Haplotypes Spanning the Complement Factor H Gene Are Protective against Age-Related Macular Degeneration

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PURPOSE. Age-related macular degeneration (AMD) is a devastating disorder that adversely affects the quality of life of nearly 2 million Americans who have advanced forms of the disease. Besides the well-known risk imparted by carrying the Y402H variant in the complement factor H (CFH) gene on chromosome 1, recent evidence of the existence of protective haplotypes spanning CFH has been reported.

METHODS. The haplo.stats program was used to test for association of the protective haplotypes after adjusting for age in the dataset of 584 sporadic cases and 248 control samples. Logistic regression modeling and likelihood ratio tests were used to investigate an interaction between a particular haplotype and smoking status. The HBAT option of FBAT was used to confirm the associations in an independent dataset of 201 families.

RESULTS. Two protective (P) haplotypes in a family-based dataset (P1 = CAATT TAG, P = 0.021; and P2 = CGGCTTAG, P = 0.018) were identified for the first time. Age-adjusted score statistics provided support for these protective haplotypes in the case–control dataset (P1 frequency in cases ~13%, in controls ~20%, P = 0.001; P2 frequency in cases ~5%, in controls ~8%, P = 0.077). There was also tentative evidence of an interaction between one of the protective haplotypes and cigarette smoking (P = 0.04 likelihood ratio test for P2-smoking interaction).

CONCLUSIONS. Replication of the association between the protective haplotypes and decreased AMD susceptibility provides increased evidence that these associations have biological meaning. The suggestion of a haplotype-smoking interaction adds to the growing body of evidence that smoking is an important environmental covariate in AMD that should be considered in genetic studies. Identification of the protective variant(s) carried within these haplotypes is critical for understanding the etiology of AMD. (Invest Ophthalmol Vis Sci. 2007;48:4277–4283) DOI:10.1167/iovs.06-1427

A ge-related macular degeneration (AMD) is a complex, late-onset disease that remains one of the primary causes of blindness in the elderly. Large, soft drusen representing undigested cellular debris manifest early in the course of the disease. Later, central visual field loss occurs as geographic atrophy and/or choroidal neovascularization develop.1 A meta-analysis of population-based data from seven studies showed that an estimated 7.3 million people in the United States have large drusen characteristic of early AMD and that 1.75 million individuals have advanced forms of the disorder.2

Both genetic and nongenetic risk factors are known to be involved in the pathogenesis of AMD. Epidemiologic studies have successfully identified many nongenetic or lifestyle risk factors for AMD, including increased age, smoking, and body mass index.3,4 The connection between smoking and risk of AMD has been particularly well documented. A recent literature review reported that 13 of 17 studies showed a statistically significant association between smoking and AMD.5 Pooled data from 9525 participants in three population-based cohort studies in the United States, The Netherlands, and Australia show an estimated odds ratio for current smokers versus never smokers at 2.83 (1.15–6.93) for geographic atrophy and 2.35 (1.30–4.27) for late AMD, which includes cases with geographic atrophy and choroidal neovascularization.6 The adjusted population attributable risk percentage for smoking is 20%, suggesting that smoking is likely to be the largest known modifiable risk factor for AMD.7

A strong genetic component has long been suspected in the etiology of AMD, based on the results of familial aggregation studies,8–10 segregation analysis,11 and twin studies.12–17 A meta-analysis of the six largest genome linkage screenings identified the chromosome regions 10q26, 1q, 2p, 3p, and 16 as the most likely regions to harbor AMD susceptibility loci.18 In Caucasian populations, the association between AMD risk and the Y402H variant in the complement factor H (CFH) gene located on chromosome 1 has been validated by multiple studies,19–27 and promising initial reports of a strong association between a polymorphism in the LOC387715 gene on chromosome 10 and AMD risk have also been replicated.7,24,28,29 A recent meta-analysis estimates that the odds ratio for rs10490924 in LOC387715 as 2.5 (95% confidence interval [CI], 2.2–2.9) in heterozygotes for the risk allele and 7.3 in homozygotes (95% CI, 5.7–9.4).30 In addition, genetic and lifestyle risk factors for AMD have been tied together by the discovery that the effect of the LOC387715 variant on AMD risk is modified by cigarette smoking.7 The most recently reported major AMD susceptibility loci are the factor B (BF) and complement component 2 (C2) genes in the major histocompatibility complex class III region of chromosome 6.29,31

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The precise role of CFH in the AMD process is still being elucidated, but it is thought that the nonsynonymous substitution of histidine for tyrosine in exon 9, which lies in the protein’s heparin binding site, may disrupt the ability of CFH to attenuate complement response and lead to damage of ocular tissue.22 Recently, data supporting the existence not only of a risk haplotype, but also of protective haplotypes in the CFH gene have been reported.19,32,33 Hageman et al.19 used a tag single nucleotide polymorphism (SNP) approach to select eight SNPs in the CFH gene for haplotype analysis in a large case–control sample of individuals of European-American descent ascertained at the University of Iowa (Iowa City, IA) and Columbia University (New York, NY). Two haplotypes had protective effects, with the SNPs that distinguish the protective haplotypes concentrated between exons 2 and 11. Using a subset of four of these eight SNPs, Okamoto et al.32 confirmed a protective haplotype in a Japanese case–control dataset. Whereas this haplotype had a frequency of 12% in cases versus 21% in controls in the individuals of European-American descent and an odds ratio of 0.54 (0.41–0.73) in the Japanese sample, the haplotype had a frequency of 18% in cases and 35% in controls, and an odds ratio of 0.63 (0.26–0.67) in the Japanese sample. Where this haplotype had a frequency of 18% in cases and 35% in controls, and an odds ratio of 0.63 (0.26–0.67). Although this haplotype had a frequency of 18% in cases and 35% in controls, and an odds ratio of 0.63 (0.26–0.67). Although this haplotype had a frequency of 18% in cases and 35% in controls, and an odds ratio of 0.63 (0.26–0.67). Although this haplotype had a frequency of 18% in cases and 35% in controls, and an odds ratio of 0.63 (0.26–0.67). Although this haplotype had a frequency of 18% in cases and 35% in controls, and an odds ratio of 0.63 (0.26–0.67).

Our goal was to study the presence of the risk and protective haplotypes in CFH and to examine the possibility of a haplotype-smoking interaction, as smoking appears to be one of the most important environmental contributors to AMD, and previous studies have shown that the effect of genetic risk factors for AMD may be modified by smoking.7

### MATERIALS AND METHODS

#### Study Participant Ascertainment

Multiplex families and an independent dataset of unrelated cases and controls, all of Caucasian, non-Hispanic descent, were ascertained at Vanderbilt University Medical Center and Duke University Medical Center. All patients and controls underwent an eye examination and had stereoscopic fundus photographs graded according to a modified version of the Age-Related Eye Disease Study (AREDS) grading system, as described elsewhere.34,35 Briefly, grades 1 and 2 represent controls. Grade 1 controls have no evidence of drusen or small nonextensive drusen without pigmentary abnormalities, whereas grade 2 controls may show signs of either extensive small drusen or nonextensive intermediate drusen and/or pigmentary abnormalities. Grade 3 AMD cases have extensive intermediate drusen or large, soft drusen with or without drusenoid retinal pigment epithelial detachment. Grade 4 AMD cases exhibit geographic atrophy and grade 5 individuals have exudative AMD, which includes nondrusenoid retinal pigment epithelial detachment, choroidal neovascularization, and subretinal or sub-RPE hemorrhage or fibrosis, or photoocoagulation scar consistent with treatment of AMD.

<table>
<thead>
<tr>
<th>Grade</th>
<th>Diagnostic Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>No drusen or small nonextensive drusen (less than 63 μm diameter), without RPE pigment abnormalities</td>
</tr>
<tr>
<td>2</td>
<td>Extensive small drusen (total extent greater or equal to an area of a circle 125 μm in diameter) or nonextensive intermediate drusen (drusen greater than 63 μm but not greater than 125 μm in diameter), and/or RPE abnormalities associated with AMD (including focal hypopigmentation, focal hyperpigmentation, pigment clumping, and focal retinal pigment epithelial atrophy not large enough to be considered geographic atrophy)</td>
</tr>
<tr>
<td>3</td>
<td>Extensive intermediate drusen or any large soft drusen (greater than 125 μm in size), includes drusenoid retinal pigment epithelial detachments (RPED)</td>
</tr>
<tr>
<td>4</td>
<td>Geographic atrophy (area of RPE atrophy with sharp margins, usually with visible choroidal vessels, minimum diameter 300 μm), with or without involvement of the center of the macula</td>
</tr>
<tr>
<td>5</td>
<td>Serous (nondrusenoid) or hemorrhagic retinal pigment epithelial detachments, choroidal neovascularization, subretinal or sub-RPE hemorrhage or fibrosis, or photoocoagulation scar consistent with treatment of AMD</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Grade (%)</th>
<th>3 (35.4)</th>
<th>4 (14.6)</th>
<th>5 (50.0)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (SD)</td>
<td>73.3 (10.3)</td>
<td>76.5 (7.6)</td>
<td>66.1 (9.1)</td>
</tr>
<tr>
<td>Female (%)</td>
<td>67.6</td>
<td>64.0</td>
<td>56.5</td>
</tr>
<tr>
<td>Ever smoked (%)</td>
<td>49.5 (unaffected)</td>
<td>60.4</td>
<td>51.0</td>
</tr>
</tbody>
</table>

### Table 2. Study Population Characteristics

#### Independent Case–Control Dataset

<table>
<thead>
<tr>
<th>Grade (%)</th>
<th>3 (29.8)</th>
<th>1 (68.1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (SD)</td>
<td>76.5 (7.6)</td>
<td>66.1 (9.1)</td>
</tr>
<tr>
<td>Female (%)</td>
<td>64.0</td>
<td>56.5</td>
</tr>
<tr>
<td>Ever smoked (%)</td>
<td>60.4</td>
<td>51.0</td>
</tr>
</tbody>
</table>
dataset. Approval for the study was obtained from the appropriate institutional review boards at VUMC and DUMC, all study participants gave informed consent, and the research adhered to the tenets of the Declaration of Helsinki.

**DNA Analysis**

A commercial system was used to extract genomic DNA from whole blood samples according to standard protocol (Purgene; Gentra Systems, Minneapolis, MN). Genotyping assays (TaqMan Assays-on-Demand or, when necessary, Assays-By-Design; Applied Biosystems, Inc., Foster City, CA) were used for genotyping SNPs in CFH (GenBank accession no. DQ233256.1 GI:77744438; http://www.ncbi.nlm.nih.gov/Genbank; provided in the public domain by the National Center for Biotechnology Information, Bethesda, MD), in both the family-based and case–control datasets. The Y402H polymorphism rs1061170 was genotyped by direct sequencing, as previously described. DNA samples from The Fondation Jean Dausset-Centre d’Etude du Polymorphisme Humain (CEPH) families were duplicated between and across plates for use as a quality control, and laboratory personnel were blinded to the affection status of the individuals being genotyped.

**Statistical Methods**

Haploview was used to examine the linkage disequilibrium (LD) patterns between SNPs in each dataset, to verify that all SNPs were in Hardy-Weinberg equilibrium, and to perform single marker tests of association. Of the 33 SNPs we genotyped within the region of complement activation (RCA) gene cluster, alleles at eight tagging SNPs in the CFH gene identical with those of Hageman et al. were used to define haplotypes (rs3753394, rs529825, rs800292, rs3766404, rs1061170, rs203674, rs3753396, and rs1065489). Of the 36 haplotypes observed at least once, only four haplotypes had an overall frequency greater than 5%; these four haplotypes accounted for 81.9% of the observed haplotypes. We estimated haplotype frequencies and tested association of each haplotype with a frequency of at least 5% in our case–control dataset with age-adjusted score statistics using the haplo.stats software package. We calculated score statistics in the overall AMD dataset (grade 3, 4, and 5 AMD cases compared with grade 1 and 2 controls) and also in a subset of grade 5 neovascular AMD cases compared with grade 1 controls. Empiric probabilities were determined through simulation by using the default simulation options in haplo.stats (P precision criterion \( P_{\text{threshold}} = 0.25 \), so that simulations continued until the sample standard error was less than \( P_{\text{threshold}} \) times the probability, minimum number of simulations = 1000, and maximum number of simulations allowed = 20,000). To determine whether the potential protective haplotypes were underrepresented in cases after taking into account the overrepresentation of the risk haplotype in cases, the frequency of the haplotype of interest in cases was compared to the expected frequency of the haplotype given no unequal transmission according to the \( \chi^2 \) test. This test has been used to show that the frequency of the APOE epsilon 2 allele is reduced in Alzheimer’s disease, even after allowing for the increased frequency of the risk APOE epsilon 4 allele in cases of Alzheimer’s. A logistic regression model considering P1, P2, and risk haplotypes with the neutral haplotype as the baseline gave similar results. Haplotypes were tested for association with AMD in the family-based dataset by using the HBAT option of the FBAT software package.

In the case–control dataset of all AMD individuals we used the haplo.glm module of haplo.stats to build a regression model to study haplotype–smoking interactions. According to the recommendation of Lake et al., we modeled only haplotypes with a frequency greater than 5%. Four haplotypes in our dataset met this criterion, and one was selected as the baseline haplotype. Though the selection of the baseline neutral haplotype is somewhat arbitrary, we chose the TGGTTTGT haplotype (Fig. 1) because it was the most frequent haplotype occurring in the same percentage of cases and controls (15.5% in controls and 13.8% in cases), and its association score statistic comparing all AMD individuals (grades 3, 4, and 5) to all controls (grades 1 and 2) indicated neither a protective nor a risk effect (haplotype score = −0.997, \( P = 0.311 \)). Smoking status was obtained from patient ques-

![Figure 1](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/932943/ on 09/20/2018)
tionnaires and dichotomized by classifying individuals who had smoked >100 cigarettes in their lifetime as “smokers.” The full model contained age, smoking, the three most frequent haplotypes (later denoted as protective haplotype P1, protective haplotype P2, and the risk haplotype), a coefficient for the combined effect of the haplotypes with a frequency less than 5%, and four haplotype-smoking interaction terms. The neutral haplotype was used as the referent group in this model. A separate coefficient was used to estimate the effect of the rare haplotypes instead of pooling them with the baseline haplotype, to ensure that any effect of the rare haplotypes would not confound the neutral baseline haplotype. We followed up the P2-smoking interaction with a likelihood ratio test to see whether a model containing the P2-smoking interaction term is significantly different from a model that does not contain this interaction. First, we used a full model containing all haplotypes with a frequency greater than 5%, age, smoking, and the haplotype-smoking interaction terms compared with a reduced model without the four interaction terms. Because we were particularly interested in only the effect of the P2-smoking interaction, we also performed a likelihood ratio test comparing the full model to a reduced model missing only the P2-smoking interaction. Finally, a stratified regression analysis was used to examine the effect of P2 separately in smokers and nonsmokers after adjustment for age.

RESULTS

Association of Protective Haplotype

Two protective haplotypes defined by alleles at eight SNPs in the CFH gene were studied. P1 corresponds to alleles CAATT-TAG and is equivalent to H2 from Hageman et al.19 P2 corresponds to CCGGTCCAG and matches H4 from Hageman et al. There are minor differences in the nomenclature between our results and those of Hageman et al. because of differences in which strand of DNA was genotyped. Also, in Figure 3 of Hageman et al. at SNP rs203674 the G and T alleles should be reversed (personal communication, Hancox L, 2005). A risk haplotype CGGTCGAG, corresponding to the H1 haplotype of Hageman et al. 2005 and containing the C allele defining haplotype CGGTCGAG, corresponding to the H1 haplotype of reversed (personal communication, Hancox L, 2005). A risk haplotype, was also defined. Figure 1 presents the pair-wise link-

Table 3. Single-Marker Analysis of the Eight Haplotype-Defining SNPs

<table>
<thead>
<tr>
<th>SNP</th>
<th>Associated Allele</th>
<th>Cases Frequency</th>
<th>Controls Frequency</th>
<th>$\chi^2$</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs7535394</td>
<td>C</td>
<td>0.741</td>
<td>0.733</td>
<td>0.095</td>
<td>0.758</td>
</tr>
<tr>
<td>rs529825</td>
<td>G</td>
<td>0.849</td>
<td>0.739</td>
<td>24.286</td>
<td>8.30E–07</td>
</tr>
<tr>
<td>rs800292</td>
<td>G</td>
<td>0.851</td>
<td>0.747</td>
<td>22.87</td>
<td>1.73E–06</td>
</tr>
<tr>
<td>rs3766404</td>
<td>T</td>
<td>0.932</td>
<td>0.899</td>
<td>4.637</td>
<td>0.031</td>
</tr>
<tr>
<td>rs1061170</td>
<td>C</td>
<td>0.568</td>
<td>0.419</td>
<td>30.208</td>
<td>3.88E–08</td>
</tr>
<tr>
<td>rs203674</td>
<td>G</td>
<td>0.606</td>
<td>0.464</td>
<td>25.918</td>
<td>3.56E–07</td>
</tr>
<tr>
<td>rs3753596</td>
<td>A</td>
<td>0.844</td>
<td>0.834</td>
<td>0.225</td>
<td>0.635</td>
</tr>
<tr>
<td>rs1065489</td>
<td>G</td>
<td>0.841</td>
<td>0.829</td>
<td>0.289</td>
<td>0.591</td>
</tr>
</tbody>
</table>

The age-adjusted score statistic for the P2 haplotype was confirmed in the overall family-based analysis (Table 4). Logistic regression modelling confirmed this result (P1, P = 0.014, in a logistic regression model considering P1, P2, and risk haplotypes with the neutral haplotype as the baseline). The association of P1 was also confirmed in the independent overall family-based dataset (P = 0.02, Table 5). Because of the low frequency of this haplotype in the families, it was not possible to get a reliable test of association of P1 in our families exhibiting primarily neovascular AMD.

The age-adjusted score statistic for the P2 haplotype trended toward statistical significance in the overall case–control dataset (P = 0.077) and in a restricted analysis of individuals with neovascular AMD (P = 0.069, Table 4). In the overall case–control dataset, the P2 haplotype was overrepresented in controls after accounting for the underrepresentation of the risk haplotype (P < 0.001, Supplementary Table S1, http://www.iovs.org/cgi/content/full/48/9/4277/DC1). Logistic regression analyses agreed well with a logistic regression model considering P1, P2, and risk haplotypes with the neutral haplotype as the baseline (P2, P = 0.059). The protective effect of the P2 haplotype was present in the overall family-based dataset (P = 0.018) and bordered on significance when the analysis was restricted to individuals with neovascular AMD (P = 0.08; Table 5).

Confirmation of the Risk Haplotype

The risk haplotype was very strongly associated with AMD in the overall case–control dataset and in cases with neovascular AMD ($P \leq 10^{-5}$ in both analyses; Table 4). The association was confirmed in the overall family-based analysis (P = 0.04) and trended toward significance in the family-based dataset.
Protective Haplotype–Smoking Interaction

Because smoking is a known risk factor for AMD and the frequencies of the haplotypes in cases and controls varied by smoking status (Table 6), we built a regression model incorporating smoking and haplotype-smoking interactions. The full regression model considered age, smoking, haplotype, and haplotype-smoking interactions. The main effects of age, smoking, the risk haplotype, and the pooled rare haplotypes were statistically significant (Table 7). The interactions between P2 and smoking ($P = 0.008$) and the pooled rare haplotypes and smoking ($P = 0.032$) appear to be significant, but a more formal likelihood ratio test is needed for a true assessment of their significance. The likelihood ratio test between the full model with age, smoking, haplotypes with a frequency greater than 5%, and the haplotype-smoking interactions compared to a model without the four haplotype-smoking interaction terms trended toward significance ($\chi^2 = 8.37, df = 4, P = 0.08$).

Because the frequency of P2 was higher in smoking controls than in smoking cases (10.0% vs. 5.3%), while the frequencies are similar in nonsmokers (4.3% nonsmoking controls vs. 6.4% nonsmoking cases), and because of the low probability for the P2-smoking interaction term in the overall regression model, the P2-smoking interaction is of particular interest. To test specifically for a P2-smoking interaction, we performed another likelihood ratio test, comparing the full model with a reduced model without the P2-smoking interaction. The other haplotype-smoking interaction terms were retained in the reduced model. This test was significant ($\chi^2 = 4.22, df = 1, P = 0.04$). Furthermore, a stratified regression analysis testing the effect of P2 in smokers versus nonsmokers after adjustment for age and controlling for the effects of the other common haplotypes showed that the protective effect of P2 bordered on significance in smokers ($P = 0.070$), but not in nonsmokers ($P = 0.196$), lending additional confidence to the haplotype-smoking interaction.

**DISCUSSION**

We have confirmed the presence of protective haplotype P1, not only in a case-control dataset, but also, for the first time, in a family-based dataset. P2 was significantly associated with AMD in the family-based dataset and was marginally significant in the case-control dataset. As expected, the risk haplotype containing the risk allele of Y402H was verified to be strongly associated in both the case-control and family-based datasets. Age-adjusted score statistics for the protective haplotypes in the overall analysis were quite similar to those obtained when only the neovascular AMD cases were studied, suggesting that the protective variant(s) on these haplotypes may be protective for the broad AMD phenotype. Most interesting, a possible protective haplotype-smoking interaction was discovered, though further confirmation of this interaction in independent datasets is necessary to draw firm conclusions.

Although statistical analyses and confirmation in multiple independent datasets of both families and singleton cases and controls has provided strong support for a protective effect of these haplotypes, functional experiments are needed to determine which variant(s) on these haplotypes are exerting the protective influence and the underlying biological mechanism by which they are operating. The CFH protein is composed of 20 short consensuses repeats (SCRs) and contains binding sites for complement component C3b, heparin, C-reactive protein (CRP), and sialic acid. CFH functions as a brake on the alternative complement cascade by preventing the activation of C3 to C3a and C3b and by inactivating existing C3b. Sialic acid, heparin, and CRP all facilitate the interaction between CFH and C3b and thus lead to a stronger inhibitory signal. Of the eight SNPs defining the protective haplotypes, three are nonsynonymous amino acid substitutions (rs800292 I62V, rs1061170 Y402H, and rs1065489 D936E), one is a synonymous substitution (rs3753396 Q672), one is a putative promoter variant, and three are intronic changes. The valine-to-isoleucine substitution at rs800292 is located within SCR 1 of CFH, which is one of three C3b binding sites within the protein. The putative promoter polymorphism rs3753394 may influence transcription. Thus, both of these variants may have functional consequences for the CFH protein.

However, the protective effect does not seem to be explained by any of the eight polymorphisms alone (Fig. 1). The P1, P2, and risk haplotypes all carry the same allele for the putative promoter variant, and P1 and P2 carry different alleles.
for the I62V, intron 1, and intron 6 polymorphisms, and so none of these variants alone seems plausible as the source of the protective effect. In addition, both protective haplotypes share the alleles TTAG at the last four SNPs of the haplotype, but the A and G alleles at the last two SNPs in P1 and P2 are also found on the risk haplotype, and the T allele at the intron 10 SNP is also found on the neutral haplotype. Therefore, these variants are unlikely to exert a strong protective effect.

As expected, both protective haplotypes carry the nonrisk T allele at the Y402H polymorphism, but several lines of evidence suggest that the absence of the Y402H risk allele is not sufficient to explain the protective effect. First, the nonrisk T allele is also present on the neutral haplotype. Moreover, the frequency of both P1 and P2 was increased in controls, even after allowing for the decreased frequency of the risk haplotype in controls. Finally, in the Japanese the Y402H C allele did not contribute significantly to increased risk for AMD,45,46 but other risk and protective haplotypes in CFH were present,32 which suggests that despite the strong single-locus Y402H effect in Caucasians, additional non-Y402H variation in CFH modifies susceptibility to AMD.

This work, as well as the previous report of an interaction between smoking and the LOC387715 variant rs10490924 on chromosome 10 that modifies AMD risk,7 demonstrate the importance of considering known lifestyle risk factors for AMD when performing genetic studies. Smokers have decreased plasma levels of CFH,7 but little is known about the biological function of LOC387715 and how smoking affects it. Future molecular work aimed at unraveling the complex interplay between smoking, CFH, LOC387715, and AMD will surely provide intriguing results.

One concern with haplotype analysis in general is that when using case–control data, the haplotype for an individual is often not directly observed and may have to be inferred using the person’s genotype data. The haplo.glm function avoids the need to infer haplotypes directly by using a weighted regression approach that incorporates the posterior probability of an individual’s having a particular haplotype into the model.40 The interpretation of haplo.glm results is more difficult when significant haplotype ambiguity exists. This haplotype ambiguity is reduced as the proportion of individuals in the dataset with high conditional haplotype probabilities increases. In our case, 86% of individuals had posterior probabilities greater than 0.95, suggesting that, at worst, only modest haplotype ambiguity was present.

Multiple testing is a significant issue in genetic studies and cannot be ignored. We limited the problem of multiple testing in this study in several ways. First, we tested only haplotypes with a frequency greater than 5% for association with AMD, rather than test all observed haplotypes. Second, we believe that confirmation of the protective and risk haplotypes in two independent datasets of different types (case-control and family-based) increases confidence that these associations are true-positive results. Finally, only two haplotype-smoking interactions were tested by likelihood ratio methods (an overall test of all haplotypes interacting with smoking and a specific test of the P2-smoking interaction), not all possible haplotype-smoking interactions.

In summary, our data provide support for the presence of two protective haplotypes in the CFH gene. A potential interaction between one of the protective haplotypes and smoking was also identified. Further work is needed to characterize the variant(s) on the haplotypes that are exerting the protective influence and how cigarette smoking may modify this effect.

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