Association of Apolipoprotein E Polymorphism, Low-Density Lipoprotein Cholesterol, and Coronary Artery Disease

H.J. Lenzen,1 G. Assmann,1,2 R. Buchwalsky,3 and H. Schulte2

We determined the frequencies of genetic apolipoprotein E isoforms in 570 survivors of myocardial infarction, all with demonstrable coronary heart disease, as compared with 624 healthy persons. In controls, E-4/E-3 heterozygosity was associated with total cholesterol concentrations of 1985 (SD 364) mg/L and low-density lipoprotein (LDL)-cholesterol concentrations of 1306 (SD 332) mg/L. Significantly lower values, 1811 (SD 312) mg/L and 1121 (SD 274) mg/L, respectively, were observed for E-3/E-2 heterozygous persons. In survivors of myocardial infarction, the respective values were significantly higher than in controls, differing between E-4/E-3 and E-3/E-2 heterozygous patients by 233 and 220 mg/L, respectively. Moreover, E-4/E-3 heterozygosity was accompanied by earlier age of myocardial infarction (48.8 ± 7.4 years) as compared with E-3/E-2 heterozygosity (53.4 ± 6.9 years) and E-3/E-3 homozygosity (51.2 ± 7.7 years). Evidently, apolipoprotein E polymorphism can contribute to total and LDL-cholesterol concentrations in serum, thereby affecting risk of coronary heart disease and myocardial infarction.

Additional Keyphrases: atherosclerosis · genetics · myocardial infarction

Apolipoprotein E (apo E) is part of all lipoprotein classes—chylomicrons and very-low-VLDL, low-LDL, and high-density lipoproteins (HDL)—and exists in three major isoforms (apo E-2, apo E-3, apo E-4), which can be separated by isoelectric focusing in polyacrylamide gels and then made visible.1 Synthesized under the control of independent alleles at a single gene locus, apo E is subject to posttranslational modifications (I–IV). The frequencies of the genotypes in the European and North American population are very similar: 9.8% apo E-2/2; 53.7% apo E-3/3; 23.3% apo E-4/4; 11.0% apo E-2/3; 3.9% apo E-2/4; 22.3% apo E-3/4 (3). Other known isoforms of apo E—apo E-1 (6), apo E-2* (7), apo E-2** (9), apo E-2 (Bethesda) (9), apo E-3* (10), apo E-313 (11), apo E-5 (12), and apo E-Suiza (13)—are very rare. Though several tests of apo E phenotypes and lipid status in patients suffering from coronary artery disease (CAD) have shown a complex relationship between apo E genotypes and lipoprotein concentrations, no reliable information about the relations of genetic apo E polymorphism and CAD could be obtained (14–21).

Therefore, we analyzed apo E polymorphism, lipid status, and anamnestic data of 570 survivors of myocardial infarction (MI) who were affected by coronary atherosclerosis. For controls, we used 624 male factory workers free of coronary heart disease.

Materials and Methods

Patients and Controls

Sera were collected during fasting from a group of 630 male survivors of MI who underwent coronary angiography at the Schüchtermann-Klinik4 for diagnostic reasons. All patients had had a myocardial infarction three months or more earlier. Patients with valve disease, vessel transposition, and cardiomyopathy were excluded. In the remaining 570 of these patients (CAD+/MI+ patients) we observed stenoses on one or several coronary arteries (see below). Sixty patients had either no or minimal coronary lesions (CAD–/MI+ patients). All patients gave informed consent, and the procedures were in accord with the institutional guidelines.

For controls we tested 624 male, age-matched factory employees who took part in the "Prospective Cardiovascular Münster (PROCAM) Study." These control persons were not affected by CAD, as judged from the anamnestic data (no signs of angina pectoris) and electrocardiograms (no abnormalities).

Procedures

Coronary angiography. Coronary angiograms were analyzed by two experienced cardiologists who did not know the genetic E polymorphism of the individual at the time of investigation. Coronary arteriography, performed by the techniques of either Judkins (22) or Sones (23), was recorded on 16-mm cine-angiographic film. If the lumina of the right or left coronary arteries, the circumflex branch, or the anterior descending branch showed stenoses of more than 50%, the patient was classified as "CAD+/MI+.

Isolation of apo VLDL. One milliliter of serum (if triglycerides exceeded 1.00 g/L) or 2 mL (if triglycerides were <1.00 g/L) was centrifuged in a type 25 rotor (Beckman Instruments, Palo Alto, CA) for 24 h (4 °C, 25 000 rpm). The top layer, about 300 μL, was aspirated and replaced with 100 μL of a mixture containing, per liter, 9 g of NaCl and 0.2 g of NaN3. The mixture was again ultracentrifuged, under the same conditions. If phenotyping could not be started at the same day, the washed VLDL samples were stored at −20 °C. The samples were mixed with 100 mL of a 9 g/L solution of NaCl and then delipidated at −20 °C with ether/ethanol (1/3 by vol) and pure diethyl ether.

Polyacrylamide gel isoelectric focusing. The apolipoproteins were dissolved in 50 mL of a pH 8.2 buffer containing, per liter, 10 mmol of Tris HCl, 15 g of sodium dicyl sulfate, 20 g of ampholytes (pH 3–7), 360 g of purified urea, 100 g of β-mercaptoethanol, and 130 g of sucrose. This mixture was
incubated at 36 °C for 1 h. For isoelectric focusing we used the Model 220 slab-gel system (Bio-Rad Lab., Richmond, CA) according to Pagan et al. (24), modified for application in large series (22, 23). The amphoteries were a mixture of one part (by vol) of Servalyte AG 3–5, 1.8 parts of Servalyte AG 5–7 (Serva Feinbiochemica, D-6900 Heidelberg, F.R.G.), one part of Ampholine 3.5–5.0, and 1.8 parts of Ampholine 5.0–7.0 (LK B, S-Bromma, Sweden). The gel solution contained, per liter, 2.1 g of N,N-methylenebisacrylamide (BioRad), 650 mg of tetramethylenediamine (Bio-Rad), 75 g of acrylamide (LKB), 15 g of amphoteries, and 6 g of purified urea. The electrophoresis was run overnight (17 h) at 200 V, with a power limit of 2.25 W per plate, then in the morning for 1 h at 600 V. For staining we used a modification of the method of Malik and Berrie (25).

Lipid analyses. Total cholesterol and triglycerides were determined enzymatically with commercially available testkits (Boehringer, D-6800 Mannheim, F.R.G.). HDL-cholesterol was determined after precipitation with commercially available reagents (Boehringer). LDL-cholesterol was estimated by the method of Friedewald (26).

Statistical methods. Differences of age and lipid parameters between the apo E phenotypes were tested by one-way analysis of variance. The significance level was set at 0.05.

Results

The prevalence of the genetic apo E phenotypes was not significantly different between CAD+/MI+ patients and controls (Table 1).

However, the alleles e2 and e4 had a major impact on total cholesterol and LDL cholesterol concentrations in both controls and CAD+/MI+ patients (Figure 1 and Table 2).

In controls, total cholesterol concentrations in serum were 170 mg/L higher in E-4/E-3 heterozygotes (1985 ± 364 mg/L, mean ± SD) than in E-3/E-2 heterozygous individuals (1811 ± 312 mg/L; p < 0.01). E-3/E-3 homozygous persons showed intermediate values (1952 ± 330 mg/L). Serum LDL-cholesterol concentrations in controls were 185 mg/L higher in E-4/E-3 heterozygotes (1306 ± 332 mg/L) than in E-3/E-2 heterozygous persons (1121 ± 274 mg/L; p < 0.001).

In E-3/E-3 heterozygous LDLC-cholesterol concentrations were intermediate (1262 ± 304 mg/L).

E-4/E-3 heterozygous CAD+/MI+ patients similarly showed 233 mg/L greater total cholesterol concentrations (2281 ± 540 mg/L, p < 0.01) and 220 mg/L more LDL-cholesterol (1668 ± 498 mg/L, p < 0.01) than did E-3/E-2 heterozygous (2048 ± 410 and 1448 ± 357 mg/L, respectively). Intermediate values were found for E-3/3 heterozygous patients (2155 ± 492 and 1568 ± 463 mg/L, respectively) (Figure 1, Table 2). Serum HDL-cholesterol and triglyceride concentrations did not vary significantly by the various apo E phenotypes in controls and in CAD+/MI+ patients.

The reported ages at which myocardial infarction had occurred and the ages at which coronary angiography was performed differed between E-4/E-3 and E-3/E-2 heterozygous CAD+/MI+ patients by 4.6 years (p < 0.01), and 4.1 years (p < 0.01), respectively.

Significant differences were found, too, in the prevalence of apo E isoforms in patients with early (<50 years) and late (>50 years) infarction (Table 3). Only 23.7% of the E-3/E-2 heterozygous persons suffering from myocardial infarction were younger than 50 years, 76.3% were older. In E-4/E-3 heterozygous patients this age dependency was reversed: 60% suffered from infarction before reaching 50 years of age and only 40% later.

Owing to the low prevalence of E-4/E-4 homozygosity and E-2/E-2 homozygosity in controls and CAD+/MI+ patients, we could not obtain meaningful data concerning differences in lipid status and manifestation of CAD in these subjects.

Discussion

As demonstrated in our study, the genetic polymorphism of apo E significantly influenced total and LDL cholesterol concentrations as well as the clinical manifestation of CAD (Figure 1, Tables 2 and 3). These results agree with previous observations by Cumming and Robertson (15) that E-4/E-3 heterozygous men were younger at the first incident of MI than patients with other phenotypes. Our results also are in accordance with our previous studies of CAD patients, in which we demonstrated that the e2 allele was more frequent in CAD− patients than in CAD+ patients (17). These former studies were done in patients who, at the time of coronary angiography, had not had a myocardial infarction. However, our current data differ from observations of Utzmann et al. (19), who found a lower frequency of the apo E4 isoform in MI patients than in unaffected controls. The origin of this difference may possibly relate to the heterogeneity of MI survivors because, as observed here, only part of those patients are affected by advanced coronary atherosclerosis. Our results concerning LDL-cholesterol concentrations agree with the data of Robertson and Cumming (15), who found lower concentrations of LDL-cholesterol in serum from healthy E-3/E-2 heterozygous men than in that from E-3/E-3 homozygotes.

Interestingly, in both controls and CAD+/MI+ patients the average differences in LDL cholesterol concentrations between E-4/E-3 heterozygotes and E-3/E-2 heterozygotes were about 200 mg/L. Thus, the higher values of LDL
Table 2. Apo E Phenotypes, Serum Lipoproteins, and Age at Myocardial Infarction in CAD+/MI+ Patients, and Control Data

<table>
<thead>
<tr>
<th>Apolipoprotein E phenotypes</th>
<th>E-4/E-3</th>
<th>E-3/E-2</th>
<th>E-3/E-3</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients (n = 570)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age at coronary angiography, yr</td>
<td>51.7 ± 7.9</td>
<td>55.8 ± 6.6</td>
<td>53.0 ± 7.7</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Age at MI, yr</td>
<td>48.8 ± 7.4</td>
<td>53.4 ± 6.9</td>
<td>51.2 ± 7.7</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Cholesterol, mg/L</td>
<td>2281 ± 540</td>
<td>2048 ± 410</td>
<td>2155 ± 492</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>LDL-cholesterol, mg/L</td>
<td>1668 ± 498</td>
<td>1448 ± 357</td>
<td>1568 ± 483</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Triglycerides, mg/L</td>
<td>1398 ± 784</td>
<td>1356 ± 677</td>
<td>1210 ± 741</td>
<td>n.s.</td>
</tr>
<tr>
<td>HDL-cholesterol, mg/L</td>
<td>342 ± 89</td>
<td>329 ± 80</td>
<td>349 ± 110</td>
<td>n.s.</td>
</tr>
<tr>
<td>Cholesterol/HDL-cholesterol</td>
<td>7.12 ± 2.48</td>
<td>6.54 ± 2.00</td>
<td>6.80 ± 2.07</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Control subjects (n = 624)

| Age, yr                      | 37.0 ± 11.6 | 38.8 ± 12.8 | 38.2 ± 11.5 | n.s. |
| Cholesterol, mg/L            | 1985 ± 384  | 1811 ± 312  | 1952 ± 330  | <0.01 |
| LDL-cholesterol, mg/L        | 1306 ± 332  | 1121 ± 274  | 1262 ± 304  | <0.001 |
| Triglycerides, mg/L          | 1258 ± 518  | 1321 ± 604  | 1243 ± 726  | n.s. |
| Cholesterol/HDL-cholesterol  | 4.88 ± 1.46  | 4.38 ± 1.09  | 4.59 ± 1.24  | <0.05 |

*p*Comparison between apolipoprotein phenotypes E-4/E-3 and E-3/E-2. n.s., not significant.

Table 3. Relationship between Age at MI and Apo E Phenotypes in CAD+/MI+ Patients

<table>
<thead>
<tr>
<th>Prevalence (%) of patients with apo E phenotypes</th>
<th>E-4/E-3</th>
<th>E-3/E-2</th>
<th>E-3/E-3</th>
<th>Age, yr</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;50</td>
<td>60.0</td>
<td>23.7</td>
<td>37.8</td>
<td>40.0</td>
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<tr>
<td>&gt;50</td>
<td>40.0</td>
<td>76.3</td>
<td>62.2</td>
<td></td>
</tr>
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</table>

Difference between E-3/E-2 and E-3/E-3 not significant; all other comparisons are significant (p < 0.001).

Cholesterol in CAD+/MI+ patients than in controls (Figure 1) cannot be explained by apo E polymorphism alone. Rather, additional genetic factors (e.g., polymorphism of apo B or LDL receptors) or environmental influences affecting the expression of LDL receptors, or both, must account for the observed high concentrations of LDL cholesterol in CAD+/MI+ patients. The influence of apo E polymorphism on the manifestation of CAD is probably a consequence of the significant differences in LDL cholesterol concentrations associated with the various apo E phenotypes.

At present, the precise metabolic effect of the various apo E alleles on LDL cholesterol concentrations is poorly understood. Because expression of hepatic apo B,E receptors exerts major influence on LDL cholesterol concentrations in serum, one may speculate that partial downregulation of the receptors occurs in the presence of apo E-4-containing lipoproteins and partial upregulation in the presence of apo E-2-containing lipoproteins. In this context we note that apo E-2 and apo E-3 both form stable complexes with apo AII (apo E–A-II complex) (27); because of its lack of cysteine, apo E-4 cannot form such complexes (28). This difference in the primary structure of apo E isoforms might cause a relative enrichment of apo E-2 and apo E-3 in HDL (as apo E–A-II complexes) and a relative enrichment of E4 in triglyceride-rich lipoproteins. Given the short turnover time of triglyceride-rich lipoproteins and the high affinity binding of apo E-4 and apo E-3-containing lipoproteins to apo E receptors (chylomicron remnants) and to apo B,E receptors (intermediate-density lipoproteins), the increased influx of cholesterol into the liver via these lipoproteins might lead to a downregulation of the apo B,E receptors. The prolonged half life of circulating HDL (containing apo E–A-II complexes) might lead to partial upregulation of the apo B,E receptors. Though this theory at present lacks experimental proof, we have repeatedly observed in our screening studies of VLDL apolipoproteins a relative enrichment of apo E-4 over apo E-3 and apo E-2.

Current studies at our laboratory are aimed to further substantiate the observed relationship of apo E isoforms, concentrations of LDL-cholesterol in serum, and coronary artery disease.

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References


