Apolipoprotein E Polymorphism and Heterozygous Familial Hypercholesterolemia

Sex-Specific Effects

Jean Ferrières, Charles F. Sing, Madeleine Roy, Jean Davignon, Suzanne Lussier-Cacan

Abstract

The impact of apolipoprotein (apo) E polymorphism on interindividual variation in plasma lipid, lipoprotein, and apolipoprotein levels was studied in a sample of familial hypercholesterolemic (FH) patients (147 women, 116 men) with the same mutation, a >10-kilobase deletion of the low-density lipoprotein (LDL) receptor gene. Each trait was adjusted for concomitants (age, age squared, height, weight, weight squared) for each sex separately before the apoE genotypic effects were estimated. The relative contribution of concomitants to sample variability was found to be very different in women and in men. Allelic variation in the apoE gene was shown to explain a statistically significant portion of the variability in adjusted lipid traits. Moreover, the contribution of apoE polymorphism was different between sexes. In women, there was significant variability (P < .01) among apoE genotypes for total cholesterol, LDL cholesterol, and total and LDL apoB. In men, significant variability (P < .01) was observed among apoE genotypes in very-low-density lipoprotein (VLDL) cholesterol and triglyceride levels. Women with the e3/2 genotype had significantly lower means for total cholesterol, LDL cholesterol, and LDL apoB than women with the e3/3 genotype (P < .05). In men, the mean VLDL cholesterol was significantly higher for the e2/2 genotype and was significantly lower for the e4/2 genotype than the mean for the e3/3 genotype (P < .05). Overall, the greatest influence was associated with the e2 allele, and the LDL cholesterol-lowering effect of this allele was present only in FH women. No statistically significant apoE effect was shown on lipoprotein(a) levels in either sex. Less than 20% of the variability for any of the lipid traits among FH patients with the >10-kb deletion is explained by the predictors considered in this study. Most of the remaining variability in lipid traits must be explained by other genetic or environmental factors. In conclusion, this study documents large effects of the e2 allele and the sex-specific apoE genotype influence in a large sample of FH patients with the same LDL receptor mutation. (Arterioscler Thromb. 1994;14:1553-1560.)

Key Words • epidemiology • apolipoprotein E polymorphism • apolipoprotein E alleles • familial hypercholesterolemia • lipoprotein(a)

Coronary artery disease (CAD) is a complex disease explained by multiple interactions among the effects of many genetic and environmental factors. One of the intermediate traits between causes and the development of disease, plasma cholesterol, has long been recognized as a risk factor for CAD. Familial hypercholesterolemia (FH) is an autosomal codominant disease characterized at the molecular level by one of a number of mutations in the low-density lipoprotein (LDL) receptor gene and high levels of plasma LDL cholesterol (LDL-C). The average age of onset of symptomatic CAD in heterozygous FH is reported to be 43 years in men and 53 years in women. In populations in which a founder effect has occurred, such as in French Canadians, shared mutations may be found in a large proportion of FH patients. Indeed, one of these mutations, a >10-kilobase deletion at the 5′ end of the LDL receptor gene involving the promoter and exon 1, has been reported in 60% of French-Canadian FH patients. Despite the inherited nature of the disease, FH shows great variability in phenotypic expression. Even in a sample of heterozygous FH patients with the >10-kb deletion (10kb-FH), the range of interindividual variation in lipid traits is as great as in healthy control subjects. Such interindividual phenotypic variation in LDL-C levels in these heterozygous FH patients could be explained by variation in unmeasured genetic and environmental factors.

The apolipoprotein (apo) E gene locus on chromosome 19 is polymorphic, with three common protein isoforms, E2, E3 and E4, which are encoded by three different alleles, e2, e3, and e4. The E2 and E4 variants differ from the more common E3 isoform by a single amino acid substitution. Whereas apoE3 has cysteine at position 112 and arginine at position 158, apoE4 has arginine and apoE2 cysteine at both sites. These substitutions affect ligand binding of triglyceriderich lipoproteins to the chylomicron remnant and LDL receptors. Population studies have shown that subjects with the e2 allele usually have lower LDL-C levels and those with e4 higher LDL-C levels compared with subjects with the allele e3. ApoE polymorphism is one of the major factors affecting the variability of plasma lipid levels, accounting for 4% to 8% of the total variance in LDL-C levels observed in populations, and it is considered to be an important factor in determining the development of atherosclerosis in a given environment. In FH patients with heterogeneous LDL receptor genotypes, the response to therapy by probucol or lovastatin is influenced by apoE polymorphism. In a sample of healthy Dutch subjects, it
has been shown that variation in apoE polymorphism is associated with variation in plasma levels of lipoprotein(a) [Lp(a)]. This effect parallels the association of apoE polymorphism with LDL-C levels. However, other studies did not detect a significant association between apoE polymorphism and Lp(a) levels.

The availability of a large number of FH patients with the same LDL receptor defect allows the evaluation of factors modulating phenotypic expression of the disease. The objective of this study was to estimate the impact of apoE polymorphism on plasma lipid, lipoprotein, and apolipoprotein traits in a sample of FH patients with the same LDL receptor gene mutation. We found that apoE polymorphism influenced interindividual variation in certain traits and that these effects were sex specific.

Methods

Subjects

Subjects with heterozygous FH were selected from untreated patients referred to the lipid clinic of the Clinical Research Institute of Montreal. We studied 263 FH adult patients (aged >18 years) within 167 families; they were all heterozygous for the >10-kb deletion of the LDL receptor gene, confirmed by Southern blot analysis. All of these 10kb-FH patients had elevated LDL-C levels (LDL-C >4.2 mmol/L). They all had either tendon xanthomas or a family history of tendon xanthomas in a first-degree relative. Analyses were conducted both on the total sample and on unrelated 10kb-FH individuals.

Laboratory Methods

After a 12-hour fast, blood samples for lipoprotein, and apolipoprotein determinations were obtained in tubes containing 1.5 mg/mL Na2 EDTA and were centrifuged within 2 hours. Plasma lipoproteins were separated under standard conditions by a combination of ultracentrifugation at d=1.006 g/mL to isolate very-low-density lipoproteins (VLDL) and heparin-manganese precipitation of apoB-containing lipoproteins in the d=1.006 g/mL infranatant to determine LDL and high-density lipoprotein (HDL) concentrations according to the protocol of the Lipid Research Clinics. Plasma and lipoprotein cholesterol and triglycerides (TG) were measured enzymatically by means of an automated analyzer (Roche Cobas Mira S, F. Hoffmann-La Roche). Total and LDL apoB concentrations were measured by electroimmunoassay with a commercial serum (Behringwerke) and a frozen serum pool as secondary standards. ApoE phenotypes were determined after isoelectric focusing of VLDL apolipoproteins. We observed six apoE phenotypes, from which six apoE genotypes were inferred. Lp(a) was measured in plasma by means of a commercially available enzyme-linked immunosorbent assay (Macra, Terumo).

Statistical Analysis

Epidemiological studies have shown that women and men are expected to have different frequency distributions for plasma lipids, lipoproteins, apolipoproteins, and comorbidities (age, height, weight). Analyses were therefore performed separately for women and men. Evidence for statistically significant quantitative trait differences between women and men was evaluated by Student's t test. Allele frequencies were estimated by the gene counting method. Differences in apoE genotype frequencies between 10kb-FH women and men were evaluated for statistical significance by the x2 test. Multiple regression was used to estimate the linear effects of concomitants and apoE genotypes for each sex separately. The percentages of the total sum of squares attributable to concomitants and to apoE genotype differences after adjustment for the effects of concomitants were estimated by the same regression analysis. To explain as much of the variability attributable to concomitants as possible, different regression equations were evaluated. We found that regression models including statistically significant contributions of age, age squared, height, weight, and weight squared explained the greatest amount of variation in the lipid, lipoprotein, and apolipoprotein levels of women and men. Therefore, a model including all these concomitants was used for each trait in both sexes as the most complete regression model. Sex-specific means were added to the residuals from the sex-specific regression analyses (data adjusted for linear effects of concomitants) before carrying out Scheffe’s multiple-comparison procedures to investigate differences among genotype means. We present the comparison of the adjusted genotype means with the e/3 mean, representing the wild genotype. Because the distributions of Lp(a), TG, and VLDL cholesterol (VLDL-C) values were highly skewed, a natural logarithmic transformation was performed. However, the inferences obtained from the analysis of untransformed and transformed values were not different. Consequently, only the analyses of untransformed data are presented.

To summarize the sex-specific impact of variation in the apoE gene on lipid traits in 10kb-FH patients, we computed multivariate measures of trait mean levels and intragenotypic trait variances for the three common genotypes according to the procedure presented by Reilly et al. To remove scale differences among traits, values for each age-, height-, and weight-adjusted trait were standardized to approximate a normal frequency distribution with a mean of 0 and a standard deviation of 1. The euclidian distance (||/i||) from the origin of the sex-specific hyperspace provides a composite measure of the shift in the adjusted mean levels of total cholesterol, VLDL-C, LDL-C, HDL cholesterol (HDL-C), TG, total apoB, LDL apoB, and Lp(a)) associated with each genotype. The sum of the intragenotypic variances (the total variance) provides a measure of the total interindividual variation associated with each genotype. Finally, to assess the impact of each of the three apoE alleles on age, height-, and weight-adjusted lipid trait levels we calculated the average excess statistic. The average excess computation is different from that of the average effect in that observed frequencies within genotype classes are used rather than expected frequencies, under the hypothesis of Hardy-Weinberg equilibrium. We compared the average excesses in 10kb-FH patients with those observed in a sample of individuals selected for health within the same population. This sample consisted of 374 men and 201 women.

The statistical analysis system, developed by the SAS Institute, was used. Unless otherwise designated, the level of statistical significance was set at .05.

Results

Description of the Sample

Descriptive statistics for the concomitants and dependent traits for women and men are given in Table 1. The mean age of the 147 women was significantly greater than the mean age of the 116 men. Mean height and weight were significantly higher in men than in women. Despite the differences in age and measures of body size, women and men had similar mean levels of total cholesterol, LDL-C, TG, total apoB, LDL apoB, and Lp(a). Women had significantly higher levels of HDL-C, whereas men had significantly higher mean levels of VLDL-C. Except for VLDL-C, greater interindividual variability in lipid traits was observed in women than in men.

Effects of Concomitants on Trait Variability

The percentages of the total sums of squares associated with independent concomitants in selected regression models were found to be different for women and
TABLE 1. Description of Independent Concomitants and Dependent Measures of Plasma Levels of Lipids, Lipoproteins, and Apolipoproteins in a Sample of Familial Hypercholesterolemic Patients With the >10-kb Deletion

<table>
<thead>
<tr>
<th>Trait</th>
<th>Mean±SD</th>
<th>Range</th>
<th>Mean±SD</th>
<th>Range</th>
<th>t Test* Means</th>
<th>F Test Variances</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Concomitants</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>43.6±14.0</td>
<td>19-74</td>
<td>40.3±11.4</td>
<td>18-66</td>
<td>.038</td>
<td>.021</td>
</tr>
<tr>
<td>Height, cm</td>
<td>158.6±5.7</td>
<td>148-175.5</td>
<td>171.6±6.4</td>
<td>143-187</td>
<td>.001</td>
<td>.145</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>60.8±11.7</td>
<td>38.7-106.5</td>
<td>74.6±12.5</td>
<td>40.8-107.2</td>
<td>.001</td>
<td>.447</td>
</tr>
<tr>
<td><strong>Dependent traits</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TC, mmol/L</td>
<td>9.08±1.75</td>
<td>5.61-13.96</td>
<td>9.14±1.45</td>
<td>6.45-15.68</td>
<td>.759</td>
<td>.034</td>
</tr>
<tr>
<td>VLDL-C, mmol/L</td>
<td>0.84±0.56</td>
<td>0.15-3.57</td>
<td>1.12±0.96</td>
<td>0.14-7.01</td>
<td>.005</td>
<td>.001</td>
</tr>
<tr>
<td>LDL-C, mmol/L</td>
<td>7.20±1.59</td>
<td>4.42-12.41</td>
<td>7.19±1.27</td>
<td>4.76-11.58</td>
<td>.964</td>
<td>.012</td>
</tr>
<tr>
<td>HDL-C, mmol/L</td>
<td>1.04±0.29</td>
<td>0.29-2.2</td>
<td>0.83±0.21</td>
<td>0.49-1.58</td>
<td>.001</td>
<td>.001</td>
</tr>
<tr>
<td>TG, mmol/L</td>
<td>1.50±1.06</td>
<td>0.39-7.45</td>
<td>1.63±0.84</td>
<td>0.56-5.25</td>
<td>.252</td>
<td>.009</td>
</tr>
<tr>
<td>Total apoB, mg/dL</td>
<td>224.0±54.9</td>
<td>119-439</td>
<td>234.7±38.6</td>
<td>146-349</td>
<td>.077</td>
<td>.001</td>
</tr>
<tr>
<td>LDL apoB, mg/dL</td>
<td>197.9±42.2</td>
<td>112-347</td>
<td>206.5±37.8</td>
<td>75-309</td>
<td>.099</td>
<td>.236</td>
</tr>
<tr>
<td>Lp(a), mg/dL</td>
<td>25.8±30.2</td>
<td>1-164</td>
<td>22.8±23.5</td>
<td>1-97</td>
<td>.356</td>
<td>.005</td>
</tr>
</tbody>
</table>

TC indicates total cholesterol; VLDL-C, very-low-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides; apo, apolipoprotein; and Lp(a), lipoprotein(a).

Effects of ApoE on Trait Variability

The percentages of trait variability attributable to concomitants and the apoE genotypes are given in Table 2. We found that the combined influence of variation in concomitants and the apoE genotype was similar between sexes for HDL-C (13% and 11.2% for women and men, respectively), TG (17.9% and 18.6%, respectively), and total apoB (16% and 15.5%, respectively). However, the contribution of apoE genotypic variation in women was different from that in men for certain traits. The most notable influence (P<.01) of apoE genotypes on variability in women was for total cholesterol, LDL-C, and total and LDL apoB and in

TABLE 2. Percentage of Sample Variability Associated With Concomitants and Apolipoprotein E Polymorphism in a Sample of Familial Hypercholesterolemic Patients With the >10-kb Deletion

<table>
<thead>
<tr>
<th>Trait</th>
<th>Women (n=147)</th>
<th>Men (n=116)</th>
<th>Comparison Between Sexes (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>c</td>
<td>Apo E/c</td>
<td>Total</td>
<td>Mean±SD</td>
</tr>
<tr>
<td>TC</td>
<td>3.9</td>
<td>11.0†</td>
<td>14.9†</td>
</tr>
<tr>
<td>VLDL-C</td>
<td>12.6‡</td>
<td>5.8</td>
<td>18.2‡</td>
</tr>
<tr>
<td>LDL-C</td>
<td>2.6</td>
<td>14.5§</td>
<td>17.1‡</td>
</tr>
<tr>
<td>HDL-C</td>
<td>10.4‡</td>
<td>2.6</td>
<td>13.0†</td>
</tr>
<tr>
<td>TG</td>
<td>14.4§</td>
<td>3.5</td>
<td>17.9‡</td>
</tr>
<tr>
<td>Total apoB</td>
<td>4.7</td>
<td>11.3‡</td>
<td>16.0‡</td>
</tr>
<tr>
<td>LDL apoB</td>
<td>2.8</td>
<td>13.6§</td>
<td>16.4‡</td>
</tr>
<tr>
<td>Lp(a)</td>
<td>2.8</td>
<td>2.4</td>
<td>5.2</td>
</tr>
</tbody>
</table>

c indicates percentage sum of squares attributable to regression of a trait on concomitants: age, age2, height, weight, weight2; Apo E/c, percentage sum of squares attributable to apolipoprotein (apo) E genotype given prior adjustment for concomitants; Total, percentage sum of squares attributable to concomitants and apoE genotype; TC, total cholesterol; VLDL-C, very-low-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides; and Lp(a), lipoprotein(a).

*p=.056; †P<.05; ‡P<.01; §P<.001.
men, for VLDL-C and TG. The influence of the apoE polymorphism in LDL-C variation in this 10kb-FH sample was large in women and negligible in men. In no case did concomitants and apoE (adjusted for concomitants) both make a significant contribution to interindividual variability.

Genotype and Allele Frequencies

The genotype and allele frequencies in our sample are summarized in Table 3. The most common genotype was e3/3 in women and men, and the rarest were e4/4 in women and e4/2 and e2/2 in men. The relative frequencies of genotypes were not significantly different between sexes. Estimates of the relative frequencies of genotypes for the total group were 0.093 for e2, 0.753 for e3, and 0.154 for e4. These relative apoE allele frequencies in 10kb-FH did not differ significantly from those observed in the healthy sample within the same geographic area.40

Effects of ApoE Genotypes and Alleles on Mean Trait Levels

Adjusted trait means for the six apoE genotypes for women and men are displayed in Table 4. Two patterns of differences among adjusted trait means for apoE genotypes were observed. In women, significant overall differences in total cholesterol, LDL-C, and total and LDL apoB were observed, as already established from the contribution of apoE given concomitants (Table 2). In men, there were significant differences among apoE genotype means for total cholesterol, VLDL-C, TG, and LDL apoB, as previously established (Table 2). When individual genotype means were compared with the e3/3 genotype mean, lower total cholesterol, LDL-C, and LDL apoB for the e3/2 genotype (Scheffé's test, P<.05) were found to explain the differences in women, whereas in men, the mean VLDL-C for the e2/2 genotype was significantly higher and for the e4/2 genotype was significantly lower (Scheffé's test, P<.05). To preclude the possibility that genetic relatedness of members of the sample could bias these inferences, the analysis of genotypic variation was also carried out on the reduced sample of 167 unrelated 10kb-FH patients. The same inferences about the e3/2 genotype were obtained in women, whereas significant e2/2 and e4/2 genotype effects were not observed in the reduced sample in men (data not shown).

Composite Effects of ApoE Genotypes on Trait Levels

The euclidian distance and the total variance are presented in Fig 1 for the e3/2, e3/3, and e4/3 genotypes, separately for women and men. The rank of the genotypes with respect to \|/\| is the same between sexes. Patients with the e3/2 genotype are farthest from the origin, whereas those with the e3/3 are closest to the origin. On the other hand, the rank of the genotypes with respect to total variance is different between women and men. Women with the e3/2 genotype have the least amount of total interindividual variation, whereas e3/2 men have the greatest amount of interindividual variation.

In Fig 2 we compared the average excesses computed from the adjusted trait means in 10kb-FH patients with those for a sample of individuals selected for health within the same population.40 In both studies the e2 allele was associated with lower LDL-C levels in both women and men, with a greater effect in women. In men from the healthy sample and in women from the 10kb-FH sample, the presence of the e2 allele was associated with lower and the e4 allele with higher TG levels. Conversely, TG levels were higher in 10kb-FH men carrying the e2 allele and lower in those with the e4 allele, as has been reported in several studies of samples from different populations.14

Discussion

Our study takes advantage of a unique opportunity to investigate the causes of phenotypic variation in a large sample of individuals, all carriers of the French-Canadian >10-kb deletion of the LDL receptor gene. This has enabled us to show the sex-specific effects of apoE polymorphism on lipid traits in 10kb-FH patients. This analysis of apoE allele effects in 10kb-FH patients further documents that lipid metabolism is influenced by variation in many factors, both genetic and environmental.36,37,62,43

We found that the relative contribution of variation in age and measures of body size to the prediction of interindividual variation is sex and trait specific. For example, the relative contribution of concomitants to sample variability was larger in women than in men for VLDL-C (12.6% versus 3.0%) and TG (14.4% versus 5.3%) and smaller for total apoB (4.7% versus 10.7%) and Lp(a) (2.8% versus 9.3%) (Table 2). These striking differences between sexes were not observed in a sample of healthy subjects from the same population.40 However, concomitants did contribute significantly to variation of a greater number of lipid traits in men than in women in a smaller healthy sample, also from the same population.43 In 10kb-FH patients different traits are influenced to a different degree by concomitants, especially body weight. These results suggest that the genetic and environmental factors that influence variation in body size may act differentially in individuals.
TABLE 4. Mean Values of Plasma Levels of Lipids and Apolipoproteins Adjusted for Linear Effects of Concomitants According to Apolipoprotein E Genotypes In a Sample of Familial Hypercholesterolemic Patients With the >10-kb Deletion

<table>
<thead>
<tr>
<th>Trait/Sex</th>
<th>Count</th>
<th>Women</th>
<th>Men</th>
</tr>
</thead>
<tbody>
<tr>
<td>Women</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Women</td>
<td>7.56±0.63</td>
<td>7.54±0.60*</td>
<td>9.29±1.80</td>
</tr>
<tr>
<td>Men</td>
<td>11.52±4.22</td>
<td>9.21±1.21</td>
<td>9.16±1.25</td>
</tr>
<tr>
<td>VLDL-C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Women</td>
<td>1.51±0.72</td>
<td>0.65±0.31</td>
<td>0.79±0.40</td>
</tr>
<tr>
<td>Men</td>
<td>3.69±3.16†</td>
<td>1.39±1.41</td>
<td>1.08±0.59</td>
</tr>
<tr>
<td>LDL-C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Women</td>
<td>4.99±0.34</td>
<td>5.74±0.91†</td>
<td>7.47±1.65</td>
</tr>
<tr>
<td>Men</td>
<td>6.64±1.54</td>
<td>6.96±1.05</td>
<td>7.27±1.30</td>
</tr>
<tr>
<td>HDL-C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Women</td>
<td>1.06±0.25</td>
<td>1.13±0.25</td>
<td>1.03±0.23</td>
</tr>
<tr>
<td>Men</td>
<td>0.98±0.47</td>
<td>0.86±0.23</td>
<td>0.81±0.18</td>
</tr>
<tr>
<td>TG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Women</td>
<td>1.91±1.19</td>
<td>1.15±0.38</td>
<td>1.43±0.79</td>
</tr>
<tr>
<td>Men</td>
<td>3.13±1.66</td>
<td>1.67±0.57</td>
<td>1.86±0.86</td>
</tr>
<tr>
<td>Total apoB</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Women</td>
<td>191.4±29.3</td>
<td>181.6±33.3</td>
<td>225.5±51.5</td>
</tr>
<tr>
<td>Men</td>
<td>203.8±19.2</td>
<td>237.1±27.5</td>
<td>237.8±36.8</td>
</tr>
<tr>
<td>LDL apoB</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Women</td>
<td>168.8±22.5</td>
<td>158.3±30.5*</td>
<td>203.3±44.6</td>
</tr>
<tr>
<td>Men</td>
<td>142.4±17.1</td>
<td>196.8±45.6</td>
<td>211.6±35.0</td>
</tr>
<tr>
<td>Lp(a)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Women</td>
<td>47.5±23.3</td>
<td>24.3±23.8</td>
<td>28.1±30.6</td>
</tr>
<tr>
<td>Men</td>
<td>35.6±11.4</td>
<td>25.9±24.2</td>
<td>19.5±21.3</td>
</tr>
</tbody>
</table>

TC indicates total cholesterol; VLDL-C, very-low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglycerides; apo, apolipoprotein; and Lp(a), lipoprotein(a). Values are mean±SD. Lipids and lipoproteins are in millimoles per liter. Apolipoproteins and Lp(a) are in milligrams per deciliter.

*P<.05, †P<.01 for differences compared with e3/3 (Scheffe’s test).

with a mutation that has a large effect on LDL-C metabolism.

The comparison of apoE allele effects between 10kb-FH patients and other samples can only be performed if the apoE allele frequencies are similar because of the inverse relation between the size of a genotype effect and the frequency of the genotype. Significant differences in the relative frequencies of the apoE alleles among populations have been documented by numerous studies. In contrast, the relative apoE allele and genotype frequencies in the sample of 10kb-FH patients we studied do not differ significantly from those observed in healthy subjects selected from the same population and are similar in women and men (Table 3). This finding suggests that the observed differences in lipid trait levels between the 10kb-FH patients and healthy control subjects cannot be significantly influenced by differences in apoE genotype frequencies. The differences in the contribution of apoE polymorphism to explanations of interindividual variability in women and men in 10kb-FH patients compared with healthy individuals (discussed below) must be attributable, by and large, to differences in phenotypic expression of the different apoE alleles and genotypes.

The joint contribution of concomitants and of apoE polymorphism to total phenotypic variation is remarkably similar across lipid traits and between sexes for each trait (Table 2). The relative contributions of these combined sources to interindividual variation in lipid levels are approximately the same as was estimated for a healthy sample from the same population. This result suggests that the frequency distribution of interactions between the genetic and environmental factors indexed by concomitants and genetic variation in the apoE gene may be the same regardless of the context of sex and the presence of the >10-kb deletion in the LDL receptor gene. As Zerba and Sing have pointed out, it
is not the separate contribution of causative factors but the interaction between them that determines the phenotypes of a quantitative trait.

Despite the fact that the total amount of variation explained by concomitants and apoE polymorphism does not appear to depend on the trait considered, sex, or sample studied, the relative contribution of apoE after adjusting for concomitants is trait, sex, and population specific. For example, the association of variability in the apoE gene, after adjusting for variation in concomitants, was found to be larger for LDL-C (14.5% versus 1.7%) and smaller for VLDL-C (5.6% versus 25%) in women than in men (Table 2). Overall, the estimated effects of apoE polymorphism were primarily on total cholesterol and LDL-C in women and VLDL-C and TG in men. These findings suggest that in 10kb-FH patients there are apoE genotype by sex interaction effects on lipid metabolism. Such interaction would not be detected if the analysis was carried out on a pooled sample of women and men. Convincing evidence for sex-specific effects of variation in the apoE gene on lipid traits adjusted for variation in concomitants has also been reported by several studies of the population at large.\textsuperscript{18,40,46,47}

In the graphic representations of the apoE effects, we illustrated the influence of the apoE gene on levels (Figs 1 and 2) and variability (Fig 1) of lipid traits in 10kb-FH patients. Fig 1 emphasizes the fact that the gene coding for apoE is a "variability gene" (influences trait variance) as well as a "level gene" (influences trait levels).\textsuperscript{37,42} The two figures also show the sex specificity of the impact of the apoE gene on variation in lipid traits in 10kb-FH patients, as has been reported in previous studies of non-FH samples.\textsuperscript{18,40,46,47} All of these observations stress that evaluation of the apoE gene effects must take into account sex and the genetic and environmental context of the individuals of the study sample indexed by age, height, and weight.

The most striking and unexpected finding of our study was the association of the e2 allele with lower total cholesterol, LDL-C, and LDL apoB levels compared with the effects of the e3 allele only in women. Whereas the e4 allele loses its LDL-C-raising effect, the e2 allele maintains a strong effect in lowering this trait in 10kb-FH women. This lowering effect of the e2 allele on total cholesterol and LDL-C in FH was shown earlier by our group in a smaller sample of patients with the >10-kb deletion\textsuperscript{48} and in a recent study of a sample of FH patients with one of the three founder LDL receptor gene mutations identified in the Afrikaner population.\textsuperscript{49} In these two studies, analyses were carried out irrespective of sex. The importance of the LDL-C-lowering effect of the e2 allele has recently been confirmed by the high frequency of the allele observed in a sample of subjects with hypercholesterolemia.\textsuperscript{50} Evidence that the influence of the e2 allele is greatest in women has also been established by several studies of the population at large.\textsuperscript{18,40,47}

Several studies have reported higher levels of Lp(a) in FH patients than in control subjects.\textsuperscript{51-53} Lp(a) was found to be a powerful predictor of CAD in FH in two studies,\textsuperscript{52,54} whereas no such relation was found in another study.\textsuperscript{53} Recently the e2 allele was shown to be associated with a 24.8% decrease in mean plasma Lp(a) levels in a sample of 303 healthy Dutch subjects.\textsuperscript{21} In our study of 10 kb-FH patients, we did not find any significant influence of apoE polymorphism on Lp(a) levels in women or in men. Recent studies have evaluated the role of apoE polymorphism on Lp(a) levels in different diseases. In a group of 337 myocardial infarction survivors, plasma Lp(a) concentrations were not influenced by apoE genotype.\textsuperscript{24} Similar negative results were obtained in 450 children with type I diabetes.\textsuperscript{26} In 120 patients with FH, Berglund et al\textsuperscript{25} did not find any relation between Lp(a) levels and apoE genotype. If plasma Lp(a) levels are influenced by apoE polymorphism, as shown by De Knijff et al,\textsuperscript{22} it would suggest that the LDL receptor is involved in Lp(a) catabolism. Whether Lp(a) is catabolized via the LDL receptor or sample studied, the relative contribution of apoE concomitants, was found to be larger for LDL-C (14.5% versus 1.7%) and smaller for VLDL-C (5.6% versus 25%) in women than in men (Table 2). Overall, the estimated effects of apoE polymorphism were primarily on total cholesterol and LDL-C in women and VLDL-C and TG in men. These findings suggest that in 10kb-FH patients there are apoE genotype by sex interaction effects on lipid metabolism. Such interaction would not be detected if the analysis was carried out on a pooled sample of women and men. Convincing evidence for sex-specific effects of variation in the apoE gene on lipid traits adjusted for variation in concomitants has also been reported by several studies of the population at large.\textsuperscript{18,40,46,47}

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apoB was found to be lower than that of LDL, suggesting that the LDL receptor does not contribute greatly to the normal clearance of intact Lp(a).\textsuperscript{50} FH is characterized by a high incidence of CAD.\textsuperscript{5} However, FH displays great variability in phenotypic expression,\textsuperscript{3,4} which could be explained by the variability of the underlying mutation.\textsuperscript{8,49} Other genetic and environmental influences could also intervene. As already shown in small samples\textsuperscript{48,49} and confirmed by our study in a large group of patients who all had the same LDL receptor gene mutation, apoE polymorphism affects lipid levels in FH. The present study was an attempt to understand the causes of variation of lipid levels in FH, without the heterogeneity in LDL receptor mutations generally found in clinically defined samples. Whether apoE polymorphism is associated with CAD is presently unresolved.\textsuperscript{19} Since our study has shown that apoE genotypes influence lipid levels even in FH, adjustment for lipid levels should be performed to evaluate the separate effects of apoE polymorphism on CAD risk in FH, as has been done in non-FH populations.\textsuperscript{41,62}

In conclusion, the availability of a large sample of 10kb-FH patients allowed us to show the sex-specific effects of the apoE gene and that the $e_2$ allele effect on total cholesterol and LDL-C levels is greater in 10kb-FH women than in 10kb-FH men. ApoE plays a central role in lipid metabolism, but the magnitudes of apoE gene effects are largely determined by the characteristics of the members of the sample studied. Even in 10 kb-FH patients whose mean values for lipid traits deviate considerably from those of the general population, the concomitants and apoE genotypic variability explain as much variation as in normolipidemic individuals. Most of the variation of these lipid traits is still not explained by concomitants and apoE. Furthermore, apoE genotypes influence lipid traits differently in 10kb-FH patients and in normolipidemic individuals. Since the apoE allele frequencies were found to be similar in 10kb-FH patients and in a healthy sample within the same population, we conclude that the apoE genotypes have different penetrance functions in normolipidemic subjects and in 10kb-FH patients. Finally, the effects of apoE genotypes on interindividual variation in lipid traits are context and sex dependent and suggest that gene-gene and gene-environment interactions determine the phenotypes of disease.\textsuperscript{5,45} Our study further emphasizes that analyzing pooled samples of women and men can only result in inappropriate inferences about the impact of the apoE gene on variation in lipid trait levels.

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