Age-Dependent Association of Apolipoprotein E Genotype With Coronary and Aortic Atherosclerosis in Middle-Aged Men
An Autopsy Study

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Background—Apolipoprotein E (apoE) polymorphism is one of the genetic determinants of serum cholesterol values. The apoE ε4 allele has been associated with advanced coronary heart disease (CHD) diagnosed by angiography, but the role of the apoE genotype in atherosclerosis has not been confirmed at vessel-wall level, nor is any age-dependent effect of the apoE genotype on the development of CHD known.

Methods and Results—The right and left anterior descending coronary arteries (RCA and LAD) and the aorta from 700 male autopsy cases (Helsinki Sudden Death Study) in 1981-1982 and 1991-1992 (average age 53 years, range 33 to 70 years) were stained for fat, and all areas covered with fatty streaks, fibrotic plaques, and complicated lesions were measured. In the RCA and LAD, the apoE genotype was significantly associated with the area of total atherosclerotic lesions in men <53 years old but not with that in older men (P=0.0085 and P=0.041, respectively, for age-by-genotype interaction). Men <53 years old with the ε4/3 genotype showed 61% larger total atherosclerotic lesion area in the RCA (P=0.0027) and 26% larger area in the LAD (P=0.12) than did men with the ε3/3. The apoE ε4/3 was also associated with atherosclerotic lesions in the abdominal (P=0.014) and thoracic (P=0.12) aorta, but this effect, unlike that of the coronary arteries, was not age-related.

Conclusions—In men, the apoE ε4 allele is a significant genetic risk factor for coronary atherosclerosis in early middle age. This suggests that at older age, other known risk factors of CHD play a more important role in the atherosclerotic process than apoE polymorphisms. (Circulation. 1999;100:608-613.)

Key Words: apolipoproteins ■ atherosclerosis ■ coronary disease

Because apolipoprotein E (apoE) plays an important role in lipid metabolism, it is an important determinant in morbidity and mortality from atherosclerotic diseases.1 The apoE gene is polymorphic, resulting in 3 common alleles (ε2, ε3, and ε4) and 6 different genotypes (ε2/2, ε3/2, ε4/2, ε3/3, ε4/3, and ε4/4).2-3 The apoE ε4 allele is associated with high serum total and LDL cholesterol concentrations,4-7 which in turn are well-established risk factors for coronary heart disease (CHD).8-9 A recent meta-analysis of 14 studies showed the apoE ε4 allele to be associated with CHD in both men and women.10 In all these studies, the phenotype of CHD was diagnosed either by clinical observation or by coronary angiography.11-16 These diagnostic methods detect only advanced coronary artery disease and coronary narrowings and are inappropriate for study of the effect of apoE polymorphism on the early phase of atherosclerosis characterized by fatty change and raised lesions. Although this question could be solved by direct examination of the coronary arteries, to the best of our knowledge only 2 autopsy studies exist.17,18 In 1 autopsy study, the association of apoE polymorphism with atherosclerosis was found only in aorta17 and in another study only in coronary arteries.18 Thus, although the apoE genotype has been shown to be associated with risk of elevated serum cholesterol levels and CHD, the association between atherosclerosis of the coronary artery wall and apoE polymorphism has not yet been confirmed unequivocally.

A Swedish twin study concluded that genetic susceptibility to death from CHD decreases at older ages.19 If apoE is an important genetic factor in the pathogenesis of atherosclerosis, it should exert its strongest effect in youth or early middle age. Such an association between genetic influence and age...
has never been studied with regard to apoE polymorphism and arterial atherosclerosis.

We studied an autopsy series of middle-aged Finnish men to investigate the association of apoE genotype with autopsy-confirmed atherosclerosis in the coronary arteries and aorta. We also investigated whether and how any effect of apoE polymorphism on atherosclerotic plaques varies with age.

**Methods**

**Subjects**

The Helsinki Sudden Death Study (HSDS) was launched to study the lifestyle and genetic factors predisposing to sudden death in Finnish middle-aged men who lived in Helsinki and its surroundings. The HSDS consists of 2 autopsy series collected at 10-year intervals. The first series (A series, n = 400) was collected during 1981-1982 and the second series (B series, n = 300) during 1991-1992. The 2 autopsy series included 700 men 33 to 70 years old (mean 53.07 years, SD 9.58 years, median 54 years) subjected to a medicolegal autopsy. Each autopsy, the arteries were dissected free, opened, and attached to a card, and then fixed in buffered formalin. The vessel wall was stained with the Sudan IV fat-staining method. The definition of atherosclerosis was based on the protocols of 2 international studies: the International Atherosclerosis Project, Standard Operating Protocol 1962,22 and the WHO Study Group in Europe.23 An area stained red with Sudan IV and showing no other types of changes underlying it was classified as fatty streak. An elevated plaque that exhibited no ulceration or thrombosis was considered a fibrotic lesion. Any plaque area with ulceration or thrombosis was classified as a complicated lesion. The area involved with fatty streaks, fibrotic plaques, and complicated lesions was measured by computer-assisted planimetry, which is standard planimetric equipment connected to a personal computer. It measures the exact area of a single lesion in square millimeters. The areas of different types of lesions were expressed in percentages by dividing the lesion area by the total area of the artery wall and multiplying by 100%. The total atherosclerotic lesion area of the arterial wall was the total areas of fatty streak and fibrotic lesions. Because the complicated lesions in the arterial wall were always incorporated into either the fatty streak or the fibrotic lesion area, or both, the complicated-lesion area was analyzed separately.

Of the series of 700 men, arterial samples for the planimetric measurements were available from 596 men for the analysis of the RCA and from 440 men for the analysis of the LAD. Planimetric data of the atherosclerosis in the thoracic (n = 256) and abdominal (n = 259) aorta were available only for the B series.

**Statistical Analyses**

Data analysis was based on ANCOVA. Data were analyzed in square-root form, but the results are displayed as crude data. In the analysis, only the most common apoE genotypes, ε3/2, ε3/3, and ε4/3, were included because in this way, the ε3/3 group provides an internal control to separate the effect of the ε2 and ε4 alleles. Furthermore, to study the effect of age, the series was divided according to the average age, 53.1 years (median 54 years), of the series into 2 subgroups: men <53 and men ≥53 years old. ApoE genotype status and age subgroup were used as factors in the ANCOVA, and in addition, the series status (A or B) was taken as a factor in the ANCOVA to eliminate its possible confounding effect. The possible confounding effect of BMI was controlled for, being taken as a covariate. If a significant interaction of age and apoE was observed, a second ANCOVA was performed for each age subgroup separately. In this analysis, age as a continuing variable was a second covariate in addition to BMI. Finally, the differences between 3 genotypes were analyzed with the Scheffé post hoc test. Computation was carried out with Statistica Version 5.1 (StatSoft Inc) on a personal computer.

**DNA Extraction and ApoE Genotyping**

In the A series, DNA was extracted from paraffin-embedded samples of cardiac muscle by the method of Isola et al.20 In the B series, DNA was isolated from frozen (−70°C) cardiac samples by the standard phenol-chloroform method, and 25 ng of purified DNA was used for apoE genotyping by polymerase chain reaction and HhaI restriction enzyme digestion as described by Hixson and Vernier.21 The digested fragments were electrophoresed on 12% polyacrylamide gel and visualized by silver staining. ApoE genotype could be successfully determined in 671 cases.

**Measurements of Area Involved With Atherosclerotic Lesions**

We measured the areas of the different types of atherosclerotic lesions in the RCA and LAD and thoracic and abdominal aortas. At autopsy, the arteries were dissected free, opened, and attached to a card, and then fixed in buffered formalin. The vessel wall was stained with the Sudan IV fat-staining method. The definition of atherosclerosis was based on the protocols of 2 international studies: the International Atherosclerosis Project, Standard Operating Protocol 1962,22 and the WHO Study Group in Europe.23 An area stained red with Sudan IV and showing no other types of changes underlying it was classified as fatty streak. An elevated plaque that exhibited no ulceration or thrombosis was considered a fibrotic lesion. Any plaque area with ulceration or thrombosis was classified as a complicated lesion. The area involved with fatty streaks, fibrotic plaques, and complicated lesions was measured by computer-assisted planimetry, which is standard planimetric equipment connected to a personal computer. It measures the exact area of a single lesion in square millimeters. The areas of different types of lesions were expressed in percentages by dividing the lesion area by the total area of the artery wall and multiplying by 100%. The total atherosclerotic lesion area of the arterial wall was the total areas of fatty streak and fibrotic lesions. Because the complicated lesions in the arterial wall were always incorporated into either the fatty streak or the fibrotic lesion area, or both, the complicated-lesion area was analyzed separately.

**TABLE 1. Characteristics of the Study Subjects**

<table>
<thead>
<tr>
<th></th>
<th>&lt;53 Years Old</th>
<th>≥53 Years Old</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ε3/2</td>
<td>ε3/3</td>
</tr>
<tr>
<td></td>
<td>(n=21)</td>
<td>(n=170)</td>
</tr>
<tr>
<td>Age, y</td>
<td>43.19±5.20</td>
<td>43.89±5.05</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>24.75±3.70</td>
<td>24.93±4.72</td>
</tr>
<tr>
<td>Cause of death, n</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cardiac disease</td>
<td>7</td>
<td>48</td>
</tr>
<tr>
<td>Other disease</td>
<td>2</td>
<td>37</td>
</tr>
<tr>
<td>Intoxication</td>
<td>6</td>
<td>51</td>
</tr>
<tr>
<td>Other violent cause</td>
<td>6</td>
<td>40</td>
</tr>
<tr>
<td>Unknown</td>
<td>0</td>
<td>3</td>
</tr>
</tbody>
</table>

Values are mean±SD.

*Differences between means was tested by ANOVA.

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Coronary Atherosclerosis and ApoE Genotype

A significant apoE genotype–by-age interaction was observed on fatty streak (P = 0.027) and total atherosclerotic (P = 0.0085) area involvement in the RCA (see Table 3). The apoE genotype was associated with the RCA atherosclerosis only in men <53 years old, and in this age subgroup, the carriers of ε4/3 genotype had on average a 54% increase in the area of fatty streaks and a 61% increase in the total atherosclerotic area involvement compared with the carriers of ε3/3 (P = 0.0471 and P = 0.0027 for ε3/3 versus ε4/3; Scheffé post hoc test). The carriers of ε3/2 tended to have lower values than the carriers of ε3/3, but the differences were statistically insignificant. The association of apoE with fibrotic lesion area was highly significant (P = 0.009) in men <53 years old, although the interaction of apoE and age was insignificant (P = 0.17). ApoE ε4/3 genotype was also associated with the highest values of the fibrotic lesion area involvement in men <53 years old (P = 0.0089 for ε3/3 versus ε4/3; Scheffé post hoc test). ApoE had no significant effect on the mean percentage area of complicated lesions in the RCA.

In the LAD, there was a significant interaction of apoE and age on the total atherosclerotic lesion area (P = 0.041) and a borderline interaction on fatty streaks (P = 0.10) (see Table 3). ApoE genotype was significantly associated with the LAD atherosclerosis only in men <53 years old, and the ε4/3 carriers tended to have higher mean percentage area of fatty streak and total atherosclerotic lesion involvement than the ε3/3 group (P = 0.1371 and P = 0.1190 for ε3/3 versus ε4/3; Scheffé post hoc test). The carriers of the ε4/3 genotype had on average a 26% increase in the total atherosclerotic area involvement compared with the carriers of ε3/3.

Aortic Atherosclerosis and ApoE Genotype

The association of apoE genotype with aortic atherosclerosis was not age-dependent (P = NS for apoE genotype–by-age interaction). In the abdominal aorta, apoE genotype was significantly associated with fibrotic lesion (P = 0.010) and total atherosclerotic lesion (P = 0.014) area (see Table 4). The ε4/3 genotype was associated with greater fibrotic and total atherosclerotic lesion area involvement than ε3/3 (P = 0.3095 and P = 0.0340 for ε3/3 versus ε4/3; Scheffé post hoc test) or ε3/2 (P = 0.0240 and P = 0.0578 for ε3/2 versus ε4/3; Scheffé post hoc test). ApoE genotype had a borderline association with fatty streaks in both thoracic and abdominal aorta. The carriers of the ε4/3 genotype consistently had the highest area of atherosclerotic lesion involvement in both parts of the aorta. In addition, the covariate age had a major effect on the

Results

ApoE Genotypes, Allele Frequencies, and Characteristics of the Subjects

ApoE genotype and allele frequencies for both age subgroups are shown in Table 2, and they are consistent with other studies of Finnish populations.5,6 There were no differences in genotype and allele frequencies between the age subgroups. Age and BMI did not vary between different apoE genotypes, as shown in Table 1.

Coronary Atherosclerosis and ApoE Genotype

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Table 2. ApoE Genotype and Allele Frequencies by Age Subgroups

<table>
<thead>
<tr>
<th>ApoE genotype, n (%)</th>
<th>&lt;53 Years Old</th>
<th>≥53 Years Old</th>
<th>All Men</th>
</tr>
</thead>
<tbody>
<tr>
<td>ε2/2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ε3/2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ε4/2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ε3/3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ε4/3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total, n</td>
<td>303</td>
<td>368</td>
<td>671</td>
</tr>
</tbody>
</table>

Table 3. Mean Percent Area of Atherosclerotic Lesions in RCA and LAD Coronary Arteries by apoE Genotype and Age Subgroups

<table>
<thead>
<tr>
<th></th>
<th>&lt;53 Years Old</th>
<th>≥53 Years Old</th>
<th>P, ANCOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>RCA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fatty streak</td>
<td>3.60±4.03</td>
<td>5.12±7.11</td>
<td>7.89±9.13‡</td>
</tr>
<tr>
<td>Fibrotic</td>
<td>1.39±2.17</td>
<td>2.63±5.51</td>
<td>4.62±6.97‡</td>
</tr>
<tr>
<td>Total†</td>
<td>4.99±5.54</td>
<td>7.75±10.02</td>
<td>12.50±11.26‡</td>
</tr>
<tr>
<td>Complicated</td>
<td>0.43±0.84</td>
<td>1.32±3.63</td>
<td>1.51±3.99</td>
</tr>
<tr>
<td>LAD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fatty streak</td>
<td>5.60±6.81</td>
<td>4.92±6.40</td>
<td>6.77±7.06</td>
</tr>
<tr>
<td>Fibrotic</td>
<td>4.52±5.78</td>
<td>5.25±6.56</td>
<td>6.08±7.02</td>
</tr>
<tr>
<td>Total†</td>
<td>10.13±10.67</td>
<td>10.17±8.00</td>
<td>12.85±9.29</td>
</tr>
<tr>
<td>Complicated</td>
<td>0.42±1.16</td>
<td>0.35±1.27</td>
<td>1.02±3.52</td>
</tr>
</tbody>
</table>

Values are mean±SD.
*P values for apoE from the ANCOVA performed for 2 age subgroups separately. Age and BMI as covariates.
†Total atherosclerosis is fatty streak and fibrotic lesions combined.
‡Significant (P<0.05) difference for ε3/3 vs ε4/3 in Scheffé post hoc test.
aortic atherosclerosis. ApoE polymorphism was not related to complicated lesions of the aorta.

The Figure shows the effect of apoE genotype on the mean percentage area of total atherosclerotic lesions in thoracic and abdominal aorta and in RCA and LAD coronary arteries. In aorta, the effect of apoE genotype on the lesion area was similar in both age subgroups, and the men $>53$ years old had higher values than younger men. In the coronary arteries, however, age modulated the effect of apoE genotype on the lesion area: apoE was associated with the lesion area only in men $<53$ years old but not in the subgroup of older men. In other words, the association of the apoE polymorphism with the area of total atherosclerotic lesions varied with age in the coronary arteries but not in the aorta.

**Discussion**

The 2 main findings of our study are that apoE polymorphism seems to associate with atherosclerotic lesion area in both the
coronary arteries and the aorta, and moreover, in the RCA and LAD, the association of apoE polymorphism is age-dependent. Our study demonstrates, at the vessel-wall level, that the apoE e4 allele is a significant risk factor for CHD and confirms the results of several clinical and angiographic studies.21–30 We could find no significant protective role for the e2 allele in coronary or aortic atherosclerosis, although the carriers of the allele tended to have less lesion involvement than the e3/3 group.

ApoE polymorphism is known to be associated with high serum lipid levels,5–7 which are important factors particularly in development of early so-called type 1 to IV lesions that only minimally reduce the lumen.24 Therefore, it can be hypothesized that in the process of atherosclerosis, apoE polymorphism affects mainly the first steps of the pathogenesis of atherosclerosis. Coronary angiography is usually performed only after clinically evident symptoms appear. Thus, for investigation of early stages of (coronary) atherosclerosis without significant stenosis, other kinds of studies are needed, such as autopsy series. Only 2 previous autopsy studies have investigated the association of apoE with atherosclerosis at the vessel-wall level, with different results.17,18 In the Pathobiological Determinants of Atherosclerosis in Youth (PDAY) study,17 the apoE e4 allele was significantly associated with total lesion area in thoracic and abdominal aorta of young men. However, the PDAY group could find no relation between apoE polymorphism and atherosclerotic lesions in the RCA.17 Conversely, an autopsy study on 130 Alaskan Natives reported an association between the e4 allele and atherosclerosis in the LAD and RCA, but not in the aorta.18 In our autopsy study, we found an association between the apoE e4 allele and atherosclerosis both in the coronary arteries and in the aorta. Differences between results of these 2 autopsy studies and ours can be explained by different kinds of study structure, study population, or age distribution, as well as by differences in methods. In the PDAY study, subjects were young men 15 to 34 years old, whereas the Alaskan Natives were both men and women 9 to 85 years old. Our subjects were middle-aged men, 33 to 70 years old, prone to atherosclerosis and sudden death. In both the PDAY and the Alaskan Natives studies, the percentage area of atherosclerotic lesions was graded visually, whereas we used computer-assisted planimetry. In our technique, we measured the area of atherosclerotic lesions in the arteries. In addition, Finns are particularly suitable for genetic studies of coronary atherosclerosis because of their high, although declining, prevalence of CHD25,26 as well as their genetic homogeneity, a result of geographic isolation and the founder effect.27

Our study suggests that the effect on coronary atherosclerosis of the apoE e4 allele is age-dependent. This finding is in agreement with the conclusions of the Swedish twin study that when people die of CHD at younger ages, genetic mechanisms play a greater role than in deaths at an older age.19 A prospective population-based study on the elderly found no relationship between apoE e4 and CHD incidence or mortality, which also supports our finding.28 In addition, there is evidence that the effect of the e4 allele on serum cholesterol is also age-dependent and that it decreases at older ages.29

Our findings imply that this decrease in effect also applies to the effect of apoE polymorphism on coronary atherosclerosis as confirmed by autopsy.

Why, then, does the apoE e4 allele seem to be associated with coronary atherosclerosis only in the younger age subgroup? If a trait is age-dependent, with risk increasing with age (as in CHD), selection for younger affected individuals probably increases the genetic contribution to the disease status of the individual in question. With age, accumulation of other risk factors and phenocopies dilutes the effect of initial participants in the pathogenetic cascade. So, when younger subjects are selected for the study, the proportion of individuals at genetic risk rises and trait differences should be seen more clearly, as did occur in our study. Further, at older age, the extent of the atherosclerosis reduces the variability, and therefore, an association of a single genetic factor with the disease might be prevented.

The association of apoE polymorphism with aortic atherosclerosis, in turn, was not age-dependent. This might be due to differences in the hemodynamic conditions between the coronary artery and the aorta. The mechanical forces determine, in large part, the susceptibility of a lesion to progress.24 In the coronary arteries, the lesions may be more progression-prone than in the aorta. This could also partly explain the lack of association of apoE polymorphism with coronary atherosclerosis at older age. It has been shown that the fatty streak lesions in the coronary arteries increase throughout life, but in the aorta, the lesions tend to remain at the same level after middle age.30 This suggests that the development of atherosclerotic lesions in the coronary arteries differs from that in the aorta.

In conclusion, in the present autopsy study of middle-aged men, the apoE e4/3 genotype had a larger area of atherosclerosis both in the coronary arteries and in the aorta than the e3/3 genotype. In the LAD and RCA, the association was age-dependent. The fact that the apoE e4 allele was associated with coronary atherosclerosis only before the age of 53 years suggests that at older age, other known or at present still unknown risk factors may play a more important role in the atherosclerotic process than apoE polymorphisms. We thus conclude that apoE polymorphism is one of the genetic factors that participate in the process of atherosclerosis in men. It might prove useful to determine the apoE genotypes of men at high risk of CHD already at early middle age so as to take preventive measures against coronary atherosclerosis, particularly for those carrying the e4 allele. The e4 allele is known to be associated with sensitivity to dietary interventions to lower serum cholesterol.31,32

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References

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