Aging and Genetic Variation of Plasma Apolipoproteins

Relative Loss of the Apolipoprotein E4 Phenotype in Centenarians

J. Louhija, H.E. Miettinen, K. Kontula, M.J. Tikkanen, T.A. Miettinen, R.S. Tilvis

Abstract We determined the common polymorphism of apolipoprotein E (E2, E3, and E4), apolipoprotein B Xba I polymorphism, and apolipoprotein C-III Sst I polymorphism in almost all Finnish centenarians alive in 1991 (n=179/185). Plasma lipid and lipoprotein levels in different apolipoprotein genotypes were also measured. In comparison with younger Finnish populations studied previously, the frequency of the apolipoprotein E e2 allele was almost twice as high (7.0% versus 4.1%; P<.05) and that of the e4 allele only approximately one third as high (8.4% versus 22.7%; P<.001) in the centenarians. Plasma cholesterol and high-density lipoprotein cholesterol levels tended to be lowest in the group with the e2 allele (4.33 mmol/L and 1.41 mmol/L, respectively), intermediate in those with the e3 allele (4.57 mmol/L and 1.48 mmol/L, respectively), and highest in those with the e4 allele (4.82 mmol/L and 1.60 mmol/L, respectively). The frequencies of the apolipoprotein B X1 and X2 alleles (Xba I restriction site absent or present, respectively) among the centenarians and among the young Finns were not significantly different, whereas the apolipoprotein C-III S2 allele (Sst I restriction site present) occurred more often in the centenarians (frequency, 12.9%) than in the youngest reference population (frequency, 8.8%; P<.05). Centenarians with the apolipoprotein B X2X2 genotype and apolipoprotein E4 phenotype had a higher mean plasma cholesterol level than those with the X1X1 genotype and E2 phenotype (5.24 versus 3.43 mmol/L; P<.05). We conclude that genetic variation of apolipoproteins influences blood lipid levels up to very high ages and that the e4 allele may affect life expectancy adversely. (Arterioscler Thromb. 1994;14:1084-1089.)

Key Words • centenarians • serum apolipoproteins • serum cholesterol • apolipoprotein gene polymorphisms • longevity

Apolipoproteins occupy a central position in lipid metabolism. Apolipoprotein E (apoE) is present in chylomicrons and very-low-density lipoprotein and their lipolytic degradation products, ie, chylomicron remnants and intermediate-density lipoproteins.1,2 It also plays a role in cholesterol absorption and in the receptor-mediated uptake of lipoprotein particles by the liver. Apolipoprotein B (apoB) is essential for the synthesis and secretion of chylomicrons in the intestine and of VLDL in the liver and serves as the ligand allowing the low-density lipoprotein (LDL) receptor to recognize LDL.1-2 Apolipoprotein C-III (ApoC-III) is a major protein constituent of triglyceride-rich lipoproteins and may also affect the activities of lipoprotein and hepatic lipases.1,3

Common genetic polymorphisms of apoE, apoB, and apoC-III have been found to influence serum lipid levels in several populations (for reviews, see References 2 through 6). Three major apoE isoforms (E2, E3, and E4), encoded by three separate alleles (e2, e3, and e4), in different combinations determine six different phenotypes.5-7 Several studies have indicated that the e4 allele is associated with elevated serum total cholesterol, LDL cholesterol, and apoB levels5,6,8-11 and also with increased risks of coronary artery disease and myocardial infarction.12-15 In the Finnish population, in which the incidence of coronary heart disease is high, the frequency of apoE4 is higher and that of apoE2 lower than in most other populations studied.8,11 The apoB Xba I polymorphism is due to a single nucleotide variation in exon 26 of the apoB gene.16 Although not involving a difference in the apoB primary structure, this polymorphism has been suggested to affect the concentration of serum lipoproteins and also the risk of atherosclerotic vascular disease.2,4 An association between the apoB X2 allele (Xba I restriction site present) and elevated serum LDL cholesterol level has been established in healthy Finnish children17 and adults,18 as well as in Finnish patients with heterozygous familial hypercholesterolemia.19 The apoC-III gene lies in close proximity to the apolipoprotein A-I (apoA-I) gene on chromosome 11.20 In many although not all studies, an extra Ssr I restriction site (S2 allele) in the 3' untranslated region of the apoc-III gene has been associated with raised serum triglyceride concentrations and an increased risk of coronary artery disease.2,4 The S2 allele is present in approximately 60% of Finnish adults with severe primary hypertriglyceridemia but in only 16% of normalipemnic control subjects.21

Although severe and relatively rare forms of primary dyslipidemias, such as familial hypercholesterolemia, are known to be associated with shortened life expectancy, much less is known about the relevance of the
more common genetic variation of lipid-regulatory genes to longevity. Previous studies have suggested a lower apoE4 frequency in the elderly.22,23 We hypothesized that any of the three apolipoprotein alleles that had been unequivocally linked to elevated blood lipid levels (see above) in young and adult Finns may affect life expectancy adversely. To test the validity of this hypothesis, we determined the frequencies of these three alleles in the Finnish centenarians and compared them with those in groups of younger Finns studied earlier. The study design also permitted us to test whether the previously established relations between plasma lipid levels and apolipoprotein polymorphisms also hold true for the oldest people in the population.

Methods

Subjects

At the beginning of the Finnish Centenarian study in March 1991, all persons born in 1891 or earlier and alive in December 1990 (n=271) were identified from the Finnish national population registry. By December 1991 one of us (J.L.) visited all centenarians alive at the time of the examination (n=185). Of the centenarians, four refused to participate in the study and two were not willing to give a blood sample. Of the 179, 25% were living at home, 44% in homes for the elderly, and 31% in hospitals. Of the centenarians alive, 70% (6.3/100 000 population) lived in southern Finland, 20% (4.7/100 000 population) in central Finland, and 10% (3.3/100 000 population) in northern Finland.

Every subject was studied in the morning as early as possible. A venous blood sample was drawn after an overnight fast into a tube containing EDTA. A portion of the EDTA-anticoagulated blood was frozen for subsequent preparation of leukocytic DNA24 in a low-speed transportable centrifuge to prepare plasma. Ice-cooled plasma samples were sent to Helsinki within 24 hours for further analyses. This study was approved by the Ethical Committee of the Helsinki University Central Hospital.

Determination of Plasma Lipids, Lipoproteins, and Apolipoproteins

Cholesterol and triglyceride concentrations were determined by enzymatic methods (Boehringer Mannheim) using an AutoAnalyzer II apparatus (Technicon Instruments). High-density lipoprotein (HDL) cholesterol was determined after precipitation of apoB-containing lipoproteins by heparin/manganese chloride, followed by separation of HDL2 and HDL3 by addition of dextran sulfate.25 LDL cholesterol was calculated with the Friedewald formula.26 The concentrations of apoA-I, apoC-III, apoA-II, and apoB were measured with commercial immunoassay kits (Orion Diagnostica).

Determination of ApoE Phenotypes

ApoE phenotyping was chosen instead of apoE genotyping because the former technique was used in studies of the reference populations (see below). We have previously found that these two techniques yield discrepant results in only 2.5% of cases.27 ApoE phenotyping was carried out with a modification28 of the method of Havekes et al29 based on isoelectric focusing of delipidated plasma followed by immunoblotting with anti-apoE antiserum. Before the isoelectric focusing the plasma was treated with cysteamine as described by Weisgraber et al.29

The apoE phenotype distribution and allele frequencies in centenarians were compared with those in 615 adult (age range, 20 to 55 years) and 1577 young (age range, 3 to 18 years) Finns described previously.4,11 In the former study the participants were mainly from the vicinity of Helsinki, the capital of the country, but the latter study included volunteers from both the southwestern and eastern parts of Finland. The mean serum cholesterol, LDL cholesterol, HDL cholesterol, and triglyceride concentrations in the adult reference population were 5.59, 3.52, 1.53, and 1.20 mmol/L, respectively, and the corresponding values in the young reference population were 4.80, 2.96, 1.44, and 0.87 mmol/L, respectively.

DNA Analysis

DNA was digested with the restriction enzymes Xba I or Sst I (Promega), fractionated by gel electrophoresis on 0.6% to 0.8% agarose, and transferred to nitrocellulose filters. Hybridization, washing, and autoradiography were carried out as described previously.18,20 The DNA probes pB23 and pSV2 2.2-kb apoA-I were gifts from Dr J.L. Breslow (New York, NY). The apoB alleles with and without the polymorphic Xba I site are designated as X2 and X1, respectively, and the apoC-III alleles with and without the polymorphic Sst I site as S2 and S1, respectively.

In 38 cases the amount of available DNA did not permit Southern blot analysis of the apoB and apoC-III polymorphisms. In these cases, the variable nucleotides were identified by a solid-phase minisequencing technique according to Syvänen et al.30 The conditions for determination of the apoB Xba I genotypes have been described previously.21 For determination of the apoC-III Sst I genotypes, three oligonucleotide primers were synthesized (nucleotide numbering refers to the sequence given in Reference 32): polymerase chain reaction (PCR) primer 1, nucleotides 313 to 333; PCR primer 2 (biotinylated), nucleotides 445 to 465 (complementary strand); and sequencing primer, nucleotides 349 to 369.

The apoB and apoC-III allele frequencies in centenarians were compared with those in 307 young (age range, 9 to 21 years) and 179 adult (age range, 20 to 66 years) Finns.18,21 The apoC-III Sst I alleles in the adult series were determined during the course of the present study by means of the Southern blot technique. The adult reference subjects were living in Helsinki and its surroundings, whereas the young subjects were from both the western and eastern parts of the country. The mean serum cholesterol, LDL cholesterol, HDL cholesterol, and triglyceride values in this adult reference group were 5.76, 3.76, 1.74, and 1.17 mmol/L, respectively, in the young group they were 4.74, 2.89, 1.47, and 0.84 mmol/L, respectively.

Statistical Analysis

Statistical analyses were carried out using the BMDP software package (BMDP Statistical Software Inc). Plasma lipid and lipoprotein levels in different apolipoprotein genotypes were compared by ANOVA (BMDP 7D). The statistical significances of gene allele and genotype differences were tested by cross tabulation (BMDP 4F). Their confidence intervals were calculated with a computer program based on the method of Gardner and Altman.33

Results

The Finnish centenarians were characterized by relatively low plasma total (4.6 mmol/L) and LDL cholesterol (2.4 mmol/L) levels and high HDL cholesterol (1.5 mmol/L) levels (Table 1). No significant sex differences were found in the mean plasma lipid or apolipoprotein levels. The sex difference in the phospholipid levels, even though statistically significant, was in relative terms similar to those in the HDL and LDL cholesterol levels. For subsequent analysis of plasma lipoproteins in the different genotypes, the data for the two sexes were combined.

The frequency of the apoE e2 allele was significantly higher and that of the e4 allele lower among the centenarians than in the younger Finnish populations.
studied earlier (Table 2). The most remarkable finding of the present study was the 63% reduction in the prevalence of the apoE e4 allele from the oldest Finnish age group (Table 2). There were no significant differences between women and men in the prevalences of the different apoE phenotypes or alleles, loss of the e4 allele from this age group having taken place in both sexes (Table 3).

The apoC-III S2 allele occurred among the centenarians with a relatively high frequency (12.9%) that showed a difference bordering on significance (P=.05) when compared with either of the younger reference cohorts (Table 2). In contrast, there were no significant differences in apoB XbaI allele frequencies between the centenarians and the younger populations (Table 2).

Plasma total and lipoprotein cholesterol concentrations, divided into three groups according to apoE phenotypes, showed a tendency for the concentrations to be highest in the centenarians with at least one e4 allele and lowest in those with at least one e2 allele (Table 4). However, the differences reached statistical significance for HDL cholesterol only (Table 4). In fact, all individuals with apoE3/E4 and apoE4/E4 phenotypes had plasma HDL cholesterol levels higher than 1.0 mmol/L, while their total cholesterol levels ranged from 3.6 to 7.2 mmol/L.

Plasma cholesterol levels tended to increase in the order of the apoB XbaI genotypes: X1X1 < X1X2 < X2X2 (Table 4). Since both the apoE E4 phenotype and the apoB X2 allele seemed to have a cholesterol-elevating effect, the combined influence of the alleles at the two loci was examined. Centenarians with the apoB X2X2 genotype and apoE E4 phenotype had a significantly higher mean plasma cholesterol level (5.24...
The most striking finding of the present investigation was the significantly diminished prevalence of the apoE e4 allele in centenarians: the percentage frequency of this allele (8.4%) was less than half that found in middle-aged (22.7%) or young (19.4%) people. It is noteworthy that in the men aged older than 100 years the apoE e4 allele was almost absent (frequency, 3.6%; Table 3). Although our young and middle-aged control subjects were not fully matched with the centenarians regarding place of residence, it appears unlikely that differences in the geographic origin of the subjects can have influenced our data. First, the youngest reference group was randomly selected from the national population register.\(^{11,17}\) The middle-aged reference cohorts represented a random healthy population with full working ability.\(^{8,18}\) Second, earlier studies have shown that the distribution of the apoE phenotypes is very similar in southern,\(^{9}\) western,\(^{11}\) and eastern\(^{10}\) Finland. Third, the choice of subjects living in the Helsinki region as adult control subjects was based on the assumption that Finns living in the vicinity of the cap it are representative of the whole Finnish population; in fact, previous studies on blood and polymorphic serum protein markers are in harmony with this assumption.\(^{24}\)

No conclusive explanation for the relative absence of the apoE e4 allele from the oldest age group can be provided. Although the following is not true of all populations examined,\(^5,6\) in Finland the apoE phenotype E4 has been associated with an elevated serum LDL cholesterol level\(^8\) and is known to be more common among patients with angiographically confirmed coronary disease than in the general population\(^5\) or in patients with cardiac disease who have normal coronary angiograms.\(^35\) In addition, the apoE e4 allele may promote accelerated atherosclerosis by mechanisms other than that operating via increased serum LDL cholesterol levels.\(^36-38\) and some of these mechanisms may be important for life expectancy. Our data confirm the results of Davignon et al\(^{22}\) and Eggertsen et al,\(^{23}\) who both were able to show a decrease of the e4 allele frequency in elderly people compared with younger age groups. Thus, the frequency of the e4 allele in Canadian octogenarians (mean age, 85 years) was 8.7%, a figure significantly lower than the corresponding frequency (15.2%) in a population with a mean age of 36 years.\(^22\) The frequencies of the e4 allele in elderly (60 to 86 years) and younger (<40 years) Swedish people were 14.7% and 21.8%, respectively.\(^{23}\) Recently the apoE phenotype E4 has also been suggested as a genetic marker for increased risk to develop Alzheimer’s dis-

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**Table 4. Serum Lipid Fractions in Finnish Centenarians in Relation to Their Apolipoprotein Genotypes**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>n*</th>
<th>Cholesterol</th>
<th>LDL-C</th>
<th>HDL-C</th>
<th>HDL-C</th>
<th>Triglycerides</th>
</tr>
</thead>
<tbody>
<tr>
<td>ApoE</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>e2/e2 + e2/e3</td>
<td>24</td>
<td>4.33±1.10</td>
<td>2.14±0.88</td>
<td>1.41±0.36</td>
<td>0.48±0.30†</td>
<td>1.72±0.07</td>
</tr>
<tr>
<td>e1/e3 + e3/e3</td>
<td>125</td>
<td>4.57±0.93</td>
<td>2.33±0.84</td>
<td>1.48±0.41</td>
<td>0.55±0.29†</td>
<td>1.66±0.81</td>
</tr>
<tr>
<td>e3/e4 + e4/e4</td>
<td>30</td>
<td>4.82±0.90</td>
<td>2.57±0.75</td>
<td>1.60±0.41</td>
<td>0.67±0.30†</td>
<td>1.42±0.92</td>
</tr>
<tr>
<td>ApoB</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X1X1</td>
<td>57</td>
<td>4.48±0.86</td>
<td>2.30±0.68</td>
<td>1.46±0.36</td>
<td>0.55±0.23</td>
<td>1.68±0.89</td>
</tr>
<tr>
<td>X1X2</td>
<td>84</td>
<td>4.62±0.99</td>
<td>2.40±0.78</td>
<td>1.48±0.40</td>
<td>0.55±0.31</td>
<td>1.70±0.74</td>
</tr>
<tr>
<td>X2X2</td>
<td>30</td>
<td>4.73±1.04</td>
<td>2.53±0.98</td>
<td>1.58±0.49</td>
<td>0.62±0.35</td>
<td>1.38±0.49</td>
</tr>
<tr>
<td>ApoC-III</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S1S1</td>
<td>130</td>
<td>4.60±0.92</td>
<td>2.40±0.75</td>
<td>1.49±0.42</td>
<td>0.55±0.30</td>
<td>1.64±0.79</td>
</tr>
<tr>
<td>S1S2</td>
<td>38</td>
<td>4.62±1.02</td>
<td>2.35±0.89</td>
<td>1.55±0.36</td>
<td>0.61±0.26</td>
<td>1.59±0.62</td>
</tr>
<tr>
<td>S2S2</td>
<td>3</td>
<td>4.63±1.84</td>
<td>2.62±1.31</td>
<td>1.20±0.37</td>
<td>0.31±0.32</td>
<td>1.78±1.19</td>
</tr>
</tbody>
</table>

LDL-C indicates low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; ApoE, apolipoprotein E; ApoB, apolipoprotein B; and ApoC-III, apolipoprotein C-III. Values are mean±SD, expressed in millimoles per liter.

*DNA samples for apoB and apoC-III genotyping were available from 171 subjects only.

†P<.05, statistical difference among three groups.

mmol/L) than those with the X1X1 genotype and E2 phenotype (3.43 mmol/L; \(P<.05\)) subjects with other genotype/phenotype combinations had intermediate plasma cholesterol levels (Figure).

In contrast, the Sst I polymorphism of the apoC-III gene was not associated with significant variations in plasma cholesterol or triglyceride levels in the centenarians (Table 4). Neither did we find any significant association between the polymorphisms of the apolipoprotein genes examined and plasma phospholipid, apoA-I, apoA-II, or apoB concentrations (data not shown).

**Discussion**

The most striking finding of the present investigation was the significantly diminished prevalence of the apoE e4 allele in centenarians: the percentage frequency of this allele (8.4%) was less than half that found in middle-aged (22.7%) or young (19.4%) people. It is noteworthy that in the men aged older than 100 years the apoE e4 allele was almost absent (frequency, 3.6%; Table 3). Although our young and middle-aged control subjects were not fully matched with the centenarians regarding place of residence, it appears unlikely that
ease.\textsuperscript{39-41} Whether these findings could partly explain the loss of the e4 allele from centenarians remains to be examined.

In contrast, we did not observe any significant differences in the distribution of the apoB XbaI alleles between the centenarians and the younger Finnish populations (Table 2). Previous studies on several populations have suggested that the X1 allele may be a genetic marker for increased risk of atherosclerotic vascular disease.\textsuperscript{42-44} The underlying mechanism is unknown and may involve a linkage disequilibrium between the X1 allele and a putative atherosclerosis-promoting functional change in the apoB gene or its immediate vicinity. However, this linkage may not be present in all populations, since our previous study showed only an insignificantly higher frequency of the X1 allele in Finnish patients with angiographically confirmed coronary constrictions compared with patients with other cardiac diseases but no angiographic evidence of atherosclerosis (61% versus 54%).\textsuperscript{35}

The higher frequency of the apoC-III S2 allele among the centenarians than among the younger Finns (Table 2) was a somewhat unexpected finding. We have previously detected the S2 allele in approximately 60% of Finnish patients with severe primary hypertriglyceridemia but in only 16% (\textit{P}<.001) of healthy normolipidemic subjects.\textsuperscript{21} Furthermore, it occurs in 23% of Finnish patients with coronary disease, among whom those with high serum triglyceride levels seem to carry the S2 allele more often than those with normal triglyceride levels (43% versus 12%; \textit{P}<.05).\textsuperscript{21} We also found a significant association between the S2 allele and high serum LDL cholesterol levels in young Finns aged 9 to 21 years.\textsuperscript{17} Collectively, these earlier data and our present findings suggest that the apoC-III S2 allele may constitute a genetic marker for a severe and relatively rare form of hypertriglyceridemia among Finns, which does not, however, influence life expectancy to any significant extent at the population level.

Previous studies on Finns have shown that both the apoE e4 and the apoB X2 allele are associated with serum total and LDL cholesterol levels that are significantly higher than average in childhood,\textsuperscript{11,17,45} and adulthood.\textsuperscript{8,18} In our centenarians we found a similar although statistically nonsignificant association between the presence of one or other of these alleles and high serum cholesterol levels in centenarians (Table 4). When the alleles at both loci were taken into account, the association between a high serum cholesterol level and apoE e4 plus apoB X2 reached statistical significance (Figure). This result is similar to that observed in a younger sample of the Finnish population.\textsuperscript{45} Our data suggest that the cholesterol-elevating effects of the apoE e4 allele and apoB X2 allele are lifelong.

A further point of interest concerning these centenarians was their relatively high total HDL cholesterol level. According to cross-sectional and follow-up studies, serum total cholesterol and LDL cholesterol decrease in the oldest age group, but a corresponding decline is less evident for HDL cholesterol.\textsuperscript{46,67} The high levels of HDL in all our centenarians support the view that high HDL cholesterol is associated with longevity and that it may exert a protective influence against cardiac disease even in those with apoE phenotypes that are associated with higher than average levels of LDL cholesterol.

In conclusion, we show that, of the three common apolipoprotein polymorphisms previously recognized to be associated with high serum lipid levels and/or a higher than average risk of atherosclerotic cardiovascular disease, apoE emerges as a factor in which genetic variation may affect life expectancy at the population level. Thus, among Finns the apoE phenotype E2/2 or E2/3 implies a fourfold greater likelihood of reaching the age of 100 years than the phenotype E3/4 or E4/4 (odds ratio, 4.0; 95% confidence interval, 1.9 to 8.3). However, because fewer than 0.01% of Finns with the E2 phenotype live longer than 100 years, it may be inadvisable to call the e2 allele a "longevity gene."

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