Familial hypercholesterolemia associated with severe hypoalphalipoproteinemia in a Moroccan family

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Introduction

Familial hypercholesterolemia (FH) is an autosomal dominant genetic disorder characterized by elevated levels of low-density-lipoprotein cholesterol (LDL-C), tendon xanthomas and increased risk of premature coronary heart disease (CHD). The FH phenotype results from defects in the LDL receptor gene (LDLR), and also defects in other genes like apolipoprotein B (apoB) (familial defective apo B) or proprotein convertase subtilisin/kexin type 9 (PCSK9) (Soria et al. 1989; Abifadel et al. 2003). High-density-lipoprotein cholesterol (HDL-C) levels are significantly reduced in many FH families. However, the metabolic basis of this hypoalphalipoproteinemia (HALP) has not been clearly understood. It has been reported that FH heterozygotes with HALP are prone to develop more severe premature artery disease (de Sauvage Nolting et al. 2003). Indeed, the latest guidelines for the diagnosis and management of FH consider levels of HDL-C less than 40 mg/dl as one of the major cardiovascular risk factors in the FH population (Civeira 2004).

In this report, we describe a Moroccan FH family with associated HALP. After screening of the LDLR gene, we identified a novel frameshift mutation in exon 5′ of the LDLR gene (756del7). To elucidate the inheritance of the HALP in this family, we analysed some other genes involved in HDL metabolism, such as apoAI, lecithin:cholesterol acyltransferase (LCAT) and lipoprotein lipase (LPL). We also screened N370S and L444P, the most frequent mutations in the β-glucocerebrosidase gene (GBA) that have been associated with HALP (Pocovi et al. 1998). This study revealed the IVS3-23C→A mutation in LCAT gene, although it did not appear to cosegregate with HALP phenotype in this family.

Materials and methods

Subjects

At the first consultation, the proband, 15 years old, presented extra vascular lipid deposits and lipid profile characteristics of FH homozygotes. However, no evident signs of atherosclerosis had been revealed. Because LDL apheresis is not available in Morocco, a high dose of statin was prescribed to the patient. Proband’s relatives were recruited and eleven were available for clinical examination and blood analyses. All subjects gave their informed consent prior to their inclusion in the study. At the age of 22 years, the proband died by myocardial infarction.

Lipid analysis

We analysed serum TC, TG and HDL-C by enzymatic methods and apo A-I and apo B by an immunoturbidimetric method (Brustolin et al. 1991). We calculated LDL-C by Friedewald formula (Friedewald et al. 1972).

Genetic analyses

DNA isolation: Genomic DNA from white blood cells was isolated using a salting-out procedure (Miller et al. 1988).

Keywords. familial hypercholesterolemia; hypoalphalipoproteinemia; LDLR gene; LCAT gene; SSCP; DNA sequencing.
**LDLR gene:** We analysed LDLR gene by Southern blot (El Messal et al. 2003) and PCR–SSCP. For SSCP analysis, PCR products were added to 95% formamide buffer, denatured at 95°C for 5 min, immediately chilled on ice and electrophoresed at 1050 V, at 25°C or 15°C in a MDE gel with or without 5% glycerol, respectively, in 0.6 x TBE buffer for 15 h, on an automated DNA sequencer equipped with a water jacket (ALF-Express™, Pharmacia Biotech). We sequenced a PCR fragment of LDLR gene (exon 5) using Big Dye Terminator Cycle Sequencing Kit (Perkin Elmer) and a CEQ8000 DNA automated sequencer (Beckman Coulter). For mutation confirmation, PCR products were electrophoresed at 96 V at room temperature, for 3 h, in 2% agarose gel in 1× TAE.

**Apo B gene:** We screened for R3500Q and R3531C mutations in apoB gene as described by Rabèes et al. (1997).

**Apo E gene:** We determined the apoE genotype by PCR–restriction analysis with HhaI as described by Hixon and Vernier (1990).

**Apo AI gene:** We analysed the promoter region, exons and exon–intron junctions of apoAI gene by PCR–SSCP (Recalde et al. 2002).

**LCAT gene:** We analysed the promoter region, exons and exon–intron junctions of LCAT gene by PCR–SSCP and sequenced the PCR fragment (exon 4) as described previously (Recalde et al. 2002). Mutation confirmation was carried out by PCR-restriction enzyme digestion with MspI of exon 4 and subsequent 3% Nusieve agarose gel electrophoresis.

**GBA gene:** We screened the GBA gene for the presence of the most frequent mutations, N370S and L444P, by PCR and restriction enzyme digestion with Xhol and NciI, respectively (Beutler et al. 1990; Tsuji et al. 1987).

**LPL gene:** We analysed the promoter region, exons and exon–intron junctions of LPL gene by direct sequencing in an ABI PRISM 3100 Genetic Analyser. Sequence of primers used were reported previously by Abifadel et al. (2004).

### Results

**Family analysis**

The biochemical and clinical features of the proband and his relatives are presented in table 1. The proband (II-5) and his sisters II-7 and II-8 (figure 1) showed biochemical and clinical features of homozygous FH. Among the other recruited relatives, only the proband’s sisters II-2 and II-6 would be heterozygous FH patients (table 1), according to proband’s

![Pedigree of the analysed individuals indicating LDL-C levels, FH genotype, HDL-C levels and HALP genotype. Proband (deceased) is indicated by an arrow; wt, wild type allele; mut, mutant allele. LDL-C and HDL-C levels were obtained before any medical treatment.](image-url)