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A pooled case-control study of the apolipoprotein E (APOE) gene in age-related maculopathy

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Abstract Age-related maculopathy (ARM) is a multifactorial disorder known to have a substantial genetic component. The ε4 allele of the apolipoprotein E gene (APOE-4) has previously been reported to have a protective effect on ARM risk, while the APOE-2 allele may increase disease risk. This study combined four independent data sets (three US and one European) of Caucasian ARM patients and controls in order to obtain better statistical power to examine the role of APOE in ARM. APOE genotype and allele frequencies were compared for 617 ARM cases and 1260 controls, adjusting for age and sex differences between the two groups via multiple logistic regression. The protective effect of the APOE-4 allele on ARM risk was confirmed (age- and sex-adjusted odds ratio (OR) for APOE-4 carriers 0.54, 95% confidence interval (CI) 0.41–0.70, $p < 0.0001$). The effect of APOE-4 did not differ significantly between males and females and was observed consistently for both atrophic and neovascular ARM. Evidence for an increased risk of ARM due to the APOE-2 allele was found for men, but not for women (OR for men 1.54, 95% CI 0.97–2.45; OR for women 0.74, 95% CI 0.52–1.06, $p = 0.01$ for interaction of sex and APOE-2 carrier status). These data confirm that the APOE-4 allele, or an allele in linkage disequilibrium with it, reduces the risk of ARM. They also suggest that the effect of the APOE-2 allele may vary by gender, and that APOE-2 may confer an increased risk only to males.

Key words Age-related macular degeneration; candidate genes; epidemiology; APOE

Introduction Age-related maculopathy (ARM) accounts for the majority of severe central vision loss in the Western world. Nearly 9% of individuals over the age of 65 years and nearly 28% of those over the age of 75 years have early signs of the disease, and approximately 7% of individuals older than 75 years are severely affected.[1,2] The absolute number of affected people is expected to triple within the next 20 to 30 years due to population aging. There are two clinically distinct late stages of the disorder, atrophic (dry) and neovascular (exudative, wet) ARM, which are also referred to as age-related macular degeneration (AMD). Vision loss has a more acute onset and is more severe in the neovascular form. Laser photocoagulation treatment is an option only for a minority of patients with neovascular ARM and has a limited success rate in terms of vision improvement.[3] Photodynamic therapy has been reported to be beneficial for patients with predominantly classic neovascular lesions, but visual acuity did not improve signifi-

cantly for patients with minimally classic neovascular lesions.[4] ARM is thus a major burden on public health, and there is a pressing need to learn more about its pathogenesis in order to try to develop better prevention and treatment strategies.

The etiology of ARM is multifactorial. Environmental risk factors for ARM seem similar to those for cardiovascular disease, smoking being the factor most consistently identified.[5] A substantial role of genetic risk factors has been demonstrated in numerous studies, including segregation analysis,[6] twin studies,[7,8] and epidemiological studies of familial aggregation.[9–12] Klaver and associates estimated a population-attributable risk for genetic factors of 23%, and a lifetime risk ratio of 4.2 for late ARM in first-degree relatives of ARM patients compared to relatives of controls.[9] Seddon and co-workers estimated the odds ratio associated with a first-degree family history of ARM to be 2.4.[10] While ARM shares clinical features with other genetic disorders of the retina and macula, involvement of most of the genes responsible for these diseases has so far not been established in candidate gene studies of ARM. However, the ATP-binding cassette transporter gene (ABCR/ABCA4), which causes autosomal-recessive Stargardt disease, continues to be examined in ARM patients, and evidence for an effect of ABCR variants on ARM risk has been reported by an international consortium of research groups.[13]

A gene that is not specifically related to hereditary retinal disorders has recently received some attention as a likely candidate gene for ARM. Independent case-control studies from six different countries (Netherlands,[14,15] France,[16] United States,[17] Italy,[18] Iceland,[19] and Australia[20]) reported evidence for a protective effect of the ε4 allele of the apolipoprotein E gene (APOE-4) in ARM.[14–20] Two studies[15,18] also suggested a modestly increased risk of ARM due to the presence of the APOE-2 allele, although this effect did not reach statistical significance in the respective data sets. Several findings make APOE a plausible candidate gene for ARM. Soft drusen, the earliest clinical sign of ARM, contain protein and lipid deposits,[21] and the apoE protein is known to play a central role in lipid transport and distribution. Expression of the APOE gene was demonstrated in ARM-relevant tissue, such as soft drusen and basal laminar deposits.[15] The apoE protein is also known to be involved in maintenance and repair of neuronal cell membranes of the peripheral and central nervous system,[22,23] and may play a similar role in the repair of retinal detachments.[24] Finally, APOE is an established susceptibility gene for other neurodegenerative diseases, such as Alzheimer disease where the APOE-4 allele increases risk in a dose-dependent fashion[25] and the APOE-2 allele renders a protective effect.[26]

Even though the majority of the evidence supports an APOE association with ARM, the data are not entirely consistent. For example, an absence of the APOE-4 allelic association was reported in a data set of Chinese patients.[27] Therefore, the goal of our study was to pool independent data sets of ARM patients and controls from different study groups in order to increase statistical power for detecting an APOE effect, both for the APOE-4 as well as the less frequent APOE-2 allele.

Materials and methods

STUDY POPULATION The groups that pooled their data for this analysis are located in five different study regions: Duke University Medical Center (DUMC) in Durham, NC, USA; Vanderbilt University Medical Center (VUMC) in Nashville, TN, USA; the University of Pittsburgh (UP), Pittsburgh, PA, USA; the University of California in Los Angeles (UCLA), CA, USA; and Erasmus University (EUR) in Rotterdam, The Netherlands. DUMC and VUMC are currently conducting a large collaborative study of genetic and environmental risk factors for ARM, whereas UP, UCLA, and EUR are involved in independent studies of ARM.

The DUMC/VUMC patients (n = 223) were ascertained in the southeastern United States as part of a genetic study that recruits both multiplex (2+ family members affected with ARM) and singleton (one family member affected with ARM) families. The families were identified through a proband from the respective clinic population or through a proband's referral to the study site from local ophthalmologists. Stereoscopic fundus photographs were available for all cases, and grading of ARM severity was performed by three of the authors (EAP, AAMADLP) according to slightly modified criteria from the International ARM Study Group,[28] as described previously.[29] Specifically, early ARM was defined as the presence of extensive (total extent ≥ area of a circle with 350 μm diameter), intermediate (63 μm ≤ x < 125 μm), or any large (≥125 μm) soft drusen. Drusenoid retinal pigment epithelium (RPE) detachments without fluid were included as early ARM, whereas serous or hemorrhagic RPE detachments were considered a symptom of late (neovascular) ARM. Controls were ascertained at DUMC from the same overall clinic population as the ARM patients. The majority of the control group (n = 309, 89.8%) was composed of spouses of Alzheimer disease and dementia patients ascertained through the Joseph and Kathleen Bryan Alzheimer Disease Research Center (ADRC). These spouses were questioned about their ocular disease history and were free of obvious signs of advanced visual impairment at the time their blood sample was obtained. However, they were not specifically examined for early signs of ARM via fundus photography. In addition, the DUMC control group also included 35 (10.2%) spouses of ARM patients who underwent a complete ophthalmologic exam and were found to be unaffected, either lacking any ARM features or having only small (<63 μm) or nonextensive intermediate drusen.

At UP, all cases (n = 210) included in this analysis were ascertained as part of a large affected relative pair study whose goal was the identification of genomic regions likely to harbor ARM susceptibility genes through a genome-wide screen.[30] Therefore, all cases had at least one relative (typically sibling) with a confirmed diagnosis of ARM. Probands were ascertained by a combination of enrollment within the local clinic population and a screening program of patients identified by private ophthalmologic practices. Stereoscopic fundus photographs were available for all cases, and grading of ARM severity was performed as described previously by one of the authors (MBG).[30] While

there was agreement between DUMC/VUMC and UP criteria on the definition of advanced (atrophic or neovascular) ARM, early ARM was adjudicated by one of the authors (MBG) to match the DUMC/VUMC criteria. No data set of unrelated control individuals from UP was available.

At UCLA, ARM patients (n = 98) were ascertained from a single retinal physician's academic practice (KWS), based on the same grading system employed by DUMC/VUMC. Stereoscopic fundus photographs were available for the majority of cases, and patients with possible neovascular ARM also had an intravenous fluorescein angiogram performed. The control subjects (n = 73) were ascertained from a similar age group and clinic population at Cedars-Sinai Medical Center, primarily to act as a control group for this and other ARM studies. They were also evaluated for the presence of cataracts and glaucoma. While no fundus photographs were taken for the control individuals, they all underwent a comprehensive dilated funduscopic examination using slit biomicroscopy with a 78 diopter lens (AN, MCK). The subjects included in this analysis were found to have no signs of any ARM features, not even small drusen < 63 μm.

At EUR, cases and controls were ascertained as part of the Rotterdam Study, a large population-based study of Rotterdam residents older than 55 years that has been described previously.[2] A total of 6476 study participants underwent a complete ophthalmologic examination, including fundus photography. Grading of ARM was performed according to the International ARM Study Group criteria.[28] Cases were all subjects with atrophic or neovascular ARM, based on the same criteria as those used by the other groups, for whom APOE genotypes were available at the time of data pooling (n = 86). No cases with early ARM were included in this analysis. Controls were a randomly selected subset of study subjects with either absence of any ARM features, or only small (<63 μm) drusen, or only isolated hyper/hypopigmentation. All controls included in this analysis (DUMC/VUMC, UCLA, EUR) were at least 55 years of age. Note that all cases (n = 617) and controls (n = 1260) were of Caucasian ethnicity, with most subjects from the US sites having a northern European ancestral origin. Basic characteristics of the study population are summarized in Table 1, and the distribution of clinical subtypes of ARM by site and overall is shown in Table 2. All investigations were performed according to the guidelines of the Declaration of Helsinki.

METHODS Genomic DNA was extracted from whole blood after isolation of peripheral blood leukocytes.[31] APOE genotyping was performed as previously described (DUMC/VUMC, UP, UCLA;[32] EUR[33]). Briefly, after PCR, the DNA was digested with the HhaI restriction enzyme, which yields a characteristic pattern of digested fragments for each of the three APOE alleles corresponding to haplotypes of the two single-nucleotide polymorphisms in exon 4 of the gene. For the statistical analysis, the Statistical Analysis System software (SAS, SAS Institute, Cary, NC, USA) was used. Initially, χ^2 tests of Hardy-Weinberg equilibrium (HWE) for APOE genotypes were performed. APOE allele frequencies were estimated by allele counting in

TABLE 1. Age, sex, APOE genotype, and allele frequencies of cases and controls, by study site.

	Cases					*Controls*			
	DUMC/ VUMC	*UP*	*UCLA*	*EUR*	*All*	*DUMC/ VUMC*	*UCLA*	*EUR*	*All*
Total no. individuals	223	210	98	86	617	344	73	843	1260
Mean age at exam ± SD	74.8 ± 7.9	75.5 ± 7.3	73.9 ± 9.5	81.1 ± 8.2	75.8 ± 8.3	69.5 ± 6.8	75.3 ± 7.8	68.2 ± 8.0	69.0 ± 7.9
(yrs) (range)	(47–96)	(52–91)	(49–94)	(56–96)	(47–96)	(55–86)	(58–93)	(55–94)	(55–94)
Females (n, %)	159 (71.3)	137 (65.2)	67 (68.4)	58 (67.4)	421 (68.2)	179 (52.0)	50 (68.5)	521 (61.8)	750 (59.5)
APOE genotype (n, %)									
2/2	4 (1.8)	1 (0.5)	1 (1.0)	0 (0.0)	6 (1.0)	4 (1.2)	0 (0.0)	9 (1.1)	13 (1.0)
2/3	25 (11.2)	25 (11.9)	21 (21.4)	19 (22.1)	90 (14.6)	37 (10.8)	11 (15.1)	114 (13.5)	162 (12.9)
2/4	5 (2.2)	2 (1.0)	1 (1.0)	2 (2.3)	10 (1.6)	8 (2.3)	1 (1.4)	17 (2.0)	26 (2.1)
3/3	146 (65.5)	159 (75.7)	60 (61.2)	56 (65.1)	421 (68.2)	209 (60.8)	47 (64.4)	466 (55.3)	722 (57.3)
3/4	41 (18.4)	23 (11.0)	15 (15.3)	9 (10.5)	88 (14.3)	76 (22.1)	13 (17.8)	219 (26.0)	308 (24.4)
4/4	2 (0.9)	0 (0.0)	0 (0.0)	0 (0.0)	2 (0.3)	10 (2.9)	1 (1.4)	18 (2.1)	29 (2.3)
APOE allele (n, %)									
2	38 (8.5)	29 (6.9)	24 (12.2)	21 (12.2)	112 (9.1)	53 (7.7)	12 (8.2)	149 (8.8)	214 (8.5)
3	358 (80.3)	366 (87.1)	156 (79.6)	140 (81.4)	1020 (82.7)	531 (77.2)	118 (80.8)	1265 (75.0)	1914 (75.9)
4	50 (11.2)	25 (6.0)	16 (8.2)	11 (6.4)	102 (8.3)	104 (15.1)	16 (11.0)	272 (16.1)	392 (15.6)
Total no. Alleles	446	420	196	172	1234	688	146	1686	2520

DUMC, Duke University Medical Center Durham, NC, USA; VUMC, Vanderbilt University Medical Center Nashville, TN, USA; UP, University of Pittsburgh Pittsburgh, PA, USA; UCLA, University of California, Los Angeles CA, USA; EUR, Erasmus University, Rotterdam, The Netherlands.

Type of ARM	*DUMC/VUMC*	*UP*	*UCLA*	*EUR*	*All*
Early ARM[a]	34 (15.3)	35 (16.7)	24 (24.5)	0	93 (15.1)
Atrophic	33 (14.8)	35 (16.7)	16 (16.3)	33 (38.4)	117 (19.0)
Neovascular	156 (69.9)	140 (66.7)	58 (59.2)	53 (61.6)	407 (65.9)
Total	223	210	98	86	617

DUMC, Duke University Medical Center, Durham, NC, USA; VUMC, Vanderbilt University Medical Center, Nashville, TN, USA; UP, University of Pittsburgh, Pittsburgh, PA, USA; UCLA, University of California, Los Angeles, CA, USA; EUR, Erasmus University, Rotterdam, The Netherlands.
[a]Defined as extensive (total extent ≥ area of a circle with 350 μm diameter), intermediate (63 μm ≤ x < 125 μm), or any large (≥125 μm) soft drusen, with or without drusenoid (nonserous) RPE detachment.

TABLE 2. Clinical subtypes of ARM patients, by study site (n, %).

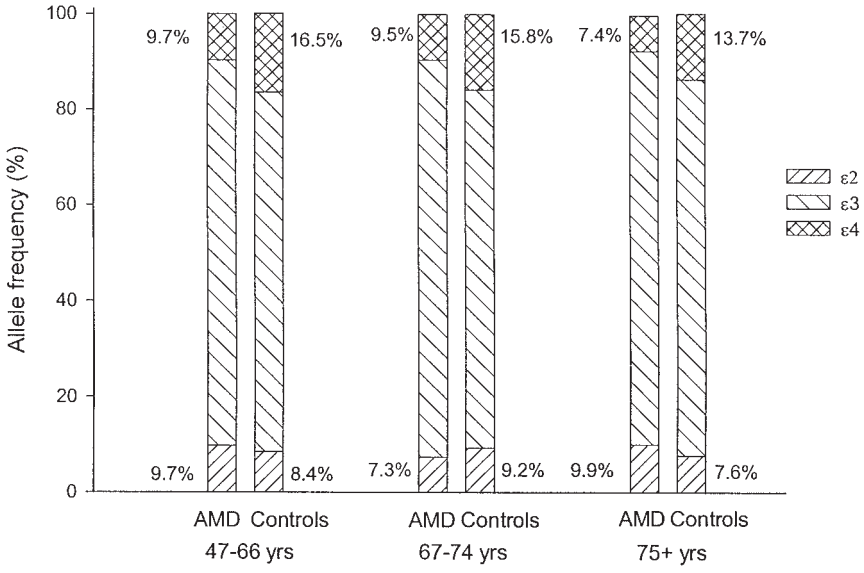

Fig. 1. APOE allele frequencies for ARM cases (n = 617) vs. controls (n = 1260), by age (at exam) group.

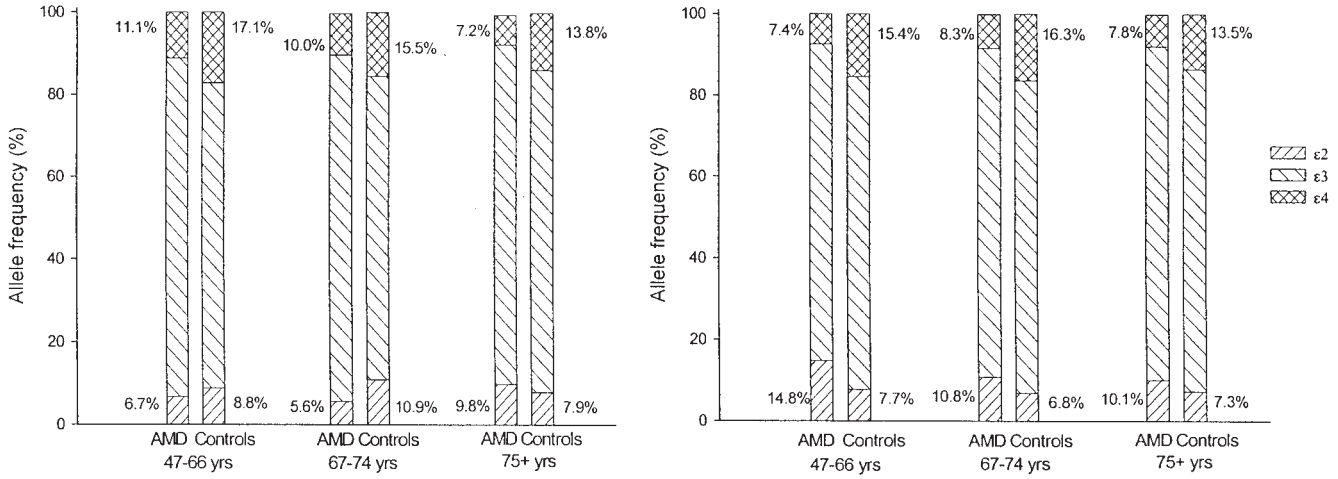

Fig. 2. APOE allele frequencies for female (left) and male (right) ARM cases vs. controls, by age (at exam) group.

the respective patient and control populations. Then, χ^2 tests comparing allele frequencies between ARM cases and controls were computed within strata defined by age groups chosen to have similar sample sizes (cases and controls combined). Age at examination was used for both cases and controls. Since the ocular changes associated with ARM reflect a clinical continuum, the use of age at exam was considered preferable to the assignment of an ambiguous age of onset for cases. In addition to χ^2 tests, multiple logistic regression analysis was used to estimate odds ratios for APOE genotypes, which were adjusted for the effects of age and sex and used the most common APOE-3/3 genotype as the referent group. Subgroup analyses for atrophic and neovascular ARM were performed as described below. Due to the lack of a control group from UP, the standard approach of using dummy variables for study site in the logistic regression model to assess effect heterogeneity across sites was not feasible. Therefore, homogeneity tests across study sites for variables of interest were carried out separately for case and control groups. Note that two of the four data sets included here (UP, UCLA) had not been previously analyzed with a case-control approach; the other two had.[15,17]

Results A comparison of mean age at exam, gender, and APOE genotype and allele frequencies across study sites, separately for patients (from four sites) and controls (from three sites), is shown in Table 1. Overall, there was a significant difference in mean age at exam ($p < 0.0001$) and gender distribution ($p < 0.0001$) between cases and controls. Therefore, it was crucial to adjust all analyses for the potential confounding effect of these variables. The χ^2 statistic for assessing homogeneity across sites of APOE allele frequencies among cases was significant (6 degrees of freedom (df), $p = 0.01$), mostly due to a higher frequency of the APOE-4 allele in the DUMC/VUMC patients (11.2%) compared to UP (6.0%) and EUR (6.4%), with UCLA (8.2%) being intermediate. However, no significant differences in APOE allele distribution were found between the DUMC/VUMC, UCLA, and EUR control groups ($p = 0.41$), indicating that a pooled analysis of our data was appropriate. For patients, the distribution of clinical subtypes of ARM was similar across study sites (Table 2), apart from the absence of early ARM in the EUR data set.

In the pooled data set, there was no evidence for deviations from HWE in either cases ($\chi^2 = 1.62$, 3 df, $p = 0.65$) or controls ($\chi^2 = 3.74$, 3 df, $p = 0.29$). An overall comparison of allele frequencies between cases and controls is shown, by age of exam, in Figure 1. The APOE-4 allele was less common in cases than controls in all three age groups (9.7% vs. 16.5% in age group 47–66 years, 9.5% vs 15.8% in age group 67–74 years, 7.4% vs. 13.7% in age group 75+ years). Global allele frequency distributions were significantly different between cases and controls for the two older age groups ($p = 0.004$ and $p = 0.0004$, respectively). These results were very similar in subgroups of only female or only male cases and controls (Figure 2). For the APOE-2 allele, we observed consistently higher frequencies in male ARM cases than male controls across all age groups (14.8% vs. 7.7% in age group 47–66 years, 10.8% vs 6.8% in age group 67–74 years, 10.1% vs. 7.3% in age group

Genotype[a]	*ARM cases (n = 617) vs. controls (n = 1260)*
APOE-3/3	(reference)
APOE-4/*	0.54 (0.41–0.70), p < 0.0001
APOE-2/*, female	0.74 (0.52–1.06)
APOE-2/*, male	1.54 (0.97–2.45), p = 0.01 for interaction of APOE-2 and sex

[a]Genotypes with the APOE-2 allele were combined, and those with the APOE-4 allele were combined. Subjects with the APOE-2/4 genotype were included in both the APOE-2/* and APOE-4/* groups.

TABLE 3. Odds ratio estimates (95% confidence intervals, p-values) for APOE genotypes, based on logistic regression for cases and controls from all study sites, with adjustment for age at exam (continuous) and sex.

75+ years), whereas no consistent trend was seen for female patients vs. female controls (Figure 2).

Age- and sex-adjusted odds ratios for the different APOE genotypes, with the homozygous APOE-3/3 genotype as the referent group, were also computed with a multiple logistic regression model and are summarized in Table 3. A significantly lower risk of ARM for carriers of the APOE-4 allele was found (OR 0.54, 95% CI 0.41–0.70, p < 0.0001). In addition, there was significant evidence for an interaction between APOE-2 carrier status and sex. The OR for female carriers of the APOE-2 allele was 0.74 (95% CI 0.52–1.06), whereas the OR for male carriers was 1.54 (95% CI 0.97–2.45). The ratio of the sex-specific odds ratios was significantly different from 1.0 (p = 0.01 for interaction on the multiplicative odds scale). This confirmed the results of the APOE allele frequency comparison and suggests that an increased risk of ARM due to the APOE-2 allele may only be conferred to males.

The protective effect of the APOE-4 allele and the interaction of sex and APOE-2 carrier status in the pooled data set remained essentially unchanged when patients with early ARM (n = 93) and controls who had not been specifically examined for early signs of ARM (n = 309) were removed (data not shown). When subgroups of atrophic (n = 117) and neovascular ARM patients (n = 407) were analyzed separately, the protective APOE-4 effect was found in both groups, with an OR of 0.40 (95% CI 0.23–0.72, p = 0.002) for atrophic and an OR of 0.61 (95% CI 0.45–0.82, p = 0.001) for neovascular ARM. The interaction of sex and APOE-2 carrier status was only observed in the subgroup of neovascular patients, compared to all controls (p = 0.03 for interaction on the multiplicative odds scale), which could be due to a lack of statistical power in the smaller subgroup of atrophic ARM patients. The overall APOE allele distribution did not differ significantly between atrophic and neovascular ARM patients (p = 0.34).

Discussion We examined the relationship of APOE genotypes and risk of ARM in the largest case-control data set available to date (617 cases, 1260 controls). Our results confirm the previously reported protective effect of the APOE-4 allele on ARM risk both for the overall study population as well as for atrophic and neovascular subgroups. In addition, there was evidence for a potential increase in ARM risk due to the APOE-2 allele for males, whereas this effect was absent in females. A similar sex-by-genotype interaction has been proposed for

the risk of coronary artery disease, where the APOE-4 allele was found to be an independent risk factor for coronary events in men, but not in women.[34] The mechanism for this sex-specific effect is currently unknown.[35] A risk-increasing effect of APOE-2 and a protective effect of APOE-4 has also been reported for chronic, and possibly acute, renal failure.[36]

The case-control approach to candidate gene analysis has been criticized recently because of its potential for detecting spurious associations due to population stratification. This putative shortcoming has been a driving force in the development of family-based association analysis methods.[37] The main advantage of these family-based methods is their robustness to population stratification, albeit at the cost of some loss of power.[38,39] Population stratification can cause spurious association if certain subgroups (e.g., those characterized by different ethnic backgrounds) differ in both the frequency of the disease and the allelic risk factor under study. For a study such as the present one, this concern may arise due to the pooling of different study populations (different geographic origin, clinic-based versus population-based ascertainment). We have attempted to minimize the possibility of population stratification by restricting the analysis to individuals of Caucasian ethnicity. In addition, APOE allele frequencies in the three control groups combined here (DUMC/VUMC, UCLA, EUR) were compared and found not to be significantly different from each other. APOE allele frequencies also agreed well with those of other published Caucasian control populations in similar age groups.[40] It has been argued that the difference between subgroups in terms of both disease and risk factor prevalence must be quite large to cause spurious associations,[39,41] and empirical studies have indicated that the potential bias resulting from uncontrolled population stratification is smaller than anticipated.[42] Therefore, concern about population stratification may have been overemphasized, and it is important to realize that the case-control study is the most powerful approach to candidate gene analysis in the absence of extensive population substructure. The fact that APOE-4 frequencies in ARM patients from all four study sites were consistently lower than those in control subjects further supports the significance of our results.

We believe that age and sex are the most important potential confounding variables for the APOE-AMD association. By controlling for the effect of these variables in a logistic regression model, we have ensured that the observed association is not due to the heterogeneity of cases and controls in terms of their age or sex distribution. A potential confounding factor that we were unable to include in our analysis is the presence of atherosclerosis in ARM patients and controls. Atherosclerosis has been suggested as a risk factor for ARM. The APOE-4 allele is a known risk factor for atherosclerosis, and thus, disease prevalence and frequency of the APOE-4 allele may differ in atherosclerotic and nonatherosclerotic subgroups of our study population. However, it is unlikely that this type of confounding would produce an effect of the APOE-4 allele on ARM risk that is opposite from that in atherosclerosis, i.e. protective rather than associated with increased risk. Logistic regression analysis of the EUR data[15] adjusted

for the presence of lower-extremity arterial disease, which did not significantly alter the risk estimates for APOE genotypes. This supports the hypothesis that the inclusion of this variable would not affect the results presented here.

We also examined whether our conclusions would be altered if we did not include (i) the patient population from UP, for which no corresponding control group was available; (ii) the early ARM cases, which could be argued to introduce heterogeneity within the pooled population of patients, particularly since the EUR data did not include early ARM cases; and (iii) the controls on whom no fundus photographs documenting absence of ARM were obtained (UCLA and 309 (out of 344) DUMC/VUMC controls). The comparison of the remaining 349 cases and 878 controls via logistic regression resulted in an odds ratio for APOE-4 carriers of 0.66 (95% CI 0.47–0.92, $p = 0.01$) and the interaction of sex and APOE-2 carrier status remained significant ($p = 0.03$). Therefore, our conclusions based on this reduced data set are identical to those reached with the overall analysis.

It cannot be ruled out that the functional gene modulating risk of ARM is not APOE, but rather a gene whose allele is in linkage disequilibrium with an APOE allele. However, the fact that we have observed opposite effects in two different alleles of the same gene supports the hypothesis that APOE itself is the etiologically relevant polymorphism. In addition, recent laboratory studies have shown that the apoE protein is strategically located where drusen accumulate. It is a ubiquitous component of ocular drusen, irrespective of an individual's clinical phenotype, and the adult human retina is the site with the body's second-highest apoE production, after the liver.[43] Interestingly, a high degree of compositional similarity between ocular drusen and atherosclerotic plaques, but dissimilarity of drusen and the types of amyloid plaques characteristic of Alzheimer disease, have been reported.[44] In mice, the absence of functional apoE protein has been associated with increased amounts of age-dependent debris in Bruch's membrane.[45,46] ApoE-deficient mice were also shown to have abnormal retinae with lower cell numbers in inner and outer nuclear layers, thicker Bruch's membrane, and abnormal elastic lamina, especially when fed a high-cholesterol diet.[47] Therefore, apoE may play an important role in maintaining normal retinal function. For example, a potential role may include the removal of spent lipids from photoreceptors, which are known to have a very high turnover rate and to achieve total membrane renewal within 10 days by constantly shedding their outer lipid-rich segments.[48] Alternatively, apoE may be capable of reducing oxidative damage to RPE cells via regulation of nitric oxide production.[49,50]

Without a doubt, the role of apoE protein in the human retina will continue to be examined in future laboratory studies. While the number of epidemiologic studies of APOE and ARM carried out to date is still substantially smaller than, for example, the number of studies that examined the relationship between APOE and Alzheimer disease, they have thus far led to very consistent results for Caucasian study populations.[14–20] In contrast to the increased risk of Alzheimer disease conferred by the APOE-4 allele, this same allele appears to have a

protective effect on ARM risk. The only study that did not confirm this protective effect was based on Chinese patients and controls.[27] This lack of association could be due to a different APOE-associated risk in different ethnic groups. Alternatively, it may be due to a lack of statistical power since the frequency of the APOE-4 allele is lower in Chinese than in Caucasian populations. The potential effect of the less frequent APOE-2 allele on ARM risk has been more difficult to estimate. The pooled case-control study presented here has raised the possibility that this allele confers an increased ARM risk to males, but not females. However, this effect is in need of replication in additional studies of similarly large size. Future studies should also include the investigation of polymorphisms in the APOE regulatory region. For Alzheimer disease, several of these promoter polymorphisms have been reported to regulate transcriptional activity of the APOE gene and to correlate with disease risk independently of the APOE-2 and APOE-4 effects.[51,52] If a similar association of APOE promoter polymorphisms and ARM risk were shown to exist, this would provide additional evidence that APOE itself modulates susceptibility to ARM.

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