Short Report

A Novel Mutation in Exon 4 of the Low Density Lipoprotein (LDL) Receptor Gene in an Iranian Familial Hypercholesterolemia Patient

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ABSTRACT

Familial hypercholesterolemia (FH) is an autosomal co-dominant disorder of lipid metabolism, caused by mutations in LDL receptor gene. The penetrance of FH is almost 100%, meaning that half of the offspring of affected parents born with disease. The patients are at risk of premature coronary heart disease (CHD). There is no report about the molecular basis of FH in Iran. Identification of mutations allows unequivocal diagnosis in potentially affected relatives. To characterize genetic aberrations in Iranian FH patients, after ruling out the most common mutation producing familial defective ApoB-100 (R3500Q), we screened exon 4 in LDL receptor gene in 30 heterozygous FH patients by single strand conformation polymorphism (SSCP). A new missense mutation (445G>T) was found in proband and his mother. This causes a Gly to Cys change in repeat 3 of LDL binding domain. This nucleotide change was not found in 50 normal individuals. *Iran. Biomed. J. 9 (3): 139-142, 2005*

INTRODUCTION

Familial hypercholesterolemia (FH) is one of the most common hereditary diseases, from which more than 10 million people suffer all over the world [1]. As most patients do not know anything about their disease till the first manifestation (e.g. angina pectoris, cardiac and cerebral infarctions) usually in their productive ages, early diagnosis is a necessity. Etiology of the FH is due to mutations in low density lipoprotein receptor (LDL-R) gene, which causes a variety of defects from LDL-R protein production to its malfunctioning. This influences LDL-R cell cycling from LDL particle capture to LDL-R recycling [2]. The consequence is an increase of LDL cholesterol levels to about twice the normal level in heterozygote individuals.

The LDL-R gene contains 18 exons and 17 introns. More than 700 mutations have already been reported till now [3]; the list is growing continuously [4-5]. Missense mutations and small deletions or insertions are the most type of aberrations in LDL-R gene. Exon 4 is one of the hot spot sites in LDL-R gene and is the largest [6]. At present, the diagnosis of FH is based on clinical and laboratory tests; but there are some overlaps with normal population leading to delays in treatment, which should be started as soon as possible [7]. Definite diagnosis is based on LDL-R function test or mutation findings [8].

As part of a project aimed at characterizing mutations in LDL-R gene underlying FH in Iran, we used SSCP to analyze exon 4 (459 bp). Considering the fact that the sensitivity of single strand conformation polymorphism (SSCP) falls dramatically for fragments more than 300 bp, the exon was divided into three fragments including flanking regions.

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MATERIALS AND METHODS

Thirty unrelated adult heterozygote patients were selected based on Simon Broome criteria [1]. Blood samples were collected in tubes containing EDTA and genomic DNA was extracted by Genomic Prep Blood DNA Isolation Kit (Amersham-Biosciences, USA). Presence of R3500Q mutation in apo-lipoprotein B (ApoB) gene causing familial defective ApoB-100 (FDB) was excluded by a method reported previously [9].

To amplify exon 4, three primer pairs (FH 51 and 52 for 5 segment; FH 53 and 54 for mid segment and FH 55 and 169 for 3 segment) were used based on previously defined procedures in literature [10]. PCR conditions were 1.5 mM $MgCl_2$, 96^oC, 5 min × 1, (96^oC 1 min, 57^oC 1 min, 72^oC 1 min) × 35, 72^oC 5 min, using *Taq* DNA polymerase (Cinagen, Iran) and almost 10-15 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^oC for 5 minutes followed by rapid chilling at 0^oC water.

SSCP was carried on MultiPhoreII electro-phoresis 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 microliter of previously chilled samples were loaded into the wells and run at 5 mA constant current mode for 15 hours at 5^oC. Afterwards, the gels were visualized by silver staining method; 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 Thermosequenase II didexoxy termination kit on an ALF automated sequencer (Amersham-Biosciences, USA).

To confirm the presence of substitution of G by T, an allele specific PCR based test was designed using allele specific oligonucleotide (ASO) (Mutant, Forward): 5 CCC GGT GCT CAC CTG TT 3, ASO (Wild, Forward): 5 CCC GGT GCT CAC CTG TG 3 primers and a previously designed reverse primer FH54 5 GAG CAG GGG CTA CTG TCC 3. PCR was performed according to the conditions mentioned above and PCR products were checked using 2.5% agarose gel. In order to exclude the presence of nucleotide substitution in normal individuals as single nucleotide polymorphism

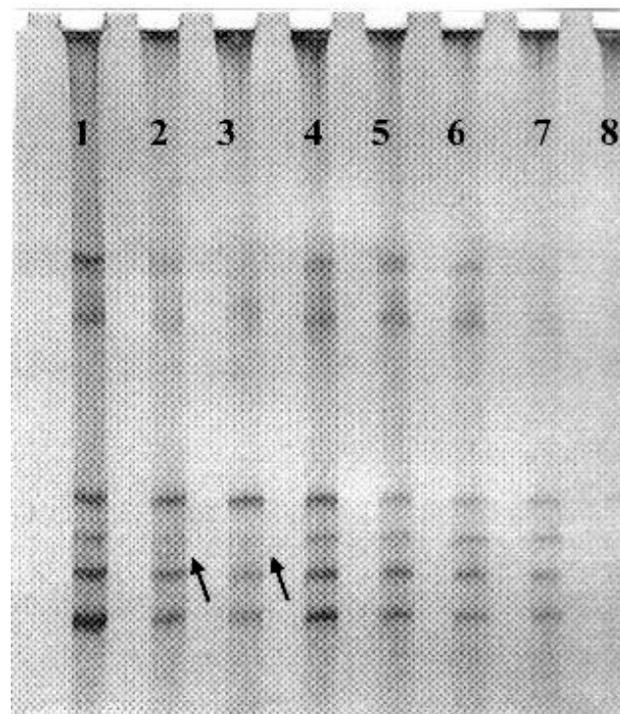(SNP), 50 control subjects were examined with the same method.

Fig. 1. SSCP of exon 4 (5` site) shows new bands in proband (lane 2) and his mother (lane 3). Lanes 1 and 4-8, wild type exon 4 (5` site) in other patients. Gel consists of 12.5% acrylamide/bisacrylamide (39:1).

RESULTS AND DISCUSSION

In the present study, 30 clinically diagnosed heterozygous FH patients were checked for presence of mutations in exon 4 of LDL-R gene. Analyzing the 5 segment of exon 4 by SSCP, an extra band in the proband and his mother was seen (Fig. 1). This PCR fragments were sequenced and a novel mutation was found (445G>T) (Gene Bank accession No.: AY504954) in both of them (Fig. 2). Allele specific PCR primers were designed to discriminate between G and T alleles. The analysis confirmed substitution of G by T (Fig. 3). On the other hand, the reaction took place only in presence of wild primers in 50 normal individuals. The mutation reported here (445G>T) causes a Gly (G) to Cys (C) change at codon 149 in repeat 3 of ligand binding domain. The domain is highly conserved and it has a compact structure that is easily disrupt even if only one single amino acid is changed. It consists of 7 cysteine rich repeats which are arranged in head-to-tail fashion and their high content of cysteines, mediates folding of the domain into a rigid structure. Repeats 2-7 participate in binding of lipoprotein [11]. Presumably, presence an additional cysteine residue interferes correct
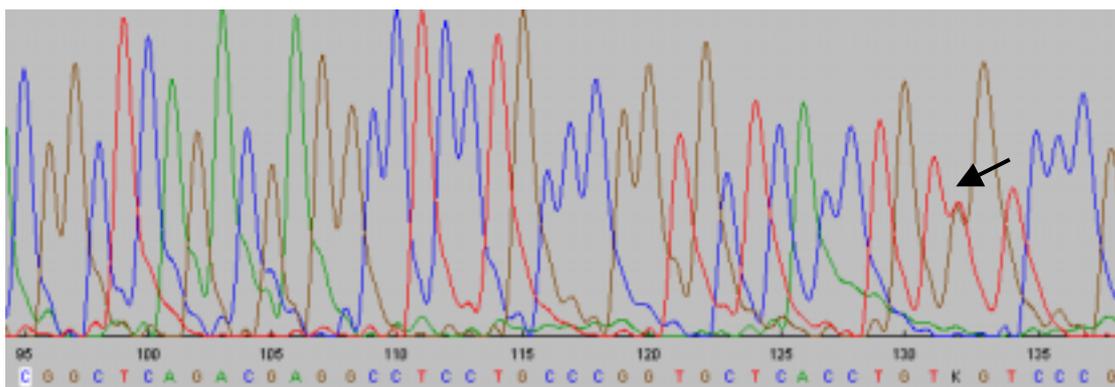
Fig. 2. Sequencing of Exon 4 (5` site). Heterozygosity is shown (G + T = K)

formation of disulfide bonds and subsequent conformational change which is essential for holding the ligand.

The G149C mutation segregates with high lipid levels in proband and his mother. Furthermore, no other mutation in the rest of the gene could be detected in this subject by SSCP and sequencing (data not shown). Therefore, the G149C mutation seems to be pathogenic. On the other hand, excluding the mutation in normal people boosts the hypothesis that this change is the reason of hypercholesterolemia due to defect in LDL particle capture by ligand binding domain of LDL-R. In future, producing a cell line carrying 445G>T mutation in LDL-R gene may show the percent of ligand capture impairment of mutant LDL-R.

Despite the high levels of morbidity and mortality associated with FH, the disease remains considerably under diagnosed, and this causes patients not to receive adequate treatment that may

reduce their risk of developing premature coronary heart disease (CHD). Global estimates suggest 200,000 heterozygous FH individuals die of preventable heart attacks each year [12]. As Iran population is about 1 percent of world population, it can be estimated the rate of such mortality in Iran is about 2,000 per year. Genetic characterization of FH patients, in addition to make a database for epidemiologic studies, can facilitate diagnosis the FH especially by family targeted screening procedure, where the causing mutation can be directly searched in subject's relatives [13]. On the other hand, the low-density lipoprotein receptor (LDL-R) is the prototype of a large family of structurally homologous cell surface receptors, which function as endocytic and signaling receptors in a wide variety of cellular processes. Finding new mutations may help better understanding of their functions as well.
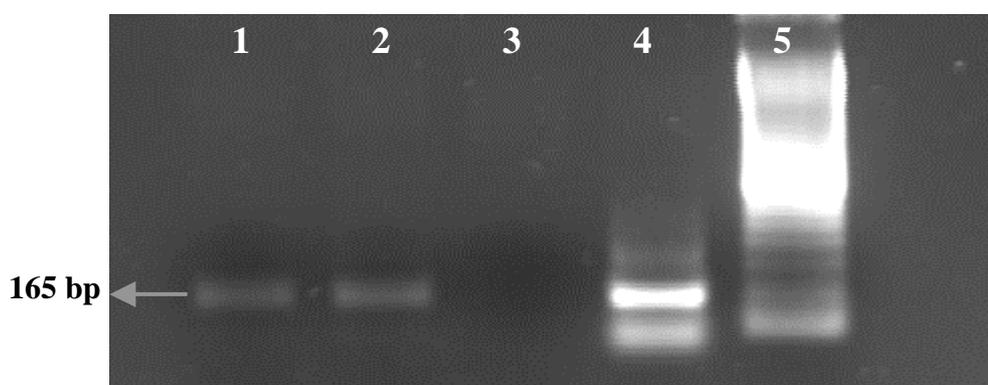

165 bp

Fig 3. Allele specific PCR on agarose 2.5%. Lane 1, Mutant sample with Mutant primer. Lane 2, Mutant sample with Wild primer; Lane 3, Wild sample with mutant primer; Lane 4, Wild sample with wild primer. Lane 5, 100 base pair ladder markers (Amersham-Biosciences, USA).

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