Neovascular Age-Related Macular Degeneration and Its Association With LOC387715 and Complement Factor H Polymorphism

R. Keith Shuler, Jr, MD; Michael A. Hauser, PhD; Jennifer Caldwell, COA; Paul Gallins, MS; Silke Schmidt, PhD; William K. Scott, PhD; Anita Agarwal, MD; Jonathan L. Haines, PhD; Margaret A. Pericak-Vance, PhD; Eric A. Postel, MD

Objective: To compare phenotypes of 2 age-related macular degeneration (AMD) susceptibility genes: LOC387715 and complement factor H (CFH).

Methods: Phenotypes of 755 AMD cases were characterized. The number of LOC387715 (T allele at rs10490924, or A69S) and CFH (T1277C at rs1061170, or Y402H) risk alleles were determined in each case. Individuals were divided into 5 groups by genotype: group 1, LOC–/– CFH–/–; group 2, LOC–/– CFH–/– or LOC+/– CFH+/– or LOC+/+ CFH+/+; group 3, LOC+/– CFH+/– or LOC+/+ CFH+/+; group 4, LOC+/– CFH+/– or LOC+/+ CFH+/+, or LOC+/+ CFH+/+, or LOC+/+ CFH+/+, and group 5, LOC+/+ CFH+/+.

Results: Signs of neovascular AMD including grade (P = .002), pigment epithelial detachment (P = .001), and subretinal hemorrhage (P < .001) demonstrated significant association with groups 2, 4, and 5 vs groups 1 and 3. Group 5 had a significantly younger mean age (72.3 years) compared with other groups (P = .002).

Conclusions: The AMD cases possessing the LOC387715 (rs10490924) variant may have a higher risk of neovascular AMD. Individuals with AMD who are homozygous for both variants might be at greater risk for earlier onset of neovascular AMD.


A GE-RELATED MACULAR DEGENERATION (AMD) is the leading cause of irreversible central vision loss in older Americans. The clinical characteristics of AMD are generally divided into nonneovascular and neovascular forms. Previously described phenotypic characteristics associated with neovascular AMD include white race, increasing age, increased body mass index, hypertension, hyperopia, intraocular pressure, lens opacity, and large drusen.

Recent articles have shown that a common polymorphism of the complement factor H gene (CFH) (T1277C at rs1061170, or Y402H) is associated with macular soft drusen as well as an increased risk of advanced AMD, including geographic atrophy and neovascular AMD. One recent article suggested that the CFH variant increases the risk for geographic atrophy in particular. The CFH Y402H polymorphism also is associated with peripheral reticular pigmentedary changes.

A second putative AMD susceptibility gene, LOC387715 (T allele at rs10490924, or A69S), has recently been described. Biological characterization of this gene is limited; however, smokers with this LOC387715 variant have a substantially greater risk for advanced AMD, especially the neovascular form, compared with nonsmokers with this variant.

Clarifying the phenotype-genotype relationships in AMD might provide clues to the involved molecular mechanisms and may eventually guide treatment recommendations for specific subtypes of AMD. Here we compare the phenotypes associated with 2 recently described AMD risk genes: LOC387715 and CFH variants.

Methods: Patients were identified in the clinic populations of the Duke University Eye Center, Durham, NC, and the Department of Ophthalmology, Vanderbilt University, Nashville, Tenn, or from referrals to the study centers by local ophthalmologists. Information was collected and protected in compliance with the Health Insurance Portability and Accountability Act of 1996 regulations, institutional review board approval was obtained, and all of the patients provided informed consent.

The clinical criteria, grades, and grading methods used to define AMD have previously been described. Age-related findings including drusen, retinal pigment epithelial (RPE) changes, neovascularization, and geographic atrophy were used to diagnose AMD in individuals aged 55 years or older.

Data collection for each individual was performed using a standardized protocol. Com-
complete ocular, medical, and family ocular histories were obtained. Most study participants completed these questionnaires in person with the clinical study coordinator (J.C.). Age and sex were recorded. Height, weight, and blood pressure were measured during the clinical encounter. Separate questionnaires were used to obtain lifelong health habits such as smoking, sunlight exposure, and dietary supplementation as well as current dietary practices. Patients completed these questionnaires typically at home or much less frequently by telephone.

The measurement of the proportion of smokers in each group was constructed as a binary “ever or never” variable based on a participant’s response to the question, “Have you smoked at least 100 cigarettes in your lifetime?” Additional smoking history information, including regular cigarette smoking, was assessed as previously described. Each participant received a complete ophthalmic evaluation that included slitlamp examination, biomicroscopy with a handheld 90-diopter (D) lens or fundus contact lens, and (20-D) indirect ophthalmoscopy of the peripheral retina.

The study protocol included a minimum of 3 standard fields of 35-mm color fundus photographs as well as stereo photographs of the disc and macula. Two of us (A.A. and E.A.P.) used the previously described modified Age-Related Eye Disease Study grading system to grade macular findings. Each eye of every individual received a grade. The overall grade for a participant was based on the more severely affected eye; if multiple features were assessed as previously described. Each participant received a complete ophthalmic evaluation that included slitlamp examination, biomicroscopy with a handheld 90-diopter (D) lens or fundus contact lens, and (20-D) indirect ophthalmoscopy of the peripheral retina.

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The AMD cases were divided into 5 groups depending on genotype. Group 1 lacked the CFH rs1061170 variant and the LOC387715 rs10490924 variant (LOC–/– CFH–/–). Group 2 was heterozygous or homozygous for the LOC387715 rs10490924 risk variant and lacked the CFH rs1061170 variant (LOC+/– CFH–/– or LOC+/+ CFH–/–). Group 3 lacked the LOC387715 rs10490924 variant and was heterozygous or homozygous for the CFH rs1061170 variant (LOC–/– CFH+/+ or LOC–/– CFH+/+). Group 4 carried at least 1 risk allele at both loci but was not homozygous for both AMD risk polymorphisms (LOC+/– CFH+/+ or LOC+/+ CFH+/+ or LOC+/– CFH+/+). Group 5 was homozygous for both AMD risk loci (LOC+/+ CFH+/+).

Groups were independently analyzed for association with all of the phenotypic characteristics investigated as well as age and sex. Statistical analyses were performed using SAS software version 8.2 (SAS Institute, Inc, Cary, NC). Conservatively assuming that all of the 16 comparisons are statistically independent, the Bonferroni correction requires an α level of approximately .0031 to achieve statistical significance. For categorical variables, phenotypic differences among the 5 genotype-defined groups were compared with a global χ² test. For continuous variables, analysis of variance was used.

### RESULTS

The data set contained 755 unrelated AMD cases divided into 5 groups by genotype: group 1, 43 cases; group 2, 91 cases; group 3, 230 cases; group 4, 336 cases; and group 5, 55 cases (Table 1). Table 1 compares the general characteristics of the groups. The proportion of women in the 5 groups did not vary significantly (P=.19). The ETDRS visual acuities also demonstrated no statistical difference between groups (P=.99). The proportion of smokers did not differ significantly between groups (P=.38). Group 5 had a mean ±SD age of 72.3 ± 6.3 years, making that group significantly younger than the other 4 groups (P=.002) (Table 1).

There were significant differences in the proportions of each grade between groups (P=.002), specifically in the proportion of grade 5 cases between groups 2 and 3 (P=.002) (Table 2). Two signs of neovascular AMD, pigment epithelial detachment (P=.001) and subretinal hemorrhage (P<.001), demonstrated a statistically significant association.
tion with groups possessing at least 1 LOC387715 allele (Table 3). In each of these comparisons, there was a measurable difference between groups 2 and 3, with group 2 having a much larger proportion of patients with the respective signs of neovascular AMD (Table 3).

Another sign of neovascular AMD, subretinal fluid \( (P = .006) \), very nearly reached statistical significance (Table 3). Additionally, the difference in AMD grade between eyes \( (P = .004) \) also very nearly reached statistical significance.

There was no significant difference found between groups with respect to the existence or type of cataract \( (P = .57) \), RPE hyperpigmentation \( (P = .66) \), RPE hypopigmentation \( (P = .99) \), geographic atrophy \( (P = .30) \), macular drusen (existence, type, or extent as defined by grade \( \text{small drusen} \ (P = .97) \), medium drusen \( (P = .53) \), or large drusen \( (P = .26) \) \), extramacular drusen \( (P = .22) \), disciform scarring \( (P = .22) \), other signs of choroidal neovascular membrane \( (P = .44) \), peripheral drusen \( (P = .14) \), and peripheral reticular pigmentary change \( (P = .33) \) (Table 3).

The CFH (rs1061170) and LOC387715 (rs10490924) variants are the most significant AMD risk genes described to date.\(^5-7,10\) This analysis suggests that individuals with AMD possessing 1 or more risk alleles at LOC387715...
rs10490924 are more likely to develop neovascular AMD compared with those with AMD who lack this variant. Individuals with AMD who are homozygous for both risk variants might be at greater risk for earlier onset of neovascular AMD; however, our data do not prove this.

Several studies4-8 including our earlier articles, have demonstrated that the C allele at CFH rs1061170 is associated with an increased risk of both forms of advanced AMD, neovascular and geographic atrophy. Our most recent analysis has demonstrated that the C allele at LOC387715 is somewhat more strongly associated with geographic atrophy (odds ratio, 3.2; P<.001) than neovascular AMD (odds ratio, 2.5; P<.001), although the confidence intervals overlap. Consistent with our previous study, the current phenotype analysis, which incorporates joint genotypes at CFH and LOC387715, suggests that the T allele at LOC387715 rs10490924 is more strongly associated with neovascular features whereas the C allele at CFH rs1061170 is more likely to lead to geographic atrophy (grade 4 disease). Prospective studies of the progression in individuals diagnosed with grade 2 or 3 disease at baseline are needed to confirm this hypothesis.

The age at examination was significantly earlier for group 5, and the standard deviation was smaller for this group. Because individuals often are initially evaluated at the onset of symptoms, these data suggest that individuals homozygous for both risk genes may develop symptomatic disease earlier. The relatively smaller variation regarding the age at examination in the AMD cases homozygous for the CFH rs1061170 and LOC387715 rs10490924 variants may suggest a more severe phenotype, not in terms of disease grade but rather a more consistent and earlier onset of disease. However, smoking history cannot be excluded as a factor contributing to this group’s earlier age at onset as discussed later. It is possible that being homozygous for both risk variants combined with cigarette smoking creates the conditions for the “perfect storm.”

The joint effect of 2 AMD risk genes does not account for all of the signs of AMD. Rather, multiple gene interactions as well as dietary and environmental factors in the setting of the aging process all contribute to the phenotype. Therefore, it is not surprising that only some of the signs of neovascular AMD investigated were significantly associated with the LOC387715 rs10490924 variant. Data collected for each individual represent a single point in time; therefore, an individual with neovascular AMD may demonstrate different signs at different times or never develop certain signs of neovascular AMD. Because these data for each patient represent a point in time, we did not emphasize the near significance of the difference in grade between eyes within a participant.

There is a continuum of disease in AMD that usually occurs at different rates in each eye. Patients often are initially evaluated when vision decreases in 1 eye, and symptoms in the second eye follow sometime later. Our study was not longitudinal; therefore, we could not determine whether this difference reached statistical significance or was lost. It would be interesting to investigate whether the difference in grade between eyes reached statistical significance and whether it could be related to the institution of any therapy.

Our previous article11 suggested that smokers with the LOC387715 rs10490924 variant have a substantially greater risk for advanced AMD, especially the neovascular form, compared with nonsmokers with this allele. In the current study, the proportion of smokers did not differ significantly between groups. Although this study did not aim to evaluate the risk of developing AMD, the relatively similar proportions of smokers across genotype groups suggests that a higher proportion of smokers alone did not account for the increased prevalence of neovascular AMD features in the groups possessing the LOC387715 rs10490924 variant.

Within the data set, pack-years of cigarette smoking were significantly different only between groups 3 and 5 (mean pack-years, 32.8 vs 48.2, respectively; P=.04; overall group difference, P=.27). This illustrates that one needs to be careful in concluding that the earlier appearance of neovascular AMD in group 5 is solely due to genetic factors. Instead, this observation is likely due to the combination of heavy cigarette smoking and the double homozygous state of both risk variants.

Age-related macular degeneration is a multifactorial disease, and this analysis does not aim to model the joint effect of smoking, CFH, and LOC387715 on the risk of developing AMD, which was done in our previously reported case-control study.19 Rather, our goal was to more carefully characterize the phenotype-genotype relationship of individuals with AMD who possess various combinations of risk alleles at these 2 genes.

Because these 2 sequence variants were already demonstrated to confer a greater risk of severe AMD, this study included only individuals with AMD. Our aim was to analyze the phenotypic characteristics of those individuals with AMD who possess 1 or both of these 2 risk alleles. However, it is important to realize that not every individual who possesses 1 of these risk variants is diagnosed with AMD; therefore, it is also relevant to determine the prevalence of these risk variants in the general population or in individuals without AMD. There were 200 of 208 individuals without signs of AMD (grade 1 and grade 2 combined) who were genotyped for both the CFH rs1061170 variant and the LOC387715 rs10490924 variant in our data set. Regarding the CFH variant, 29.8% of the individuals possessed no variant alleles (TT genotype), 53.8% possessed 1 variant allele (TC genotype), and 16.4% possessed 2 variant alleles (CC genotype). Of the same 200 individuals with respect to the LOC387715 variant, 56.3% had no variant alleles (GG genotype), 35.6% had 1 variant allele (GT genotype), and 8.2% had both variant alleles (TT genotype). The breakdown into the 5 genotype combinations considered in this study was as follows: group 1, 34 individuals (17.0%); group 2, 27 individuals (13.5%); group 3, 74 individuals (37.0%); group 4, 62 individuals (31.0%); and group 5, 3 individuals (1.5%). Limited conclusions can be drawn from this sample size, but it does appear that a minority of individuals without AMD possess either or both of these risk variants.

Determination of high-risk genotypes and phenotypes could prove valuable to the clinician. If these associations are confirmed, rapid and cost-effective assays can be developed for determining whether an indi-
individual has the T allele at LOC387715 (rs10490924) and/or the C allele at CFH (rs1061170) and preventative and therapeutic treatments may be recommended based on an individual’s phenotype as well as genotype. A more thorough understanding of the genotype-phenotype relationship in AMD may eventually improve therapeutic recommendations, provide a more accurate diagnosis, make the investigation of the nongenetic components more straightforward, and allow for a better understanding of the mechanism of this complex disease.

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Correspondence: Eric A. Postel, MD, Duke University Eye Center, Box 3802, Durham, NC 27710 (poste002@mc.duke.edu).

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