Apolipoprotein E Gene Polymorphisms and Retinal Vascular Signs

The Atherosclerosis Risk in Communities (ARIC) Study

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Objective: To examine the association between apolipoprotein E (APOE) gene polymorphisms and retinal microvascular signs.

Methods: Population-based, cross-sectional study. Participants from the Atherosclerosis Risk in Communities Study (n=10,036; aged 49-73 years) had retinal photographs taken in 1 randomly selected eye. Photographs were graded for presence of retinal microvascular signs using a standardized protocol; a computer-assisted method was used to measure retinal vessel diameter. DNA from blood samples was analyzed for common APOE alleles.

Results: After adjusting for age, sex, systolic blood pressure, total serum cholesterol, triglycerides, and other co-variates, APOE ε4 was associated with nondiabetic retinopathy in white (multivariate-adjusted odds ratio, 1.3; 95% confidence interval, 1.0-1.6) and black (multivariate-adjusted odds ratio, 1.4; 95% confidence interval, 1.0-2.1) individuals. Other retinal microvascular signs were not strongly associated with APOE polymorphisms. Neither retinal arteriolar nor venular diameter was associated with APOE polymorphisms in white or black individuals.

Conclusions: Apolipoprotein E ε4 was weakly associated with retinopathy in persons without diabetes. Other signs were less consistently associated with APOE polymorphisms.

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RETINAL MICROVASCULAR signs (eg, isolated microaneurysms, hemorrhages, and arteriovenous nicking) are common fundus findings in adults, even in those without diabetes. Population-based studies have shown that these signs are detectable in 3% to 14% of individuals aged 40 years and older without diabetes. \(^1\) \(^2\) Recent studies show that these signs are associated with subclinical and clinical stroke independent of traditional risk factors \(^3\) \(^9\) and may mirror microvascular disease in the brain and possibly other organs. \(^10\) Although studies show that these retinal signs are often associated with hypertension, cigarette smoking, and other cardiovascular risk factors, a substantial proportion of retinal microvascular signs in the population are not explained by known environmental risk factors, indicating possible genetic influence. \(^11\) \(^12\)

Apolipoprotein E (APOE) is a major apolipoprotein that is widely expressed in the central nervous system. \(^13\) \(^14\) The APOE gene is polymorphic with 3 codominant alleles (ε2, ε3, and ε4), which code for 6 APOE phenotypes. Apolipoprotein E polymorphisms have been linked with incident ischemic stroke, \(^15\) an association believed to be mediated by its effects on lipid metabolism and large-vessel atherosclerosis. \(^16\) \(^19\) However, whether the APOE gene also has similar effects on the small vessels or microvasculature is less clear. As some studies have shown that APOE polymorphisms are associated with magnetic resonance imaging-defined cerebral white matter lesions, which are manifestations of cerebral microvascular disease, \(^20\) \(^21\) we hypothesized that APOE alleles may influence disease processes at the microvascular level. In the current study, we examine the association of APOE polymorphisms with retinal microvascular signs in a large population-based cohort of white and black middle-aged Americans.

METHODS

STUDY POPULATION

The Atherosclerosis Risk in Communities (ARIC) study is a population-based cohort study that included 15,792 women and men aged 45 to 64 years at recruitment in 1987-1989. \(^22\) The study population was selected by...
probability sampling from 4 US communities: Forsyth County, North Carolina; Jackson, Miss; suburbs of Minneapolis, Minn; and Washington County, Maryland. The Jackson sample included only African American individuals; in the other field centers, samples were representative of the populations in these communities (mostly white in Minneapolis and about 15% African American in Washington County and Forsyth County). Of the 13,792 participants at baseline, 14,346 (93% of survivors) returned for a second examination 3 years later in 1990-1992, and 12,887 (86% of the survivors) returned for a third examination 3 years after the second examination in 1993-1995. Differences between participants and nonparticipants at baseline examination have been described elsewhere.23 The study population is derived from individuals who participated at the third examination when retinal photography was first performed. Of the 12,887 participants who were seen for this examination, we excluded the following: 478 participants who did not have APOE data or who did not give consent for DNA analyses; 224 who did not have retinal photographs; 1734 who had ungradable photographs; 21 with retinal vein occlusions; 103 with other missing data; 211 who were not seen at the second examination; 38 whose race was neither black nor white (owing to small numbers); and 42 black residents in Minneapolis and Maryland (so that race and field center could be adjusted in the following 5 categories: Jackson; white participants in Forsyth County; African American participants in Forsyth County; Minneapolis; and Maryland). For this current analysis, 10,036 participants remained, whose data were used. Characteristics of participants with and without gradable retinal photographs have been previously reported.24,25 In general, participants with gradable photographs were younger and more likely to be white but did not differ from participants with ungradable photographs on sex or smoking status. Institutional review boards at each study site and at the Fundus Photograph Reading Center at the University of Wisconsin approved the study. Informed consent was obtained from all participants and the study was conducted in accordance with the Declaration of Helsinki.

ASSESSMENT OF RETINAL MICROVASCULAR SIGNS

The retinal photography procedure and grading of retinal microvascular signs have been described in detail elsewhere.24,25 Retinal photographs of 1 randomly selected eye from each participant were taken at the third examination. If the selected eye was considered too difficult or not possible to photograph with adequate quality (eg, the pupil could not be dilated to ≥4 mm, we were unable to adequately fixate for proper photographic field definition, or opacities of the ocular media prevented a reasonably clear view of the retina), the other eye was photographed instead. This occurred infrequently (2.4% of the cohort). Following 5 minutes of dark adaptation, a 45° photograph was taken (centered on the region of the optic disc and the macula) using an autolocus camera.

Trained graders, masked to participant characteristics, examined the photographs for the presence of focal retinal microvascular abnormalities, including (1) retinopathy lesions (ie, presence of microaneurysms, retinal hemorrhages, cotton wool spots, or hard exudates), (2) arteriovenous (AV) nicking, and (3) focal arteriolar narrowing. The grading and definition of these lesions were based on a standard protocol described in other reports.24 For analysis of retinopathy, we excluded persons with diabetes, because the pathogenesis of diabetic retinopathy is different. Thus, the retinopathy lesions in this study are referred to as nondiabetic retinopathy.

To quantify retinal arteriolar and venular diameters, the fundus photographs were digitized and the diameters of all arterioles and venules moving through a specified area surrounding the optic disc were measured using a computer-assisted approach. Individual vessel diameters were combined into summary measures of arteriolar and venular diameters of the eye, based on formulas by Parr and Hubbard.24 Quality control procedures for retinal photography and grading have been previously reported.24 In general, intragrader and intergrader \( \chi^2 \) statistics for various microvascular signs ranged from 0.61 to 1.00. For retinal vessel diameter measurements, intragrader and intergrader reliability coefficients were 0.84 and 0.79, respectively. Intraindividual reliability coefficients were also generally high.24

APOE GENOTYPING

Genomic DNA was isolated from venous blood leukocytes using a standard procedure.27 Apolipoprotein E genotyping was performed for the entire cohort at the Human Genetics Center at the University of Texas, Houston. The APOE variants at codons 130 and 176 (formerly 112 and 138) were each detected separately using the TaqMan assay (Applied Biosystems, Foster City, Calif). We separately analyzed the 3 common alleles of APOE (\( \epsilon_2 \), \( \epsilon_3 \), and \( \epsilon_4 \)) and its 6 common genotypes.

OTHER VARIABLES

Participants underwent standardized evaluations at each examination.22 Blood pressures were taken with a random-zero sphygmomanometer; the mean of the last 2 of 3 measurements was used for analyses. Hypertension and diabetes history, cigarette smoking, alcohol consumption, and use of antihypertensive and antidiabetic medications were ascertained from a questionnaire. Hypertension was defined as a participant having a systolic blood pressure of ≥140 mm Hg or higher, a diastolic blood pressure of 90 mm Hg or higher, or having used antihypertensive medication within the previous 2 weeks. Diabetes was defined as having a fasting glucose of 126 mg/dL or higher (≥7.0 mmol/L), a nonfasting glucose of 200 mg/dL or higher (≥11.1 mmol/L), or a self-reported history of physician-diagnosed diabetes or pharmaceutical treatment for diabetes. Total plasma cholesterol and triglycerides were measured by enzymatic methods; high-density lipoprotein cholesterol was measured after dextran-magnesium precipitation of the non–high-density lipoproteins; and glucose was measured by the modified hexokinase/glucose-6-phosphate dehydrogenase procedure. Height and weight were measured to record body mass index, calculated as weight in kilograms divided by height in meters squared. All variables were based on the third examination at the time of retinal photography.

STATISTICAL ANALYSIS

We examined the prevalence of retinal microvascular signs among carriers of the 3 main APOE alleles (\( \epsilon_2 \), \( \epsilon_3 \), and \( \epsilon_4 \)) and its 6 genotypes (\( \epsilon_2/\epsilon_2 \), \( \epsilon_2/\epsilon_3 \), \( \epsilon_2/\epsilon_4 \), \( \epsilon_3/\epsilon_3 \), \( \epsilon_3/\epsilon_4 \), and \( \epsilon_4/\epsilon_4 \)); however, for ease of interpretation, only the results of the allele analyses are given. We defined \( \epsilon_2 \) allele carriers to include genotypes \( \epsilon_2/\epsilon_2 \), \( \epsilon_2/\epsilon_3 \), and \( \epsilon_2/\epsilon_4 \); and \( \epsilon_4 \) allele carriers to include genotypes \( \epsilon_3/\epsilon_4 \) and \( \epsilon_4/\epsilon_4 \). Because allele frequencies are different in white and black individuals, we performed analyses separately. We tested whether the distribution of APOE alleles was in Hardy-Weinberg equilibrium using \( \chi^2 \) tests comparing observed with expected proportions. As hypertension is strongly associated with retinal microvascular signs, we performed subgroup analyses in persons with and without hypertension. Analysis of covariance was used to obtain adjusted mean retinal vessel diameters. We calculated odds ratios (ORs) and confidence

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Baseline characteristics of participants carrying different APOE alleles are given in Table 1. White carriers of the ε2 allele had higher fasting blood glucose, higher total plasma triglycerides, and lower total plasma cholesterol than carriers of the ε3 and ε4 alleles. White carriers of the ε4 allele had the lowest fasting blood glucose levels and highest total plasma cholesterol and were less likely to have diabetes than carriers of the ε2 allele and those with the ε3/ε3 genotype. A similar pattern was observed in black participants, except for black ε2 carriers, who had similar total plasma triglycerides as black ε3 and ε4 carriers. In both white and black participants, mean systolic and diastolic blood pressure did not differ significantly between the 3 APOE allele groups. Allele frequencies were 0.08 (ε2), 0.77 (ε3), and 0.15 (ε4) in white individuals, and 0.11 (ε2), 0.66 (ε3), and 0.23 (ε4) in black individuals, which are similar to previously published figures. APOE variation was in Hardy-Weinberg equilibrium in both white (P = .86) and black (P = .19) participants.

The association between focal retinal signs and APOE alleles is given in Table 2. In white individuals, ε4 carriers had a slightly lower prevalence of retinal AV nicking (multivariate-adjusted OR, 0.8; 95% CI, 0.7-1.0) when compared with the ε3/ε3 reference group, whereas in black participants, ε2 carriers had a slightly higher prevalence of AV nicking (multivariate-adjusted OR, 1.3; 95% CI, 1.0-1.8). Focal arteriolar narrowing was more prevalent in white ε2 carriers compared with white ε3/ε3 carriers (multivariate-adjusted OR, 1.3; 95% CI, 1.0-1.5), but no association was observed between APOE alleles and focal arteriolar narrowing in black participants. In both white and black participants without diabetes, signs of nondiabetic retinopathy were more common in ε4 carriers than carriers of the ε3/ε3 genotype (in white participants, multivariate-adjusted OR, 1.3; 95% CI, 1.0-1.6; in black participants, multivariate-adjusted OR, 1.4; 95% CI, 1.0-2.1). We repeated the aforementioned multivariate analyses with additional adjustment for pack-years of cigarette smoking and diastolic blood pressure; the results remained unchanged.

No statistically significant differences in retinal arteriolar or retinal venular diameters were associated with APOE polymorphisms in white or black participants (Table 3). When stratified by hypertensive status (Table 4), a lower prevalence of AV nicking was associated with the ε4 allele in white individuals both with and without hypertension, though the association was only statistically significant in white participants without hypertension (multivariate-adjusted OR, 0.8; 95% CI, 0.7-1.0). White ε2 carriers were slightly more likely to have focal arteriolar narrowing than ε3/ε3 carriers, regardless of whether or not they had hypertension (multivariate-adjusted OR, 1.3; 95% CI, 1.0-1.6; in both those with and without hypertension). In the subgroup of black participants without hypertension, ε2 carriers were more likely to have focal arteriolar narrowing than ε3/ε3 carriers (multivariate OR, 2.0; 95% CI, 1.1-3.5). This association was not seen in the subgroup of black participants with hypertension, but the interaction between APOE polymorphisms and hypertension on focal arteriolar narrowing in black individuals was not significant. Nondiabetic retinopathy was slightly more common in both black and white carriers of the ε4 allele regardless of hypertension status, though these associations were marginally nonsignificant (P = .06).
In white individuals without diabetes, we found that blot hemorrhage (a common retinopathy lesion) was not associated with APOE polymorphisms (adjusted OR, 0.7; 95% CI, 0.3-1.4; comparing ε2 carriers [n = 10; 0.6%] with ε3/ε4 carriers [n = 34; 0.8%]), but microaneurysms were associated with APOE polymorphisms (adjusted OR, 1.6; 95% CI, 1.1-2.5; comparing ε4 carriers [n = 37; 2.1%] with ε3/ε4 carriers [n = 53; 1.3%]). In black individuals without diabetes, blot hemorrhages were associated with APOE polymorphisms (adjusted OR, 3.1; 95% CI, 1.0-10.3; comparing ε4 carriers [n = 11; 1.8%] with ε3/ε4 carriers [n = 4; 0.6%]), but microaneurysms were not (adjusted OR, 1.3; 95% CI, 0.7-2.4; comparing ε4 carriers [n = 8; 1.3%] with ε3/ε4 carriers [n = 2; 0.6%]). APOE ε4 allele was associated with retinopathy signs in both white and black participants without diabetes. The ε2 allele was associated with focal arteriolar narrowing in both white and black individuals. In white participants, the ε4 allele was weakly associated with a lower prevalence of AV nicking; whereas in black participants, the ε2 allele was weakly associated with a higher prevalence of AV nicking.

There are few studies for direct comparison. Our findings for retinopathy in both black and white individuals without diabetes are consistent with findings from an earlier study of 88 persons with hypertension in which the ε4 allele was found to be associated with retinopathy lesions (P < .05)30 and left ventricular hypertrophy (P < .001) when compared with non-ε4 carriers.30 The Cardiovascular Health Study (approximately 2000 individuals), using similar methodology, also recently found that white and black ε4 carriers had a higher rate of retinopathy than ε3 carriers (T.Y.W., unpublished data, 2006). These findings suggest that ε4 may confer a slightly increased susceptibility to retinal microvascular damage. An associ-

### Table 2. Focal Signs and APOE Alleles in White and Black Individuals

<table>
<thead>
<tr>
<th>Focal Sign</th>
<th>APOE Allele</th>
<th>White Individuals</th>
<th>Black Individuals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of Carriers</td>
<td>No. (%)</td>
<td>Age, Sex, and Center Adjusted</td>
</tr>
<tr>
<td>AV nicking</td>
<td>ε2</td>
<td>1216</td>
<td>179 (14.7)</td>
</tr>
<tr>
<td></td>
<td>ε3/ε3</td>
<td>4731</td>
<td>680 (14.4)</td>
</tr>
<tr>
<td></td>
<td>ε4</td>
<td>1995</td>
<td>247 (12.4)</td>
</tr>
<tr>
<td>Focal arteriolar narrowing</td>
<td>ε2</td>
<td>1216</td>
<td>215 (17.6)</td>
</tr>
<tr>
<td></td>
<td>ε3/ε3</td>
<td>4731</td>
<td>713 (15.1)</td>
</tr>
<tr>
<td></td>
<td>ε4</td>
<td>1995</td>
<td>315 (15.7)</td>
</tr>
<tr>
<td>Nondiabetic retinopathy†</td>
<td>ε2</td>
<td>1067</td>
<td>42 (3.9)</td>
</tr>
<tr>
<td></td>
<td>ε3/ε3</td>
<td>4135</td>
<td>169 (4.1)</td>
</tr>
<tr>
<td></td>
<td>ε4</td>
<td>1790</td>
<td>91 (5.1)</td>
</tr>
</tbody>
</table>

### Table 3. Mean Retinal Vessel Diameter and APOE Alleles in White and Black Individuals

<table>
<thead>
<tr>
<th>Retinal Vessel Index</th>
<th>APOE Allele</th>
<th>White Individuals</th>
<th>Black Individuals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of Participants</td>
<td>Age, Sex, and Center Adjusted</td>
<td>Multivariate Adjusted*</td>
</tr>
<tr>
<td>Mean retinal arteriolar diameter (95% CI), µm</td>
<td>ε2†</td>
<td>1216</td>
<td>161.0 (160.1-161.9)</td>
</tr>
<tr>
<td></td>
<td>ε3/ε3</td>
<td>4731</td>
<td>161.4 (160.1-161.9)</td>
</tr>
<tr>
<td></td>
<td>ε4</td>
<td>1995</td>
<td>161.8 (160.1-162.5)</td>
</tr>
<tr>
<td>P value§</td>
<td>.44</td>
<td>.47</td>
<td>.1</td>
</tr>
<tr>
<td>Mean retinal venular diameter (95% CI), µm</td>
<td>ε2‡</td>
<td>1216</td>
<td>192.0 (191.1-192.9)</td>
</tr>
<tr>
<td></td>
<td>ε3/ε3</td>
<td>4731</td>
<td>191.4 (190.9-191.9)</td>
</tr>
<tr>
<td></td>
<td>ε4‡</td>
<td>1995</td>
<td>191.1 (190.4-191.8)</td>
</tr>
<tr>
<td>P value§</td>
<td>.29</td>
<td>.18</td>
<td>.49</td>
</tr>
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</table>

### COMMENT

In this study of a middle-aged mixed-race adult population, we report on the relationship between APOE allele polymorphisms and retinal microvascular signs. In general, we found few strong or consistent associations. The

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between focal arteriolar narrowing and hypertension.35

We observed that white carriers of the ε2 allele were more likely to have focal retinal arteriolar narrowing than carriers of the ε3/ε3 genotype, regardless of hypertensive status, whereas in black carriers, ε2 was associated with this sign only in those free of hypertension. These associations could have arisen because of the pathophysiology underlying any potential relationship between focal arteriolar narrowing and the ε2 allele is not known, except for a possible but controversial link between ε2 and hypertension33,34 and a strong link between focal arteriolar narrowing and hypertension.35 We are unable to explain the inconsistent associations between AV nicking and APOE alleles in white and black individuals. We found no relationship between APOE alleles and retinal arteriolar or venular diameter in white or black participants, which is consistent with a recent genomic analysis that did not detect a link between the APOE gene and retinal vessel diameter.12 Apolipoprotein E polymorphisms are reported to modulate arteriolar smooth muscle proliferation and differentiation13 as well as vascular endothelial function36; these mechanisms may underlie, in part, the differences in retinal microvascular signs we observed with different APOE alleles.

Our results are consistent with some37,38 but not all39,40 reports that APOE polymorphisms may affect the risk of retinal diseases, such as age-related macular degeneration. Retinal vascular signs are promising as markers of systemic microcirculatory damage and may be useful in cardiovascular risk prediction.5,7 Our findings may have research implications for future clinical studies, as they suggest that (1) APOE polymorphisms have weak, if any, effects on the retinal microcirculation, in contrast to their stronger effects on the systemic macrocirculation,16-19 and (2) studies investigating links between retinal vascular signs and cardiovascular disease do not need to account for the effects of APOE on the retinal vasculature.

This study has several significant strengths, including a large sample size, a study population randomly drawn from communities, standardized assessment of retinal microvascular signs from photographs, and detailed information on a variety of risk factors. In particular, this is the first study of which we are aware that systematically examines the relationship between APOE polymorphisms and retinal microvascular signs, both of which may have important roles in cardiovascular risk stratification. The large sample size of our study and the mostly negative associations we report make it unlikely that strong associations between retinal signs and APOE polymorphisms were missed. Important limitations of our study should be considered when interpreting the findings. First, the positive associations could have been due to chance and should be confirmed in other populations. Second, residual confounding or unmeasured confounders could have biased our findings, though such confounders would have to be highly prevalent and strongly associated with both APOE and retinal vascular signs to explain our results.41 We adjusted for most known major confounding variables in our analyses. Finally, grading was performed from a single retinal photograph taken through a nonpharmacologically dilated pupil in 1 eye in each participant. This underestimates the prevalence of retinal microvascular signs. Such underestimation and the resulting miscategorization is likely to have biased the observed associations toward the null.42

In summary, in this ARIC study population we found that APOE alleles were, in general, weakly and inconsis-
ently associated with retinal microvascular signs. The most consistent associations were observed in white and black individuals without diabetes, where carriers of the e4 allele were more likely to have retinopathy.

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Author Contributions: Dr Liew had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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