

Original Article

Familial Hypercholesterolemia in Iran: A Novel Frameshift Mutation in Low Density Lipoprotein Receptor (LDLR) Gene

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ABSTRACT

Background and Objective: Familial hypercholesterolemia (FH) is an autosomal trait, which is caused by mutations in Low Density Lipoprotein Receptor (LDLR) gene. FH penetrance is about 100% and worldwide prevalence for heterozygous subjects is almost 1 in 500 and for homozygous 1 in 1,000,000. The patients are at risk of premature coronary heart disease (CHD) due to defective LDLR and hence cholesterol metabolism disorder. The aim of this study was identifying genotype of possible mutation in an Iranian FH patient.

Materials and Methods: Promoter and all 18 exons including exon-intron boundaries of LDLR gene were scanned. Polymerase chain reaction - single strand conformation polymorphism (PCR-SSCP) was used as mutation scanning method. DNA sequencing was used to identify any nucleotide change(s).

Results: A new frameshift mutation (660-661InsCC) was found in proband.

Conclusion: This mutation causes a truncated, non-functional protein, which results in hypercholesterolemia. The mutation can be screened in proband's relatives to find other FH patients.

Keywords: Hypercholesterolemia, LDL Receptors, Polymorphism, Single-Stranded Conformational

Introduction

One of the most common monogenic inherited metabolic diseases is familial hypercholesterolemia (FH), with an average worldwide prevalence for heterozygous subjects of about 1 in 500 and for homozygous 1 in 1,000,000 (1). FH is caused by mutations in the low-density lipoprotein receptor (LDLR) gene which spans 45 kb on the distal short

arm of chromosome 19 (p13.1-p13.3) and is divided into 18 exons and 17 introns (2). After removing 21 amino acid signal peptide chain by signal peptidase, the LDL receptor mature peptide contains 839 amino acids, which is targeted to the Golgi apparatus where glycosylation takes part (3). The mature protein appears at the cytoplasmic membrane and facilitates uptake of circular low-density lipoprotein (LDL)

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into liver via its ligand, ApoB-100 protein. The LDL-LDLR complex is internalized in cytoplasmic vesicles where LDL dissociates and degrades mainly to cholesterol and amino acids; however, the LDLR remains intact, goes back to the cell surface, and enters a recycling pathway (4). More than 800 mutations at various sites in the LDL receptor gene have been reported, including nonsense and missense mutations, deletions and insertions, which affect the synthesis, post-translational processing, and ligand binding activity, internalization, or recycling of the LDL receptor (5-7). Mutations in the LDL receptor gene diminish the number of cellular LDLR or make their function defective, resulting in a lifelong elevation of serum LDL cholesterol to levels two- to three-fold higher among FH heterozygous than non-FH subjects. The most important clinical manifestation of FH is premature CHD. Stenosis was detectable by coronary angiography in 692 FH heterozygotes on average after 17 and 25 years of age in the males and females, respectively (8). At present, the diagnosis of FH is based on clinical and laboratory tests but the phenotype of the disease is not constant, even among patients within a family (9). In addition, there are some overlaps in clinical manifestation with normal population leading to delays in treatment, which should be started as soon as possible. With early diagnosis and adequate long-term pharmacological treatment, many FH patients could achieve reductions in LDL cholesterol levels, and increase their life expectancy by 10-30 years. Definite diagnosis is based on LDL-R function test or mutation findings (10).

The aim of this study was finding underlying mutation in a FH proband to use it in screening of other proband's family and relatives in future as well as completing Iranian FH mutations database.

Materials and Methods

Based on Simon Broome criteria (11), a clinically FH diagnosed 32 year Iranian man attended to hospital by severe heart attack was investigated for having mutation in LDLR gene. Peripheral blood sample was collected in tube containing EDTA. Genomic DNA was extracted by Genomic Prep Blood DNA Isolation kit (Amersham-Biosciences, USA). Familial defective ApoB-100 (FDB) (12), a clinically similar disorder to FH, was excluded by ruling out

the presence of R3500Q mutation in apolipoprotein B (ApoB) gene using a method reported previously (13). PCR-SSCP method was used to scan LDLR gene mutation(s) based on previously methods (14). In brief, to amplify promoter, exons and intron-exon boundaries of LDLR gene, 22 previously designed primer pairs were used (three pairs for exon 4 -5', mid and 3' side- and two pairs for exon 10 -5' and 3' side, as the size of these exons are out of SSCP sensitivity) (15). PCR conditions were 1.5 mM MgCl₂, 96°C, 5 min ×1, (96°C 1 min, 57°C 1 min, 72°C 1 min) × 35, 72°C 5 min, using Taq DNA polymerase (Cinagen, Iran) and almost 30 ng of genomic DNA in each reaction. Following PCR amplification, 5 µl of PCR product was mixed with 10 µl SSCP loading solution (80% formamide/ 0.25% xylene cyanol FF/ 40% sucrose) (Amersham-Biosciences, USA). DNA was made single stranded by heating at 96°C for 5 minutes followed by rapid chilling at 0°C water. SSCP was carried on MultiPhoreII electrophoresis unit (Amersham- Biosciences, USA) using gel-casting system. Gels consisted of 12.5% acrylamide/ bisacrylamide (39:1) (Sigma, Germany) with no glycerol and the buffer system was 1X TBE, pH 8.3. Ten micro liters of previously chilled samples were loaded into the wells and run at 5 mA constant current mode for 15 hours at 5°C. The gels were visualized by a modified silver staining method: 10 minutes in 10% ethanol, 30 seconds distilled water wash, 5 minutes in 1% nitric acid, 30 seconds distilled water wash, 30 minutes in 0.2% silver nitrate, and 3 minutes distilled water wash. Developing solution consisted of 500 micro liters formaldehyde in 200 ml of 2.5% sodium carbonate. After visualization, the developing process was stopped by using 5% citric acid for 5 minutes. Aberrantly migrated samples were reamplified and PCR products purified by GFX PCR DNA and Gel Band Purification Kit (Amersham-Biosciences, USA) followed by DNA sequencing using ABI Thermosequenase II didexoxy termination kit on an ABI 3700 automated sequencer (Applied Biosystems, USA).

Results

In this study, a clinically diagnosed familial hypercholesterolemia patient was checked for having mutation in LDLR gene. Mutation scanning by PCR-

SSCP showed extra band in 3' segment of exon 4 in the proband (Fig. 1). Sequencing of this PCR fragment revealed a novel frameshift mutation (660-661InsCC) (Gene Bank accession No.: AY875868) (Fig. 2).

Fig. 1. SSCP of exon 4 (3' site) shows new bands in proband (arrow). Lanes 1-4 and 6 show wild type exon 4 (3' site) from other patients. Gel: acrylamide/Bis 39/1, 12.5%; TBE x1, pH 8.3; run: 14 hours at 5 mA; temperature, 5°C; visualization: Silver Staining.

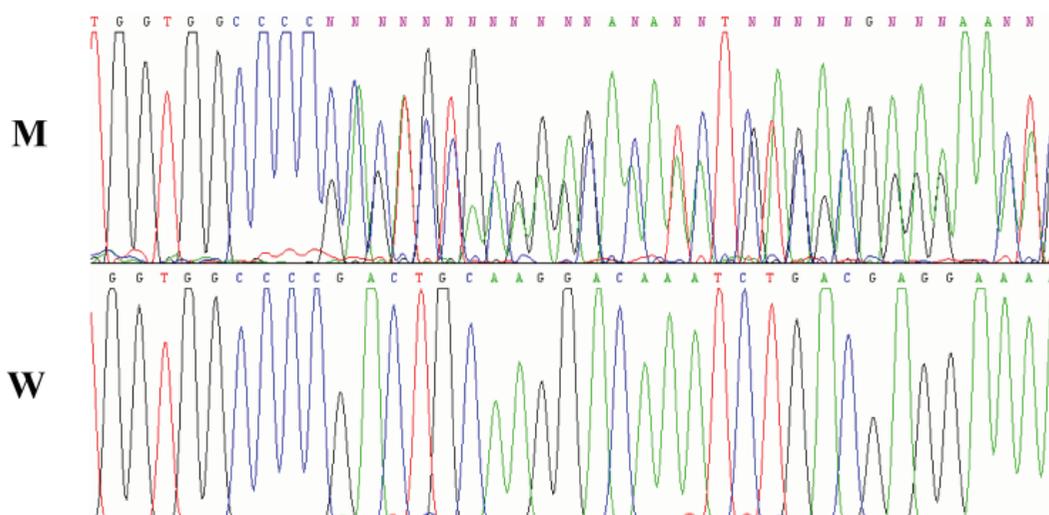
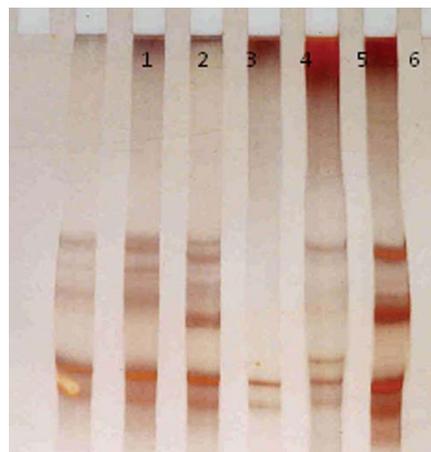


Fig. 2. Electropherogram of exon 4 (3' end), shows 660-661InsCC mutation; W, wild allele; M, mutant allele. (Electropherograms by ABI full automated sequencer).

Discussion

The diagnosis of FH is generally made based on clinical features, but clinically diagnosed FH has been shown to carry mutations in few genes including LDLR and apoB-100. According to diagnostic criteria with this proband we inferred that the hypercholesterolemia might be caused by mutation(s) in mentioned genes. We excluded the most prevalent mutation (R3500Q) in apoB-100 gene and subsequently scanned whole LDLR gene for presence of any possible mutation(s).

Single strand conformation polymorphism (SSCP) was used to scan the mutation in LDLR gene in a clinically diagnosed FH patient. Gene scanning prevents expensive direct sequencing of entire exons. We used PCR-SSCP as it is a rapid, simple, and cost-effective scanning method for mutation detection. Its sensitivity is more than 80% (16). The mutation reported here (660-661InsCC) causes an (CC) insertion into exon 4 of the LDLR gene, which

changes the translational frame and result in a non-functional, truncated protein due to encountering translational machine with a stop codon in few further codons. This defective LDLR lacks all essential protein domains (ligand binding domain, EGF precursor homology domain, O-linked carbohydrate domain, membrane-spanning domain and cytoplasmic domain). Furthermore, no other mutation in the rest of the gene could be detected in this subject by SSCP and sequencing (data not shown). So this frameshift mutation is seems to be definitely the cause of hypercholesterolemia in this patient. This conclusion is not far from imagination as the pathological effect of such mutations can be predicted on the basis of their position with respect to previously reported mutations with an estimated reduction of the receptor activity, or like in this case, premature termination of translation (17). There are many reports on finding frameshift mutations in LDLR gene, which have been related to familial hypercholesterolemia (18-21). In

most of them interpretation have been only based on DNA sequencing and producing non-functional LDLR protein (22). In a study in The Netherlands, a few of many novel mutations have been reported in various exons of LDLR gene, which cause truncated peptide (23). Detection of a novel exon 4 low-density lipoprotein receptor gene deletion in a Swiss family showed that absence of exon 4 and producing truncated peptide correlates with severe familial hypercholesterolemia (24). In addition, in a Japanese study a familial hypercholesterolaemia family was found with a 327insC mutation in the LDLR gene, which halts the translational machine, by a newly formed stop codon (25).

Conclusion

The mutation reported here was confirmed to be novel after comparing with databases available for the reported mutations of LDLR gene on the web (26). Finding this mutation in mentioned proband can help FH diagnosis of his other family and relative members. This can be easily achieved by screening exon 4 of LDLR gene for 660-661InsCC using direct PCR-sequencing.

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