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A "de novo" mutation of the LDL-receptor gene as the cause of familial hypercholesterolemia

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Abstract

Familial hypercholesterolemia (FH) is a common genetic disorder caused by mutations of the LDL-receptor gene and transmitted as a co-dominant trait. However, there are some forms of hypercholesterolemia which have a recessive type of transmission. We have identified a subject with the clinical phenotype of heterozygous FH whose parents had normal plasma lipid values, suggesting a recessive type of transmission. The analysis of the LDL-receptor gene revealed that the patient was heterozygous for a G > C transversion in exon 4, which results in a serine for cysteine substitution at position 88 (C88S) of the receptor protein. Since this novel mutation was not found in the proband's parents and non-paternity was excluded, we concluded that the patient was a carrier of a "de novo" mutation. Haplotype analysis of LDL-receptor locus indicated that this "de novo" mutation occurred in the paternal germ line. The C88S mutation is the likely cause of LDL-receptor defect as it was present in the proband's hypercholesterolemic son and was not found in 200 chromosomes of control subjects.

Keywords: Familial hypercholesterolemia; "de novo" mutation; LDL-R gene

1. Introduction

Monogenic hypercholesterolemias represent a heterogeneous group of disorders which includes: (i) familial hypercholesterolemia (FH) due to a large variety of mutations of LDL-receptor gene [1–3]; (ii) familial defective Apo B-100 (FDB) caused by some mutations of apoB gene resulting in defective binding of LDL-apoB100 to the LDL-receptor [4,5]; (iii) autosomal recessive hypercholesterolemia (ARH), a recently characterised disorder due to mutation of a putative LDL-receptor adaptor protein [6]. Linkage studies in some large families with monogenic hypercholesterolemia have suggested the presence of other genes which have not been identified yet [7].

On clinical grounds, the criteria for diagnosis of FH and FDB are based on the presence of: (i) LDL cholesterol levels above the 95th percentile of the distribution in the general population; (ii) tendon xanthomatosis in the proband or in a first-degree relative, or alternatively, severe hypercholesterolemia in some prepuberal children of the proband's family; (iii) a vertical transmission of hypercholesterolemia and a bimodal distribution of plasma LDL cholesterol levels in the proband's family.

The diagnosis of FDB is usually based on the finding of four mutations of Apo B gene, all causing single amino acid substitutions (R3500Q, R3500W, R3531C and R3480W) [4,5], known to reduce Apo B binding to LDL-receptor. The molecular diagnosis of FH is based on the demonstration of: (i) a mutation in the LDL-receptor gene which has previously been shown to cause a functional defect in cultured cells or has been documented in other FH patients (see http://www.ucl.ac.uk/fh); (ii) a novel mutation which unequivocally co-segregates with FH in the proband's family and has not been found in at least 100 randomly selected control subjects. Since FH is characterised by a striking allelic heterogeneity (>700 mutations of LDL-receptor gene

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have been reported so far), in most cases, the molecular diagnosis of FH requires the sequence of the entire LDL-receptor gene.

Although less frequent, primary hypercholesterolemia may be transmitted as a recessive trait. In the few reported families in which recessive transmission is suspected for the presence of consanguinity, probands have LDL cholesterol levels which are higher than those usually observed in heterozygous FH and resemble those found in FH homozygotes [6,8,9].

An apparently recessive mode of transmission of FH may be observed in the case of the occurrence of a "de novo" mutation of the LDL-receptor gene [10–12]. In the present study, we describe a family in which, because of the presence of a "de novo" mutation, an apparently recessive mode of transmission coexists with the classical co-dominant transmission of FH.

2. Materials and methods

2.1. Proband

The proband was a 47-year-old white male who at the age of 43 had suffered from a myocardial infarction. He had smoked 40 cigarettes per day since the age of 20 but quit at the age of 43. Coronary angiography, performed at age 44, showed a critical stenosis (95%) in the left anterior descending artery that was successfully treated by PTCA. During routine blood examination at age 35, he was found to have elevated plasma LDL cholesterol level (9.10 mmol/l), but he did not take any lipid-lowering drug until the age of 43. At the age of 38, he underwent cholecystectomy for gallstone disease. At the present, tendon xanthomas, xanthelasmas and arcus corneae are not detectable. The proband's parents were apparently unrelated.

2.2. Analysis of LDL-receptor gene

Genomic DNA of the proband and his family members was extracted from peripheral blood leukocytes by a standard procedure [13]. PCR amplifications of promoter region, all exons and exon–intron boundaries of LDL-receptor gene were performed as reported previously [14], using the primers reported by Jensen et al. [15]. PCR amplified fragments were sequenced directly by using CEQ™ 2000XL Analysis System (Beckman Coulter, Inc., Fullerton, CA).

For the screening of the LDL-R mutation found in the proband, we amplified the 5′ half of exon 4 (355 bp) by using the following primers: 5′-GTTGGGAGACTTCACACGGTGATGG-3′(forward primer), 5′-ACTTAGGCAGTGGAACTCGAAGGCC-3′(reverse primer); the amplification conditions were: 94 °C for 5 min, followed by 30 cycles of denaturation at 94 °C for 30 s, annealing at 65 °C for 30 s, extension at 72 °C for 1 min, final extension at 72 °C for 7 min. The amplification product was digested with 6 U of Aat II (Roche Diagnostic) at 37 °C for 4 h. The presence of the mutation, which introduces an Aat II restriction site, generates two fragments (293 and 62 bp, respectively).

2.3. Haplotype analysis at LDL-R locus

The haplotype co-segregating with the mutation was identified by the analysis of the polymorphic tetranucleotide microsatellite D19S394, which is 250 kb telomeric to the LDL-R gene and of the intragenic Ava II polymorphism located in exon 13.

The PCR amplification of D19S394 microsatellite was carried out with a modification of the method described by Day et al. [16]. Briefly, the amplification mix included 200 ng of genomic DNA, 1.25 U of Platinum Taq polymerase (Gibco BRL-Life Technologies, Italia, Srl), 200 μM each of the dNTPs, 1 × solvent enhancer provided with the polymerase, 5 pmol of each primer (forward, 5′-AGACTACAGTGAGCTGTGG-3′ and reverse 5′-GTGTTCCTAACTACCAGGC-3′, without any fluorescent label) and 1.5 mM of $MgSO_4$. The PCR reaction consisted in 35 cycles (95 °C for 30 s/56 °C for 30 s/68 °C for 1 min) that were preceded by a 2 min denaturation at 95 °C and followed by a 7 min extension at 68 °C. The PCR product was directly analyzed with the Agilent 2100 Bioanalyzer (Agilent Technologies, Waldbronn, Germany) for high-voltage capillary electrophoresis on microchips and laser-induced fluorescence (LIF) detection, as previously described [17]. Allele sizes provided by this instrument equipped with Agilent DNA500 LabChip (for sizing dsDNA fragments up to 500 bp) and its specific DNA sizing ladder were rounded to the nearest conventional allele value and expressed as the number of variable repeats found in each allele.

The Ava II (C>T) polymorphism was analyzed with the method described by Humphries et al. [18].

2.4. Paternity testing

A small aliquot of genomic DNA was amplified by PCR [19,20]. Three multiplex reactions were performed for the following markers: Hum F13B, TPOX, FGA, CSF1PO, F13A01, D8S1179, LPL, TH01, VWA31/A, FES/FPS, D18S51, D19S253, D21S11.

An aliquot of the amplification reaction (0.5–2 μl) was mixed with 6 fmol of commercial internal standard GS 2500 (ABD, USA), denatured at 90 °C for 2 min and loaded onto a 6% polyacrylamide denaturing gel. Samples were electrophoresed at constant power (36 W) for 5 h in an Applied Biosystem Automated DNA sequencer 373A Leon and sized by Genescan 672 software (ABD, USA) employing the Local Southern method.

Informed consent was obtained from all subjects investigated. The study protocol was approved by the institu-

Table 1
Clinical and biochemical features of proband's family

	Proband	Father	Mother	Spouse	Son
Age (years)	47	76	72	44	9
BMI (kg/m^2)	25.3	23.1	32.0	19.5	20.3
Cholesterol	10.78	3.51	4.99	5.37	9.51
LDL cholesterol	8.97	1.99	2.97	2.77	7.14
HDL cholesterol	1.34	1.21	1.19	1.76	1.63
Triglycerides	1.30	0.83	2.31	1.86	1.66
Apo AI	144	129	148	170	156
Apo B	168	52	78	75	152

Lipid values (mmol/l), apolipoproteins (mg/dl).

tional human investigation committee of each participating institution.

3. Results

Table 1 shows the clinical features and plasma lipids in the proband's family. While serum total cholesterol, LDL cholesterol and Apo B in the proband's parents were within the normal level, the values found in the proband were consistent with the diagnosis of primary hypercholesterolemia.

Table 2
Parental diagnosis

Chromosome	Marker (STRs)	Alleles			Allele shared with father
		Father	Mother	Proband	
1	F13B	10/8	9/6	10/9	10
2	TPOX	8/8	11/8	8/11	(8)
4	FGA	21/22	23/22	21/23	21
5	CSF1PO	10/11	11/12	10/11	10
7	F13A01	6/3.2	15/4	6/15	6
8	D8S1179	16/15	14/13	16/14	16
8	LPL	11/9	13/10	11/13	11
11	TH01	9.3/9.3	9/6	9.3/9	9.3
12	VWA31/A	15/16	18/17	15/18	15
15	FES/FPS	11/10	12/10	11/12	11
18	D18S51	17/17	16/17	17/16	(17)
19	D19S253	12/11	7/11	12/7	12
21	D21S11	30/28	32.2/32.2	30/32.2	30

High LDL cholesterol and Apo B levels were also observed in the proband's 9-year-old son, suggesting a father to son transmission of the disorder.

The sequence of LDL-R gene revealed that the proband was heterozygous for G>C transversion in exon 4, which converts the codon TGC for cysteine at position 88 into a codon TCC for serine (Fig. 1). This mutation, which involves the repeat 3 of the binding domain, was found in the proband's son but not in the proband's parents.

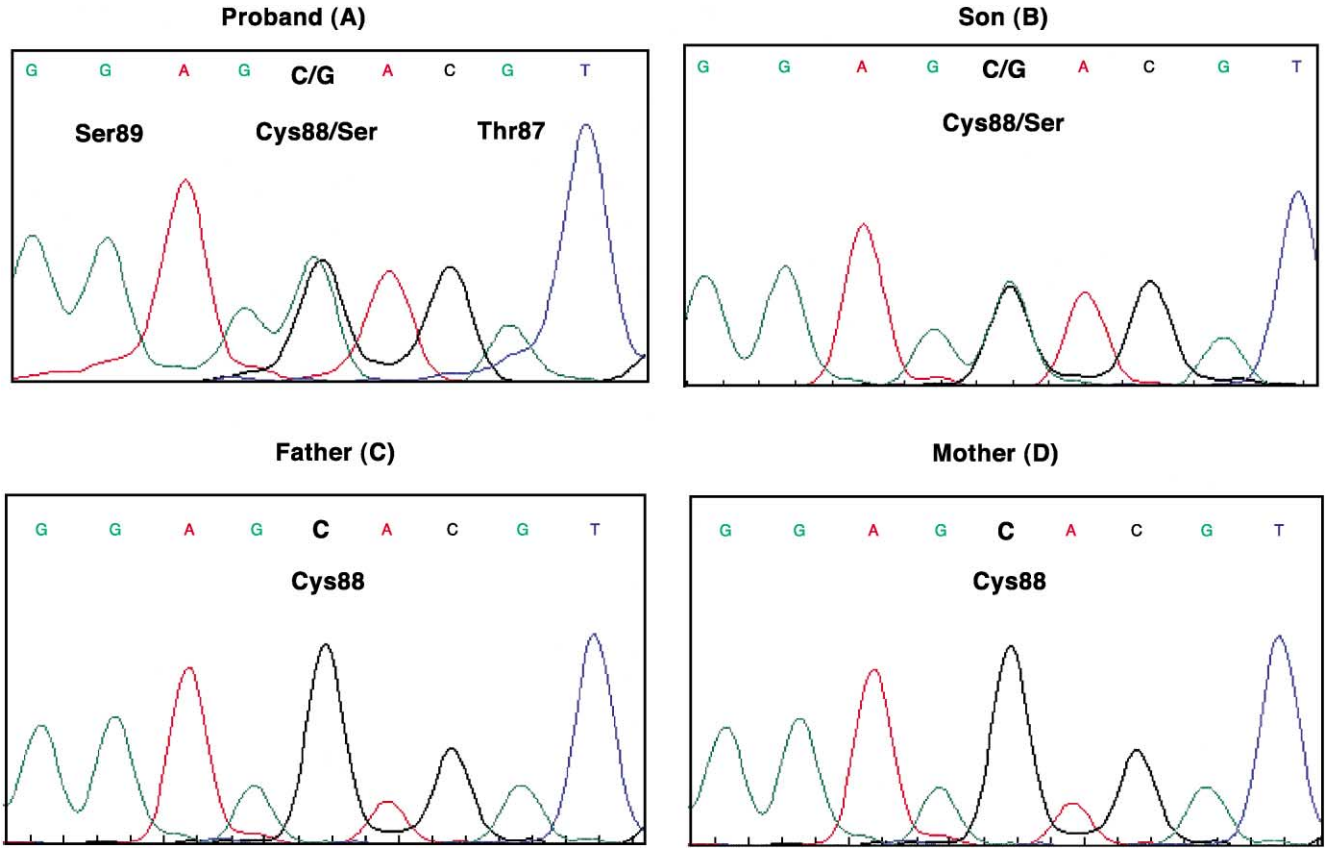

Fig. 1. Partial sequence (antisense) of exon 4 showing the C>G transversion at nucleotide 326 of the LDL receptor cDNA in the proband (panel A) and his son (panel B). The mutation is not present in proband's parents (panels C and D).

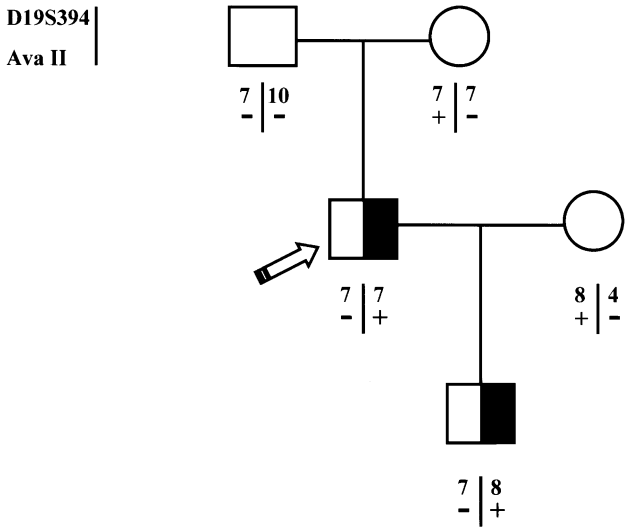

Fig. 2. Proband's pedigree. The alleles of the microsatellite D19S394 (number of repeats) and of the Ava II polymorphism are shown below each subject. The haplotype co-segregating with the mutation, seven repeats and Ava II (−), indicates that the mutational event occurred in the paternal germ line.

Paternity testing was performed using a battery of 13 short tandem repeats located in 12 different chromosomes (Table 2). The result of this analysis defined a hypothesis of paternity with a casual sharing of 1.5×10^{-8}. The likelihood of paternity was therefore 99.998%.

The analysis of genetic markers within or close to LDL-R gene allowed the identification of the haplotype co-segregating with the mutation. The segregation of this haplotype in the pedigree indicated that the mutational event occurred in the germ line of the proband's father (Fig. 2). This mutation was not found in DNA samples from 100 randomly selected control subjects.

4. Discussion

In this study, we report a "de novo" mutation of LDL-receptor gene in a subject with severe hypercholesterolemia and a history of myocardial infarction. Since non-paternity was excluded, the fact that the proband's parents had normal cholesterol raised three possible hypotheses: (i) the proband's parents were carriers of mutations in some genes causing recessive hypercholesterolemia [6]; (ii) one of the proband's parents was a carrier of an LDL-gene mutation, the effect of which was abolished by the concomitant presence of an LDL-lowering gene (lack of penetrance); (iii) the presence of a "de novo mutation" of LDL-receptor gene. The finding that neither of the parents was a carrier of the mutation of LDL-receptor gene found in the proband and the observation that this mutation was present in the proband's son substantiated the hypothesis of a "de novo" germ line mutation, transmitted as a co-dominant trait to the next generation.

To the best of our knowledge, this is the fifth report of a "de novo" mutation of the LDL-receptor gene associated with the clinical phenotype of FH. The first "de novo" mutation was an 18 bp duplication in exon 4 found in an FH-Afrikaner male subject who transmitted this mutation to his daughter [10]. The second mutation (a deletion of exons 14 and 15) was detected in a Finnish FH-compound heterozygote [11].

The third mutation was reported by our group in an 11-year-old boy with no family history of familial hypercholesterolemia. In the latter case, the mutation was T>A transversion in exon 1, which causes an arginine for tryptophane substitution at the −12 position of the signal peptide (W-12R) of the LDL-receptor [12]. The fourth mutation (a splicing defect, 313+1 g>a) was found in an African FH subject [21].

The mutation discovered in our proband eliminates a cysteine in the third repeat of the ligand binding domain, where cysteine residues play a crucial role in the proper folding of this domain. It is likely that this substitution is the cause of FH in our proband for four reasons: (i) cysteine at position 88 is highly conserved in LDL-receptor of various species (e.g. rabbit and xenopous) [22,23]; (ii) substitutions of this cysteine with other amino acids (C88R, C88Y and C88F) have been reported in FH patients (see http://www.ucl.ac.uk/fh); (iii) C88S was not found in 100 randomly selected control subjects; (iv) C88S is present in the proband's hypercholesterolemic son. It is possible that C88S reduces LDL binding and, in view of the misfolding of the LDL-R binding domain, causes an increased intracellular degradation of LDL-receptor protein [1].

The fact that only few "de novo" mutations of LDL-receptor gene have been documented so far is surprising in view of the large number of mutations of this gene found to date and its presence in several hot spots for mutations (CG dinucleotides, direct and inverted short repeats in coding sequences and the relatively large number of Alu sequences). It is possible that this low number of reported "de novo" mutations reflects a selection bias, since sporadic cases of primary hypercholesterolemia, in the absence of a positive family history of FH (tendon xanthomas, hypercholesterolemia, premature cardiovascular disease, etc.) or of sufficient family data, are not diagnosed as FH, or they are labelled as polygenic hypercholesterolemia. Since most of these cases are probably not genotyped at the LDL-receptor locus, their precise etiology remains to be defined. In view of these considerations, we do not have an estimate of the frequency of "de novo" mutations of the LDL-receptor gene causing FH in the population.

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