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Case Study

A case of hypocholesterolemia and steatosis in a carrier of a *PCSK9* loss-of-function mutation and polymorphisms predisposing to nonalcoholic fatty liver disease

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Abstract: We report a new case of hypobetalipoproteinemia in a 44-year-old man of Peruvian origin exhibiting a heterozygous *PCSK9* missense mutation (c.946 G>T, p. Gly316Cys). *In vitro* functional studies demonstrated that this mutation leads to a loss of function of PCSK9 on low-density lipoprotein receptor degradation. This patient exhibited liver steatosis; he was neither diabetic, nor obese or alcoholic, but is a carrier of 2 polymorphisms, p.Ile148Met (rs738409) and p.Glu167Lys (rs58542926) on *PNPLA3* and *TM6SF2* gene, respectively, previously shown to be associated with nonalcoholic steatosis and fibrosis evolution. These data suggested that if a resistance to hepatic steatosis mediated by the PCSK9 deficiency exists, as demonstrated in mice, it is not sufficient to prevent hepatic fatty accumulation in the case of genetic factors predisposing to nonalcoholic fatty liver disease.

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Introduction

Hypobetalipoproteinemia is defined by levels of low-density lipoprotein (LDL) and apolipoprotein B below the fifth percentile for age and sex. Five different inherited disorders lead to genetic hypobetalipoproteinemia including 3 diseases with codominant transmission. Heterozygous loss-of-function (LOF) mutation on *APOB*, *PCSK9*, or *ANGPTL3* gene (combined familial hypolipidemia) was found in asymptomatic patients, but some carriers of *APOB* mutation develop liver steatosis.[1,2] In the present report, we will focus on a novel *PCSK9* LOF mutation.

The crucial role of PCSK9 protein in LDL catabolism was discovered after the association of gain-of-function (GOF) mutations with autosomal dominant hypercholesterolemia in families where mutations in *LDLR* and *APOB* were excluded.[3] PCSK9 is secreted in the plasma by the liver and then binds the low-density lipoprotein receptor at the surface of hepatocytes and targets it for lysosomal degradation.[4] Further studies showed that genetic variations in this gene significantly regulate plasma levels of LDL cholesterol (LDLc): GOF was associated with increased LDLc, whereas LOF mutations were associated with 30% to 40% reduction of plasma LDLc and protection against coronary heart disease.[3–6] Due to these associations, PCSK9 was quickly considered as a key target of lipid-lowering therapy, and different pharmacologic strategies for inhibiting PCSK9 were developed.[4] Large phase III clinical trials have consistently shown that 2 inhibitory PCSK9-specific monoclonal antibodies, alirocumab and evolocumab, are highly effective in reducing LDLc and to some extent lipoprotein (a).[7] As this protein is a very promising therapeutic target, it is important to enhance our knowledge about its various functions, targets, and the consequences of LOF, thanks to the description of patients carrying naturally occurring mutations.[8]

We herein report a new case with hypobetalipoproteinemia and liver steatosis with a mutation of *PCSK9* gene. We discuss our results in the context of liver steatosis in this patient.

Case report

A 44-year-old man (body mass index of 26 kg/m^2) was referred to our department in 2013 because of an important liver steatosis detected by ultrasound during a routine medical assessment. The patient did not take any medication (or supplements) and did not report any comorbidity. He was working in a municipal administration suggesting no potential exposure to toxic products. Living in Belgium since more than 15 years, he belongs to the Amerindian ethnic groups originating from a very isolated village in the Peruvian mountains. Therefore, relevant medical information about his family was not available. To the patient awareness, his parents (living in Peru) were healthy, and no familial history of steatosis, diabetes, or metabolic disorder was reported. His alcohol consumption was strictly established to 1 or 2 alcohol units per week. The abdomen was normal, and no hepatomegaly was noted. Routine laboratory tests demonstrated that amounts of alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, and gamma-glutamyltransferase were normal; fasting glucose was 4.55 mmol/L; and glycosylated hemoglobin level was 5.4%. Total cholesterol was measured at 2.67 mmol/L and LDLc at 1.14 mmol/L, which correspond to percentiles 2 and 3, respectively, revealing a hypocholesterolemia phenotype. Levels of high-density lipoprotein cholesterol and triglycerides remained in the normal range at 1.30 and 0.53 mmol/L, respectively. The results of the routine laboratory tests remained the same during the follow up (Table 1). Serologic status for hepatitis A, B, and C virus were negative, whereas previous immunization was observed for Epstein-Barr virus and *Cytomegalovirus*.

After obtaining an informed written consent from the patient, coding regions and intron-exon boundaries of genes causing hypocholesterolemia were sequenced directly on genomic DNA. Because nonalcoholic fatty liver disease occur in patients with familial hypobetalipoproteinemia,[2] screening for possible mutation(s) in *APOB* encoding was performed as previously described[9] and revealed no abnormality. Further investigations identified a heterozygous form of a mutation (Chr1(GRCh37):g.55521812 G>T) in *PCSK9*. This mutation consisted in a c.946 G>T transversion in exon 6 (NM_174936.3), predicting a Gly316-to-Cys (p.G316C) amino acid change. This mutation was reported (rs554488891) in the ExAC database with a frequency of 0.0044% (all populations); in fact, this mutation was only reported in the ExAC controls of Latino origin with a frequency of 0.046% and was not found in controls from other populations (http://exac.broadinstitute.org/variant/1-55521812-G-T). *In silico*, this missense mutation in a highly conserved residue was predicted to be deleterious for a normal PCSK9 function using the interactive Biosoftware Alamut.

To assess the impact of this mutation, *in vitro* experiments were performed. Thus, HEK293 cells were transiently transfected with bicistronic recombinant pIRES2 cDNA plasmids (Clontech Labs), allowing the independent expression of a fluorescent EGFP from an internal ribosome entry site (IRES) and C-terminally V5-tagged wild-type (WT) human PCSK9[10] or its G316C mutant under the control of a CMV promoter. The WT PCSK9 and the GOF PCSK9-L108R[11] served as controls. At 40 hours after transfection, levels of total PCSK9 were measured by an *in house* PCSK9 enzyme-linked immunosorbent assay[12] both in the media and in the PCSK9-transfected cells (Fig. 1). Interestingly, we did not observe any PCSK9 in the media of cells expressing PCSK9-G316C, whereas 3- to 4-fold higher levels of PCSK9 compared with cells were detected in the control media of cells expressing WT PCSK9. As expected, there was a 36% higher level of PCSK9 in the media of cells expressing the GOF L108R compared with WT PCSK9.[11] In contrast, 468 ng proPCSK9-G316C protein levels were detected in cell lysates and none in the media compared with WT