The apolipoprotein E polymorphism and cardiovascular diseases—an autopsy study

Neena Theresa Kumara,⁎, Knut Liestølb, Else Marit Løberg⁠a, Henrik Mikael Reims⁠c, Sverre-Henning Brorson⁠c, Jan Mæhlen⁠a

⁎ Corresponding author. Department of Pathology, Oslo University Hospital-Ullevål, P.O. Box 4956, Nydalen, 0424 Oslo, Norway. Tel.: +47 22 11 89 10, +47 22 11 89 13; fax: +47 22 11 82 39.
E-mail address: n.t.kumar@medisin.uio.no (N.T. Kumar).

Abstract

Background: Numerous studies have addressed the association between the apolipoprotein E polymorphism and cardiovascular disease, but only a few reports are based on findings at autopsy. In the present retrospective study, we have used autopsy findings from a general hospital population to further investigate this issue. Methods and results: We collected information from 1522 consecutive autopsy reports (886 men, mean age 65.7 years; 636 women, mean age 69.7 years) conducted at Oslo University Hospital, Norway, in the period from 1996 to 2000. Cause of death and signs related to cardiovascular disease including the degree of atherosclerosis in the aorta and the coronary arteries, signs of myocardial infarction, heart weight, and signs of cerebrovascular disease were recorded. The patients were genotyped, and the apolipoprotein E allele frequencies (ɛ2, 8.0%; ɛ3, 72.6%; and ɛ4, 19.4%) were not statistically different from a group of healthy controls. Approximately 35% of the patients died from a cardiovascular disease. Genotypes differed significantly (P < 0.05), with more ɛ4-carriers (34.3% vs. 29.6%) and fewer ɛ2-carriers (11.8% vs. 13.9%) among patients who died from cardiovascular disease compared to those who died from other causes. A similar distribution of genotypes was seen in patients recorded with myocardial infarction or cerebrovascular disease. There was an association between the presence of ɛ4 and atherosclerosis in the aorta and coronary arteries, but this did not reach statistical significance. Among patients with signs of coronary heart disease, standardized heart weights were significantly higher in ɛ2-carriers compared to ɛ4-carriers. Conclusion: The present autopsy study suggests that the risk of developing and dying from cardiovascular disease, including coronary heart disease and cerebrovascular disease, is influenced by the apolipoprotein E polymorphism. © 2012 Elsevier Inc. All rights reserved.

Keywords: Apolipoprotein E; Cardiovascular disease; Atherosclerosis; Autopsy

1. Introduction

Cardiovascular disease (CVD), including coronary heart disease (CHD), is still the leading cause of death in Western countries [1]. The pathogenesis is likely to involve a complex interaction among environmental factors, lifestyle, and genetics. Since the early report [2] of lipoprotein(a) as an inheritable factor in the development of CVD, numerous studies, including recent large genomewide association studies, have addressed the genetic basis of CHD and its risk factors [3–5]. During the last decades, the contribution of the apolipoprotein E (apoE) gene polymorphism has been widely discussed [6–13]. ApoE was first described as a protein component of very low density lipoprotein by Shore and Shore in 1973 [14], and in 1977, Utermann et al. [15] discovered that the apoE polymorphism had effects on dysbetalipoproteinemia. The apoE gene is located at chromosome 19q13.2 and is polymorphic with three common alleles designated ɛ2, ɛ3, and ɛ4. The three protein isoforms encoded by these alleles are called E2, E3, and E4, respectively, and are synthesized and secreted mainly in the liver [10]. ApoE plays a part in the lipoprotein metabolism, ultimately altering circulating levels of cholesterol [16]; the ɛ4-allele is associated with increased
total cholesterol levels, and the ε2-allele with decreased levels [11]. Thus, the apoE genotype might be expected to influence the development of atherosclerosis and atherosclerotic vascular diseases.

A meta-analysis carried out in 2004 [17] suggests that the apoE ε4-allele is a significant risk factor for CHD, increasing the risk by about 40%, but some recent studies do not confirm such a strong effect [18,19]. Still, there are studies indicating no associations between genotype and the risk of CHD [20]. Results regarding the importance of different apoE genotypes in the development of stroke are ambiguous [21].

In almost all studies of the relation between CHD and apoE genotype, end points have been based on clinical observations or coronary angiography [17,22]. Only a few studies have evaluated atherosclerosis and other signs of CVD at autopsy [23–26]. The main purpose of the present study was to further examine the relationship between apoE polymorphism and the presence of atherosclerotic disease and its clinical and pathological manifestations in a general population by analyzing findings in a consecutive hospital-based autopsy series.

2. Methods

2.1. Study population

The study was carried out at the Department of Pathology, Oslo University Hospital, Norway. We obtained data collected among patients subjected to ordinary medical autopsy at Oslo University Hospital during the period August 1996 to December 2000. The patients were sampled consecutively. From August 1996 to March 1999, we included all patients aged 20 years and above, and from April 1999 to December 2000, we included patients aged 20 to 75 years. To be included, blood samples allowing for determination of apoE genotype had to be available, and this was the case for about 85% of the patients. Of the 1606 patients where the genotype had been established, patients without complete autopsy were excluded. Due to possible selection bias caused by a low autopsy rate among the very old and because nonagenarians and centenarians are more likely to suffer from multimorbidity, patients older than 89 years of age (n=28) were also excluded. This left a total of 1522 persons consisting of 886 men and 636 women. The gender distribution and mean age for three age groups are shown in Table 1.

As controls for the overall allele frequencies in the autopsied patients, we used healthy, young blood donors from Oslo described earlier [27].

2.2. Registration of data

All the autopsies were carried out at the same academic pathology department by the pathologists on duty and according to the same protocol based on

<table>
<thead>
<tr>
<th>Autopsy cases</th>
<th>Men (n=1522)</th>
<th>Women (n=1522)</th>
<th>Age (n=1522)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autopsy cases</td>
<td>886 (58%)</td>
<td>636 (42%)</td>
<td>65.7 (14.3)</td>
</tr>
<tr>
<td>&lt;60</td>
<td>270 (30%)</td>
<td>144 (23%)</td>
<td>47.7 (9.1)</td>
</tr>
<tr>
<td>60–72</td>
<td>289 (33%)</td>
<td>178 (28%)</td>
<td>66.7 (3.7)</td>
</tr>
<tr>
<td>&gt;72</td>
<td>327 (37%)</td>
<td>314 (49%)</td>
<td>79.2 (4.1)</td>
</tr>
</tbody>
</table>

Data shown as n (%) or mean (S.D.).

recommendations by the Armed Forces Institute of Pathology [28]. Standardized information was extracted from the autopsy reports by three experienced pathologists prior to apoE genotyping.

Based on the patients’ medical records, information about disease including angina pectoris, hypertension, heart failure, diabetes mellitus, neurodegenerative disease, cancer, respiratory disease, alcoholism, and renal disease were registered.

Autopsy findings, including the patient’s height and weight, together with heart weight, coronary atherosclerosis, aortic atherosclerosis and its complications, myocardial infarction (MI), cardiac dilation and/or hypertrophy, and valve disease, were registered. Findings of cerebrovascular and neurodegenerative disease, cancer with or without metastases, lung disease, alcohol-related disease, and renal disease were also registered.

The degree of coronary atherosclerosis was classified as none, moderate, or severe. Cases with severe stenosis were classified as severe atherosclerosis, regardless of the general amount of coronary atherosclerosis. The exact degree of stenosis was not assessed due to longitudinal opening of the coronary arteries. The degree of atherosclerosis in the aorta was classified as mild, moderate, severe, or severe with complications. The grading was based on the visual findings stated in the autopsy report. We have separately assessed the interobserver agreement using autopsied hearts and aortas, and found Kappa values of 0.52 and 0.63 for coronary and aortic atherosclerosis, respectively. According to these assessments, changes classified as mild, moderate, and severe correspond to approximately <20% (mild), 20%–60% (moderate), and ≥60% (severe) atherosclerotic surface area. MI was categorized as none, recent, old, or both recent and old. This information was based on microscopic findings. Cerebral findings were categorized as infarction, hemorrhage, and neurodegenerative changes, also based on microscopic findings.

We evaluated and registered the underlying, immediate, and contributing causes of death determined at autopsy (based on The Systematized Nomenclature of Medicine classification system [29]). Cases in which the original conclusion was considered questionable were discussed among the investigators in order to reach consensus. We also discussed other problems or divergences to achieve the most consistent information in our database.
2.3. ApoE genotyping

Ten milliliters of blood was extracted from the femoral vein at autopsy and frozen at −25°C. Genomic DNA was extracted from the samples using QIAamp Blood Kit (Qiagen GmbH, 40724 Hilden, Germany). DNA was amplified by polymerase chain reaction (PCR) in a thermal cycler (GeneAmp 2400). The method used was a seminested PCR described elsewhere [30]. The amplification resulted in a 188-base-pair PCR product, which was subjected to digestion by a restriction enzyme (HhaI) to distinguish between the different alleles of apoE. The restriction fragments were separated by polyacrylamide gel (15%) electrophoresis at 200 V for 5.5 h.

2.4. Statistical analysis

The relations between apoE genotypes and signs of CVD were analyzed after dividing the cases into three groups: I, genotype ε2/εX including ε2/ε2 and ε2/ε3; II, genotype ε3/ε3; and III, genotype ε4/εX including ε3/ε4 and ε4/ε4. Thus, cases with ε2/ε4 were excluded, leaving 1477 subjects in the statistical analysis. There were no notable autopsy findings regarding CVD in the excluded ε2/ε4 group. The three categories were then treated as ordered (assuming ε3/ε3 intermediate to the two other groups), and Kendall’s test was used. The test results were confirmed using logistic regression with adjustment for age. Within-group differences in standardized heart weight between the different genotype categories were also compared with Kendall’s test. The allele frequencies in the autopsy cases and the control group were compared with χ² test. All tests were two-sided, and results with P<.05 were considered significant. We used the JMP 8.0 statistical system.

To avoid small groups in the analyses of atherosclerosis in the coronary arteries and the aorta, cases were merged into two groups labeled “mild/moderate” and “severe.” In the coronary arteries, the group “mild/moderate” included cases graded as “none” and “moderate,” as the grade “mild” was not recorded. In the aorta, the group “severe” included cases graded as “severe” and “severe with complications.”

Since heart weight depends on body weight and gender, we standardized heart weight by first using regression to compute expected heart weight based on body weight and gender among the cases dying from causes other than CVD and cancer. The standardized heart weight was then defined as the ratio between the measured heart weight and the expected heart weight.

2.5. Ethics

The study was approved by the Regional Ethical Committee in Norway (Regional komité for medisinsk og helsefaglig forskningsetikk Sør-Øst).

3. Results

3.1. ApoE and causes of death

The apoE allele frequencies in the autopsy cases did not differ significantly from those in the control group (Table 2). The ε2 frequency in the control group was lower compared to that in the autopsy cases.

The underlying causes of death in the autopsied patients are shown in Table 3. To evaluate the effect of apoE on causes of death, we first extracted all patients with dementia and histopathological evidence of Alzheimer or Lewy body disease as immediate, underlying, or contributing cause of death. These 44 cases had a genotype distribution that differed significantly from the other cases, with more genotypes including the ε4-allele and less genotypes including the ε2-allele (Table 4). The remaining patients were divided into two groups: those with CVD as the cause of death and those with other causes of death. The CVD group had a significantly different genotype distribution compared to those without CVD, with more genotypes including the ε4-allele and less genotypes including the ε2-allele (Table 4).

In more than half of the cases who died from CVD, CHD was the underlying or immediate cause of death and the only registered CVD. The remaining cases had noncoronary CVD as the underlying or immediate cause of death, or any CVD as the contributing cause of death. There was no significant difference in the genotype distribution between these two groups (data not shown).

Among cases without CVD, about half of them died of cancer, and the remaining died of other diseases (Table 3).

3.2. ApoE and CVD

The genotype distribution differed significantly between patients with MI (including recent, old, or both) and patients

<table>
<thead>
<tr>
<th>Allele</th>
<th>Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>ε2/ε2</td>
<td>ε2/ε3</td>
</tr>
<tr>
<td>ε3/ε3</td>
<td>ε3/ε4</td>
</tr>
<tr>
<td>ε4/ε4</td>
<td>ε4/εX</td>
</tr>
</tbody>
</table>

Data shown as n or %.

* Differences in allele frequencies between the control group and all the autopsy cases are not statistically significant.
without MI, with more genotypes including the ε4-allele and less genotypes including the ε2-allele in patients with MI (Table 5).

Patients with cerebral infarction (n=209) and intracerebral hemorrhage (n=41) were grouped together as having cerebrovascular disease. The genotype distribution differed significantly between those with cerebrovascular disease and those without cerebral findings, with more genotypes including the ε4-allele and less genotypes including the ε2-allele in the group with cerebrovascular disease (Table 5). There was no significant difference between patients with cerebral infarction and intracerebral hemorrhage (data not shown).

Genotypes including the ε4-allele consistently tended to be slightly more frequent and genotypes including the ε2-allele less frequent among patients with severe atherosclerosis in the aorta and the coronary arteries (Table 6). However, these differences were not statistically significant. Results in the non-CVD group among females are uncertain since few women in this group had severe atherosclerosis.

The mean heart weight differed between the groups based on cause of death, with the highest weight among those dying from CHD and the lowest among those dying from cancer (Table 7). In the CHD group, there was a marked and significant (P<0.01) difference in standardized heart weight between the apoE genotypes, with the highest weights in ε2-carriers. A similar, but nonsignificant trend was seen in the rest of the CVD patients. Consistent with this finding, the percentage of hearts visually assessed as hypertrophic during autopsy showed a similar distribution among the genotype groups in patients dying from CHD or other CVDs: 28% in ε2/εX, 20% in ε3/ε3, and 17% in ε4/εX. Moreover, there was a nonsignificant trend towards a higher prevalence of hypertension in ε2-carriers among these patients: 26% in ε2/εX, 20% in ε3/ε3, and 19% in ε4/εX.

### 4. Discussion

In this autopsy study, we have described the association between the apoE polymorphism and cause of death, as well as its association with manifestations of CHD, aortic atherosclerotic disease, and cerebrovascular disease. Specifically, the ε4-allele was more common and the ε2-allele was less common among patients with CVD as either the underlying or contributing cause of death than among patients with other causes of death. Moreover, the ε4-allele was more common and the ε2-allele was less common among patients with MI or cerebrovascular disease verified at autopsy than in patients without these findings. Atherosclerosis of the coronary arteries and the aorta also tended to

### Table 3
Underlying cause of death (n=1522)

<table>
<thead>
<tr>
<th>CVD*</th>
<th>Cancer</th>
<th>Infection</th>
<th>Lungb</th>
<th>Alcohol</th>
<th>Accident</th>
<th>Neurodegenerative disease</th>
<th>Diabetes mellitus</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>540 (36)</td>
<td>443 (29)</td>
<td>125 (8)</td>
<td>79 (5)</td>
<td>66 (4)</td>
<td>41 (3)</td>
<td>24 (2)</td>
<td>10 (0)</td>
</tr>
<tr>
<td>&lt;60</td>
<td>108</td>
<td>113</td>
<td>52</td>
<td>11</td>
<td>43</td>
<td>20</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>60–72</td>
<td>168</td>
<td>157</td>
<td>23</td>
<td>28</td>
<td>15</td>
<td>10</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>&gt;72</td>
<td>264</td>
<td>173</td>
<td>50</td>
<td>40</td>
<td>8</td>
<td>11</td>
<td>13</td>
<td>2</td>
</tr>
</tbody>
</table>

Data shown as n (%) or n.

- CVDa Includes CHD, fatal aortic atherosclerotic disease, and cerebrovascular disease except subarachnoidal hemorrhage.
- Lungb Includes respiratory diseases except cancer and infection.
- Diabetes mellitusc Includes diabetes mellitus (type 1 and 2) coded as underlying cause of death, whereas a total of 175 patients were registered to suffer from diabetes mellitus.

### Table 4
Apo E genotype frequencies according to underlying, immediate, and contributing cause of death (n=1477)

<table>
<thead>
<tr>
<th>Cause of death</th>
<th>n</th>
<th>ε2/εX</th>
<th>ε3/ε3</th>
<th>ε4/εX</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dementia</td>
<td>44</td>
<td>6.8</td>
<td>31.8</td>
<td>61.4</td>
<td>&lt;.005</td>
</tr>
<tr>
<td>CVD</td>
<td>694</td>
<td>11.8</td>
<td>53.9</td>
<td>34.3</td>
<td>&lt;.05</td>
</tr>
<tr>
<td>No CVD</td>
<td>739</td>
<td>13.9</td>
<td>56.4</td>
<td>29.6</td>
<td></td>
</tr>
</tbody>
</table>

Data shown as n or %.

- *P value is given for comparison of within-group genotype distributions in patients with dementia vs. patients without dementia.
- bP value is given for comparison of within-group genotype distributions in patients with CVD vs. patients without CVD (in this comparison, cases with dementia are excluded).

### Table 5
Apo E genotype frequencies among patients with and without MI and cerebrovascular disease (n=1476)*

<table>
<thead>
<tr>
<th>n</th>
<th>ε2/εX</th>
<th>ε3/ε3</th>
<th>ε4/εX</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>MI</td>
<td>541</td>
<td>11.3</td>
<td>53.0</td>
<td>35.7</td>
</tr>
<tr>
<td>No MI</td>
<td>935</td>
<td>13.6</td>
<td>55.3</td>
<td>31.1</td>
</tr>
<tr>
<td>CVD</td>
<td>250</td>
<td>11.6</td>
<td>49.2</td>
<td>39.2</td>
</tr>
<tr>
<td>No CVD</td>
<td>66</td>
<td>12.1</td>
<td>56.1</td>
<td>31.8</td>
</tr>
</tbody>
</table>

Data shown as n or %. NS, not significant.

- *One case missing information about findings in brain; another in heart.
- bP value is given for comparison of within-group genotype distributions in patients with MI vs. patients without MI.
- eP value is given for comparison of within-group genotype distributions in patients with cerebrovascular disease vs. patients without cerebral findings.
- Includes primary brain tumors, metastases, infections, and injuries.
- P value is given for comparison of within-group genotype distributions in patients with “Other cerebral diseases” vs. patients without cerebral findings.
be more severe in ε4-carriers than in non-ε4-carriers, although this difference did not reach statistical significance. Finally, we found a strong association between dementia and ε4.

By using autopsy reports, we were able to obtain information about pathological findings, including atherosclerosis of the coronary arteries and the aorta, as well as heart weight, myocardial infarcts, and cerebrovascular disease, some of which may have remained undetected in a clinical trial. Furthermore, studies based on clinical findings and coronary angiography usually include only patients with symptoms or clinical manifestations of cardiac disease. In contrast, our study includes nearly all adult patients who were subjected to ordinary medical autopsy at our hospital during a 4-year period.

It has been suggested that ε4-carriers are more susceptible to serious CHD than non-ε4-carriers [8], and in a prospective study of elderly Finnish men [7], there was a doubling of the relative ε4-allele frequency among patients who died of CHD compared with survivors during a 5-year follow-up. In the present study, we found that both CVD morbidity and mortality were positively associated with the ε4-allele and negatively associated with the ε2-allele. In a meta-analysis, Song et al. [17] found the CHD risk to be 40% higher in ε4-carriers compared to ε3-homozygous persons, whereas the CHD risk was not significantly influenced by the ε2-allele. In a more recent meta-analysis [18] focusing only on larger studies, the authors concluded that ε2-carriers had a 20% lower risk of CHD, whereas ε4-carriers had a slightly higher risk compared with ε3-homozygous persons. The association between ε4 and CHD seems to be stronger in fatal cases than in nonfatal cases [31]. Gerdes et al. [32] found that although the apoE genotype did not predict increased risk of a nonfatal coronary event, the mortality rate after an MI in ε4-carriers was almost twice the mortality rate in non-ε4-carriers during 5.5 years of follow-up. Consequently, if death occurs earlier in ε4-carriers suffering from CHD, this may reduce the degree of heart hypertrophy at death and thus provide one possible explanation for the descending gradient in standardized heart weight from ε2-carriers to ε4-carriers among those dying from CVD. High heart weights may also be related to longer periods with hypertension. We only have information on hypertension from medical records, and the information may not be complete. There was, however, a nonsignificant trend towards a higher prevalence of registered hypertension in ε2-carriers among patients dying from CVD.

Our findings regarding the relationship between apoE genotype and MI provide autopsy-based support to the INTERHEART genetic case–control study [33], which showed an increased MI risk in patients with the ε4-allele and a decreased risk in those with the ε2-allele. Our observation is also consistent with the findings in a multicenter population-based study of an association between the presence of the ε4-allele and increased MI risk [34].

Furthermore, we found an association between the ε4-allele and cerebrovascular disease, including cerebral infarction and intracerebral hemorrhage. This is in agreement with some previous studies [35,36], although it is not a consistent finding [21]. Cerebral infarction may be due to thrombotic occlusion of cerebral arteries or to embolism originating in the heart or large vessels. We did not record the degree of atherosclerosis in the carotid arteries, but ε4-carriers have previously been found to have increased carotid intima–media thickness [37]. About one fifth of ischemic

Table 6
ApoE genotype frequencies and atherosclerosis in the coronary arteries and the aorta

<table>
<thead>
<tr>
<th></th>
<th>Coronary</th>
<th>Aorta</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ε2/εX</td>
<td>ε3/εX</td>
</tr>
<tr>
<td>CVD</td>
<td>11</td>
<td>54</td>
</tr>
<tr>
<td>Severe</td>
<td>12</td>
<td>49</td>
</tr>
<tr>
<td>No CVD</td>
<td>17</td>
<td>56</td>
</tr>
<tr>
<td>Severe</td>
<td>8</td>
<td>63</td>
</tr>
</tbody>
</table>

Data shown as %. Differences in within-group genotype distributions between patients with mild/moderate atherosclerosis and patients with severe atherosclerosis are not statistically significant.

Table 7
ApoE genotypes, mean heart weight, and standardized heart weight by cause of death

<table>
<thead>
<tr>
<th></th>
<th>CHD b</th>
<th>Other CVD b</th>
<th>Cancer</th>
<th>Other</th>
<th>All</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart weight (g)</td>
<td>518 (7)</td>
<td>469 (7)</td>
<td>355 (4)</td>
<td>394 (5)</td>
<td>434 (4)</td>
</tr>
<tr>
<td>Standardized heart weight</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ε2/εX</td>
<td>1.33 (0.05)</td>
<td>1.21 (0.04)</td>
<td>0.94 (0.03)</td>
<td>0.98 (0.03)</td>
<td>1.10 (0.02)</td>
</tr>
<tr>
<td>ε3/εX</td>
<td>1.27 (0.02)</td>
<td>1.18 (0.02)</td>
<td>0.94 (0.01)</td>
<td>1.00 (0.01)</td>
<td>1.09 (0.01)</td>
</tr>
<tr>
<td>ε4/εX</td>
<td>1.22 (0.03)</td>
<td>1.15 (0.02)</td>
<td>0.92 (0.02)</td>
<td>1.00 (0.02)</td>
<td>1.08 (0.01)</td>
</tr>
<tr>
<td>P</td>
<td>&lt;01</td>
<td></td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean (SE). NS, not significant.

a P values are given for comparison of within-group differences in standardized heart weight between patients with different genotypes.
b Among patients dying from CVD (“CHD” and “Other CVD” pooled together), no significant differences between the genotype groups were found for gender (% female: ε2/εX: 37%, ε3/εX: 42%, ε4/εX: 35%) or mean age (ε2/εX: 70.9, ε3/εX: 70.4, ε4/εX: 69.7).
strokes are cardioembolic in origin [38]. Thus, some of the association between ε4 and stroke in the present study may be secondary due to heart disease.

To our knowledge, only four other autopsy studies [23–26], all based on forensic materials, have addressed the effect of the apoE polymorphism on atherosclerosis. In a study of 700 Finnish men, Ilveskoski et al. [23] concluded that the ε4-allele was a significant genetic risk factor for coronary atherosclerosis in early middle age, but not in older men. The ε4-allele was also associated with atherosclerosis in the aorta, but this was not age related. In a study [26] of 130 Alaskan men and women, ε4 was significantly associated with atherosclerosis in the coronary arteries, but not in the aorta. Conversely, in a study of 720 young males, Hixson [25] found a significant effect of ε4 on atherosclerosis in the aorta, but not in the coronary arteries. In a German study [24] that included 121 autopsies revealing serious coronary atherosclerosis at young age (<45 years), the ε4-allele was associated with a higher degree of stenosis in the coronary arteries. Moreover, 18.1% of patients who died from coronary atherosclerosis were ε4-carriers, compared to 8.1% of those who had coronary atherosclerosis as a secondary finding [24]. The main findings in these studies and the present study are summarized in Table 8. Some of the apparent differences may reflect different demographic or lifestyle factors between study populations, but may also be explained by random variation. In addition, differences could be attributable to methodological differences in the assessment of atherosclerosis. Our results are based on visual grading without staining of fat deposits in vessel walls. By contrast, three of the studies cited above [23,25,26] used Sudan IV staining to define the size of arterial fat deposits and may therefore have had the opportunity for a more precise measurement and also to detect a greater number of early atherosclerotic lesions. Despite significantly different genotypes in patients with MI and cerebrovascular disease, we failed to detect a significant relationship between genotype and the severity of atherosclerosis. Due to the multifactorial pathogenesis of atherosclerosis, one single factor such as apoE polymorphism may not be strictly correlated to the severity of atherosclerotic vascular changes. Also, the risk of myocardial infarction is believed to depend more on plaque vulnerability (plaque type) than on the plaque size [39,40]. We did not record atherosclerosis in the cerebral arteries or, as mentioned, in the carotid arteries, both of which are possible risk factors giving rise to cerebrovascular disease. Finally, the use of a semiquantitative visual grading system for atherosclerosis may have weakened the statistical relationship between apoE polymorphism and the severity of atherosclerosis, whereas findings of myocardial infarction or cerebrovascular disease may be more robust. Irrespective of these considerations, the present and the previous autopsy studies all suggest an effect of apoE on atherosclerotic disease.

In investigating apoE’s biological role, several studies have found a strong relation between ε4 and Alzheimer’s

<table>
<thead>
<tr>
<th>Study population</th>
<th>Method of assessment of atherosclerosis</th>
<th>CVD associated with genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Coronary atherosclerosis</td>
<td>Aortic atherosclerosis</td>
</tr>
<tr>
<td>Hixson [25]</td>
<td>Sudan IV staining, visual grading</td>
<td>NS a</td>
</tr>
<tr>
<td>Scheer et al. [26]</td>
<td>Sudan IV staining, visual grading</td>
<td>Yes b</td>
</tr>
<tr>
<td>Ilveskoski et al. [23]</td>
<td>Sudan IV staining,</td>
<td>Yes c</td>
</tr>
<tr>
<td></td>
<td>visual grading by</td>
<td></td>
</tr>
<tr>
<td></td>
<td>computer-assisted</td>
<td></td>
</tr>
<tr>
<td></td>
<td>planimetry</td>
<td></td>
</tr>
<tr>
<td>Heide et al. [24]</td>
<td>Visual grading (maximum</td>
<td>Yes d</td>
</tr>
<tr>
<td></td>
<td>degree of stenosis)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Visual grading (area of</td>
<td></td>
</tr>
<tr>
<td></td>
<td>atherosclerosis)</td>
<td></td>
</tr>
<tr>
<td>Present study</td>
<td></td>
<td>NS e</td>
</tr>
</tbody>
</table>

NS, not significant.

a The ε4-allele was significantly associated with atherosclerosis in the aorta; no significant association in the coronary arteries (RCA).

b The ε4-allele was significantly associated with atherosclerosis in the coronary arteries (RCA and LAD); NS association in the thoracic aorta; no association in the abdominal aorta.

c Genotype ε4/ε3 compared to ε3/ε3: significant difference for coronary (RCA and LAD) atherosclerosis in men <53 years of age; no association for men >53 years of age; significant not age-related effect in the aorta.

d The ε4-allele was significantly associated with a higher degree of coronary stenosis.

e Genotypes including the ε4-allele were more frequent and genotypes including the ε2-allele were less frequent; NS for coronary (all three arteries) and aortic atherosclerosis.

Table 8 Comparison of autopsy studies assessing the effect of apoE polymorphism on atherosclerosis [23–26]
dementia [6,10]. Consistent with this, we found that genotypes including ε4 were more common than genotypes including ε2 in patients with dementia.

There are some limitations to the study. Due to the retrospective study design, we were not able to systematically obtain information regarding lipid levels, smoking habits, alcohol use, blood pressure, or medication, some of which may interact with the apoE-genotype [8,32,41]. Further, the assessment of atherosclerosis was based on semiquantitative visual grading made during autopsy, and the definitions of the amount atherosclerosis corresponding to “mild,” “moderate,” and “severe” were not utilized prospectively. However, a test of the interobserver agreement at our department with regard to this method attained good correlation between observers. Besides, our results demonstrated the expected age gradient and gender difference, as well as the expected association between severe atherosclerosis and death from CVD (data not shown). These considerations suggest that the method is adequate and allows for discrimination between mild/moderate and severe atherosclerosis.

About 50% of all patients dying at Oslo University Hospital were subjected to autopsy in the observation period. This corresponds to nearly 10% of all registered deaths in Oslo in the same time span. The probability for a hospital autopsy to be carried out may be influenced by the patient’s age at death, disease characteristics, and several other factors. For example, the effect of apoE on development of dementia could influence the probability of dying in hospital since patients with dementia may be more likely than others to receive terminal care in nursing homes. Such selection would, however, only be expected to be seen at higher ages, whereas the effects of apoE on CVD described here and elsewhere are observed in younger patients. Further, the causes of death in our autopsy series are comparable to the registered causes of death in Oslo in the same 5-year period [42]. These considerations suggest that although there may be some selection bias, our study population is fairly representative of the general population. Moreover, the allele frequencies for our autopsy cases are similar to those in a group of 800 healthy blood donors. The ε2 frequency in the control group may be slightly lower by chance since it was also low compared to what is reported in the general populations in other Nordic studies [43,44].

The heritability of CVD has been estimated to exceed 50%, and it is believed that over 100 genes are involved [4]. Thus, the apoE polymorphism is only one of multiple genetic and environmental influences in the development of atherosclerotic CVD. Although the apoE locus has been suggested to be the major single gene influencing longevity [45], the magnitude of the differences in CVD manifestations between apoE genotypes appears to be relatively modest. The mechanisms by which apoE may influence the development of CVD are not completely understood. The three isoforms have different low-density lipoprotein receptor binding affinities and lipid binding preferences [10,46], and studies have shown a clear relationship between ε2 and lower cholesterol levels and between ε4 and higher cholesterol levels [13,47]. It has been estimated that the apoE polymorphism is responsible for approximately 17% of the genetic variability in total plasma cholesterol [47]. Although some studies have found that the effect of apoE on CHD risk is attenuated when adjusted for cholesterol levels [19], other studies indicate ε4 as a risk factor independent of cholesterol levels [48]. Further, animal studies have suggested an antiatherosclerotic effect of apoE [49], suggesting that the concentration of apoE itself may be important in the development of CVD. In fact, ε2-carriers tend to have higher plasma concentrations of apoE than ε4-carriers [50,51]. In addition, apoE may modulate CVD risk through effects on immunological or inflammatory processes as well as through oxidative mechanisms [16,52,53].

In conclusion, the results of the present autopsy study show that the risk of developing and dying from CVD, including CHD and cerebrovascular disease, is influenced by the apoE polymorphism. Specifically, CVD morbidity and mortality are consistently increased among ε4-carriers, whereas the ε2-allele appears to be protective. However, the effects on CVD morbidity and mortality appear to be relatively modest. Thus, although screening of apoE genotype could be appropriate in certain subpopulations [32,54], we do not believe that our findings justify genetic analysis of the apoE polymorphism in the general population.

References


