Apolipoprotein E Polymorphism and Coronary Artery Disease

Increased Prevalence of Apolipoprotein E-4 in Angiographically Verified Coronary Patients

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Several studies have indicated that genetic polymorphism of apolipoprotein (apo) E is related to coronary artery disease (CAD). We therefore determined the apo E phenotype in 91 consecutive Finnish men with angiographically confirmed CAD. The apo E phenotype distribution differed significantly from that observed in the Finnish population (p<0.05). In the patient group, the frequency of the ε4 allele was 0.324, which is 1.4-fold higher than in the normal Finnish population and twice as high as in other Caucasian populations. Serum lipoproteins and postheparin plasma lipase activities did not display any significant variation according to apo E phenotype. These studies confirm and extend, in a population with high ε4 allele frequency, the previous data on the impact of the ε4 allele on the risk of CAD and suggest that the high ε4 allele frequency in the Finnish population may be one factor contributing to Finns' increased susceptibility to CAD.

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Apolipoprotein E (apo E), a normal constituent of plasma chylomicrons, very low density lipoproteins (VLDL), and high density lipoproteins (HDL), binds with high affinity to receptors in liver and extrahepatic cells, thereby mediating the uptake of apo E-containing lipoproteins, especially the cholesterol-enriched remnants of triglyceride-rich lipoproteins. In addition, it is considered that an HDL subfraction containing apo E plays an important role in the reverse transport of cholesterol from peripheral cells to the liver.

The genetic polymorphism of apo E is due to three common alleles, ε2, ε3, and ε4, at a single autosomal gene locus. These alleles determine the six phenotypes E2/2, E3/3, E4/4, E4/2, E4/3, and E3/2. The isoprotein apo E-2, a product of the ε2 allele, is a mutant differing from the most common isoprotein apo E-3 by a single amino acid interchange (Arg158→Cys158). This mutation leads to a protein that has a much lower affinity for the cellular apolipoprotein receptor. The other mutant, apo E-4, differs from the parent apo E-3 by a single Cys112→Arg112 interchange. Low density lipoprotein (LDL) metabolism is associated with the apo E polymorphism, and as much as 16% of the genetic variance in LDL concentration can be accounted for by allelic differences at the apo E gene locus. Thus, subjects with the phenotype E3/2 have, on the average, 20% lower, and E4/3 subjects have 10% higher levels of LDL cholesterol than do subjects with phenotype E3/3. Since LDL metabolism is closely associated with atherogenesis, it is plausible to presume that the apo E genotype may convey a susceptibility to atherosclerosis.

The apo E gene frequency has been determined in survivors of myocardial infarctions and in subjects with angiographically verified coronary artery disease (CAD). These studies indicated that the ε4 allele is associated with early development of coronary heart disease (CHD), whereas the ε2 allele is less common in coronary patients. In a recent study, the Finns were shown to have a markedly higher ε4 allele frequency than other Caucasian populations (0.227 vs. 0.11 to 0.175), whereas the ε2 allele frequency was low (0.041 vs. 0.08 to 0.17). It was suggested that this might be one of several factors responsible for the high incidence of CHD in Finland. To evaluate the significance of the apo E polymorphism as a risk factor for CAD, we determined apo E phenotypes in 91 consecutive male patients who underwent coronary angiography before coronary bypass surgery.

Methods

Subjects

Patients were recruited for the study between 1985 and 1986 from the First Department of Medicine, University of Helsinki. All patients younger than 60 years of age who were to have coronary angiography because of long-standing angina were contacted at their admittance; the purpose of the study was explained, and informed consent was obtained. The study protocol was approved by the Ethical Committee of Meilahti Hospital. The study...
Table 1. Presence of Risk Factors for Coronary Artery Disease in Subjects with Different Apo E Phenotypes

| Risk factor          | Apo E phenotype |
|----------------------|-----------------|
|                      | 4/4 (n=8)       | 4/3 (n=41) | 3/3 (n=34) | 4/2 (n=2) | 3/2 (n=6) |
| Smoking*             | 50 (4)          | 58 (24)   | 62 (21)    | 100 (2)   | 67 (4)    |
| Hypertension*        | 50 (4)          | 44 (18)   | 65 (22)    | 50 (1)    | 50 (3)    |
| Hypercholesterolemia*| 25 (2)          | 7 (3)     | 15 (5)     | 0 (0)     | 17 (1)    |
| Diabetes             | 0 (0)           | 5 (2)     | 0 (0)      | 0 (0)     | 0 (0)     |

The values are the percentage of numbers in the group with each phenotype. The numbers in parentheses indicate the number of affected individuals.

*The observed distribution of this risk factor among the different apo E phenotype subjects did not differ significantly (p>0.05) from the expected distribution by the \( \chi^2 \) test.

Apo = apoprotein.

Table 2. Use of Drugs among Subjects with Different Apo E Phenotypes

| Apo E phenotype |
|-----------------|
|                  |
| 4/4 (n=8)       |
| 4/3 (n=41)      |
| 3/3 (n=34)      |
| 4/2 (n=2)       |
| 3/2 (n=6)       |
| Beta-blockers*  | 100 (8)         |
|                | 80 (33)         |
|                | 82 (23)         |
|                | 100 (2)         |
|                | 50 (3)          |
| Diuretics*      | 38 (3)          |
|                | 29 (12)         |
|                | 32 (11)         |
|                | 0 (0)           |
|                | 33 (2)          |
| Ca** antagonists* | 50 (4)         |
|                | 66 (27)         |
|                | 50 (17)         |
|                | 100 (2)         |
|                | 67 (4)          |
| Prazosin*       | 13 (1)          |
|                | 0 (0)           |
|                | 3 (1)           |
|                | 0 (0)           |
|                | 0 (0)           |
| Clofibrate*     | 0 (0)           |
|                | 7 (3)           |
|                | 0 (0)           |
|                | 0 (0)           |
|                | 17 (1)          |

Values are the percentage of numbers in the group with each phenotype. The values in parentheses are the numbers of subjects on the drug.

*The observed distribution of use of this drug did not differ from the expected (p>0.05 by the \( \chi^2 \) test).

The \( \chi^2 \) test was not performed because of the small number of subjects taking this drug.

Apo = apoprotein.

group included 91 men with positive angiography findings (see below). The mean age of the patients was 52 years (range, 37 to 60 years), and all had a well-documented history of coronary symptoms for at least 2 years. The presence of the common CAD risk factors in the different apo E phenotypes is shown in Table 1. The use of cardiovascular drugs was common but similar among our patients belonging to different apolipoprotein E phenotypes (Table 2). Blood samples for lipoprotein and apoprotein analysis were taken at 8 A.M. after an overnight fast. This was followed by an intravenous injection of 100 IU/kg of heparin for the collection of postheparin plasma into precooled tubes 5 and 15 minutes later.

**Coronary Angiography**

CAD was confirmed by selective arteriography. The standard Judkins technique for selective coronary angiography in multiple views was performed. A transmural narrowing of 50% or more was defined as significant. A total of 51 subjects had triple-vessel disease, 30 had double-vessel disease, and 10 had single-vessel disease. In the single-vessel group, all patients had severe narrowing (≥75%) of the left anterior descending vessel. All patients underwent bypass surgery.

**Lipid and Lipoprotein Analyses**

Serum total and lipoprotein cholesterol and triglyceride concentrations were determined by enzymatic methods with commercially available kits (Boehringer GmbH, FRG, No. 236691 and No. 297771, respectively) in a Kone Olii-C analyzer (Kone Limited, Espoo, Finland). Apo A-I and A-II were determined by immunoturbidimetry by using monospecific gamma globulins (Boehringer GmbH, FRG, No. 726478 and No. 726486). Apo B concentration was determined by radial immunodiffusion on commercially available plates (Behringwerk GmbH, FRG). The separation of lipoprotein fractions was carried out by sequential ultracentrifugation with a Kontro TF T 45.8 rotor operated at 10,000 rpm and 4°C in a Beckman L7-70 ultracentrifuge. *Centrifugation times of 16 hours, 20 hours, and 72 hours at densities of 1.006 g/ml, 1.063 g/ml, and 1.21 g/ml were used for the separation of VLDL, LDL, and HDL, respectively.

**Table 3. Prevalence of Apolipoprotein E Phenotypes in Patients with Positive Coronary Angiograms**

| Apo E phenotype | CAD patients | Finnish population |
|-----------------|--------------|-------------------|
| E4/4            | 8            | 5                 |
| E4/3            | 41           | 30                |
| E4/2            | 2            | 2                 |
| E3/3            | 34           | 49                |
| E3/2            | 8            | 5                 |
| E2/2            | 0            | 0                 |

*Expected phenotype distribution obtained using the \( \chi^2 \) test frequencies in the Finnish population (\( p<0.05 \)).

CAD = coronary artery disease, Apo = apoprotein.
Table 4. e4 Allele Frequencies in Normal Finns and in Patients with Coronary Heart Disease

| Population            | e4         | e3         | e2         |
|-----------------------|------------|------------|------------|
| Normal Finns          | 0.227±0.013| 0.733±0.017| 0.041±0.015|
| Finns with CAD        | 0.324±0.037*| 0.632±0.044*| 0.044±0.039*|

Values include ±SD. *p<0.001, NS=not significantly different from the corresponding allele frequency in normal Finns by t-test. The data on normal Finns is from reference 16.

Table 5. Plasma Lipoproteins, Apoproteins A and B, and Postheparin Plasma Lipoprotein Lipase and Hepatic Lipase Activities according to Apolipoprotein E Phenotype in Men with Coronary Heart Disease

| Phenotype | E4/4 (n=8) | E4/3 (n=41) | E3/3 (n=34) | E4/2 (n=2) | E3/2 (n=8) |
|-----------|------------|------------|------------|------------|------------|
| Triglyceride (mmol/l) | 2.07 (0.19) | 2.03 (0.17) | 2.27 (0.20) | 2.27 (0.48) | 2.33 (0.48) |
| Cholesterol (mmol/l)   | 6.58 (0.33) | 6.05 (0.16) | 6.64 (0.18) | 6.32 (0.37) | 5.72 (0.59) |
| VLDL cholesterol      | 0.60 (0.09) | 0.61 (0.05) | 0.69 (0.05) | 0.58 (0.13) | 0.56 (0.10) |
| LDL cholesterol       | 4.80 (0.64) | 4.29 (0.14) | 4.62 (0.18) | 4.38 (0.29) | 3.87 (0.59) |
| HDL cholesterol       | 1.20 (0.12) | 1.16 (0.04) | 1.14 (0.04) | 1.36 (0.05) | 1.14 (0.18) |
| Apo A-I (g/l)         | 0.83 (0.06) | 0.94 (0.03) | 0.97 (0.03) | 1.00 (0.11) | 0.92 (0.07) |
| Apo A-II (g/l)        | 0.26 (0.01) | 0.25 (0.01) | 0.24 (0.01) | 0.27 (0.05) | 0.22 (0.02) |
| Apo B (g/l)           | 1.18 (0.13) | 1.16 (0.05) | 1.15 (0.07) | 1.12 (0.07) | 1.02 (0.07) |
| LPL (umol/h/ml)       | 17.5 (1.5)  | 22.6 (1.4)  | 20.9 (1.0)  | 19.0 (0.2)  | 16.2 (2.4)  |
| HL (umol/h/ml)        | 33.3 (4.2)  | 29.0 (2.1)  | 32.6 (2.6)  | 23.4 (1.2)  | 32.8 (2.8)  |

The values are means ±SEM. The differences for all parameters between the different apo E phenotypes were tested by one-way analysis of variance and were nonsignificant (p>0.05) in all tests. VLDL=very low density lipoprotein, LDL=low density lipoprotein, HDL=high density lipoprotein, apo=apoprotein, LPL=lipoprotein lipase, HL=hepatic lipase.

Appliably, the lipoprotein lipase (LPL) and hepatic lipase (HL) activities of postheparin plasma were determined by an immunochemical assay.20

Applioprotein E Phenotyping

This was done by isoelectric focusing as described.21

Statistical Methods

The standard deviations of the gene frequencies were calculated as described earlier.19 The differences between serum lipoproteins, apolipoproteins, and postheparin plasma lipase activities of various apo E phenotype subjects were tested by one-way analysis of variance. A nonpaired t test was used to analyze the differences between the allele frequencies of CAD patients and normal Finns. The deviation of the observed phenotype distribution from the expected was tested using the χ² test.

Results

Of the six common apo E phenotypes, five were observed in the CAD patients; only the phenotype E2/2 was not represented (Table 1). In this respect, our CAD material did not differ from that in the average Finnish population. However, the observed phenotype frequencies were significantly different from the expected distribution as judged from the χ² test of the goodness-of-fit of CAD data to the expected frequencies based on the Finnish allele frequencies (χ²=10.6, p<0.05, 5×2 comparison) (Table 3). This was mainly due to an increase in the number of CAD patients with the phenotypes E4/4 and E4/3 and a corresponding decrease of the E3/3 phenotypes as compared with the distribution of the E phenotypes in the Finnish population. This enrichment of apo E-4 phenotypes was independent of age and severity of coronary heart disease (i.e., number of narrowed vessels) as judged from the e4 allele frequencies of 0.337 in
triglycerides and the postheparin plasma lipases, LPL and HL, were similar in the different apo E phenotype groups (Table 5).

Discussion

Our observation of a significantly increased frequency of the $e_4$ allele in patients with angiographically verified CAD is in accordance with some previous reports. However, Utermann et al. were not able to confirm this finding. One reason for the unambiguous enrichment of the $e_4$ allele among our CAD patients is probably that clinical criteria required before a patient was subjected to coronary angiography were strict. All patients had had a well-documented history of coronary symptoms for at least 2 years. Another aspect is the young age of the patients in the present study: the mean age was only 52 years. Lenzen et al. observed significant differences in the prevalence of apo E phenotypes in patients with early and late onset of infarction. They noted that 60% of E3/E4 patients had suffered infarction before the age of 60 and only 40% later, while in E3/E2 patients this age dependency was reversed. In this respect, our increased prevalence of $e_4$ allele is in good accordance with earlier studies in young CAD patients. Apolipoprotein E2-containing phenotypes are more rare in Finns than in other Caucasians. The $e_2$ allele frequency was also low in the CAD patients and did not differ from that in the general population. Thus, the impact of $e_2$ allele on the CAD risk seems to be of minor importance in Finns, unlike in other populations.

Several recent studies have demonstrated associations between the $e_4$ allele and increased LDL cholesterol levels and the $e_2$ allele and low LDL cholesterol in normal subjects; a similar association has been found in patients with myocardial infarction. An analogous trend was observed in the current study, although the range of serum LDL cholesterol was relatively narrow, but this association was not statistically significant. Recent calculations show that the variation of serum cholesterol according to the apo E phenotype covers only 2.5% of the variation in the CAD risk. Accordingly, the apo E phenotypes probably can influence the CAD risk through mechanisms not related to changes in fasting plasma lipoproteins (see below). Notably, only one subject with E4/E3 phenoype had hyper-beta-apoproteinemia, thus ruling out the contribution of this disorder to the present results. However, in the different apo E phenotypes, the genetic variation of apo B was more consistent than that of LDL cholesterol (Figure 1). This applies especially to the E4/E3 phenotypes where the mean LDL cholesterol was lower than expected, probably since patients in this group were on clofibrate treatment. In fact, the survivors of myocardial infarction in Finns have, at present, LDL cholesterol values quite similar to those of matched control subjects, whereas their LDL protein content is elevated. Thus, LDL protein appears to be a better discriminator than LDL cholesterol for CAD in the Finnish population.

The VLDL and HDL lipoproteins and the postheparin plasma LPL and HL activities were similar among the...
different phenotypes in the CAD patients. Also, previous studies have shown that serum HDL cholesterol and triglyceride concentrations do not vary significantly according to apo E phenotype.15,16 This suggests that the apo E phenotype dependent variation in LDL concentration probably is not due to alterations in the VLDL-intermediate density lipoprotein-IDL cascade but, rather, is caused by altered catabolism of LDL. This lipoprotein class is catabolized through receptor-mediated uptake in liver and extrahepatic tissues. These apo B/E receptors also bind apo E,28 and it is conceivable that the different binding affinities for the genetic isoforms of apo E to these receptors can up- or down-regulate them and, consequently, changes in LDL metabolism15,26,29 result. Moreover, recent studies have suggested that even the absorption of dietary cholesterol20 and the clearance of dietary lipids21 are associated with the apo E phenotype. This variation in lipid metabolism according to apo E phenotype does not need to be reflected in changes of serum lipids and lipoproteins determined in fasting serum samples. Overall, it is tempting to suggest that these variations of lipoprotein metabolism according to the apo E phenotype probably influence the atherosclerotic process and, due to the high ε4 allele frequency, might also contribute to the increased prevalence of CAD in Finland.

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