Phenotypic consequences of a deletion of exons 2 and 3 of the LDL receptor gene

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Abstract Screening for structural alterations of the low density lipoprotein (LDL) receptor gene by Southern blot analysis revealed an abnormal band pattern in one subject with a clinical diagnosis of homozygous familial hypercholesterolemia (FH). The molecular defect was further characterized by polymerase chain reaction and cDNA sequencing. These analyses identified a 4.8 kb in-frame deletion of exons 2 and 3, where exon 1 was spliced to exon 4. This deletion is expected to produce a receptor that has lost the two first cysteine-rich repeats of the ligand-binding domain. Previously published data of in vitro site-directed mutagenesis has shown that binding of LDL to such a receptor is reduced to 70% of normal. A mild phenotype in our FH homozygote is consistent with that observation. In contrast, heterozygotes carrying this deletion have a relatively more severe phenotype that is comparable to that of heterozygotes carrying a null-allele. A severe phenotype was also found in a compound heterozygote carrying this deletion. Possible mechanisms for this phenotypic variability are discussed.—Rødningen, O. K., S. Tonstad, J. D. Medh, D. A. Chappell, L. Ose, and T. P. Leren. **Phenotypic consequences of a deletion of exons 2 and 3 of the LDL receptor gene. *J. Lipid Res*. 1999.** 40: **213–220.**

Supplementary key words deletion • familial hypercholesterolemia • homozygous FH • low density lipoprotein receptor

Familial hypercholesterolemia (FH) is one of the most common autosomal dominant diseases (1, 2). It is characterized by hypercholesterolemia, tendon xanthomas and premature coronary heart disease (2, 3). FH heterozygotes and FH homozygotes have levels of total serum cholesterol that are 2- to 3-fold or 5- to 6-fold higher than normal, respectively. As a consequence, FH heterozygotes usually contract coronary heart disease in their fourth or fifth decade, while FH homozygotes suffer from coronary heart disease in childhood or in their early teens (2).

The molecular defects underlying FH are mutations in the gene encoding the low density lipoprotein receptor (LDLR) that disrupt the synthesis of normal cell surface LDLRs (2, 4). As a consequence, the LDLR-mediated endocytosis of low density lipoprotein (LDL) is reduced. This causes hypercholesterolemia both through decreased catabolism and increased synthesis of LDL (5).

The LDLR gene spans 45 kb and consists of 18 exons and 17 introns (6). The 5.3 kb mRNA, of which only 2.5 kb are translated, encodes the 860 amino acid receptor protein (7).

Structural alterations of the LDLR gene detectable by Southern blot analysis have been found to cause FH in approximately 15% of the patients (2, 4). In order to screen for structural alterations of the LDLR gene among Norwegian FH patients, we have performed Southern blot analysis with a panel of restriction enzymes and probes. On the basis of this study, a 4.8 kb deletion that deletes exons 2 and 3 was detected. The effects of this mutation on mRNA and lipid levels are presented.

MATERIALS AND METHODS

Subjects

Fifty-seven unrelated FH subjects were studied. Of these one was a FH homozygote and 56 were FH heterozygotes. All subjects were Caucasians of Norwegian descent, most of them living in the Southeastern part of Norway. FH was diagnosed according to the criteria of Goldstein, Hobbs, and Brown (2).

Serum lipids and clinical manifestations

The FH homozygote proband was a male born in 1979. Tendon and skin xanthomas were observed from the age of 5. At the age of 10 he had a total serum cholesterol value of 13.9 mmol/l, and after 4 weeks on lovastatin 40 mg, a cholesterol value of 13.0 mmol/l was recorded. Thus, this medication had only a marginal effect on the levels of total serum cholesterol.

Abbreviations: FH, familial hypercholesterolemia; LDL, low density lipoprotein; LDLR, low density lipoprotein receptor; PCR, polymerase chain reaction; VLDL, very low density lipoprotein.

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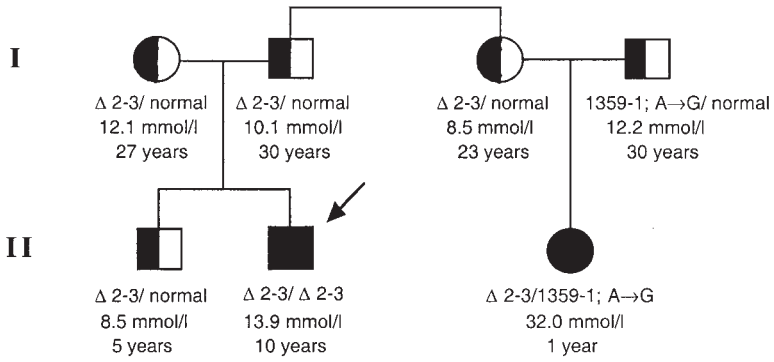

Fig. 1. Pedigree of the family with the Δ2–3 deletion. The FH heterozygotes are indicated by half-filled symbols and the FH homozygote and the compound heterozygote by filled symbols. The FH homozygote proband is indicated by an arrow. Also indicated are the mutations possessed by each individual, as well as their age and levels of total serum cholesterol obtained before any lipid lowering therapy was started.

Both parents have been diagnosed as FH heterozygotes according to standard clinical criteria (2). The parents had values for total serum cholesterol in the range of 10–12 mmol/l before lipid-lowering drug therapy was started. The parents were apparently unrelated, but they were from the same geographic part of Northern Norway. The pedigree of the family as well as lipid levels and ages are shown in **Fig. 1**.

Analyses of structural alterations by Southern blot analysis

Eight μg of DNA was used for digestion with 20 U of each of the six different restriction enzymes used for haplotype analysis described below. The restriction fragments were electrophoresed in a 1% agarose gel, blotted on to a GeneScreen membrane (NEN Research Prod., Boston, MA), and hybridized with cDNA probes encompassing exons 1–10 or exons 11–18 (8).

Haplotype analysis at the LDLR locus

Seven restriction fragment length polymorphisms (RFLPs) were used as haplotype markers at the LDLR locus. These were: SphI polymorphism in intron 6 (9), StuI polymorphism in exon 8 (10), AvaII polymorphism in exon 13 (11), ApaLI polymorphism (5′ ApaLI) in intron 15 (12), NcoI polymorphism in exon 18 (13), PstI polymorphism in the 3′ flanking region (14), and ApaLI polymorphism (3′ ApaLI) in the 3′ flanking region (12). The methods used to analyze these RFLPs, have been previously described (8, 15). Haplotypes were constructed under the assumption that there had been no recombination within the LDLR locus.

Analyses of exons 1, 2, 3, 4 and 5 of the LDLR gene by polymerase chain reaction (PCR)

Exons 1, 2, 3, 4, and 5 of the LDLR gene were amplified by PCR using sets of primers flanking each exon as described by Leitersdorf et al. (16). The reaction mixtures for the PCRs contained 40 pmoles of each of the two exon-specific primers, 1 U Taq DNA polymerase (0.5 U for amplification of exon 5), 200 μm dNTPs, and the buffer supplied with the enzyme (Boehringer Mannheim, Germany). The reaction volume was 100 μl. Three percent DMSO was included for amplifications of exons 2 and 3. A DNA Thermal Cycler 480 (PE Applied Biosystems, Foster City, CA) was used for the thermal cyclings. The conditions for the thermal cyclings were identical for the PCRs except for the annealing step that was 1 min at 50°C for exons 1 and 3, 1 min at 53°C for exon 2, 1 min at 62°C for exon 4, and 1 min at 58°C for exon 5. Initial denaturation for 7 min at 94°C was followed by 25 cycles of denaturation for 2 min at 94°C, annealing as described above, and extension for 2 min at 72°C. The thermal cyclings were completed by terminal extension at 72°C for 10 min. The PCR products were analyzed by 8% polyacrylamide gel electrophoresis (PAGE).

Fibroblast cultures

Fibroblast cultures were established from the proband and a healthy normocholesterolemic subject, as well as from a true FH homozygote possessing another mutation in the LDLR gene (FH-Elverum, 17). Cells of FH-Elverum homozygotes have previously been proven to be LDL receptor-negative (18). Skin biopsies were obtained from the proximal part of the volar side of the forearm. Cells were grown in monolayer in 75 cm^2 stock flasks containing 15 ml Amniomax −100 (Gibco, Life Technologies, Rockville, MD) at 37°C in a humidified atmosphere of 5% CO_2. The cells were harvested when they had almost reached confluency and after 6 to 15 passages.

Northern blot analysis

Northern blot analysis was used to study mRNA of the LDLR gene from cultured skin fibroblasts from the FH homozygote proband and from a healthy normocholesterolemic subject. mRNA was isolated using Dynabeads oligo $(dT)_{25}$ (Dynal A.S., Oslo, Norway) as described by Rødningen et al. (19), and loaded onto a 1% agarose gel containing 1 m formaldehyde. After fractionation by electrophoresis, mRNA was transferred to a GeneScreen membrane (NEN Research Prod., Boston, MA) in accordance with the instructions given by the manufacturer.

The probes used for hybridization were prepared by amplification of genomic DNA. The LDLR probe consisting of 475 nucleotides was prepared using primers flanking exon 13 (5′-GTC ATC TTC CTT GCT GCC TGT TTA G-3′) and exon 14 (5′-ACG CAG AAA CAA GGC GTG TGC CAC A-3′), respectively (16). The β-actin probe, used for hybridization to control for differences in the amounts of mRNA loaded on the gel, was prepared by amplification of genomic DNA encompassing nucleotides 1358 to 2126 of the β-actin gene (20).

Labelling of probes and hybridization conditions have been previously described (19). The autoradiograms were scanned to determine the amounts of mRNA. This was done using a Shimadzu dual wavelength flying spot scanner model CS-9000, with the single lane zig-zag scan method. Levels of LDLR mRNA were normalized using β-actin as an internal control.

RT-PCR and cDNA sequencing

mRNA was isolated from fibroblasts using the Micro Fast Track mRNA isolation kit (Invitrogen, Leek, The Netherlands). Approximately 0.5 μg mRNA was used in a one-step RT-PCR using primers in exons 1 and 4. The 5′ primer (5′-GAA ATT GCG CTG GAC CGT CGC CTT G-3′) had biotin at its 5′ end, and the 3′ primer (5′-GAA ACT CGT CCT GGG AGC ACG TCT T-3′) had the sequence for the −21 M13 universal primer at its 5′ end. The reaction mixtures for the PCRs contained 40 pmol of each primer, 1.5 U Taq DNA polymerase, 1 × PCR-buffer (Boehringer Mannheim, Germany), 200 μm dNTPs, and 10 U MMLV RNase H^- RT (BRL, Life Technologies, Rockville, MD) in a 100 μl reaction vol-

ume. A DNA Thermal Cycler 480 (PE Applied Biosystems, Foster City, CA) was used for the thermal cyclings. The conditions for the thermal cyclings were: 42°C for 1 h followed by 30 cycles of 1 min at 95°C, 2 min at 55°C, and 2 min at 72°C. The thermal cyclings were completed by terminal extension at 72°C for 10 min. cDNA sequencing was performed using Sequenase and solid-phase sequencing (21), and analyzed on a Model 373A Automated DNA Sequencer (PE Applied Biosystems, Foster City, CA).

^{125}I-labeled LDL and ^{125}I-labeled VLDL degradation assay

LDL with a density of 1.02–1.05 g/ml and very low density lipoprotein (VLDL) with S_f 100–400 were isolated by ultracentrifugation of plasma from fasted normolipidemic human subjects with the most common apoE phenotype (E3/3) as described previously (22). Lipoproteins were iodinated to a specific activity of 100–400 μCi/ng by the iodine monochloride method (23).

Fibroblasts from the FH homozygote proband and from a healthy normocholesterolemic subject, as well as from a FH-Elverum homozygote, were maintained in DMEM (Gibco, Life Technologies, Rockville, MD) containing 20% fetal calf serum (FCS) at 37°C in a humidified atmosphere of 5% CO_2. For the degradation assay, cells were grown to 70% confluency in DMEM supplemented with 20% FCS and 2 mm glutamine. LDL receptors were up-regulated by incubation for 48 h prior to the assay with media containing 2 mg/ml lipoprotein-deficient serum, the final 24 h in presence of 1 μg/ml of lovastatin (24).

Steady-state LDL degradation was measured after incubating cells with iodinated LDL or VLDL for 5 h at 37°C. At the end of the incubation, the culture media were collected and long chain proteins were precipitated by adding trichloroacetic acid (TCA) to a final concentration of 15%. The lipids were removed from the supernatant by a chloroform–methanol extraction. The aqueous phase was counted for radioactivity. Degradation was defined as radioactivity present in the incubation medium that was soluble in TCA and not extractable in chloroform–methanol. Radioactivity was converted to units of protein mass using the specific activity of iodinated lipoprotein. Total protein in each well as determined by the Lowry assay (25) varied by less than 15% within each experiment. Results were corrected for total cellular protein.

Detection of the 1359-1; G→A mutation

A 5′ primer (5′-ATG CCC TTC TCT CCT CCT GCC TC-3′), encompassing nucleotides −3 to −25 of intron 9, and a 3′ primer (5′-AGC CCT CAG CGT CGT GGA TAC GC-3′), encompassing nucleotides +3 to +25 of intron 10, were used to amplify a 278 bp fragment with a natural DdeI restriction site. The 1359-1; G→A mutation (26) will destroy this restriction site. The reaction mixtures for the PCRs contained 20 pmol of each primer, 0.5 U Taq DNA polymerase, 1 × PCR-buffer (Boehringer Mannheim, Germany), and 200 μm dNTPs in a final volume of 50 μl. A Gene Amp PCR Systems 2400 (PE Applied Biosystems, Foster City, CA) was used for the thermal cyclings. The conditions for the thermal cyclings were: 7 min at 94°C followed by 25 cycles of 45 s at 95°C, 45 s at 55°C, and 45 s at 72°C. The thermal cyclings were completed by terminal extension at 72°C for 10 min. Twenty μl of the reaction products was cut with 15 U DdeI under the conditions recommended by the manufacturer (New England Biolabs, Beverly, MA). The restriction fragments were analyzed by 8% PAGE. In the absence of the mutation, the 278 bp fragment was cut into fragments of 257 bp and 21 bp, and in the presence of the mutation, the fragment remained uncut.

RESULTS

Southern blot analysis of the LDLR gene

DNA from 57 unrelated FH subjects was subjected to Southern blot analysis using six different restriction enzymes and cDNA probes encompassing exons 1–10 or 11–18.

Using the restriction enzyme SphI and a cDNA probe encompassing exons 1–10, invariant bands of 9.4 kb and 5.3 kb were detected. In addition, normal subjects, homozygous for absence or presence of the polymorphic SphI site in intron 6, had bands of 3.7 kb and 3.2 kb, respectively (**Fig. 2**).

In one subject, who was a FH homozygote clinically, the invariant 9.4 kb band was lacking and an abnormal band of 4.6 kb was observed (**Fig. 3**). The 4.6 kb band was also observed in the father, mother, and brother of the proband, who were all clinically heterozygotes. These data suggested that a structural alteration of the LDLR gene caused FH in this family. The father and brother were both heterozygous for the polymorphic SphI site, while the mother and the FH homozygote were homozygous for the presence of the polymorphic SphI site. This should indicate that neither the polymorphic SphI site in intron 6 nor the invariant SphI site in intron 5 was affected by the structural alteration (Fig. 2).

Analysis of the structural alteration by PCR and cDNA sequencing

Based upon the probe that was used and the restriction map of the gene, the FH homozygote must have been

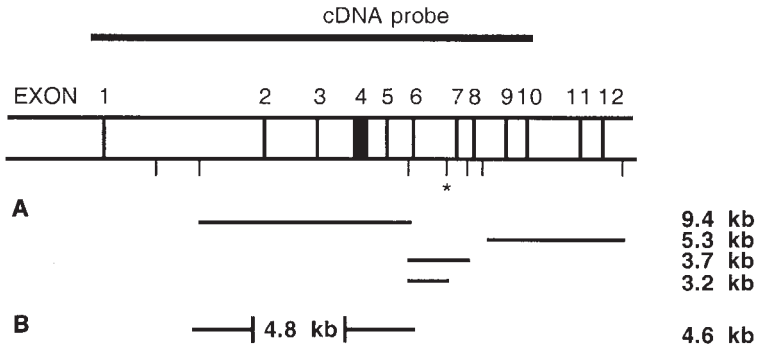

Fig. 2. Analysis of restriction fragments of the LDLR gene using Southern blot analysis and the restriction enzyme SphI. Above the schematic drawing of the LDLR gene (adopted from ref. 6) is shown the cDNA probe used to analyze the polymorphic SphI site in intron 6 by Southern blot analysis. The exons are indicated by vertical bars and SphI restriction sites are shown below the LDLR gene. The polymorphic SphI site is indicated by an asterisk. In A, the fragment lengths (kb) detectable by Southern blot analysis are indicated. Neither fragments shorter than approximately 0.8 kb nor the large fragment spanning exon 1 are visualized under the conditions used for the analysis. In B, the predicted 4.8 kb deletion spanning exons 2 and 3, resulting in a 4.6 kb restriction fragment, is indicated.

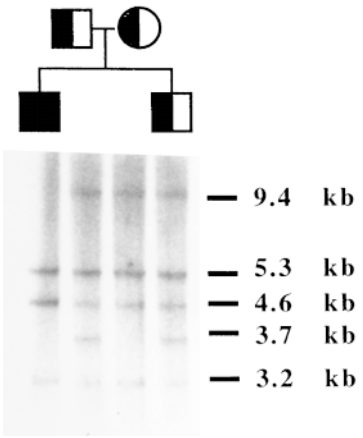

Fig. 3. Southern blot analysis of the polymorphic SphI site in intron 6. Southern blot analysis of the polymorphic SphI site in intron 6 was performed in all members of the family. Shown here are the results of Southern blot analysis of the proband, his FH heterozygous brother, and parents. The half-filled symbols represent FH heterozygotes and the closed symbol represents the FH homozygote proband. The observed fragment lengths are indicated.

lacking the normal 9.4 kb band spanning exons 2 to 5. To further characterize the molecular defect, exons 1 to 5 were individually amplified by PCR. The results showed that in contrast to exons 1, 4 and 5, exons 2 and 3 could not be amplified from genomic DNA of the FH homozygote (**Fig. 4**). Thus, exons 2 and 3 were deleted, and as determined by sequencing of cDNA, exon 1 was spliced to exon 4 (**Fig. 5**). Thus, the homozygote was a true homozygote for a 4.8 kb in-frame deletion that deletes exons 2 and 3. The mutation was denoted Δ2–3.

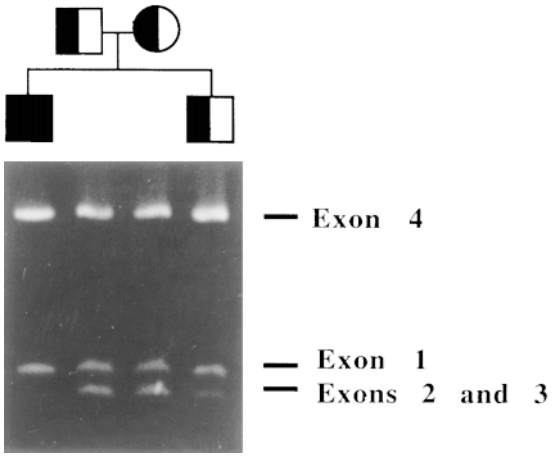

Fig. 4. Analysis of exons 1–5 of the LDLR gene by PCR. Exons 1–5 of the LDLR gene were separately amplified by PCR from DNA of the FH homozygote and his first degree relatives. Twenty-μl samples of the reaction products from amplifications of exons 1–4 from each subject were pooled and loaded in one lane. The PCR products were also analyzed individually in control experiments (data not shown). Exon 5, which is of the same size as exons 2 and 3, could be successfully amplified from all four subjects (data not shown). The half-filled symbols represent FH heterozygotes and the filled symbol represents the FH homozygote proband.

Northern blot analysis of LDLR mRNA

Northern blot analysis of mRNA from fibroblasts of the FH homozygote revealed a single major transcript slightly shorter than the normal (**Fig. 6**). This finding is consistent with a 246 bp deletion of exons 2 and 3. No other transcripts were detected.

As determined by scanning of the autoradiogram, the ratio of the amount of mRNA of the LDLR gene to that of the β-actin gene was 50% higher in the FH homozygous cell line as compared to a normal cell line.

Measurement of LDLR activity by a ^{125}I-labeled LDL and ^{125}I-labeled VLDL degradation assay

Degradation of LDL by fibroblasts from the FH homozygote proband possessing the Δ2–3 mutation was approximately 0.3 times that of normal fibroblasts (1287 ng/mg protein and 3320 ng/mg protein, respectively, using 10 μg/ml ^{125}I-labeled LDL), while degradation of LDL by fibroblasts from an FH homozygote possessing FH-Elverum (17) was 0.1 times that of normal fibroblasts (354 ng/mg protein and 3320 ng/mg protein, respectively, using 10 μg/ml ^{125}I-labeled LDL) (**Fig. 7A**). All three cell lines responded to the LDL receptor up-regulation by a 10- to 15-fold increase in LDL degradation. On the other hand, VLDL degradation by fibroblasts from the proband was identical to that of normal cells (1443 ng/mg protein and 1587 ng/mg protein, respectively, using 10 μg/ml ^{125}I-labeled VLDL), whereas VLDL degradation was reduced in the LDL receptor-negative FH-Elverum mutant (466 ng/mg protein at 10 μg/ml ^{125}I-labeled VLDL) (Fig. 7B). Thus deletion of exons 2 and 3 resulted in receptors that had normal VLDL degradation but impaired LDL degradation.

Haplotype analysis at the LDLR locus

Haplotype analysis at the LDLR locus was performed in family members of the FH homozygote proband using seven RFLPs within or closely linked to the LDLR gene. The deletion segregated with haplotype: SphI: +, StuI: +, AvaII: +, 5′ ApaLI: +, NcoI: +, PstI: +, 3′ ApaLI: +.

Frequency of the Δ2–3 mutation among Norwegian FH heterozygotes

In order to study the frequency of the Δ2–3 mutation among Norwegian FH heterozygotes, one FH subject from each of 41 additional unrelated FH families was analyzed by Southern blot analysis. None of these had the abnormal 4.6 kb band. Thus, this mutation appears to be present in less than 1% of Norwegian FH patients.

Analyses of the 1359-1; G→A mutation

Screening for the Δ2–3 mutation in additional family members revealed that subject II-3 (Fig. 1), who was diagnosed as a FH homozygote according to standard clinical criteria (2), was heterozygous for the Δ2–3 mutation. Sequencing each of the 18 exons and the immediate flanking regions of the LDLR gene (21, 27, 28), identified heterozygosity for a G→A splice acceptor mutation at position −1 of intron 9 (1359-1; G→A). Thus, this subject was a

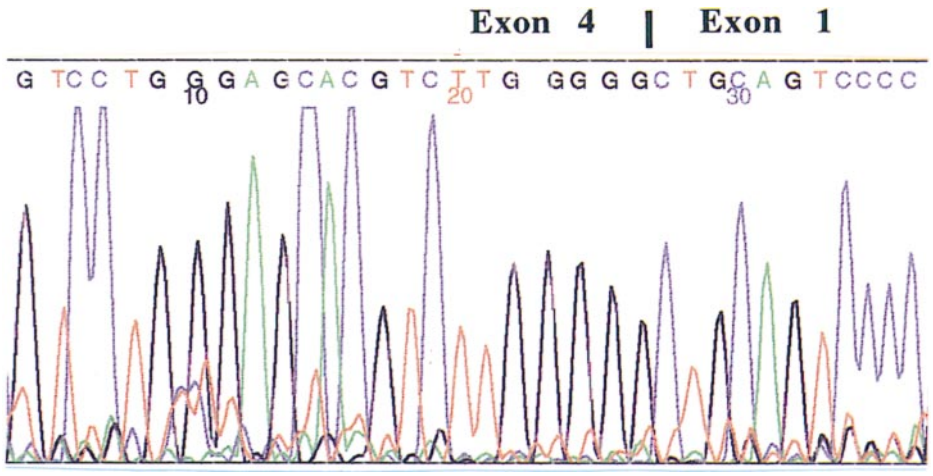

Fig. 5. Characterization of the LDLR cDNA by sequencing. A 5′ primer in exon 1 and a 3′ primer in exon 4 were used to amplify a 98 bp fragment by RT-PCR of mRNA isolated from the FH homozygote proband. The 3′ primer had at its 5′ end the sequence for the −21 M13 universal primer, and the 5′ primer was biotinylated. Solid phase automated sequencing of the antisense strand was performed using dye primers (−21 M13) and a Model 373A Automated DNA Sequencer (PE Applied Biosystems, Foster City, CA). The boundary between exon 1 and exon 4 is indicated.

compound heterozygote. The 1359-1; G→A mutation has been previously described in the Dutch population (26). A PCR-based assay was developed, and the 1359-1; G→A mutation was also detected in the father of the compound heterozygote; subject I-4 in Fig. 1.

DISCUSSION

Screening for structural alterations of the LDLR gene by Southern blot analysis revealed a 4.8 kb deletion of exons 2 and 3 in an FH homozygous subject. A 5 kb deletion of exons 2 and 3 has previously been described in the French Canadian population (16, 29) and the Japanese population (30). By comparing the results of the published restriction fragment analyses, it appears that the Norwegian deletion is different from the two other deletions. However, as the deletion breakpoints have not been identified, further studies are necessary in order to decide whether this is indeed a novel deletion.

Sequencing of cDNA confirmed that exon 1 was spliced to exon 4. As introns in this region of the gene disrupt the coding sequence without distorting the reading frame, deletion of exons 2 and 3 is predicted to result in a receptor protein that is 82 amino acids shorter than normal. The functional consequence will be a receptor that lacks the first and second repeat of the ligand binding domain (6, 7). The deletion appears to account for the mutation in less than 1% of Norwegian FH patients, and occurs on a chromosome with haplotype 3 according to the nomenclature of Rødningen et al. (8). This haplotype accounts for 43.1% of the defective haplotypes in Norwegian FH patients (8), and must therefore also harbor many other mutations.

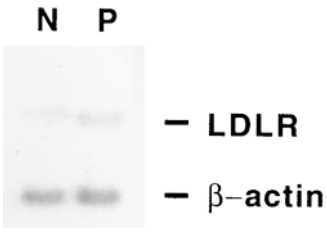

Fig. 6. Analysis of the LDLR mRNA by Northern blot analysis. Northern blot analysis of mRNA from a normal subject (N) and the FH homozygote proband (P) showing the fragments hybridized to the LDLR probe encompassing exons 13–14 and the β-actin probe.

The value for total serum cholesterol of the homozygous proband was 13.9 mmol/l before plasma exchange therapy was started. Because FH homozygotes typically have values for total serum cholesterol in the range of 20–25 mmol/l (2), these data indicate that deletion of exons 2 and 3 causes only a moderate reduction in receptor-mediated endocytosis of LDL. A relatively mild phenotype has also previously been observed in four true homozygotes for a 10 kb deletion involving exons 2 and 3 (31, 32).

The notion that deletion of exons 2 and 3 causes a mild phenotype is also supported by the results of in vitro site-directed mutagenesis experiments of transfected mammalian cells (33–35). These studies have shown that deletion of exons 2 and 3 reduced the binding of LDL by only 30%. Thus, the first two repeats of the LDLR ligand binding domain are only of minor importance for the binding of LDL.

These findings are in line with the results of the ^{125}I-labeled LDL degradation assay in our study. Whereas cells from a true homozygote carrying a null allele had a value for degradation of ^{125}I-labeled LDL that was 0.1 times that of normal cells, the corresponding value from cells of the proband who was homozygous for Δ2–3 was 0.3 times that of normal cells. This indicates that the mutant receptor is

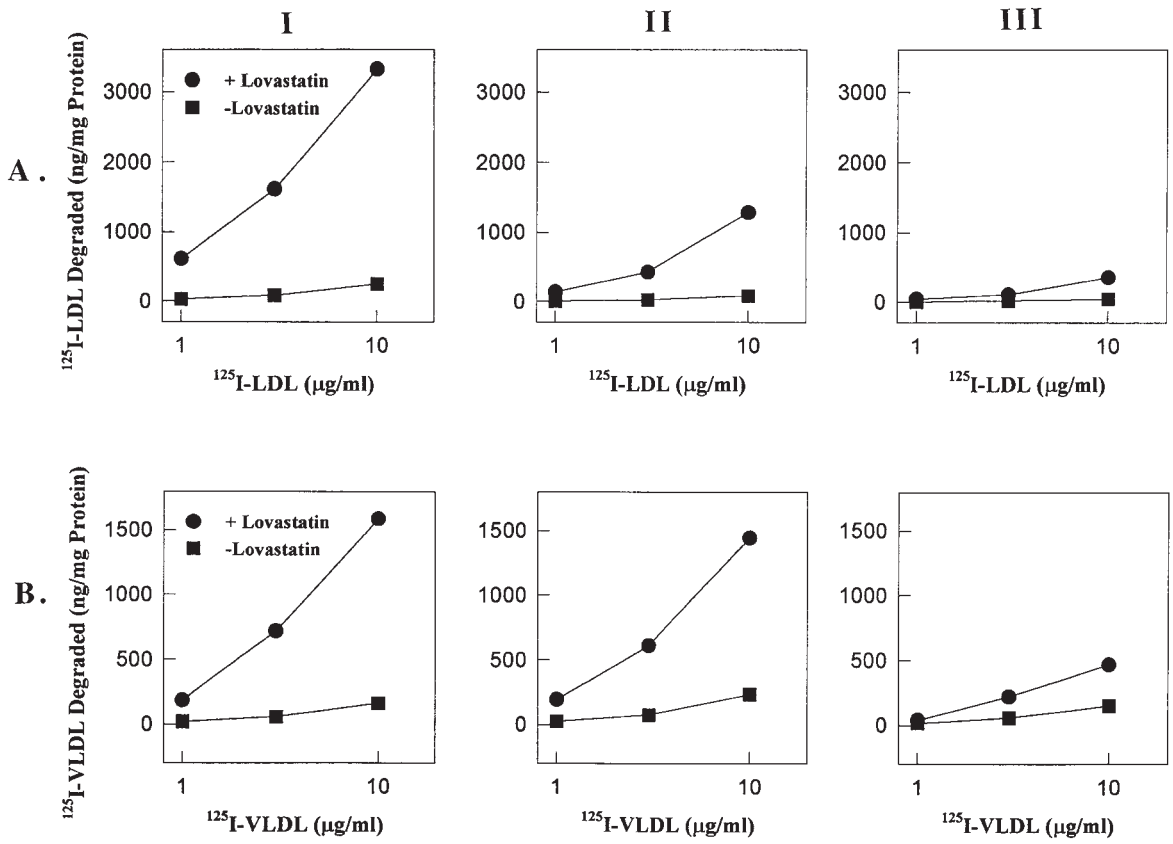

Fig. 7. ^{125}I-labeled LDL and ^{125}I-labeled VLDL degradation by fibroblast lines. Fibroblasts were cultured from a normal subject (I), the FH homozygous proband (II) and from a receptor-negative FH patient possessing the FH-Elverum mutation (III). Cells were incubated with the indicated concentration of ^{125}I-labeled LDL (**A**) or ^{125}I-labeled VLDL (**B**) and degradation was measured in the presence (circle symbol) or absence (square symbol) of lovastatin treatment as described in the Methods section.

able to bind, internalize, and degrade LDL. These results are also in line with those of Mabuchi et al. (32). They found that the receptor activity of a true homozygote for a 10 kb deletion of exons 2 and 3 (FH Tonami-2) was approximately 40% of normal.

In contrast to the reduced degradation of LDL to cells from our homozygous proband, degradation of VLDL was completely normal. These findings of normal degradation of VLDL but impaired degradation of LDL are consistent with the findings of Russell, Brown, and Goldstein (35) indicating that binding of LDL is mediated by repeats 2–7 while only repeat 5 is crucial for binding of VLDL.

The data of Russell et al. (35) and those of ours are at variance with those of Sass et al. (36). Based upon ligand blotting of cell protein extracts using either ^{125}I-labeled LDL or ^{125}I-labeled βVLDL, they concluded that the mutant receptor caused by a 5 kb deletion of exons 2 and 3 was unable to bind both LDL and VLDL. The explanation for this discrepancy is unknown, but could possibly be due to the different assays used for measurement of LDL receptor activity.

In contrast to the mild phenotype in our homozygous proband, the heterozygous parents had a severe phenotype with levels of total serum cholesterol in the range of 11–12 mmol/l. The two other heterozygous relatives possessing the Δ2–3 mutation also had relatively high levels of total serum cholesterol. These findings are in agreement with those of Sass et al. (36), who found that FH heterozygous French Canadians carrying a 5 kb deletion of exons 2 and 3, had unexpectedly high values of total serum cholesterol. Thus, heterozygotes for this deletion seem to be relatively more severely affected than the homozygotes.

The FH heterozygous subject possessing the 1359-1; A→G mutation also had a high value of serum cholesterol, suggesting that this mutation severely affects the function of the receptor. His daughter, who was a compound heterozygote for Δ2–3 and 1359-1; A→G, was also severely affected, with a total serum cholesterol level of 32.0 mmol/l at the age of 1.

At face value a compound heterozygote possessing one mild and one severe mutation would be expected to have a phenotype in between that of a true homozygote possessing a mild mutation and a true homozygote possessing a severe mutation. However, in the compound heterozygote, the 1359-1; A→G mutation apparently has a dominant negative effect over the Δ2–3 mutation. On the other hand, in the heterozygous parents of the proband, it seems that the Δ2–3 mutation exerts a dominant negative effect. Thus, these findings may suggest that the two allelic receptor proteins do not function independently of each other.

One model for how the two allelic receptor proteins might interact is that the receptor functions as a dimer, as has been previously suggested (37, 38). A subnormal function of heterodimers would explain the clinical features of our patients. A mild phenotype would then be expected in the homozygous proband with homodimers made up of two mildly defective proteins, whereas a relatively severe phenotype would be expected in patients who are heterozygotes or compound heterozygotes, irrespective of whether the mutations themselves are mild or severe. However, other mechanisms for the phenotypic variability, including environmental or protective genetic factors, must also be considered.

Another particular feature of our homozygous proband is the lack of response to statin therapy, whereas his heterozygous parents responded well. The latter finding is presumably due to up-regulation of the normal allele. In FH homozygotes with mutant LDLRs that are severely defective in binding of LDL, an increased level of transcription by a statin will not be expected to result in increased LDLR-mediated catabolism of LDL. This expectation is also supported by clinical experience. However, if homozygotes like our proband produce a receptor that has just a moderate reduction in the ability of binding LDL, one would expect the increased level of transcription by a statin to result in a significant reduction of the level of total serum cholesterol. However, only a marginal effect of 40 mg lovastatin was observed in our homozygote.

Northern blot analysis of mRNA from fibroblasts from the homozygote cultured in the absence of a statin revealed that mRNA was at least as abundant as that of a normal cell line. A 50% overexpression of the deleted allele was also found in 7 French-Canadian heterozygotes with a 5 kb deletion of exons 2 and 3 (36). Taken together with the clinical findings, the Northern blot analysis could indicate that the transcription might already be at its maximum. Apparently, the observation that cultured fibroblasts from the proband responded to lovastatin by a 10- to 15-fold increase in LDL degradation is at variance with this notion. However, the discrepancy between the in vivo and in vitro effects of lovastatin could possibly be due to the higher doses of lovastatin used for the in vitro study.

Although further studies are needed to prove that the LDL receptor functions as a dimer, such a model could explain unexpected clinical manifestations of FH as reported in this study. Our study also underscores the importance of characterizing the naturally occurring mutations in order to study the normal lipid metabolism.

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