

Non-alcoholic fatty liver disease associated with hypobetalipoproteinemia: report of three cases and a novel mutation in APOB gene

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ABSTRACT

Background: Non-alcoholic fatty liver disease, the leading cause of chronic liver disease in children, is defined by hepatic fat infiltration >5% of hepatocytes, in the absence of excessive alcohol intake, evidence of viral, autoimmune or drug-induced liver disease. Conditions like rare genetic disorders must be considered in the differential diagnosis.

Case Report: Two male brothers, and a non-related girl, all overweight, had liver steatosis. One of the brothers and the girl had elevated transaminases; all three presented with low total cholesterol, low density lipoproteins and very low density lipoproteins cholesterol levels, hypotriglyceridemia and low apolipoprotein B. A liver biopsy performed in the brother with cytotoxicity confirmed steatohepatitis and the molecular study of apolipoprotein B gene showed a novel homozygous mutation (c.9353dup p.Asn3118Lysfs17). Patients with cytotoxicity lost weight, however liver steatosis persists.

Conclusion: Fatty liver disease might be a consequence of hypobetalipoproteinemia. Evidence is scarce due to low number of reported cases.

Key-words: APOB gene, Children, Familial hypobetalipoproteinemia, Non-alcoholic fatty liver disease, Non-alcoholic steatohepatitis.

FÍGADO GORDO NÃO-ALCOÓLICO ASSOCIADO A HIPOBETALIPOPROTEINEMIA: APRESENTAÇÃO DE TRÊS CASOS CLÍNICOS E DE UMA NOVA MUTAÇÃO NO GENE APOB

RESUMO

Introdução: O fígado gordo não alcoólico, a principal causa de doença hepática crónica na criança, é definida por infiltração de gordura hepática em >5% dos hepatócitos, na ausência de ingestão alcoólica excessiva, evidência de doença vírica, autoimune ou induzida por drogas. Doenças genéticas raras são condições a considerar no seu diagnóstico diferencial.

Caso Clínico: Apresentam-se os casos de dois irmãos do sexo masculino, e de uma criança do sexo feminino, todos com excesso de peso e esteatose hepática. Um dos irmãos e a criança do sexo feminino apresentavam também elevação das transaminases; todos os casos associados a baixos níveis de colesterol total, lipoproteínas de baixa densidade e lipoproteínas de muito baixa densidade, hipotrigliceridemia e baixos níveis de apolipoproteína B. A biópsia hepática realizada num dos irmãos com citólise confirmou a presença de esteatohepatite e o estudo molecular do gene da apolipoproteína B mostrou a presença de uma nova mutação em homozigotia (c.9353dup p.Asn3118Lysfs17). Os doentes com citólise perderam peso, no entanto, a esteatose hepática manteve-se.

Conclusão: A doença hepática não-alcoólica poderá ser uma consequência da hipobetalipoproteinemia. A evidência científica é escassa, dado o baixo número de casos descritos.

Palavras-chave: Gene APOB, Criança, Hipobetalipoproteinemia familiar, Fígado gordo não alcoólico, Esteatohepatite não alcoólica.

Nascer e Crescer 2016; 25(2): 104-7

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Abbreviations:

ABL - Abetalipoproteinemia	GGT - Gamma-glutamyl transferase
ALT - Alanine aminotransferase	HBL - Hypobetalipoproteinemia
ApoB - Apolipoprotein B	HDL - High density lipoproteins
ANGPTL3 - Angiopoietin-Like 3	HLA B51 - Human leukocyte antigen serotype B51
ASCAs - Anti-saccharomyces cerevisiae antibodies	LDL - Low density lipoproteins
AST - Aspartate aminotransferase	MTP - Microsomal triglyceride transfer protein
BMI p - Percentile of body mass index	NAFLD - Non-alcoholic fatty liver disease
CMRD - Chylomicron retention disease	NASH - Non-alcoholic steatohepatitis
FHBL - Familial hypobetalipoproteinemia	SAR1B - Secretion Associated, Ras Related GTPase
	VLDL - Very low density lipoproteins

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is defined by hepatic fat infiltration >5% of hepatocytes in the absence of excessive alcohol intake, evidence of viral, autoimmune or drug-induced liver disease.¹⁻³ A subgroup of patients can also have liver cell injury, varying degrees of inflammation (steatohepatitis), fibrosis, and cirrhosis.⁴

The major risk factors for NAFLD are obesity, type 2 diabetes mellitus and metabolic syndrome². However, other diseases should be considered in the differential diagnosis such as hypobetalipoproteinemia, a rare genetic disorder of lipid metabolism, in which liver steatosis and increased liver transaminases may be associated.^{5,6}

Hypobetalipoproteinemia (HBL) is characterized by very low levels (<5th percentile) of total cholesterol, LDL cholesterol or apolipoproteina B (ApoB) in plasma.⁷ This condition can be secondary to vegetarian diets, intestinal fat malabsorption, severe liver disease, malnutrition and hyperthyroidism.⁷ It can also be associated with inherited disorders such as abetalipoproteinemia (ABL; OMIM 200100) caused by a variety defects in Microsomal Triglyceride Transfer Protein (MTP) gene, chylomicron retention disease (CMRD; OMIM 246700), caused by Secretion Associated Ras Related GTPase 1B (SAR1B) gene mutations, and Familial Hypobetalipoproteinemia (FHBL).⁸⁻¹⁰

FHBL, the most common form of HBL is a genetically heterogeneous disorder. Familial hypobetalipoproteinemia 1 (FHBL1; OMIM 615558) is caused by mutations in APOB gene, and familial hypobetalipoproteinemia 2 (FHBL2; OMIM 605019) is caused by mutations in Angiopoietin- Like 3 (ANGPTL3) gene.^{8,9} The best characterized cases are due to nonsense mutations of the APOB gene leading to production of truncated ApoB forms, associated with reduced concentrations. There are two forms of ApoB, namely ApoB-48, a component of chylomicrons synthesized by enterocytes and ApoB-100 a component of VLDL and LDL particles synthesized by the liver; these are two isoforms derived from differential splicing of RNA from a single APOB gene.^{6,10} APOB gene is located on the short arm of human chromosome 2, and it consists of 29 exons, of which exon 26, is the largest and encodes more than one half of the full-length protein.⁹

FHBL homozygotes or compound heterozygotes clinical features include severe fat malabsorption, neurologic symptoms, acanthocytosis, retinitis pigmentosa and/or NAFLD. FHBL heterozygotes often have no clinical expression or have fatty liver disease.⁶

Although there seems to be cardiovascular protection in these patients, the long-term consequences of fatty liver disease are not yet known. We report three cases of NAFLD related to FHBL and a novel mutation in the APOB gene.

CASE REPORT

Case 1

A caucasian 12-year-old male had recurrent oral aphthosis with a few years of evolution with recent elevated transaminases and steatosis. His parents were nonconsanguineous and his 8-year-old brother was healthy.

There was no history of alcohol consumption or drug use. No other symptoms were reported such as genital aphthosis, uveitis or joint pain. On physical examination he had oral aphthosis, mild hepatomegaly, and no other signs of liver disease, nor peripheral stigmata of lipidic or endocrine disorders. Neurologic and ophthalmologic examinations were normal. Body mass index was in percentile 95 (BMI p95).

Complete blood count, blood smear, erythrocyte sedimentation rate, electrolytes, creatinine and urea were normal. However, he had persistent elevation of transaminases and his lipid profile revealed low total cholesterol, LDL-cholesterol and VLDL-cholesterol levels, hypotriglyceridemia with low apolipoprotein (Apo) B levels (Table 1). Abdominal ultrasonography confirmed hepatomegaly and moderated steatosis.

Table 1 – Baseline clinical and analytical characteristics of FHBL patients

Characteristic	Case 1	Case 2	Case 3	Reference Interval
Sex	Male	Male	Female	
Age (years)	12	8	9	
Body weight (Kg)	60	40	40	
BMI percentile (Kg/m ²)	95	>95	95	
AST (U/l)	61	32	51	10-34
ALT (U/l)	110	29	56	10-44
GGT (U/l)	21	16	26	10-66
Total cholesterol (mg/dl)	58	67	81	
LDL-cholesterol (mg/dl)	<1	<1	25	
HDL-cholesterol (mg/dl)	52	54	47	
VLDL-cholesterol (mg/dl)	2,2	ND	9	
Triglycerides (mg/dl)	10	<7	44	
ApoA (mg/dl)	157	127	125	90-170
ApoB (mg/dl)	35	2	29	56-162
Vitamin A (umol/L)	1,10	N	0,80	1,05-2,79
25-OH-Vitamin D (nmol/L)	120,2	N	59	75-500
Vitamin E (umol/L)	12	Low	10,2	14-23
APOB gene	c.9353dup (p.Asn3118-Lysfs17)	ND	No abnormalities	

BMI percentile, percentile of body mass index; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glutamyl transferase; LDL, low density lipoproteins; HDL, high density lipoproteins; N, normal, ND, not done, VLDL, very low density lipoproteins; Apo, apolipoprotein

Infectious, autoimmune and metabolic causes of liver disease were excluded: hepatitis A, B and C virus were negative; tests for anti-nuclear, anti-smooth muscle cell, anti-liver-kidney microsomal type 1 antibodies, anti-liver cytosol 1 and anti-soluble liver antigen were negative, serum alpha 1-antitrypsin, serum ferritin, serum ceruloplasmin and 24-hour urinary copper were normal. Autoimmune thyroiditis with normal function and morphology was diagnosed. Celiac disease was excluded. He had slightly positive anti-Saccharomyces cerevisiae antibodies (ASCAs) and HLA B51.

The hypothesis of hypobetalipoproteinemia was evocated and a lipid profile in both parents and brother was performed. Low total cholesterol levels were found in brother and borderline values in the father.

A liver biopsy was performed showing micro and macrovesicular steatosis and no features suggesting autoimmune disease and liver copper was normal.

The molecular study of APOB gene revealed the novel mutation in homozygosity, c.9353dup (p.Asn3118Lysfs17), confirming the diagnosis of familial hypobetalipoproteinemia. This nonsense mutation has not been reported previously and consists of one base pair duplication in c.9353 position. In addition it was also detected a homozygous variant c.8851G>A (p.Glu2951Lys) that has also never been described before, and so far is expected to be benign by its analysis.

Fat-soluble vitamins were determined (Table 1) and he started vitamin supplementation.

Presently he is asymptomatic, and lost weight (BMI p85), however the transaminases remain high and steatohepatitis persists on ultrasound. Neurologic and ophthalmologic evaluations are normal, without evidence of retinitis pigmentosa.

Case 2

This patient is the 8-year-old male brother of case 1.

He was obese (BMI p>95) with no other significant past medical history. He was asymptomatic and his physical examination was normal. No hepatomegaly was detected on the examination and no signs of liver disease or extrahepatic disease were found. Neurologic and ophthalmic examinations had no abnormalities. His laboratory tests showed normal hemoglobin levels, as well as liver transaminases, electrolytes, creatinine, urea and thyroid function. His lipidogram revealed low total cholesterol and LDL-cholesterol levels, hypotriglyceridemia with low apolipoprotein (Apo) B (Table 1). Hepatomegaly and high degree steatosis were demonstrated by abdominal ultrasonography. Fat-soluble vitamins were determined (Table 1) and he started vitamin E supplementation.

He remains asymptomatic, obese (BMI p>95), with normal values of transaminases; hepatomegaly and steatosis persists on ultrasound.

The parents of these two patients (case 1 and case 2) have normal transaminases; liver ultrasound was not performed. Although not fulfilling diagnostic criteria for hypobetalipoproteinemia, the father's lipid profile shows borderline values of total cholesterol (120 mg/dl), LDL-cholesterol (59 mg/dl) and triglycerides (35 mg/dl). The mother has a normal lipid profile. Until now and due to financial constraints, neither case 2 nor both parents performed molecular study.

Case 3

A caucasian 9-year-old female had elevated liver transaminases. She was overweight (BMI p95), and had no history of alcohol consumption or drug use. Her parents were nonconsanguineous and healthy, as well as her 16-year-old sister. One cousin died at 4 years old with an unknown liver disease.

She was asymptomatic with a normal physical examination. No hepatomegaly or splenomegaly were detected, and no other clinical signs of liver disease. Neurologic and ophthalmologic examination were normal.

Her laboratory tests including complete blood count, blood smear, erythrocyte sedimentation rate, electrolytes, creatinine, urea and thyroid function tests were normal. Transaminases were elevated and her lipid profile showed low total cholesterol, LDL-cholesterol and VLDL-cholesterol levels, hypotriglyceridemia and low apolipoprotein (Apo) B levels (Table 1). Abdominal ultrasonography revealed diffuse steatosis.

Hepatitis A, B and C virus were negative; tests for anti-nuclear, anti-smooth muscle cell, anti-liver-kidney microsomal type 1 antibodies, anti-liver cytosol 1 and anti-soluble liver antigen were negative; serum alpha 1-antitrypsin, serum ferritin, serum ceruloplasmin, 24-hour urinary copper and liver copper were normal. Liver biopsy revealed periportal macrovesicular steatosis in 30% of hepatocytes.

The diagnosis of hypobetalipoproteinemia was suggested and a molecular study of APOB gene showed no mutations in the analyzed regions. Her mother and sister's cholesterol values were normal.

Fat-soluble vitamins were determined (Table 1) and she started vitamin supplementation.

Currently she remains asymptomatic, with a BMI p75, transaminases values are normal, but steatosis persists on ultrasound.

DISCUSSION

Non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH) have recently emerged as the leading cause of chronic liver disease in children, being a reflection of the worldwide annual increment of obesity.⁴ While much of these can be explained by traditional risk factors of metabolic disease, an important subset is unexplained by these factors and require evaluation for secondary causes of NAFLD/NASH⁴. A large set of causes may underlie NAFLD/NASH namely Wilson disease, celiac disease, viral and autoimmune hepatitis, diabetes mellitus, drugs hepatotoxicity, glycogen storage diseases, alfa-1 antitrypsin deficiency, all excluded in our three cases.^{1,2} Autoimmune thyroiditis was present in case 1.

The three patients were overweight / obese and this could be the cause for NASH per se; however as described above, when case 1 lost weight the transaminases values remained high and the steatosis persisted, and when case 3 lost weight, the transaminases returned to normal but steatosis persisted. These features were an indicator of the presence of a cause for NAFLD/NASH, other than obesity, justifying the need for complementary studies.

In case 1, the presence of oral aphthosis, positive ASCAS antibodies, and autoimmune thyroiditis made mandatory the exclusion of autoimmune liver disease, which was done by applying the scoring system for diagnosis of autoimmune hepatitis, including liver histology. Another important cause of NAFLD/NASH is Wilson disease, excluded by serum ceruloplasmin, urinary copper excretion in a 24-hour period,

and search for Kayser–Fleischer rings in all patients, and liver copper in cases 1 and 3.^{11,12} Although case 1 and 3 had low plasma levels of fat-soluble vitamins, suggesting their intestinal malabsorption, none of the patients had delayed growth or steatorrhea, therefore it was not considered necessary to proceed the study of the intestinal mucosa.

FHBL is a disorder of lipoprotein metabolism characterized by decreased levels of ApoB due to mutations in the APOB (and other) genes, inducing low levels of cholesterol and triglyceride in plasma. These truncated ApoB forms lead to the impaired capacity to transport triglycerides from the liver, which associated with the low rates of hepatic production of normal lipid cause accumulation of triglycerides and other components in the liver, contributing to the development of NAFLD/NASH.⁵

All the three cases fulfilled biochemical criteria for HBL (total cholesterol < 100 mg/dl, LDL-cholesterol < 50 mg/dl, ApoB < 50 mg/dl) and had NAFLD or NASH.

In case 1, the molecular study of APOB gene revealed the novel homozygous mutation c.9353dup (p.Asn3118Lysfs17). This mutation also leads to a truncated protein, reinforcing the idea that it is a disease-causing mutation. Yet, at contrary of the previously described mutations, even in homozygosity was associated with a slight phenotype. In case 2 the molecular study was not performed. In case 3 no mutations were found in the analyzed regions of APOB gene.

The absence of identified mutations can not exclude the disease, because mutations outside the analyzed regions of the gene or present in other genes, can exist. The majority of the cases of FHBL previously described in the literature had mutations in the APOB gene resulting in a truncated ApoB, but in some cases of FHBL were described mutations in other genes (MTP, ANGPTL3). Only about 0.5% of subjects with HBL have plasma-detectable ApoB truncations.^{10,13}

Long term consequences of NASH associated with HBL are unknown. Therefore, the report of new cases, as well as their follow-up, is crucial to the knowledge of the natural history of this disease. A remarkable variability was described in the fat content of the liver even in FHBL cases caused by the same genetic defect and some groups of FHBL patients have no fat accumulation in the liver, for still unclear.¹⁴

In conclusion, clinicians should be aware of this disorder, and a lipid profile should be assessed in patients with NAFLD/NASH.

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Recebido a 07.09.2015 | Aceite a 22.12.2015