em></td>
<td><em>P &lt; 0.0005</em></td>
<td><em>P &lt; 0.0005</em></td>
<td><em>P &lt; 0.0005</em></td>
<td><em>P &lt; 0.0005</em></td>
</tr>
</tbody>
</table>

* Comparison with control frequencies, χ² test.

Data are expressed as the number of subjects (% of the entire group).

### Table 2. Genotype Frequencies for the Y402H Polymorphism in the Complement Factor H Gene in Cases and Controls Depending on Smoking History

<table>
<thead>
<tr>
<th>Pack Years of Cigarette Smoking</th>
<th>0</th>
<th>0.1–20</th>
<th>&gt;20</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Genotype</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>51 (47.7)</td>
<td>22 (13.5)</td>
<td>38 (38.4)</td>
</tr>
<tr>
<td>CT</td>
<td>33 (30.8)</td>
<td>84 (50.9)</td>
<td>49 (49.5)</td>
</tr>
<tr>
<td>CC</td>
<td>23 (21.5)</td>
<td>59 (35.8)</td>
<td>12 (12.1)</td>
</tr>
<tr>
<td><strong>Significance</strong></td>
<td><em>P &lt; 0.0005</em></td>
<td><em>P &lt; 0.0005</em></td>
<td><em>P &lt; 0.0005</em></td>
</tr>
</tbody>
</table>

Data and significance are as described in Table 1.
damage host cells and tissues. The substitution of a positively charged histidine for a noncharged hydrophobic tyrosine at residue 402 has the potential to impair this function, being located within binding regions for both heparin and C-reactive protein (CRP). It may be relevant that serum levels of CRP tend to be elevated in AMD. Smoking can activate the alternative complement pathway in vitro through the modification of C3, and plasma levels of CRP are reduced in smokers, providing a possible explanation for smoking’s being a risk factor for AMD.

Further evidence implicating CFH in the pathogenesis of AMD comes from the observation that drusen show immunofluorescence for the C5b-C9 complex and other components of the complement cascade, supporting the hypothesis that local inflammation and activation of the complement cascade contribute to the pathogenesis of AMD. Smoking can activate the alternative complement pathway in vitro through the modification of C3, and plasma levels of CFH are reduced in smokers, providing a possible explanation for smoking’s being a risk factor for AMD.

The data presented herein and published previously leave no doubt that the Y402H polymorphism is an important determinant of risk for end-stage AMD. However, because more than half the population carry at least one copy of the C allele and no doubt that the Y402H polymorphism is an important determinant of risk for end-stage AMD. However, because more than half the population carry at least one copy of the C allele and most will never have AMD, there must be further genetic or environmental factors that influence risk. Other components of the complement system are obvious candidate susceptibility genes and merit investigation.

Acknowledgments

The authors are grateful to all the clinicians who supported the study, to clinic staff and medical photographers at the participating clinics, to Tunde Peto and colleagues for grading the fundus photographs, to the staff at Whatman International, Ltd., for DNA extraction, and to all the patients and their families who kindly participated in the study.

References


**TABLE 3. Odds Ratios for AMD Associated with the Complement Factor H Gene Y402H Genotype**

<table>
<thead>
<tr>
<th>AMD Type</th>
<th>Smoking History</th>
<th>No of Controls (n)</th>
<th>No. of Cases (n)</th>
<th>Odds of AMD (95% CI) Compared with the TT Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMD</td>
<td>Any</td>
<td>262</td>
<td>443</td>
<td>3.1 (2.0–4.6) P &lt; 0.0005 6.3 (3.8–10.4) P &lt; 0.0005</td>
</tr>
<tr>
<td></td>
<td>Any</td>
<td>262</td>
<td>106</td>
<td>2.6 (1.4–5.0) P = 0.004 6.0 (2.9–12.5) P &lt; 0.0005</td>
</tr>
<tr>
<td></td>
<td>Never smoked</td>
<td>107</td>
<td>165</td>
<td>2.7 (1.7–4.2) P = 0.0005 5.1 (2.9–8.9) P &lt; 0.0005</td>
</tr>
<tr>
<td>AMD</td>
<td>0.1–20 pack years</td>
<td>99</td>
<td>126</td>
<td>5.5 (2.7–11.3) P &lt; 0.0005 5.7 (2.6–12.5) P &lt; 0.0005</td>
</tr>
<tr>
<td>AMD</td>
<td>&gt;20 pack years</td>
<td>56</td>
<td>152</td>
<td>2.4 (1.2–4.6) P = 0.01 6.0 (2.6–13.9) P &lt; 0.0005</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.9 (0.9–4.0) P = 0.09 12.0 (4.0–35.7) P &lt; 0.0005</td>
</tr>
</tbody>
</table>

Odds Ratios for AMD Associated with the Complement Factor H Gene Y402H Genotype

P<0.0005 P<0.0005


APPENDIX

Remaining Members of the Genetic Factors in AMD Study Group

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