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A loss-of-function variant in *OSBPL1A* predisposes to low plasma HDL cholesterol levels and impaired cholesterol efflux capacity

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ABSTRACT

Background and aims: Among subjects with high-density-lipoprotein cholesterol (HDL-C) below the 1st percentile in the general population, we identified a heterozygous variant *OSBPL1A p.C39X* encoding a short truncated protein fragment that co-segregated with low plasma HDL-C.
Methods: We investigated the composition and function of HDL from the carriers and non-carriers and studied the properties of the mutant protein in cultured hepatocytes.
Results: Plasma HDL-C and apolipoprotein (apo) A-I were lower in carriers versus non-carriers, whereas the other analyzed plasma components or HDL parameters did not differ. Sera of the carriers displayed a reduced capacity to act as cholesterol efflux acceptors ($p < 0.01$), whereas the cholesterol acceptor capacity of their isolated HDL was normal. Fibroblasts from a *p.C39X* carrier showed reduced cholesterol efflux to lipid-free apoA-I but not to mature HDL particles, suggesting a specific defect in ABCA1-mediated efflux pathway. In hepatic cells, GFP-OSBPL1A partially co-localized in endosomes containing fluorescent apoA-I, suggesting that OSBPL1A may regulate the intracellular handling of apoA-I. The GFP-OSBPL1A-39X mutant protein remained in the cytosol and failed to interact with Rab7, which normally recruits OSBPL1A to late endosomes/lysosomes, suggesting that this mutation represents a loss-of-function.
Conclusions: The present work represents the first characterization of a human *OSBPL1A* mutation. Our observations provide evidence that a familial loss-of-function mutation in *OSBPL1A* affects the first step of the reverse cholesterol transport process and associates with a low HDL-C phenotype. This suggests that rare mutations in *OSBPL* genes may contribute to dyslipidemias.

1. Introduction

Plasma high-density-lipoproteins (HDL) comprise a heterogeneous population of particles which differ in density, particle size, charge, and composition. Epidemiological studies have consistently shown an inverse correlation between plasma HDL cholesterol (HDL-C) as well as apolipoprotein A-I (apoA-I) levels and cardiovascular disease (CVD) risk [1,2]. However, recent failures of drugs that target HDL-C levels [3,4], together with evidence from Mendelian randomization studies indicate that HDL may not be causally

Abbreviations: ABCA1, ATP-binding cassette transporter A-1; ER, Endoplasmic reticulum; GFP, Green fluorescent protein; GST, Glutathione-S-transferase; HDL-C, High-density-lipoprotein cholesterol; LE, Late endosomes; OSBP, Oxysterol-binding protein; OSBPL1A, Oxysterol-binding protein-like 1A; wt, Wild-type.

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related to cardiovascular disease [5,6]. A number of common and rare genetic variations have been shown to be associated with altered plasma HDL-C concentrations. The best recognized among these are cholesteryl ester transfer protein (*CETP*) and endothelial lipase (*LIPG*) loss-of-function variants which cause markedly raised HDL-C levels, but do not protect against coronary heart disease (CHD) [6–8]. Additional genetic variants associated with elevated HDL-C levels include *TRIB1*, *APOA1/C3/A4/A5* gene cluster, *ANGPTL4 and GALNT2* [6].

Loss–of–function mutants in *APOAI*, *ABCA1* and *LCAT* cause extremely low HDL-C levels [9]. Whether these mutations cause increased CVD risk is still a matter of debate [10,11]. On the other hand, a hypothesis on the importance of HDL functionality has emerged, suggesting the presence of distinct functional HDL subpopulations [12,13]. In a previous study, we discovered an increased number of rare non-synonymous variants in lipid-related genes in a cohort of subjects with extreme plasma HDL-C concentrations (<1st or >99th percentile) [14]. Among the identified variants in subjects with low HDL-C (<1%) there was a nonsense mutation p.C39X in *OSBPL1A*, which encodes a homolog of Oxysterol-binding protein, OSBP [15,16].

The human *OSBPL* genes constitute a family with 12 members the protein products of which are characterized by a carboxy-terminal OSBP-related ligand-binding domain (ORD). These proteins are suggested to act as sterol and phospholipid transporters or sensors at organelle interfaces [17,18]. OSBPL1A targets late endocytic compartments (LE) via the small GTPase Rab7 and the endoplasmic reticulum (ER) through VAMP-associated proteins (VAPA and -B) [19,20]. OSBPL1A regulates the interactions of LE with ER membranes in a sterol-specific manner. OSBPL1A controls the motility, subcellular distribution and tethering of the endosomes by bridging between the ER and LE and through regulation of dynein/dynactin and homotypic fusion and protein sorting (HOPS) complexes [21–23].

The role of OSBPL1A in lipid metabolism remains to be elucidated. Overexpression of human *OSBPL1A* in mouse macrophages disturbed cholesterol efflux to HDL and enhanced atherogenesis in low-density lipoprotein receptor knock-out animals [24]. On the other hand, knock-down of *Osbpl1a* in mouse macrophages impaired the efflux of endocytosed LDL cholesterol to extracellular apoA-I, indicating that the function of Osbpl1a in LE may involve cross-talk with ATP-binding cassette transporter A1 (Abca1)-mediated pathways of cholesterol efflux, and could thus impact the biogenesis of HDL in the liver and the intestine [22].

In the present study, we characterize the composition and function of HDL from heterozygous carriers of an *OSBPL1A p.C39X* mutation. We further demonstrate an abnormal subcellular localization and a functional defect of the mutant protein, suggesting that this *OSBPL1A* loss of function mutation affects cholesterol removal from cells associated with the low HDL-C phenotype in the mutant carriers.

2. Materials and methods

2.1. Study subjects

A cohort of individuals with very high (n = 40) and very low (n = 40) plasma HDL-C levels (<1st and >99th percentile for age and gender) from the general population was studied to identify the genetic background underlying the HDL-C phenotype as described [14]. Coding sequence and exon-intron boundaries of 195 lipid-related genes and 78 lipid-unrelated genes were sequenced using Agilent Sureselect custom capture library on the Illumina HiSeq 2000 platform. In 1 individual with low HDL a nonsense mutation in *OSBPL1A* (NM_080597.2) was identified and further family expansion was done to evaluate co-segregation of each mutation with the low HDL-C phenotype. Genomic DNA was extracted from 10 ml whole blood on an AutopureLS system (Gentra Systems, Minneapolis, MN) according to the manufacturer's protocol. The primers to amplify the coding and intron-exon boundaries were designed using Primer 3 software (exon 1 *p.C39X*; c.112-115dup: forward 5′- TCC AAT CTG TGG GGT TCT TC-3′, reverse 5′- CCC CTT TCT GTA TTA GCA GGT G-3′ and sequenced as described elsewhere [25].

Written informed consent was obtained from all individuals and the study was approved by the Medical Ethical Committee of the Amsterdam Medical Center.

2.2. Analytical procedures

Blood was obtained after overnight fasting in EDTA-containing tubes and directly placed on ice. Plasma was isolated by centrifugation at 4 °C, 1700 × g for 15 min and stored at −80 °C for further analysis. Plasma and HDL phospholipids were analyzed using the Phospholipids B kit (Wako Chemicals, Osaka, Japan) or Pureauto S PL-kit (Daiichi Pure Chemicals, Tokyo, Japan); triglycerides using the Triglycerides GPO-PAP-kit (Roche Diagnostics, Mannheim, Germany); free cholesterol using the Wako Free Cholesterol C kit (Wako Chemicals) and total cholesterol using the Cholesterol CHOD-PAP kit (Roche Diagnostics). Human apolipoprotein E (apoE) was detected by ELISA as described previously with some modifications [26]. Quantitation of human apoA-I was performed with a sandwich ELISA. Briefly, the wells were coated with a polyclonal rabbit antibody against human apoA-I. The bound apoA-I protein was detected with a HRP-conjugated rabbit anti-human apoA-I immunoglobulin G (IgG) [26]. Protein concentration of isolated HDL fraction was determined using bovine serum albumin as standard [27].

2.3. Isolation of high-density lipoprotein (HDL)

HDL was isolated from serum samples by sequential ultracentrifugation using Beckman Optima TL Table-Top ultracentrifuge (Beckman Coulter, Brea, CA) and KBr for density adjustment [28]. Serum (0.5 ml) was first adjusted to the density (d) of 1.019 g/ml and the centrifuge tube filled with a d = 1.019 g/ml KBr solution to the total volume of 3 ml. The samples were centrifuged at 5 °C for 2 h at 500,000 × g. After centrifugation remnant particles as well as very low and intermediate density lipoproteins were recovered in the top 1 ml fraction and the bottom was adjusted to a density of 1.063 g/ml using solid KBr, filled to 3 ml with d = 1.063 g/ml KBr solution and centrifuged again (5 °C, 3 h, 500,000 × *g*). The top 1 ml fraction contained low-density lipoproteins (LDL). To isolate the HDL fraction, the LDL bottom fraction was adjusted with solid KBr to a density of 1.21 g/ml, the vials filled with KBr 1.21 g/ml density solution and then centrifuged (5 °C, 18 h, 500,000 × *g*). Total HDL was obtained in top 1 ml fraction. The isolated HDL was dialyzed against phosphate-buffered saline (PBS, pH 7.4) and stored at −80 °C before analysis.

2.4. Measurement of preβ-HDL levels

The ability of human plasma samples to generate preβ-HDL was analyzed by incubating plasma for 17 h at 37 °C in the presence of a Lecithin-Cholesterol Acyltransferase (LCAT) inhibitor (1 mM iodoacetate). The formed preβ-HDL particles were quantified by resolving the post-incubation plasma samples with two-dimensional crossed immunoelectrophoresis, as previously reported [29]. Briefly, the crossed immunoelectrophoresis consisted of an agarose electrophoresis in the first dimension for separation