Serum Low Density Lipoprotein Cholesterol Level and Cholesterol Absorption Efficiency Are Influenced by Apolipoprotein B and E Polymorphism and by the FH-Helsinki Mutation of the Low Density Lipoprotein Receptor Gene in Familial Hypercholesterolemia

Helena Gylling, Katriina Aalto-Setälä, Kimmo Kontula, and Tatu A. Miettinen

The aim of the present study was to evaluate the effect of variation of different gene loci separately and in concert on lipid metabolism in heterozygous familial hypercholesterolemia (FH). We assayed a unique low density lipoprotein (LDL) receptor gene defect (designated as FH-Helsinki), the Xba I polymorphism of the apolipoprotein (apo) B, phenotypes of the apo E, and determined the levels of serum lipoproteins, the efficiency of cholesterol absorption, and the values for several parameters of cholesterol metabolism in 51 unrelated patients with heterozygous FH. The genetic parameters were distributed independently of each other. Gender distribution and the prevalence of coronary artery disease were similar in the different apo E phenotypes, in the apo B genotypes, and in patients with and without the FH-Helsinki mutation. However, the FH-Helsinki mutation was associated with an increased body mass index. Serum LDL cholesterol was significantly elevated in patients with the FH-Helsinki mutation and the apo B X2 allele. Apo E phenotypes were not related to serum lipids per se, but the highest serum LDL cholesterol levels were measured in patients with the FH-Helsinki gene, apo E4 phenotype, and at least one X2 allele. Patients with the FH-Helsinki mutation and apo E4 phenotype had the highest cholesterol absorption efficiency. Cholesterol absorption was not related to serum lipids or lipoproteins, but LDL cholesterol was most elevated in patients with the most efficient cholesterol absorption. We conclude that in FH, diverse genetic factors exert individual and additive influences on serum LDL cholesterol levels. (Arteriosclerosis and Thrombosis 1991;11:1368-1375)

Several genetic factors contribute to the regulation of serum cholesterol level in humans. Familial hypercholesterolemia (FH) is among the best-characterized entities of the genetic abnor-
levels. Thus, studies with the Xba I restriction fragment length polymorphism of the apo B gene have revealed that in many populations, serum total and LDL cholesterol as well as apo B levels are higher in subjects with the X2 allele (restriction site present) than in those homozygous for the X1 allele (restriction site absent).17-20 Similar results have been reported for patients with FH.21 Moreover, apo E polymorphism is associated with variations in serum total and LDL cholesterol levels in various population studies.22-28 Apo E phenotype also affects the intestinal absorption efficiency of cholesterol in healthy men29 and in patients with FH but does not appear to be associated with serum cholesterol in the latter. To evaluate the effect of variation of the different gene loci separately and in concert on lipid metabolism in FH, we assayed the FH-Helsinki LDL receptor mutation, the Xba I polymorphism of the apo B gene, and phenotypes of apo E and determined the efficiency of cholesterol absorption, levels of serum lipoproteins, and several parameters of cholesterol metabolism in patients with the heterozygous form of FH.

Methods

Patients

The study group consisted of 51 consecutive unrelated outpatients, 36 females and 15 males with heterozygous FH, who were attending the lipid clinics of Helsinki University Central Hospital. The age was 43.3±1.6 years (mean±SEM), ranging from 15 to 64 years. The diagnostic criteria of FH were 1) serum total cholesterol greater than 8 mmol/l and serum LDL cholesterol greater than 5 mmol/l, 2) the presence of tendon xanthomas, and 3) the presence of hypercholesterolemia and/or tendon xanthomas in at least one first-degree relative. In all cases, analyses were performed before hypolipidemic drug interventions. The patients had been requested earlier to consume a cholesterol-lowering diet (30% of daily calories as fat and daily cholesterol intake <300 mg). They were advised to adhere to this diet during the experimental period. A dietary recall for a subgroup of 24 subjects showed that the cholesterol intake was less than 300 mg/day. The body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared.

None of the study group had heart failure; renal, liver, or thyroid disease; or diabetes mellitus. All patients volunteered for the study, which had been approved by the Ethical Committee of the hospital.

DNA Analysis

DNA was isolated from frozen whole-blood samples with the technique described by Bell et al.31 For determination of the apo B gene polymorphism, DNA samples were digested with the restriction enzyme Xba I ( Promega, Madison, Wis.; 2–5 units/µg DNA), fractionated by gel electrophoresis on 0.6% agarose, and transferred to nitrocellulose filters. The apo B cDNA probe pB2332 used in the hybridization analysis was a kind gift of Jan L. Breslow, New York, N.Y. The method has been described in detail recently.20,21 The allele resulting in the formation of an 8.6-kb Xba I fragment is designated as X1, and that generating a 5-kb fragment as X2.

Analysis of the 8-kb deletion of the LDL receptor gene (FH-Helsinki) specific for the Finnish population was conducted as described recently.14,16 In brief, DNA samples were digested with the restriction enzyme Pvu II or BamHI, fractionated by gel electrophoresis on 0.6% agarose, and transferred to nitrocellulose filters. Hybridization of the filters was performed under standard conditions and with the use of an LDL receptor cDNA probe that was prepared from plasmid pLDLR3 (donated by D.W. Russell, Dallas, Tex.) that contained exons 11–17 of the LDL receptor gene.16 The FH-Helsinki mutation is characterized by the appearance of a unique 11-kb or 8-kb restriction fragment when DNA digestion is accomplished with Pvu II or BamHI, respectively.16

Apolipoprotein E Phenotyping

Apo E phenotyping was performed by isoelectric focusing.28

Serum Lipids

The concentration of serum total cholesterol and triglycerides was analyzed enzymatically by use of commercial kits (Boehringer-Mannheim Diagnostica GmbH, Mannheim, FRG). Lipoproteins were separated by ultracentrifugation in density classes: d<1.006 g/ml (very low density lipoprotein, VLDL); d<1.019 g/ml (intermediate density lipoprotein, IDL); d<1.063 g/ml (LDL); and d>1.063 g/ml (high density lipoprotein, HDL) mainly as described in the Manual of Laboratory Operations of the Lipid Research Clinics Program.33

Cholesterol Absorption and Fecal Sterols

Cholesterol absorption and fecal sterols were studied in 29 random outpatients with the same distributions of sex, age, BMI, and serum lipoprotein as well as of apo B and apo E polymorphism as the remaining 22 patients. The patients were following their cholesterol-lowering diet, without any recommendation to change it. Cholesterol absorption was measured with the peroral double-isotope feeding method.34 Therefore, the patients consumed three capsules a day, each containing carbon-14–labeled cholesterol, tritiated sitosterol, and 200 mg Cr2O3 to correct the fecal flow. After a stabilization period of 7 days, a 3-day stool collection was performed and analyzed for radioactivities, fecal sterols,35–37 and Cr2O3.38

Coronary Artery Disease

A careful physical examination was completed, including an electrocardiogram (ECG) at rest and an exercise ECG on a bicycle up to a heart rate exceeding 85% of the age-predicted maximum. The criteria detecting patients with coronary artery disease
**TABLE 1. Clinical Characteristics, Apolipoprotein E Phenotypes, and Apolipoprotein B Xba I Genotypes in Familial Hypercholesterolemia With and Without the Finnish Low Density Lipoprotein Receptor Gene Mutation**

<table>
<thead>
<tr>
<th>Variable</th>
<th>FH-Helsinki mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes (n=20)</td>
</tr>
<tr>
<td></td>
<td>No (n=31)</td>
</tr>
<tr>
<td>Sex</td>
<td>F/A/M</td>
</tr>
<tr>
<td></td>
<td>16 F/4 M</td>
</tr>
<tr>
<td></td>
<td>20 F/11 M</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>39.4±2.7</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>71.9±4.0</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.65±0.02</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>26.3±1.2*</td>
</tr>
<tr>
<td>Coronary artery disease (No.)</td>
<td>4</td>
</tr>
<tr>
<td>Apo E phenotype (No.)</td>
<td></td>
</tr>
<tr>
<td>3/2</td>
<td>1</td>
</tr>
<tr>
<td>3/3</td>
<td>8</td>
</tr>
<tr>
<td>4/3</td>
<td>10</td>
</tr>
<tr>
<td>4/4</td>
<td>1</td>
</tr>
<tr>
<td>Apo B Xba I genotype (No.)</td>
<td></td>
</tr>
<tr>
<td>X2X2</td>
<td>3</td>
</tr>
<tr>
<td>X1X2</td>
<td>10</td>
</tr>
<tr>
<td>X1X1</td>
<td>7</td>
</tr>
<tr>
<td>Demographic data show mean±SEM.</td>
<td></td>
</tr>
</tbody>
</table>

FH, familial hypercholesterolemia; F, female; M, male; Apo, apolipoprotein.

*Significantly different from patients without the FH-Helsinki mutation (p<0.05). Significances of mean values tested with Student’s t test and the absolute numbers with Fisher’s exact test.

(CAD) included at least one of the following: 1) angina pectoris with typical chest pain and ischemic alterations on the exercise ECG; 2) a history of myocardial infarction verified from hospital records, including a typical clinical picture, ECG alterations, and enzymatic changes; or 3) the presence of a pathological Q wave on the ECG at rest.

**Statistical Methods**

Statistical significances between genetic categories were tested with the two-tailed Student’s t test and the Fisher’s exact χ² test. Univariate correlations were calculated by Pearson’s coefficient of correlation, and multiple stepwise regression analysis (BMDP Statistical Software, Inc., Los Angeles, Calif., program 2r) was used to differentiate the parameters affecting serum LDL cholesterol levels. p<0.05 was considered statistically significant.

**Results**

Twenty of the 51 (39%) patients examined had the FH-Helsinki mutation (gross deletion at the 3’ end of the LDL receptor gene) as shown in Table 1. The apo E 2/2 phenotype was not identified, apo E 3/2 was found in 4%, apo E 3/3 was found in 49%, apo E 4/3 was found in 14%, and apo E 4/4 was found in 6% of the patients. The apo B X2X2 genotype was found in 22%, X1X2 in 53%, and X1X1 in 25% of the patients. The apo E and apo B genotypes and the FH-Helsinki mutation were distributed independently of each other.

Sex, age, and prevalence of CAD were similar in the different apo E phenotypes and apo B genotypes (data not shown) and in patients with and without the FH-Helsinki mutation (Table 1). However, the FH-Helsinki mutation was associated with an increased BMI (Table 2). When the study group was divided into quartiles according to BMI (12 patients in each group; for three patients, weight or height was divided into quartiles according to BMI (12 patients in each group; for three patients, weight or

**TABLE 2. Body Mass Index, Serum Lipids, Lipoprotein Cholesterol, Cholesterol Absorption, and Parameters of Cholesterol Metabolism in Patients With Familial Hypercholesterolemia With Different Genetic Characteristics**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Apo E phenotype</th>
<th>Apo B genotype</th>
<th>FH-Helsinki mutation</th>
<th>r values with cholesterol absorption (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3/2, 3/3</td>
<td>4/3, 4/4</td>
<td>X1X1</td>
<td>X1X2</td>
</tr>
<tr>
<td></td>
<td>(n=27)</td>
<td>(n=24)</td>
<td>(n=13)</td>
<td>(n=27)</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>23.6±0.9</td>
<td>25.4±0.8</td>
<td>23.6±1.1</td>
<td>25.2±0.8</td>
</tr>
<tr>
<td>Serum cholesterol (mmol/l)</td>
<td>10.3±0.3</td>
<td>10.4±0.5</td>
<td>9.3±0.3</td>
<td>10.3±0.4</td>
</tr>
<tr>
<td>VLDL cholesterol (mmol/l)</td>
<td>0.5±0.1</td>
<td>0.3±0.1</td>
<td>0.3±0.1</td>
<td>0.4±0.1</td>
</tr>
<tr>
<td>IDL cholesterol (mmol/l)</td>
<td>0.5±0.1</td>
<td>0.3±0.1</td>
<td>0.3±0.1</td>
<td>0.4±0.1</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/l)</td>
<td>7.4±0.2</td>
<td>7.8±0.4</td>
<td>7.0±0.3</td>
<td>7.4±0.3</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/l)</td>
<td>1.4±0.1</td>
<td>1.4±0.1</td>
<td>1.3±0.1</td>
<td>1.4±0.1</td>
</tr>
<tr>
<td>LDL/HDL cholesterol</td>
<td>5.7±0.4</td>
<td>6.2±0.5</td>
<td>5.6±0.4</td>
<td>5.9±0.5</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>1.5±0.1</td>
<td>1.3±0.1</td>
<td>1.2±0.1</td>
<td>1.5±0.1</td>
</tr>
<tr>
<td>Cholesterol absorption (%)</td>
<td>47.5±2.2±1</td>
<td>53.7±3.1</td>
<td>51.8±3.2</td>
<td>50.8±1.9</td>
</tr>
<tr>
<td>Fecal bile acids (mg/kg/day)</td>
<td>4.7±0.6</td>
<td>5.0±0.4</td>
<td>5.8±0.6</td>
<td>4.8±0.5</td>
</tr>
<tr>
<td>Neutral sterols (mg/kg/day)</td>
<td>9.6±0.7</td>
<td>9.7±0.9</td>
<td>10.5±2.1</td>
<td>9.7±0.6</td>
</tr>
<tr>
<td>Total fecal sterols (mg/kg/day)</td>
<td>14.7±1.2</td>
<td>14.3±1.0</td>
<td>16.3±2.6</td>
<td>14.5±0.8</td>
</tr>
</tbody>
</table>

Values are mean±SEM. FH, familial hypercholesterolemia; VLDL, very low density lipoprotein; IDL, intermediate density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein.

*Significantly different from patients without the FH-Helsinki mutation (p<0.05); significantly different from apo B X1X1 (p<0.05); significantly different from apo E 4/3, 4/4 (p<0.05); §significant correlation (p<0.05); ¶significances of the mean values were tested with Student’s t test.
TABLE 3. Genetic Categories of Familial Hypercholesterolemic Patients With the Highest and Lowest Serum Total and Low Density Lipoprotein Cholesterol Levels and Respective Cholesterol Absorption Efficiencies

<table>
<thead>
<tr>
<th>Variable</th>
<th>F+ apo E4+ X1X2 or X2X2 (n=8)</th>
<th>F+ apo E4+ X1X2 (n=6)</th>
<th>F+ apo E4+ X1X2 (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol (mmol/l)</td>
<td>12.3±0.8§</td>
<td>11.5±0.8§</td>
<td>8.6±0.5</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/l)</td>
<td>9.3±0.3§</td>
<td>8.8±0.5§</td>
<td>6.1±0.6</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/l)</td>
<td>1.2±0.1</td>
<td>1.2±0.1</td>
<td>1.7±0.3</td>
</tr>
<tr>
<td>LDL/HDL cholesterol</td>
<td>7.8±0.7§</td>
<td>7.8±1.0†</td>
<td>4.3±0.8</td>
</tr>
<tr>
<td>Cholesterol absorption (%)</td>
<td>57.7±2.1†</td>
<td>57.0±2.7†</td>
<td>46.9±3.6</td>
</tr>
</tbody>
</table>

Values are mean±SEM.

Apo, apolipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein.

*F+ denotes presence of the familial hypercholesterolemia (FH)-Helsinki gene, F− its absence. Apo E4+ signifies phenotype 4/3 or 4/4. X1X1, X1X2, and X2X2 show the apo B Xba I genotypes.

1p<0.05, 2p<0.01, §p<0.001, F+ subgroups compared with F−. Significances of mean values were tested with Student’s t test.

Serum Lipids and Lipoproteins

Subjects homozygous or heterozygous for the apo B X2 allele had significantly higher serum total and LDL cholesterol levels than those homozygous for the X1 allele (Table 2). Moreover, the patients with the FH-Helsinki gene had higher serum LDL and lower HDL levels than did those without this mutation. Serum VLDL and IDL cholesterol or triglycerides did not bear any relation to any of the genetic groups examined.

In agreement with our earlier observation, apo E phenotypes per se were not associated with serum lipid levels. However, the highest serum total (12.3±0.8 mmol/l, mean±SEM) and LDL (9.3±0.3 mmol/l) cholesterol levels were measured in eight patients with the FH-Helsinki gene (F+), the apo E4 phenotype (E4+), and at least one X2 allele (X2+) (Table 3). Of these subjects, those homozygous for the X2 allele were the most hypercholesterolemic (Figure 1; total and LDL cholesterol levels of 14.8±1.6 and 11.1±0.9 mmol/l, respectively), but the number of subjects was limited to two, thus limiting meaningful comparison to other groups. The remaining six subjects characterized by the presence of the apo E4 phenotype and the apo B X1X2 genotype (F+E4+X2+) had significantly higher total and LDL cholesterol levels (11.5±0.8 and 8.8±0.5 mmol/l, respectively) than did the five other subjects with similar apo E and apo B gene characteristics but without FH-Helsinki (F−E4+X2+; 8.6±0.5 and 6.1±0.6 mmol/l; Table 3).

Total and LDL cholesterol levels of subjects with other gene combinations fell between those of the F+E4+X2+ and F−E4+X2+ groups. Thus, another group with either the apo E 4/3 or 4/4 phenotype and apo B X2X2 genotype (without the FH-Helsinki gene) also had marked hypercholesterolemia, with a mean LDL cholesterol level of 8.6±0.7 mmol/l. The lowest LDL cholesterol level next to that of the group F−E4+X2+ (Table 3) (6.9±0.4 mmol/l; four subjects) was found in patients with the apo B X1X1 genotype without the FH-Helsinki gene but, somewhat unexpectedly, in combination with the apo E4.
phenotype. Multiple stepwise regression analysis including BMI and the variables shown in Table 2 showed that 14% of the variability of serum LDL cholesterol level could be explained by the FH-Helsinki mutation and 12% could be explained by the apo B genotype; further steps with the remaining variables were nonsignificant.

The mean serum HDL cholesterol level was lowest in patients with the apo B X1X2 genotype and the FH-Helsinki mutation, independent of apo E phenotypes. The LDL to HDL cholesterol ratio was highest in groups with the highest LDL cholesterol levels and lowest in the group with the lowest LDL cholesterol content (Table 3); other mean LDL to HDL cholesterol ratios fell between these two means.

**Cholesterol Absorption and Metabolism**

Cholesterol absorption efficiency percent was not related to serum lipids or lipoproteins, but it was inversely associated with neutral sterol excretion (Table 2). The apo B genotypes did not associate, whereas the apo E phenotypes and the occurrence of the FH-Helsinki mutation did associate, with the intestinal absorptive efficiency of cholesterol (Table 2). Thus, patients with the LDL receptor gene deletion and apo E 4/3 or 4/4 phenotype had the highest absorptive efficiency, with a mean±SEM of 57.7±2.1%, which differed significantly from that of other groups (Figure 2). Cholesterol absorption efficiency was significantly lower in subjects with the lowest LDL cholesterol levels than in those with the highest LDL levels (Table 3).

Bile acid synthesis and fecal total cholesterol elimination (sum of bile acids and neutral sterols) did not vary significantly according to any of the genetic subgroups, although the values were lowest in patients with one or two apo B X2 alleles (Table 2).

**Discussion**

The present study shows that in FH, diverse genetic factors exert individual and additive influences on serum LDL cholesterol level. The FH-Helsinki mutation was correlated with high LDL and low HDL cholesterol, obesity, and an enhanced intestinal absorptive efficiency of cholesterol. The X2 allele was associated with high serum total and LDL cholesterol but was not associated with cholesterol absorption. The DNA site responsible for the Xba I polymorphism of the apo B gene may be in linkage disequilibrium with a hitherto-unknown DNA locus that independently modulates serum LDL cholesterol level. The apo E4 phenotype was not related to LDL cholesterol level but was associated with high absorption of cholesterol. The highest total and LDL cholesterol levels could be observed in patients with a combination of apo E4 phenotype and the apo B X2 allele, the FH-Helsinki mutation, and high intestinal absorption of cholesterol.

Several mutations of the LDL receptor gene have been described, varying from single-base substitutions to large deletions.1 The LDL receptor gene is located on the short arm of chromosome 19, where it is linked to the gene for apo E, apo C-I, and apo C-II.3,9 The receptor molecule is a single-chain glycoprotein of 839 amino acids,1 according to the functional phenotype of the mutant protein, four different classes of mutations at the LDL receptor locus have been identified.1 The FH-Helsinki mutation deletes about 9,500 base pairs from the 3’ end of the LDL receptor gene, deleting exons 16, 17, and part of 18.14-16 This region encodes the membrane-spanning and cytoplasmic domains of the protein. Molecular lesions located in this area belong to the class 4 category of mutations, which are relatively rare and usually lead to a receptor molecule that is able to bind LDL but is unable to internalize bound LDL.1

In fact, the FH-Helsinki mutation interferes with both the receptor-mediated binding and the internalization of LDL in fibroblast cultures.16 In our previous study we did not see differences in serum lipoprotein levels among 23 FH patients with the FH-Helsinki gene and in 23 other FH patients with a hitherto-unknown LDL receptor gene mutation.16 Genotypes of apo B and apo E were not determined in the earlier study; it is possible that in the present series, patients with the FH-Helsinki gene had a relatively higher prevalence of the apo E4 and/or apo B X2 allele than did those in the earlier investigation. We emphasize, however, that any conclusions concerning the metabolic effects of the FH-Helsinki
mutation in comparison with other LDL receptor mutations must be made cautiously until the molecular characteristics of the latter are determined.

The possibility that the 31 patients of the present series without the FH-Helsinki allele would represent genetic defects other than those affecting the LDL receptor gene is highly unlikely. First, the criteria for patient selection were very strict, including the presence of tendon xanthomas. Second, another relevant genetic defect with similar phenotypic characteristics, familial defective apolipoprotein B, can be virtually excluded by its documented absence among Finns with moderate or severe hypercholesterolemia.

The variation in serum LDL cholesterol concentration depends mainly on its catabolism via LDL apo B receptors in the liver; the simultaneous impairment to catabolize LDL by reduced receptor activity also causes some overproduction of LDL from IDL.14-44 In the present series, whether analyzed by univariate or multivariate analysis, serum LDL was significantly higher in subjects with the FH-Helsinki deletion. This mutation was the primary factor, explaining 14% of the variability of serum LDL cholesterol in the multivariate model. The mechanisms involved can only be speculated.

First, it can be assumed that the functional defect caused by the FH-Helsinki deletion was severe enough to totally deplete the receptor function. Thus, heterozygous FH subjects with this mutation bind and take up LDL at half the normal rate, while the yet-uncharacterized mutations in the remaining FH patients may not be equally deleterious. Second, the apo B X2 allele was associated with elevated serum total and LDL cholesterol, a finding consistent with results obtained from population studies, moderately hypercholesterolemic subjects,17-20 and our earlier studies of FH.21 When the apo B X1 allele alone was present (genotype X1X1), serum LDL cholesterol was similar in subjects with and without the FH-Helsinki mutation, and no apo E phenotype influence could be observed either. The ability of the apo B X2 allele to modulate serum LDL cholesterol level is further substantiated by the fact that the LDL cholesterol level of the 13 subjects carrying both the FH-Helsinki mutation and the X2 allele was higher than in those 25 patients without the FH-Helsinki mutation (8.7±0.4 versus 7.2±0.3 mmol/l, mean±SEM). Apo B X2 homozygosity has been described to impair LDL catabolism in normal subjects.45,46 Whether this implies that the apo B protein encoded by the X2 allele is functionally more impaired when interacting with the receptor encoded by the FH-Helsinki gene than with those encoded by other mutant LDL receptor genes should be studied further.

Third, the intestinal efficiency of cholesterol absorption was enhanced in subjects with the FH-Helsinki mutation (Table 2). The basic mechanism is totally unexplored. The enhanced efficiency of cholesterol absorption associated with the FH-Helsinki mutation could, however, have an additive impact on serum LDL cholesterol level. According to the homeostasis of cholesterol metabolism,1 dietary cholesterol inhibits hepatic cholesterol synthesis and down-regulates LDL receptor activity, thereby increasing serum total and LDL cholesterol.47,48 The highest serum LDL cholesterol level in the present series was measured in subjects with the apo e4 and apo B X2 alleles combined with the FH-Helsinki mutation. Apo E polymorphism is associated with variations of absorptive efficiency of cholesterol in the normal population29 and in FH.30 Moreover, in the normal population, cholesterol absorption is positively and hepatic cholesterol synthesis is negatively related to serum total and LDL cholesterol.29 However, in FH this association does not exist,30 and neither could an independent association between apo E polymorphism and serum LDL cholesterol be demonstrated in the present series in accordance with earlier observations.46 The fact that apo E may yet exert an important modulative role together with other genetic factors is substantiated by the data shown in Figure 2. The FH-Helsinki mutation seems to be associated with enhanced fractional cholesterol absorption, especially in subjects with the apo E 4/3 and 4/4 genotypes. The mechanism of this combined effect of these two alleles (FH-Helsinki and e4) on cholesterol absorption remains to be examined.

Serum HDL cholesterol is, in general, low in FH,50-53 In the present study, serum HDL cholesterol was significantly lower in patients with the FH-Helsinki mutation. Of factors affecting serum HDL cholesterol,54 gender distribution was similar, and none of the female subjects were taking estrogens. The number of smokers and the level of alcohol consumption were low and were not different between subjects with and without the FH-Helsinki mutation. The subjects with the mutation were more obese, and BMI and serum HDL cholesterol were related in the entire series (r=-0.371, p<0.01). Whether the association of low HDL cholesterol level with the presence of the FH-Helsinki mutation is totally a consequence of obesity remains open because in multivariate analysis the association disappeared.

The association between obesity and the FH-Helsinki mutation is intriguing. First, it had been shown earlier that in FH families, affected family members are shorter than the normolipemidic ones55 and family members with mild, nonxanthomatic hypercholesterolemia are shorter than those with normal serum lipids.56 Although not significantly, the subjects with the FH-Helsinki mutation were shorter than the other FH patients without the mutation. Second, there is increasing evidence that inheritance is a remarkable factor in obesity.97 The exact basis of inheritance, as well as the possible gene or genes responsible for obesity, is undetermined. According to the present results, there could be some linkage between the LDL receptor gene and the inheritance pattern of obesity.
Acknowledgments

The expert technical and secretarial assistance of Eeva Gustafsson, Marja Aarnio, Liisa Kuhta, Soile Aarnio, Leena Kaipiainen, Anja Saolainen, Pia Hoffström, Elii Toivainen, Meeri Lappalainen, and Kaija Kettunen is greatly acknowledged.

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KEY WORDS • familial hypercholesterolemia • low density lipoprotein receptor gene • low density lipoprotein cholesterol • cholesterol absorption • apolipoprotein E • apolipoprotein B
Serum low density lipoprotein cholesterol level and cholesterol absorption efficiency are influenced by apolipoprotein B and E polymorphism and by the FH-Helsinki mutation of the low density lipoprotein receptor gene in familial hypercholesterolemia.

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Arterioscler Thromb Vasc Biol. 1991;11:1368-1375
doi: 10.1161/01.ATV.11.5.1368

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