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Apolipoprotein E polymorphism and carotid artery intima-media thickness in a random sample of middle-aged men

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Abstract

Genetic polymorphism of apolipoprotein E (apoE) is an important factor in the development of coronary artery disease but the results concerning apoE genotype and carotid artery atherosclerosis remain controversial. We investigated a random sample of 189 Finnish middle aged men (mean age 54 years, range 50–59) to assess the role of apoE in the process of carotid atherosclerosis. Intima-media thickness (IMT) of the carotid artery wall was measured at three standardised segments (common carotid artery, bifurcation and internal carotid artery) by B-mode ultrasonography. Overall mean IMT value was also calculated. The carriers of E3:2 (n = 20) genotype had significantly lower (P < 0.01) total cholesterol and LDL cholesterol concentrations than carriers of E3:3 genotype (n = 109) or the E4 allele (n = 60). ApoE polymorphism was associated with common carotid artery IMT (P = 0.034) when adjusted for age and body-mass index (model 1). The carriers of E3:2 had on average 9% (95% CI 0.8–16%, P = 0.028) lower common carotid IMT values than the carriers of E3:3. After further adjustment with LDL and HDL cholesterol, systolic blood pressure, lipoprotein (a), apolipoprotein B and pack-years of smoking (model 2) the association was not statistically significant. The overall mean IMT varied significantly with apoE genotype (P = 0.03 for model 1 and P = 0.07 for model 2), and it was also lowest in the carriers of E3:2 genotype. This suggests that apoE E3:2 genotype is a protective factor in the development of carotid artery atherosclerosis in randomly selected middle-aged men. The favourable effect might be mediated at least partly by the lowering effect of E3:2 genotype on serum cholesterol. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Apolipoprotein E; Polymorphism; Carotid atherosclerosis; Ultrasonics; Intima-media thickness

1. Introduction

Cardiovascular diseases remain important causes of morbidity and mortality in Western countries. Much attention has been paid to the classic risk factors for atherosclerosis. However, they explain only a part of the susceptibility, and it would be valuable to find the predisposing genes. One candidate gene is apolipoprotein E (apoE), which is a polymorphic glycoprotein having an important influence on the lipid metabolism [1–3]. The common three isoforms of the protein are designated E2, E3 and E4, and codominant inheritance leads to six phenotypes (E2:2, E3:2, E4:2, E3:3, E4:3 and E4:4) of which E3:3 is the most common [4].

Several studies in different populations have shown that the carriers of the apoE E4 allele have higher total cholesterol (TC) and low density lipoprotein (LDL) cholesterol values and the E2 in turn is associated with lower values [2,5–9]. Further, the E4 allele is associated with coronary heart disease (CHD) in patients with angina pectoris symptoms, and with coronary artery disease (CAD) in patients with angiographically verified
coronary stenosis [5,10–12]. Increased incidence of myocardial infarction has also been reported in patients with the E4 allele [13]. On the other hand, there are also studies suggesting that the E2 allele protects from CAD and from myocardial infarction [5,14,15]. The role of apoE polymorphism has also been studied in stroke but the results are controversial, and besides the ‘bad allele’ E4, the protective E2 isoform has also been associated with stroke [16,17].

Age, high systolic blood pressure, cigarette smoking, and high LDL cholesterol concentration all increase the carotid artery intima-media thickness (IMT) measured by B-mode ultrasonography [18,19]. Genetics is suggested to determine a large part of the variability of the carotid IMT in healthy people [20]. Further, it has been shown that there is also an association between CAD and carotid artery disease determined by measuring the carotid artery IMT [21]. Since apoE polymorphism has an effect on serum LDL cholesterol concentration and on the risk for CAD, it may also play a role in the development of atherosclerosis in the carotid artery. There are only a few studies concerning the relationship between carotid atherosclerosis and apoE, and both E3/2 genotype and the E4 allele have been associated with carotid artery intima-media thickness (IMT) measured by ultrasonography [22,23]. However, a lack of association of apoE polymorphism with carotid IMT has also been reported [24]. The possible protective effect of the E2 allele, as it has on the development of CAD, has not been studied in detail previously.

To clarify the association between apoE polymorphism and carotid atherosclerosis we examined a random sample of 189 middle aged men who underwent carotid artery ultrasonography.

2. Materials and methods

2.1. Subjects

The original study population was comprised of 300 men aged 50–59 years who were randomly selected from ten age-cohorts (n = 9058) living in the city of Tampere in Southern Finland. The men were invited to participate in the study by letter. A total of 223 men agreed to participate (74%), while 33 refused and 44 did not answer or could not be reached. Finally, data was collected on 219 participants. The Ethics Committee of the Urho Kaleva Kekkonen (UKK) Institute approved the study, and in addition all participants gave their written informed consent.

Detailed medical histories were collected, with particular emphasis on cardiovascular and metabolic diseases and chronic medications. A total of 65 men had chronic medication, of which 45 were treated for hypertension, ten for diabetes mellitus, nine for ischemic heart disease, four for hyperlipidemia and 17 men for other diseases. Smoking status was assessed using a validated questionnaire and measured as pack–years of smoking.

Weight and height were recorded and the body mass index (BMI) was calculated (kg/m²). Blood pressure was recorded from the dominant arm with a mercury sphygmomanometer after 15 min of supine rest. Three measurements in supine, two in standing and one in sitting position were performed and the averages of six measurements were recorded as resting systolic and diastolic blood pressure values.

2.2. Laboratory methods

Blood was drawn after a 12-h overnight fast. Alcohol and exercise were not allowed for 24 h before sampling. Sera for all determinations but lipoproteins was divided into aliquots and stored immediately at −70°C until analysed. Lipoprotein fractions were assessed from fresh samples by ultracentrifugation [25] and cholesterol was measured from serum and lipoprotein fractions using an enzymatic method (CHOD-PAP, Boehringer Mannheim, Germany). Triglycerides were measured by enzymatic method (GPO-PAP, Boehringer Mannheim, Germany). Apolipoprotein B (apoB) was analysed by using immunonephelometric method (Behring, Behringwerke AG, Marburg, Germany) and lipoprotein (a) (Lp(a)) by using a two-site immunoradiometric assay (Pharmacia, Uppsala, Sweden).

2.3. ApoE phenotyping

ApoE phenotyping was performed using delipidated plasma, isoelectric focusing, cysteamine treatment and immunoblotting, as described by Menzel and Utermann [26], with minor modifications described by Lehtimäki et al. [8]. The verification of correct apoE phenotypes in gel was based on comparison to previously known apoE genotype standards.

2.4. Carotid ultrasonography

Quantitative carotid artery ultrasonography was performed using a standardised protocol (MIDAS) [27]. A high-resolution B-mode ultrasound with a 10 MHz transducer was used (Biosound Phase 2, Biodynamics, Indianapolis), to examine left and right carotid arteries. The examinations were recorded on S-VHS videotapes (Panasonic AG 7530A, Panasonic, Japan) and the tapes were then read off-line at the ultrasound reading center, Wake Forest University, NC. All recordings and measurements were performed by the same certified sonographer and reader, respectively.

Carotid arteries from both sides were imaged by performing a circumferential scan including the longitudinal views of the lateral, posterior, and anterior angles.
Identification of the arteries was accomplished by Doppler analysis. The protocol involved the scanning of the distal 10 mm of the common carotid artery, the bifurcation and the proximal 10 mm of the internal carotid artery. The distance between the media-adventitia interface and the lumen-intima interface represents the IMT. The maximum IMT of the near and far wall was measured at 12 well-defined arterial segments. The single largest IMT was determined by selecting the largest IMT among the individual maximum IMTs in the 12 standard arterial walls, i.e. the near and far walls of the common, bifurcation, and internal carotid artery at both sides. The mean maximum IMT (M_{max}, overall mean) was calculated as the mean of 12 maximum IMTs identified at 12 standard sites [27]. To assess the intraobserver variability of the recording and measurement of IMT, a repeated scan of a total of 15 randomly selected participants were performed 1 week later. The mean absolute difference between repeated measurements was 0.052 mm in the MMax IMT of the 12-site and the single largest mean difference, 0.11 mm, was detected in the near wall of the left internal carotid artery. These figures are comparable to previously reported data [28].

2.5. Statistical methods

All calculations were done using SPSS Version 8.0 for Windows on a PC. Non-normally distributed data such as triglyceride and Lp(a) concentrations and carotid IMT were analysed in logarithmically transformed form but the results are expressed as crude. ApoE phenotypes were designated as E2 (E3/E3 homozygous). There were four men with phenotype E4/2 and they were excluded from the analyses. From the total of 219 men, 26 men were excluded because of missing data, and in the end 189 men were included in the final analysis of clinical characteristics and carotid IMT.

The means of the continuous variables in the three apoE phenotype groups (E2, E3 and E4) were compared by using the analysis of covariance (ANCOVA) with age as a covariate. The ANCOVA was also used to identify the possible associations of apoE phenotype with the carotid IMT. Differences between groups were analysed using Sidak post-hoc tests. Age and BMI were used as covariates in model 1, and in model 2 apoE was covaried with systolic blood pressure, pack-years of smoking, LDL cholesterol, HDL cholesterol, apoB and Lp(a) concentrations in addition to the covariates of model 1. Data are expressed as mean ± S.D. P-values < 0.05 were considered statistically significant.

3. Results

3.1. ApoE genotype, serum lipids and apoproteins

ApoE allele frequencies among all 219 men were 0.06 for ε2, 0.75 for ε3 and 0.19 for ε4. This is consistent with larger studies of Finnish populations [7,8]. The genotype distribution was in Hardy–Weinberg equilibrium.

Table 1 shows clinical characteristics of all 189 participants by apoE genotype groups. ApoE was statistically significantly associated with serum total and LDL cholesterol concentrations (P = 0.004 and P = 0.002; ANCOVA, age as a covariate), as expected. The E3/2 genotype group had lower total and LDL cholesterol concentrations than E3 or E4 groups (P < 0.01 for E2 versus E3 or E4, post-hoc analysis). ApoB concentration was also associated with apoE genotype (P = 0.003). Other clinical variables did not vary significantly with apoE genotype group but the E2 group tended to be less addicted to smoking than E3 or E4 (P = 0.18).

Table 1

<table>
<thead>
<tr>
<th>Clinical characteristics by apoE genotype groups*</th>
<th>E2 (n = 20)</th>
<th>E3 (n = 109)</th>
<th>E4 (n = 60)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>54.2 (3.0)</td>
<td>54.2 (3.0)</td>
<td>54.2 (2.9)</td>
<td>0.99</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>27.5 (3.1)</td>
<td>26.7 (3.7)</td>
<td>27.7 (3.6)</td>
<td>0.16</td>
</tr>
<tr>
<td>Systolic blood pressure, mmHg</td>
<td>127 (11)</td>
<td>131 (16)</td>
<td>132 (19)</td>
<td>0.57</td>
</tr>
<tr>
<td>Diastolic blood pressure, mmHg</td>
<td>83.1 (8.5)</td>
<td>83.9 (9.8)</td>
<td>83.8 (11.9)</td>
<td>0.96</td>
</tr>
<tr>
<td>Total cholesterol, mmol/l</td>
<td>4.84 (0.65)</td>
<td>5.50 (0.96)</td>
<td>5.59 (0.76)</td>
<td>0.004*</td>
</tr>
<tr>
<td>LDL-cholesterol, mmol/l</td>
<td>2.95 (0.57)</td>
<td>3.59 (0.85)</td>
<td>3.67 (0.73)</td>
<td>0.002*</td>
</tr>
<tr>
<td>HDL-cholesterol, mmol/l</td>
<td>1.28 (0.25)</td>
<td>1.25 (0.25)</td>
<td>1.17 (0.30)</td>
<td>0.13</td>
</tr>
<tr>
<td>VLDL-cholesterol, mmol/l</td>
<td>0.61 (0.28)</td>
<td>0.66 (0.40)</td>
<td>0.75 (0.48)</td>
<td>0.32</td>
</tr>
<tr>
<td>Triglycerides, mmol/l</td>
<td>1.26 (0.50)</td>
<td>1.50 (0.80)</td>
<td>1.63 (1.02)</td>
<td>0.39</td>
</tr>
<tr>
<td>Lp(a), mg/l</td>
<td>303 (306)</td>
<td>268 (378)</td>
<td>231 (278)</td>
<td>0.59</td>
</tr>
<tr>
<td>Apolipoprotein B, g/l</td>
<td>1.09 (0.24)</td>
<td>1.32 (0.31)</td>
<td>1.34 (0.26)</td>
<td>0.003*</td>
</tr>
<tr>
<td>Pack-years of smoking, years</td>
<td>6.4 (9.3)</td>
<td>12.2 (13.8)</td>
<td>12.3 (13.1)</td>
<td>0.18</td>
</tr>
</tbody>
</table>

* Values are mean (± S.D.). Differences between means was tested by analysis of covariance and age was used as covariate when appropriate. Sidak post-hoc tests: **P < 0.01 for E2 versus E3 or E4.
Table 2
Carotid artery IMT in different segments by apoE genotype

<table>
<thead>
<tr>
<th></th>
<th>E2 (n = 20)</th>
<th>E3 (n = 109)</th>
<th>E4 (n = 60)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Common</td>
<td>0.95 (0.12)</td>
<td>1.05 (0.17)</td>
<td>1.03 (0.16)</td>
</tr>
<tr>
<td>Bifurcation</td>
<td>1.30 (0.28)</td>
<td>1.45 (0.39)</td>
<td>1.41 (0.33)</td>
</tr>
<tr>
<td>Internal</td>
<td>1.09 (0.50)</td>
<td>1.19 (0.48)</td>
<td>0.99 (0.30)</td>
</tr>
<tr>
<td>M_{max}</td>
<td>1.11 (0.22)</td>
<td>1.23 (0.26)</td>
<td>1.15 (0.21)</td>
</tr>
</tbody>
</table>

* Values are mean (± S.D.).

3.2. ApoE genotype and carotid IMT

The unadjusted carotid IMT means in different segments by apoE genotype group are shown in Table 2, and Table 3 shows the mean percentual differences of carotid artery IMT between apoE genotype groups. The ANCOVA showed a statistically significant association of apoE genotype with common carotid artery IMT when adjusted for age and BMI (P = 0.034; ANCOVA model 1) (Table 3). The carriers of the E3/2 genotype had on average 9% (95% CI 0.8–16%) lower IMT values than carriers of the E3/3 (P = 0.028) when adjusted for the covariates model 1. The difference in common carotid IMT between E3/2 and E4 group was statistically insignificant (P = 0.09). ApoE had no significant effect on common carotid IMT when adjusted for other risk factors (P = 0.23; model 2).

From all three segments the IMT of the carotid bifurcation was the thickest, as expected. However, it did not vary with apoE genotype, although the carriers of E3/2 tended to have lower IMT values (Table 2).

The internal carotid artery IMT was associated with apoE (P = 0.006; ANCOVA model 2), but surprisingly, the participants homozygous for E3 had on average 18% (95% CI 4–33%) higher IMT than those with the E4 allele, when adjusted for the effects of covariates in model 2 (Table 3). The difference between the E2 and E3 groups did not reach statistical significance (P = 0.73).

Table 3
The mean percentual differences of carotid artery IMT between apoE genotype groups in different segments

<table>
<thead>
<tr>
<th></th>
<th>E2 versus E3 % difference (95% CI)</th>
<th>E2 versus E4 % difference (95% CI)</th>
<th>E3 versus E4 % difference (95% CI)</th>
<th>P-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Common</td>
<td>Model 1: -8.9 (-16.9 - 0.8)</td>
<td>Model 1: -7.8 (-16.0 - 0.9)</td>
<td>Model 1: 1.2 (-4.4 - 7.1)</td>
<td>0.034*</td>
</tr>
<tr>
<td></td>
<td>Model 2: -5.5 (-13.2 - 2.8)</td>
<td>Model 2: -3.5 (-12.5 - 5.5)</td>
<td>Model 2: 2.1 (-3.3 - 7.7)</td>
<td>0.23</td>
</tr>
<tr>
<td>Bifurcation</td>
<td>Model 1: -9.7 (-21.3 - 2.5)</td>
<td>Model 1: -7.6 (-20.6 - 6.8)</td>
<td>Model 1: -2.4 (-6.5 - 12)</td>
<td>0.20</td>
</tr>
<tr>
<td></td>
<td>Model 2: -7.1 (-19.6 - 5.6)</td>
<td>Model 2: -4.4 (-17.1 - 11)</td>
<td>Model 2: 2.9 (-5.9 - 12)</td>
<td>0.40</td>
</tr>
<tr>
<td>Internal</td>
<td>Model 1: -9.6 (-25.8 - 6.5)</td>
<td>Model 1: 6.9 (-12.3 - 30)</td>
<td>Model 1: 18 (4.7 - 34)</td>
<td>0.004**</td>
</tr>
<tr>
<td></td>
<td>Model 2: -7.0 (-23.12)</td>
<td>Model 2: 9.3 (-11.34)</td>
<td>Model 2: 18 (4.1 - 33)</td>
<td>0.006**</td>
</tr>
<tr>
<td>M_{max}</td>
<td>Model 1: -10 (-19.0 - 7.0)</td>
<td>Model 1: -4.6 (-15.1 - 7.1)</td>
<td>Model 1: 5.7 (-1.7 - 14)</td>
<td>0.033***</td>
</tr>
<tr>
<td></td>
<td>Model 2: -6.9 (-17.3 - 3.9)</td>
<td>Model 2: -1.2 (-12.11)</td>
<td>Model 2: 6.1 (-1.1 - 14)</td>
<td>0.07</td>
</tr>
</tbody>
</table>

* Sidak post-hoc tests: *P = 0.028 for E2 versus E3 and P = 0.09 for E2 versus E4; **P < 0.01 for E3 versus E4; and ***P = 0.07 for E2 versus E3.

b Values are mean percentual between-group differences and their 95% confidence intervals adjusted for multiple comparisons by Sidak method.

c ANCOVA; model 1: age and BMI as covariates; model 2: model 1 plus systolic blood pressure, LDL-C, HDL-C, apoB, Lp(a) and pack-years of smoking as covariates. Data was analysed in logarithmically transformed form.

The M_{max} carotid artery IMT varied statistically significantly with apoE genotype in the ANCOVA using model 1 (P = 0.03) (Table 3). The association of apoE genotype group with M_{max} IMT was due to difference between E3/2 and E3/3 carriers, the E3/2 genotype being associated with on average 10% (95% CI –0.7–19%) lower IMT values than E3/3 (adjusted for age and BMI). However, this difference reached only borderline statistical significance (P = 0.07). The E2 group did not differ significantly from E4 group (P = 0.70), and the difference between E3 and E4 groups was also statistically insignificant (P = 0.19). After adjustment for other risk factors the association of apoE genotype with M_{max} IMT still reached borderline significance (P = 0.07; ANCOVA model 2).

4. Discussion

Our study of randomly selected middle-aged men showed that the carriers of the apoE E3/2 genotype had lower intima-media thickness (IMT) values in common carotid artery and lower M_{max} IMT values than the E3 homozygotes. However, these associations weakened after adjustments for carotid artery disease risk factors including serum lipids. Contrary to our hypothesis, we found no relationship between the E4 allele and increased carotid artery IMT.

In contrast with our results and those produced by Kogawa et al. [24] and Sass et al. [29], the E4 allele was associated with carotid IMT in an Italian study in asymptomatic adults [30] and in a Finnish study in type 2 diabetics and controls [31]. Terry et al. also found that the E4 allele is associated with high overall mean IMT [23]. However, in their study carriers of the E2 allele had significantly lower IMT values in their common carotid arteries than carriers of the E3 or E4 allele.
which supports our finding that the E2 allele has a protective role. In contrast to other studies of apoE polymorphism and carotid IMT, in a multicenter case-control study the E2 allele (E3/2 genotype) was found to increase the risk for carotid artery disease, i.e. the E2 allele was a risk factor for carotid atherosclerosis [22]. The differences in subjects and study design could have accounted for controversial results in the present and previous studies. First, it has been shown in autopsy studies that men have more plaques in their carotid arteries than women [32] and parallel results have been detected also by ultrasound [33]. The subjects of all previous studies [22–24,29–31] were both men and women while we studied only men. This discrepancy might offer one explanation of the different results. Second, the selection of study subjects can cause selection biases. The subjects of one previous study were selected from among patients who had undergone coronary angiography [23]. This may have led to selection of patients with more advanced atherosclerosis, because coronary angiography is not performed without symptoms. Another study excluded patients with evident cerebrovascular or coronary heart disease symptoms [22], which in turn leads to selection of more healthy study subjects. We avoided selection biases by choosing a random sample from 10 age-cohorts of Finnish male population. On the other hand, our study cannot control for the possible confounding effect of advanced atherosclerotic diseases because coronary angiography was not performed on our subjects. Third, in our sample the E2 group was less addicted to smoking (Table 1). Although the difference was statistically insignificant ($P = 0.18$) it could have biased the outcome. Therefore, smoking was taken as a covariate in the analysis and the results were adjusted for it. Use of medication may also be a confounding factor. About 30% of the men in our sample had chronic medication. The reason why we did not exclude the men with medication was that our study is supposed to represent a random sample of Finnish male population. A selection like this would have led to more healthy participants. In addition, the proportion of men with medication was identical (25–30%) in each of the genotype groups (E2–E4). Therefore, the medication is unlikely to be a confounding factor in the study.

The observation that the internal carotid IMT was significantly higher in men carrying E3/3 genotype when compared with the E4 group was contrary to our hypothesis that it is the E4 allele that increases carotid IMT. None of the clinical characteristics or lipid parameters explains the higher IMT values of internal carotid artery in the E3 group (Table 1). It is possible that some unknown factors not considered herein confounded the results. Therefore, the value of this finding remains unclear. However, one previous study also found that the E3 allele is associated with the highest carotid IMT values [20].

The E2 allele is associated with lower total and LDL cholesterol values [2,5–9] which was also shown in our study (Table 1). In the present study, the E3/2 genotype was associated with lower IMT in common carotid artery when compared with the E3/3 and the favourable effect of the E3/2 was even more pronounced in the $M_{\text{max}}$ IMT values. The $M_{\text{max}}$ IMT is an average value of the IMT of 12 standard sites giving a reliable picture of the involvement of early atherosclerotic changes in the whole carotid artery tree [27]. The significant association of the E3/2 with this measurement increases the reliability of our finding.

The protective effect of the E3/2 genotype on the development of carotid atherosclerosis could be mediated by differences in the lipid metabolism because the association of E3/2 genotype with IMT disappeared after adjustment with carotid artery disease risk factors including LDL cholesterol. The E2 allele has been found to protect from stroke [34] which speaks for a protective role of the E3/2 genotype. The studies of CAD and apoE polymorphism also support the protective role of E2 allele in the atherosclerosis of the carotid artery. It has been well documented that the apoE E4 allele is a risk factor for advanced CHD or CAD [5,10–15,35], and there are also two prospective studies showing that the E4 is associated with increased incidence of coronary deaths [13,36]. The E2 allele has in turn been found to be protective from CAD [5,14,15] although the rare E2 homozygotes with hyperlipoproteinemia type III have markedly increased risk of myocardial infarction [37]. In addition, an autopsy study by Hixson and colleagues found that the carriers of the E2 had less atherosclerosis in their aortas when compared to the E3/3 carriers [38]. We found no association of E4 allele with increased IMT of the carotid artery, which implies that the role of the apoE E4 allele in the development of carotid atherosclerosis might be different from its role in CAD.

In conclusion, our study of 189 randomly selected middle-aged men showed that the common carotid and $M_{\text{max}}$ IMT values were lower in the carriers of E3/2 genotype than in the E3/3 carriers. The association weakened after adjustment with serum cholesterol and with other risk factors, suggesting that the protective effect of the E3/2 genotype in our sample is mediated at least partly through cholesterol metabolism.

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