Apolipoprotein E and Carotid Artery Atherosclerosis
The Rotterdam Study

A.J.C. Slooter, MD, PhD; M.L. Bots, MD, PhD; L.M. Havekes, PhD; A. Iglesias del Sol, MD; M. Cruts, PhD; D.E. Grobbee, MD, PhD; A. Hofman, MD, PhD; C. Van Broeckhoven, PhD; J.C.M. Witteman, PhD; C.M. van Duijn, PhD

Background and Purpose—Carotid artery atherosclerosis is a strong predictor for future stroke. It is yet unclear whether the apolipoprotein E polymorphism (*APOE*) is related to atherosclerosis in the carotid arteries. The aim of the present study was to investigate the role of *APOE* in carotid artery atherosclerosis.

Methods—A population-based cross-sectional study was performed on 5401 subjects. Atherosclerosis was noninvasively assessed by the common carotid artery intima-media wall thickness and the presence of plaques in the carotid arteries. The relationship of the 6 *APOE* genotypes with these 2 indicators was studied with linear and logistic regression analysis, respectively, with adjustments for age and sex.

Results—Carriers of the *E2E3* genotype had a thinner intima-media wall thickness (mean difference, −0.02 mm; 95% CI, −0.03 to −0.01 mm) and fewer plaques (odds ratio for >3 plaques at 6 sites, 0.6; 95% CI, 0.4 to 0.8) than the most common group, *E3E3*. The *E4E4* group had slightly more atherosclerosis, but this was not statistically significant. Adjusting for the level of the apolipoprotein E protein (apoE) in serum or total or HDL cholesterol did not essentially change these findings.

Conclusions—Our results suggest that APOE*4 is not an important risk factor for carotid artery atherosclerosis. The inverse relationship of *E2E3* with carotid artery atherosclerosis seems to be independent of serum apoE and total and HDL cholesterol levels. However, the low frequency, together with the small effects, implies that any protective effect of *E2E3* on carotid artery atherosclerosis is limited. **(*Stroke*. 2001;32:1947-1952.)**

Key Words: apolipoproteins ■ atherosclerosis ■ carotid arteries ■ genetics ■ polymorphism (genetics)

High levels of total and LDL cholesterol and low levels of HDL cholesterol predispose to the development of atherosclerosis.[1] Serum levels of these lipids are partly determined by the apolipoprotein E genotype (*APOE*). The *APOE* gene has 3 common alleles, APOE*2, APOE*3, and APOE*4, which fully determine the protein isoforms apoE2, apoE3, and apoE4, respectively, and partly determine the level of apolipoprotein E protein (apoE) in serum.[2–4] Compared with APOE*3 homozygotes, the most common genotype, APOE*2, is associated with lower levels of total and LDL cholesterol and with higher levels of HDL cholesterol, while APOE*4 has opposite effects.[2–4] ApoE plays a pivotal role in the transport of lipoproteins[4] and is involved in numerous processes in the arterial wall.[5]

Research on the *APOE* polymorphism and stroke has been inconclusive, but many of the studies were heterogeneous and small.[6–14] Because ultrasonographically assessed atherosclerosis in the carotid arteries strongly predicts a future stroke,[15] studies of these traits can efficiently be used to study stroke risk factors. Additionally, studies on *APOE* and carotid artery atherosclerosis have yielded inconsistent results.[16–21] Again, several investigations were relatively small. Since most studies were not population-based[16,19–21] and excluded subjects at high risk of atherosclerosis,[16–21] selection bias may have occurred. None of the previous studies evaluated an association between *APOE* and atherosclerotic plaques, while not more than 1 study explored the role of serum apoE level in carotid atherosclerosis.[21]

A putative relation between *APOE* and atherosclerosis could result either from differences in function of the isoforms or from differences in serum levels. The aim of this study was to investigate, in the general population of elderly, the association of the *APOE* genotype with atherosclerosis in carotid arteries, while considering the role of serum apoE level and total and HDL cholesterol.

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From the Department of Epidemiology and Biostatistics, Erasmus Medical Center, Rotterdam, Netherlands (A.J.C.S., M.L.B., A.I. del S., D.E.G., A.H., J.C.M.W., C.M. van D.); TNO Prevention and Health, Gaubius Laboratory and Department of Cardiology and Internal Medicine, University Hospital Leiden, Leiden, Netherlands (L.M.H.); Julius Center for Patient Oriented Research (M.L.B., D.E.G.) and Department of Neurology (A.J.C.S.), University Medical Center, Utrecht, Netherlands; and Molecular Genetics Laboratory, Flanders Interuniversity Institute for Biotechnology, Born-Bunge Foundation, Department of Biochemistry, University of Antwerpen, Antwerp, Belgium (M.C., C. Van B.).

Reprint requests to Professor C.M. van Duijn, Department of Epidemiology and Biostatistics, Erasmus Medical Center, Dr Molewaterplein 50, PO Box 1738, 3000 DR Rotterdam, Netherlands. E-mail vanduijn@epib.fgg.eur.nl

Stroke **is available at http://www.strokeaha.org**

TABLE 1. Characteristics of Study Population According to *APOE* Genotypes

	E2E2 (n=46)	*E2E3* (n=704)	E2E4 (n=137)	*E3E3* *(n=3122)*	*E3E4* *(n=1258)*	*E4E4* (n=134)
Age, y	71.4 (8.8)	69.3 (9.1)	69.2 (8.7)	69.2 (8.9)	69.1 (8.8)	68.1 (7.6)
Men	44% (20)	36% (256)	43% (59)	41% (1289)	41% (518)	45% (60)
Systolic blood pressure, mm Hg	142.5 (25.0)	139.2 (21.8)	138.5 (21.8)	140.0 (22.6)	137.6 (21.5)*	140.6 (22.8)
Diastolic blood pressure, mm Hg	74.8 (11.7)	73.5 (11.2)	73.0 (12.0)	73.7 (11.8)	72.7 (11.4)*	74.0 (11.4)
Diabetes mellitus	9% (4)	11% (79)	10% (13)	11% (328)	9% (111)	7% (9)
Body mass index, kg/m^2	26.9 (4.4)	26.5 (3.7)	26.5 (3.5)	26.3 (4.1)	26.1 (3.7)	26.1 (3.6)
Current smoking	22% (10)	23% (157)	25% (33)	23% (718)	23% (278)	18% (23)
Total cholesterol, mmol/L	6.42 (1.65)	6.38 (1.30)‡	6.54 (1.11)	6.62 (1.21)	6.79 (1.19)‡	6.81 (1.04)
HDL cholesterol, mmol/L	1.34 (0.31)	1.41 (0.39)‡	1.36 (0.40)	1.35 (0.36)	1.32 (0.36)*	1.31 (0.38)
ApoE level, mmol/L	2.21 (1.35)*	1.18 (0.53)‡	1.11 (0.45)†	0.81 (0.32)	0.64 (0.25)‡	0.45 (0.15)‡

Values are unadjusted means (SD) or proportions (numbers). In total, 44 subjects (0.8%) had missing data on blood pressure, 1 (0.02%) on diabetes mellitus, 82 (1.5%) on smoking, 32 (0.6%) on total cholesterol, and 39 (0.7%) on HDL cholesterol. Serum apoE was measured in a random sample (n=1194).

*Significantly different from *E3E3* group: $P<0.05$.

†Significantly different from *E3E3* group: $P<0.005$.

‡Significantly different from *E3E3* group: $P<0.0005$.

Subjects and Methods

Population

This study is part of the Rotterdam Study, a population-based, single-center cohort study on chronic and disabling diseases in the elderly. The design of the study has been described previously.[22] Informed consent was obtained from all subjects, and the study was approved by the medical ethics committee of Erasmus Medical Center. All inhabitants of a suburb of Rotterdam, aged at least 55 years, including people living in homes for the elderly, were invited to participate. In total, 7983 participants (response rate, 78%) were included (1990–1993). Blood was taken from 7041 participants of the Rotterdam Study (88%). *APOE* genotyping was performed in all subjects who donated blood (n=6852; 86% of the cohort). Failure to determine the *APOE* genotype resulted mainly from inadequate storage of buffy coats from leukocytes and was random. Ultrasonography of the carotid arteries was performed in 5854 participants (73%), of whom 5401 subjects had a known *APOE* genotype. Of these, 4273 persons had complete assessment on atherosclerotic plaques at both sides of the common carotid arteries, the carotid artery bifurcations, and the internal carotid arteries. Missing ultrasonography data were mainly due to limited availability of ultrasonographers in 1990 and 1991 and were random. In a random sample of the first 1200 participants, serum apoE level could be determined in 1194.

Investigations

To assess the presence of atherosclerosis in the carotid arteries, ultrasonography was performed with a 7.5-MHz linear-array transducer and a duplex scanner (ATL UltraMark IV). The intima-media thicknesses of the distal part of both common carotid arteries were measured and averaged, as described before.[23] In addition, both common carotid arteries, both carotid artery bifurcations, and both internal carotid arteries were evaluated for the presence of atherosclerotic plaques, defined as a focal widening relative to adjacent segments with protrusion into the lumen.[23] Information on health status, drug use, and smoking behavior was obtained with a computerized questionnaire. Height and weight were measured, and body mass index was calculated.

Laboratory Analysis

APOE genotyping and the determination of the serum apoE level were performed on coded samples from nonfasting subjects, without knowledge of the other measurements. Genotyping was performed with the use of a polymerase chain reaction, as described elsewhere.[24] Serum apoE levels were determined by enzyme-linked immunosorbent assay.[25] Serum total and HDL cholesterol were determined with an automated enzymatic procedure.[26]

Data Analysis

The association between the *APOE* genotype and carotid artery atherosclerosis was explored in the following multivariate models. Differences in common carotid artery intima-media wall thickness were studied in a multiple linear regression model. The relative prevalence of a plaque (ie, at least 1 plaque at either the left or the right side) in the common carotid arteries, the carotid bifurcations, and the internal carotid arteries was estimated by odds ratios (OR) with 95% CI with the use of multiple logistic regression models. Moreover, because plaques were assessed at 3 sides in the carotid arterial tree, at both the left and the right sides, the minimum number of plaques was 0, and the maximum number was 6. Because there is not further strong evidence to assume an association between *APOE* and atherosclerosis at 1 particular side, the study population was categorized into those without any plaques, persons with 1 to 3 plaques, and subjects with 4 to 6 plaques to explore a possible dose-response relationship. In the analyses of the ORs for 1 to 3 plaques, subjects with 4 to 6 plaques were excluded. Similarly, when we analyzed the outcome for 4 to 6 plaques, we excluded subjects with 1 to 3 plaques. The *E3E3* group was used as a reference in all analyses.

To adjust for confounding, age and sex were included in all models. To explore any effects of *APOE*, independent of total and HDL cholesterol, we added these factors to the model. Furthermore, we built models with the *APOE* genotypes and the following cardiovascular risk factors: systolic blood pressure, diastolic blood pressure, diabetes mellitus, body mass index, smoking, and total and HDL cholesterol to study effects of *APOE* irrespective of these variables. Moreover, to investigate the role of serum apoE level in the association of the *APOE* genotypes and atherosclerosis, the study population was restricted to subjects in whom the level of apoE in serum was determined, and models were compared with and without serum apoE level (entered as a continuous variable).

In addition, to explore an association of the level of serum apoE and carotid artery plaques, logistic regression analysis was used, and linear regression models were used to study the relationship of apoE level and intima-media thickness. Serum apoE levels was entered as a continuous variable, and these analysis were also adjusted for age and sex.

The percentage of explained variance was estimated by the squared adjusted multiple correlation coefficient.[27] The Pearson's χ^2

TABLE 2. Difference in Common Carotid Artery Intima-Media Thickness by *APOE* Genotypes

	E2E2	*E2E3*	*E2E4*	*E3E3*	*E3E4*	*E4E4*
All						
Model 1	−0.04 (−0.08 to −0.00)*	−0.02 (−0.03 to −0.01)*	−0.01 (−0.03 to 0.02)	0 (reference)	0.00 (−0.01 to 0.01)	0.01 (−0.01 to 0.04)
Model 2	−0.04 (−0.08 to 0.00)	−0.01 (−0.03 to −0.00)*	0.00 (−0.03 to 0.02)	0 (reference)	0.00 (−0.01 to 0.01)	0.01 (−0.02 to 0.03)
	n=46	n=704	n=137	n=3122	n=1258	n=134
Men						
Model 1	−0.08 (−0.15 to −0.01)*	−0.03 (−0.05 to −0.01)*	0.02 (−0.02 to 0.06)	0 (reference)	0.00 (−0.02 to 0.01)	0.03 (−0.01 to 0.06)
Model 2	−0.07 (−0.14 to −0.01)*	−0.03 (−0.05 to −0.01)*	0.02 (−0.02 to 0.06)	0 (reference)	0.00 (−0.02 to 0.01)	0.02 (−0.01 to 0.06)
	n=20	n=256	n=59	n=1289	n=518	n=60
Women						
Model 1	−0.01 (−0.06 to 0.04)	−0.01 (−0.02 to 0.00)	−0.02 (−0.05 to 0.01)	0 (reference)	0.00 (−0.01 to 0.01)	0.00 (−0.03 to 0.03)
Model 2	−0.01 (−0.07 to 0.04)	−0.01 (−0.02 to 0.01)	−0.02 (−0.05 to 0.01)	0 (reference)	0.00 (−0.01 to 0.01)	0.00 (−0.04 to 0.03)
	n=26	n=448	n=78	n=1833	n=740	n=74

Values are mean difference with *E3E3* group in mm with 95% CIs, adjusted for age and sex (model 1) or age, sex, and total and HDL cholesterol (model 2).
*Significantly different from *E3E3* group: $P<0.05$.

statistic was used for categorical data, and an ANOVA was applied to continuous, normally distributed variables. In case of missing data for possible confounders or intermediate factors, the most likely value was imputed on the basis of age and sex.

Results

The distribution of the *APOE* polymorphism in our study population was in Hardy-Weinberg equilibrium (*E2E2*, 0.9% [n=46]; *E2E3*, 13.0% [n=704]; *E2E4*, 2.5% [n=137]; *E3E3*, 57.8% [n=3122]; *E3E4*, 23.3% [n=1258]; and *E4E4*, 2.5% [n=134]; $\chi^2=1.5$, $df=3$, $P=0.35$). Descriptive statistics are presented in Table 1. Total and HDL cholesterol differed across the *APOE* genotypes ($F_{5,5363}=11.4$, $P<0.0005$ and $F_{5,5356}=5.5$, $P<0.0005$, respectively). Compared with the *E3E3* group, total cholesterol was higher in *E3E4* and in *E4E4* carriers and lower in carriers of *E2E2* or *E2E3*. HDL cholesterol, by contrast, was highest in subjects with the *E2E3* genotype and lower in persons with *E3E4* or *E4E4*. The *APOE* genotype explained 27% of the variance in serum apoE levels. Serum apoE level was highest in *E2E2* carriers, intermediate in subjects with *E3E3*, and lowest in carriers of the *E4E4* genotype ($F_{5,1188}=88.1$, $P<0.0005$). No other major differences were observed between subjects with the various *APOE* genotypes.

APOE and Intima-Media Thickness

The median common carotid artery intima-media wall thickness in the *E3E3* group was 0.77 mm (10th centile, 0.63; 90th centile, 1.00 mm). Compared with this reference group, carriers of *E2E2* or *E2E3* had a thinner intima-media wall thickness, particularly among men (Table 2). Among men we found further that carriers of *E2E4* or *E4E4* had a slightly increased common carotid artery intima-media wall thickness than persons with *E3E3*, but this was not statistically significant ($P=0.32$ and $P=0.21$, respectively). Findings were comparable after stratification according to age. When total and HDL cholesterol were added to the model, or when we further adjusted or stratified on the cardiovascular risk factors as defined above, our observations did not change.

APOE and Carotid Artery Plaques

We found similar relationships of *APOE* and plaques in the common carotid arteries, the carotid artery bifurcations, and the internal carotid arteries. Carriers of the *E2E3* genotype had a lower prevalence of plaques at all 3 sites (age- and sex-adjusted ORs, all 0.8) compared with the *E3E3* group. Carriership of an APOE*4 allele was not associated with the presence of plaques. We further analyzed the total number of plaques at all sides to increase statistical power. As shown in Table 3, there was no association between *APOE* and the intermediate number of plaques. However, the prevalence of >3 plaques was decreased in carriers of *E2E3* (OR, 0.6; 95% CI, 0.4 to 0.8; $P=0.003$) and increased in persons with *E4E4*, albeit this was not statistically significant (OR, 1.4; 95% CI, 0.8 to 2.4; $P=0.21$). In men we found an increased OR for the *E2E2* genotype, but this was not statistically significant either (OR, 2.7; 95% CI, 0.6 to 12.1; $P=0.19$). No other major differences were observed across sex or categories of age. Adjusting for total cholesterol and HDL cholesterol or for the aforementioned cardiovascular risk factors did not essentially change our findings (latter not shown).

Serum ApoE Level and Atherosclerosis

When we restricted the study population to persons with a known serum apoE level (n=1194), we found that serum apoE level was not associated with common carotid artery intima-media thickness (age- and sex-adjusted $\beta=0.002$; SE=0.009; $P=0.80$). Furthermore, similar associations were found of *APOE* with the presence of a plaque at 1 of the 3 locations in the carotid arterial tree, and we therefore analyzed the total number of plaques at all sides. We found that increasing serum apoE levels slightly increased the OR for <4 plaques (OR=1.3 for every mmol/mL increase; 95% CI, 0.9 to 2.1), but this was not statistically significant ($P=0.21$). The OR for >3 plaques was 1.8 for every mmol/mL increase (95% CI, 0.9 to 3.4; $P=0.08$). Analyses with both the *APOE* genotype and serum apoE levels in the model slightly strengthened the relationships of both determinants with carotid plaques and intima-media thickness (data not shown).

TABLE 3. OR for Plaques in Carotid Arteries in Relation to *APOE* Genotypes

No. of Plaques	*E2E2*	*E2E3*	*E2E4*	*E3E3*	*E3E4*	*E4E4*
All						
1–3, model 1	1.0 (0.4 to 2.2)	0.9 (0.7 to 1.1)	0.9 (0.6 to 1.4)	1 (reference)	1.1 (0.9 to 1.2)	0.7 (0.4 to 1.1)
1–3, model 2	1.0 (0.4 to 2.3)	0.9 (0.8 to 1.2)	0.9 (0.6 to 1.4)	1 (reference)	1.0 (0.9 to 1.2)	0.7 (0.4 to 1.1)
Affected/observations	14/31 (45%)	254/558 (46%)	44/107 (41%)	1103/2506 (44%)	434/966 (45%)	35/105 (33%)
All						
4–6, model 1	1.0 (0.3 to 3.0)	0.6 (0.4 to 0.8)†	0.9 (0.5 to 1.7)	1 (reference)	1.0 (0.8 to 1.3)	1.4 (0.8 to 2.4)
4–6, model 2	1.2 (0.4 to 3.7)	0.6 (0.5 to 0.9)*	1.0 (0.5 to 1.8)	1 (reference)	1.0 (0.8 to 1.2)	1.3 (0.7 to 2.2)
Affected/observations	6/31 (19%)	64/558 (12%)	19/107 (18%)	438/2506 (18%)	164/966 (17%)	26/105 (25%)
Men						
1–3, model 1	0.7 (0.2 to 3.5)	1.0 (0.7 to 1.4)	0.7 (0.3 to 1.4)	1 (reference)	1.0 (0.8 to 1.3)	1.0 (0.8 to 1.3)
1–3, model 2	0.9 (0.2 to 4.2)	1.0 (0.7 to 1.4)	0.7 (0.3 to 1.4)	1 (reference)	1.0 (0.8 to 1.3)	1.0 (0.5 to 1.9)
Affected/observations	4/13 (31%)	103/208 (50%)	18/46 (39%)	472/1041 (45%)	180/386 (47%)	20/49 (41%)
Men						
4–6, model 1	2.7 (0.6 to 12.1)	0.7 (0.4 to 1.1)	0.9 (0.4 to 2.1)	1 (reference)	0.9 (0.7 to 1.3)	1.5 (0.7 to 3.4)
4–6, model 2	3.3 (0.7 to 15.1)	0.7 (0.4 to 1.1)	0.9 (0.4 to 2.1)	1 (reference)	0.9 (0.6 to 1.3)	1.5 (0.7 to 3.3)
Affected/observations	6/13 (46%)	31/208 (15%)	11/46 (24%)	226/1041 (22%)	76/386 (20%)	14/49 (29%)
Women						
1–3, model 1	1.1 (0.4 to 2.8)	0.8 (0.7 to 1.1)	1.1 (0.6 to 1.9)	1 (reference)	1.1 (0.9 to 1.3)	0.5 (0.3 to 1.0)
1–3, model 2	1.1 (0.4 to 2.9)	0.9 (0.7 to 1.2)	1.1 (0.6 to 2.0)	1 (reference)	1.0 (0.8 to 1.3)	0.5 (0.3 to 1.0)
Affected/observations	10/18 (56%)	151/350 (43%)	26/61 (43%)	631/1465 (43%)	254/580 (44%)	15/56 (27%)

Values are ORs with 95% CIs adjusted for age and sex (model 1) or age, sex, and total and HDL cholesterol (model 2).
*Significantly different from *E3E3* group: $P<0.05$.
†Significantly different from *E3E3* group: $P<0.005$.

Discussion

In this large population-based study, we found that the *E2E3* genotype was inversely related to plaques in the carotid arteries and with the intima-media thickness of the common carotid arteries. These inverse associations did not weaken when adjustments were made for serum apoE and total or HDL cholesterol. We did not find an association between *E3E4* and carotid artery atherosclerosis. Atherosclerosis was slightly more prevalent in the *E4E4* group, although these associations never reached statistical significance. Inconsistent findings were observed in the *E2E2* and *E2E4* groups.

A limitation of this study is the cross-sectional design because it could be hypothesized that selective survival may have occurred. However, when we restricted the study population to persons in whom mortality may be less important (ie, younger persons with no evidence of vascular diseases or vascular risk factors), similar findings were obtained (not shown). Furthermore, inconsistent findings have emerged from studies on *APOE* and survival. In the Rotterdam Study, *APOE* was not related with mortality during follow-up.[28] This indicates that selective survival did not play a major role.

An advantage of this study is the population-based approach that included institutionalized subjects with a high response rate. Of concern is the exclusion of persons with missing data. However, when we compared subjects from our study population with other, excluded participants in the Rotterdam Study, we did not observe consistent differences with regard to cardiovascular risk indicators (not shown). Selection bias seems therefore not to be likely, and our findings can be generalized to the general elderly population.

We estimated the presence of atherosclerosis by established, validated techniques.[29–31] To assess the prevalence of atherosclerosis, we investigated not only the intima-media thickness of the common carotid arteries but also plaques at 3 sites of the carotid arterial tree. However, some measurement error may have occurred, and we could not differentiate between homogeneous and heterogeneous plaques.[32,33] Since all laboratory analyses were performed without knowledge of the other measurements, any misclassification is most likely nondifferential and will therefore most likely result in an underestimation of the true effect. Furthermore, our study population was more than twice as large as all previous studies on *APOE* and carotid artery atherosclerosis together (n=5401 versus n=2148).[16–21]

All possible relationships of the *APOE* genotype with atherosclerosis have been reported before.[16–21,34] First, the presence of atherosclerosis was found to be increased in APOE*4 carriers and to be decreased in carriers of the APOE*2 allele.[16,17,34] This was observed in an autopsy study in young males who died of external causes[34] and in studies in which carotid artery atherosclerosis was assessed by ultrasonography: 1 in patients referred for coronary angiography[16] and 1 in a population-based study of subjects free from cardiovascular disease.[17] Second, an increased risk of atherosclerosis has been found in relation to APOE*2. This was suggested by the Atherosclerosis Risk in Communities (ARIC) Study among subjects free from cardiovascular disease and confirmed in a study on subjects referred to an atherosclerosis prevention clinic, who had no plaque or stenosis in the carotid wall and who were free from cardio-

vascular disease.[20] Third, no association between *APOE* and atherosclerosis has been reported. These findings refer to 2 investigations on ultrasonographically assessed intima-media thickness of subjects without evidence for vascular disease.[19,21] All previous studies thus excluded persons with highest risk of vascular disease, and because few of them were population-based,[17,18] these findings are subject to selection bias and cannot be generalized to the general population.

Our observation that APOE*2 carriers have in serum higher levels of apoE and HDL cholesterol and lower levels of total cholesterol and that APOE*4 has opposite effects is in agreement with earlier reports.[2–4] However, adjustment for serum apoE level or for total and HDL cholesterol did not weaken the inverse association of *E2E3* with carotid artery atherosclerosis. Because the effects of *E2E3* on atherosclerosis seem therefore not to result from its effects on serum apoE or cholesterol levels, the *E2E3* genotype may protect from atherosclerosis through alternative pathways.

Atherosclerosis is associated with increased oxidative stress, since the oxidative modification of LDL seems to be a crucial step in its development.[35] The various apoE isoforms have been found to exert different antioxidant effects.[36] Furthermore, because apoE seems to have several other properties with relevance to vessel wall homeostasis, including the modulation of platelet aggregability and the proliferation and migration of smooth muscle cells and lymphocytes,[5,37] it is possible that these properties may differ across the apoE isoforms.

In conclusion, we found that APOE*4 was only weakly and nonstatistically significantly related to atherosclerosis in the carotid arteries. By contrast, an inverse relationship between the *E2E3* genotype and carotid artery atherosclerosis was observed, which was independent of serum apoE and total and HDL cholesterol levels. However, the low *E2E3* frequency, together with the small effects, implies that any protective effect is limited.

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