The Familial Hypercholesterolemia (FH)–North Karelia Mutation of the Low Density Lipoprotein Receptor Gene Deletes Seven Nucleotides of Exon 6 and Is a Common Cause of FH in Finland

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Abstract

A mutation of the LDL receptor gene very common among Finnish patients with heterozygous familial hypercholesterolemia (FH) was identified. This mutation, designated as FH–North Karelia, deletes seven nucleotides from exon 6 of the LDL receptor gene, causes a translational frameshift, and is predicted to result in a truncated receptor protein. Only minute quantities of mRNA corresponding to the deleted gene were detected. Functional studies using cultured fibroblasts from the patients revealed that the FH–North Karelia gene is associated with a receptor-negative (or binding-defective) phenotype of FH. Carriers of the FH–North Karelia gene showed a typical xanthomatous form of FH, with mean serum total and LDL cholesterol levels of 12 and 10 mmol/liter, respectively. This mutation was found in 69 (34%) out of 201 nonrelated Finnish FH patients and was especially abundant (prevalence 79%) in patients from the eastern Finland. These results, combined with our earlier data on another LDL receptor gene deletion (FH–Helsinki), demonstrate that two "Finnish-type" mutant LDL receptor genes make up about two thirds of FH mutations in this country, reflecting a founder gene effect. This background provides good possibilities to examine whether genetic heterogeneity affects the clinical presentation or responsiveness to therapeutic interventions in FH. (*J. Clin. Invest.* 1992. 90:219–228.) Key words: DNA • low density lipoprotein cholesterol • low density lipoprotein receptor • messenger RNA • polymerase chain reaction

Introduction

The LDL receptor occupies a central role in the regulation of cholesterol homeostasis (1, 2). Localized on the plasma membrane, it promotes the cellular uptake of lipoprotein particles containing apo B and/or apo E and thus determines the catabolic rate of serum LDL and its precursors, intermediate-density lipoprotein and VLDL particles (1, 2). The human LDL receptor is a transmembrane glycoprotein composed of 839 amino acids; and its structural gene, containing 18 exons and 17 introns, is located on the short arm of chromosome 19 (3, 4). Overexpression of the human LDL receptor gene in transgenic mice was shown to dramatically lower its circulating ligand, under conditions both with (5) and without diet-induced hypercholesterolemia (6).

Mutations of the LDL receptor gene result in familial hypercholesterolemia (FH),[1] a disease clinically characterized by lifelong elevation of serum LDL cholesterol level, deposits of cholesterol in skin and tendons as well as the walls of the coronary arteries and aorta, and an increased risk of myocardial infarction (2). About 1 in 500 persons carries one mutant LDL receptor gene (heterozygous FH), whereas only 1 in 1,000,000 has inherited two mutant alleles, one from each parent (homozygous FH) (2). Heterozygous FH patients typically develop coronary symptoms by the age of 50 (male patients) to 60 yr (female patients), whereas homozygous FH patients develop severe heart disease in childhood and only rarely survive past age 30 (2). The fact that efficient therapeutic alternatives can today be offered for both heterozygous and homozygous FH patients (2, 7–9) underscores the importance of early diagnosis and intervention in FH.

Cellular and molecular analysis of the LDL receptor defects has revealed wide variations of phenotypic characteristics of the mutant proteins as well as heterogeneity at the level of DNA structure. Thus, five different functional classes of mutations (defect in receptor synthesis, transport, ligand binding, internalization, or recycling of receptor) may be caused by a wide array of DNA alterations (small and large deletions, insertions, and nonsense and missense types of single nucleotide alterations; for reviews, see references 2, 10, and 11). This compromises attempts to develop molecular genetic methods for unequivocal individual diagnosis of suspected yet doubtful cases of FH. There are, however, several exceptions to this diverse molecular basis of FH. Among Lebanese Christian Arabs (12), French Canadians (13), and South African Afrikaners (14), one or two mutant LDL receptor genes explain the majority of the FH cases in the respective populations. In view of the high prevalence of the heterozygous form of FH in these populations, about 1 in 270 to 1 in 100 (15–17), a founder gene effect is apparent.

The exact prevalence of FH in Finland is currently unknown. We have previously characterized an LDL receptor gene mutation, FH–Helsinki, that appears unique to the Fin-

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1. *Abbreviations used in this paper:* FH, familial hypercholesterolemia; PCR, polymerase chain reaction; SSCP, single-strand conformational polymorphism.

nish population and is present in about one third of the Finnish patients with FH (18, 19). The 9.5-kb FH–Helsinki deletion extends from intron 15 to exon 18 and results in a truncated receptor protein with an internalization-defective phenotype of FH (19). Screening for the occurrence of the FH–Helsinki gene in different areas of the country revealed its very low prevalence among FH patients in eastern Finland (20). This finding, suggestive for the enrichment of some other mutation(s) in this part of Finland, prompted us to systematically characterize DNA samples from proven FH cases living in eastern Finland.

Methods

Patients

Venous EDTA-anticoagulated blood samples (10–20 ml) were collected from 201 unrelated heterozygous FH patients attending five different lipid outpatient clinics: 106 were from the University Central Hospital of Helsinki (southern Finland), 19 from the University Central Hospital of Turku (southwestern Finland), 18 from the University Central Hospital of Oulu (northwestern Finland), 19 from the University Central Hospital of Kuopio (eastern Finland), and 39 from the Central Hospital of North Karelia, Joensuu (eastern Finland). The diagnostic criteria for FH included (*a*) serum total cholesterol level over 8 mmol/liter, (*b*) the presence of tendon xanthomas in the proband or in his/her first-degree relative, and (*c*) the presence of hypercholesterolemia in at least one of the first-degree relatives of the proband.

To follow the inheritance of the mutant LDL receptor gene in informative families, venous blood samples were also collected from a total of 26 relatives of six FH probands from the Joensuu area. In addition, blood samples of 34 healthy normolipidemic controls from the Helsinki area were screened for the occurrence of the specific FH mutation reported in the present study.

Amplification of the exons of the LDL receptor gene by the polymerase chain reaction

Polymerase chain reaction (PCR) primers, homologous to the intron sequences flanking the 18 exons of the LDL receptor gene and containing 25 nucleotides, were designed according to the sequences published by Leitersdorf et al. (21). The primers were synthesized on a DNA synthesizer (model 381A; Applied Biosystems, Inc., Foster City, CA) by the β-cyanoethyl phosphoramidite method (22). 100 ng of leukocyte DNA, prepared by the method of Bell et al. (23), was subjected to the PCR (24). As an example, the PCR mixture for exon 6 contained 0.2 mM of each dNTP, 0.1 μl of [α-^{32}P]dCTP (3,000 Ci/mmol, 10 mCi/ml), 10 mM Tris-HCl (pH 8.4), 50 mM KCl, 1.5 mM $MgCl_2$, 0.1 mg/ml gelatin, 1 μM each of the primers SP64 (5′-TCCTTCCTCTCTC-TGGCTCTCACAG-3′) and SP67 (5′-GCAAGCCGCCTGCACCGAG-ACTCAC-3′), and 1 U of DNA polymerase (AmpliTaq; Perkin-Elmer Cetus, Norwalk, CT) in a volume of 10 μl. 30 cycles of a reaction of 1 min at 95°C and 6 min at 68°C were run in a DNA thermal cycler (Techne PHC-2; Techne Ltd., Cambridge, UK). For amplification of the other 17 exons, similar conditions were used except for minor variations in the primer annealing/extension temperatures. The presence of PCR products and their sizes were initially determined on 2% agarose gels followed by ethidium bromide staining.

Analysis of single-strand conformation polymorphism

The PCR product was diluted 100-fold in 0.1% SDS and 10 mM EDTA; and an equal volume of 95% formamide, 20 mM EDTA, 0.05% bromophenol blue, and 0.05% xylene cyanol was added. The samples were denatured at 100°C for 5 min, cooled on ice, and analyzed for single-strand conformation polymorphisms (SSCPs) (25) on nondenaturing 5% polyacrylamide gels (42 × 33 × 0.04 cm) using a buffer containing 90 mM Tris-borate (pH 8.4), 4 mM EDTA, and 10% glycerol. After electrophoresis at room temperature, the gel was transferred to 3 MM paper (Whatman Laboratory Products Inc., Clifton, NJ), dried in a vacuum slab dryer, and subjected to autoradiography on Konica X-ray film at −70°C.

Size analysis of PCR products and separation of the normal and mutant alleles

The radiolabeled PCR products were prepared as above for SSCP and size-fractionated on denaturing 5% polyacrylamide gel containing 7 M urea. The bands were visualized by autoradiography as described above. For sequence analysis, individual bands of different size originating from the same sample were excised from the dried denaturing gel and soaked in 100 μl of water overnight. An aliquot (5 μl) of the eluate was amplified with PCR for 30 cycles in a volume of 100 μl as described above, with the exception that no labeled dCTP was added.

DNA sequencing

The double-stranded PCR products were fractionated by electrophoresis on a 2% agarose gel, followed by purification with the liquid nitrogen "freeze-squeeze" method (26) and precipitation with ethanol. Both strands of the purified DNA fragment were sequenced by a modification (27) of the dideoxy chain termination method of Sanger et al. (28) using the PCR primers as sequencing primers and reagents from the Sequenase kit (U.S. Biochemical Corp., Cleveland, OH). The sequencing reactions were electrophoresed on 5% polyacrylamide gels containing 7 M urea, and the results were visualized by autoradiography.

Preparation of RNA and Northern blot analysis

Human fibroblasts were obtained from incisional skin biopsy specimens and grown in monolayer at 37°C in 5% CO_2. Maximal expression of LDL receptors was induced by incubation in lipoprotein-deficient serum for 16 h before study. Total RNA was isolated by the LiCl–urea technique (29) or the method described by Chomczynski and Sacchi (30) and enriched in poly(A)-containing RNA by oligo(dT)-cellulose affinity chromatography (31). Aliquots of RNA were electrophoresed under denaturing conditions in 0.9% agarose gels containing 0.66 M formaldehyde and transferred onto nylon membranes (Hybond-N, Amersham, Bucks., UK) according to standard procedures (32). The filters were hybridized with a mixture of 5′- and 3′-end LDL receptor cDNA probes labeled with [α-^{32}P]dCTP by random hexanucleotide priming (33). These probes were released from the plasmid pLDLR-3 (34) (kindly provided by Drs. M. S. Brown, J. L. Goldstein, and D. W. Russel, University of Dallas, TX) and contained exons 2–7 (a 1,015-bp PstI/BamHI fragment) and exons 11–17 (a 971-bp BamHI/XhoI fragment), respectively. After hybridization, the blots were washed and exposed to autoradiography films essentially as described previously (35).

RNA amplification and sequence analysis

A specific DNA copy of the LDL receptor RNA sequence was synthesized with use of three 20-mer oligonucleotide primers derived from the published normal sequences of exon 6 (primer E6: 5′-GCATCAC-CCTGGACAAAGTC-3′), exon 7 (primer E7: 5′-TCTTAAGGTCATT-GCAGACG-3′), and exon 9 (primer E9: 5′-CATCTTCCTGACCTC-GTGCC-3′) of the LDL receptor gene (34). First-strand cDNA synthesis was accomplished by extension with the downstream primer E9 in a 20-μl reaction containing 0.5 μg of total RNA, 30 pmol of PCR primer, and 10 U of reverse transcriptase (Promega Biotec, Madison, WI) in standard PCR buffer. The reverse transcription mixture was then included into a 100-μl PCR, with 50 pmol of each of the primers E6 and E9, and 2.5 U of AmpliTaq polymerase. Other conditions were as described above for genomic DNA except that labeled dCTP was omitted. 40 cycles of PCR amplification were performed at 95, 55, and 72°C for 1 min each. PCR products of the appropriate size were recovered from 2% agarose gels and subjected to reamplification with the primers E6 and E7. After addition of labeled dCTP, the nested PCR was carried out in a 50-μl volume for 20–35 cycles as described above. The PCR products were analyzed for size by denaturing polyacrylamide gel electrophoresis. For quantitative estimate of the transcript levels, band in-

tensities were determined by laser scanning densitometry (UltroScan, LKB Produkter, Uppsala, Sweden). The resolved bands were recovered from the gels and sequenced as described for genomic DNA (see above).

LDL receptor assays

Functional properties of the mutant LDL receptor were examined in cultured fibroblasts as described previously for the FH–Helsinki mutation (19). Briefly, cells from stock cultures were seeded at a concentration of 1×10^5 cells per dish into 60-mm petri dishes and maintained in monolayer culture in DME medium containing 10% (vol/vol) FCS. On day 3, the cells were switched to fresh DME supplemented with 10% (vol/vol) human lipoprotein–deficient serum and used for experiments 48 h thereafter. Cell surface binding, internalization, and proteolytic degradation of ^{125}I-labeled LDL (16 μg protein/ml) by cell monolayers were determined in duplicates at 37°C after incubation for 5 h in the absence or presence of a 20-fold excess of unlabeled LDL. The amounts of LDL receptor–mediated binding, internalization, and degradation were calculated by subtracting the values for ^{125}I-labeled ligand binding, internalization, and degradation in the presence of excess unlabeled LDL from those in its absence. The results were correlated with the total protein content of the cells, as measured by the method of Lowry et al. (36), and are given as percent values±SE relative to two control cell lines analyzed in parallel.

Assays for serum lipids

Serum lipid analyses were carried out either before any hypolipidemic drug intervention or after at least 6 wk drug-free washing-out period. Blood samples were taken after 12 h of fasting. Serum cholesterol (37) and triglyceride (38) levels were determined by enzymatic methods using commercial kits obtained from Boehringer Mannheim (Mannheim, FRG). The concentration of serum HDL was measured enzymatically after precipitation of LDL and VLDL fractions with dextran sulfate and $MgCl_2$ (39), and serum LDL cholesterol level was calculated using the formula of Friedewald et al. (40).

Assay for the FH–Helsinki gene

Most of the patients of the present series were screened for the presence of the FH–Helsinki mutation of the LDL receptor gene during a previous study (20). In the case of 13 probands from the Joensuu area this information was missing; these samples were analyzed by a Southern blot technique using the restriction enzyme BamHI and a 3′-end LDL receptor cDNA probe as described previously (19).

Results

Identification of a deletion in exon 6 of the LDL receptor gene

Preliminary Southern blot analyses with human LDL receptor cDNA probes covering the entire coding region of the gene failed to identify restriction fragments unique to the North Karelian FH patients, thus excluding the occurrence of any major LDL receptor gene rearrangements in these individuals. For subsequent experiments, DNA samples from 10 FH patients living in the North Karelia region were randomly selected. All 18 exons of the LDL receptor gene were amplified using primer pairs flanking the exon sequences. After PCR, the amplification products were initially examined by agarose gel electrophoresis. In all samples, a single band of the appropriate size was seen, and no differences in size were detected between FH individuals and controls (data not shown).

However, upon subjecting these amplified DNA fragments to SSCP analysis, nine samples were identified that displayed allelic polymorphism, i.e., generated aberrantly migrating DNA fragments derived from amplification of exon 6. The PCR–SSCP procedure was repeated on the DNA samples in which the variant allele was detected and on several other FH and control samples. The control samples yielded three different fragments under these conditions: a fast-migrating band, most likely resulting from duplex formation despite the initial denaturation, and two bands with a slower rate of migration, representing the two strands of the amplified DNA (Fig. 1 *A*). DNA samples from a number of North Karelian FH patients yielded variant SSCP bands that were reproducible and never generated in the control samples (Fig. 1 *A*). Analysis of these samples on a nondenaturing gel without prior denaturation revealed the presence of two DNA fragments of different size and a third band of slower electrophoretic mobility in all of the FH samples, whereas only one band was observed in the control samples (data not shown). This result indicated the presence of a deletion in one of the patients' alleles, the third band being a heteroduplex DNA fragment formed between the normal and the mutant LDL receptor alleles during PCR. Analysis of the same samples on 5% denaturing gels unequivocally confirmed the presence of a small deletion in one of the LDL-receptor alleles (Fig. 1 *B*).

Nucleotide sequence of the mutant allele

To precisely define the deletion in exon 6, the PCR products originating from the normal and mutant allele were sequenced and compared with each other. The normal sequence GAA CCC ATC AAA G coding for Glu-287, Pro-288, Ile-289, and Lys-290, identical to that published for the normal LDL receptor cDNA (34), was found to be replaced in the mutant DNA fragment by the sequence GAA AAG as a result of a 7-bp deletion close to the 3′-end of exon 6 (Fig. 2). The exact position of the deletion cannot be determined because the 5′-end of the deletion could have started at the last A of Glu-287 or at the first C of Pro-288, resulting in a seven-nucleotide deletion of either ACCCATC or CCCATCA, respectively. In either case, a deletion of one A and CCCATC results in elimination of the codons for Pro-288 and Ile-289. Furthermore, a translational frameshift obligatorily takes place after amino acid 287 (glutamine) of the mutant LDL receptor protein. The PCR product from the FH patient migrating in a way similar to that from a control subject revealed a nucleotide sequence identical to that published for exon 6 of the normal LDL receptor gene. Identical results were obtained when both strands of the PCR product were sequenced. No other differences in the coding region of the LDL receptor sequence were detected either by SSCP analysis of several North Karelian FH patients or by sequencing of almost the entire coding region of the LDL receptor gene from one North Karelian FH patient.

Analysis of the mRNA corresponding to the deleted allele

Poly(A)-enriched RNA preparations from fibroblasts of nine North Karelian FH patients with the seven-nucleotide deletion were subjected to RNA hybridization blot analysis and compared with the corresponding RNA samples from fibroblasts of an FH patient with the FH–Helsinki deletion and fibroblasts of a healthy control subject. All the samples from the North Karelian patients displayed a single 5.3-kb mRNA species identical to that present in fibroblast RNA of the control subject, whereas, in accord with our previous studies (35), an additional 4.5-kb mRNA species was present in the RNA sample from the FH–Helsinki patient (data not shown). On the basis of these data alone, it is not possible to conclude whether a putative

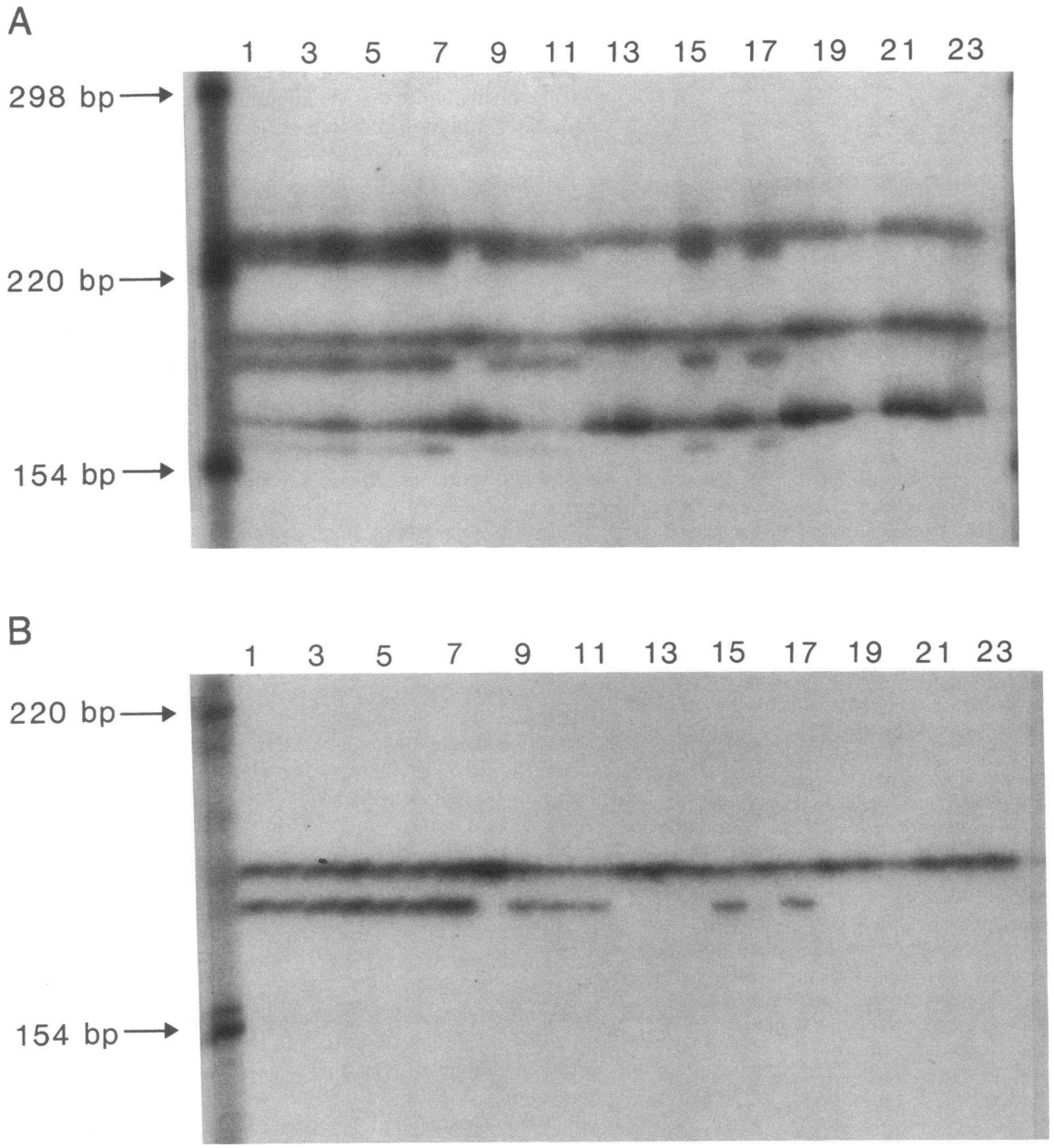

Figure 1. (*A*) PCR–SSCP analysis of DNA from several unrelated FH patients for exon 6 mutations of the LDL receptor gene. DNA samples from 10 FH patients from North Karelia (lanes *1–10*), ten FH patients from the Helsinki area (lanes *11–20*), 3 normolipidemic non-FH individuals (lanes *21–23*), and a buffer blank (lane *24*) were subjected to PCR–SSCP analysis using a pair of PCR primers flanking exon 6 of the LDL receptor gene. Allelic polymorphism is displayed by samples *1–7, 9–11, 15,* and *17.* (*B*) Size analysis of PCR-amplified DNA fragments from the same individuals on a denaturing polyacrylamide gel. Molecular size markers were generated by digestion of the plasmid pBR322 with HinfI and EcoRI.

mutant mRNA species, seven nucleotides shorter than the normal, was superimposed with the normal mRNA in the samples from the North Karelian patients or whether it was totally absent.

To further survey the presence of mRNA transcribed from the mutant LDL receptor allele, PCR amplification products generated from total fibroblast RNA samples of two healthy controls and six heterozygous FH individuals were analyzed. Using primers that generate fragments spanning exon–intron boundaries, we readily identified a smaller transcript present only in the samples from the FH patients (Fig. 3). The electrophoretic analysis of the amplified PCR products showed only a minor amount of abnormally sized products, suggesting that the mutant mRNA was present in markedly smaller quantities than that corresponding to the normal allele (Fig. 3).

To get an estimate of the amount of the mutant mRNA relative to the normal mRNA, samples were withdrawn from PCR at every 5 cycles after the 20th amplification cycle until the 35th cycle. After electrophoretic separation of the PCR products, the resultant autoradiograms were subjected to multiple scans by laser densitometry. The ratio of the band intensities remained constant over these 15 cycles, with the amount of

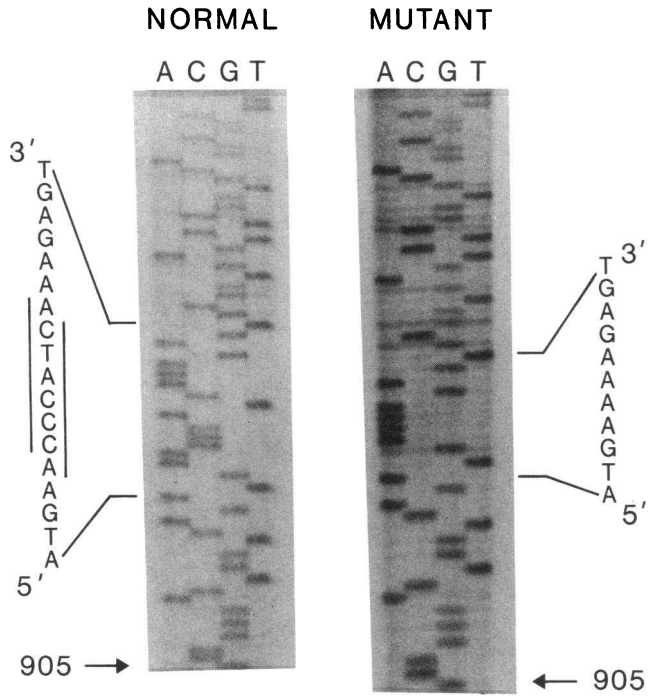

Figure 2. Partial DNA sequences of the normal and mutant alleles of the LDL receptor gene demonstrating a seven-nucleotide deletion close to the 3′-end of exon 6. The vertical lines along the right and left side of the normal sequence indicate the two alternative deletions resulting in the same seven-nucleotide deletion in the mutant allele. The position of nucleotide 905 is according to the nucleotide numbering presented by Yamamoto et al. (34).

the mutant product persisting at ~ 5% of that of the normal product. However, errors of quantitative PCR, as applied herein, could result from unequal efficiency and completeness of reverse transcription as well as unequal efficiency of two-step amplification of the two cDNAs present in unknown amounts. Even with these precautions, it is unlikely that the abundance of the mutant mRNA could be higher than 5% of that of the normal mRNA.

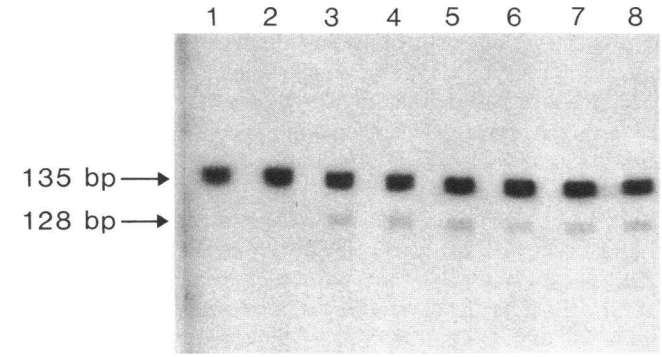

Figure 3. Size analysis of the PCR products originating from fibroblast RNA of two normolipidemic control subjects (lanes *1* and *2*) and six heterozygous FH patients (lanes *3–8*) on a denaturing polyacrylamide gel. A two-step PCR system was used with primers E6 and E9, derived from the normal exon 6 and exon 9 sequences, respectively, in the first step, and primer E6 and a nested 3′-end primer E7 in the second step. The sizes of the final PCR products corresponding to the normal allele and deleted allele are expected to be 135 and 128 bp, respectively; comparison to the migration rate of the molecular size markers was in full accordance with this prediction.

Sequence analysis of the PCR products originating from both RNA species confirmed the presence of the seven-nucleotide deletion also in the mRNA product of the mutant allele (Fig. 4, *left*). The sequencing data additionally demonstrate that splicing of intron 6 occurs in an intact way compared with the processing of the normal mRNA (Fig. 4, *left*). This confirms our assumption of a translational frameshift beyond the amino acid 287 of the LDL receptor protein. As our data are compatible with normal processing of introns downstream of exon 6, translation is predicted to be terminated by a newly formed termination codon at amino acid position 346 in the eighth exon (Fig. 4, *right*). Whether any mutant protein corresponding to this nucleotide sequence is actually synthesized awaits additional studies.

Phenotypic analysis of the LDL receptor function in the patients with the deleted gene

Fibroblasts from eight patients with the exon 6 DNA deletion were cultured; and their ability to bind, internalize, and degrade radiolabeled LDL particles was examined. The LDL-binding capacity of the fibroblasts from the FH patients was only 50.1±3.1% of that of the cells from normal controls, and the abilities of the patients' fibroblasts to internalize and degrade labeled LDL particles were similarly reduced to 41.1±1.9 and 41.6±1.9% of normal, respectively (Fig. 5). These data indicate that the truncated mutant protein, whether synthesized or not, is devoid of any functional LDL receptor activity.

Inheritance of the deleted allele in families with FH

DNA samples were collected from available family members of six probands with the established carrier status of the deleted allele and subjected to PCR amplification of the exon 6 of the LDL receptor gene. The expected 173- and 166-bp bands corresponding to the normal and mutant gene, respectively, were visualized on 5% denaturing polyacrylamide gels by autoradiography. An unequivocal cosegregation of serum total and LDL cholesterol level with the deleted allele was demonstrated in each family examined (Fig. 6). In these kindreds, the subjects with the LDL receptor gene deletion had mean (±SD) serum and LDL cholesterol levels of 10.0±2.3 and 8.3±2.2 mmol/liter, respectively, whereas the corresponding concentrations in the unaffected family members were 4.9±1.1 and 3.2±1.0 mmol/liter, respectively.

Geographical distribution of the FH patients with the exon 6 deletion

A total of 201 heterozygous FH patients were screened for the presence of the deleted LDL receptor gene using the method described above. 69 patients (34%) were demonstrated to carry this mutant allele, 66 (33%) were shown to possess the FH–Helsinki gene, and 66 (33%) had FH caused by yet unknown type of LDL receptor gene mutation (Fig. 7). None of the 34 healthy normolipidemic control subjects from the Helsinki area were found to carry either of the mutant genes. There were striking differences in the geographical distribution of the two fully characterized gene mutations in that the exon 6–deleted allele was present in 46 (79%) out of 58 FH patients from eastern Finland (Kuopio, n = 11; and Joensuu, n = 35) (Fig. 7). Because this mutation was especially enriched and the first pro-

band identified in the Joensuu (North Karelia) region, it is designated as the FH–North Karelia gene. The FH–North Karelia gene was found in only 2 (5%) out of 37 patients from the western areas (Turku and Oulu) of the country. The geographical distribution of the FH–Helsinki gene was more uniform, ranging from 26 to 61% in the areas examined, with the notable exception of Joensuu, where its prevalence in FH patients was only 3% (Fig. 7). A definite diagnosis of FH by DNA techniques can now be provided in ~ 90% of the FH cases in the eastern Finland (Fig. 7).

Serum lipid levels

In each category of FH mutations (FH–North Karelia, FH–Helsinki, or the group with yet-unknown types of LDL receptor gene mutations) the serum total and LDL cholesterol levels were in a range typical of heterozygous familial hypercholesterolemia (Table I). There were no significant differences in the mean plasma lipid levels between the patients with the FH–Helsinki and FH–North Karelia mutations (Table I). Mean serum LDL cholesterol levels were higher in FH patients with these two established LDL receptor gene mutations than in the category with a yet-unknown type of mutation (Table I). These results may indicate that some of the individuals belonging to the latter category were in fact incorrectly diagnosed as FH patients.

Discussion

Most of the mutations of the LDL receptor gene characterized so far have involved major gene rearrangements or single nucleotide alterations (10, 11). Previously, three minor deletions and two minor insertions have been reported. In a black South African patient (patient TT), Leitersdorf et al. (41) identified a deletion of 6 bp in exon 2 that results in the omission of two amino acids from the first cysteine-rich ligand-binding repeat. Another in-frame deletion eliminates three nucleotide pairs from exon 4 of the LDL receptor gene and is a very common cause of FH among Israeli and South African Ashkenazi Jews (42). The Watanabe heritable hyperlipidemic rabbit, an animal model for FH, carries a mutant LDL receptor gene, which is characterized by a deletion of 12 nucleotides that eliminates four amino acids from the third cysteine-rich repeat of the ligand-binding domain (43). A small 4-bp insertion described by Lehrman et al. (44) produces a frameshift of translation; this mutation, present in exon 17, yet maintains the main architecture of the LDL receptor but strikingly alters its cytoplasmic domain, resulting in an internalization-defective phenotype. The FH–Nashville mutation, phenotypically a null allele, is characterized by an insertion of 4 bp in exon 8 with a resulting frameshift of translation (11).

The FH–North Karelia deletion described in the present study affects exon 6 of the LDL receptor gene encoding the seventh cysteine-rich repeat of the receptor protein. Our data suggest that this gene may not be able to direct the synthesis of any functional receptor protein. First, the level of the mRNA corresponding to the mutant allele is very low, at least in cultured skin fibroblasts. Second, the frameshift of translation would eliminate a large number of the cysteine residues encoded by exons 6–8 of the normal LDL receptor gene (Fig. 4, *right*). Previous studies have shown that abnormal spacing of the cysteine residues may affect the normal maturation of the LDL receptor (41, 43, 45). Third, the protein product of the

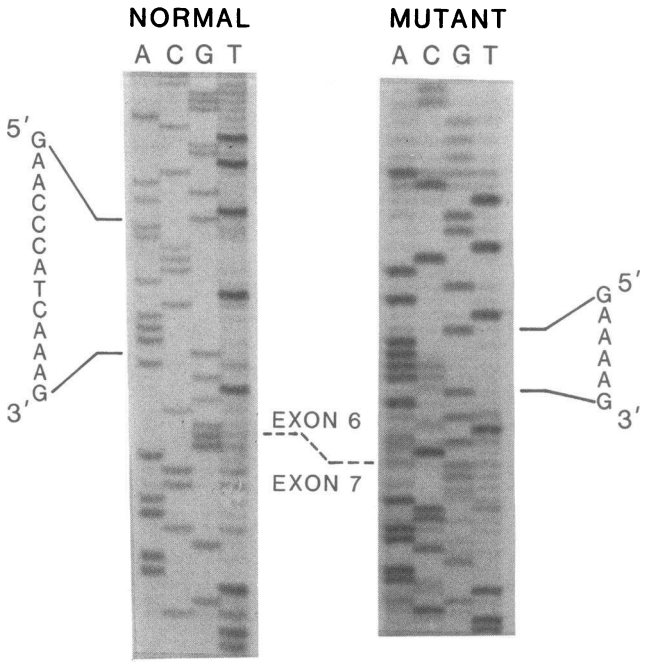

Figure 4. (*Left*) Partial cDNA sequences of the normal and deleted LDL receptor gene. PCR-amplified DNA fragments corresponding to the two bands visualized in Fig. 3 were separately subjected to reamplification with the primers E6 and E7 and sequenced. Dotted line indicates the boundary between the exons 6 and 7. (*Right, opposite page*) Partial nucleotide and predicted amino acid sequences of the normal LDL receptor gene (reference 34) and the mutant gene with the seven-nucleotide deletion. One of the two alternative deletions that can generate the abnormal sequence found in the genomic DNA and cDNA is shown by underlining and bold nucleotide lettering. Positions of the nucleotides and amino acids are indicated by numbering above and below the sequences, respectively. Cysteine residues are indicated by black dots.

mutant allele, even if present after the posttranslational processing, might be secreted extracellularly due to the lack of a hydrophobic transmembrane domain. Our data from the functional studies of the LDL receptors in fibroblasts of the patients are in accordance with these theoretical predictions. Although the results do not permit definitive conclusions on the phenotypic characteristics of the FH–North Karelia gene, they are compatible with a receptor-deficient or binding-defective phenotype and argue against an internalization-defective phenotype (Fig. 5).

Previous studies on FH have suggested that low mRNA abundance is principally associated with large deletions of the LDL receptor gene (46). There are exceptions to this rule, however. Lehrman et al. (44) reported that a nonsense mutation at codon 792 of the LDL receptor gene appeared to be associated with a reduction of the corresponding mRNA level to only 25–30% of normal. Hummel et al. (47) identified a family of rhesus monkeys with spontaneous elevation of LDL cholesterol and deficiency of LDL receptors. These monkeys were shown to be heterozygous for a point mutation which introduces a premature termination codon in exon 6 of the LDL receptor gene, potentially resulting in a truncated receptor protein. Quantitative analysis of liver RNA showed that the abundance of the LDL receptor mRNA was reduced by ~ 50% in these animals. The present study demonstrates that the level of

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                                                                    960
                                                                     |
NORMAL   GACTGCCGGGACTGGTCAGATGAACCCATCAAAGAGTGCGGGACCAACGAATGCTTGGAC
         AspCysArgAspTrpSerAspGluProIleLysGluCysGlyThrAsnGluCysLeuAsp
         280 •                          •                 •
MUTANT                                   AAGAGTGCGGGACCAACGAATGCTTGGAC
                                Glu-------LysSerAlaGlyProThrAsnAlaTrpThr
                                287
                                                                   1020
                                                                     |
NORMAL   AACAACGGCGGCTGTTCCCACGTCTGCAATGACCTTAAGATCGGCTACGAGTGCCTGTGC
         AsnAsnGlyGlyCysSerHisValCysAsnAspLeuLysIleGlyTyrGluCysLeuCys
         300         •          •                      •     •
MUTANT   AACAACGGCGGCTGTTCCCACGTCTGCAATGACCTTAAGATCGGCTACGAGTGCCTGTGC
          ThrThrAlaAlaValProThrSerAlaMetThrLeuArgSerAlaThrSerAlaCysAla
          298                                                    •
                                                                   1080
                                                                     |
NORMAL   CCCGACGGCTTCCAGCTGGTGGCCCAGCGAAGATGCGAAGATATCGATGAGTGTCAGGAT
         ProAspGlyPheGlnLeuValAlaGlnArgArgCysGluAspIleAspGluCysGlnAsp
         320                                •                 •
MUTANT   CCCGACGGCTTCCAGCTGGTGGCCCAGCGAAGATGCGAAGATATCGATGAGTGTCAGGAT
          ProThrAlaSerSerTrpTrpProSerGluAspAlaLysIleSerMetSerValArgIle
          318
                                                                   1140
                                                                     |
NORMAL   CCCGACACCTGCAGCCAGCTCTGCGTGAACCTGGAGGGTGGCTACAAGTGCCAGTGTGAG
         ProAspThrCysSerGlnLeuCysValAsnLeuGluGlyGlyTyrLysCysGlnCysGlu
         340         •          •                      •     •
MUTANT   CCCGACACCTGCAGCCAGCTCTGCGTGA
          ProThrProAlaAlaSerSerAlaSTOP
          338                   345
```

Figure 4 (*continued*)

the mRNA corresponding to the FH–North Karelia gene is also strikingly reduced, at least in the skin fibroblasts (Fig. 3). This could be due to improper processing of the primary transcript of the mutant gene or diminished stability of the mature mRNA. An undetectable or decreased steady-state mRNA abundance appears to be a common phenomenon for genes with nonsense mutations. Thus, nonsense mutations of the genes encoding the vitamin D receptor (48), insulin receptor (49), β-globin (50, 51), dihydrofolate reductase (52), and triosephosphate isomerase (53) have all been found to result in greatly reduced levels of the corresponding mRNAs. The exact mechanisms whereby premature termination codons affect mRNA abundance are not known. It has been suggested that nonsense codons located within exons close to the 5′ end of the

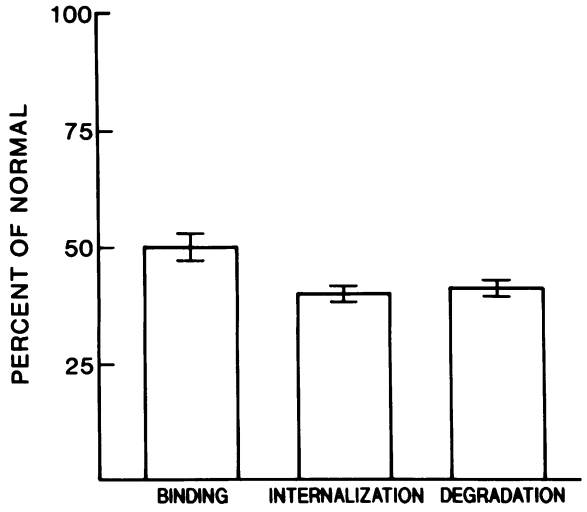


Figure 5. Relative values (mean±SE) for high-affinity binding, internalization, and degradation of ^{125}I-LDL by fibroblasts of the heterozygous FH patients (n = 8) with the deleted LDL receptor gene.

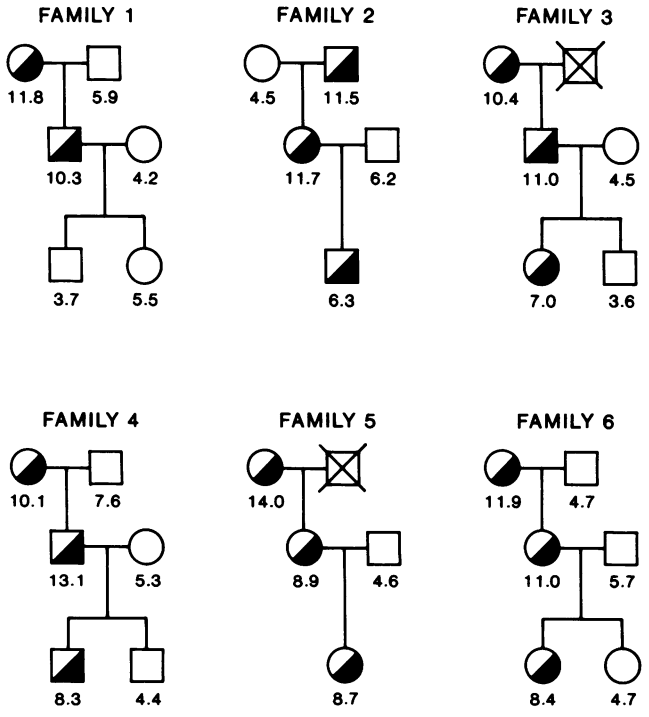
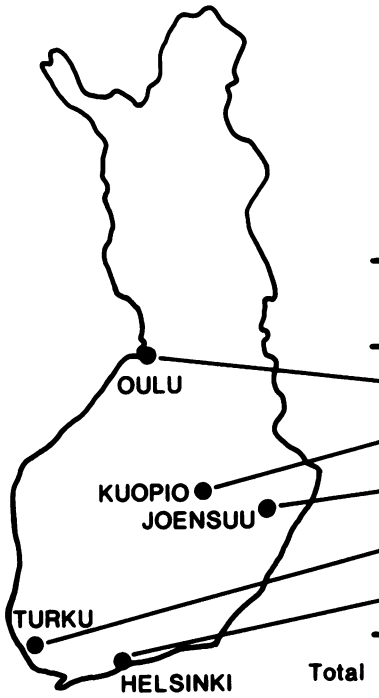


Figure 6. Demonstration of cosegregation of the FH phenotype with the deleted allele in six North Karelian pedigrees. Individuals heterozygous for the seven-nucleotide deletion are indicated by half-filled symbols. Numbers below each symbol represent concentrations of serum cholesterol (in mmol/liter).



	Type of mutation			
	FH-North Karelia	FH-Helsinki	Unknown	Total
OULU	2 (11.1%)	11 (61.1%)	5 (27.8%)	18
	11 (57.9%)	6 (31.6%)	2 (10.5%)	19
KUOPIO / JOENSUU	35 (89.7%)	1 (2.6%)	3 (7.7%)	39
	0 (0.0%)	5 (26.3%)	14 (73.7%)	19
TURKU	21 (19.8%)	43 (40.6%)	42 (39.6%)	106
HELSINKI				
Total	69 (34.3%)	66 (32.8%)	66 (32.8%)	201

Figure 7. Prevalence and geographical distribution of different LDL receptor gene mutations in Finland. The percentages in parentheses indicate the proportion of the mutation categories in a given subregion or the whole country.

gene may reduce the steady-state level of the mRNA by inhibiting the processing of downstream exons (54) or by leaving the mRNA in abnormal polyribosome context without protection of the free mRNA segments by ribosomes (55). Another theory maintains that there is a nuclear scanning apparatus searching for nonsense codons in newly synthesized exons; any RNA species containing such a codon is targeted for degradation before it reaches the cytoplasm (54).

Previous studies using blood group and serum markers have supported the notion that the Finnish population is genetically relatively homogeneous (56, 57). The data of this communication provide some alternative views on this concept. The oldest settlement located in the southwestern part of Finland was inhabited by a relatively few ancestral Finns, a branch of the Finnish–Ugrian race, during the first centuries A.D. (58). It is possible that at this time another independent branch originating from the same pool of people commenced inhabitation of Karelia in the eastern part of the country (58). Soon thereafter, groups of the former settlement started slowly migrating into the neighboring inland to reach the present-day Tavastland. It was not earlier than in the 16th century when the settlement of the northeastern (Savonia, Kainuu) and northern parts (Ostrobothnia) of the country effectively started; at this time the total population hardly exceeded 250,000. The present evidence suggests that the Ostrobothnia (Oulu) region was mainly inhabited by people originating from Tavastland, whereas the Savonia (Kuopio) area was occupied by the Karelians and possibly to a small extent by Tavastians (58). With this background the enrichment of the FH–North Karelia gene in the eastern parts of the country and its rarity in the western regions (Fig. 7) may find its explanation, provided the mutation was established in the original South Karelian population. In contrast, our data give no firm clue as to the origin of the FH–Helsinki mutation. It may have arisen in the ancient Tavastian population, which could explain its very common occurrence in the Oulu region and moderate prevalence in the Kuopio region (Fig. 7). Not unexpectedly, the mutation spectrum in Helsinki city and its surroundings is more complicated, as the inhabitants of the capital area represent a mixture of Finnish tribes. Furthermore, the data compiled in Fig. 7 favor the hypothesis that a yet-unknown LDL receptor gene mutation may be enriched in the southwestern (Turku) area of the country. It should be emphasized that the figure for the combined prevalence of the FH–North Karelia and FH–Helsinki genes (135 out of 201 patients, or 67%; Fig. 7) may be a slight overestimate considering the whole Finland, as FH patients from the eastern areas of the country were more efficiently recruited for the particular purposes of the present study. Both the FH–North Karelia and FH–Helsinki genes appear unique to the Finnish population in that there are no reports on their occurrence in other populations.

In conclusion, the present study describes a novel type of LDL receptor gene mutation, FH–North Karelia, characteristic of the Finnish population. DNA sequencing data as well as studies on cultured fibroblasts suggest that the FH–North Karelia gene does not produce functional LDL receptors. This mutation and the FH–Helsinki gene described previously by us

Table I. Serum Lipid Levels in FH Patients Subclassified According to the Type of Mutation

Variable	Type of mutation		
	North Karelia	Helsinki	Unknown
No. of patients (females/males)	69 (40/29)	66 (26/40)	66 (35/31)
Age (*yr*)	47±11	47±12	51±12
Cholesterol (*mmol/liter*)	12.1±2.1*	11.7±1.8	11.2±2.3
LDL cholesterol (*mmol/liter*)	10.1±1.9*	9.9±1.9*	9.1±2.2
HDL cholesterol (*mmol/liter*)	1.12±0.31*	1.17±0.31	1.26±0.31
Triglycerides (*mmol/liter*)	1.61±1.02	1.51±0.88	1.63±0.85

Values are means±SD. * $P < 0.05$ compared with the group with unknown type(s) of LDL receptor gene mutation(s).

together make up > 60% of the mutant genes responsible for FH in the Finnish population. This background greatly facilitates the diagnosis of FH by molecular genetic methods in Finland. As the two mutant genes result in different phenotypic characteristics of the LDL receptor function, the mutation spectrum of the Finnish FH patients offers good possibilities to investigate whether variable defects of the LDL receptor modify the clinical severity and drug responsiveness of this disease.

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