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Short communication

A combined LDL receptor/LDL receptor adaptor protein 1 mutation as the cause for severe familial hypercholesterolemia

Muhidien Soufi [a,*,1], Stephan Rust [b,1], Michael Walter [c], Juergen R. Schaefer [a]

[a] Department of Internal Medicine, Cardiology, Philipps University, D-35033 Marburg, Germany
[b] Leibniz-Institut für Arterioskleroseforschung, D-48149 Münster, Germany
[c] Institute of Laboratory Medicine, Clinical Chemistry and Pathobiochemistry, Charité Berlin, D-12200 Berlin, Germany

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ABSTRACT

Familial hypercholesterolemia (FH) results from impaired catabolism of plasma low density lipoproteins (LDL), thus leading to high cholesterol, atherosclerosis, and a high risk of premature myocardial infarction. FH is commonly caused by defects of the LDL receptor or its main ligand apoB, together mediating cellular uptake and clearance of plasma LDL. In some cases FH is inherited by mutations in the genes of PCSK9 and LDLRAP1 (ARH) in a dominant or recessive trait. The encoded proteins are required for LDL receptor stability and internalization within the LDLR pathway. To detect the underlying genetic defect in a family of Turkish descent showing unregular inheritance of severe FH, we screened the four candidate genes by denaturing gradient gel electrophoresis (DGGE) mutation analysis. We identified different combinatory mixtures of *LDLR*- and *LDLRAP1*-gene defects as the cause for severe familial hypercholesterolemia in this family. We also show for the first time that a heterozygous LDLR mutation combined with a homozygous LDLRAP1 mutation produces a more severe hypercholesterolemia phenotype in the same family than a homozygous LDLR mutation alone.

1. Introduction

Within the past decades several monogenic traits of inherited hypercholesterolemia have been identified and mutational analysis of the predisposing genes was performed (Abifadel et al., 2003; Goldstein and Brown, 2001; He et al., 2002). To date four genetic causes of hypercholesterolemia have been unraveled. The first identified gene in which mutations are the molecular basis for autosomal familial hypercholesterolemia (FH) was the low density lipoprotein receptor gene (LDLR MIM# 606945) (Brown and Goldstein, 1986). The fundamental work of Goldstein and Brown discovered that the LDLR pathway is the main cellular source for the uptake of low density lipoprotein derived cholesterol (LDL) and that impaired LDLR function causes familial hypercholesterolemia (FH) (Goldstein and Brown, 1979; Hobbs et al., 1992). In FH the receptor mediated LDL clearance is decreased which results in a high risk for severe atherosclerosis and early onset coronary heart disease (CHD) in affected patients. The second genetic cause of autosomal hypercholesterolemia is familial defective apolipoprotein B (FDB). FDB results from a missense mutation (R3550Q) in the apolipoprotein B-100 gene (ApoB MIM# 144010) that causes defective LDLR binding (Innerarity et al., 1990; Marz et al., 1992), thus also leading to the decreased removal of LDL particles from the circulation.

The third genetic cause, which predisposes to dominant hypercholesterolemia, is caused by mutations in the proprotein convertase subtilisin kexin type 9 (PCSK9 MIM# 607786) gene (Abifadel et al., 2009). PCSK9 is a secreted protein and member of the serine protease family. Upon extracellular binding to the LDL receptor, PCSK9 promotes degradation of internalized LDLR in lysosomes. Two classes of PCSK9 mutations have been identified. Gain of function PCSK9 mutations decreases cellular LDL receptor levels, which results in the reduced removal of plasma LDL from the circulation, thus leading to a high risk for atherosclerosis and coronary heart disease (CHD), similar to LDLR mutations. By contrast, loss of function PCSK9 mutations, increases LDLR mediated plasma LDL clearance resulting in a protection from atherosclerosis and CHD development (Cohen et al., 2005). Besides the previously mentioned three loci for autosomal dominant forms of hypercholesterolemia, a fourth locus for autosomal recessive hypercholesterolemia (ARH) has been identified (Eden et al., 2001). ARH is caused by mutations in the LDL receptor adaptor protein 1 (LDLRAP1 MIM# 603813) gene, which encodes an adaptor protein that binds to the NPXY motif in the cytoplasmic tail of the LDLR and governs the clustering of the LDLR into clathrin-coated pits. This protein is thus required for the internalization of the LDLR/LDL

Abbreviations: ARH, autosomal recessive hypercholesterolemia; CHD, coronary heart disease; Dab2, disabled-2; DGGE, denaturing gradient gel electrophoresis; FDB, familial defective apolipoprotein B; FH, familial hypercholesterolemia; HDL, high density lipoprotein; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; LDL, low density lipoprotein; LDLRAP1, LDL receptor adaptor protein 1; LDLR, LDL receptor; PCSK9, proprotein convertase subtilisin kexin type 9; PTB, phosphotyrosine-binding.

* Corresponding author. Tel.: +49 6421 5863578; fax: +49 6421 5867019.
E-mail address: soufi@staff.uni-marburg.de (M. Soufi).

[1] Both authors equally contributed to the study.

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complex (Cohen et al., 2003). ARH is a rare disorder with the highest incidence in the Mediterranean region (Arca et al., 2002). Clinically ARH resembles FH. Patients with ARH reach serum LDL cholesterol levels similar to those observed in patients with homozygous FH, however, in the mean the levels are slightly lower and the prevalence for developing early onset CHD is lower compared to FH patients with strong homozygous LDLR mutations (Pisciotta et al., 2006). Although substantial progress has been made in understanding the genetic basis for monogenic hypercholesterolemia, it is still unclear why there is sometimes a very high variation in plasma LDL values within members of one particular FH family (Rader et al., 2003). This emphasizes that there might be additional yet unidentified loci for hypercholesterolemia, or that combined mutations in the genes for LDLR, apoB-100, PCSK9 and LDLRAP 1 predispose to hypercholesterolemia.

A first analysis of the LDL-receptor in the present case revealed a mutation, but segregation did not fit with the inheritance of the hypercholesterolemia. Therefore the four known major candidate genes were screened for mutations.

2. Material and methods

2.1. Clinical history of patients and laboratory measurements

Lipid studies were carried out in all four members of a family and informed consent was given by the patients for genetic screening of their disorder. The work was approved by the local ethics committee of the Philipps University Marburg and all procedures were in accordance with the Helsinki Declaration of 1975, as revised in 1996. Parents were first cousins. The father and his sons in the age of 1 and 2 years had very high levels of total cholesterol and LDL cholesterol, thus meeting the diagnostic criteria for homozygous familial hypercholesterolemia (FH). The mother had total cholesterol and LDL cholesterol levels that are typical for heterozygous FH. The father had an arcus lipoides corneae and tendon xanthomas (Fig. 1A). In addition early onset angiographic proven CHD in the age of 24 was diagnosed. At the age of 28 he suffered from myocardial infarction and coronary artery bypass surgery was performed. Aggressive lipid lowering therapy with 3-hydroxy-

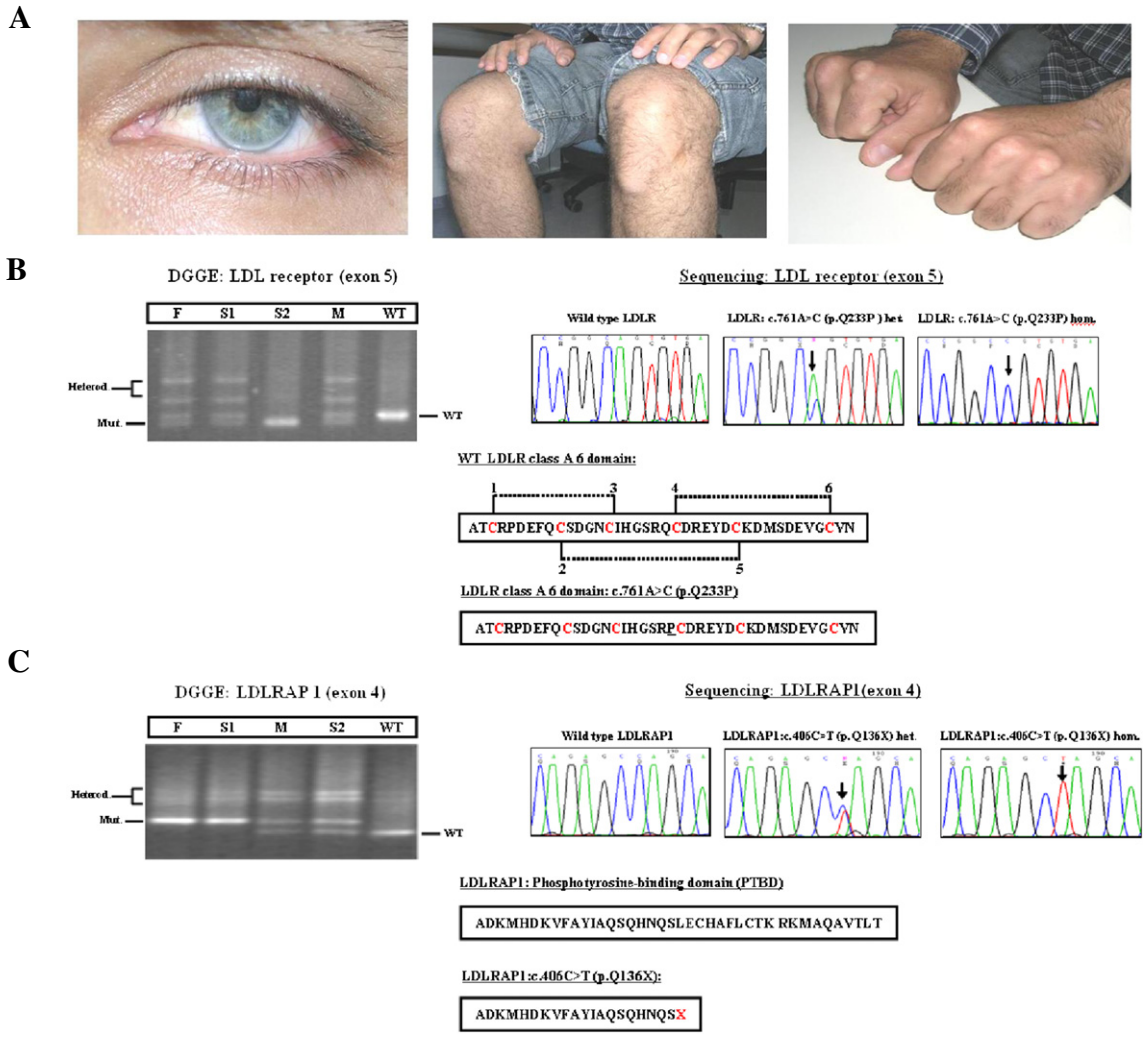

Fig. 1. A: Phenotypic signs of the affected father from the Turkish FH-Family. Shown is an arcus lipoides cornea and xanthomas over the extensor tendons of the knees and hands. B: DGGE mutation analysis of exon 5 from the LDLR gene of the Turkish FH-family and one control subject. A heteroduplex banding pattern is seen in the father (F), mother (M) and first affected son (S1), indicating the presence of a heterozygous LDLR mutation in the family. The second affected son (S2) showed a homoduplex band in the denaturing gradient gel indicating a true homozygous LDLR mutation. The mutation results in the substitution of glutamine (Q) by proline (P) at residue 233 (p.Q233P) in the 6th LDL-receptor class A domain of the LDLR binding site. C. DGGE mutation analysis of exon 4 from the LDLRAP1 gene. A single homoduplex band is seen in the father and his son (F, S1), indicating the presence of an identical mutation on both alleles. The mother (M) and second son (S2) are heterozygous carriers of the mutation. The mutation shifts codon 136 (CAG) coding for glutamine into a stop codon (p.G136X) and truncates the conserved phosphotyrosine-binding domain of LDLRAP1 that interacts with the LDLR.