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Hepatic NPC1L1 promotes hyperlipidemia in LDL receptor deficient mice

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ABSTRACT

Background and aims: Niemann-Pick C1-like1 (NPC1L1), a crucial cholesterol absorption receptor expressed in human intestine and liver. But in mouse it is only expressed in intestine. Previous studies elucidated that expression of human NPC1L1 in mouse liver led to increase of plasma cholesterol due to activation of absorption from bile. However, hepatic NPC1L1 function was not elucidated in LDL receptor deficient mouse (LDLR−/−) in which LDL was a main lipoprotein as in human.

Methods and results: L1-Tg/LDLR−/− mouse was created by crossing liver-specific NPC1L1 transgenic mouse (L1-Tg) with LDLR−/−. L1-Tg/LDLR−/− mice developed hyperlipidemia when fed with atherogenic diet (AD) containing 0.2% cholesterol for 21days. Compared with control mice, biliary cholesterol level in L1-Tg/LDLR−/− mice was significantly lower, plasma cholesterol level was significantly higher in L1-Tg/LDLR−/− mice under both chow diet and AD feeding. New finding in this study is augmentations of plasma TAG L1-Tg/LDLR−/− mice fed with AD. Results were shown that very low density lipoprotein (VLDL) secretion was elevated in L1-Tg/LDLR−/− mice after AD fed. The increase of VLDL secretion was further confirmed by higher expression of hepatic triacylglycerol hydrolase (TGH) and microsomal triglyceride transfer protein (MTP).

Conclusion: L1-Tg/LDLR−/− mouse is a humanized model to study cholesterol absorption and transportation. The results obtained from L1-Tg/LDLR−/− mouse indicated no feedback mechanism to inhibit NPC1L1 function in liver and hepatic expression of NPC1L1 correlated with VLDL secretion in hypercholesterolemia state.

1. Introduction

High plasma cholesterol level is considered as a main cause of atherosclerotic cardiovascular diseases. Homeostasis of plasma cholesterol is regulated by biliary excretion, intestinal absorption and de novo biosynthesis. Niemann-Pick C1-like1 (NPC1L1) is a cholesterol transmembrane absorption receptor [1]. In human it is expressed in small intestine and in liver. Intestinal NPC1L1 is located in the brush border membrane of absorptive enterocytes, contributes to exotic cholesterol transportation from diet into blood eventually. In liver, NPC1L1 localizes to the canalicular membrane, contributes to cholesterol absorption from bile [2,3]. The biological importance of the hepatic NPC1L1 is to prevent massive loss of cholesterol in mammals.

In contrast to humans, mouse NPC1L1 is expressed only in intestine [4]. In order to study hepatic function of NPC1L1, transgenic mice expressing human NPC1L1 in the liver (L1-Tg) has been created [5]. The L1-Tg mice showed a dramatic reduction of biliary cholesterol, but significantly increase of plasma cholesterol level. The function of hepatic NPC1L1 could be inhibited by ezetimibe [5]. These results suggest that hepatic NPC1L1 plays an important role in regulation of biliary cholesterol excretion. This finding was also confirmed by Karano and colleagues using adenovirus mediated gene transfer to express NPC1L1 in the liver of normal mice [6]. Furthermore, the L1-Tg mouse was used as animal model to study the macrophage reverse cholesterol transport (RCT) [7] and transintestinal cholesterol efflux (TICE) [8].

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As a hydrophobic substrate, cholesterol is transported via lipoprotein particles including chylomicrons (CM), very low density lipoprotein (VLDL), low density lipoprotein (LDL) and high density lipoprotein (HDL) in circulatory system of mammals. Among these lipoproteins, LDL is the predominant lipoprotein particles in human plasma. Cholesterol and other lipids are delivered to peripheral tissues via LDL [9]. However, HDL is the major lipoprotein in mouse. The high HDL level is considered as a main contributor to lower incidence of cardiovascular diseases in murine [10]. Due to the difference of lipoproteins profile between human and mouse, LDL receptor deficient (LDLR−/−) mice are widely used in the research of human cardiovascular disease [11,12]. Compared to wild-type mice, the LDL level in LDLR−/− mice can be increased seven-to nine fold when fed a high cholesterol diet [13,14].

In this study, we investigate the role of human NPC1L1 in the physiological conditions resemble human lipoprotein profile and hypercholesterolemia, by liver-specifically expressing NPC1L1 in LDLR−/− mice (L1-Tg/LDLR−/−).

2. Materials and methods

2.1. Animal and diet

LDLR−/− mice purchased from The Jackson Laboratory (Bar Harbor, ME, USA) were bred with L1-Tg mice [5] to produce L1-Tg/LDLR−/− mice. Genetic background of both LDLR−/− and L1-Tg mice was B6D2F1 [5]. All the mice were housed in a specific pathogen-free animal facility at 22 °C in a 12-h dark/light cycle with free access to water and regular chow diet (Prolab RMH 3000; LabDiet, Brentwood, MO, USA), Considering the slow development of atherosclerosis in LDLR−/− mice [13,15] with chow diet feeding, we treated the both LDLR−/− and L1-Tg/LDLR−/− with a synthetic low-fat, atherogenic diet (AD), containing 10% energy from palm oil and 0.2% (w/w) cholesterol obtained from the Wake Forest University Health Sciences department, USA. After the 21day treatment, started from age of 8 weeks, the mice were anesthetized by isoflurane in induction chamber and then euthanized by cervical dislocation. After the collection of gallbladder bile, livers were removed, weighed, and snap-frozen in liquid nitrogen. All animal procedures were approved by the institutional animal care and use committee at Animal and avian Science Department in University of Maryland, college park, USA. In this study only male mice were used and LDLR−/− mice were used as controls. The sequences of the primers used to amplify hNPC1L1 from mouse liver for qPCR were listed in the Supplemental Table 1.

2.2. Immuno-histochemical, H&E and sirius red staining analysis

Liver samples from LDLR−/− and L1-Tg/LDLR−/− mice were fixed with 4% paraformaldehyde, sectioned into 5 μm thick slices, and processed for immunohistochemistry utilizing rabbit anti-human NPC1L1 antibody 69B as a primary antibody and peroxidase-labeled polymer of goat anti-rabbit Ig antibody (Envision system, Dako, Glostrup, Denmark) as the secondary antibody. Color development was performed with DAB, and the samples were counterstained by hematoxylin. Fixed liver slides also processed for H&E and Sirius red staining to analysis inflammation and fibrosis in mouse liver. The methods were described previously [15,16].

2.3. Hepatic, biliary and plasma lipid analysis

Hepatic, biliary and plasma lipid were measured as described previously [17–19]. To determine plasma lipoprotein distribution, 20 μL of plasma from a group of 6 LDLR−/− and 6 L1-Tg/LDLR−/− mice was pooled, and lipoprotein classes were separated by gel filtration chromatography as described previously [19]. Phospholipid (PL) content was measured using Phospholipids B (Wako Life Sciences, Inc. Richmond,VA) enzymatic assay kit [18,19].

2.4. VLDL secretion analysis

L1-Tg/LDLR−/− and control mice (n = 6) were fasted for 4 h and then injected with tyloxapol retro-orbitally (500 mg/kg body weight). Blood sample were collected in heparin tubes at 0, 30, 60, 90. 120, 150 and 180 min. Plasma triacylglycerol (TAG) was measured using L-Type Triglyceride M kit (Wako Life Sciences, Inc. Richmond, VA).

2.5. Immunoblot analysis

A total of 50–100 mg of liver was homogenized in 0.5 ml ice cold lysis buffer (2x Ripa buffer-2, Alfa Aesar, Tewksbury, MA, USA: J60629) containing protease inhibitor cocktail (Sigma-Aldrich, St.Louis, MO, USA P-8340) with Potter–Elvehjem homogenizer. Then homogenate was centrifuged at 14,000 g for 20 min at 4 °C. After centrifugation, the supernatants were collected and frozen at −80 °C. Extracted proteins were immunoblotted as described previously [1,20] with polyclonal rabbit anti-human NPC1L1 antibody 69B (kindly provided by J.C. Cohen and H.H. Hobbs, University of Texas Southwestern Medical Center, Dallas, Texas, USA); TGH polyclonal antibody (Thermo fisher, Wilmington, DE, USA: PA5-19740); SR-B1 antibody (Abcam, Cambridge, MA, USA: ab137829); MTP antibody (BD bioscience, Billerica, MA,USA: 612022).

Plasma lipoproteins was immunoblotted as described [5] with a rabbit anti-rat apoB, apoE and apoA1 serum (kindly provided by J. Herz, University of Texas Southwestern Medical Center, Dallas, Texas, USA). Blots were quantified by densitometry with ImagJ software (NIH, Bethesda, MD).

2.6. Quantitative real-time PCR (qPCR)

RNA extraction from liver of L1-Tg/LDLR−/− and control mice (n = 6) and qPCR were performed as described [21] using RNeasy Mini Kit 250 (QIAGEN, Germantown, MD, USA: 74106) and SYBR® Green PCR Master Mix Kit (Life Technologies, Frederick, MD, USA: 4309155). GAPDH was used as an internal control and mRNA expression levels were calculated based on the ΔΔ-CT. mRNA levels for each gene represent the amount relative to that of control mice which was arbitrarily standardized to 1. Sequences of the primers used for qPCR are available in Supplemental Table 1.

2.7. Analysis of fecal neutral sterol excretion

L1-Tg/LDLR−/− and control mice (n = 6) were housed independently to collect feces during AD diet treatment. Fecal neutral sterol excretion was measured as described previously [22].

2.8. Statistics

Data are presented as the mean ± SEM. Groups were compared by unpaired 2-tailed student *t*test using Graphpad Prism software.

3. Results

3.1. Creation of L1-Tg/LDLR−/− mouse

L1-Tg/LDLR−/− mice were created by crossing L1-Tg mice [5] with LDLR−/− mice. Real-time PCR analysis showed that NPC1L1 mRNA expression is extremely high in liver of L1-Tg/LDLR−/− mice compared to LDLR−/− mice (Fig. 1A). Immunoblotting analysis

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