Characterization of low density lipoprotein receptor (LDLR) gene mutations in Albania

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Abstract

Introduction: Familial hypercholesterolaemia (FH) is a clinical syndrome characterised by elevated serum total cholesterol (TCHOL) levels due to an increase in low-density lipoprotein (LDL) cholesterol, by tendon xanthomata and clinical manifestations of ischaemic heart disease in early life. Typically, it results from mutations in the low-density lipoprotein receptor (LDLR) gene. So far, more than 800 mutations have been reported for the *LDLR* gene and account for FH. The nature of *LDLR* gene mutations varies among different ethnicities. Until now no mutations of LDLR have been reported in the Albanian population.
Material and methods: We assessed the contribution of the *LDLR* gene mutations as causes of FH in an Albanian population. Fifty probands with a clinical diagnosis of FH were included. We analysed all the exons and the promoter of the *LDLR* gene by using restriction isotyping or direct sequencing.
Results: Twenty-one patients were heterozygous for the 1646G>A mutation (FH Genoa) in exon 11 and 9 patients were heterozygous for the 81T>C mutation in exon 2 of the *LDLR* gene.
Conclusions: This report describes two *LDLR* gene mutations accounting for FH in Albania (1646G>A, 81T>C).

Key words: familial hypercholesterolaemia, low density lipoprotein receptor gene, Albania.

Introduction

The low-density lipoprotein receptor (LDLR; MIM# 143890) is a 160 kD, cell surface transmembrane protein that facilitates the uptake of plasma LDL primarily into hepatocytes, where it is further catabolised [1]. A genetic defect caused by mutations in the *LDLR* gene on chromosome 19 results in malfunctioning or insufficient numbers of *LDLR* molecules, which accounts for the reduced clearance of LDL molecules from the plasma via the receptor-mediated pathway. Low-density lipoprotein is then catabolised via a non-receptor mediated pathway. This abnormality leads to premature development of atherosclerosis. The worldwide heterozygote frequency for FH is about 1/500. The prevalence of homozygosity is much less, about 1/1000,000. The most common clinical features of FH are tendon

xanthomata, xanthelasma palpebrarum, corneal arcus and ischaemic heart disease. The clinical diagnosis is only confirmed by identification of the mutation in the *LDLR* gene. However, to our knowledge no mutation has been reported in the Albanian population until now. Knowledge of the mutations in the *LDLR* gene helps to confirm the clinical diagnosis and also to identify younger affected members in a known FH family. Furthermore, presymptomatic diagnosis and early therapeutic intervention could prevent premature atherosclerosis. Thus, we undertook the present study to characterise the mutations of the *LDLR* gene in Albanian pedigrees with a clinical diagnosis for heterozygous FH.

Material and methods

Patient recruitment

A total of 50 unrelated patients (33 males and 17 females) aged from 11 to 70 years old attending the lipid clinic of the University Hospital Centre "Mother Teresa" with a clinical diagnosis of heterozygous FH participated in the study. The diagnosis of FH was based on William's clinical criteria [2]. In detail, patients with plasma LDL-C levels above 190 mg/dl and with a family history of tendon xanthomata were considered as FH patients and were recruited into our study. Hypercholesterolaemic patients with the apolipoprotein (apo) B3500 gene mutation were excluded by sequencing of the apo B gene and other secondary causes of hypercholesterolaemia were excluded by history, physical examination and the appropriate laboratory tests including thyroid function (TSH), creatinine, proteinuria and alkaline phosphatase.

Detection of low-density lipoprotein receptor gene mutations

In all cases, DNA analysis for the LDLR gene was performed. Whole blood was collected from all patients after informed consent. DNA was extracted from the whole blood specimens as described previously [3]. PCR was carried out using 50 ng DNA isolated from each individual. All *LDLR* gene exons and the promoter were amplified as described previously [4]. PCR was carried out using an MJR PTC-100 thermal cycler. Restriction enzyme isotyping was first used for detecting previously described *LDLR* gene mutations in northwestern Greece, an area which is next to Albania [5, 6]. Automated sequencing was performed using the Applied Biosystems "Big Dye Terminator Cycle Sequencing Ready Reaction Kit" as instructed by the manufacturer (Applied Biosystems). Both forward and reverse sequencing were performed to confirm our findings. The sequenced products were then separated by capillary electrophoresis using an ABI PRISM 3130 Genetic Analyzer. Sequencing Analysis and Sequence Navigator software were used to analyse the collected data and compare them with the published reference sequence of the *LDLR* gene.

Results

This study led to the identification of 2 LDLR gene mutations, which to our knowledge are the first described in the Albanian population. Twenty-one patients were heterozygous for the 1646G>A mutation (FH Genoa) in exon 11 and 9 patients were heterozygous for the 81T>C mutation in exon 2. No patient was found to be homozygous for the above 2 mutations. The frequency of the above mutations is shown in Table I, and the lipid profile of the patients carrying the same mutation is shown in Table II. There were no significant differences in the values of serum lipid parameters or in the prevalence of tendinous xanthomas, xanthelasma palpebrarum, corneal arcus and ischaemic heart disease among heterozygous patients carrying these 2 *LDLR* gene mutations.

Table I. *LDLR* gene mutations found in Albanian pedigrees (n = 50)

Exon	Nucleotide change	Amino acid change	Number of patients	Percentage of study group	Detection assay
2	81T>C	C5W	9	18%	Direct sequencing
11	1646G>A	G528D	21	42%	Direct sequencing

Table II. Lipid profile of the study population

Mutation	Age*	Total cholesterol**	Triglycerides**	HDL** cholesterol	LDL** cholesterol
81T>C	41 ±11	358 ±48	130 ±27	54 ±12	282 ±38
1646G>A	43 ±12	362 ±75	128 ±48	56 ±15	280 ±49

**years, **mean value ±SD [mg/dl]*

Discussion

We undertook the present study to characterise the mutations in the *LDLR* gene in Albanian pedigrees with heterozygous FH. Forty-two percent of the patients possessed the 1646G>A *LDLR* gene mutation, located in exon 11. The LDLR activity is known to be reduced to 2%. The same mutation has previously been described in an Italian FH family [7] and in Greek FH patients [5, 6]. The 81T>G LDLR (5C>W) gene mutation, located in exon 2, has a frequency of 18% in the Albanian population. The activity of the *LDLR* resulting from the 81T>G mutation is reported to be reduced to 5-15%. The mutation was first described in Americans [8] and also in Croatian and English FH patients [Database of *LDLR* gene mutations in FH: http://www.ucl.ac.uk/fh]. Furthermore, the mutation 81T>G has also been previously described in the population of northwestern Greece [9].

Although the clinical criteria for the diagnosis of heterozygous FH were met in all participants, not all of the participants were genetically diagnosed as FH heterozygotes. This finding can be explained by the fact that we did not check for other genetic syndromes that mimic FH such as familial hypercholesterolaemia type III and autosomal recessive hypercholesterolaemia.

The identification of these gene mutations will improve genetic diagnosis of FH in the Albanian population. From a public health point of view, the knowledge of these mutations and their distribution may favour the development of tailed information and screening programmes based on these data [10]. The improvement of genetic diagnosis is of paramount importance for the early diagnosis of new members of an affected family or new probands in Albania. Therapeutic intervention at this early stage is effective for the primary prevention of coronary artery disease. Combination therapy with a statin and ezetimibe is now available for more plasma LDL-C reductions [11]. Furthermore, the fact that gene therapy is not far away makes the knowledge of the pathogenic mutations in various populations of paramount importance.

In conclusion, to our knowledge, this study describes for the first time details of the *LDLR* gene mutations causing FH in an Albanian population. However, this is a preliminary publication and more research is needed for the Albanian FH data to be extended and effective in preventing cardiovascular disease.

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