

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

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Case Report

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Abstract

Background: Autosomal recessive hypercholesterolemia (ARH) is a rare monogenic disorder resulting from mutations of the *LDLRAP1* gene, which leads to elevated LDL-C levels. Here, using whole exome sequencing (WES), we describe a 22-year-old Iranian female who carries a novel nonsense mutation in *LDLRAP1*.

Methods: Genetic investigations were performed for the patient and her family. She showed LDL-C level of 720 mg/dL since the age of 11 years. At the age of 13 years old, aortic valve repair surgery was performed due to severe aortic valve stenosis (AVS). At the age of 17, along with prescription of rosuvastatin plus ezetimibe, coronary *angiography* displayed the presence of serious stenotic lesions of the coronary arteries and also aortic valve, making the patient eligible for coronary artery bypass grafting (CABG) and aortic valve replacement (AVR).

Results: Genetic analysis showed the presence of a previously unreported homozygous *LDLRAP1* gene variant, c.649G>T, generating a nonsense mutation at amino acid 217, shortening the ARH protein from 308 to 217 amino acid, which removes AP-2 binding domain of ARH, as an important part in LDL uptake.

Conclusion: During a 10-year treatment, we observed a 74% reduction in LDL-C level. Despite the treatment with maximal dose of rosuvastatin plus ezetimibe, the results of coronary angiography demonstrated severe supra-aortic stenosis (SVAS) resulted in significant stenotic lesions of the coronary arteries and aortic valve. This highlights the importance of WES in early diagnosis of ARH, and it is proposed to prevent or at least delay the onset of the cardiovascular events.

Introduction

Familial hypercholesterolemia (FH, OMIM number #143890), as a life-threatening monogenic disorder which is 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 *LDLR*, *APOB* and *PCSK9* genes (2). However, mutations in low-density lipoprotein receptor (LDLR) adaptor protein-1 (*LDLRAP1*) gene cause autosomal recessive inheritance pattern of FH called autosomal recessive hypercholesterolemia (ARH, OMIM number #603813). *LDLRAP1* protein, encoded by *LDLRAP1* gene, is required for receptor-mediated endocytosis of LDL-C (2). ARH is a rare disorder with the estimated prevalence of less than 1 in a population of one million. This disease is considered as 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 caused by two defective *LDLR*

genes with approximated prevalence of 1 individuals per million (8). Considering ARH patients may develop aggressive and premature atherosclerotic cardiovascular disease (ASCVD) as a result of hypercholesterolemia during early adulthood, lipid-lowering therapy must be initiated at 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 homozygous state in exon 7 of *LDLRAP1* gene causing severe ARH.

Case Report

The patient is a 20-year-old female from a small village in Ilam, Iran. When she was 10 years old, the earliest clinical manifestations became apparent and she was referred to a physician due to the existence of tendon xanthomas on her hands, elbows and knees. The patient was aware of her severe hypercholesterolemia since she was 11 years old, with an LDL-C concentration of 720 mg/dL. A daily pharmacological treatment of 4 g cholestyramine was initiated. The patient reported that she had undergone subaortic web resection and aortoplasty due to her uncontrolled hyperlipidemia at the age of 13. She referred to our cardiovascular center at the age of 17 years for the study of her hypercholesterolemia. Bilateral corneal arcus, xanthomas and xanthelasmas were present. The plasma lipid profile was performed revealing severe hypercholesterolemia: total cholesterol (TC) 520 mg/dL, low-density lipoproteins-cholesterol (LDL-C) 446 mg/dL, high-density lipoproteins-cholesterol (HDL-C) 57 mg/dL and triglycerides (TG) 93 mg/dL. Secondary causes of hypercholesterolemia, including renal diseases, diabetes mellitus and thyroid disease were ruled out. Liver enzymes levels were normal. Her physical examination revealed blood pressure of 110/70 mmHg and body mass index (BMI) of 29 kg/m². The family history displayed that the parents were cousins and had given birth 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 years) was at 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 years from coronary artery disease and his lipid profile is unavailable (Fig. 1A). Genetic analysis determined a homozygous mutation c.649G>T (p.Glu217Ter) in the *LDLRAP1* gene of the patient. We identified her as ARH based on these findings. *According to the results of coronary angiography*, the patient was candidate for coronary artery bypass grafting (CABG) and aortic valve replacement (AVR) *but* she rejected this surgery for personal reasons. A pharmacological treatment with rosuvastatin (60 mg/day) plus ezetimibe (10 mg/day) was initiated. The treatment process and the consequent lipid responses are shown in Table 1. Undergoing this treatment, the cutaneous *xanthomas decreased* markedly and also a remarkable reduction of plasma LDL-C was determined (from 402.5 ± 31.1 to 103.8 ± 26.02 mg/dL) associated with a significant increase in alanine aminotransferase (ALT, from 16.25 ± 6.05 to 49.2 ± 25.2 UI/L). The changes in lipid levels obtained in baseline situation (mean of 4 determinations) and along cholesterol-lowering treatment (mean of monthly determinations) is shown in Table 1. After 3 years of treatment, despite using maximal recommended rosuvastatin and ezetimibe doses, the results of coronary angiography demonstrated severe supra-avalvular aortic stenosis (SVAS) resulted in significant stenotic lesions of the

coronary arteries and aortic valve. Hence, it was suggested that the patient should have 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. A gene panel-based next-generation sequencing (NGS) was performed to find casual variant(s) in the known involved genes of FH, including *LDLR*, *LDLRAP1*, *PCSK9* and *APOB* (4). *Sanger sequencing* was used to validate the presence of the new variant identified by NGS (Fig. 1B). A novel homozygous variant, c.649G>T, in the *LDLRAP1* gene, was detected in the patient. The same mutation c.649G>T was identified in the *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 (ESP). This variant was not found in the literature as well. The sequence variant was submitted to ClinVar (Accession number: VCV000981055.1; <https://www.ncbi.nlm.nih.gov/clinvar/variation/981055/>) database. The identified mutation c.649G>T leads to the formation of stop codon at amino acid residue 217 of LDLRAP1 protein (p. Glu217Ter). The results of NGS analysis did not identify any pathogenic changes in *LDLR*, *PCSK9* and *APOB*.

Discussion

ARH disease is a genetic disorder of lipid metabolism caused by disruptive mutations in both alleles of *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 LDLRAP1 protein with 308 amino acids. According to 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 the *LDLRAP1* gene. 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 couldn't perform genetic analysis for him, the possibility of having large deletion including exon 7 of the *LDLRAP1* gene cannot be excluded for him (5). In other word, the patient might be a compound heterozygous for large deletion including exon 7 and c.649G>T (p.Glu217Ter).

In silico analysis were performed by available software tools, including CADD, SIFT and MutationTaster to predict pathogenicity of the variant. The results of the analysis predicted this variant as damaging due to generation of premature stop codon in exon 7 of the *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 (iPSC)-derived hepatocyte-like cells (HLCs) (7). This study demonstrated that the LDL-uptake by the HLCs disrupted due to this mutation. Hence, our findings confirmed the pathological effect of the mutation.

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

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

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

Declarations

Declaration of conflict of interest

The authors declare no conflicts of interest.

Acknowledgments

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Tables

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

Plasma parameter	Before drugs	R60 + E10	Percent change	P*
TC mg/dL	482.25 ±28.4	169.7 ±32.1	-64.8	< .0001
LDL-C mg/dL	402.5 ±31.1	103.8 ±26.02	-74.2	< .001
HDL-C mg/dL	61.5 ±5.4	48.1 ±6.8	-21.8	< .01
TG mg/dL	81.5 ±13.6	62.8 ±11.5	-22.9	< .052

TC, total cholesterol; LDL-C, LDL-cholesterol; HDL-C, HDL-cholesterol; TG, triglycerides. Values are mean ± SD. *Comparisons were performed by student's test for paired data.

Figures

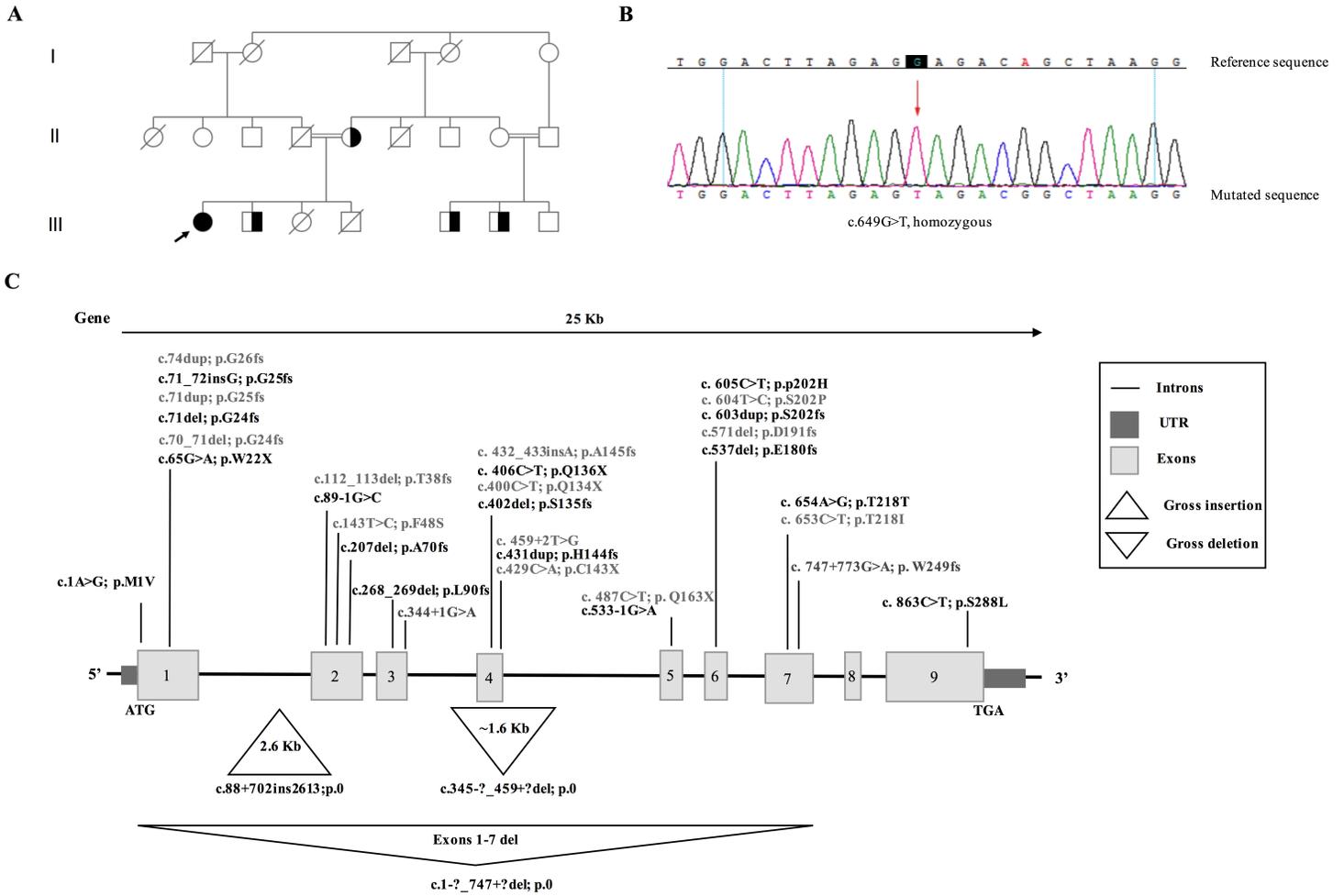


Figure 1

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.