Clínica e Investigación en Arteriosclerosis 35 (2023) 244–247

Sociedad Española de Arteriosclerosis

CLÍNICA E INVESTIGACIÓN EN ARTERIOSCLEROSIS

www.elsevier.es/arterio

BRIEF REPORT

A case of hypocholesterolemia under research

Ana Camacho[a], **María José Ariza**[b,*], **Nuria Amigó**[c,d], **Patricia Macías Guillén**[b], **Miguel Ángel Sánchez Chaparro**[b,e], **Pedro Valdivielso**[b,e]

[a] *Servicio de Medicina Interna, Hospital Infanta-Elena, Huelva, Spain*
[b] *Laboratorio de Lípidos y Arteriosclerosis, Departamento de Medicina y Dermatología, Centro de Investigaciones Médico Sanitarias (CIMES), Instituto de Investigación Biomédica de Málaga (IBIMA-Plataforma Bionand), Universidad de Málaga, Málaga, Spain*
[c] *Biosfer Teslab Metabolomics Interdisciplinary Laboratory, Instituto de Investigación Sanitaria Pere Virgili (IISPV), Reus, Tarragona, Spain*
[d] *Centro de Investigación Biomédica en Red de Diabetes y Enfermedades Metabólicas Asociadas (CIBERDEM), Madrid, Spain*
[e] *Servicio de Medicina Interna, Hospital Universitario Virgen de la Victoria, Málaga, Spain*

Received 10 May 2023; accepted 16 May 2023
Available online 19 September 2023

KEYWORDS
Primary hypocholesterolemia;
Asymptomatic hypobetalipoproteinemia;
Apolipoprotein B;
Diagnostic test;
Loss of function variant;
PCSK9

Abstract Primary hypocholesterolemia (or hypobetalipoproteinemia) is a rare disorder of lipoprotein metabolism that may be due to a polygenic predisposition or a monogenic disease. Among these, it is possible to differentiate between symptomatic and asymptomatic forms, in which, in the absence of secondary causes, the initial clinical suspicion is plasma ApoB levels below the 5th percentile of the distribution by age and sex. Here we describe the differential diagnosis of a case of asymptomatic hypocholesterolemia. We studied proband's clinical data, the lipid profile of the proband and her relatives and the clinical data of the family relevant to carry out the differential diagnosis. We performed a genetic study as the diagnostic test. The information obtained from the differential diagnosis suggested a heterozygous hypobetalipoproteinemia due to *PCSK9* loss-of-function variants. The diagnostic test revealed, in the proband, the presence of a heterozygous *PCSK9* frame-shift variant of a maternal origin. Plasma levels of LDL cholesterol and PCSK9 of the patient and her relatives were compatible with the segregation of the variant revealed. In conclusion, the diagnostic test performed confirmed the suspected diagnosis of the proband as asymptomatic familial hypobetalipoproteinemia due to a loss-of-function variant in the *PCSK9* gene.

DOI of original article: https://doi.org/10.1016/j.arteri.2023.05.003
* Corresponding author.
E-mail address: mariza@uma.es (M.J. Ariza).

PALABRAS CLAVE
Hipocolesterolemia primaria;
Hipobetalipoproteinemia asintomática;
Apolipoproteína B;
Prueba diagnóstica;
Variante de pérdida de función;
PCSK9

Un caso de hipocolesterolemia a estudio

Resumen Las hipocolesterolemias primarias (o hipobetalipoproteinemias) constituyen un trastorno infrecuente del metabolismo de las lipoproteínas que pueden obedecer a una predisposición poligénica o a una enfermedad monogénica. Entre estas, es posible diferenciar entre formas sintomáticas y asintomáticas, en las que, en ausencia de causas secundarias, la sospecha clínica inicial son concentraciones plasmáticas de ApoB por debajo del percentil 5 de la distribución por edad y sexo. E esta nota clínica describimos del diagnóstico diferencial de un caso de hipocolesterolemia asintomática. Estudiamos los datos clínicos de la paciente índice, así como su perfil lipídico y el de los familiares junto con los datos clínicos de estos que son relevantes para realizar el diagnóstico diferencial. Se realizó un estudio genético como prueba diagnóstica. El diagnóstico diferencial realizado sugirió una hipobetalipoproteinemia heterocigota por variantes de pérdida de función en *PCSK9*. La prueba diagnóstica puso de manifiesto, en la paciente índice, la presencia de una variante de cambio de pauta de lectura en *PCSK9,* en heterocigosis, de origen materno. Las concentraciones plasmáticas de colesterol de LDL y PCSK9 de la paciente y los familiares, fueron compatibles con la segregación de dicha variante. En conclusión, la prueba diagnóstica realizada permitió confirmar el diagnóstico de sospecha en el caso estudiado de hipobetalipoproteinemia familiar asintomática a causa de una variante de pérdida de función en el gen *PCSK9*.

Introduction

Primary hypocholesterolaemia is a rare disorder of lipoprotein metabolism that may be due to a polygenic predisposition or a monogenic disease.[1,2] We present a case of hypocholesterolaemia that was presented at the XVIII National Meeting on Hypertriglyceridaemia and VIII Meeting of the Lipid Units of the SEA (Toledo), in a closed case format.

Closed clinical case

A 33-year-old woman referred to the lipid unit for hypocholesterolaemia (LDL<50 mg/dl cholesterol). No personal history of allergies, toxic habits, or surgical interventions. Diagnosed with asthma on treatment with formoterol/budesonide on demand. Her family history included only her father with obesity, diabetes, hypertension, and primary polycythaemia, treated with hydroxyurea, atorvastatin 20 mg, metformin 850 mg/12 h, enalapril 20, bisoprolol 10, and trifusal 1 tablet/day. In the anamnesis, the patient was asymptomatic, without diarrhoea or other gastrointestinal symptoms. On examination, she weighed 58 kg, height 1.51 m, waist circumference 79 cm, blood pressure 130/75 mmHg, no xanthelasmas or xanthomas were found. Neck with good carotid pulses, no murmurs. Cardiac and pulmonary auscultation without abnormalities. Abdomen at the same level as the thorax, soft and depressible, no palpable megalias. Extremities with preserved pulses.

The lipid profile of the patient and her relatives is shown in Table 1; neither the index case nor her relatives showed abnormalities of the liver, thyroid, or renal profile.

An abdominal ultrasound was performed and reported as a normal sized liver with homogeneous echogenicity with no data indicative of hepatic steatosis. The rest of the examination showed no abnormalities.

A diagnostic test was performed.

Differential diagnosis

Based on the information available to us, this would appear to be a case of hypobetalipoproteinaemia, which is defined by the presence of plasma ApoB levels below the 5th percentile for age and sex.[3] These analytical disturbances may be secondary or primary. Secondary disturbances include very strict vegetarian diets, chronic alcoholism, or various diseases, such as intestinal malabsorption, liver disease, malnutrition, or hyperthyroidism, and can be secondary to hypolipidemic treatment.[4] None of these situations is present in our case, it seems reasonable, therefore, to rule them out.

Primary monogenic causes include abetalipoproteinaemia, familial hypobetalipoproteinaemia, chylomicron retention disease, familial combined hypolipidaemia, and hypobetalipoproteinaemia due to loss of PCSK9 function.[2] Among these, a distinction is made between symptomatic and asymptomatic primary hypobetalipoproteinaemia.

Abetalipoproteinaemia is among the symptomatic causes, an autosomal recessive hypolipidaemia (AR) caused by variants of the *MTTP* gene with very low or almost absent plasma lipid levels and deficiency of fat-soluble vitamins. It is characterised by the presence from the neonatal period of a fat malabsorption syndrome, with steatorrhoea, vomiting, growth retardation, as well as retinitis, severe ataxia, liver disease may develop and

Table 1 Fasting blood test of the index case and first-degree relatives.

Variable (mg/dl)	Index case	Father	Mother	Sister	Brother
TC	104	185	219	144	104
TG	53	363	63	49	56
HDL-C	43	28	82	58	52
Non-HDL cholesterol	61	157	137	86	52
LDL-C	50	ND	124	76	41
Apo B	55	ND	ND	ND	ND
Lp(a)	17	ND	ND	ND	ND

c-HDL, HDL cholesterol; LDL-C, LDL cholesterol; TC, total cholesterol; ND, not determined; TG, triglycerides.

acanthocytosis is characteristic. AR inherited chylomicron retention disease caused by mutations in the *SAR1B* gene and autosomal dominant (AD) homozygous or compound heterozygous familial hypobetalipoproteinaemia caused by mutation of the *APOB* gene are two diseases that are clinically and biochemically virtually indistinguishable from abetalipoproteinaemia - except for the normal triglyceride (TG) levels of chylomicron retention disease. Therefore, diagnosis of these hypobetalipoproteinaemias would require a genetic study.[1,5] In any case, these 3 diseases can reasonably be ruled out in the index case because the patient is asymptomatic.

The asymptomatic hypobetalipoproteinaemias include heterozygous familial hypobetaliproteinaemia,[4] inherited by AD due to pathogenic variants in the *APOB* gene that give rise to truncated proteins. Carrier patients are generally asymptomatic, but may develop liver disease of a steatotic nature with hypertransaminasaemia, low but not undetectable LDL-C, usually below 89 mg/dl, with normal TG concentrations. When the *APOB* variant is shorter than apoB48, fat absorption is compromised.[6] Familial combined hypolipidaemia[7] is also asymptomatic, with AD inheritance due to variants in the *ANGPTL3* gene. Homozygous cases have very low LDL-C, HDL-C, and TG levels, and heterozygotes have a reduction of approximately 50% in TC, LDL-C, and TG levels, with relatively normal HDL-C. Finally, asymptomatic forms also include familial hypobetaliproteinaemia caused by autosomal dominantly-inherited loss-of-function variants in *PCSK9*,[8] with low but detectable LDL-C levels (21%–40% lower than normal). These 3 asymptomatic hypolipidaemias appear to be associated with decreased cardiovascular risk.

Based on the above, in the case presented, we would rule out familial combined hypolipidaemia because she does not have very low TG concentrations. Furthermore, the homozygous form would have much lower concentrations of TC and LDL-C. Regarding heterozygous familial hypobetalipoproteinaemia, although patients are usually asymptomatic, they may also have liver disease, which seems to be ruled out in the patient and relatives. Therefore, our suspected diagnosis is heterozygous hypobetalipoproteinaemia due to loss-of-function variants in *PCSK9*.

Case resolution

The diagnostic test was massively parallel sequencing (Genológica, Málaga, Spain) which showed the presence of a reading frameshift variant in exon 9 of *PCSK9*: c.1378delG; p.Val460Tyrfs (rs749090549), not previously described in the literature and with an allelic frequency <.001. According to the criteria of the American College of Genetic and Genomic Medicine (ACMG), this variant is classified as a variant of uncertain significance as it has a single pathogenicity criterion, due to the reading frameshift produced, and has been found in healthy subjects (https://varsome.com/variant/hg38/rs749090549?annotation-mode=germline). To check whether this variant segregates in the family, samples were taken from the index case, the parents, and a sibling, and were analysed by the High-Resolution Melt (HMR) analysis technique,[9] at the Lipids and Arteriosclerosis Laboratory (CIMES, University of Malaga). This methodology made it possible to identify the presence of the variant in the mother and in the sibling analysed, and confirm it in the index case (Fig. 1). The concentration of circulating PCSK9 protein was measured by enzyme-linked immunosorbent assay (Human Protein Convertase 9/*PCSK9* Quantikine ELISA Kit [DPC900], R&D Systems®, Inc., Minneapolis, USA). Optical density was read at a wavelength of 450 nm using the SPECTROstar Nano microplate reader (BMG Labtech, gmbh, Offemburg, Germany). Sample concentrations were determined by interpolation from the standard curve generated with the standard samples supplied by the manufacturer with a 1:20 dilution of the serum samples (BiosferTeslab Laboratory, Reus, Tarragona, Spain). The results show that the carriers of the identified variant had lower concentrations than usual, given that, with the assay used, the normal values are 313 ± 71.5 ng/mL.

The following comments are pertinent to the present case. Familial hypobetalipoproteinaemia is rare but cannot be considered a rare disease. Recent data from the UK Biobank and the American NHBLI, on a total of 209,537 samples, indicate that 0.4% of these correspond to patients with variants that produce truncated proteins in apolipoprotein B or PCSK9, the latter being more frequent than the former.[10] Finally, in terms of vascular risk, the presence of familial hypobetalipoproteinaemia reduces the risk significantly, by 80% and 50%,[8,10] therefore prevention strategies should be maintained in these patients.

In summary, we present a case of asymptomatic familial hypobetalipoproteinaemia due to a loss-of-function variant in the *PCSK9* gene.

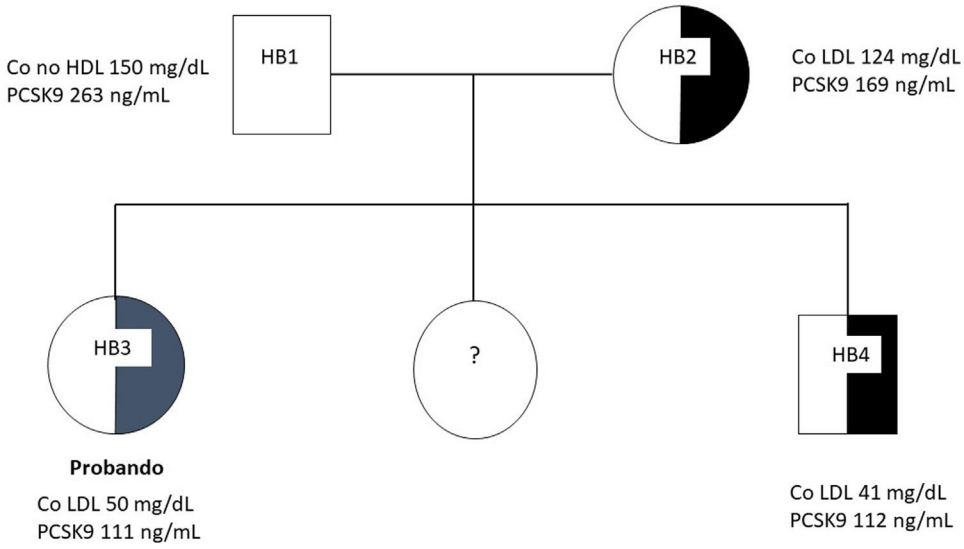

Figure 1 Genealogical tree of the family studied. Black shading indicates the presence of the PCSK9 loss-of-function variant in heterozygosis. Circulating levels of LDL cholesterol (Co) (mg/dl) and PCSK9 (ng/mL) are shown.

Funding

The group's own funds (CTS-159) from the Andalusian Plan for Research, Development and Innovation (PAIDI).

The University of Málaga/CBUA funded the open publication of this article.

Conflict of interests

The authors have no conflict of interests to declare.

Acknowledgments

We would like to thank the study index case and her family members, who consented to anonymous publication.

References

1. Bredefeld C, Hussain MM, Averna M, Black DD, Brin MF, Burnett JR, et al. Guidance for the diagnosis and treatment of hypolipidemia disorders. J Clin Lipidol. 2022:1–16, http://dx.doi.org/10.1016/j.jacl.2022.08.009.
2. Jakubowski B, Shao Y, McNeal C, Xing C, Ahmad Z. Monogenic and polygenic causes of low and extremely low LDL-C levels in patients referred to specialty lipid clinics: Genetics of low LDL-C. J Clin Lipidol. 2021;15(5):658–64, http://dx.doi.org/10.1016/j.jacl.2021.07.003.
3. Balder JW, de Vries JK, Nolte IM, Lansberg PJ, Kuivenhoven JA, Kamphuisen PW. Lipid and lipoprotein reference values from 133,450 Dutch Lifelines participants: Age- and gender-specific baseline lipid values and percentiles. J Clin Lipidol. 2017;11(4):1055–64, http://dx.doi.org/10.1016/j.jacl.2017.05.007, e6.
4. Martínez-hervás S, Real-collado JT. Hipotrigliceridemias/hipolipidemias. Clin Invest Arterioscler. 2021;33:63–8.
5. Hegele RA, Borén J, Ginsberg HN, Arca M, Averna M, Binder CJ, et al. Rare dyslipidaemias, from phenotype to genotype to management: a European Atherosclerosis Society task force consensus statement. Lancet Diabetes Endocrinol. 2020;8(1):50–67, http://dx.doi.org/10.1016/S2213-8587(19)30264-5.
6. Martín-Morales R, García-Díaz JD, Tarugi P, González-Santos P, Saavedra-Vallejo P, Magnolo L, et al. Familial hypobetalipoproteinemia: analysis of three Spanish cases with two new mutations in the APOB gene. Gene. 2013;531(1):92–6, http://dx.doi.org/10.1016/j.gene.2013.08.049.
7. Di Costanzo A, Di Leo E, Noto D, Cefalù AB, Minicocci I, Polito L, et al. Clinical and biochemical characteristics of individuals with low cholesterol syndromes: comparison between familial hypobetalipoproteinemia and familial combined hypolipidemia. J Clin Lipidol. 2018;11(5):1234–42, http://dx.doi.org/10.1016/j.jacl.2017.06.013.
8. Cohen JC, Boerwinkle E, Mosley TH Jr, Hobbs HH. Sequence variations in PCSK9, low LDL, and protection against coronary heart disease. N Engl J Med. 2006;354(12):1264–72, http://dx.doi.org/10.1056/NEJMoa054013.
9. Ariza MJ, Pérez-López C, Almagro F, Sánchez-Tévar AM, Muñiz-Grijalvo O, Álvarez-Sala Walter LA, et al. Genetic variants in the LPL and GPIHBP1 genes, in patients with severe hypertriglyceridaemia, detected with high resolution melting analysis. Clinica Chimica Acta. 2019;500:136–71, http://dx.doi.org/10.1016/j.cca.2019.10.011.
10. Dron JS, Patel AP, Zhang Y, Jurgens SJ, Maamari DJ, Wang M, et al. Association of rare protein-truncating DNA variants in APOB or PCSK9 with low-density lipoprotein cholesterol level and risk of coronary heart disease. JAMA Cardiol. 2023;8(3):258–67, http://dx.doi.org/10.1001/jamacardio.2022.5271.