Lack of an Association of Apolipoprotein E Gene Polymorphisms With Familial Age-Related Macular Degeneration

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Background: Previously, the ε4 allele of apolipoprotein E (APOE) was reported to have a significant association with a decreased risk of age-related macular degeneration (AMD). In addition, the ε2 allele of APOE was reported to be possibly associated with an increased risk of AMD.

Objective: To determine if APOE polymorphisms, previously reported to be associated with AMD, affect its expression in medium to large families, as well as in unrelated patients with AMD.

Methods: The APOE genotype was determined by HhaI restriction digests of polymerase chain reaction–amplified products in a collection of 259 affected and 207 unaffected individuals from 56 AMD families. Genotypes were determined similarly in a set of 104 unrelated AMD patients and in 113 unaffected control subjects. Diagnosis of AMD was based on clinical examination and evaluation of fundus photographs. Evidence of an association between alleles of APOE and AMD in families was tested by the following 4 statistical methods: χ² analysis of simple allele counting, logistic regression analysis adjusting for age, construction of likelihood ratios of haplotype frequencies, and the pedigree disequilibrium test.

Results: None of the statistical methods used showed a significant association between the common alleles of APOE and AMD in our collection of families or in the set of unrelated AMD patients.

Conclusions: No evidence was found to support an association between AMD in medium to large families and the ε4 or ε2 alleles of APOE. Neither was any evidence found for an association of APOE polymorphisms with the set of unrelated patients with AMD. However, a trend for a decreased risk of AMD associated with APOE ε4 was observed in the set of unrelated patients with a family history of AMD.

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associated with Down syndrome. Drusen, a hallmark of AMD, are deposits found between the basal lamina of the retinal pigment epithelium and the inner collagenous layer of the Bruch membrane. Interestingly, APOE has been shown to be a ubiquitous component of drusen, and to accumulate in the cytoplasm of overlying retinal pigment epithelial cells.

The ε4 allele of APOE was initially reported to be associated with a decreased risk of exudative AMD. In other studies, the ε4 allele was reported to be associated with a decreased risk of late AMD. Recent studies suggest that the association of the APOE ε4 allele with a decreased risk for AMD occurs primarily in those individuals with a family history of AMD. In this article, we investigated the relationship between APOE and AMD in medium- to large-sized families with 3 to 12 living affected members (mean, 5 affected members). In addition, we compared the APOE genotype and allele frequencies in a series of unrelated AMD patients with a control group of individuals without AMD.

### METHODS

Apolipoprotein E genotypes were determined in 4 study populations. The first population consisted of affected individuals (n = 259) in families with AMD. Affected status was defined as geographic atrophy, neovascularization, or extensive drusen. The second population consisted of unaffected individuals (n = 207) from this same set of families. All families (n = 56) had a minimum of 3 living affected members. The families were recruited as part of an ongoing study of the genetics of AMD. The third population consisted of unrelated affected individuals (n = 104) with late AMD (geographic atrophy or neovascularization) who were recruited from clinical practices at the Casey Eye Institute (Portland, Ore). These were further subdivided on the basis of a family history of AMD (any first- or second-degree relative, living or deceased, reported to have had AMD). The fourth population consisted of unrelated control subjects (n = 88) recruited from the clinical practices and clinics at the Casey Eye Institute, and spouses (n = 23) in AMD families. In all individuals, the diagnosis of AMD was established on the basis of clinical examination and fundus photographs. In accordance with Institutional Review Board of Oregon Health & Science University approval, informed signed consent was obtained from all study participants.

Subjects in all 4 study populations were white. Unrelated patients with AMD, control subjects, and in most cases, family probands, resided in the northwest portion of the United States. Although females were overrepresented in all populations (Table 1), there was no significant difference in their numbers between unrelated affected individuals and controls ($P = .36$), or between affected and unaffected family members ($P = .21$). There was a significant difference, however, in the mean age of unrelated AMD patients vs control subjects (78.4 vs 72.5 years, respectively) and affected vs unaffected family members (73.2 vs 59.0 years, respectively) by t test analysis.

Approximately 20 mL of blood was collected from each subject, and DNA was extracted by standard techniques. The APOE genotype was determined by means of HhaI restriction analysis of a 237-base-pair polymerase chain reaction (PCR) product, which was generated using the primers 5’-GGGACGGCCTGTCCAA-3’ and 3’-ATAAAGATTCCCCGGCGTGTGACT-3’. The second primer carried a 9-base noncomplementary 5’ extension that allowed the APOE genotype to be more readily discerned because of greater separation between 2 gel bands associated with genotypes involving the APOE ε2 and ε4 alleles. HhaI digests of PCR products were loaded on a 10% nondenaturing polyacrylamide gel and electrophoresed at 600 volts for 1 hour in a GenePhor apparatus (Amer sham Pharmacia, Piscataway, NJ) at 15°C.

Genotype and allele frequencies deviating from those expected under the null hypothesis were tested using the Pearson χ² analysis. To adjust for the effect of age, logistic regression was performed using the APOE ε4 (or ε2) carrier status as the independent variable (ie, each person carrying 1 or 2 copies of the ε4 allele is coded as having the ε4 carrier status) and age as a covariate.

In families, the ILINK program was used to estimate haplotype frequencies of relevant APOE AMD haplotypes, first by allowing for allelic association, and then under the assumption of independent assortment. The maximum likelihood estimates were used to construct a likelihood ratio test for the association of ε4 (or ε2) with AMD. Under the hypothesis of association, the frequencies of the 4 possible haplotypes are estimated directly using maximum likelihood. Under the hypothesis of no association, the haplotype frequencies are calculated from the independently estimated allele frequencies. The resulting likelihood ratio test has 1 degree of freedom, and hence, 2ln (likelihood) is distributed as a χ² statistic with 1 degree of freedom ($\chi^2_1$). All estimates used our standard model for the inheritance of AMD, which accounts for the age-dependent penetrance of AMD.

In addition, the association of APOE ε4 or ε2 with AMD was evaluated using the pedigree disequilibrium test (PDT). Statistical analyses were performed using SYSTAT 8.0 for Windows, and SPSS 10.0 for Macintosh (SPSS Inc, Chicago, Ill). Since previous published reports had shown an effect of APOE, P values are denoted as 1-sided where appropriate.

### RESULTS

FAMILIAL CASES OF AMD

We detected no significant difference in the frequency of APOE alleles (by simple allele counting) (Table 2)
between those affected with AMD and those unaffected in the 56 families with 3 or more affected individuals. The frequency of the 4 allele of APOE was 12.4% in affected and 14.3% in unaffected family members. This is not significantly different by $\chi^2$ analysis ($P = .15$; 1-sided). Neither is the frequency of APOE 2 significantly different between affected and unaffected family members ($P = .44$; 1-sided). Similarly, there was no significant difference in the frequency of any of the 6 possible genotypes between affected and unaffected family members (Table 2).

Logistic regression, adjusted for age, failed to reveal any association between the 4 carrier status of APOE and AMD in this same set of families. The odds ratio for APOE 4 was 0.91 (95% confidence interval [CI] 0.50-1.63; $P = .37$, 1-sided). Logistic regression also did not suggest any association between the 2 allele of APOE and familial AMD. The odds ratio for APOE 2, adjusted for age, was 1.22 (95% CI, 0.74-2.0; $P = .22$, 1-sided).

A possible association between either APOE 4 or APOE 2 and AMD in families was also evaluated by comparing the likelihood of observing the data while allowing for allelic association to the likelihood (Table 3) of observing the data with no association (ie, random segregation of the APOE allele and the AMD locus). Since the directly estimated haplotype frequency (AMD, 4 = 12.5%) is greater than that assuming no association (AMD, 4 = 9.42%), a weak causative rather than protective effect is suggested for APOE 4 in AMD families. However, this association is not significant ($\chi^2 = 0.63; P = 0.43$). Also, since the directly estimated haplotype frequency (AMD, 2 = 0.0025%) is less than that assuming no association (AMD, 2 = 4.53%), a weak protective rather than causative effect is suggested for APOE 2 in AMD families. However, neither is this association significant ($\chi^2 = 2.0; P = .15$).

The PDT sum statistic was used to observe any differences in transmission of either the APOE 4 or 2 allele to affected and healthy offspring. This test also failed to show any evidence of an association with AMD in families.
Neither was there a significant difference in the relative frequencies of any of the 6 genotypes, between the group of unrelated patients and controls. By $\chi^2$ analysis, genotypes in both the unrelated AMD patient and control groups were in Hardy-Weinberg equilibrium with their respective allele frequencies (unrelated AMD cases: $\chi^2 = 1.8$, $P = .60$; control subjects: $\chi^2 = 1.4$, $P = .70$).

A logistic regression analysis also failed to show an association between the $e4$ allele of APOE and AMD among unrelated patients. Without adjusting for the effects of sex and age, the odds ratio was 0.67 (95% CI, 0.34-1.30; $P = 0.12$, 1-sided). The influence of sex on the analysis of the model was not significant ($P = .70$), however the influence of age was highly significant ($P < .001$). The age-adjusted odds ratio was slightly lower at 0.61 (95% CI, 0.29-1.27; $P = .09$, 1-sided), but it was still not significant. It is possible that the differences in age between the cases and controls in our sample reduced the power of the association analysis.

Because the inclusion of spouses ascertained from AMD families could affect the conformity of our control study population, the analysis was repeated without spouses. However, the genotype and allele frequencies did not change significantly (data not shown). This analysis also failed to demonstrate a significant difference in allele frequencies between unrelated AMD patients and control subjects.

Finally, unrelated AMD cases were subdivided further into 2 groups: those that mentioned a family history of AMD (any first- or second-degree relative), and those that did not mention one. Unrelated patients with a family history of AMD are distinct from our families with AMD since this set of unrelated patients does not meet our requirement of 3 living affected members. Although the frequency of the $e2$ and $e4$ genotypes of APOE did not differ significantly between either group of patients and controls, unrelated AMD patients with a positive history had a frequency of APOE $e4$ of only 6.8%, compared with 12.4% for control subjects (Figure.

We found no statistically significant associations between APOE gene polymorphisms and familial AMD using 4 different methods of analysis. Specifically, in contrast to previous reports, we found no evidence for either a protective effect of APOE $e4$ or a causative effect of APOE $e2$ on AMD risk. Also, we found no statistically significant associations between APOE gene polymorphisms and AMD among unrelated patients. However, there was a trend toward a lower APOE $e4$ frequency (ie, a protective effect as seen previously) among unrelated patients with a positive family history of AMD. It is worth noting that this trend is much weaker than the deviations observed in previous studies. For example, the age-adjusted odds ratio of 0.61 in our logistic regression analysis is less extreme than the odds ratio of 0.43 in the study of Klaver et al or 0.3 in the study of Simonelli et al.

Our failure to find a significant association between APOE and risk for AMD in unrelated AMD cases could result from either the effect being smaller or nonexistent in our AMD population, or the effect being more difficult to detect because of a lower frequency of the APOE $e4$ allele in our control population. Using the APOE $e4$ frequency of 15.6% among control subjects and 6.8% among AMD cases as in Klaver et al, our power to detect an association in our unrelated patient population was predicted to be 0.91 (1-sided test at $P = .05$). However, given our actual frequencies of APOE $e4$ in control subjects (12.4%) and unrelated AMD patients (9.1%), our power was only 0.29.

Stratification of the unrelated cases by the criterion for a positive family history of AMD notably reduced the frequency of the APOE $e4$ allele among unrelated patients to a value equivalent to that reported in Klaver et al (0.068). However, the association still wasn’t significant, due in part to our smaller sample size. Recently, the association between AMD and APOE $e4$ has been reported to involve familial AMD cases of those younger than 70 years as opposed to sporadic cases. Interestingly, this collection of AMD families included single confirmed cases of AMD who reported an additional family member with AMD. Therefore, the families collected in this recent study more closely resemble our set of unrelated AMD patients with a family history of AMD, than our collection of medium to large AMD families.

Although one cannot rule out insufficient power as an explanation for the lack of an association between APOE and AMD in our families, another plausible explanation stems from our selection bias for families with 3 or more living individuals (average, 5 individuals) affected with AMD. Age-related macular degeneration in such families may be caused by genes associated with greater genetic risk than that associated with APOE, and that thus have masked its effects. Conceivably, the association of APOE $e4$ with AMD may be observed most readily in small families similar to our set of unrelated patients with a family history of AMD. Investigation of the APOE polymorphisms in such families seems warranted to further define the role of this gene in the etiology of AMD.
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REFERENCES


Uthoff reported two cases of fracture of the skull with effusion of blood at the base. The hemorrhages passed along the optic nerves both within the dural heath and without. In both cases, some hours after the injury, the picture of optic neuritis appeared with great venous congestion and pronounced edema of the disc. At the autopsy in both cases much blood was found in the dilated nerve sheaths.