Apolipoprotein E Polymorphism in a Healthy Swedish Population: Variation of Allele Frequency with Age and Relation to Serum Lipid Concentrations

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We analyzed blood samples from 407 healthy Swedish individuals, 244 men and 163 women, ages 17 to 86 years, for apolipoprotein (apo) E isoforms and serum triglycerides, low-density lipoprotein (LDL), high-density lipoprotein (HDL), and total cholesterol. Parallel genotyping by means of polymerase chain reaction (PCR)-amplified DNA was performed in 200 subjects. Identical results were obtained by genotyping and phenotyping in 95% of all subjects analyzed. The apo E allele frequencies were 7.8% for ε2, 71.9% for ε3, and 20.3% for ε4. Compared with other Caucasian populations, the present population had a high relative allele frequency of ε4. The ε4 frequency decreased with increasing age and was significantly lower in individuals >60 years of age (14.7%). When controlling for age and sex, there were strong correlations between total serum and LDL cholesterol and the various ε alleles. The ε4 and ε3 alleles correlated positively with serum cholesterol and the ε4 allele correlated positively with LDL cholesterol. In contrast, HDL cholesterol and serum triglycerides did not show any correlation to the allele types. Thus, the results demonstrate a considerable age variation of the ε allele frequency among healthy Swedes and an influence of apo E alleles on serum and LDL cholesterol concentrations.

Indexing Terms: isoelectric focusing • polymerase chain reaction • lipoproteins

Apolipoprotein (apo) E is important in the clearance of remnant lipoproteins because of its recognition by hepatic lipoprotein receptors (I–3). Three common alleles, ε2, ε3, and ε4, at the apo E gene locus code for three protein isoforms, apo E2, E3, and E4, respectively, resulting in six different phenotypes (3–6). Because of charge differences based on amino acid substitutions, the different phenotypes can be separated by isoelectric focusing (3, 4, 6–9). Several less frequently occurring apo E isoforms have been described (10–13). Posttranslational modification of apo E may occur through various degrees of sialylation (5, 14). Structural and (or) charge differences between different apo E isoforms influence the interaction between receptors and lipoproteins, thereby affecting lipoprotein metabolism and plasma lipid concentrations (1–3, 6, 15–18). It is well known that homozygosity for apo E2 is a prerequisite for development of type III hyperlipoproteinemia (19, 20). In addition, the ε4 allele has been associated with high concentrations and the ε2 allele with low concentrations of low-density lipoprotein (LDL) cholesterol (6, 13, 21–23). Variation at the apo E gene locus has been estimated to account for as much as 4–8% of the total variation in serum cholesterol in the normal population (6).

The distribution of the apo E alleles in the general population has been studied in several countries (6, 21). In Caucasians, the relative frequency of ε2, ε3, and ε4 is similar in most populations, in general varying between 0.072 and 0.130 (ε2), 0.720 and 0.786 (ε3), and 0.128 and 0.160 (ε4). In the Finnish population, however, the relative frequency of the ε4 allele is higher, and the ε2 frequency lower (24). This may be relevant to the comparatively high frequency of hypercholesterolemia in the Scandinavian population. Limited information is available from other Nordic countries. In a recent study of Swedish patients with familial hypercholesterolemia we found a high frequency of the ε4 allele (25); this led us to investigate the apo E allele variation and its impact on serum lipid concentrations in the healthy Swedish population.

Subjects and Methods

Subjects

Blood samples were obtained from 407 healthy normal individuals, 244 men and 163 women, ages 17 to 86 years. The participants were mostly medical students or hospital staff members. The elderly participants were recruited from senior citizen groups outside the hospital. Informed consent was obtained from all subjects, and the study was approved by the hospital ethics committee. Blood was drawn in EDTA-containing tubes after the subjects had fasted overnight. The samples were immediately centrifuged at 1500 × g for 20 min at 4 °C, plasma was removed, and phenylmethylsulfonyl fluoride (Sigma Chemical Co., St. Louis, MO) was added to a final concentration of 0.01 mmol/L. Unless immediately analyzed, plasma was frozen at −20 °C. For genotyping, parallel samples of venous blood were collected in EDTA-containing tubes and frozen at −70 °C. Serum was prepared from samples drawn simultaneously and used for lipid determinations as described below.

Materials

The following items were purchased: nitrocellulose membranes BA 85 (Schleicher & Shuell, Dassel, Ger-

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3 Nonstandard abbreviations: apo, apolipoprotein; LDL, low-density lipoprotein; PCR, polymerase chain reaction; and HDL, high-density lipoprotein.

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many); sheep anti-apo E antiserum (Immuno AG, Vienna, Austria); biotinylated anti-sheep IgG and Vectastain ABC kit (Vector Laboratories, Burlingame, CA); Ampholine pH 4.0–6.0 (Pharmacia LKB Biotechnology, Uppsala, Sweden); restriction enzyme CfoI (Boehringer Mannheim, Mannheim, Germany); Taq polymerase (Perkin-Elmer Cetus Instruments, Emeryville, CA); and Fuji New RX film (Fuji Photo Film Co., Minami Ashigara, Japan).

Oligonucleotides were synthesized on a Gene Assembler Plus DNA Synthesizer by using the β-cyanoethyl N,N-diisopropyl phosphoramidite process (Pharmacia LKB). Polymerase chain reaction (PCR) was performed in a Perkin-Elmer Cetus DNA Thermo Cycler.

Analytical Procedures

Serum cholesterol and triglyceride concentrations were determined by standard enzymatic procedures. High-density lipoprotein (HDL) cholesterol concentrations were determined after precipitation of apo B-containing particles with phosphotungstic acid (26). LDL cholesterol concentrations were calculated by using the Friedewald formula (27).

For apo E phenotyping, we used the Western blotting procedure of Menzel and Utermann (28). Briefly, plasma aliquots of 15 μL were delipidated with ethanol/ether (3:1, by vol) and the protein was pelleted by centrifugation. The pellets were washed with ether, dried under a gentle stream of nitrogen, and dissolved in 0.1 mol/L Tris/HCl, pH 10.0, containing 6 mol/L urea and 10 mmol/L dithiothreitol. The samples were then separated by isoelectric focusing in a 5% polyacrylamide gel with a pH gradient of 4–6, for 20 h at 4 °C. The proteins were electroblotted onto a nitrocellulose membrane, incubated with antibodies to apo E, and then incubated with biotinylated secondary antibodies. The apo E protein bands were then stained with a biotinylated peroxidase complex, by using either 9-aminocarbazol or the ECL Western Blotting Detection System (Amersham, Amersham, UK). For easier evaluation of the various bands, untreated and cysteamine-treated (8) samples of an E2/2 homozygote were always included for reference.

We prepared genomic DNA from peripheral blood cells by phenol/chloroform extraction of proteinase K-treated nuclei in microscale. For apo E genotyping, we used the technique described by Hixson and Vernier (29) with some minor modifications. In brief, an amplified fragment of the apo E gene containing the polymorphic sites coding for amino acid residues 112 and 168 was subjected to digestion by the restriction enzyme CfoI. The resulting pattern of fragments, characteristic for the individual genotypes, was evaluated by electrophoresis. For the PCR a preincubation period at 98 °C for 10 min was included. The temperatures for annealing, extension, and denaturation were set to 62 °C (30 s), 72 °C (2 min), and 96 °C (1 min), respectively. Usually, sufficient amounts of PCR product were obtained. After digestion with CfoI, the products were concentrated by ethanol precipitation and subjected to electrophoresis on a 12% nondenaturing polyacrylamide gel. The gel was stained with ethidium bromide and the pattern was evaluated by visual inspection. Initially, genotyping was performed by allele-specific hybridization, but because interpretation difficulties often occurred we adopted the Hixson–Vernier procedure (29). The results from genotyping and phenotyping were evaluated independently by different investigators. All subjects were analyzed for apo E phenotype, and genotype analysis was performed in 200 cases. In 10 cases (5%), results between the two methods differed; in most cases (n = 6), phenotyping resulted in E4/4 and genotyping in E4/3.

Statistical Analysis

Data are presented as means ± SD. The number of a given allele, e.g., e3, in the pair of alleles can be 0, 1, or 2, corresponding to individual relative frequencies of 0%, 50%, or 100%, respectively. A carrier of a specific apo ε allele was a subject carrying the E4, E3, or E2 phenotype in homo- or heterozygous form. For correlation studies, product-moment correlation (Pearson) analyses were performed. The correlation coefficients were tested for significance with a two-tailed Student’s t-test, as were differences of means in subgroups. When testing the distributions of the allele types according to age, the cases were divided into three age groups: <40 years, 40–60 years, and >60 years. The nonparametric Kruakal–Wallis one-way analysis of variance was used for testing significance. For analyzing the dependence of lipoproteins on ε alleles when controlling for age and sex, multiple stepwise regression analyses were performed (30).

Results

The prevalences for the apo E phenotypes are shown in Table 1. The E4-containing phenotypes, primarily E3/4 and E4/4, were more common in the present study than has been reported for most other Caucasian populations except Finns (6). This observation was further reflected in a high frequency of the e4 allele (Table 2). The high e4 allele frequency was mirrored in a lower ε3 allele frequency, whereas the ε2 allele frequency was about the same as in other populations. The highest concentrations of LDL cholesterol were found in the E3/4 and E4/4 groups, but only small differences were noted in total serum cholesterol and LDL cholesterol concentrations between the different apo E phenotypes (Table 1).

To study the variation of apo E allele frequency with age, we divided the participants into three groups, <40 years, 40–60 years, and >60 years. All groups were compared by the Kruskal–Wallis test. As shown in Table 2, the e4 allele frequency was significantly lower (P <0.018, Student’s t-test) in the older age group (>60 years) and, in parallel, a slight increase was observed in the ε3 and ε2 allele frequencies. No significant differences in allele frequency by sex were found in any of the age groups. However, in the youngest age group, men tended to have a lower e4 frequency than women (P <0.055, Student’s t-test).

To evaluate the correlations between the relative fre-
Table 1. Apo E Phenotype and Serum Lipid Concentrations in Normal Individuals

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Percent</th>
<th>Age, years*</th>
<th>Triglycerides</th>
<th>Cholesterol</th>
<th>HDL cholesterol</th>
<th>LDL cholesterol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>407</td>
<td></td>
<td>47 ± 19</td>
<td>1.2 ± 0.6</td>
<td>5.5 ± 1.2</td>
<td>1.5 ± 0.5</td>
<td>3.5 ± 1.1</td>
</tr>
<tr>
<td>E 2/2</td>
<td>4</td>
<td>1</td>
<td>58 ± 13</td>
<td>1.5 ± 0.7</td>
<td>5.7 ± 1.7</td>
<td>1.8 ± 0.6</td>
<td>3.3 ± 1.6</td>
</tr>
<tr>
<td>E 2/3</td>
<td>42</td>
<td>10</td>
<td>48 ± 18</td>
<td>1.2 ± 0.6</td>
<td>5.3 ± 1.2</td>
<td>1.6 ± 0.5</td>
<td>3.2 ± 0.9</td>
</tr>
<tr>
<td>E 2/4</td>
<td>14</td>
<td>3</td>
<td>52 ± 17</td>
<td>1.2 ± 0.7</td>
<td>5.4 ± 1.6</td>
<td>1.5 ± 0.6</td>
<td>3.4 ± 1.2</td>
</tr>
<tr>
<td>E 3/3</td>
<td>239</td>
<td>59</td>
<td>47 ± 20</td>
<td>1.1 ± 0.6</td>
<td>5.5 ± 1.2</td>
<td>1.5 ± 0.4</td>
<td>3.5 ± 1.1</td>
</tr>
<tr>
<td>E 3/4</td>
<td>65</td>
<td>16</td>
<td>48 ± 18</td>
<td>1.1 ± 0.6</td>
<td>5.7 ± 1.3</td>
<td>1.5 ± 0.5</td>
<td>3.6 ± 1.0</td>
</tr>
<tr>
<td>E 4/4</td>
<td>43</td>
<td>11</td>
<td>40 ± 16</td>
<td>1.3 ± 0.6</td>
<td>5.7 ± 1.3</td>
<td>1.5 ± 0.4</td>
<td>3.6 ± 1.2</td>
</tr>
</tbody>
</table>

* Mean ± SD.
To convert mmol/L to mg/dL, for triglycerides multiply by 88.5 and for cholesterol, by 38.7.

Table 2. Variation of Apo E Allele Frequency with Age

<table>
<thead>
<tr>
<th>Age, years</th>
<th>n</th>
<th>47 ± 19</th>
<th>28 ± 6</th>
<th>49 ± 6</th>
<th>71 ± 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>172</td>
<td>116</td>
<td>119</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apo E allele frequency, %</td>
<td>7.8</td>
<td>6.1</td>
<td>8.6</td>
<td>9.7</td>
<td></td>
</tr>
<tr>
<td>E2</td>
<td>71.0</td>
<td>72.1</td>
<td>67.7</td>
<td>75.6</td>
<td></td>
</tr>
<tr>
<td>E3</td>
<td>20.3</td>
<td>21.8</td>
<td>23.7</td>
<td>14.7*</td>
<td></td>
</tr>
</tbody>
</table>

* P < 0.018, two-tailed Student's t-test.

Table 3. Stepwise Multiple Regression Analyses on Lipoproteins with Age, Sex, and α Allele as Predictors

<table>
<thead>
<tr>
<th>Serum cholesterol</th>
<th>B</th>
<th>SEα</th>
<th>t</th>
<th>P &lt;</th>
<th>R² (adj)</th>
<th>F(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.036</td>
<td>0.003</td>
<td>13.4</td>
<td>0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td>0.414</td>
<td>0.103</td>
<td>4.03</td>
<td>0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E4</td>
<td>0.853</td>
<td>0.281</td>
<td>3.04</td>
<td>0.003</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E3</td>
<td>0.524</td>
<td>0.255</td>
<td>2.5</td>
<td>0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Constant</td>
<td>3.11</td>
<td>0.277</td>
<td>11.5</td>
<td>0.001</td>
<td>0.36</td>
<td>58.92 (0.000)</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>0.030</td>
<td>0.002</td>
<td>13.0</td>
<td>0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E4</td>
<td>0.348</td>
<td>0.131</td>
<td>2.65</td>
<td>0.009</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Constant</td>
<td>1.98</td>
<td>0.122</td>
<td>16.1</td>
<td>0.001</td>
<td>0.30</td>
<td>85.6 (0.000)</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td>0.318</td>
<td>0.042</td>
<td>7.60</td>
<td>0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>0.004</td>
<td>0.001</td>
<td>4.06</td>
<td>0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Constant</td>
<td>1.18</td>
<td>0.055</td>
<td>21.6</td>
<td>0.001</td>
<td>0.17</td>
<td>43.7 (0.000)</td>
</tr>
</tbody>
</table>

Allele types are coded 0 if not present in the pair of alleles, 0.5 if one allele is present, and 1 if the specific allele is present in both members of the pair.
B, regression coefficient; SEα, standard error of B; t, Student's t-test; R² (adj), adjusted coefficient of determination; F(P), results of F-test of model and P values of the tests. Constant, the intercept that reflects the basic concentrations when controlling for variation in age, sex, or α allele types.

For the mean, 1 year of age increases the concentration of serum cholesterol by 0.036 units when the other factors are constant. The presence of two E3 alleles in the pair increases the serum cholesterol concentration by 0.524 units. One E3 in the pair increases the serum cholesterol concentration by 0.524/2 units.

Women have a mean serum cholesterol concentration that is 0.414 units higher than in men. The values in the table can be used for prediction of serum concentrations; e.g., a 45-year-old women with E phenotype 4/3 is predicted to have a serum cholesterol concentration of 3.11 + 45 × 0.036 + 0.414 + 0.853/2 + 0.524/2.

Discussion

The distribution of the different apo E phenotypes varies between different ethnic groups (6, 13, 21).
Among Caucasians, the frequencies of apo E alleles and phenotypes are similar, with the high frequency of the e4 allele in the Finnish population as a notable exception (24). Relatively little is known about apo E polymorphism and its effect on serum lipid concentrations in other Nordic countries. The e4 allele frequency in Iceland is only slightly higher than in other Caucasian populations (21); results from Norway and Denmark also indicate a lower e4 allele frequency than in Finland (31, 32).

In the present report, we evaluated the distribution of apo E isoforms in 407 healthy Swedish individuals, ranging in age from 17 to 86 years. The relative allelic frequency in the population studied (0.203/e4; 0.719/e3; 0.078/e2) was higher for the e4 allele than in most other Caucasians, but somewhat lower than that found in Finns (24) and some non-Caucasian populations, notably Sudanese and North American blacks (21, 33). The results indicate that the e4 allele frequency might be generally higher in the Swedish and Finnish populations despite the fact that these populations differ ethnically and represent different language families. Similar allele frequencies for apo E in Swedes were also found in a recent preliminary report (34).

When comparing results from different studies, the use of a similar type of method is clearly preferable. Even if the genotyping procedure offers some advantages (35, 36), most studies reported thus far are based on results from phenotyping, because convenient methods for genotyping have been available for only a limited time. In our hands, however, there was good agreement between the two methods, as has also been reported elsewhere (36). Even if we used only the results from the genotyping procedure, the apo e4 allele frequency was 0.19, clearly demonstrating that the enrichment of this allele in the subjects did not depend on the method used.

The relation among apo E isoforms, serum lipid and lipoprotein concentrations, and coronary artery disease has been investigated extensively. In several studies with healthy individuals, homo- and heterozygotes for the e4 allele had higher amounts of LDL cholesterol, which may indicate an increased risk for coronary artery disease (3, 6, 21). However, in some populations this has not been the case, and different dietary habits have been suggested to explain this apparent discrepancy (37). In the present study, bivariate correlation analyses showed that the e2 and e3 alleles tended to decrease total serum and LDL cholesterol concentrations and the presence of e4 tended to increase these variables. However, these correlations were not statistically significant. The regression models did show significant correlations between the e alleles and serum and LDL cholesterol concentrations when controlling for age and sex (Table 3). This result shows the need to adjust for such factors when investigating the influence of apo E allele variation on serum lipid concentrations.

Of other populations with a high rate of allelic frequency for e4, the Finnish and Sudanese also showed significantly higher LDL cholesterol concentrations in e4-positive individuals (except E3/4 in Sudanese). Interestingly, serum cholesterol concentrations in eastern Finland are higher than in the western part, although the distribution of apo E phenotypes does not differ (38). Obviously, different genetic and environmental factors may influence the relation between serum or LDL cholesterol and apo E phenotypes, and factors other than the apo E phenotype affect serum cholesterol concentrations.

We also found a lower e4 allele frequency in the elderly (Table 2), in agreement with previous studies (39, 40), but we did not find any significant sex differences with regard to the e2 allele frequency as also was observed by others (39). So far, only limited information on apo E allele frequency in the elderly is available (39–42). The frequent findings of increased plasma and LDL cholesterol concentrations among e4 allele carriers suggest that the e4 allele might constitute a risk factor for development of coronary artery disease, as has been suggested (43). Our findings of a lower e4 allele frequency among the elderly would agree with such a contention. The influence of age on serum lipid concentrations (44, 45) and the striking variation of e4 allele frequency with age may blunt the results to some extent, although it seems reasonable to assume that the healthy e4 allele carriers among the elderly might have a tendency towards lower plasma lipid concentrations. However, apo E has a variety of functions, not all of them associated with lipid transport (3). Allelic variation may also be important for some of these other functions; e.g., apo E genotypic effects on arterial lesions were recently found not to be mediated entirely by changes in serum cholesterol concentrations (39).

In conclusion, the apo E isoform pattern in the Swedish population revealed a relative increase in the e4 allele frequency in comparison with most other Caucasian populations. Because the interaction between apo E isoform, serum lipid concentrations, and atherosclerosis is complex and may vary between different populations, more detailed studies are needed to explore the mechanisms and possible pathophysiological importance of apo E isoform variation.

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References


