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Autosomal recessive hypercholesterolemia: Case report

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INTRODUCTION: Autosomal recessive hypercholesterolemia (ARH; OMIM #603813) is a very rare monogenic disorder affecting less than 1 in 1000,000 people and is characterized by very high levels of low-density lipoprotein cholesterol (LDL-C), leading to aggressive and premature atherosclerotic cardiovascular disease if left untreated. Lowering of LDL-C is the main target of the treatment. We report on a 29-year-old male patient born in nonconsanguineous Lithuanian family homo(hemi-)zygous for LDLRAP1 gene variant causing ARH. This variant is not present in population databases and, to our knowledge, has not been reported in scientific literature before.

METHODS AND RESULTS: The earliest clinical sign, noticed at the age of 5 years, was painful and enlarging nodules on Achilles tendons. At the age of 10 years, xanthomas of the metacarpal joint area on both hands emerged. The first lipid panel was performed at the age of 12 years. In accordance with Dutch Lipid Clinic Network diagnostic criteria for familial hypercholesterolemia (FH), definite FH (type IIA hyperlipoproteinemia) was diagnosed and the treatment with cholestyramine 4 grams per day was initiated. As the patient was 15 years old, direct adsorption of low-density lipoprotein apheresis was started and repeated monthly. At the age of 20 years, along with lipoprotein apheresis, 10 mg of rosuvastatin daily intake was prescribed. At the age of 28 years, the dose of rosuvastatin was increased to 40 mg per day, and 10 mg of ezetimibe daily intake was added. At the age of 28 years, homozygous *LDLRAP1* gene variant NM_015627.2:c.488A>C, NP_056442.2:p.(Gln163Pro) causing autosomal recessive hypercholesterolemia was determined by genetic testing.

CONCLUSIONS: This case report implies that ARH, being an extremely rare disorder, is a severe disease. As there is limited routine testing, including genetic testing, patients suffering from both this disease and FH may remain undiagnosed. Cascade screening and genetic counseling differ for ARH as compared with FH, as the carrier of a pathogenic variant in the LDLRAP1 gene does not have marked total cholesterol and LDL-C elevations. However, genetic testing of the proband and their relatives is essential to evaluate the risk of development of FH and to provide prognosis as well as adequate, timely treatment. To improve the quality of life of patients with FH and prolong their life expectancy, national registries of FH and wider laboratory and genetic testing are undoubtedly

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necessary. A national FH screening program was set up in Lithuania, which helps to identify, monitor, and treat subjects with FH.

Introduction

Autosomal recessive hypercholesterolemia (ARH) (OMIM #603813) is a very rare monogenic disorder affecting less than 1:1000,000 of the population and is characterized by very high levels of low-density lipoprotein cholesterol (LDL-C), leading to aggressive and premature atherosclerotic cardiovascular disease (CVD).[1] ARH is caused by pathogenic variants in the LDL receptor adaptor protein 1 (*LDLRAP1*) gene, resulting in a truncated or nonfunctional adaptor protein that is involved in the uptake in the LDL receptor (LDLR) and clearance of LDL particles.[1] Homozygous or compound heterozygous pathogenic variants in other genes, such as apolipoprotein B (APOB), proprotein convertase subtilisin/kexin type 9 (PCSK9), or the LDLR gene, are found in patients with homozygous familial hypercholesterolemia (HoFH), demonstrating a clinical phenotype consistent with ARH[2]—extremely high LDL-C levels, very extensive cutaneous or tendon xanthomas, aortic stenosis, and premature atherosclerotic CVD.[1,2] However, owing to currently unknown reasons, patients with HoFH have a greatly reduced life expectancy, higher rates of premature atherosclerotic CVD (including risks of myocardial infarction before the age of 20 years), and an even worse response to lipid-lowering treatment compared to patients with ARH.[1–5] The prevalence of FH is commonly reported as 1 in 500 individuals.[6] Recent studies showed the prevalence of heterozygous FH consistent with the ratios of 1:200–1:250; by extrapolation, HoFH may affect up to 6 individuals per million (or 1 in 160,000–300,000).[7–9] According to the data collected by the Lithuanian Department of Statistics, the estimated number of patients with heterozygous FH in Lithuania should be 14,240 (1:200), 18 patients with HoFH (1:160000), and only 2 to 3 individuals with ARH (1:1000000). A national FH screening program in Lithuania has been initiated, and a cascade screening of families of the detected subjects is being performed. Because the disease affects individuals at a very early age, it is crucially important to identify patients with FH and initiate the treatment as early as possible to reduce their high cardiovascular mortality.

In this article, we aim to present a 29-year-old male patient born in a nonconsanguineous Lithuanian family, homo (hemi-)zygous for the *LDLRAP1* gene variant causing ARH. This variant is not present in population databases and, to our knowledge, has not been reported in scientific literature before.

Clinical report

We present a case report of a 29-year-old man who is the first patient in Lithuania to be diagnosed with a case of genetically proven ARH.

Clinical findings and course of the disease

When the patient was 5 year old, the earliest clinical manifestation—enlarged nodules on the Achilles tendons—became apparent. At the age of 10 years, numerous painless 1 × 0.5 cm-sized nodes, similar to those encountered in rheumatoid cases, appeared on the metacarpal joint area of both hands. At that time, a treatment for reactive polyarthritis was initiated. As the condition did not improve, at the age of 12 years, the first lipid panel was performed and it showed severe hypercholesterolemia: total cholesterol (TCh) 18.94 mmol/L (732 mg/dL), LDL-C 16.69 mmol/L (645 mg/dL), high-density lipoprotein cholesterol (HDL-C) 1.41 mmol/L (55 mg/dL), and triglycerides (TG) 1.83 mmol/L (162 mg/dL). The first plastic surgery of highly enlarged xanthomas on the Achilles tendons, knees, elbows, and hands was performed when the patient was 12 years of age. At that time, any secondary causes for the increased lipids were ruled out, and definite FH (type IIA hyperlipoproteinemia) was diagnosed according to the Dutch Lipid Clinic Network diagnostic criteria for FH. A treatment with a daily 4 gram dose of cholestyramine 4 was initiated. The patient underwent another two plastic

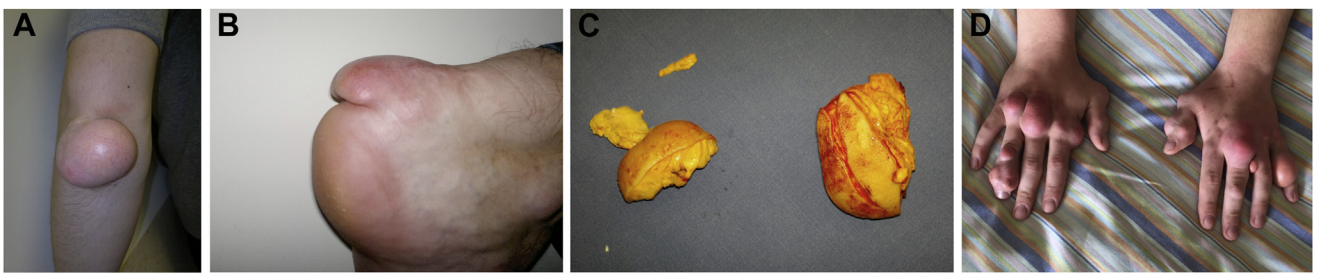

Figure 1 A) Xanthomas on the elbows (24 years). (B) Xanthomas on the Achilles tendons (24 years). (C) Histology of Xanthomas (24 years). (D) Xanthomas on the metacarpal joint area of both hands (28 years).

Table 1 Dynamics of laboratory tests, key highlights, and treatment

Patient's age (date)	TCh (mmol/L)	LDL-C (mmol/L)	HDL-C (mmol/L)	TG (mmol/L)	APOB (g/L)	APOE (g/L)	APO A1 (g/L)	APO A2 (g/L)	Lp(a) (g/L)	APOB/APOA1 (g/L)	Key highlights and treatment
12 (2002 y)	18,94	16,69	1,41	1,83	-	-	-	-	-	-	The first lipid panel was assessed. Treatment with Cholestyramine (4 grams per day) was initiated.
14 (2003 y)	17,65	12,16	1,7	1,36	-	-	-	-	-	-	Direct adsorption of low-density lipoprotein apheresis (DALI) recommended.
15 (2004 y)	11,72	9,89	1,28	1,19	-	-	-	-	0,22	-	DALI apheresis started (lipid panel shows the concentrations of serum lipids before apheresis).
17 (07/11/2006)	30,17	25,45	1,23	2,21	3,65	-	-	-	0,35	-	Maximal lipid concentrations determined before DALI apheresis.
17 (07/11/2006)	8,72	7,1	1,0	1,3	1,61	-	-	-	0,10	-	Lipid concentrations right after the DALI apheresis.
20 (2010 y)	18,7	16,95	0,93	1,79	4,41	-	-	-	0,23	-	Along with DALI apheresis, rosuvastatin (10 mg per day) was added to the treatment (lipid panel shows the concentrations of serum lipids before routine apheresis and the initiation of treatment with statins). DALI apheresis was continued from 2004 to 2014. Lost of follow-up from 2014 to 2017. Patient discontinued any treatment.
27 (03/2017)	17,64	16,03	0,89	1,56	3,58	108	1,15	0,27	0,24	3,1	Lipid panel right after the lost follow-up period (spent without any treatment). Treatment with rosuvastatin (40 mg per day) was initiated.
27 (12/2017)	8,61	7,06	0,74	1,76	1,8	63	1,06	0,21	0,13	1,7	Lipid panel after 10 mo of daily 40 mg rosuvastatin intake. A daily 10 mg dose of ezetimibe added.
28 (04/2018)	9,02	7,37	1,13	1,13	1,77	64	1,5	0,32	0,16	1,18	Five months of treatment consisting of rosuvastatin 40 mg and ezetimibe 10 mg per day.

TCh, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides; APOB, apolipoprotein B.

surgeries of xanthomas at the age of 20 and 24 years (Fig. 1A, B, C, D).

When the patient was 15 years old, direct adsorption of low-density lipoprotein (DALI) apheresis was started and repeated monthly. At the age of 20 years, along with lipoprotein apheresis, a daily intake of 10 mg of rosuvastatin was prescribed. During the period of December 2004 and February 2014, selective LDL apheresis was performed sixty times. From February 2014 to March 2017, there was a loss of the patient's follow-up, and the treatment was discontinued. At the age of 28 years, the dose of rosuvastatin was increased to 40 mg per day, and a daily dose of 10 mg of ezetimibe was added. Since the last xanthoma surgery at the age of 24 years and the DALI apheresis procedures, xanthoma regrowth was prevented. At the age of 28 years, a homo (hemi-)zygous *LDLRAP1* gene variant NM_015627.2:c.488A>C, NP_056442.2:p.(Gln163Pro), which was causing ARH, was determined on the basis of genetic testing.

Familial history

The first lipid panel of the patient's mother (at the age of 60 years) showed severe hypercholesterolemia: TCh—8.35 mmol/L (323 mg/dL), LDL-C—5.8 mmol/L (224 mg/dL), and HDL-C—1.86 mmol/L (72 mg/dL). However, no xanthomas had manifested in her case. Treatment with statins has been recommended; however, there was also a loss of follow-up, and the response to treatment is unknown. The lipid profiles of the patient's siblings were at normal ranges; mild hypercholesterolemia has been identified in the lipid panel of the patient's father (at the age of 64 years): TCh—5.32 mmol/L (206 mg/dL), LDL-C—3.72 mmol/L (144 mg/dL), and HDL-C—1.13 mmol/L (44 mg/dL). The siblings and the father of our patient refused to carry out genetic testing. The patient's grandparents' clinical information is unavailable.

Laboratory testing

As the patient was 17 years old, the highest concentrations of serum lipids were determined before the DALI apheresis: TCh—30.17 mmol/l (1167 mg/dL), HDL-C—1.23 mmol/L (48 mg/dL), LDL-C—25.45 mmol/L (984 mg/dL), and TG—2.21 mmol/L (196 mg/dL). After the DALI procedure, a considerable decrease of serum lipids was observed: TCh—8.72 mmol/L (337 mg/dL), HDL-C—1.0 mmol/L (39 mg/dL), LDL-C—7.1 mmol/L (275 mg/dL), and TG—1.3 mmol/L (115 mg/dL). Owing to a very good response to the treatment, recommendations to repeat the DALI apheresis for the rest of the patient's lifetime were provided. The dynamics of laboratory tests, the key highlights, and the response to treatment are shown in Table 1. Lipoprotein electrophoresis was assessed twice—at the age of 20 and 28 years. Both revealed a very intensive fraction of beta-lipoproteins; the fraction of alfa-lipoproteins was slightly lower; the fraction of pre-beta-lipoproteins was unaltered; no pathological migrating fractions were identified. This inferred to a type IIA hyperlipoproteinemia.

Instrumental investigation

The instrumental investigation during the period of 2010–2017 showed only minor atherosclerotic lesions of the aorta and carotid arteries. The first detailed echocardiography was performed at the age of 20 years and showed the bicuspid aortic valve with first-degree insufficiency. The signs of a significant mutual tendinopathy were revealed through an ultrasound of the muscles and tendons. Corneal lipoid rings of both eyes were identified.

Genetic testing

A next-generation sequencing analysis of the patient's genomic DNA was performed using the TruSight Cardio Sequencing panel (Illumina Inc, San Diego, CA). Next-generation sequencing data were analyzed using Illumina BaseSpace bioinformatics. A total of 174 genes were sequenced, including the main genes associated with hypercholesterolemia (*LDLR, APOB, PCSK9,* and *LDLRAP1*). Gene variants with a minor allele frequency of >5% have been excluded. ExAC[10] and Clinvar[11] databases were used to assess the prevalence and pathogenicity of the variants. An in silico analysis of the missense mutations was performed using PolyPhen-2,[12] SIFT Human Protein,[13] and Mutation Taster2.[14] The proband was found to be homo (hemi-)zygous for the *LDLRAP1* gene variant NM_015627.2:c.488A>C, NP_056442.2:p.(Gln163Pro). The *LDLRAP1* gene variant NP_056442.2:p.(Gln163Pro) is not present in global population databases and has not been reported in the literature before. This variant occurs at a position that is conserved across species (PhyloP Score is 4.53). The in silico analysis is controversial: SIFT predicts that the variant is deleterious with a score of 0, Mutation Taster predicts that the variant is disease-causing with a probability >0.9999, whereas the prediction of PolyPhen-2 is benign with a score of 0.248. Sanger sequencing confirmed the homo (hemi-)zygous variant to be the proband. New-generation sequencing using the TruSight Cardio sequencing panel genes revealed the carrier status of the pathogenic variant in the *LDLRAP1* gene NM_015627.2:c.488A>C, NP_056442.2:p.(Gln163Pro) to the mother. No additional pathogenic variants in the main genes associated with hypercholesterolemia (*APOB*, *LDLR*, *PCSK9*) were identified. Blood samples of the proband's father and siblings were not made available for genetic testing.

Discussion

We report on a patient with a homo (hemi-)zygous variant in the *LDLRAP1* gene causing ARH. Only several families with autosomal recessive FH have been described in scientific literature,[15] and this is the first case of autosomal recessive FH in Lithuania. The *LDLRAP1* gene (1p36.11) is associated with ARH (MIM#603813). This

gene encodes LDLRAP1, promoting the internalization of LDLs.[16] To date, 26 pathogenic variants had been identified in the coding sequence of the gene, leading to the impairment of cholesterol metabolism and the development of clinical signs of hypercholesterolemia.[17] Of those, only three were missense, and the remaining caused the premature termination of the protein. The *LDLRAP1* gene NM_015627.2:c.488A>C, NP_056442.2:p.(Gln163Pro) variant is localized in the gene sequence encoding the PH domain-like (IPR011993) and the PTB/PI domain (IPR006020), serving as parts of the protein binding lipids and functioning as adaptors to the signaling system involved in many physiological processes.[18] The extreme rarity of the variant, its homo (hemi-)zygous state, its relevant localization in the protein sequence, and the in silico analysis results encouraged us to classify it as probably pathogenic.

The clinical picture of ARH is mimicking the homozygous dominant entity of FH. Our patient was presented with a significant phenotypic FH manifestation at an extremely early age. The main difference of ARH is that the parents and siblings, carrying the heterozygous variant, are not suffering from the disorder, and their cholesterol level should remain within the normal range. In the proband's family, the mother was clinically diagnosed with FH. This finding could have misled to a clinical diagnosis of homozygous autosomal dominant hypercholesterolemia if wide-scale genetic testing would not identify potentially ARH-causing variants in the *LDLRAP1* gene. Still, the almost normal levels of cholesterol of the father and the carrier status of the mother (which is not sufficient to be the cause of hypercholesterolemia) confirm the molecular diagnosis of ARH. Biochemical findings of the mother of the proband could not be attributed solely to the heterozygous genotype of the *LDLRAP1* gene. A polygenic etiology has been proposed for individuals with severe hypercholesterolemia or even a clinical diagnosis of FH if no pathogenic variants in three known genes are identified.[9] Sánchez-Hernández et al. have suggested that some heterozygous *LDLRAP1* mutations may contribute to polygenic hypercholesterolemia.[1]

The homozygosity of this novel, extremely rare variant raises the suspicion regarding any possible consanguinity or uniparental disomy (of the first chromosome, in this case). Any close familial relationships between the parents of the patient were ruled out during the genetic counseling of the patient, when a three-generation genealogy was analyzed. The second proof of nonconsanguinity came with the genetic data—the patient has other heterozygous benign or intronic variants in the *LDLRAP1* gene, demonstrating the homozygosity of the c.488A>C variant *LDLRAP1* gene by state. Many autosomal recessive disorders with proven novel or extremely rear homozygous pathogenic variants in *SLC19A2*,[19] *TREX1*,[20] *POMK* (our unpublished data), identified in Lithuanian residents, indicate the relative genetic homogeneity of the Lithuanian population, arising possibly due to the distant common founder effect. Moreover, the analysis of the carrier status of autosomal recessive disorders within the Lithuanian population revealed a substantial difference in the frequency of pathogenic variants between individuals from our country and other Caucasian populations, conferring to the higher probability of the development of the disorder due to a homozygous genetic alteration.[21,22]

The possibility of uniparental (maternal) disomy of the first chromosome in the absence of any confirmation of the carrier status of the father's variant was rejected both clinically and genetically. The first chromosome, being the biggest chromosome in the genome, should inevitably carry more than one recessive pathogenic variant. In that case, we would have diagnosed at least several unrelated autosomal recessive disorders in the patient. The data of genetic testing justified clinical observations—there are many heterozygous benign or intronic variants not only in *LDLRAP1* but also in other genes located in the first chromosome (eg, *PRDM16*, *ZBTB17*).

The identification of ARH in the patient changes family counseling and makes a difference in estimating disease risk in the relatives. Cascade screening and genetic counseling differs for ARH as compared with FH. In the case of ARH, unless the patient was to marry a carrier of the pathogenic variant in the *LDLRAP1* gene, his children would not develop severe hypercholesterolemia because carriers do not have marked elevations. Therefore, genetic testing of ARH family members is required to determine their carrier status or make an early diagnosis in any newborn siblings of the probands. On the contrary, pathogenic variants in the *LDLR*, *APOB*, and *PCSK9* genes can be passed to offspring and cause autosomal dominant FH with 50 percent probability. Consequently, the testing of relatives is essential to evaluate the risk of developing FH.

The main purpose of preventive measures in ARH as well as FH patients is the reduction of cardiovascular risk.[23] Sánchez-Hernández et al., in their recent paper characterizing ARH in Spain, revealed that the LDL-C decrease for patients with ARH with appropriate lipid-lowing therapy, including high-dose statins and ezetimibe, ranged from 41 to 84%, confirming a much larger lipid response than HoFH and very similar to that observed in general population.[1] Some studies indicate that PCSK9 inhibitors, such as evolocumab, might be a beneficial therapy for patients with FH, especially the ones with statin intolerance or for individuals who fail to attain the target goal of LDL-C despite the consumption of maximum doses of statins.[24,25] However, the reduction of LDL-C levels' response to the PCSK9 inhibitor in patients with ARH is heterogeneous.[1] PCSK9 reduces LDLR expression on the cell surface. Monoclonal antibodies can inactivate PCSK9, thereby increasing LDLR expression. This enhances LDL uptake in the presence of a normal LDLRAP1 protein.[26] This may probably explain the mild response in ARH subjects who lack functional LDLRAP1.[1,26] Therefore, an inhibitor of the microsomal triglyceride transfer protein, such as lomitapide, could be an alternative treatment for ARH because it acts independently

of the LDLR.[1] When target LDL-C concentrations cannot be reached with maximum tolerated doses of pharmacotherapy, a more aggressive form of treatment with LDL apheresis could be initiated.[27] Our patient's treatment with DALI apheresis reduced his LDL-C concentration by 72%. Treatment with a maximum dose of rosuvastatin alone reduced his LDL-C by 50%, which refers to significant lipid response to statin therapy. The lack of response to ezetimibe may be explained by poor adherence to treatment. We do not have decent compliance from our patient, and we can not verify if the patient took ezetimibe as it had been prescribed. There were no financial opportunities to treat our patient with PCSK9 inhibitors; nevertheless, a response to this treatment for patients with ARH is controversial, and better results are shown for FH than ARH patients.

Conclusions

This case report implies that ARH, being an extremely rare disorder, is a severe disease. As there is limited routine testing, including genetic testing, patients suffering from both this disease and FH may remain undiagnosed. Cascade screening and genetic counseling differ for ARH as compared with FH, as the carrier of a pathogenic variant in the *LDLRAP1* gene does not have marked TCh and LDL-C elevations. However, genetic testing of the proband and their relatives is essential to evaluate the risk of development of FH and to provide prognosis as well as adequate, timely treatment. To improve the quality of life of patients with FH and prolong their life expectancy, national registries of FH and wider laboratory and genetic testing are undoubtedly necessary. A national FH screening program was set up in Lithuania, which helps to identify, monitor, and treat patients with FH.[28]

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Authors' contributions: Zaneta Petrulioniene was treating the patient, performing his follow-ups, supervising all parts of the paper's preparation, and was responsible for editing and verifying the final version of the article. Urte Gargalskaite participated in the patient's follow-ups, was responsible for collecting all the clinical data of the patient, and writing the clinical part of this paper as well as editing the paper. Violeta Mikstiene wrote the genetic part of the paper and edited it. Rimvydas Norvilas performed the genetic testing for the patient and edited the paper. Egle Skiauteryte participated in the patient's follow-ups, did a research on the existing data related to this case, wrote the introduction and discussion parts of the paper, and edited the paper. Algirdas Utkus supervised the genetic testing of the patient, consulted the patient and his family, and participated in preparing and editing the paper.

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