Association of Apolipoprotein E Alleles with Susceptibility to Age-Related Macular Degeneration in a Large Cohort from a Single Center

Sepideh Zareparsi, Adam C. Reddick, Kari E. H. Branham, Kathryn B. Moore, Laurie Jessup, Susan Thoms, Michael Smith-Wheelock, Beverly M. Yashar, and Anand Swaroop

PURPOSE. To examine the effect of apolipoprotein E (APOE) alleles on age-related macular degeneration (AMD) risk and on age at diagnosis of AMD in a large patient cohort recruited from a single center.

METHODS. The frequency of APOE alleles was analyzed in 632 unrelated AMD patients and 206 unrelated controls, all of whom were of white ancestry. The presence or absence of disease symptoms in all patients and controls was based on clinical examination and/or ophthalmic records. The association with APOE was explored in the context of AMD subtypes, family history status, possible interaction with smoking, and distribution of age at diagnosis of AMD.

RESULTS. The frequency of the ε4 allele was significantly reduced in patients compared with controls (0.10 vs. 0.14, P = 0.02). Gender- and age-adjusted odds ratios indicated that ε4 carriers have significantly lower risk of developing AMD compared to ε3ε3 subjects (OR = 0.55, 95% CI: 0.37–0.82, P = 0.004). In the cohort, AMD patients with a positive family history exhibited a significant 5.5 years earlier age at diagnosis (P = 0.001); however, APOE alleles did not appear to modulate the age at diagnosis of AMD.

CONCLUSIONS. The association between the APOE-ε4 allele and a reduced risk of AMD was established in a large cohort with sufficient statistical power. How distinct APOE alleles affect AMD susceptibility warrants further investigation. (Invest Ophthalmol Vis Sci. 2004;45:1306–1310) DOI:10.1167/iovs.03-1253

Age-related macular degeneration (AMD) is the leading cause of irreversible vision loss in the elderly population. The risk of developing AMD increases with advancing age, affecting over 30% of individuals aged 75 years or older.1,2 AMD is a progressive degenerative disease that primarily involves the retina and the retinal pigment epithelium (RPE) in the macular region.3 Early symptoms of AMD include drusen, which are accumulations of acellular debris in the basement membrane of RPE, and pigment abnormalities in RPE.4 Late stages of AMD are characterized by two distinct subtypes: a “dry” form, known as geographic atrophy (GA), involving loss of RPE and photoreceptors; and a “wet” exudative form, known as choroidal neovascularization (CNV), involving ingrowth of choroidal vessels.4 Despite the growing medical, economical, and societal impact of AMD, the specific underlying mechanisms of AMD pathogenesis are poorly understood.

AMD is a complex disease resulting from interactions between multiple genes and environmental factors.4,5 The strongest risk factors identified to date are advanced age and family history.1 First-degree relatives of AMD patients are reported to have two- to fourfold increased risk of developing AMD, compared with the first-degree relatives of unaffected individuals.6,7 Twin studies have consistently observed high levels of concordance among monozygotic twins, providing additional support for genetic predisposition.8,9 Genome-wide linkage analyses of affected family members implicate a number of chromosomal regions that may harbor AMD susceptibility genes.10–15 In a high resolution (5-cM) whole genome scan of 274 affected sib pairs, several suggestive AMD susceptibility loci were identified.16 In addition, several monogenic early-onset forms of macular degeneration (MD) show phenotypic similarities with AMD, prompting association studies to examine their contribution to AMD susceptibility. However, variations in the genes responsible for monogenic MD do not appear to have a significant causal relationship to AMD.17 To date, only sequence changes in the ABCR gene that causes Stargardt disease have been associated with AMD; however, this gene is unlikely to be a major susceptibility gene for AMD.18,19

Apolipoprotein E (APOE) is involved in lipoprotein metabolism and plays an essential role in neuronal response to injury.20 APOE is located on chromosome 19q and has three common polymorphic alleles: ε2, ε3, and ε4. Allelic variations in APOE are associated with neurodegenerative diseases, such as Alzheimer’s disease (AD)21 and Parkinson’s disease (PD).22 The ε4 allele is associated with an increased risk and earlier onset of AD whereas ε2 allele with reduced susceptibility and delayed onset. Interestingly, APOE is a component of drusen, a pathologic hallmark of AMD.23,24 Three independent studies have observed a reduced ε4 frequency in AMD patients, consistent with a protective effect.25,26,27 An increased ε2 allele frequency in AMD was also detected.25 This finding was not observed consistently by other groups, possibly due to small sample sizes and the relative rarity of the ε2 allele in the population. A recent meta-analysis, based on samples from three of the published studies, confirms the protective effect of the ε4 allele to AMD susceptibility, though similar frequencies were observed in patients with either GA or CNV.27 With
TABLE 1. Sample Characteristics and APOE Genotypes

<table>
<thead>
<tr>
<th>Sample size (n)</th>
<th>AMD Patients</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Females (%)</td>
<td>409 (65%)</td>
<td>115 (56%)</td>
</tr>
<tr>
<td>Age at diagnosis (y)</td>
<td>71.6 ± 8.4</td>
<td>74.6 ± 4.9</td>
</tr>
<tr>
<td>Age at most recent exam (y)</td>
<td>79.2 ± 7.9</td>
<td>74.6 ± 4.9</td>
</tr>
</tbody>
</table>

AMD subtypes:
- GA: 147 (23%)
- CNV: 182 (29%)
- MDL: 103 (16%)
- G&A&CNV: 200 (32%)

APOE genotype:
- ε2ε2: 1 (0.2%) 0 (0%)
- ε2ε3: 104 (16.5%) 29 (14.2%)
- ε2ε4: 10 (1.6%) 4 (2%)
- ε3ε3: 406 (64.6%) 119 (58.0%)
- ε3ε4: 106 (16.8%) 53 (25.8%)
- ε4ε4: 2 (0.3%) 0 (0%)

* Three patients and one control could not be genotyped after multiple attempts.

Regarding respect to the ε2 allele, an increased risk of AMD was observed in men but not women.27

In this report, we examined APOE allele frequencies among 652 unrelated AMD patients and 206 unrelated controls, all of whom were of white ancestry. This patient sample represents the largest group recruited from a single center, reported to date for association studies. The association between AMD and APOE alleles among the different clinical subtypes of AMD and AMD among sporadic and familial AMD patients was investigated. The effect of APOE genotypes on age at diagnosis of AMD and the interaction between APOE and smoking were also evaluated.

MATERIALS AND METHODS

The majority of the participants (91% of 652 unrelated AMD patients, 88% of 206 unrelated controls) were recruited from the clinics of the Kellogg Eye Center at University of Michigan. The remaining participants were recruited through community outreach programs and self-referral to our study. This research adhered to the tenets of the Declaration of Helsinki. All subjects signed an informed consent, approved by the Institutional Review Board. Family and medical history (including information about known and suspected risk factors for AMD) were collected.

Subjects were identified by a chart review of clinical records, searching only for a diagnosis of AMD. At the time of a clinic visit, retina clinic physicians referred their patients to this study without knowledge of the patients’ family history. Ophthalmic records, fundus photography, and/or fluorescein angiograms (when possible) were obtained for all participants. Fundus findings in each eye were classified based on a standardized set of diagnostic criteria established by the International ARM Epidermiologic Study.28 These criteria include the presence or absence of small macular drusen (<125 µm), coarse RPE changes, large macular drusen (MDL), GA, and CNV. The following patients were excluded from the study: individuals with a history of severe macular disease or vision loss before age 40; others with signs of a juvenile macular or retinal degeneration or macular damage resulting from ocular trauma, retinal detachment, high myopia, choroiditis, infective or inflammatory processes, or choroidal dystrophy; and those with inadequate documentation. Affected individuals with CNV consisted of those with CNV in both eyes and/or CNV in one eye and MDL in the other eye. Patients with GA included those with GA in both eyes and/or GA in one eye and MDL in the other eye. Patients with MDL had bilateral findings of MDL. Age at first diagnosis of AMD was collected from all patients and whenever possible, confirmed by medical records. Familial AMD was defined as having at least one reported first- or second-degree relative or extended family member affected with AMD. All of the affected family members that were enrolled in the study were examined by an ophthalmologist; however, where family history was reported but additional members were not enrolled we could not obtain clinical information. Patients with no known reported affected family member were categorized as sporadic. Proband with an unknown family history (n = 49) were excluded from analyses that involved family history. Information about smoking was collected by self-report, including the number of packs per day, age at which first started smoking, and age when stopped smoking.

Control participants, including some of the spouses of affected individuals enrolled in the study, were considered unaffected if they were under the age of 68 at their last ophthalmic examination, did not have a family history of AMD in more than one family member, and did not have any AMD findings in either eye.

Genomic DNA was extracted from peripheral blood leukocytes. APOE genotyping was performed using a standard technique.29 Allele frequencies were estimated by allele counting. χ² analyses of Hardy-Weinberg equilibrium for APOE genotypes were performed for patients and controls. Allele frequency differences were tested by a Z-test. Logistic regression was used to estimate odds ratio adjusted for gender and age. Kaplan-Meier survival analysis was used to plot genotype-specific age at onset distribution curves, and the differences between the curves were tested with log rank statistics. All analyses were performed using SPSS software (Release 8.0; SPSS Inc., Chicago, IL).

RESULTS

APOE genotypes and sample characteristics for both patients and controls are summarized in Table 1. There was a significantly higher frequency of females than males among our AMD patients compared with controls (P < 0.02). A higher preponderance of women among AMD patients is in agreement with other studies.27 The average age at diagnosis of AMD is 71.6 ± 8.4 years in our cohort, with 79.2 ± 7.9 years being the average age at the most recent ophthalmic examination. For control individuals, the average age of the ophthalmic examination when no AMD findings were observed in either eye is 74.6 ± 4.9 years. Although patients were older than controls in our study, the controls were older than the average diagnosis age of patients and still free of any signs of AMD.

APOE genotypic and allelic frequencies were in Hardy-Weinberg equilibrium in both AMD patients and controls. The frequency of the ε4 allele was significantly reduced in patients compared with controls (0.10 vs. 0.14, P ≤ 0.02) (Table 2). Although, ε2 allele frequency was slightly higher in patients

TABLE 2. APOE Allele Frequencies in Controls and AMD Patients by Family History and by AMD Subtype

<table>
<thead>
<tr>
<th>Family History</th>
<th>AMD Patients</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Familial AMD</td>
<td>268</td>
<td>0.10</td>
</tr>
<tr>
<td>Sporadic AMD</td>
<td>312</td>
<td>0.08</td>
</tr>
<tr>
<td>Smoking</td>
<td>Nonsmokers</td>
<td>275</td>
</tr>
<tr>
<td></td>
<td>Smokers</td>
<td>319</td>
</tr>
</tbody>
</table>

* P < 0.01.
than controls (0.09 vs. 0.08), this difference was not significant. We also calculated odds ratio (OR) for ε4-carriers (ε3ε4 and ε4ε4) compared to the reference group of ε3ε3 subjects. Subjects with ε2ε4 genotype were excluded to evaluate the effect of each allele, independently. Carriers of the ε4 allele had a significantly reduced risk (almost 40%) of developing AMD (OR = 0.60, 95% CI: 0.41–0.88, P = 0.009).

Whether the association between AMD susceptibility and APOE alleles is specific to an AMD subtype was examined by classifying cases into patients with GA, patients with CNV, patients with large macular drusen, and patients with both GA and CNV (Table 2). The ε4 allele frequency was reduced in all patient groups compared with controls. The ε2 allele frequency appeared higher in patients with CNV when compared to patients with GA (0.10 vs. 0.07). These findings were based on a small subset of patients within each disease subtype, hence there was not sufficient power for statistical analysis. However, these results indicated the protective effect of ε4 in both forms of late-stage AMD and suggested that ε2-carriers are probably at increased risk for developing CNV subtype.

APOE allele frequencies in patients with and without a family history of AMD were investigated. A previous study had identified the protective effect of the ε4 allele in familial AMD but not in sporadic patients. No differences in APOE allele frequencies were detected by family history (Table 2). Frequency of the ε4 allele was reduced in both familial (0.09) and sporadic (0.10) AMD patients compared with controls. Although APOE allele frequencies were examined in several studies, their effect on age at onset or diagnosis of AMD was not previously determined. In our study, familial AMD patients had a significant 3.5 years earlier age at diagnosis than sporadic patients (69.9 ± 8.5 vs. 73.4 ± 8.1, P < 0.001). Therefore in order to evaluate whether this earlier diagnosis age was due to a susceptibility gene, the effect of APOE alleles on age at diagnosis of AMD was investigated. The age at diagnosis distributions for the three groups (ε2-carriers, ε3ε3 subjects, and ε4-carriers) in a Kaplan-Meier survival analysis demonstrated no significant differences in age at diagnosis between ε3ε4/ε4ε4 patients compared with ε3ε3, or ε2ε3/ε2ε2 (Fig. 1).

One environmental risk factor that has been definitively associated with AMD is smoking. Therefore the possibility of an interaction between APOE alleles and smoking on AMD susceptibility was explored. Among AMD patients, ε4 allele frequencies were reduced in both patient groups [smokers (0.10) and nonsmokers (0.09)], compared with control individuals. Furthermore, ε2 allele frequencies were similar between smokers (0.10) and nonsmokers (0.09). Data based on four different smoking categories (nonsmokers, ≤10 packs/year, >10 and ≤30 packs/year, and >30 packs/year) were qualitatively unchanged; however, the sample sizes of each subgroup were too small to determine statistical significance.

APOE allele frequencies may vary with age because of their association with AD, heart disease, and longevity. Hence, whether differences in APOE allele frequencies between AMD patients and controls are affected by differences in age were determined. Comparison was made between ε4 allele frequency in patients and controls before age 75 years and after age 75 years (Fig. 2), as described in a recent meta-analysis report.[25] The latter studied 47- to 66-year-old patients; however, our controls are older than 66 years.] The reduced ε4 allele frequency was observed in AMD patients compared with controls, before age 75 years (0.11 vs. 0.15) and after age 75 years (0.08 vs. 0.12; Fig. 2). After adjusting for age and gender, ε4-carriers still had a significantly reduced risk of developing AMD (OR = 0.55, 95% CI: 0.37–0.82, P = 0.004).

**DISCUSSION**

This study firmly established that the APOE-ε4 allele, or a nearby allele in linkage disequilibrium, was associated with a decreased susceptibility for AMD manifestations. The protective effect of the ε4 allele did not vary by family history status or by AMD subtype; ε4 allele frequency was reduced in both familial and sporadic AMD and in advanced forms of AMD, GA, and CNV. Although familial patients have 3.5 years earlier age at diagnosis of AMD than the sporadic patients, this difference in diagnosis age (though significant between the two groups) was not apparently due to APOE alleles. Age at diagnosis of AMD was similar between ε4-carriers (ε3ε4 and ε4ε4), ε2-carriers (ε2ε2 and ε2ε3), and ε3ε3 patients. The data suggested that other as yet unidentified gene(s) may modulate age at diagnosis of AMD.

In this study, patients and controls were matched for ethnicity to avoid confounding due to population stratification. All subjects were white and a majority resided in the same geographical location (i.e., the state of Michigan). APOE allele frequencies observed in the controls in our study were similar.
to those reported by other studies on APOE and AMD and reported for the general white population. Moreover, APOE allele frequencies in our patients were similar to those reported by others. In AMD, frequency of the ε4 allele ranges from 0.07 to 0.12 and ε2 allele from 0.09 to 0.125. We did not detect a significant difference in ε2 allele frequency between patients and controls, possibly due to the insufficient statistical power. Interestingly, the association between APOE and AMD has also been replicated in a group of Italian AMD patients, but not in Chinese AMD patients. This indicates that the association between APOE and AMD may vary among different ethnic groups.

To date, there has been one published report that failed to detect the association between ε4 and AMD in white people, when examined in families with three or more affected members, and in unrelated AMD cases. Though no association was apparent among 56 AMD families, the data presented in that report demonstrate a trend for reduced ε4 frequency in unrelated AMD patients compared with controls (0.09 vs. 0.12). The lack of association could be explained by the small sample size and insufficient statistical power. It is possible that the effect of APOE was masked by the effect of other genes with a greater impact on AMD.

Although our control population was slightly younger than affected patients (74.6 ± 4.9 vs. 79.2 ± 7.9), all control subjects had an ophthalmic examination and were at least 68 years old at the time of enrollment. The average age at the most recent clinical evaluation for controls was 3 years more than the average age at diagnosis of AMD patients. The reduced ε4 allele frequency in patients compared with controls was observed in both age groups, those before 75 years and after. Our data strongly argue that the observed association is real and not due to a result of varying allele frequencies between different age groups.

APOE is associated with both risk of developing AD and age at onset of AD. Although it is now clear that APOE is associated with risk of developing AMD, the effect is in the opposite direction. The effect of APOE genotypes on age at diagnosis of AMD had not been examined before our study. An effect on age at diagnosis of AMD by APOE alleles was not detected, suggesting yet another difference in the association of APOE and these two aging-associated yet distinct neurodegenerative diseases.

Our understanding of the mechanism(s) of association between APOE alleles and AD remains limited even after 10 years. The association between APOE alleles and AMD was first reported approximately 5 years ago and was met with controversy. Our results based on a large patient cohort recruited from a single center, along with the results of the meta-analysis report, clearly demonstrate the existence of the association and should encourage further research into the role of APOE and its variants in pathogenesis of AMD. Because of a high rate of turnover in photoreceptor outer segments, especially in the macular region, APOE may play a role in cell-membrane remodeling, which is essential for normal functioning and maintenance of the retina. It is also possible that the inability of ε4 to form dimers compared with ε2 or ε3 variants may allow easier transport of lipids through Bruch’s membrane because of smaller size lipid particles. Accumulation of neutral lipids with age may then lead to the formation of a hydrophobic barrier within Bruch’s membrane. An alternative hypothesis is based on the difference in ability to clear debris through Bruch’s membrane, since unlike ε2 and ε3, ε4 contains positive charges. APOE is also suggested to reduce oxidative damage to the RPE by regulating nitric oxide production.

In summary, in the largest patient cohort recruited from a single center to date, the APOE-ε4 allele was shown to be associated with a significantly decreased risk of AMD. The age at diagnosis of AMD may be modified by a familial risk factor(s). As the search for additional AMD susceptibility loci continues, it is important to explore the interactions among various genetic and environmental risk factors. A clear understanding of AMD pathogenesis will be most readily achieved through appreciation of these complex interactions.

Acknowledgments

The authors express gratitude to numerous individuals and families who donated their time and resources. Thanks are also due to Susan Eiler, John Heckenlively, Samuel G. Jacobson, Mark W. Johnson, Ron Kurtz, Paul R. Lichter, Donald Puro, Stephen Saxe, Paul A. Sieving, Andrew K. Vine, and David Zacks for their clinical inputs and support during patient collection. Julia E. Richards and Goncalo Abecasis for constructive discussions, a large number of retina physicians in the state of Michigan and the Great Lakes region for their involvement, and the clinical staff at the Kellogg Eye Center Retina Clinic, whose everyday assistance made this work possible. The efforts of John Bahling, Suja Hirtianna, and Elena Fillipova in patient collection and sample preparation are appreciated. Sharyn Ferrara is acknowledged for administrative assistance.
References