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CASE REPORT

ADVANCED

CLINICAL CASE

A Novel Variant in *APOB* Gene Causes Extremely Low LDL-C Without Known Adverse Effects

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ABSTRACT

A novel frameshift variant was identified in *APOB* that segregates in a dominant manner with low levels of low-density lipoprotein cholesterol. Affected family members show no apparent clinical complications. There is no consensus regarding clinical management, and the long-term consequences of low levels of low-density lipoprotein cholesterol remain unknown. (**Level of Difficulty: Advanced.**) (J Am Coll Cardiol Case Rep 2020;2:775–9) © 2020 The Authors. Published by Elsevier on behalf of the American College of Cardiology Foundation. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

A 20-year-old Caucasian male patient presented to his primary care provider with complaints of palpitations. His 12-lead electrocardiogram was normal. A lipid profile revealed a low-density lipoprotein cholesterol (LDL-C) level of 8 mg/dl. The patient was referred to the Michigan Medicine Lipid Disorders Clinic for evaluation. His physical examination was notable for a weight 64 kg, height 180 cm, and body mass index 19.5 kg/m^2. The remainder of his physical examination was unremarkable, including no evidence of hepatomegaly, peripheral neuropathy, or retinopathy. A lipoprotein metabolism profile (Mayo Clinic, Rochester, Minnesota) revealed total cholesterol 77 mg/dl, LDL-C 15 mg/dl, high-density lipoprotein cholesterol 48 mg/dl, triglycerides 32 mg/dl, and apolipoprotein B (ApoB) 15 mg/dl. Thyroid-stimulating hormone level, comprehensive metabolic panel, and complete blood count were normal.

LEARNING OBJECTIVES

- To understand that genetic testing may be necessary for diagnostic accuracy when encountering patients with low levels of LDL-C.
- To understand the causes of low levels of LDL-C and how to manage patients with genetic conditions that cause low levels of LDL-C.

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The authors attest they are in compliance with human studies committees and animal welfare regulations of the authors' institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the *JACC: Case Reports* author instructions page.

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ABBREVIATIONS AND ACRONYMS

apoB = apolipoprotein B

FHBL = familial hypobetalipoproteinemia

LDL-C = low-density lipoprotein cholesterol

LoF = loss-of-function

PAST MEDICAL HISTORY

The patient's childhood development was normal, with no reported history of failure to thrive; no symptoms of malabsorption; and no neurological, hepatic, or visual abnormalities. There was no history of chronic or acute infections, recent hospitalizations, or inflammatory disease.

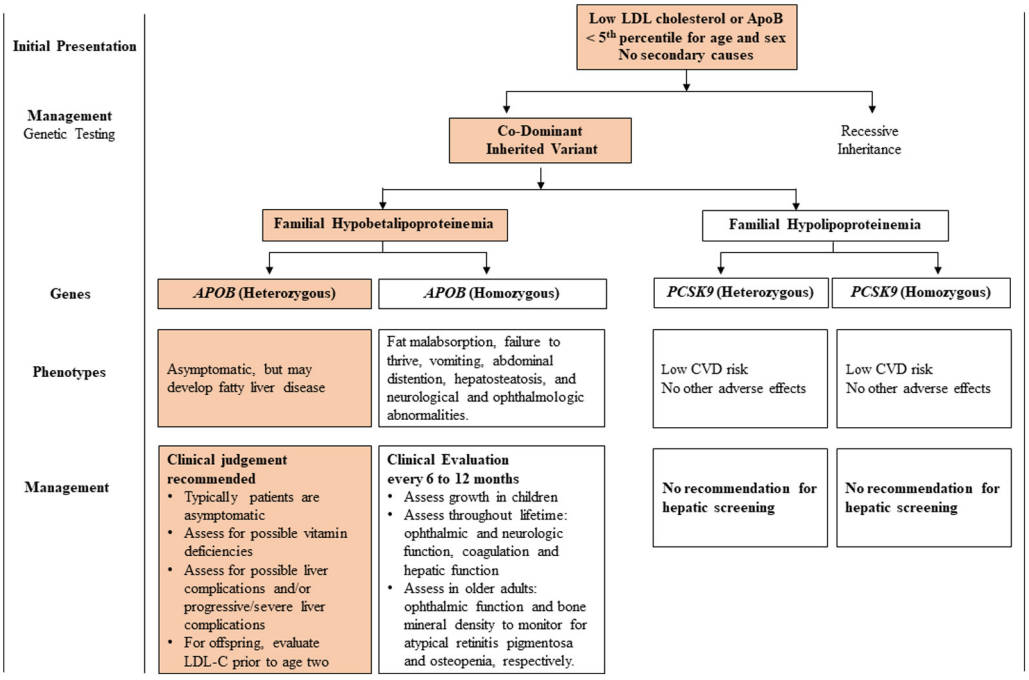
DIFFERENTIAL DIAGNOSIS

Causes of very low levels of LDL-C are listed in Table 1. Based on the history and available data, the etiology of this patient's very low LDL-C was hypothesized to be genetic (1,2). Genetic conditions causing very low LDL-C levels have varied etiologies and clinical presentations (3). They are characterized by plasma levels of total cholesterol, LDL-C, and apoB <5th percentile, with typical LDL-C values between 20 and 50 mg/dl (4). Homozygous or biallelic pathogenic mutations in genes encoding key proteins in lipoprotein metabolism and synthesis cause abetalipoproteinemia, homozygous familial hypobetalipoproteinemia (FHBL), and chylomicron retention disease. These diseases typically present with prominent multiorgan system phenotypes identified in infancy or youth, including failure to thrive, steatorrhea, and fat-soluble vitamin

TABLE 1 Differential Diagnosis of Low LDL-C

	Affected Gene	Mode of Inheritance
Primary causes		
Abetalipoproteinemia	*MTTP*	Recessive
Familial combined hypolipoproteinemia	*ANGPLT3*	Recessive
Chylomicron retention disease	*MTTP*	Recessive
Familial hypobetalipoproteinemia	*APOB*	Co-Dominant
Familial hypolipoproteinemia	*PCSK9*	Co-Dominant
Acquired/secondary causes		
Chronic parenchymal liver disease	–	–
Intestinal fat malabsorption syndromes	–	–
Exocrine pancreatic deficiency		
Cystic fibrosis		
Chronic pancreatitis		
Hyperthyroidism	–	–
Moderate to severe hypertriglyceridemia	–	–
Malignancy	–	–
Sepsis	–	–
Chronic infection	–	–
Medication effects	–	–

FIGURE 1 Low LDL-C and ApoB Less Than the 5th Percentile



This figure presents the stepwise process for clinical management for patients presenting with low levels of low-density lipoprotein cholesterol (LDL-C), low apolipoprotein B (apoB), and co-dominant inheritance. This figure was modified from Tarugi and Averna (2) and modified from Hartz et al. (1). CVD = cardiovascular disease.

deficiencies often with retinopathy, neuropathy, or coagulopathy (4).

This patient lacked any of the multiorgan system dysfunction of abetalipoproteinemia, homozygous FHBL, or chylomicron retention disease despite having an LDL-C <5th percentile. Thus, other genetic causes of low LDL-C, which typically have little or no clinical manifestations, were considered (**Figure 1**). These included heterozygous or homozygous familial hypolipoproteinemia due to mutations in *PCSK9* and heterozygous FHBL due to mutations in *APOB* (**Figure 1**) (2). Heterozygous FHBL is the most common cause of LDL-C <5th percentile, with an estimated prevalence of *APOB*-related FHBL of 1 in 1,000 to 3,000 (4,5). However, this patient presented with an LDL-C of 8 mg/dl, which is much lower than typical for heterozygous FHBL, thereby confounding the clinical picture. Contrarily, patients with homozygous or compound heterozygous FHBL have LDL-C levels and clinical conditions similar to the severe recessive disorders (i.e., abetalipoproteinemia).

INVESTIGATIONS

Cascade screening for low levels of LDL-C was performed for first- and second-degree relatives. Next, the index patient and 7 family members (**Table 2**, **Figure 2**) enrolled into the Cardiovascular Health Improvement Project, a longitudinal biorepository (HUM00052866). Saliva samples were collected, coded, and linked to electronic health records, and DNA was isolated. High-coverage whole genome sequencing (30×) was performed by Psomagen, Inc. (Rockville, Maryland). Supplemental Table 1 and the Supplemental Material provide a summary of genetic sequencing data and methods.

WHOLE GENOME SEQUENCING. Of the rare (not present in gnomAD [6] or 1000 Genomes European reference populations [7] or minor allele frequency <1%) high-impact polymorphisms (variant effect predictor annotations as transcript ablation, splice acceptor or donor, stop gained, frameshift, and stop or start lost) identified in the 8 samples, only 1 (*APOB* p.Val853fs) followed the dominant inheritance pattern with the low LDL-C status (**Table 3**, **Figure 3**). All 5 affected cases carried 1 copy of the rare mutation, and 3 unaffected control subjects carried none (LOD score = 1.51).

A similar analysis was performed for moderate-impact mutations (missense, inframe insertion and deletion, and protein altering). A total of 7 mutations were identified that showed a dominant mode of inheritance with low LDL-C. None of these 7 mutations fell into genes known to affect lipid metabolism (**Table 3**, **Figure 3**).

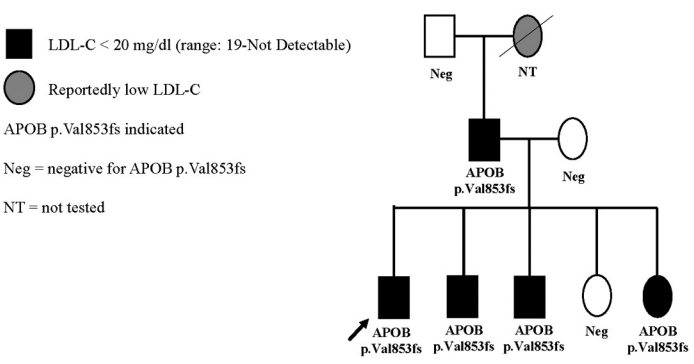
TABLE 2 Lipid Profiles for the 8 Samples

Family Member	Low-LDL Case	Total Cholesterol (mg/dl)	LDL-C (mg/dl)	Triglycerides (mg/dl)	HDL-C (mg/dl)	*APOB* p.Val853fs
Control	Not tested	–	–	–	–	Noncarrier
Case	Yes	84	19	32	59	LoF carrier
Control	No	199	102	132	71	Noncarrier
Index case	Yes	86	8	29	72	LoF carrier
Case	Yes	88	12	16	73	LoF carrier
Case	Yes	59	Undetectable	20	55	LoF carrier
Control*	No	Particle size	Particle size	Particle size	Particle size	Noncarrier
Case	Yes	73	13	25	55	LoF carrier

*Particle sizes were measured and interpreted as normal (R.B.).
HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; LoF = loss of function.

The identified *APOB* frameshift variant was not present in any of the public databases, and we were not able to find any publications or case reports in the published data. Thus, we believe that this particular variant is novel. There are multiple previously published mutations in *APOB* shown to cause low LDL-C levels, but also observed in conjunction with adverse liver effects including hepatic steatosis and hepatocellular carcinoma (1,4,8). The LDL-C levels seen for the affected cases (undetectable and between 8 and 19 mg/dl) are significantly lower (T-statistic for the difference in mean = 7.58, 2-tailed $p = 3.0 \times 10^{-8}$) than the average LDL-C level of heterozygous *APOB* loss-of-function (LoF) mutation carriers in the HUNT (9) biobank (mean LDL-C for *APOB* LoF carriers = 56 mg/dl)

FIGURE 2 Family Pedigree



This figure presents the family pedigree. Abbreviations as in **Figure 1**.

TABLE 3 Rare Mutations That Segregate With Disease and Are Predicted to Have an Impact on the Protein

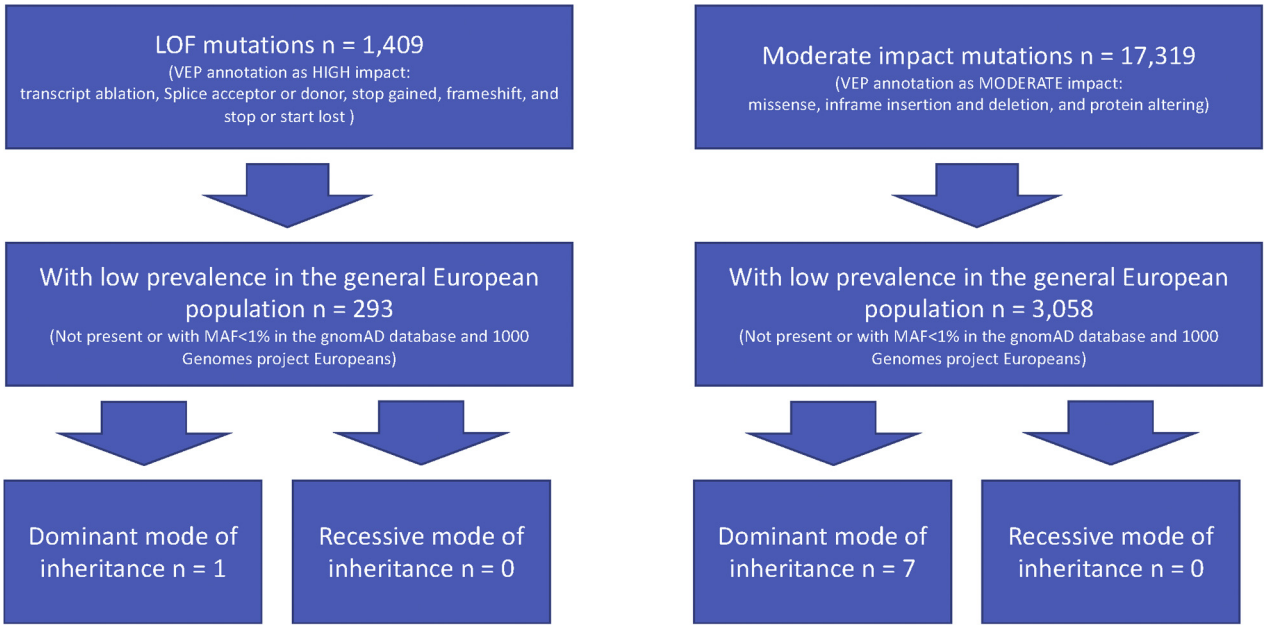
CHR	POS (hg38)	rsID	REF/ALT	Annotation (Most Severe)	Gene	Missense Prediction SIFT/PolyPhen or Consequence
1	233375929	rs35758282	A/C	Missense	*MAP3K21*	Deleterious/possibly damaging
1	240493250	rs142343894	G/C	Missense	*GREM2*	Tolerated/benign
1	248038821	rs138290082	C/T	Missense	*OR2L2*	Deleterious/probably damaging
2	21023572		CT/C	Frameshift	*APOB*	Loss-of-function
2	37222434	rs62001874	C/T	Missense	*CEBPZ*	Tolerated/benign
3	193363301	rs201387347	C/T	Missense	*ATP13A5*	Tolerated/benign
6	43339661	rs145629243	G/A	Missense	*ZNF318*	Deleterious/probably damaging
6	43450828	rs768837940	C/T	Missense	*DLK2*	Deleterious/benign

(Figure 4). The extreme LDL-C phenotype (LDL-C values <10 mg/dl) observed could be attributable to an uncharacterized property of this particular mutant ApoB protein or an unidentified genetic cause, including an unknown genetic variant or the possibility of a polygenic burden for low LDL-C levels.

MANAGEMENT

Patients with heterozygous *APOB*-related FHBL have been reported to have elevated levels of liver enzymes. Decreased secretion of mutated apoB from the liver results in reduced hepatic triglyceride export, which can lead to the development of hepatic steatosis, oral fat intolerance, and intestinal fat malabsorption (4,10). There have also been reports of patients with *APOB*-related FHBL developing cirrhosis and hepatocellular carcinoma (4,8,11). Thus, despite having normal liver function, it was recommended that the index patient and affected family members obtain liver ultrasounds and have liver enzymes measured as part of routine medical care. Additional testing including retinal examinations and serum fat-soluble vitamin levels (e.g., vitamin A) as well as formal testing for peripheral neuropathy was recommended. The patient was counseled that offspring should have lipid profiles by age 2 years, and should consult with a lipid specialist if LDL-C is <5th percentile (3).

FIGURE 3 Description of the Sequence Data Analysis



This figure presents a flow chart of the analyses identifying a loss of function (LoF) and moderate impact mutations. MAF = minor allele frequency; VEP = variant effect predictor.

DISCUSSION OF HETEROZYGOUS *APOB*-RELATED FHBL

The novel finding is that the identified *APOB* LoF variant carriers have significantly lower LDL-C levels compared with levels of the carriers from the HUNT study. Moreover, the index patient and affected family members were without known adverse clinical complications. Hepatic steatosis and elevation of liver enzymes are the main clinical manifestations (4); however, the current screening approaches, liver ultrasound and fibroscan, lack sensitivity, and subclinical disease can progress. Genetic testing is important for risk-stratifying patients into groups requiring long-term and routine follow-up, such as those with *APOB* LoF compared with *PCSK9* LoF variant carriers, the latter of which typically do not have any adverse clinical manifestations.

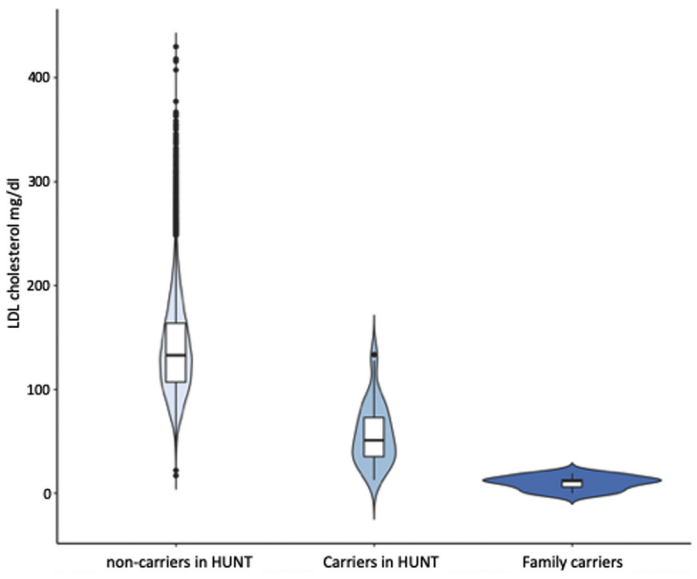
FOLLOW-UP

The index patient and affected cases were not clinically followed after the management plan was discussed due to the proximity of residence to clinic location.

CONCLUSIONS

This case report is reflective of a family with normal development without known adverse clinical complications despite very low levels of LDL-C. Given the finding of an *APOB* LoF as the cause for the low LDL-C, routine screening for liver disease and other associated compilations should be pursued long-term. Importantly, this patient and affected family members had normal development with very low LDL-C levels, and will likely gain protection against atherosclerotic cardiovascular disease.

FIGURE 4 LDL-C Distributions in *APOB* LoF Carriers and Noncarriers in HUNT Biobank Compared With the Family LoF Mutation Carriers



This figure presents the LDL-C distribution in HUNT Biobank for individuals with (n = 32) and without (n = 55,309) *APOB* LoF mutations compared with the low-LDL family members carrying the newly identified LoF mutation. Three *APOB* LoF mutations were identified in HUNT: rs745457003, chr2:21234303 T/A, and rs121918383; 12, 10, and 10 carriers respectively. Abbreviations as in Figure 1.

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KEY WORDS apolipoprotein B loss-of-function, hypobetalipoproteinemia, low-density lipoprotein cholesterol

APPENDIX For supplemental methods and a supplemental table, please see the online version of this paper.