Diagnosis of Heterozygous Familial Hypercholesterolemia
DNA Analysis Complements Clinical Examination and Analysis of Serum Lipid Levels

Pekka V.I. Koivisto, Ulla-Maija Koivisto, Tatu A. Miettinen, and Kimmo Kontula

The concordance of clinical and molecular genetic diagnoses of heterozygous familial hypercholesterolemia (FH) was studied in 65 subjects (10 propositi and 55 first-degree relatives) from 10 families with FH. Nine propositi were carriers of the FH-Helsinki deletion of the low density lipoprotein (LDL) receptor gene, prevalent in the Finnish population, while a new deletion, extending from intron 14 to intron 15 of the LDL receptor gene, was identified in one family. Serum LDL cholesterol levels used in the clinical diagnosis (<5.0 mmol/l, not FH; 5.0–5.9 mmol/l, possible FH; ≥6.0 mmol/l, FH; limits are 1 mmol/l lower for those <18 years) were derived from an authoritative recommendation. Tendon xanthomas constituted an additional criterion. With the DNA analysis as the reference, 55 (85%) subjects could be correctly classified clinically as FH patients or subjects without FH. The remaining 10 subjects were misclassified or were in the "possible FH" category. When the age- and sex-specific 95th percentile LDL cholesterol levels were used instead of the rigid values for both adults and children, the percentage of correct diagnoses rose to 95%. Common genetic polymorphisms of apolipoproteins E and B did not markedly affect LDL cholesterol levels in FH patients, whereas increasing age and obesity were associated with elevated LDL levels. In conclusion, DNA analysis is a valuable adjunct to the diagnosis of FH that is applicable to families with a known mutation of the LDL receptor gene. If DNA methods are not available, age- and sex-specific LDL levels should be used as an aid in the clinical diagnosis of FH. (Arteriosclerosis and Thrombosis 1992;12:584–592)

KEY WORDS • familial hypercholesterolemia • low density lipoprotein receptor gene • apolipoprotein B • apolipoprotein E

Recent developments in the treatment of hyperlipidemias have led to an increasing need for systematic recommendations for the recognition and management of these disorders. The diagnosis of familial hypercholesterolemia (FH) has relied on its principal clinical features: elevated serum total and low density lipoprotein (LDL) cholesterol concentrations, the presence of tendon xanthomas and premature coronary heart disease (CHD), and similar findings in a first-degree relative. FH is caused by a mutation of the LDL receptor gene, resulting in the defective functioning and/or a diminished number of LDL receptors in the hepatic and other cells, which in turn is accompanied by the slow receptor-mediated catabolism of LDL and the accumulation of LDL in the circulation.

FH is inherited in an autosomal codominant way, with the prevalence of heterozygous patients being approximately one in 500 in most populations.

The phenotypic expression of heterozygous FH depends on exogenous factors, including diet and the use of hypolipidemic drugs, as well as genetic polymorphism of the ligands for the LDL receptor, i.e., apolipoproteins E and B. In one family, a putative "cholesterol lowering gene" was found to even normalize serum LDL cholesterol levels of the known carriers of the LDL receptor gene. All these findings call for unequivocal diagnostic methods for FH.

A cell-biological diagnosis of FH may be accomplished by studying LDL binding, internalization, and degradation by cultured fibroblasts or by lymphocyte proliferation in response to mitogen administration as a function of the prevailing LDL cholesterol concentration. These methods are, however, technically very demanding, and the use of hypolipidemic drugs may interfere with lymphocyte culture assays. Use of molecular genetic techniques would constitute an ideal diagnostic approach for FH, with no interference by exogenous lipid-modulating factors. The multitude of LDL receptor gene mutations, ranging from single nucleotide changes to large deletions and insertions, limits the usefulness of the DNA approach for the diagnosis of...
FH in most populations. In some genetically homogeneous populations, such as Lebanese Christian Arabs, French-Canadians, and South African Afrikaners, one or a few LDL receptor gene mutations have been enriched by a founder gene effect. The Finnish population is another example of a genetically homogeneous population, and in fact, about 40% of the Finnish FH patients carry the same type of LDL receptor gene mutation. This mutation, designated as FH-Helsinki, deletes exons 16 and 17 and a portion of exon 18 of the LDL receptor gene and results in an internalization-defective phenotype of FH.

The present study was conducted to clarify 1) whether in given families with an established LDL receptor gene mutation the conventional clinical criteria of FH match with the molecular genetic diagnosis of FH, 2) if any variation in LDL cholesterol levels could be explained by genetic variation of apolipoproteins B and E and whether their effects contribute to any discrepancy between the clinical and the DNA diagnosis of FH, and 3) whether any evidence in favor of the existence of possible cholesterol-lowering gene(s) in FH families could be provided.

Methods

Patients and Design of the Study

Ten propositi with large deletions of the LDL receptor gene and their first-degree relatives (parents, siblings, and children) were investigated. In addition, if clinical data favored the diagnosis of FH ("FH" or "possible FH"; see below) in a relative, the first-degree relatives of the latter person were also invited to the study. Very young children (<7 years of age) and relatives living outside the Helsinki University Central Hospital district were not invited.

The propositi were identified during an ongoing screening of hypercholesterolemic subjects for the FH-Helsinki gene in Finland. The propositi and their relatives were invited by a letter in which the purpose of the study was stated. A total of 65 of the 69 invited individuals visited the outpatient clinic, where fasting blood samples were drawn and a physical examination was performed. The clinical diagnoses were set by one of the investigators following a protocol designed for this study, while the FH diagnosis by DNA analysis was accomplished by another investigator. The two judgments were kept blinded to each other until the whole study had been completed. The study protocol was approved by the Ethics Committee of the hospital.

Physical Examination

The patients were asked about previous cholesterol measurements, medications, and illnesses, with a particular attention to findings and symptoms suggestive of CHD. They were classified as having CHD if a previous diagnosis of myocardial infarction or angina pectoris had been established or if they had had exercise electrocardiographic findings compatible with those of CHD.

During physical examination the presence of tendon and subperiosteal xanthomas, corneal arcus, and xanthalasmas was registered. For xanthomas, a three-stage classification was used as follows: 1) no xanthomas, 2) suspected xanthomas (slight irregularity in a tendon or olecranon or a slightly predominant tibial tuberosity), or 3) unequivocal xanthomas. The latero–lateral thickness (in millimeters) of the Achilles tendons was measured using a ruler, by taking the tendon between the thumb and index finger and applying slight pressure, with the patient relaxed in bed with the ankle flexed at 90°.

Laboratory Methods

Serum cholesterol and triglyceride concentrations were measured by enzymatic methods using commercial kits obtained from Boehringer (Mannheim, FRG). The concentration of high density lipoprotein (HDL) cholesterol was measured enzymatically after precipitation of LDL and very low density lipoprotein (VLDL) fractions with dextran sulfate and magnesium chloride. The concentration of LDL cholesterol was calculated using the Friedewald formula.

To exclude causes of secondary hypercholesterolemia, serum aspartate aminotransferase, alkaline phosphatase, creatinine, thyroid stimulating hormone, fasting blood glucose, and urine protein were determined using standard techniques of the clinical laboratory. Findings in these tests were normal with the exception of a slightly elevated aspartate aminotransferase activity in one case and an elevated fasting blood glucose (8.1 mmol/l) in another. Both of these patients were middle-aged obese non-FH men. In addition, one male FH patient had insulin-dependent diabetes mellitus.

Clinical Diagnosis of Familial Hypercholesterolemia

Most young FH patients do not have xanthomas. The concentration of LDL cholesterol in serum is a better discriminator between FH and non-FH patients than is total cholesterol. Therefore, serum LDL cholesterol concentration was chosen as the main criterion. Serum cholesterol levels show day-to-day variation, and serum LDL cholesterol levels of FH and non-FH patients overlap. Therefore, a three-stage classification (not FH, possible FH, and FH) was used.

The LDL cholesterol limits for these categories were derived from the Policy Statement of the European Atherosclerosis Society. According to this statement, LDL cholesterol levels >4.9 mmol/l (or total cholesterol >7.8 mmol/l) are compatible with adult FH, although adult FH patients usually have total cholesterol levels >9 mmol/l. Accordingly, for the present study the LDL cholesterol limits were chosen as follows: 1) <5.0 mmol/l for "not FH," 2) 5.0–5.9 mmol/l for "possible FH," and 3) ≥6.0 mmol/l for "FH." If a patient with a serum LDL cholesterol level in the "possible FH" range had unequivocal xanthomas, he or she was diagnosed as having FH. According to the policy statement, in children and adolescents serum total cholesterol levels >6.7 mmol/l are compatible with FH. Therefore, the LDL cholesterol limits for the diagnostic categories in children (<18 years) were set 1 mmol/l lower than those for the adults. Patients taking hypolipidemic drugs were judged to have FH if they were documented to have a pretreatment serum total cholesterol concentration ≥9 mmol/l. This criterion was used in 15 cases. One patient, the sister of a propositus, with a serum LDL cholesterol concentration of 4.8 mmol/l was considered to have FH despite the protocol outlined above because
1) she had metastasized carcinoma of the breast, 2) she reported an earlier serum cholesterol level of 9.5 mmol/l, and 3) her son had clinically evident FH with xanthomatosis.

The present criteria for the diagnosis of FH are more conservative than those presented in the policy statement, according to which all serum LDL levels compatible with FH (>4.9 mmol/l in adults) together with the presence of xanthomas in a relative (which was true for all the patients of the present series) provide a definite diagnosis of FH. Our decision to include adult patients with serum LDL cholesterol levels in the range of 5.0–5.9 mmol/l in the FH group only if xanthomas were present was based on the fact that serum cholesterol concentration levels are higher in the Finnish population than in most other Western populations.23,24

DNA Analysis

Leukocytic DNA was extracted from 10 ml EDTA-anticoagulated whole blood. The assay for the FH-Helsinki mutation, which is caused by a 9.5-kb deletion at the 3' end of the LDL receptor gene, was performed as previously described.15,20 In brief, DNA was digested with BamHI, fractionated by agar gel electrophoresis, transferred to nitrocellulose filters, and hybridized by using a cDNA probe specific to exons 11–17 of the LDL receptor gene. This probe was prepared by BamHI-XhoI digestion of pLDLR3 (a gift from Drs. D.W. Russell, M.S. Brown, and J.L. Goldstein, Dallas, Tex.), which contains 971 nucleotides. The presence of the FH-Helsinki mutation is characterized by the appearance of an extra 8-kb BamHI restriction fragment in addition to the normal 17-kb BamHI restriction fragment.15

For the determination of the XbaI polymorphism of apolipoprotein B, DNA samples were analyzed essentially as described above with the exception that the enzyme XbaI was used to digest the samples and that the apolipoprotein B cDNA probe pB23 (donated by Dr. Jan L. Breslow, New York) was used in the hybridization analysis.9 The allele resulting in the formation of an 8.6-kb XbaI fragment is designated as X71 and that generating a 5-kb fragment as X2.

The common apolipoprotein E alleles e2, e3, and e4 were determined by direct DNA analysis.26 In this method, the target DNA is amplified by the polymerase chain reaction (PCR) and subsequently analyzed by digestion with the restriction enzyme HhaI, followed by polyacrylamide gel electrophoresis of the cleavage products.26

Statistical Methods

Statistical calculations were carried out using BMDP statistical software program 7D to explore statistical significances of differences between groups and program 6D to compute linear correlations (BMDP Statistical Software, Inc., Los Angeles). All the data are given as mean±SEM.

Results

Clinical Versus DNA Diagnosis of Familial Hypercholesterolemia

Altogether, 65 subjects from 10 families took part in the study. Fifty-four were adults (including the 10 propositi), and 11 were children (<18 years). A clinical diagnosis of FH was set in 28 cases, six were in the “possible FH” category, and 31 subjects were considered not to have FH (Figure 1).

DNA analysis showed that 25 of the 28 subjects with the clinical diagnosis of FH had inherited the FH-Helsinki mutation (or the “M family” mutation; see below) of the LDL receptor gene (Figure 1). The three subjects who were misclassified as FH patients on clinical grounds were adult persons. In two of them, LDL cholesterol levels (6.1 and 6.2 mmol/l) were only slightly above the classification limit (6 mmol/l), whereas the third, a woman in her 60s, had an LDL cholesterol level of 6.8 mmol/l.

Four of the six subjects in the “possible FH” category proved to have inherited the FH-Helsinki or the M family mutation (Figure 1). Only one patient with a DNA diagnosis of FH was classified in the clinical “not FH” category. This 12-year-old boy, belonging to the M family (see below), had a serum LDL cholesterol concentration of 3.0 mmol/l.

Characteristics of the Low Density Lipoprotein Receptor Gene Mutation in the M Family

In one of the 10 families (the M family), the LDL receptor gene mutation turned out to be different from the true FH-Helsinki mutation, although the propositus of this family (a 65-year-old woman) had earlier been tentatively classified as a carrier of the FH-Helsinki gene. In the FH members of this family, the extra BamHI fragment hybridizable with the LDL receptor cDNA probe was slightly larger (11 kb) than that (8 kb) in families with the FH-Helsinki mutation (Figure 2). The boundaries of this 11-kb fragment were judged to be determined by the BamHI restriction sites in intron 10 and exon 18 (Figure 2). Digestion of the DNA samples with KpnI resulted, in addition to the formation of three invariant bands (about 23, 15, and 9 kb in size) both in the member of the M family and the control subjects, in the appearance of a faint band with a molecular size of about 3 kb (Figure 2). The generation of this fragment is apparently determined by the KpnI sites in introns 14 and 16 (Figure 2). The extra 10-kb Pvu II fragment is explainable by the narrowing of the distance between the Pvu II sites in introns 10 and 16 in the M mutation (Figure 2). The 5'-end and the 3'-end boundaries of the extra 18-kb EcoRV fragment in the mutant DNA are determined by the EcoRV restriction sites in exon 13 and at the 3'-flanking area of the LDL.

![Figure 1](http://atvb.ahajournals.org/)

**Figure 1.** Diagram showing clinical versus DNA diagnosis of familial hypercholesterolemia (FH) in adults (≥18 years) and children and adolescents (<18 years). FH+, FH+/-, and FH− denote the diagnostic categories “FH,” “possible FH,” and “not FH,” respectively, and the numbers denote quantities of patients in each category.
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Figure 2. Analysis of the low density lipoprotein (LDL) receptor gene mutation in the M family; for comparison, corresponding hybridization data for the FH-Helsinki mutation are also shown. Panel A: Southern blot hybridization analysis of DNA samples from a member of the M family (M), an FH patient from a family with a known carrier status for the FH-Helsinki mutation (H), and a healthy control (C). Restriction enzymes used are shown uppermost, and positions of molecular-size markers are at right. Panel B: Relevant restriction sites for KpnI (K), BamHI (B), PvuII (P), EcoRV (E), and XbaI (X) at the 3' end of the normal LDL receptor gene and schematic illustration of the FH-M (LDL receptor gene mutation in the M family) and FH-Helsinki deletions. FH, familial hypercholesterolemia.

receptor gene, respectively (Figure 2). The abnormal 5-kb XbaI fragment is likely to result from a closer distance between the XbaI sites in intron 12 and intron 16 in the mutant gene (Figure 2). Collectively, these data indicate that the M family mutation is most likely a deletion of about 6 kb, extending from intron 14 to intron 15 and thus deleting the entire exon 15 (Figure 2).

Lipoprotein Concentrations

Serum total and LDL cholesterol concentrations according to age and results of DNA analysis are plotted in Figure 3; data for the M family are indicated by separate symbols. The 12-year-old boy clinically misclassified as a non-FH subject in the M family had the lowest serum LDL cholesterol concentration recorded for known carriers of a mutant LDL receptor gene in the present study (Figure 3). Serum LDL cholesterol concentrations in the M family were otherwise well in the range of the other FH patients (Figure 3). Therefore, in subsequent analyses of lipoprotein levels in FH and non-FH subjects, data from the M family were combined with those of the other families.

All but two of the DNA-verified FH patients who were more than 40 years of age were taking hypolipidemic medication during the course of the present study. These two patients, including a 68-year-old woman with a metastasized carcinoma of the breast and a 44-year-old man with insulin-dependent diabetes mellitus and a strict low-fat/high-carbohydrate diet, had serum LDL cholesterol levels below the FH range (Figure 3). In untreated adult DNA-verified FH patients, serum total cholesterol concentrations ranged from 6.8 to 9.7 mmol/l, and in children and adolescents the corresponding values amounted to 4.7-7.3 mmol/l. The mean serum total cholesterol concentration of the untreated FH patients was 7.5±0.4 mmol/l, and that of non-FH relatives was 5.3±0.3 mmol/l.

Serum HDL cholesterol levels did not show significant variation in different categories of the study cohort (FH patients versus non-FH subjects, children and adolescents versus adults, or male versus female pa-
Factors Influencing Serum Low Density Lipoprotein Cholesterol Levels in Patients Not Taking Hypolipidemic Drugs

The mean serum LDL cholesterol level of the FH patients with an established DNA diagnosis who were not taking hypolipidemic drugs was 2.5 mmol/l higher than that of the non-FH patients (Table 2). Children and adolescents had a serum LDL cholesterol level approximately 1.4–1.7 mmol/l lower than that of the adults. In this study gender did not significantly affect serum LDL cholesterol levels.

There was a positive correlation between serum LDL cholesterol concentration and age in the non-FH group in particular (r=0.679, p<0.001; Figure 3). Serum LDL cholesterol levels were also closely correlated with the body mass index in the non-FH subjects (r=0.596, p<0.001), in the untreated FH patients (r=0.679, p<0.005), and in both groups combined (r=0.414, p<0.01). When the patients were divided into two groups according to their body mass index, the more obese group had a higher mean serum LDL cholesterol level (Table 2).

The apolipoprotein E allele frequencies in the total cohort examined were as follows: ε2, 0.10; ε3, 0.65; and ε4, 0.25. The frequency of the ε4 allele was higher in FH patients (0.33) than in their unaffected relatives (0.17; p<0.001). The presence of the ε4 allele was not associated with significant variations of serum LDL cholesterol level in the total number of untreated subjects or either of its subgroups, i.e., subjects with or without FH (Table 2). The frequency of the ε1 allele of the apolipoprotein B gene was 0.73 and that of the ε2 allele was 0.27 in the total cohort examined. There were no significant differences in the apolipoprotein B allele frequencies of subjects with and without FH. The presence of the ε2 allele was associated with a higher mean serum LDL cholesterol concentration than its absence in the non-FH subjects; this association was not found in FH patients (Table 2).

All of the three adult non-FH subjects who were misclassified as FH patients on clinical grounds had the X1X2 genotype of apolipoprotein B. One of them possessed the ε4/ε4 genotype, whereas the others had the genotypes ε3/ε2 and ε3/ε3 of apolipoprotein E. The corresponding apolipoprotein B and E genotypes of the FH child clinically misclassified as a non-FH subject were X1X2 and ε4/ε3, respectively.

Serum Low Density Lipoprotein Cholesterol Concentrations in Familial Hypercholesterolemia Patients Taking Hypolipidemic Drugs

The mean serum LDL cholesterol concentration in the 15 adult FH patients taking hypolipidemic drugs was 4.98±0.28 mmol/l. All of them reported consumption of a hypolipidemic diet. In this group the mean serum LDL cholesterol, HDL cholesterol, or triglyceride concentrations did not show significant variation according to the apolipoprotein E or B genotypes (data not shown), but female patients had a higher mean serum triglyceride levels.
TABLE 2. Effect of Age, Gender, Obesity, and the Polymorphisms of Apolipoproteins E and B on Serum Low Density Lipoprotein Cholesterol Concentration in Patients With a DNA Diagnosis of Familial Hypercholesterolemia and Their Non–Familial Hypercholesterolemia First-Degree Relatives

<table>
<thead>
<tr>
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<th>LDL cholesterol (mmol/l)</th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>n</td>
<td>All</td>
<td>n</td>
<td>Non-FH</td>
</tr>
<tr>
<td>All</td>
<td>50</td>
<td>4.2±0.2</td>
<td>35</td>
<td>3.5±0.2</td>
</tr>
<tr>
<td>Age &lt;18 yrs</td>
<td>11</td>
<td>3.4±0.4</td>
<td>6</td>
<td>2.3±0.2</td>
</tr>
<tr>
<td>≥18 yrs</td>
<td>39</td>
<td>4.4±0.3</td>
<td>29</td>
<td>3.7±0.3</td>
</tr>
<tr>
<td>p</td>
<td>NS</td>
<td></td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>Gender Male</td>
<td>30</td>
<td>4.3±0.3</td>
<td>19</td>
<td>3.5±0.3</td>
</tr>
<tr>
<td>Female</td>
<td>20</td>
<td>3.9±0.4</td>
<td>16</td>
<td>3.4±0.4</td>
</tr>
<tr>
<td>p</td>
<td>NS</td>
<td></td>
<td></td>
<td>NS</td>
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<tr>
<td>Obesity BMI ≥25 kg/m²</td>
<td>20</td>
<td>5.0±0.3</td>
<td>15</td>
<td>4.6±0.3</td>
</tr>
<tr>
<td>BMI &lt;25 kg/m²</td>
<td>30</td>
<td>3.5±0.3</td>
<td>20</td>
<td>2.7±0.2</td>
</tr>
<tr>
<td>p</td>
<td>&lt;0.01</td>
<td></td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Apo E Present*</td>
<td>17</td>
<td>4.2±0.4</td>
<td>10</td>
<td>3.2±0.4</td>
</tr>
<tr>
<td>Apo E Absent*</td>
<td>33</td>
<td>4.1±0.3</td>
<td>25</td>
<td>3.6±0.3</td>
</tr>
<tr>
<td>p</td>
<td>NS</td>
<td></td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Apo B X2 Present†</td>
<td>26</td>
<td>4.4±0.3</td>
<td>19</td>
<td>3.9±0.3</td>
</tr>
<tr>
<td>X2 Absent†</td>
<td>24</td>
<td>3.9±0.4</td>
<td>16</td>
<td>2.9±0.3</td>
</tr>
<tr>
<td>p</td>
<td>NS</td>
<td></td>
<td>0.03</td>
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Values are mean±SEM. Only patients not taking hypolipidemic drugs were included.
LDL, low density lipoprotein; FH, familial hypercholesterolemia; BMI, body mass index; apo, apolipoprotein; NS, not significant.
*Present, at least one allele present; absent, no allele present.
†X2 present, genotypes X1X2 or X2X2; X2 absent, genotype X1X1.

HDL cholesterol level than did the male patients (1.42±0.09 versus 1.13±0.08 mmol/l, p=0.02).

Findings on Physical Examination and History of Coronary Heart Disease

Xanthomatosis was demonstrated in 17 of the 25 adult DNA-verified FH patients (68%) but in none of the non-FH patients (Figure 4). In addition, xanthomatosis was suspected in one young and six adult FH patients, as well as in two non-FH patients who both were more than 55 years of age. Thus, only two (8%) of the 25 adult FH patients were totally free of signs of xanthomatosis. Unequivocal xanthomatosis was most commonly noted in the Achilles tendons, whereas suspected xanthomas were often recorded in the subperiosteal regions (Table 3). The Achilles tendons of the adult FH patients were significantly thicker than those of the non-FH subjects (Table 3; p<0.001).
TABLE 3. Occurrence of Xanthomas and the Latero-Lateral Thickness of the Achilles Tendon in Familial Hypercholesterolemia and Non–Familial Hypercholesterolemia Subjects

<table>
<thead>
<tr>
<th></th>
<th>FH</th>
<th>Non-FH</th>
</tr>
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<tbody>
<tr>
<td>Thickness ≥20 mm</td>
<td>19 (76)</td>
<td>2 (40)</td>
</tr>
<tr>
<td>Thickness, mean</td>
<td>21.5±0.9</td>
<td>17.7±2.4</td>
</tr>
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</table>

Table shows the numbers of patients, with percentages in parentheses. Classification into FH and non-FH groups was based on DNA analysis.

FH, familial hypercholesterolemia.

Using somewhat modified criteria of an authoritative recommendation, we achieved a correct clinical diagnosis of FH in 55 (85%) of the 65 subjects examined. Four subjects were placed in an incorrect diagnostic category, and six subjects were assigned to the “possible FH” group. In the age group <18 years, only two of the five FH children were correctly diagnosed clinically because the serum LDL levels in the remaining three patients were lower than the diagnostic limits adopted. In this age group tendon xanthomas are a rarity and, therefore, virtually never assist in the diagnosis. All these data emphasize the usefulness of DNA methods as an adjunct in the diagnosis of heterozygous FH.

When FH is suspected on clinical presentation, the age of the patient should be carefully taken into account. If the Finnish age- and sex-specific 95th percentile LDL cholesterol concentration curves (Reference 27 and A. Reunanen, unpublished) were depicted in Figure 3, the serum LDL cholesterol concentrations of the present non-FH subjects would not exceed this 95th percentile level. The corresponding value would be below the age- and sex-specific 95th percentile level in only four FH patients, including two children and a 44-year-old man with insulin-dependent diabetes mellitus and a strict low-fat diet, as well as the patient with metastasized carcinoma of the breast who was discussed earlier. In fact, the introduction of the age- and sex-specific 95th percentile LDL cholesterol values instead of the rigid ones originally employed would have permitted a correct diagnosis (FH versus non-FH) in 95% of the subjects of the present study.

During the initial screening, all propositi of the present study were judged to be carriers of the FH-Helsinki mutation of the LDL receptor gene. This mutation, unique to the Finnish population, is an ≈9.5-kb-long deletion of the LDL receptor gene that eliminates exons 16 and 17 and part of exon 18, resulting in a truncated LDL receptor protein and leading to a defective internalization of LDL particles. During the progress of the present study, one of the probands and six of her affected relatives belonging to the M family were demonstrated to have an LDL receptor gene deletion reminiscent of but yet different from the FH-Helsinki mutation. Further studies revealed that this mutation is due to an ≈6-kb deletion extending from intron 14 to intron 15 (Figure 2). The determination of the exact boundaries of this deletion was, however, not possible on the basis of the current data. We have designated this mutation FH-Espoo according to the residence of the M family. Although the mean serum LDL cholesterol levels in the FH-Espoo family were not different from those in other families, a 12-year-old boy with FH-Espoo and his affected cousin had relatively low serum LDL cholesterol levels (3.0 and 4.0 mmol/l, respectively; Figure 3). The possibility that the low LDL cholesterol levels in the two young cousins could be due to a “cholesterol lowering gene” is made less likely by the fact that their fathers were carriers of the M family mutation and had high serum LDL cholesterol levels. In the families with the FH-Helsinki mutation, a total of 23 subjects were identified as carriers of the mutant gene by DNA analysis. None of them was clinically classified in the non-FH category, and only four of them, each from separate families, were classified as “possible FH” patients. Collectively, we were unable to find evidence...
for the occurrence of a putative cholesterol-lowering gene in the families examined by us.

Polymorphisms of the genes encoding the ligands for the LDL receptor, apolipoproteins E and B, have been shown to affect serum total and LDL cholesterol levels in FH patients in some but not in all studies. In the present study, in which only a relatively small group of untreated patients was available, these polymorphisms did not significantly affect serum LDL cholesterol levels in FH patients. Of the non-FH relatives, carriers of the apolipoprotein X2 allele had a higher mean serum LDL cholesterol level than did those without this allele, which is in accordance with our earlier findings in a random Finnish population sample. The overrepresentation of apolipoprotein E allele e4 in our FH patients compared with unaffected relatives is very likely a chance association because the genes for the LDL receptor and apolipoprotein E have been localized on opposite ends of chromosome 19 and should therefore not be linked. No association between FH and any apolipoprotein E phenotype has been noted in other studies.

The present study, in which the ultimate diagnosis of FH was based on DNA analysis and thus is unequivocal, confirms the findings of earlier studies of the prevalence of some physical findings in heterozygous FH patients. Tendon xanthomatosis was encountered in 68% of the adult FH patients, which compares well with earlier estimates. The simple measurement of the Achilles tendon thickness (≥20 mm) identified three quarters of the adult FH patients. This percentage is almost as high as that achieved by measuring Achilles tendon thickness radiologically. Our simple method is, however, less precise than the radiological methods, and an excess of subcutaneous fat may cause falsely elevated values. In fact, in the present series the two non-FH subjects with abnormally thick Achilles tendons were both obese.

In conclusion, our study shows that more than 10% of adult members belonging to FH families may be incorrectly classified as FH or non-FH subjects when simple clinical criteria like those in the present study are used. However, when age-, population-, and sex-specific 95th percentile values for serum LDL cholesterol are used instead of common reference limits for all adults, only one of 20 subjects is incorrectly classified as to the FH status. In doubtful cases and in particular in the evaluation of children, detection or exclusion of an LDL receptor gene mutation is a very useful adjunct to confirm or refute the diagnosis. The applicability of DNA diagnostics is especially conspicuous in populations with an enrichment of specific LDL receptor mutation(s) until convenient simultaneous screening techniques for multiple mutations of a specific gene become available.

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Diagnosis of heterozygous familial hypercholesterolemia. DNA analysis complements clinical examination and analysis of serum lipid levels.

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