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Limited mutational heterogeneity in the LDLR gene in familial hypercholesterolemia in Tunisia

A. Jelassi[a], I. Jguirim[a], M. Najah[a], A.M. Abid[b], L. Boughamoura[c], F. Maatouk[d], M. Rouis[e], C. Boileau[f,g], J.P. Rabès[f,g], M.N. Slimane[a,*,1], M. Varret[f,1]

[a] Research Unit of Genetic and Biologic Factors of Atherosclerosis, Faculty of Medecine, Monastir, Tunisia
[b] Institut de Nutrition, Tunis, Tunisia
[c] Department of Pediatric, Farhat Hachad Hospital, Sousse, Tunisia
[d] National institute of nutrition and food technology Tunis, Tunisia
[e] INSERM U545, Institut Pasteur de Lille and University of Lille II, France
[f] INSERM U781, Hôpital Necker-Enfants Malades, Université Paris-5, France
[g] UMR 7079 CNRS ,University Pierre and Marie Curie ,Paris, France

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ABSTRACT

Familial hypercholesterolemia (FH) is an autosomal dominant disease caused by mutations in the low-density lipoprotein receptor (*LDLR*), apolipoprotein B (*APOB*), and proprotein convertase subtilisin/kexin type 9 (*PCSK9*) genes. In previous studies, we have identified novel mutations in Tunisian FH families. In this study, we have extended our investigation to additional families. Five unrelated probands were screened for mutations in the *LDLR* and *APOB* genes, using direct sequencing and enzymatic restriction. We identified two novel *LDLR* mutations: a missense mutation in exon 7: p.Gly343Cys (c.1027G > T), and a nonsense mutation in exon 17: p.Lys816X (c.2446A > T). Using the PolyPhen and SIFT prediction computer programs the p.Gly343Cys is predicted to have a deleterious effect on LDL receptor activity. The missense mutation we found in exon 3, p.Cys89Trp (c.267C > G), has previously been identified in patients from United Kingdom and Spain, and is reported here for the first time in the Tunisian population. Finally, the framshift mutation in exon 10, p.Ser493ArgfsX44, is reported here for the fourth and fifth time in Tunisian families. The latter is the most frequent FH-causing mutation in Tunisia. These *LDLR* gene mutations enrich the spectrum of mutations causing FH in the Tunisian population. The framshift mutation, p.Ser493ArgfsX44, seems to be a founder mutation in this population.

1. Introduction

Familial hypercholesterolemia (FH, OMIM # 143890) is an autosomal dominant disease caused by mutations in the low-density lipoprotein receptor (*LDLR*) gene [1], and defects in the apolipoprotein B-100 (*APOB*) [2], the proprotein convertase subtilin/kexin type 9 (*PCSK9*) gene or in other unidentified genes [3]. However, the main causes of FH are the mutations in the *LDLR* gene.

FH affects approximately 1:500 persons worldwide making it one of the most frequent metabolic disorders. At the clinical level, FH is associated with tendinous and cutaneous xanthomas, xanthelasmas, corneal arcus, and/or premature coronary heart disease [1]. In Tunisia, a higher frequency was reported (about 1 per 165 persons) related to the high levels of consanguinity in this population [4]. This frequency is as high as that found in populations with a high degree of inbreeding, such as Afrikaners in South Africa [5] Christian Lebanese [6] and French Canadians [7]. In these populations, only a limited number of *LDLR* gene mutations cause the majority of affected cases as the result of founder effects. To date, even through more than 1000 mutations in the *LDLR* gene have been reported in the world [8,9] (http://www.ucl.ac.uk/fh and http://www.umd.necker.fr), only five different FH-causing mutations in the *LDLR* gene have been reported in the Tunisian population [10–12]. We have continued our study for mutations causing FH in Tunisian FH patients. We have found and report here four mutations, two of which are novel point mutations.

2. Material and methods

2.1. Patients

Twenty-seven subjects from five unrelated Tunisian families were analysed, including five probands and twenty-two first and

* Corresponding author. Tel.: +216 73 462 200; fax: +216 73 460 737.
E-mail address: naceur.slimene@fmm.rnu.tn (M.N. Slimane).
[1] These authors equally contributed to this work.

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second degree members of their families. Diagnostic criteria for FH probands were high plasma levels of total and LDL cholesterol (LDL-cholesterol > 4.9 mmol/L), family history of hypercholesterolemia, presence of xanthomas (tendon, planar, and/or tuberous) and personal and/or family history of premature CHD. Secondary cause of hypercholesterolemia, including diabetes, hypothyroidism and nephrotic syndrome were excluded.

Detailed family history data and complete medical history were obtained for each subject of this study after their informed consent. A control group was made of 100 unrelated normocholesterolemic Tunisian individuals with no history of FH or premature CHD.

2.2. Biochemical analysis

Total cholesterol (TC), triglycerides (TG), and HDL-cholesterol levels were measured by standard enzymatic methods using commercially available kits (Biomérieux, France). LDL-cholesterol was calculated with the Friedewald formula.

2.3. Genetic analysis

2.3.1. DNA sequencing of LDLR gene and screening for p.Arg3500Gln APOB mutation

Genomic DNA was isolated from frozen whole blood EDTA samples using the salting-out method [13]. The promoter and the 18 exons of the *LDLR* gene were amplified by polymerase chain reaction (PCR) as previously described [14].

Direct bidirectional sequencing analysis of purified PCR product (ExoSAP-IT, GE Healthcare) was carried out as previously described [14]. When a mutation was detected, another sequencing reaction was performed both on genomic DNA from the relative and from a new PCR product from the proband. The amino acid numbers of the LDL receptor are in agreement with the international nomenclature which starts from the initiating methionine and thus includes the 21 amino acids of the signal peptide (http://www.hgvs.org/mutnomen/). Concerning the *APOB* gene, the common mutation p.Arg3500Gln was screened in all subjects using the PCR-mediated mutagenesis method [15].

2.3.2. Confirmation of nucleotide changes

Restriction endonuclease digestion of PCR was used to confirm the p.Gly343Cys (c.1027G > T) mutation. The amplification was carried out with the same forward primer used for exon 7 sequencing and with a modified reverse primer: ATCTTCGCTGGGCCACCAGCTGGAGGC. The later introduces a HaeIII restriction site in the normal sequence that is absent when the mutation is present. The digestion was carried out overnight with 10 U of enzyme (New England BioLabs) and the digested products were run under 120 V on 3% agarose gel during 90 min. The bands were visualised on UV Transilluminator after staining with ethidium bromide.

2.4. Analysis of variants

In an attempt to analyse the effect of missense amino acid substitution on the LDL receptor, two online available computer programs were employed:

2.4.1. Polymorphism phenotyping (PolyPhen) [16]

PolyPhen is an automatic tool for prediction of possible impact of an amino acid substitution on the structure and function of a human protein. It predicts whether an amino acid substitution would be probably damaging, possibly damaging, or benign, and provides a PSIC (Position-Specific Independent Counts) score for wild type and variant mapping to known 3D structure (http://genetics.bwh.harvard.edu/pph/).

2.4.2. Sorting Intolerant from Tolerant (SIFT) [17]

SIFT predicts whether an amino acid substitution in a given peptide would be tolerated or not tolerated by performing homologue sequence analysis against a family of proteins (http://blocks.fhcrc.org/sift/SIFT.html).

Human LDL peptide sequence (P01130) in FASTA format was used in these two programs.

3. Results

3.1. Clinical analysis

Biochemical and clinical data for the five unrelated probands and their relatives are shown in Table S1 (Supplementary data). Among the relatives, we identified thirteen novel heterozygous FH patients. Cholesterol extra-vascular deposits were observed only for the homozygous FH subjects.

3.2. Genetic analysis

Following DNA screening for the p.Arg3500Gln mutation in the *APOB* gene, FDB was excluded in all the subjects studied.

With the sequencing of the 18 exons and the promotor of the *LDLR* gene, we detected four mutations: two missense (p.Cys89Trp and p.Gly343Cys), one nonsense (p.Lys816X) and one frameshift (p.Ser493ArgfsX44). Among these, p.Gly343Cys and p.Lys816X are reported here for the first time (Table 1). Cosegregation of these mutations within families is shown in Fig. 1. Each mutation described is the only sequence variation identified in the whole *LDLR* gene in each family and has not been found in 100 normocholesterolemic subjects (data not shown).

3.2.1. Family S

The mutation identified in exon 7 of the *LDLR* gene is a G to T transition at nucleotide 1027 (c.1027G > T). It changes codon GGC 343 for glycine to codon TGC for cysteine (p.Gly343Cys). The probands (3S) and two of his sisters (5S, 6S) are homozygous, whereas the mother (1S), one sister (4S) and one brother (deceased) (7S) are heterozygous. The father died before this study and was not tested. The mutation can be rapidly detected by HaeIII digestion (Fig. S1(a and b), Supplementary data).

3.2.2. Family W

The mutation identified is an A to T transition at nucleotide 2446, c.2446A > T (Fig. S1(c), Supplementary data), which is the first base of codon 816 (AAG) that encodes lysine in exon 17 and results in the appearance of a premature stop codon (TAG) (p.Lys816X) giving rise to a truncated LDL receptor. This mutation is found at the heterozygous state for the proband (4W), her father (1W) and her sister (3W), and is not found in her mother (2W) who is normocholesterolemic. High cholesterol levels are obtained for the three mutation-carriers of the family. On clinical examination, only the father presents xanthelasmas, very probably because he has been exposed a longer time than his daughters to hypercholesterolemia (and thus cholesterol deposits).

3.2.3. Family E, and So

These two families carry the same mutation, p.Ser493ArgfsX44 that was previously described in the Tunisian population. It is a complex mutation that consists in a deletion of 3 bp (TCT) that encode serine 493 and an insertion of 7 bp (AGAGACA) in exon 10 (c.1477_1479delTCTinsAGAGACA) [10]. At the protein level, it