Prevalence of familial hypercholesterolemia among young North Karelian patients with coronary heart disease: a study based on diagnosis by polymerase chain reaction

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Abstract Two deletions of the low density lipoprotein (LDL) receptor gene account for about 90% of the mutations that cause familial hypercholesterolemia (FH) in eastern Finland. The FH-Helsinki mutation deletes exons 16, 17 and a portion of exon 18, while the FH-North Karelia allele is characterized by a deletion of seven nucleotides from exon 6 of the LDL receptor gene. We developed a DNA assay based on the use of polymerase chain reaction (PCR) which simultaneously detects both of these mutations. We have screened 90 young (< 45 years) eastern Finns with symptomatic coronary heart disease (CHD) for the presence of these FH genes. One or the other of the mutations was present in 4 out of 55 survivors of acute myocardial infarction (AMI) and 4 out of 35 patients with angina pectoris (AP), but in none of 50 healthy controls of similar age. These data show a relatively high prevalence of confirmed FH in young CHD patients (AMI and MI combined: 8/90, or 9%), and also demonstrate the feasibility of PCR techniques in diagnosis of FH among populations with enrichment of specific types of LDL receptor gene mutations.—**Koivisto, U-M., L. Hämäläinen, M-R. Taskinen, K. Kettunen, and K. Kontula.** Prevalence of familial hypercholesterolemia among young North Karelian patients with coronary heart disease: a study based on diagnosis by polymerase chain reaction. *J. Lipid Res.* 1993. **34:** 269–277.

Supplementary key words low density lipoprotein receptor • DNA amplification • myocardial infarction • angina pectoris • serum lipoproteins

Coronary heart disease (CHD) is a multifactorial disorder in which several intrinsic and extrinsic factors, such as age, sex, blood pressure, serum lipids, fat intake, cigarette smoking, and physical activity may modify the individual's genetically determined risk. A number of population, case-control, and intervention studies have shown a positive correlation between serum low density lipoprotein (LDL) cholesterol level and risk of CHD whereas there is an inverse relationship between serum high density lipoprotein (HDL) concentration and occurrence of CHD (1–6).

There is evidence that certain forms of primary (inherited) dyslipoproteinemias occur much more often in patients with symptomatic CHD than in the general population. Familial hypercholesterolemia (FH) is an autosomally co-dominantly inherited disease that is characterized by greatly elevated serum LDL cholesterol levels, tendon xanthomata, and premature atherosclerosis (7). FH heterozygotes occur in the general population with a frequency of about 1 in 500 whereas FH homozygotes are very rare (7). However, a hyperlipidemic (type IIa) disorder with features of FH was the underlying disorder in 2.6% of 193 survivors of myocardial infarction reported by Pattersson and Slack (8), 4.1% of the 366 survivors below 60 yr studied by Goldstein et al. (7, 9), and 6.0% of 101 young (age less than 50 yr) survivors of the study of Nikkilä and Aro (10). These same studies suggest that familial combined (also cited as multiple-type) hyperlipoproteinemia may be an even more common inherited disorder in these patients, with a prevalence of 10–11% (9, 10); however, the genetic basis of this form of hyperlipidemia remains unknown.

We have recently demonstrated that two common mutations of the LDL receptor gene make up about two-thirds of the FH mutations in Finland, apparently reflecting a founder gene effect (11, 12). The 9.5-kb FH-Helsinki deletion extends from intron 15 to exon 18 resulting in a truncated LDL receptor protein with an internalization-defective phenotype of FH (11). The FH-North Karelia mutation deletes seven nucleotides from exon 6 of the

Abbreviations: FH, familial hypercholesterolemia; PCR, polymerase chain reaction; CHD, coronary heart disease; AP, angina pectoris; AMI, acute myocardial infarction; LDL, low density lipoprotein; HDL, high density lipoprotein.

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LDL receptor gene and is associated with a receptor-negative FH phenotype (12). One or the other of these mutations is found in about 90% of FH patients living in the eastern Finland (12). The aims of the present study were, first, to set up a simultaneous nonisotopic assay for these two LDL receptor gene mutations; and second, to determine the exact prevalence of FH in young patients with symptomatic CHD.

PATIENTS AND METHODS

Patients with acute myocardial infarction (group AMI)

Fifty five consecutive patients aged 45 yr or less treated because of acute myocardial infarction (AMI) at the Central Hospital of North Karelia, Joensuu, between December 1987 and June 1991 were enrolled for the present study. There were 53 males and 2 females. The criteria of AMI were as follows: an intensive retrosternal pain lasting at least 30 min; serial electrocardiographic changes typical of a Q-wave infarction (a transient elevation of the S-T segment more than 1 mm, inverted T waves, and the subsequent appearance of pathological Q waves in appropriate limb or precordial recordings) or changes attributable to a non-Q-wave infarction (deep, symmetrical T waves in appropriate recordings, lasting more than 24 h); and a rise in the activity of the serum creatine kinase and/or creatine kinase MB isoenzyme.

Patients with effort-induced angina pectoris (group AP)

The AP group consisted of 35 consecutive patients 45 yr or younger that were referred between April 1988 and May 1991 to the cardiological outpatient ward of the same hospital because of exercise-related retrosternal tightness or dyspnea. The discomfort was relieved quickly upon resting or intake of nitroglycerin. Other criteria included an S-T wave depression amounting to at least 1 mm in precordial electrocardiographic recordings during subjective maximal exercise testing; a perfusion defect on 99mTc-emission tomography examination during exercise; and exclusion of other cardiomyopathies and valvular diseases by two-dimensional echocardiography. Thirty two of the patients were men and 3 women.

Control subjects

The control samples were collected from 50 subjects (42 males, 8 females) of similar age. They were recruited among attendants of routine health examinations at the Joensuu Occupational Health Center between August 1988 and October 1991, and were devoid of any signs of cardiovascular disease as judged by health questionnaire, clinical examination, exercise testing, and two-dimensional echocardiography.

The population bases of the service areas for the Central Hospital of North Karelia, Joensuu (AP and AMI subjects), and the Joensuu Occupational Health Center (control subjects) are identical, comprising altogether 180,000 people living in the North Karelia county; of this number about 50,000 live in the Joensuu town and 130,000 in the surrounding communes. Analysis of the birth places of the AP, AMI, and control subjects indicated that in each group more than 75% of the subjects were born in a place located in the present-day North Karelia county.

PCR assay for the two Finnish-type LDL receptor gene mutations

Primers (P1–P5) for a single-tube polymerase chain reaction (PCR) were designed in such a way as to permit the generation of amplified DNA fragments informative enough to allow the identification of the presence of the normal allele, the FH-North Karelia gene, and the FH-Helsinki gene in any of their possible combinations (see Fig. 1). The sequences for the oligonucleotide primer P1 (5'-GCATCACCCTGGACAAAGTC-3') and P4 (5'-CTGAGACACCCGGTTACCTT-3') were derived from the coding strand, and primer P5 (5'-ATCCCAACACA-CACGACAGA-3') from the noncoding strand of the LDL receptor gene according to Yamamoto et al. (13). Primer P2 (5'-GCAAGCCGCCTGCACCGAGACTCAC-3'), homologous to intron 6 sequence flanking exon 6, was designed according to Leitersdorf et al. (14). Primer P3 (5'-AACAGTTCTTGCCCTCTTTG-3'), homologous to intron 15 sequence close to its 5' end, was designed according to the data of Aalto-Setälä et al. (11). These PCR primers were synthesized on an Applied Biosystems 381A DNA synthesizer by the β-cyanoethyl phosphoramidite method (15).

DNA was prepared from 20 ml of EDTA-anticoagulated venous blood according to Bell, Karam, and Rutter (16). DNA (100 ng per sample) was amplified in a mixture (total volume 30 μl) containing the five primers P1–P5 (final concentration 1 μM for P1–P4 and 1.5 μM for P5), 0.2 mM each of dATP, dCTP, dGTP, and dTTP, 10 mM Tris-HCl (pH 8.4), 50 mM KCl, 1.5 mM $MgCl_2$, 0.1 mg/ml gelatin, and 1 U of AmpliTaq DNA polymerase (Perkin-Elmer Cetus, Norwalk, CT). Thirty PCR cycles for 1 min each at 95°C, 55°C, and 72°C, followed by a final extension reaction for 10 min at 72°C, were performed in a DNA thermal cycler (Techne PHC-2, Techne Ltd., Cambridge, U.K.). After amplification, the samples were electrophoresed on a 12% polyacrylamide gel in a buffer containing 90 mM Tris-borate (pH 8.4) and 4 mM EDTA for 16 h (or until the xylene cyanole dye reached the bottom of the gel) at a current of 9 mA. After electrophoresis, the gels were stained with ethidium bromide and the amplified DNA fragments were visualized by ultraviolet illumination. Some preliminary experiments were carried

out using the protocol described above, with the exception that 0.2 μl of [α^{32}P]dCTP (3,000 Ci/mmol, Amersham, U.K.) was added to the PCR; after PCR, samples were diluted 30-fold and electrophoresed on polyacrylamide gel; the amplified DNA fragments were visualized by autoradiography.

A panel of DNA samples from previously established patients with a known carrier status of the FH-Helsinki (11) or FH-North Karelia gene (12) was used during set-up and optimization of the assay conditions. In addition, a DNA sample from a compound heterozygote FH patient with one FH-Helsinki and one FH-North Karelia gene was available.

Serum lipid assays

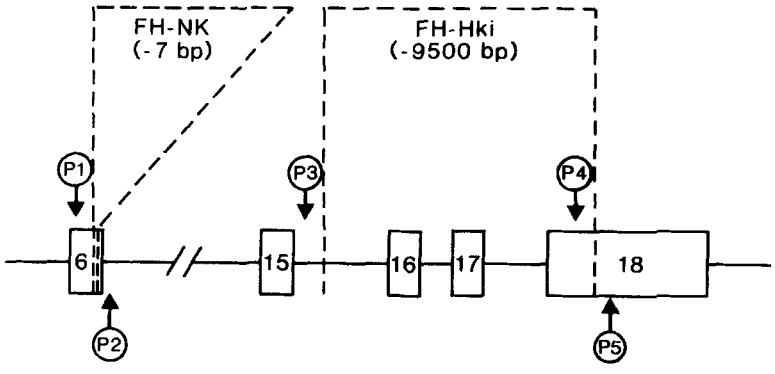
Serum lipid analyses were carried out on blood samples from subjects who had fasted for 12 h. In most cases of the AMI group, blood samples were obtained on the first morning after admission to the hospital (n = 37). If this specimen was not available, serum lipid determinations were carried out on blood samples collected from ambulatory patients 3 months after myocardial infarction (n = 18). Previous studies on patients with acute myocardial infarction have shown a good agreement between serum lipid levels measured on admission and 3 months later (17, 18). Furthermore, in the present series there were 21 cases in which both the on-admission and the 3-months' follow-up samples were available for comparison: there were no significant differences in the mean serum cholesterol, LDL cholesterol, HDL cholesterol, or triglyceride levels ($P > 0.2$ in each case) in this cohort. Data of the on-admission and the follow-up samples were therefore pooled in the statistical analysis of serum lipid levels in the different study groups. Lipoprotein fractions were isolated by sequential ultracentrifugation of the fasting serum samples (19). Cholesterol concentrations in the whole serum and in the LDL and HDL fractions as well as triglyceride concentration in the whole serum were determined by enzymatic methods (20, 21) using commercial kits from Boehringer (Mannheim, Germany).

At the time of sample collection, only three subjects, subsequently shown to possess a mutated LDL receptor gene, used hypolipidemic drugs. Pre-treatment serum total cholesterol, triglyceride, and HDL cholesterol concentrations (22) were used in these cases, and the serum LDL cholesterol concentration was derived from the Friedewald formula (23).

Statistical methods

Comparison of serum lipid levels in different categories of subjects was carried out using the Macintosh Statworks™ program (Cricket Software Inc., Philadelphia, PA). Analysis of serum cholesterol, LDL cholesterol, and HDL cholesterol levels in the three study groups did not reveal any significant deviation of normal distribution; *t*-test was used for statistical evaluation of these data. Similar analysis of serum triglyceride levels disclosed a deviation from normality approaching statistical significance in

A.



B.

PCR DESIGN		GENOTYPE					
PRIMER PAIR	PRODUCT SIZE	N N	N NK	NK NK	N Hki	Hki Hki	NK Hki
P1-P2	100	+	+	—	+	+	+
P1-P2	93	—	+	+	—	—	+
P3-P5	159	—	—	—	+	+	+
P4-P5	209	+	+	+	+	—	+

Fig. 1. Principle of the PCR assay for the two Finnish-type of LDL receptor gene mutations, FH-North Karelia (NK) and FH-Helsinki (Hki). A. Scheme of the positioning of the oligonucleotide primers (P1-P5) along the LDL receptor gene. Exons are indicated by numbered boxes, and the boundaries of the two deletions by dashed lines. Note that the figure is not drawn to scale. B. A key for interpretation of the data.

the control (P = 0.06) and AMI (P = 0.07) groups. This was eliminated by logarithmic transformation of the original data, which was thus applied to statistical comparison of all serum triglyceride data.

RESULTS

The principle of the PCR assay for the two Finnish-type LDL receptor gene mutations is summarized in **Fig. 1.** The positioning and nucleotide compositions of the PCR primers P1–P5 were based first, on efficient amplification of all relevant DNA fragments under approximately similar conditions in the same test tube; and second, on generation of DNA fragments that could be size-fractionated in a fully informative way by a single electrophoretic run. The primer pair P1–P2 amplifies a DNA fragment of either 93 or 100 bp in size, depending on whether the exon 6 -mutated FH-North Karelia allele is present or not, respectively (Fig. 1). If the DNA contains the FH-Helsinki mutation, the primers P3 and P5 are brought close enough to each other to allow the amplification of a DNA fragment 159 bp in size; otherwise the distance between these two primers (about 9.5 kbp) is too long to permit any efficient amplification of DNA. The primer pair P4–P5, directing the amplification of a 209-bp DNA fragment, is necessary in order to differentiate between patients heterozygous and homozygous for the FH-Helsinki gene (Fig. 1).

For preliminary experiments we used a collection of DNA samples with a known carrier status of the FH-Helsinki gene or the FH-North Karelia gene. The presence of these mutations was previously confirmed by a Southern blot analysis (11) or a PCR technique combined with DNA sequencing over the mutated region (12), respectively. There was complete agreement between the data from these techniques used earlier and the results of the present PCR assay. Examples of the PCR assay are shown in **Fig. 2A** (detection by autoradiography) and Fig. 2B (detection by ethidium bromide staining). DNA samples from FH patients heterozygous either for the FH-Helsinki or the FH-North Karelia mutation were readily distinguished from the samples without these mutations. In addition to the presence of the four anticipated types of PCR products in varying combinations in different DNA samples, an extra band with a slower migration rate was identified whenever one FH-North Karelia allele was present in the sample (Fig. 2). This extra band appears to result from generation of heteroduplexes (24) by annealing of the two heterologous (normal and mutant) DNA strands, determined by the primer pair P1–P2 and differing by seven nucleotides in size. A DNA sample derived from a known compound heterozygous FH patient with one FH-Helsinki and one FH-North Karelia gene also generated an expected pattern of amplified DNA fragments, including the heteroduplex band (Fig. 2). No subjects homozygous for either of the FH-Helsinki or FH-North Karelia genes are known to live in Finland at present.

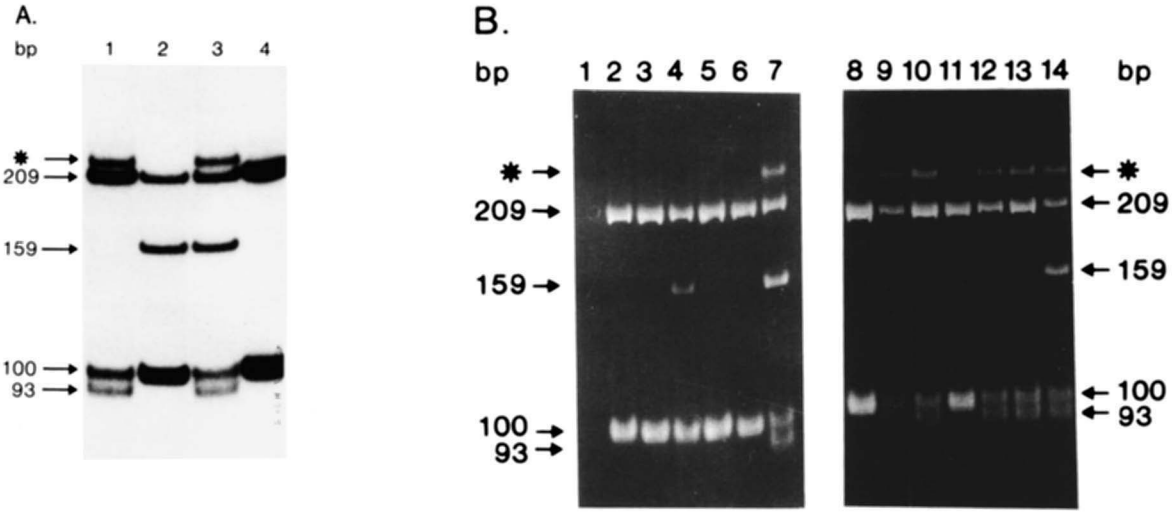


Fig. 2. A: Visualization of the ^{32}P-labeled PCR products after size-fractionation on a 12% polyacrylamide gel and autoradiography (preliminary experiments). Lane 1, a patient heterozygous for the FH-North Karelia mutation; lane 2, a patient heterozygous for the FH-Helsinki mutation; lane 3, a compound heterozygous FH patient with one FH-North Karelia and one FH-Helsinki gene; lane 4, a healthy control. The asterisk shows the position of the heteroduplex band. B: Detection of the nonlabeled PCR products after size-fractionation on a 12% polyacrylamide gel and staining with ethidium bromide (results from two separate runs). Lane 1, a buffer blank (no DNA); lanes 2, 3, 5, 6, 8, and 11, healthy controls; lane 4, patient heterozygous for the FH-Helsinki mutation; lanes 9, 10, 12, and 13, patients heterozygous for the FH-North Karelia mutation; lanes 7 and 14, the compound heterozygous FH patient with one FH-North Karelia and one FH-Helsinki gene. The asterisk shows the position of the heteroduplex band.

TABLE 1. Results of the PCR assay in the three study groups

Group	n	FH-NK Negative FH-Hki Negative	FH-NK Positive	FH-Hki Positive
Control	50	50	0	0
AP	35	31	4	0
AMI	55	51	3	1
Total	140	132	7	1

FH-NK, the FH-North Karelia mutation; FH-Hki, the FH-Helsinki mutation.

One or the other of the two FH genes was found in 8 (9%) out the 90 CHD patients screened but in none of the control subjects (**Table 1**). Seven of the mutations were of the FH-North Karelia type and only one of the FH-Helsinki type, a finding consistent with their relative prevalences among heterozygous FH patients in eastern Finland (12). None of the eight FH patients were known to be siblings or cousins with each other.

The mean serum total and LDL cholesterol concentrations were higher among the CHD patients than in controls; this difference was statistically significant in the AP group (**Table 2**). In contrast, there were no significant differences in the mean serum HDL cholesterol levels between groups of the present series (Table 2). When those patients in the AP and AMI groups that were subsequently identified as carriers of either of the two FH genes were excluded in the calculations, serum total and LDL cholesterol concentrations in the AP group (6.3 ± 1.1 and 4.0 ± 0.9 mmol/l, respectively) remained significantly ($P < 0.01$) higher than the corresponding levels in the control group (5.6 ± 1.1 and 3.4 ± 0.9 mmol/l, respectively). The corresponding lipid values in the AMI patients without either deletion were 5.9 ± 1.5 mmol/l ($P = 0.24$ vs. the controls) and 3.7 ± 1.4 mmol/l ($P = 0.19$), respectively.

Serum cholesterol levels of the CHD patients with and without a deleted LDL receptor gene were partially overlapping, indicating that among coronary patients serum total cholesterol determinations alone are not sufficient to identify heterozygous FH patients (**Fig. 3A**). A better, if not complete, separation between the two groups was achieved when serum LDL cholesterol concentration was used as a discriminant (Fig. 3B). We cannot exclude the possibility that some of the patients with serum LDL cholesterol levels about 6 mmol/l or more but without the two assayable gene deletions (Fig. 3B) could also have FH, caused by a yet undiscovered type of LDL receptor gene mutation.

DISCUSSION

In this report we describe a PCR assay that detects the two LDL receptor gene deletions that account for approximately two-thirds of the FH mutations in Finland (11, 12). This PCR technique is based on the simultaneous amplification of normal and mutant fragments over the deletion sites present in the FH-Helsinki and FH-North Karelia genes. The anticipated PCR products corresponding to the normal allele (209 and 100 base pairs in size), the FH-Helsinki gene (159 base pairs), and the FH-North Karelia gene (93 base pairs) were readily detected by polyacrylamide gel electrophoresis followed by ethidium bromide staining (Fig. 2B). When DNA samples from patients heterozygous for the FH-North Karelia gene were analyzed with this technique, annealing of the two heterologous DNA strands derived from amplification of exon 6 created the formation of a heteroduplex DNA molecule that could be visualized as an extra band on polyacrylamide gel electrophoresis, thus augmenting the identification of this allele (Fig. 2). The diagnostic value of heteroduplex formation was recently demonstrated by Triggs-Raine and Gravel (25) who applied this principle to reveal the presence of a 4-bp insertion mutation of the hexosaminidase A gene present in many Ashkenazi patients with Tay-Sachs disease, and by Meiner et al. (26) who identified a 3-bp deletion of exon 4 of the LDL receptor gene prevalent among Ashkenazi Jews. In

TABLE 2. Mean age, sex distribution, and mean (± SD) serum lipid levels in the three study groups

Parameter	Controls	AP	AMI
No. of subjects	50	33[a]	54[a]
Males/females	42/8	31/2	52/2
Age	38.7 ± 4.4	40.8 ± 4.0	40.8 ± 4.3
Total cholesterol (mmol/l)	5.6 ± 1.1	6.6 ± 1.5[b]	6.1 ± 1.7
LDL cholesterol (mmol/l)	3.4 ± 0.9	4.3 ± 1.4[b]	3.9 ± 1.6
HDL cholesterol (mmol/l)	1.33 ± 0.35	1.25 ± 0.32	1.28 ± 0.44
Triglycerides (mmol/l)	1.62 ± 1.31	2.15 ± 1.02[c]	1.92 ± 1.38

[a]Two patients from the AP group and one patient from the AMI group were omitted from the statistical comparison of the lipid levels because results from assays based on sequential ultracentrifugation were not available in these cases.

[b]$P < 0.001$; [c]$P < 0.01$, in comparison to controls.

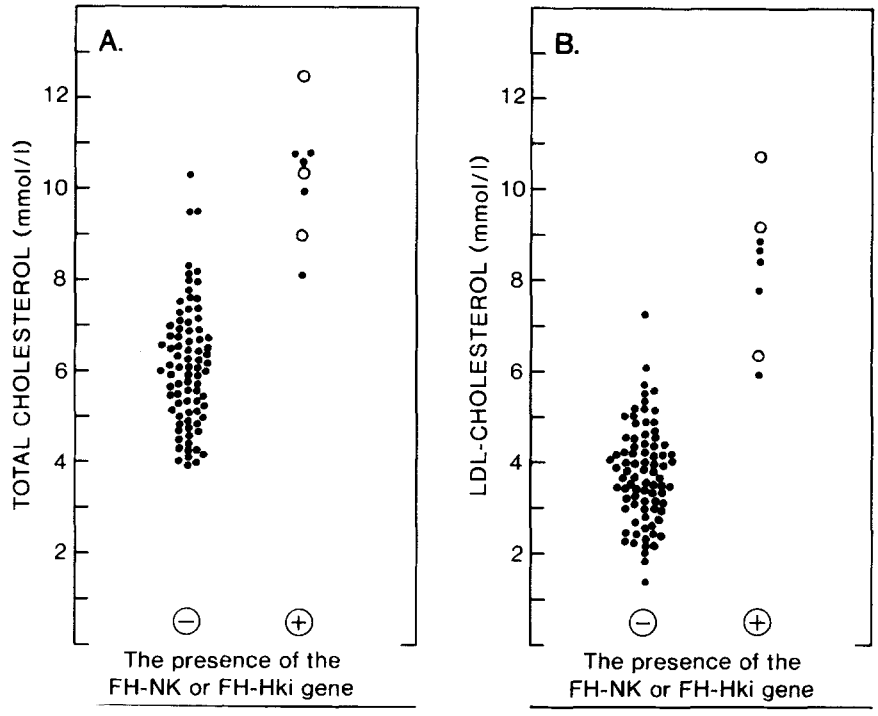


Fig. 3. Serum total cholesterol (A) and LDL cholesterol (B) concentrations in the patients with CHD (AMI and AP groups combined). The whole group (n = 90) was subcategorized into two groups (n = 82 and n = 8) according to the results of the PCR assay. FH-NK = the FH-NK mutation, FH-Hki = the FH-Hki mutation. Open symbols indicate those three cases in which separation of serum lipoproteins was based on the magnesium chloride/dextran sulfate precipitation; in the remaining cases, this was accomplished by sequential ultracentrifugation of serum.

summary, our PCR assay is reliable, easy to perform, avoids the use of radioisotopes, and is even applicable to routine clinical laboratories.

For FH diagnostics, PCR techniques are potentially most useful in populations in which one or a few mutant genes have been enriched by an apparent founder gene effect (11, 12, 27–29). Methods properly tailored according to the nature of the mutation can be designed for both major rearrangements (30, 31), small deletions (26), or single nucleotide alterations (14, 29, 32) of the LDL receptor gene. Our previous studies have shown that methods based on DNA techniques may provide an unequivocal diagnosis in cases of suspected but yet unconfirmed cases of FH (31), as well as confirm or exclude this disease in members of families with a known carrier status of a prevalent LDL receptor gene mutation (33). In fact, we experienced that without molecular genetic methods the correct categorization into FH and non-FH subjects is occasionally difficult even in first degree relatives of established heterozygous FH patients (33).

Our study demonstrates that FH is highly prevalent among symptomatic CHD patients of about 40 yr of age, at least in eastern Finland. The recorded prevalence (8/90 or 8.9%) must be considered as a minimum estimate as the two gene deletions assayed do not absolutely cover the spectrum of mutations causing FH in the study population (12). The figure is somewhat higher than previous estimates (4.1–5.9%) on FH among relatively young survivors of myocardial infarction (9, 10). Another recent study on familial dyslipidemia syndromes in familial early-onset coronary heart disease showed that FH was an underlying disorder in only 3% of the families screened (34). These disparities in our own and the previous estimates for the occurrence of FH in CHD patients may at least partially be explained by differences in patient selection: the age cut-off in the present study (45 yr) was lower than those (50–65 yr) chosen for earlier studies (9, 10, 34). It is also possible that differences in the prevalence of FH among young CHD patients may reflect similar differences in the prevalence of FH in the respective populations. Unfortunately, there is no exact information on the prevalence of heterozygous FH patients in all of Finland or different parts of it, although nothing suggests that it would be significantly higher than the average estimate (1 in 500) for many other European populations (7).

Premature coronary heart disease is by far the most important clinical manifestation of FH. According to Slack (35) CHD appears at the average age of 43 yr in male and 53 yr in female patients with the heterozygous form of FH. In the study of Stone et al. (36) acute myocardial in-

farction or sudden death occurred by the mean age of 42 yr in men and 64 yr in women. Hirobe et al. (37) reported that the clinical sequelae of CHD (angina pectoris or myocardial infarction) occurred with incidence of 42% in males under 50 and 82% in those aged over 50 yr; the corresponding figures for females were 25% and 30%, respectively. Data from the angiographic study of Mabuchi et al. (38) suggest that the process of coronary stenosis begins at 17 and 25 yr of age in the male and female heterozygotes, respectively. Except the mutant gene as such, other determinants of CHD risk among FH heterozygotes may include a low serum HDL cholesterol level (37, 39, 40), an elevated level of serum triglycerides (40, 41) and lipoprotein[a] (41), as well as a low rate of bile acid synthesis (42). According to one study there is an association between the apolipoprotein E4 phenotype and predisposition to CHD in FH (43), whereas another study showed only a trend towards such a relation (44). Finally, the nature of the mutation itself may affect the risk of CHD in FH, at least in its homozygous form, in that CHD occurs later and is less extensive in patients with some residual LDL receptor activity (receptor-defective patients) than in those with no measurable LDL receptor activity (receptor-negative patients) (1, 40).

Our data underscore the importance of considering the possibility of FH in all symptomatic CHD patients aged 45 yr or younger. This recommendation is additionally supported by the recent findings that show regression of atherosclerotic lesions in response to effective lipid-lowering therapy in FH (45–48). None of the eight CHD patients of the present series that were ultimately found to carry a mutated LDL receptor gene had previously been diagnosed as an FH heterozygote although three of them were on hypolipidemic drugs. This study prompted a more detailed questionnaire and clinical examination of these patients. Using the diagnostic criteria for heterozygous FH by the Finnish health authorities: *a)* serum total cholesterol level permanently more than 9 mmol/l; *b)* exclusion of a significant elevation of serum triglyceride concentration or other causes of secondary hypercholesterolemia; and *c)* the presence of tendon xanthomas, or the presence of familial hypercholesterolemia or premature CHD in at least one first-degree relative of the patient, a correct diagnosis of FH could be retrospectively made in only six out of the eight cases. Seven patients had at least one first-degree relative with a coronary death before the age 65, and five patients had clearcut extensor tendon xanthomas.

In conclusion, we have set up a PCR assay for two LDL receptor gene mutations very prevalent among the Finns. This convenient technique proved to yield indispensable help to accurately identify subjects with the heterozygous form of FH in a group of patients in which diagnosis of FH should immediately result in effective measures of secondary prevention.

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