Supplementary material

Bobrowska B, Zasada W, Zawada M, et al. Autosomal recessive hypercholesterolemia: the first experience in Poland. Pol Arch Intern Med. 2023; 133: 16498. doi:10.20452/pamw.16498

Autosomal recessive hypercholesterolemia (ARH)

ARH is caused by loss-of-function mutations in *LDLRAP1*, a gene located on chromosome 1, encoding an adaptor protein involved in the uptake of the LDL receptor (LDLR) and clearance of LDL particles. LDLRAP1 protein is an LDLR chaperone that binds to the LDLR, allowing the LDL/LDLR complex to be internalized in the clathrin-coated pit [1]. ARH has been considered a phenocopy of homozygous familial hypercholesterolemia (HoFH) due to LDLR mutations [2]. Patients with ARH, similarly to HoFH, develop extensive cutaneous or tendon xanthomas, aortic stenosis, and ASCVD [1,2]. Therefore, early identification of patients with ARH by performing genetic tests of the proband and their relatives is crucial to implement appropriate aggressive lipid-lowering treatment as soon as possible, which may reduce their high cardiovascular risk.

Familial history

The patient's father died of a myocardial infarction at 67. He had previously been treated for arterial hypertension, type 2 diabetes mellitus, and chronic kidney disease. He had elevated cholesterol values. The patient's mother is alive, she is now 83 years old, and her cholesterol is normal. The patient has three siblings and three children. A sister (LDL-C 4.8 mmol/l) and one daughter (LDL-C 4.1 mmol/l) are carriers of the *LDLRAP1*:c.603dupC gene variant. The lipid profile of the other siblings is normal. Two children were not included in the genetic

testing because they were abroad. Their lipid profiles were at normal ranges. None in the family has tendon xanthomas or corneal arcus.

Genetic testing

Next-generation sequencing analysis of the patient's genomic DNA was performed using the Ampliseq for Illumina On-Demand kit. After preparing the libraries, the samples were amplified and sequenced using the MiSeq sequencer (Illumina). Bioinformatic analyses were performed using BaseSpace and Variant Interpreter applications (Illumina) in relation to hg19 human genome. The HGVS 2016 guidelines presented the terminology of revealed changes. The detected genetic variants' meaning and association with the observed disease were interpreted based on currently available databases and scientific publications. The proband was homozygous for the *LDLRAP1* gene variant NM_015627.3:c.603dupC p.(Ser202LeufsTer19), rs781585299. The presence of the variant was confirmed by Sanger sequencing. According to the available literature, it is a pathogenic variant that causes extremely rare autosomal recessive hypercholesterolemia (ARH) [3,4]. Sanger sequencing revealed the carrier status of the pathogenic variant in the *LDLRAP1* gene NM_015627.3:c.[603dupC];[603=] to the sister and daughter of the patient. No additional pathogenic variants in the main genes associated with hypercholesterolemia (*APOB*, *LDLR*, *PCSK9*) were identified.

Laboratory testing

The oldest, and at the same time the highest, available lipid profile was from 2014 when the patient had been treated with atorvastatin 80 mg in combination with ezetimibe 10 mg (TCh-13.3 mmol/L, HDL-C-1.0 mmol/L, LDL-C-11.3 mmol/L, and TG-2.21 mmol/L). The patient was also treated with PCSK9 inhibitors and inclisiran in addition to standard therapy, but the

decrease in LDL cholesterol was insignificant and amounted to 7-21%. The patient tolerates high doses of statins well, but the switch from rosuvastatin to atorvastatin was due to kidney disease.

Discussion (in relation to the available literature describing ARH)

We present a patient with a homozygous variant in the *LDLRAP1* gene causing ARH, also known as familial hypercholesterolemia type 4 (FHCL4). Only several families with ARH have been described in the scientific literature [5]. ARH is caused by homozygous or compound heterozygous mutations in the *LDLRAP1* gene, locus 1p36.11, which encodes an adapter protein for the LDL receptor and regulates its expression and degradation [6]. According to the literature review, approximately 35 pathogenic variants in the coding sequence of *LDLRAP1* have been reported [7,8,9]. The mutation found in our patient is one of the best described. ARH is a phenocopy of HoFH due to mutations in the *LDLR* gene. But the clinical phenotype of ARH is milder than that of receptor-negative HoFH and resembles that observed in receptor-defective HoFH [10].

More than 90% of patients with ARH have tendon xanthomas, which are larger and more massive, and the corneal arcus is observed in about 65% of patients. ASCVD is diagnosed in about 50% of patients, but its development occurs later [10,11]. The risk of aortic valve stenosis, relatively often documented in patients with ARH [12,13], was also similar to that in LDLR-HoFH [14]. Three times more women developed this complication than men [14]. In our image, aortic valve stenosis in the moderate stage was present. The main difference of ARH is that the family members who carry the gene variant do not suffer from the disorder, and their lipid panel remains within the normal range or is only slightly elevated. Sanchez-Hernandez et al[1] have suggested that some heterozygous *LDLRAP1* mutations may contribute to polygenic hypercholesterolemia. Our two heterozygous *LDLRAP1* mutation

carriers (the sister and the daughter) had only a tiny increase in LDL-C. None of them had premature ASCVD, aortic stenosis, or disease manifestations on physical examination. Researchers from Spain describing their group with ARH revealed a decrease in LDL cholesterol in the 41 to 84% range on the high-dose statin therapy in combination with ezetimibe, which was compared to that of the general population and significantly superior to the HoFH group [1].

Unfortunately, we do not know the response to combination therapy in our patient because we do not have access to her lipid panel without any treatment. The reduction of LDL-C levels' response to the PCSK9 inhibitor in patients with ARH is heterogeneous and relatively mild [1]. That seems to be understandable considering the mechanism of action of monoclonal antibodies, which, by inactivating the PCSK9 protein, increases the amount of LDLR on the cell surface. In the case of a lack of functional *LDLRAP1*, the LDL/LDLR complex cannot be internalized appropriately into the cell. Therefore, the amount of LDLR is of no importance.

Similarly, inclisiran, a small interfering RNA preventing the formation of the PCSK9 protein, will not improve the lipid profile in ARH patients. A similar effect of the above type of drugs was found in our patient. Due to its LDLR-independent action, lomitapide, an inhibitor of the microsomal triglyceride protein transfer, may be an alternative treatment for ARH [1]. Still, its cost was too high to introduce this drug. 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 [15]. Our patient did not consent to this type of therapy.

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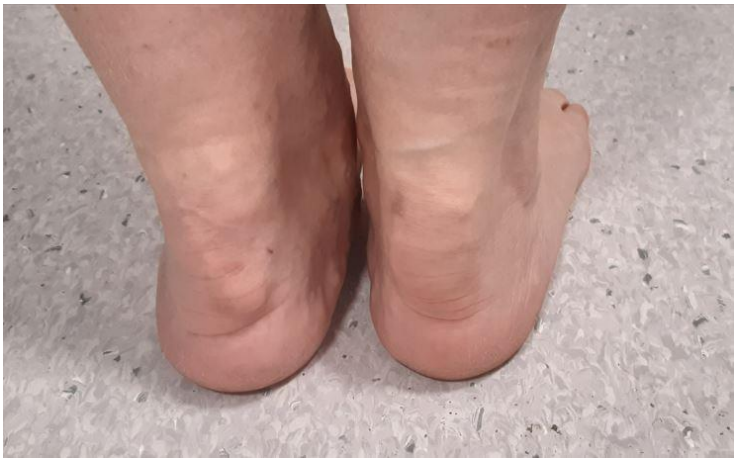

Figure S1. Xanthomas on the Achilles' tendons

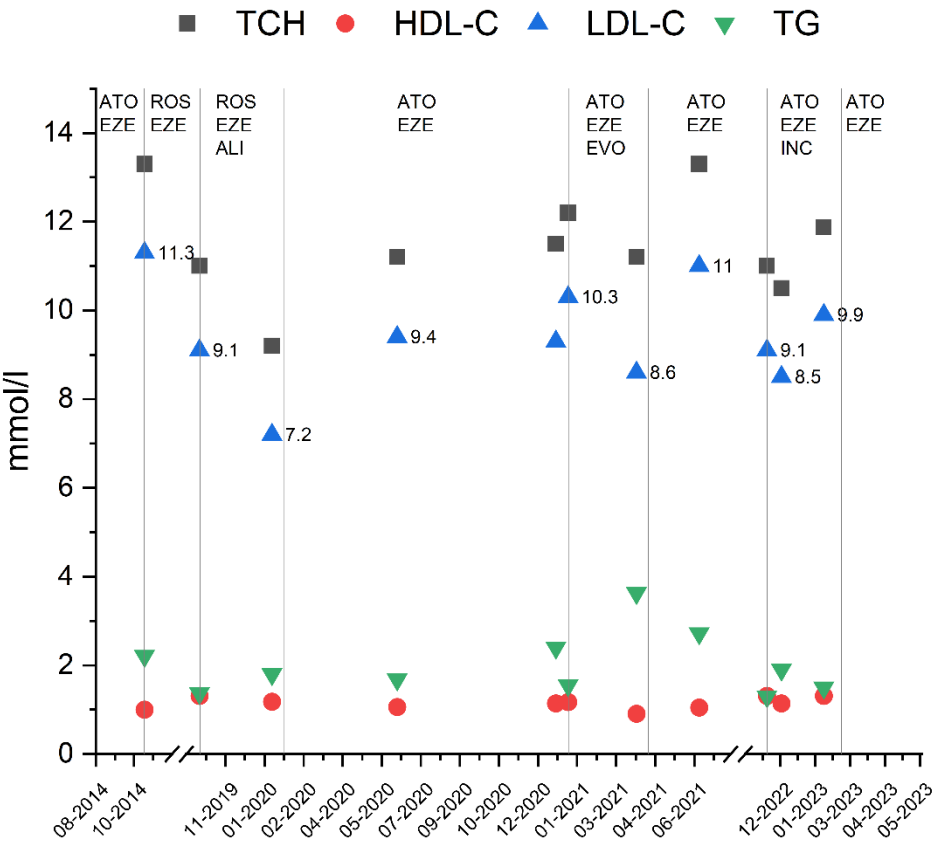

Figure S2. Cholesterol over time. ALI - alirocumab 150 mg; ATO - atorvastatin 80 mg; EVO - evolocumab 140 mg; EZE - ezetimibe 10 mg; HDL-C - high-density lipoprotein cholesterol; INC - inclisiran 284 mg; LDL-C - low-density lipoprotein cholesterol; ROS - rosuvastatin 40 mg; TCH - total cholesterol; TG - triglycerides

Table S1. The results of laboratory tests with concomitant treatment.

Date	TCH (mmol/l)	LDL-C (mmol/l)	HDL-C (mmol/l)	TG (mmol/l)	Creatine (umol/l); GFR (ml/min/1.73m2)	Glc (mmol/l)	ALT (U/l)	AST (U/l)	Creatine kinase (U/l)	TSH (uIU/ml)	Treatment
29.10.2014	13.3	11.3	1.0	2.21							ATO + EZE
18.10.2019	11	9.1	1.31	1.37	113; 45	5.23	13	19	146	0.777	ROS + EZE
15.01.2020	9.2	7.2	1.18	1.8	202; 23		11	22	145		ROS + EZE + ALI

17.06.2020	11.2	9.4	1.06	1.68	159; 31	5.3	15	24	125		ATO + EZE
29.12.2020	11.5	9.3	1.14	2.4	157; 31	5.85	12	22	111	2.88	
13.01.2021	12.2	10.3	1.17	1.55		5.15	13	22	132	1.73	
07.04.2021	11.2	8.6	0.91	3.63	168; 29		11	22	106		ATO + EZE + EVO
23.06.2021	13.3	11	1.05	2.72			13		124		ATO + EZE
21.11.2022	11	9.1	1.31	1.3	164; 29	5.48	15	24	161	2.0	
09.12.2022	10.5	8.5	1.14	1.9	118; 43		14	23	139		ATO + EZE + INC
30.01.2023	11.87	9.88	1.31	1.5	152; 31		17	24	106	1.44	

ALI - alirocumab 150 mg; ALT - Alanine aminotransferase; AST - aspartate aminotransferase; ATO - atorvastatin 80 mg; EVO - evolocumab 140 mg; EZE - ezetimibe 10 mg; GFR - glomerular filtration rate; Glc - glucose; HDL-C - high-density lipoprotein cholesterol; INC - inclisiran 284 mg; LDL-C - low-density lipoprotein cholesterol; ROS - rosuvastatin 40 mg; TCH - total cholesterol; TG - triglycerides; TSH - thyroid-stimulating hormone