



BRIEF REPORT

Familial ApoB-specific familial hypobetalipoproteinemia in a patient with non-classical congenital adrenal hyperplasia

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Available online 24 May 2024

KEYWORDS

Familial hypobetalipoproteinaemia;
Non-classical congenital adrenal hyperplasia;
APOB gene;
21-hydroxylase;
CYP21A2

Abstract Familial hypobetalipoproteinaemia is a disorder of lipid metabolism characterized by low levels of total cholesterol, low-density lipoprotein cholesterol and apolipoprotein B. ApoB-related familial hypolipoproteinemia is an autosomal condition with a codominance inheritance pattern. Non-classical congenital adrenal hyperplasia is an autosomal recessive disorder due to mutations in the *CYP21A2*, a gene encoding for the enzyme 21-hydroxylase, which results in an androgen excess production from adrenal source. We here present the case of a 25-year-old woman with NCAH showing decreased levels of total-cholesterol, low-density lipoprotein cholesterol and triglycerides. Her parent had digestive symptoms and severe hepatic steatosis with elevated liver enzymes, as well as decreased levels of total and low-density lipoprotein cholesterol. A genetic-molecular study of the proband identified a mutation in the *APOB* gene, which allowed a diagnosis of heterozygous ApoB-related hypolipoproteinaemia to be made.

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DOI of original article: <https://doi.org/10.1016/j.arteri.2023.12.002>

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PALABRAS CLAVE

Hipobetalipoproteinemia familiar;
Hiperplasia suprarrenal congénita no clásica;
Gen *APOB*;
21-hidroxilasa;
CYP21A2

Hipobetalipoproteinemia familiar ApoB específica en una paciente con hiperplasia suprarrenal congénita no clásica

Resumen La hipobetalipoproteinemia familiar es un trastorno del metabolismo lipídico caracterizado por niveles bajos de colesterol total, colesterol de lipoproteínas de baja densidad y apolipoproteína B. La hipobetalipoproteinemia familiar relacionada con la apolipoproteína B es una enfermedad autosómica con un patrón de herencia de codominancia. La hiperplasia suprarrenal congénita no clásica es un trastorno autosómico recesivo prevalente por mutación del gen *CYP21A2* que codifica para la enzima 21-hidroxilasa, lo que condiciona un exceso de producción de andrógenos a nivel suprarrenal. Se presenta el caso una mujer de 25 años con hiperplasia suprarrenal congénita no clásica que presenta concentraciones circulantes bajas de colesterol total, LDL-colesterol y triglicéridos. Progenitor con clínica digestiva y esteatosis hepática grave con elevación de enzimas hepáticas y concentraciones bajas de colesterol total y LDL-colesterol. Se realiza estudio genético del caso identificado una mutación en el gen *APOB* lo que permitió realizar el diagnóstico de hipobetalipoproteinemia familiar heterocigota.

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Introduction

Familial hypobetalipoproteinemia (HBL) is a genetic disorder characterised by decreased plasma concentrations of total cholesterol, low-density lipoprotein (LDL) cholesterol, and apolipoprotein B (ApoB) below the 5th percentile of the reference population.¹ Although in some cases the mutation is not identified, the best characterised variants are located in the *APOB* gene; these are nonsense and frameshift mutations that result in truncated proteins.² This specific HBL ApoB has an autosomal co-dominant inheritance and its prevalence is estimated at one in 1000–3000 individuals.³ The clinical presentation depends on the number of alleles affected. Heterozygous individuals are usually asymptomatic and are identified by lipid profile. They may occasionally develop hepatic steatosis or steatorrhea. In contrast, when the mutation is biallelic, the clinical manifestations are more florid with gastrointestinal, neurological, ophthalmological, and fat-soluble vitamin deficiencies. The definitive diagnosis is made with the analysis of mutations in the *APOB* gene located on chromosome 2 after demonstrating a family history, and ruling out other causes of abetalipoproteinemia such as mutations in *PCSK9* and *MTTP*. Dietary restriction of long-chain triglycerides improves gastrointestinal symptoms. Essential fatty acid and fat-soluble vitamin supplementation is recommended if deficiency is detected.

However, non-classical congenital adrenal hyperplasia (NCAH) is an autosomal recessive disorder that, in its most frequent form, affects the *CYP21A2* gene that codes for the enzyme 21-hydroxylase, involved in the synthesis of adrenal steroids. In contrast to the classical form, in NCAH the enzyme maintains a residual activity of 30%–50%,⁴ avoiding clinical cortisol and aldosterone deficiency, but presents with hyperandrogenism. Although some patients debut with precocious puberty, the clinical presentation in most cases is clinically indistinguishable from polycystic

ovary syndrome, with dermo-cosmetic involvement mainly in the form of hirsutism and/or acne, ovulatory dysfunction, and in a significant percentage of patients, multifollicular ovarian morphology on ultrasound. Diagnosis includes the demonstration of baseline 17-hydroxyprogesterone (17-OHP) concentrations above 10 ng/mL in the follicular phase of the menstrual cycle or after stimulation with 1–24 ACTH. In most NCAH subjects ACTH secretion is normal.⁴ Adrenal hyperandrogenism is postulated to be due to an enzymatic defect with increased steroidogenic precursors and peripheral androgen conversion, also accompanied by hypersecretion of ovarian origin. Genetic diagnosis includes analysis of the *CYP21A2* locus. Deletions, conversions, and point mutations comprise 95% of the gene variants associated with CAH.⁵ The presence of a mild mutation in at least one of both mutated alleles is associated with sufficient enzymatic capacity to prevent cortisol and aldosterone synthesis deficiency, whereas severe bi-allelic mutations lead to gluco- and mineralocorticoid deficiency, although the genotype-phenotype correlation is not absolute.

The exceptional concurrence of both entities in the same patient – according to the known prevalence of both conditions, 1 case per $1–3 \times 10^6$ individuals in the general population – and the diagnostic-therapeutic implications of this have not been reported.

Clinical case

A 25-year-old woman with no known drug allergies, smoker of 7–8 cigarettes/day, and a history of a 6 mm thyroid cyst in the left thyroid lobe. Her family history included a diagnosis of NCAH in her sister (*Ile172Asn/Val281Leu* genotype). The mother and father were heterozygous carriers of the mutated *Val281Leu* and *Ile172Asn* alleles, respectively. The patient was under follow-up in the reproductive endocrinology clinic at our centre for NCAH - with the same

Table 1 Analytical evolution.

Date (month/year)	09/2019	09/2020	09/2021	11/2021	06/2022	10/2022
Total cholesterol, mg/dL	139	132	114	123	149	134
HDL-cholesterol, mg/dL	113	112	89	101	118	102
LDL-cholesterol, mg/dL	20	14	15	19	24	25
Triglycerides, mg/dL	28	26	50	15	32	31
Aspartate aminotransferase, U/L (NV 4–50 U/L)	18	18	16	17	17	18
Alanine aminotransferase, U/L (NV 5–40 U/L)	12	12	12	12	10	12
γ -glutamyl transferase, U/L (NV 7–30 U/L)	13	11	10	9	12	13
Apolipoprotein B, mg/dL (NV 53–182 mg/dL)	–	–	–	<11	–	–
Vitamin A, μ g/dL (NV 30–60 μ g/dL)	–	–	50.1	–	75	77.9
Vitamin E, μ g/dL (NV 500–1800 μ g/dL)	–	–	1083	–	961	1179

NV: normal values.

genotype as her sister - diagnosed at another centre at 7 years and 10 months of age after presenting with premature pubarche with advanced bone age and acne. Hyperandrogenaemia was found, biochemically confirming the diagnosis of NCAH and subsequently the referred genotype. Treatment with hydrocortisone was started initially, with spontaneous menarche at 13 years of age. Given the lack of hyperandrogenaemia control, hydrocortisone was replaced by evening dexamethasone at 17 years of age. At the time she was transferred to our clinic aged 18, the patient was not overweight or obese, had reached her target height, reported regular menstruation, and had no dermo-cutaneous signs of hyperandrogenism. Mild suppression of the corticotropic axis was observed (serum cortisol after conventional stimulation with 1–24 ACTH of 12 μ g/dL), and therefore the glucocorticoid dose was progressively reduced until a sufficient functional reserve was observed for it to be discontinued, and she remained clinically asymptomatic. During her disease progression, at the age of 22, treatment was started with a combined oral contraceptive composed of ethinylestradiol (30 μ g) and dienogest (2 mg) for dysmenorrhoea. In various analyses performed during the 5 years of follow-up in our adult clinic, total circulating cholesterol <150–130 mg/dL was observed (this finding was already present in analyses performed at 16 years of age); LDL-cholesterol concentrations repeatedly <30 mg/dL, HDL-cholesterol between 80 and 110 mg/dL, and circulating triglycerides <40 mg/dL, for which reason referral was decided to a specialist clinic for familial dyslipidaemia.

Targeted history taking showed that her 66-year-old father, with a history of hypertension and resolved HBV, was being followed up by the gastroenterology department for chronic diarrhoea with 5–6 watery stools per day, without pathological products with accompanying urgency, with histological data of lymphocytic colitis, as well as severe non-alcoholic hepatic steatosis, and under treatment with oral budesonide, resin-cholestyramine, loperamide on demand, and calcifediol due to vitamin D deficiency. Last lipid profile showed fasting circulating concentrations of total cholesterol 135 mg/dL, HDL 54 mg/dL, LDL 45 mg/dL, and triglycerides 177 mg/dL. No known history of cardiovascular disease in the family. On re-anamnesis, the patient presented with non-specific gastrointestinal alterations with increased gastric motility as the only noteworthy finding. The analytical parameters of liver function and fat-soluble

vitamin concentrations were within the normal range. However, given the abnormalities in the lipid profile (Table 1) and the family history, familial HBL was suspected, and a DNA study was performed on the patient, focusing on the coding region and adjacent intronic regions of the *APOB*, *ANGPTL3*, *PCSK9*, and *MTTP* genes by NGS using the Roche KAPA HyperChoice technology panel developed by the Institute of Medical and Molecular Genetics (INGEMM). MLPA was also performed with the P062-D2 kit (MRC-Holland) and subsequent analysis with Coffalyser.NET software. An interruption was found in the reading frame by premature stop codon due to a nonsense substitution at cDNA level NM.000384.2:c.7600C>T; amino acid change p.(Arg2534*) in exon 26 of the *APOB* gene, in heterozygosis; considered a pathogenic variant. Given this result, it was decided to extend the genetic study to her parent, pending at the time of this publication.

The patient is currently asymptomatic and does not require treatment. The analytical values are shown in Table 1.

Discussion

We present the case of a patient with two rare genetic disorders. According to ICD 11, familial HBL (5C81.1) is considered a rare disease, while NCAH has an overall prevalence in our geographical area of around .1% of the general population, although it accounts for approximately 4% of the causes of hyperandrogenism in specialist clinics.⁶ To our knowledge, this is the first documented case of a combination of both diseases.

Several studies have investigated cardiovascular risk factors in patients with NCAH and classical CAH,⁴ although the results are highly controversial,^{7,8} and the exogenous administration of mineralo- and glucocorticoids, the dose and duration of treatment could be at the basis of some metabolic abnormalities described in adults with CAH compared to the population without functional hyperandrogenism.⁹ With regard to lipid profile, in most of the reported studies, patients with NCAH do not show notable differences with respect to controls without functional hyperandrogenism. In obese patients, an increase in triglycerides and lower HDL levels have been reported, probably related to excess weight and hyperandrogenism.^{5,6} In

any case, the patient presented a lipid pattern with excessively low LDL and triglyceride levels, which is not a classic presentation NCAH, in addition to fulfilling the criteria of Fredrikson et al.¹⁰ for the diagnosis of HBL, that is, low plasma concentrations, absence of secondary causes explain this abnormality (malabsorption, malnutrition, severe infections, severe liver disease), and a potentially affected first-degree relative.

In familial HBL, circulating concentrations of total cholesterol, LDL, ApoB, and sometimes triglycerides, are decreased below the fifth percentile of the reference population.¹ In plasma ApoB is expressed in two forms: ApoB-100 and ApoB-48; both encoded by the APOB gene located on chromosome 2. ApoB-100 is expressed in the liver and is a component of VLDL, IDL and LDL; it also serves as a ligand for the LDL receptor. ApoB-48 is expressed in the intestine as part of chylomicrons.¹¹ ApoB-specific familial HBL the mutation is located in the APOB gene; production of premature stop codons in the mRNA is the most frequent mutation.² Translation of these mRNAs leads to the formation of truncated ApoB that loses the ability to form plasma lipoproteins and, consequently, to export lipids from the liver and intestine to other organs. The detection of truncated ApoB in plasma suggests that the mutation is located from exon 26 to exon 29 of the APOB gene. Truncated ApoBs shorter than ApoB29-30, due to mutations located in the first 25 exons of the gene, are not secreted and therefore cannot be detected in plasma.¹¹ In the patient, a mutation was detected in exon 26 due to an amino acid change causing a premature codon stop and the generation of abnormal ApoB. The case's parent had severe fatty liver disease, which is a frequent clinical manifestation in heterozygous patients. Due to the low production rate of unmutated ApoB-100 and impaired triglyceride transport, the VLDL export system for lipids is impaired,² causing the accumulation of lipids in the liver. In fact, recent evidence links familial HBL to the development of cirrhosis and hepatocellular carcinoma,¹ and it is advisable to monitor liver enzymes and perform imaging tests if these are elevated. Monitoring and follow-up are important, and the prognosis has been reported to be severe when the disease appears in early childhood and excellent for the moderate form without cytolysis or steatosis. However, in general, heterozygous subjects do not require any specific treatment, except for fat-soluble vitamin supplementation, if deficiency is detected, to avoid neurological complications. Stressing the fact that this condition is benign in most cases, this heterozygosity has been related to a prolonged life expectancy in probable relation to a lower risk of cardiovascular events.

Our case is the first documented case of a combination of both genetic disorders. The remoteness of the responsible genes located on 2 different chromosomes rules out a joint inheritance pattern due to linkage disequilibrium. However, hypothesising any evolutionary advantage of such a combination may be controversial, beyond the longevity observed in some heterozygous subjects with ApoB-related familial HBL, given that there is no evidence that non-classical CAH impairs the survival of subjects with it. A potential decrease in circulating androgen and progestogen concentrations secondary to decreased cholesterol concentrations, such as that observed in the first case after statin administration in functional hyperandrogenism,¹² could be accompanied by

an increase in fertility in these women. However, this assertion is purely speculative, especially since most women with NCAH conceive spontaneously, and functional hyperandrogenism could even be an evolutionary advantage per se.¹³ However, studies that are not available in successive generations of this family may shed some light on the possible intra-familial association of these mutations, as has been observed in other diseases.¹⁰

Conclusions

We present the case of a patient with 2 genetic disorders inherited from her parents with an exceptional association. If discordant clinical signs or symptoms are detected in the first known disease, other concomitant genetic disorders should be considered. Parental genetic study and family genetic follow-up in offspring is essential to assess this association.

Funding

This research has not received specific support from public sector agencies, commercial sector, or non-profit organisations.

Conflict of interests

The authors have no conflict of interests to declare.

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