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Mutation-in-Brief

A case of autosomal recessive hypercholesterolemia with a novel mutation in the *LDLRAP1* gene

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Introduction

Familial hypercholesterolemia (FH, OMIM number #143890), a life-threatening monogenic disorder characterized by high levels of low-density lipoprotein cholesterol (LDL-C), is classified into dominant and recessive types (1). The dominant form of FH may result from mutations in the *LDLR*, *APOB,* and *PCSK9* genes (2). However, mutations in the low-density lipoprotein receptor (LDLR) adaptor protein-1 (*LDLRAP1*) gene cause an autosomal recessive inheritance pattern of FH called autosomal recessive hypercholesterolemia (ARH, OMIM number #603813). The LDLRAP1 protein, encoded by the *LDLRAP1* gene, is required for receptor-mediated endocytosis of LDL-C (2). ARH is a rare disorder with an estimated prevalence of less than 1 in a population of one million. This disease is considered a phenocopy of the most severe form of FH, homozygous familial hypercholesterolemia (HoFH; OMIM number 143890). Hence, most ARH patients are clinically indiscernible from HoFH, which is caused by two defective *LDLR* genes with an approximate prevalence of one individual per million (8). Considering that ARH patients may develop aggressive and premature atherosclerotic cardiovascular disease (ASCVD) due to hypercholesterolemia during early adulthood, lipid-lowering therapy must be initiated during childhood (3). Early identification of ARH patients through genetic analysis of the proband and their relatives can provide prognosis and subsequently appropriate, timely treatment. In this report, we describe a novel variant, c.649G>T, p.Glu217Ter, in the homozygous state in exon 7 of the *LDLRAP1* gene, causing severe ARH.

Case Report

The patient was a 20-yr-old woman from a small village in Ilam, Iran. When she was 10 yr old, the earliest clinical manifestations became apparent, and she was referred to a physician because of the existence of

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tendon xanthomas on her hands, elbows, and knees. The patient was aware of her severe hypercholesterolemia since she was 11 yr old, with an LDL-C concentration of 720 mg/dL. Daily pharmacological treatment with 4 g cholestyramine was initiated. The patient reported that she had undergone subaortic web resection and aortoplasty due to uncontrolled hyperlipidemia at 13 yr of age. She was referred to our cardiovascular center at 17 yr of age for the study of hypercholesterolemia. Bilateral corneal arcus, xanthomas, and xanthelasmas were present. The plasma lipid profile revealed severe hypercholesterolemia: total cholesterol (TC), 520 mg/dL; low-density lipoprotein-cholesterol (LDL-C), 446 mg/dL; high-density lipoprotein-cholesterol (HDL-C), 57 mg/dL; and triglyceride (TG), 93 mg/dL. Secondary causes of hypercholesterolemia, including renal disease, diabetes mellitus, and thyroid disease, were ruled out. The liver enzyme levels were normal. Physical examination revealed a blood pressure of 110/70 mmHg and a body mass index (BMI) of 29 kg/m^2. Family history revealed that the parents were cousins and had given birth to four children (two males and two females), two of whom had passed away for unknown reasons. The lipid profile of the patient's mother (at the age of 44 yr) was in the normal range: TC, 198 mg/dL; LDL-C, 105 mg/dL; HDL-C, 62 mg/dL; and TG, 150 mg/dL. The patient's father had died at the age of 42 yr from coronary artery disease, and his lipid profile was unavailable (**Fig. 1A**). Genetic analysis revealed a homozygous mutation c.649G>T (p.Glu217Ter) in the *LDLRAP1* gene of the patient. Based on these findings, the patient was diagnosed with ARH. According to the results of coronary angiography, the patient was a candidate for coronary artery bypass grafting (CABG) and aortic valve replacement (AVR); however, the patient refused this surgery for personal reasons. Pharmacological treatment with rosuvastatin (60 mg/d) and ezetimibe (10 mg/d) was initiated. The treatment process and the consequent lipid responses are shown in **Table 1**. While undergoing this treatment, the cutaneous xanthomas decreased markedly, and a substantial reduction in plasma LDL-C was noted (from 402.5 ± 31.1 to 103.8 ± 26.02 mg/dL). This reduction was associated with a markedly increase in alanine aminotransferase (from 16.25 ± 6.05 to 49.2 ± 25.2 UI/L). The changes in lipid levels obtained at baseline (mean of four determinations) and during cholesterol-lowering treatment (mean of monthly determinations) are shown in **Table 1**. After 3 years of treatment, despite using maximal recommended rosuvastatin and ezetimibe doses, the results of coronary

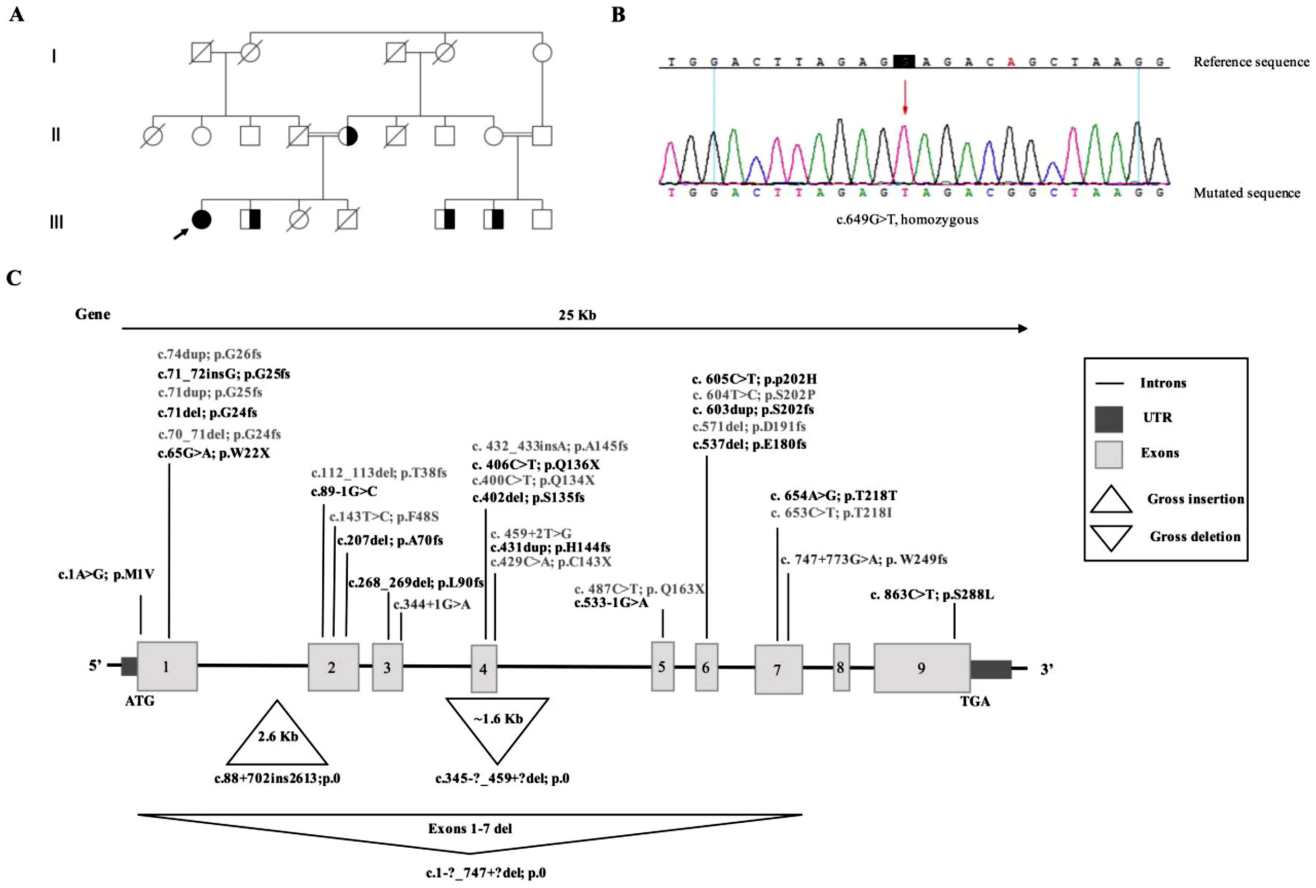

Fig. 1. A: Pedigree of the family with ARH. The proband indicated by the black arrow carries the novel mutation c.649G>T in *LDLRAP1*. The mother and brother were heterozygotes for the same mutation. B: Chromatogram indicating the novel mutation in exon 7 of *LDLRAP1* in the affected individual. C: Updated version of mutations in ARH patients determined to date.

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Table 1. Evolution of lipid profile of the patient before and during treatment with rosuvastatin (60 mg/d) plus ezetimibe (10 mg/d)

Plasma parameter	Before drugs	R60 + E10	Percent change	P*
TC mg/dL	482.25 ± 28.4	169.7 ± 32.1	–64.8	< 0.0001
LDL-C mg/dL	402.5 ± 31.1	103.8 ± 26.02	–74.2	< 0.001
HDL-C mg/dL	61.5 ± 5.4	48.1 ± 6.8	–21.8	< 0.01
TG mg/dL	81.5 ± 13.6	62.8 ± 11.5	–22.9	< 0.052

TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides Values are mean ± SD. * Comparisons were performed using Student's t-test for paired data.

angiography demonstrated that severe supravalvular aortic stenosis (SVAS) resulted in significant stenotic lesions of the coronary arteries and aortic valve. Hence, it was suggested that the patient should undergo CABG and AVR, but she refused to do so again.

Mutational Analysis

After obtaining informed consent from the proband's mother, we extracted genomic DNA from the peripheral blood samples of the proband when she was 17 yr old. Gene panel-based next-generation sequencing (NGS) was performed to identify causal variant(s) in the known genes involved in FH, including *LDLR*, *LDLRAP1*, *PCSK9*, and *APOB* (4). Sanger sequencing was used to validate the presence of the new variant identified via NGS (**Fig. 1B**). A novel homozygous variant (c.649G>T) in the *LDLRAP1* gene was detected in this patient. The same mutation was identified in *LDLRAP1* in heterozygosity for her mother and living brother. The identified nonsense variant was absent in HGMD, dbSNP version 147, ClinVar databases, Iranome, and Exome Sequencing Project. This variant has not been found in the existing literature. The sequence variant was submitted to the ClinVar database (Accession number: VCV000981055.1; https://www.ncbi.nlm.nih.gov/clinvar/variation/981055/). c.649G>T leads to the formation of a stop codon at amino acid residue 217 of the LDLRAP1 protein (p.Glu217Ter). The results of NGS analysis did not identify any pathogenic changes in *LDLR, PCSK9,* and *APOB.*

Discussion

ARH is a genetic disorder of lipid metabolism caused by disruptive mutations in both alleles of the *LDLRAP1* gene (4). The *LDLRAP1* gene is 25-kb long and contains nine exons. It is located on the short arm of chromosome 1 (1p36.11) and encodes the LDLRAP1 protein with 308 amino acids. According to the currently available information in the ClinVar database and literature review, 34 pathogenic variants in the coding sequence of *LDLRAP1* have been reported (**Fig. 1C**) (1, 3, 5, 6). In this clinical report, we described a novel variant, c.649G>T, p.Glu217Ter, in exon 7 of *LDLRAP1*. The patient is most likely homozygous for c.649G>T (p.Glu217Ter) in the *LDLRAP1* gene, since the parents are consanguineous. However, as the patient's father had died many years ago and we could not perform genetic analysis for him, the possibility of a large deletion, including exon 7 of the *LDLRAP1* gene, cannot be excluded (5). In other words, the patient might be compound heterozygous for large deletions, including exon 7 and c.649G>T (p.Glu217Ter).

In silico analysis was performed using available software tools, including CADD, SIFT, and MutationTaster, to predict the pathogenicity of the variant. The results of the analysis predicted that this variant was damaging due to the generation of a premature stop codon in exon 7 of *LDLRAP1*. Furthermore, we recently determined the functional consequence of this mutation by disease modeling of the novel *LDLRAP1* variant c.649G>T in patient-specific induced pluripotent stem cell-derived hepatocyte-like cells (HLCs) (7). This study demonstrated that LDL-uptake by HLCs was disrupted by this mutation. Hence, our findings confirmed the pathological effects of this mutation.

HoFH, the most severe form of FH, is considered a phenocopy of ARH. Therefore, most ARH patients are clinically indiscernible from HoFH. The risk of aortic valve stenosis (AVS) and ASCVD is similar between them. However, SVAS is a rare finding in ARH patients (6), which is typically observed in HoFH.

The lipid-lowering response of ARH patients is much better than that of HoFH patients (8), specifically LDLR-negative patients. However, there is great variability in the LDL-C lowering response to statin treatment in ARH patients, ranging from 20% to 90% (6). In our ARH patient, a 74% reduction in plasma LDL-C concentration was achieved through a high dose of rosuvastatin plus ezetimibe.

In spite of conventional therapies, the cardiovascular prognosis of ARH is poor (3). Despite treatment with a high dose of rosuvastatin plus ezetimibe, our patient presented with severe cardiovascular involvement, AVS, and SVAS at a very early age. Consequently, our findings highlight the importance of early identification of ARH patients via genetic analysis to improve prognosis and determine appropriate treatment.

Conflict of interests: The authors declare no conflicts of interest.

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