

Partial duplication of the EGF precursor homology domain of the LDL-receptor protein causing familial hypercholesterolemia (FH-Salerno)

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Abstract A novel mutation of low density lipoprotein (LDL)-receptor gene was found in an Italian familial hypercholesterolemia (FH) patient during a screening of 300 FH patients. The proband as well as her daughter were found to be heterozygotes for the mutation. Binding, internalization, and degradation of ¹²⁵I-labeled LDL by the proband's fibroblasts were reduced to approximately 50% compared to values found in control cells. DNA analysis by Southern blotting showed that the mutant allele was characterized by an insertion of about 10 kb, which resulted from a duplication of exons 9-14 of the LDL-receptor gene. In addition, Northern blot analysis of the proband's RNA showed, besides the normal-sized LDL-receptor mRNA (5.3 kb), an additional mRNA of about 6.2 kb. The junction between exon 14 and the duplicated exon 9 was amplified by polymerase chain reaction (PCR) from the cDNA. The sequence of the amplified fragment showed that exon 14 joined the duplicated exon 9 without changing the reading frame. The derived amino acid sequence indicated that the mutated receptor protein had a partial duplication of the EGF precursor homology domain. Ligand and immunoblotting revealed that proband's fibroblasts contained one-half of the normal amount of LDL-receptor protein (molecular mass 130 kDa) and an abnormally large receptor of approximately 160 kDa. The amount of this abnormal receptor as detected by two monoclonal antibodies (10A2 and 4B3) was found to be approximately 30% that of the normal LDL-receptor present in the same cells. Treatment of the proband's cells with pronase greatly reduced the amount of both normal and abnormal receptors detected, indicating that both receptors were present on the cell surface. Pulse-chase experiments using [³⁵S]methionine indicated that the receptor was processed to the mature form (195 kDa), although at a rate slightly slower than the normal receptor (160 kDa) present in the same cells. However, the mature abnormal receptor was degraded much more rapidly (half life 4.6 h) than the normal receptor present in the proband's cells (half life 11.9 h) or in normal cells (half life 12.4 h). **■** In conclusion, the mutant allele present in our proband produces an abnormally large receptor protein that is normally processed to the mature form but is degraded more rapidly than the normal counterpart. As the proband's family originated from the city of Salerno, in southern Italy, the mutation was named FH_{Salerno}. — **Bertolini, S., D. D. Patel, D. A. Coviello, N. Lelli, M. Ghisellini, R. Tiozzo, P. Masturzo, N.**

Elicio, B. L. Knight, and S. Calandra. Partial duplication of the EGF precursor homology domain of the LDL-receptor protein causing familial hypercholesterolemia (FH-Salerno). *J. Lipid Res.* 1994. 35: 1422-1430.

Supplementary key words familial hypercholesterolemia • LDL-receptor gene • exon duplication • ligand blotting • immunoblotting • receptor synthesis and degradation

Familial hypercholesterolemia (FH) is an inherited disease with an autosomal dominant trait; the heterozygote frequency is about one in 500 and the homozygote frequency less than one in a million of the Western population. FH is characterized by a selective elevation in the plasma level of low density lipoprotein (LDL) (two- to threefold in heterozygotes, sixfold or more in homozygotes and in compound heterozygotes), tendon and skin xanthomas, arcus corneae in young people, and premature coronary heart disease (CHD) in the fourth and fifth decade of life in the heterozygous patients and in the first or second in the homozygous form (1). The disease is caused by mutations in the gene coding for the LDL receptor, which is located on the short arm of chromosome 19; the gene spans 45.5 kb and consists of 18 exons and 17 introns (1). The final product of the LDL receptor gene is a cell surface glycoprotein of 839 amino acids (aa) with five domains: ligand binding domain (292 aa) encoded by exons 2-6, EGF precursor homology domain (400 aa) encoded by exons 7-14, O-linked sugars domain (58 aa) encoded

Abbreviations: FH, familial hypercholesterolemia; LDL, low density lipoprotein; LPDS, lipoprotein-deficient serum; FCS, fetal calf serum; PCR, polymerase chain reaction; EGF, epidermal growth factor; CHD, coronary heart disease; RFLP, restriction fragment length polymorphism.

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by exon 15, membrane-spanning domain (22 aa) encoded by exons 16–17, and cytoplasmic domain (50 aa) encoded by exons 17–18 (1–3).

A large number of mutations of the LDL receptor gene (deletions, insertions, nonsense and missense mutations) have been characterized at the DNA level (4, 5). Some of these mutations have been defined at the protein level, allowing the identification of five classes of functional defects of the receptor. These classes include defect in synthesis, intracellular transport, ligand binding properties, internalization, and recycling of the receptor (4, 5).

In the present study we describe a novel LDL-receptor gene mutation that causes familial hypercholesterolemia in an Italian kindred. The proband was heterozygous for a mutation that consists of a duplication of six exons (from exon 9 through exon 14) that surprisingly does not produce a shift in the reading frame. As a result, the allele bearing the duplication encodes for an abnormally large receptor protein. This reaches the cell surface but is degraded more rapidly than its normal counterpart.

SUBJECTS AND METHODS

Subjects

The proband (I.C.) was a 55-year-old woman who had experienced a myocardial infarction when she was 50; her father and brother had died from myocardial infarction when they were 52 and 57, respectively. The proband had a history of high plasma cholesterol concentration but not of tendon xanthomas, arcus cornealis, and/or xanthelasma. She had three children (two males and one female) who were checked for plasma cholesterol and lipoprotein levels. All the subjects gave their informed consent for this study.

Biochemical analysis

Plasma lipoproteins were separated by preparative ultracentrifugation in a Beckman 50.4 Ti rotor (6) and cholesterol and triglyceride were assayed by enzymatic methods (Boehringer, Mannheim, Germany) (6). Plasma A-I and B apolipoproteins were measured by immunonephelometry (Behringwerke AG, Marburg, Germany).

Southern blot analysis

Genomic DNA was extracted from peripheral blood leukocytes by a standard procedure (7). DNA (5–15 μg) was digested using 5–10 U/ μg of several restriction enzymes (PvuII, EcoRI, XbaI, BglII, KpnI, BamHI, NcoI, ApaI, and StuI), separated by agarose gel electrophoresis, and transferred to nylon membranes (Hybond-N, Amersham International, UK). Two cDNA clones of the LDL-receptor gene (pLDLR-2HH1 and pTZ1) were a kind gift from Dr. D. W. Russell (Dallas, TX). pLDLR-2HH1 is a plasmid that contains a BamHI insert

of 1.9 kb corresponding to the last 8 exons of the gene; pTZ1 is a plasmid that contains the whole coding sequence of the gene in a HindIII insert of 2.6 kb. Exon-specific cDNA probes were obtained by digesting both inserts with restriction enzymes as previously specified (8). Pre-hybridization and hybridization of the filters were carried out as reported previously (8). Dried filters were subjected to autoradiography on Hyperfilm-MP (Amersham International, UK) for 24–48 h at -80°C . Densitometric scanning of X-ray films was performed by using a laser densitometer (Ultrosan XL, Pharmacia LKB Biotech., Sweden).

Northern blot analysis

Total cellular RNA was isolated by extraction in guanidine-thiocyanate (9) from cultured skin fibroblasts that had been maintained in a lipoprotein-deficient serum (LPDS) for 15 h. RNA (15 μg) was denatured in 50 μl 50% formamide, and separated by electrophoresis in 1.2% agarose gels and transferred to Hybond-N membranes as previously reported (8). Membranes were hybridized with either the full-size cDNA probe or a probe specific for exons 15–17 (8). The cDNA clone pHF β A-1 of human β -actin was used to normalize the RNA filters. Pre-hybridization and hybridization were performed as previously described (8).

Reverse transcription and PCR amplification

RNA (10 μg) from cultured fibroblasts of the proband (I.C.) and a normal subject were reverse-transcribed in a 50- μl reaction mixture, at 37°C for 1 h, using a cDNA synthesis kit (Gibco-Brl, UK), and an oligonucleotide exon 9-specific primer (9 reverse, "9rv": 5' CTGCAGATCATCTCTGGA 3'). The single-stranded cDNA was ethanol-precipitated and the polymerase chain reaction (PCR) was performed in the same test tube. The RNA-DNA hybrids were used to amplify the junction of the duplicated region using an exon 14-specific primer (14 forward, "14fr": 5' CCACTCGCCCAAGTTTACC 3'), and the antisense exon 9-specific primer used for the synthesis of the first strand cDNA ("9rv", see above). The first cycle was used to synthesize the second strand of the cDNA, and it was carried out at 93°C for 7 min (denaturation), at 60°C for 1 min (annealing), and at 72°C for 3 min (extension); the following 25 cycles were carried out at 93°C for 1 min, at 60°C for 30 sec, and at 72°C for 3 min. In the last cycle the time for the extension was 7 min. An asymmetric PCR was performed to obtain single stranded DNA (10); the molar ratio of the two primers used was 100:1. One-tenth of the amplified material was fractionated by gel electrophoresis on 1.5% agarose to check the amount of the single-stranded DNA and its size; the remaining amplified material was purified using Centricon-100 microconcentrator (Amicon Div., Danvers, MA), and sequenced directly without further subcloning.

Sequencing

Sequencing of single-stranded DNA was performed by the dideoxynucleotide chain-termination method (11), using the enzyme Sequenase (United States Biochemical Corp., Cleveland, OH) and ^{35}S dATP as labeled nucleotide (NEN-DuPont, UK). The primers used to sequence both strands were the same as those used in the PCR reaction (see above).

Fibroblast culture and LDL-receptor activity

A skin biopsy was taken, after informed consent, from the proband and from four control subjects. Explants were cultured in 25-cm² flasks in DMEM (Dulbecco's modification of Eagle's Medium), 100 IU/ml of penicillin, and 50 $\mu\text{g}/\text{ml}$ of streptomycin, 2 mM glutamine, 15% fetal calf serum, and 95% air-5% CO₂. The assay of ^{125}I -labeled LDL binding, internalization, and degradation by cultured skin fibroblasts was performed as previously described (12).

Receptor content and synthesis

For ligand blotting, cell monolayers that had been incubated for 48 h in LPDS were washed three times with phosphate-buffered saline, scraped from the dishes with a cell scraper, and centrifuged at 2000 *g* for 5 min. The cell pellet was solubilized with 200 mM Tris-maleate, pH 6.5, 2 mM CaCl₂, 0.5 mM PMSF (phenylmethylsulfonyl fluoride), 25 μM leupeptin, and 40 mM β -octyl-D-glucoside. The cell extracts were centrifuged at 300,000 *g* (Beckman TLA 100.1 rotor) at 4°C for 40 min and the pellet was discarded. Protein concentration in the supernatant was measured by the method of Lowry et al. (13). For blotting experiments the samples did not contain reducing agents and were not heated prior to application to polyacrylamide gel. Proteins were separated by 7% SDS-PAGE according to Laemmli (14). Electrophoretic transfer of proteins to nitrocellulose filters was performed in a buffer containing 10 mM NaHCO₃ and 3 mM Na₂CO₃ at pH 9.9 in 20% (v/v) methanol (15). Ligand blotting with ^{125}I -

labeled LDL was performed as described by Daniel et al. (16). For immunoblotting, cells were harvested and solubilized as described by van Driel et al. (17) and the proteins were separated and detected with specific monoclonal antibodies to the LDL receptor (monoclonal antibodies 4B3 and 10A2) as described before (18). Antibody 4B3 is known to bind to the extreme NH₂-region of the receptor protein (B. L. Knight, D. D. Patel, and A. K. Soutar unpublished observations) which is outside the duplicated region of the abnormal receptor protein found in the proband's fibroblasts. Methods used for the immunoprecipitation of ^{35}S -labeled LDL receptor protein from cultured cells have been described previously (19). Monoclonal antibody 4B3 was used throughout these experiments.

RESULTS

Biochemical findings

Lipoprotein analysis, carried out in proband I.C. and four members of her family (Table 1), confirmed the presence of severe hypercholesterolemia previously documented in the proband, and revealed a mild increase in the level of plasma cholesterol and apoB in her daughter (D.P.).

Genomic DNA analysis

The proband's DNA was first screened with probes complementary to the 3' end of the gene in order to detect gross rearrangements as well as common RFLPs. Digestion with PvuII and StuI and hybridization with a probe specific for exons 11-18 revealed the presence of abnormal fragments (10 and > 24 kb, respectively) (Fig. 1A and Table 2). Abnormal fragments of variable size were also seen when DNA was digested with a number of other restriction enzymes (XbaI, BglII, EcoRI, BamHI, and KpnI) (Table 2). These preliminary results indicated that the proband was heterozygous for a gross rearrangement in the LDL-receptor gene. This was also detected in her

TABLE 1. Biochemical findings in the proband and her relatives

Subject	Name	Age	Total CH	LDL CH	HDL CH	TG	Apolipoprotein	
							A-I	B
		<i>yr</i>			<i>mg/dl</i>			
Proband	IC	55	403	339	49	88	152	187
Husband	DD	63	168	119	30	110	124	81
Son	DF	18	143	94	42	38	138	64
Son	DG	27	212	162	37	77	133	93
Daughter	DP	23	260	203	47	55	150	125

All data represent the mean value of three determinations performed on different days; CH, cholesterol; TG, triglycerides.

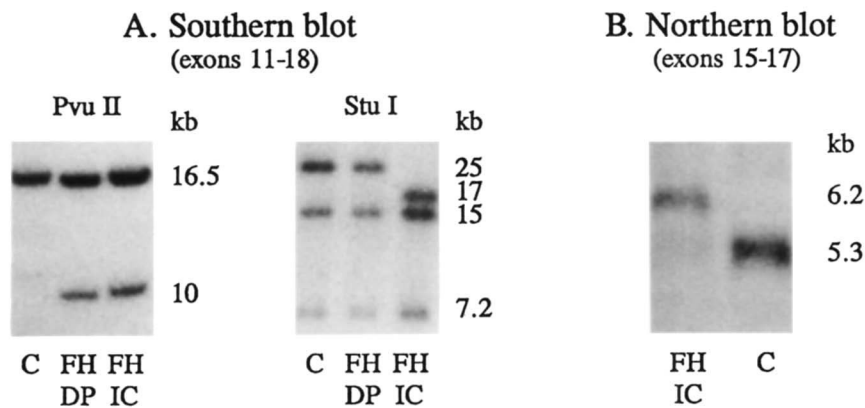


Fig. 1. A) Southern blot analysis of PvuII and StuI digests of DNA from the proband (FH-IC), her hypercholesterolemic daughter (FH-DP), and one control subject. The hybridization was performed with a cDNA probe specific for exons 11-18 of the LDL-receptor gene. B) Northern blot analysis of mRNA isolated from skin fibroblasts of the proband (FH-IC) and a control subject (C). The hybridization was performed with a cDNA probe specific for exons 15-17 of LDL-receptor gene.

hypercholesterolemic daughter but not in her two normolipidemic sons.

Densitometric analysis of the filters revealed that the hybridization intensity of the normal fragments seen in the proband was identical to that found in controls; this observation was consistent with the presence of a double gene dose. On the other hand, the relatively low intensity of the additional bands seen in the same lanes was com-

patible with the presence of a single gene dose (Fig. 1A). Moreover, the hybridization of NcoI digests with a probe specific for exons 11-18 showed no abnormal fragment but revealed a higher intensity of the normal band of 7.2 kb (data not shown); this finding was compatible with three copies of this fragment. From an examination of the restriction map of the normal gene (2) our results, summarized in Table 2, suggested an insertional event whereby

TABLE 2. Southern blot analysis of genomic DNA from IC and DP patients

Restriction Enzyme	Probe (Exons)	Band Size (kb)	
		Normal (5'→3')	Abnormal
PvuII	11-18	16.5, 3.6	10
XbaI	11-15	23, 1.4, 10.5	8.5
	8-9, 8-10	23	8.5
BglII	12-18	21	10
	8-9, 8-10	13	10
EcoRI	12-18	23	10
	15-17	23	
	8-9, 8-10	8	10
KpnI	11-18	20, 9, 14	10
	15-17	9, 14	
	8-9, 8-10	20	10
BamHI	11-15	17	7.4
	1-11	16	7.4 (faint)
NcoI	11-18	7.2 ^a , 9, 3.4/13	
	1-11	9.4, 7.5, 7.2 ^a	2.8
ApaLI	11-18	5.8, 1.7, 3.8, 2.8, 19.6	5.5 (faint)
	1-11	12, 5.8	5.5
StuI	11-18	15, 7.2	25
	11-15	15	25
	1-11	8.4, 1.6, 15	25
	8-9, 8-10	15	25

^aNormal size, but intensity higher than normal.

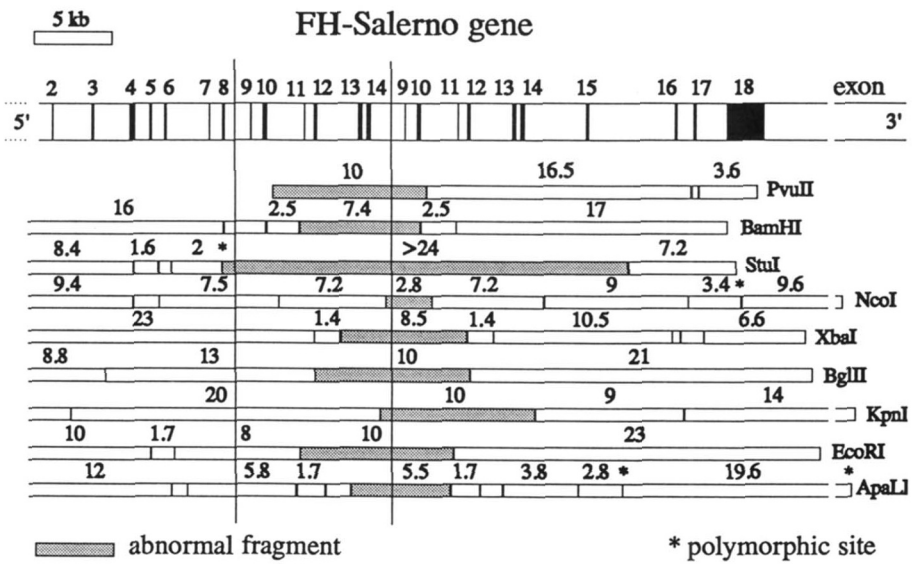


Fig. 2. Restriction map of the insertion-bearing allele of the LDL receptor gene in FH_{Salerno} (proband I.C.).

the restriction sites included between intron 8 and the 5' half of intron 14 were repeated about 10 kb downstream (Fig. 2).

The hybridization of EcoRI and KpnI digests with a probe complementary to exons 15–17 did not reveal any abnormal band (Table 2), thus excluding the involvement of exons 15–18 in the observed rearrangement (Fig. 2). In order to define the 5' end of the insertion more accurately, several DNA digests (XbaI, BglII, KpnI, EcoRI) were hybridized with a probe complementary to exons 8–9. As the abnormal bands were still detectable with this probe (Table 2), we inferred that exon 9 was involved in the insertion (Fig. 2).

The restriction patterns taken together indicated that proband I.C. and her daughter were heterozygous for the presence of an insertion of about 10 kb due to a duplication of exons 9–14, as illustrated in Fig. 2.

Molecular characterization mutant mRNA

Northern blot analysis revealed two LDL-receptor mRNA species in the proband's fibroblasts: one with a normal size of 5.3 kb and the other with a larger size of about 6.2 kb (Fig. 1B).

To ascertain whether the sequence corresponding to exon 14 was followed by that of the exon 9 bis in the mutated mRNA, (i.e., the duplicated exon 9) the cDNA spanning the junction of these exons was amplified by asymmetric PCR and sequenced. By using "14fr" and "9rv" primers, we obtained an amplified fragment of 246 nt that was sequenced directly. The sequence of this fragment showed that exon 14 was normally transcribed, and its last nucleotide "G" joined the first two nucleotides "GC" of the duplicated exon 9 giving the "GGC" codon

specific for glycine. The same codon is produced by the normal junctions between exons 14 and 15 and between exons 8 and 9 (2). The abnormal junction between exon 14 and exon 9 bis produced no change of the reading frame of the duplicated region and did not introduce a new amino acid (Fig. 3).

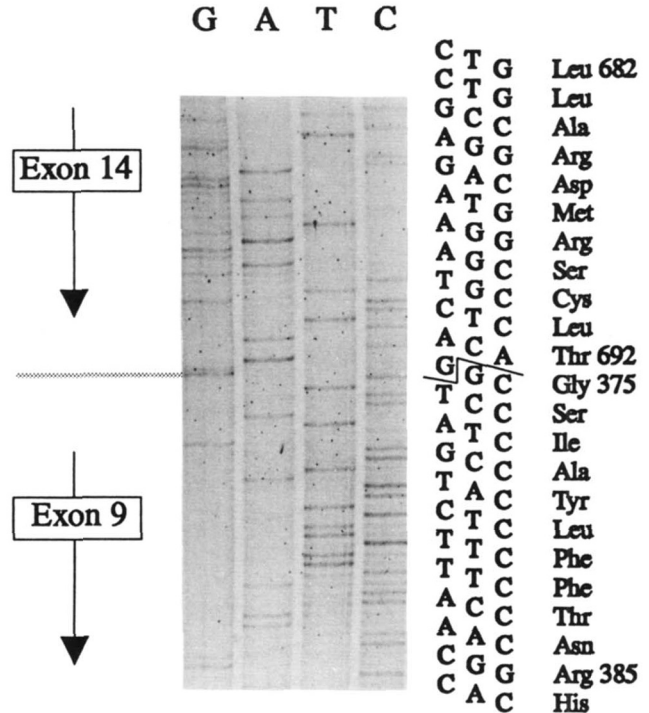


Fig. 3. The cDNA nucleotide sequence corresponding to the junction between exon 14 and duplicated exon 9. The deduced amino acid sequence corresponding to the junction is indicated.

Assay of LDL receptor activity

At 37°C the binding of ¹²⁵I-labeled LDL to the proband's fibroblasts was 48% of that found in the control cell line (at a medium concentration of 50 μg/ml of ¹²⁵I-labeled LDL). Internalization and degradation were reduced approximately to the same extent (51% and 38%, respectively). A similar reduction of ¹²⁵I-labeled LDL binding was observed at 4°C (data not shown).

Ligand and immunoblotting

The binding of ¹²⁵I-labeled LDL to detergent-solubilized cell proteins separated by SDS-PAGE revealed the presence of a major protein with a molecular mass of 130 kDa in normal fibroblasts. In the proband's fibroblasts the intensity of the 130 kDa protein was approximately one-half that found in control cells; in addition, two other proteins with a molecular mass of 158 kDa and 190 kDa were detectable (data not shown).

Immunoblotting with anti-LDL receptor monoclonal antibodies showed only a single abnormally large protein in the proband's fibroblasts, of 155 kDa molecular mass, as well as the normal protein (Fig. 4A). The intensity of this band, compared to that of the normal receptor band in the same cells, was 28% with antibody 10A2 (Fig. 4A, lanes b and c) and 30% with antibody 4B3 (Fig. 4A, lanes d and e). The intensity of both bands was greatly reduced after treatment of the cells with pronase to remove receptor exposed on the cell surface (Fig. 4A, lane a).

Incorporation of [³⁵S]methionine

To examine the synthesis and breakdown of the abnormal receptor, cells were incubated with [³⁵S]methionine

and the radioactivity incorporated into LDL-receptor protein was determined after immunoprecipitation with a specific anti-receptor antibody (4B3) (Fig. 4B). After a 2-h incubation the proband's cells showed two bands by SDS-PAGE under reducing conditions, with relative molecular masses of 150 kDa and 195 kDa, (lane g) in addition to the two present in normal cells (120 kDa and 160 kDa) (lanes f and g). During chase-incubation with non-radioactive methionine, radioactivity was lost from the 120- and 150-kDa bands, suggesting that they were the respective precursors of the 160- and 195-kDa mature forms (lane h). A more detailed investigation showed that the abnormal precursor was processed more slowly than the normal (Fig. 5). However, the lag was not great and the same amount of radioactivity was incorporated into the normal and abnormal mature receptors in the same cells. Experiments using longer periods of chase revealed that the mature abnormal 195-kDa receptor was degraded more rapidly than both normal receptor in normal cells and the mature normal 160-kDa receptor in the same cells (Fig. 4B, lanes i and j). The half life for the loss of radioactivity from the 195-kDa receptor protein was 4.6 h compared to 12.4 h for the normal receptor in normal cells and 11.9 h for the normal receptor in the proband's cells (Fig. 6).

DISCUSSION

Most of the major rearrangements of LDL-receptor gene causing FH are deletions; up until now more than

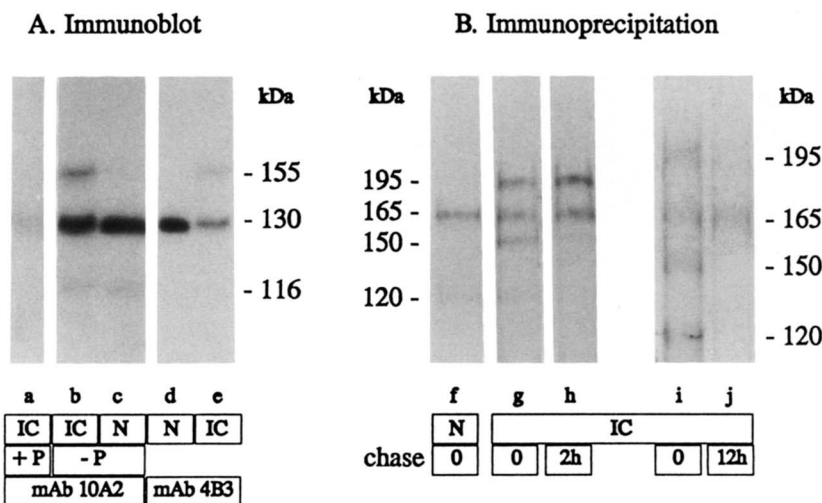


Fig. 4. For immunoblotting, cells were pre-incubated in medium containing 10% LPDS for 40–48 h. A) Immunoblotting with monoclonal antibody 10A2 (a, b, c) or 4B3 (d, e) of extracts from normal (N) (c, d) or proband I.C. (a, b, e) fibroblasts. In lane a the cells had been pre-incubated for 1 h at 4°C with 5 μg/ml pronase. B) Immunoprecipitation of ³⁵S-labeled receptor protein. Cells were pre-incubated for 16 h with 10% LPDS and for 2 h with [³⁵S]methionine followed by the indicated chase periods with 200 μM nonradioactive methionine. Lanes shown are for normal cells with no chase (f) and proband I.C. cells with no chase (g, i), 2 h chase (h) or 12 h chase (j). The values for relative molecular mass were obtained by comparison with known standards.

Time course of the incorporation of [³⁵S] methionine

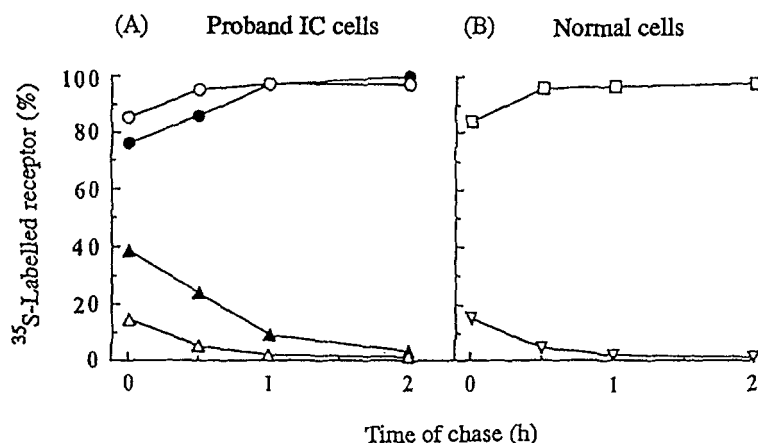


Fig. 5. Cells were pre-incubated for 16 h in medium containing 10% LPDS and for 2 h with [³⁵S]methionine. The radioactivity was then chased for 2 h with 200 μM unlabeled methionine and the LDL-receptor protein was immunoprecipitated and analyzed by electrophoresis and autoradiography. The autoradiographs were scanned to determine the densities of the bands. Results, expressed as % of total normal bands after a 2-h chase, are from two 90-mm dishes at each time point and represent the content of (A) high molecular weight mature receptor (●) and normal mature (○) receptor, and high molecular weight precursor (▲) and normal precursor (△) in proband I.C. cells, and (B) normal mature (□) and normal precursor (▽) in normal cells.

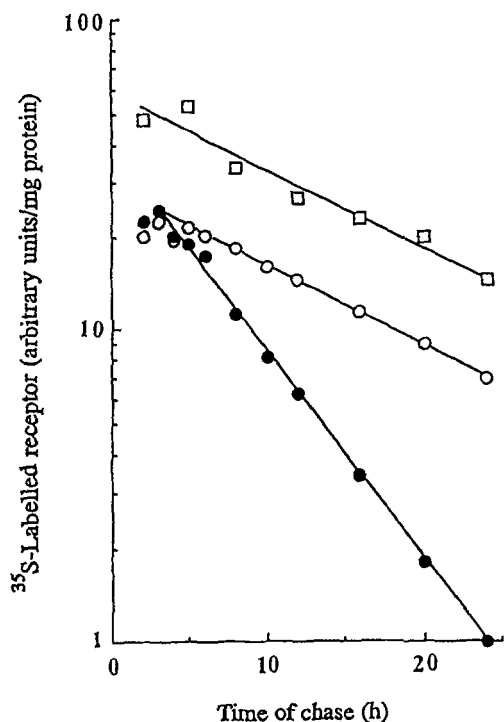


Fig. 6. Cells were pre-incubated for 16 h in medium containing 10% LPDS and for 2 h with [³⁵S]methionine. The radioactivity was then chased for the indicated period with 200 μM unlabeled methionine and the LDL-receptor protein was immunoprecipitated and analyzed by electrophoresis and autoradiography. The autoradiographs were scanned to determine the densities of the bands. Values shown are from 90-mm dishes of cells and represent the radioactivity in the mature receptor of normal cells (□) and the mature normal (○) and high molecular weight receptor (●) of proband I.C. cells.

50 different deletions have been reported (4, 5). By contrast, insertions due to exon duplication seem quite rare (5). Two of the eight insertions reported so far (4, 5, 20) were found by our group in Italian FH patients; FH_{Viterbo} is a 5.5-kb insertion with a duplication of exon 16 and 17, which was found in a homozygous patient (8); FH_{Bologna-2} is a duplication of exon 13-15 which was found in a heterozygous patient (21). The insertion described in the present study is the second largest insertion reported so far. It was detected by Southern blotting (Table 2 and Fig. 2) which, in most instances, revealed the presence of an abnormal band (with an hybridization intensity compatible with a single allele), in addition to the presence of normal bands with an hybridization intensity consistent with a double dose allele. The insertion-bearing allele produces an mRNA, whose size (6.2 kb) is compatible with the insertion of 954 nucleotides corresponding to the transcript of exons 9-14. The sequence analysis of the junction between exon 14 and exon 9 bis (the duplicated exon 9) showed that the processing of the abnormal mRNA was unaffected by the mutation and that the reading frame and the amino acid sequence of the duplicated region did not change. Therefore, the molecular defect in our proband could be defined as the replication of the residues 375-692 in the LDL-receptor protein. The predicted receptor protein should have one complete EGF precursor homology domain followed by the same domain devoid of the first two repeats (A and B); the other domains should be unaffected (Fig. 7). The most likely mechanism for explaining the mutation in proband I.C.

is an unequal crossing-over involving introns 8 and 14. Both these introns contain Alu sequences that are oriented in the same direction (22, 23); these Alu repeats could be involved in a recombination event.

In view of these results, one should expect the molecular mass of the mutated protein to be approximately 160 kDa under nonreducing conditions. Indeed, ligand blotting experiments showed that the proband's fibroblasts contained not only a reduced amount of the normal LDL-receptor (130 kDa) but also a protein of 155–158 kDa that could be regarded as the product of the mutated allele.

Immunoblotting confirmed the presence of the abnormally large receptor protein in proband's cells. Results with antibodies against two different epitopes (one of which is known to be outside the duplicated region) indicated that there was only about 30% as much of the abnormal receptor as of the normal receptor in these heterozygous cells. This did not result from a major defect in receptor synthesis. Immunoprecipitation of ^{35}S -labeled receptor protein showed that the abnormal receptor was synthesized as a precursor of apparent molecular mass of 150 kDa which was processed to a mature 195-kDa protein. Although the abnormal receptor showed a slightly delayed processing (Fig. 5), none was apparently

degraded before it reached the mature form. In contrast, the mature form itself was degraded more rapidly than the normal, at a rate that could account for its reduced content in the cells.

The LDL-receptor assay in skin fibroblasts demonstrated that cells from proband I.C. had reduced binding, internalization, and degradation of LDL. This finding is puzzling in view of the fact that the mutation-bearing allele encodes a receptor that, apart from its increase in size, has all the functional domains present in the normal LDL-receptor protein and binds LDL apparently normally on ligand blots. The observation that the rate of degradation of the mature abnormal receptor was almost three times faster than its normal counterpart (Fig. 6) as well as the immunoblot data (Fig. 4A) suggest that, in steady state conditions, about one-third of the abnormal receptor produced is available on the cell surface. If the abnormal receptor could bind LDL normally, we would have expected a binding activity approximately 65% of the normal, which is slightly higher than the 48% that we have observed in several independent assays.

The presence of a readily distinguishable mutant receptor in a heterozygous cell line provides a rare opportunity to assess the effect of any interaction between receptors in

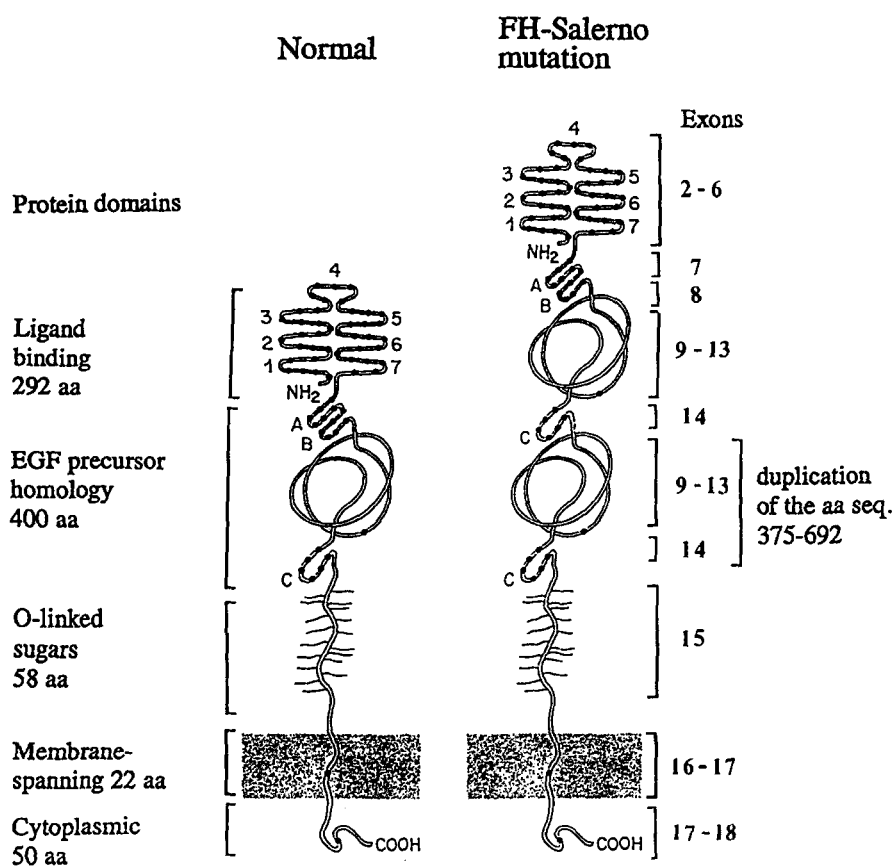


Fig. 7. Schematic models of normal LDL-receptor and mutant FH-Salerno receptor (from Lerhman et al. 22), modified.

influencing receptor function. There was little evidence that any such interaction affected processing and degradation of receptors in the proband's fibroblasts. The slightly delayed processing and greatly accelerated breakdown of the abnormal receptor clearly did not affect the processing or breakdown of the normal receptor, which was the same as that in normal cells (Fig. 6). This would argue against the presence of receptor heterodimers, during processing in the Golgi or at the point that determines degradation.

In conclusion, the duplication of exons 9-14 of the LDL-receptor gene found in our FH heterozygous patient I.C. did not alter the reading frame of mutant mRNA. This mRNA encodes an abnormally large receptor protein that reaches the cell surface, binds LDL, but is degraded more rapidly than the mature normal receptor. In this context the mutant allele of proband I.C. represents a new class of functional defects of LDL-receptor protein, that could be named "accelerated degradation." It would be interesting to know whether this situation leads to a recycling defect. To answer this question with certainty, one needs to have cells from a homozygote proband or cells transfected with a cDNA containing the 9-14 duplication. Unfortunately, neither of these cell types is available at present.

Since the family of our proband originated from a district close to the city of Salerno in southern Italy, the mutant allele reported in this study has been designated FH_{Salerno}. This mutation was discovered during a genetic screening of 300 Italian FH patients. ■

This work was supported by a grant from CNR Target Project on Aging to S.B. (no. 92.00248.PF40) and a grant from CNR Target Project on Genetic Engineering to S.C. The authors wish to thank Dr. Anne K. Soutar of the MCR Lipoprotein Team, Hammersmith Hospital, for reading the manuscript.

Manuscript received 28 October 1993 and in revised form 4 February 1994.

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