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ORIGINAL ARTICLE

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Screening for point mutations in the LDL receptor gene in Bulgarian patients with severe hypercholesterolemia

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Abstract Familial hypercholesterolemia (FH) is a common, autosomal dominant disorder of lipid metabolism, caused by defects in the receptor-mediated uptake of LDL (low-density lipoproteins) due to mutations in the LDL receptor gene (*LDLR*). Mutations underlying FH in Bulgaria are largely unknown. The aim of the present study was to provide information about the spectrum of point mutations in *LDLR* in a sample of 45 Bulgarian patients with severe hypercholesterolemia. Exons 3, 4, 6, 8, 9, and 14, previously shown to be mutational hot spots in *LDLR*, were screened using PCR-single-strand conformation polymorphism (SSCP). Samples with abnormal SSCP patterns were sequenced. Three different, hitherto undescribed point mutations (367T > A, 377T > A, 917C > A) and two previously described mutations (858C > A and 1301C > T) in eight unrelated patients were identified; four of the detected point mutations being missense mutations and one, a nonsense mutation. One of the newly described point mutations (917C > A) is a base substitution at a nucleotide position, at which two other different base substitutions have already been reported. Thus, all three possible base substitutions at this nucleotide position have been detected, making it a hot spot for point mutations causing FH. This is the first such mutational hot spot described in exon 6 of *LDLR*.

Keywords Familial hypercholesterolemia · LDL receptor · Point mutations

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Introduction

Familial hypercholesterolemia (FH, MIM#143890) is a common autosomal dominant disorder of lipid metabolism characterized by an elevation in the level of low-density lipoproteins (LDL), the formation of tendon and skin xanthomata, and the development of premature atherosclerosis. It is caused by defects in the receptor-mediated uptake of LDL due to mutations in the LDL receptor gene (*LDLR*) (Goldstein et al. 1995). The prevalence of heterozygous FH in most studied populations is approximately 0.2%. Except for the few populations dominated by founder mutations, the mutations underlying FH are very heterogeneous, and most affected families are expected to have unique *LDLR* mutations.

To-date, more than 920 different *LDLR*mutations have been described worldwide (http://www.umd.necker.fr and http://www.ucl.ac.uk/fh). Of these, about 90% are point mutations (single base substitutions and small insertions and deletions), the rest being gross rearrangements. Analysis of point mutations distribution reveals clustering in certain coding regions of the *LDLR*.

Currently, FH diagnosis is generally based on clinical presentation and lipid profile. However, clinical presentation of the disease is variable, even among

patients with the same genetic mutation, and clinical symptoms of the disease may not always be apparent, especially in young adults and children. Furthermore, lipid values for heterozygous FH patients and unaffected relatives may overlap (Williams et al. 1993). Therefore, a specific molecular diagnosis of these types of hypercholesterolemia is desirable in order to definitively diagnose FH patients and track the mutation within the affected family. In addition, recent studies have shown a relation between the presence of specific mutations and response to lipid lowering therapy (Jeenah et al. 1993; Leitersdorf et al. 1993; Couture et al. 1998; Sun et al. 1998; Heath et al. 1999). Thus, the molecular approach helps in stratifying the patient's risk individually and possibly selecting the most effective treatment.

Mutations underlying FH in Bulgaria are largely unknown, but a broad spectrum of mutations in *LDLR* is expected, given the genetic heterogeneity of the Bulgarian population—well known from studies of other genes (Kalaydjieva et al. 1992; Bronzova et al. 1994; Angelicheva et al. 1997). The present report provides the first data about the point mutation spectrum in six exons of *LDLR*, known to harbor more than 60% of the mutations in other population groups. A sample of 45 Bulgarian unrelated patients with severe hypercholesterolemia was studied, using single-strand conformation polymorphism (SSCP) analysis (Orita et al. 1989; Jordanova et al. 1997), followed by direct sequencing, and three different, hitherto undescribed and two previously described mutations were found in eight unrelated patients.

Materials and methods

Subjects

This study comprises a group of 45 unrelated patients (20 female and 25 male) with severe hypercholesterolemia (total plasma cholesterol level above 8.5 mmol/l prior to treatment). The lipid profile of our group is shown in Table 1. All participants gave their informed consent to participate in the study, and the study protocol has been approved by the IRB of the Medical University of Sofia, Bulgaria. None of the 45 subjects studied was carrier of the most common single mutation resulting in hypercholesterolemia, *APOB* R3500Q, or any other mutations in the receptor-binding region of APOB (Horvath et al. 2001).

DNA analysis

Genomic DNA was extracted from peripheral blood cells as described previously (Miller et al. 1988). Exons 3, 4, 6, 8, 9, and 14 of *LDLR* were amplified by PCR using primers according to Jensen et al. (1996) and subjected to mutation screening using a modified, nonradioactive, high-sensitivity SSCP method (Jordanova et al. 1997). Samples exhibiting mobility shifts were sequenced by radioactive labeling with the Sequenase 2.0 PCR Product Sequencing Kit (Amersham/USB) in both directions using the amplification primers.

Results and discussion

We analyzed 45 patients with severe hypercholesterolemia. Exons 3, 4, 6, 8, 9, and 14, shown to be mutational hot spots in the *LDLR*, were screened using PCR-SSCP. These exons constitute a region over which more than 60% of all known point mutations in the *LDLR* are located (http://www.umd.necker.fr and http://www.ucl.ac.uk/fh).

DNA sequencing of the samples showing aberrant SSCP patterns detected five different mutations in three exons (exons 4, 6, and 9) in eight unrelated patients, all of them in a heterozygous state. The mutations detected in our sample are summarized in Table 2. Three of the mutations (367T > A, 377T > A, and 917C > A) are described for the first time and the rest (858C > A and 1301C > T) have been previously found in other populations (Hobbs et al. 1992; Webb et al. 1996; Day et al. 1997; Mavroidis et al. 1997; Nauck et al. 2001; Wang et al. 2001). These nucleotide changes constitute four missense mutations (S102T, F105Y, S265R, and T413M) and one nonsense mutation (S285X).

Table 1 Lipid profile of 45 Bulgarian hypercholesterolemic subjects studied for point mutations in the *LDLR*. Values are mean ± SD

Subjects	TC (mmol/l)	LDL-C (mmol/l)	HDL-C (mmol/l)	TG (mmol/l)	Age (years)
$n = 45$	10.81 ± 2.23	7.68 ± 2.51	1.26 ± 0.55	3.9 ± 2.98	47.2 ± 9.7

Table 2 Point mutations in the *LDLR* identified in Bulgarian hypercholesterolemic subjects

Nucleotide change	Codon change	Amino acid change	Location	Number of carriers (proportion ± SE)	Reference
367T > A	TCT > ACT	S102T	Exon 4	2 (0.04 ± 0.03)	This study
377T > A	TTC > TAC	F105Y	Exon 4	1 (0.02 ± 0.02)	This study
858C > A	AGC > AGA	S265R	Exon 6	3 (0.07 ± 0.04)	Hobbs et al. (1992), Webb et al. (1996), Day et al. (1997), Mavroidis et al. (1997), and Nauck et al. (2001)
917C > A	TCA > TAA	S285X	Exon 6	1 (0.02 ± 0.02)	This study
1301C > T	ACG > ATG	T413M	Exon 9	1 (0.02 ± 0.02)	Wang et al. (2001)

Table 3 Genotype-phenotype comparisons in the Bulgarian heterozygous familial hypercholesterolemia subjects

Mutation	TC (mmol/l)	LDL-C (mmol/l)	HDL-C (mmol/l)	TG (mmol/l)	Age (years)
367T > A, S102T	8.59	6.71	1.22	2.91	43
367T > A, S102T	8.50	7.05	1.13	1.75	60
377T > A, F105Y	9.44	7.70	1.44	0.58	60
858C > A, S265R	11.31	8.53	0.72	4.50	50
858C > A, S265R	11.00	9.06	0.80	2.50	49
858C > A, S265R	9.20	6.60	1.78	1.80	64
917C > A, S285X	12.90	9.15	2.18	2.89	36
1301C > T, T413M	9.30	6.78	1.21	2.82	50
$x \pm SD$	10.02 ± 1.55	7.70 ± 1.08	1.31 ± 0.49	2.47 ± 1.14	51.5 ± 9.44

Three new mutations in *LDLR* were found in Bulgarian FH patients. Screening of 120 chromosomes from healthy Bulgarians by SSCP did not reveal carriers of these mutations, suggesting that these are more likely disease causing mutations rather than polymorphisms.

The missense mutation S102T was identified in two unrelated patients. This sequence variation is not supposed to lead to a change in charge or hydrophobicity because of the appreciable structural similarity between the wild type and mutant amino acids (Ser102 substituted by Thr). This is why we expect only a partial impact of the change on LDLR protein conformation, and therefore, on its ligand-binding ability. Indeed, both carriers of this mutation exhibit levels of total serum cholesterol and LDL cholesterol in the lower range of our group: TC, 8.59 and 8.50 mmol/l; LDL-C, 6.71 and 7.05 mmol/l, respectively (Table 3). Also, they present a milder phenotype of familial hypercholesterolemia—no apparent xanthomas in both of them, and coronary heart disease in one of them.

The mutation F105Y in exon 4 leads to the replacement of the hydrophobic amino acid phenylalanine by the hydrophilic amino acid tyrosine in repeat 3 of the LDLR binding domain. This change in hydrophobicity may affect the conformation of this domain. The clinical symptoms of FH are more severe in comparison with the carriers of the closely located mutation S102T, with higher values of serum TC and LDL-C (Table 3), advanced coronary heart disease, and prominent arcus corneae.

The newly identified nonsense mutation in exon 6, S285X, gives rise to a premature stop codon in repeat seven of the LDLR-binding domain so that the mutant receptor protein would lack all domains carboxy-terminal to the ligand-binding domain. Accordingly, the S285X allele has to be classified as a null-allele. Indeed, the carrier of this newly described mutation in *LDLR* has most severe clinical manifestations of familial hypercholesterolemia—highest levels of total serum cholesterol and LDL cholesterol among all mutation carriers, 12.90 and 9.15 mmol/l, respectively (Table 3), severe coronary heart disease, and multiple xanthomas.

Our analysis of both most updated and comprehensive *LDLR* mutation databases (http://www.ucl.ac.uk/fh; http://www.umd.necker.fr) identified 480 unique, single base substitutions in the coding sequence. Interestingly, one of the newly described mutations in this study (S285X) is caused by a substitution at a nucleotide position where other mutations have already been reported (Hobbs et al. 1992; Schuster and Humphries 1994; Lombardi et al. 1995; Pimstone et al. 1997; Jensen et al. 1999; Lombardi et al. 2000; Kuhrova et al. 2001, 2002). The mutation S285X identified in our study is caused by a substitution of cytosine by adenine (917C > A), whereas the two other substitutions at the same position identified earlier by other groups are another nonsense mutation 917C > G (Kuhrova et al. 2001, 2002), and a missense mutation 917C > T (S285L, known as FH Amsterdam) (Hobbs et al. 1992; Schuster and Humphries 1994; Lombardi et al. 1995; Pimstone et al. 1997; Jensen et al. 1999; Lombardi et al. 2000). Consequently, nucleotide position 917 of *LDLR* can be described as a hot spot for point mutations causing FH. This is the first reported mutational hot spot in exon 6 with all three possible base substitutions at the same nucleotide position; nine other such hot spots have been reported so far throughout the other 17 exons of the gene.

In summary, we have detected point mutations explaining elevated LDL levels in 8 out of 45 analyzed patients with hypercholesterolemia (frequency 0.18 ± 0.06). These eight patients were found to be carriers of five different point mutations. None of the patients was detected to be a carrier of more than one nucleotide alteration.

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