Atherosclerosis 207 (2009) 452–457

Contents lists available at ScienceDirect

Atherosclerosis

journal homepage: www.elsevier.com/locate/atherosclerosis

Characterization of a new *LCAT* mutation causing familial LCAT deficiency (FLD) and the role of *APOE* as a modifier gene of the FLD phenotype

Alexis Baass*, Hanny Wassef, Michel Tremblay, Lise Bernier, Robert Dufour, Jean Davignon

Hyperlipidemia and Atherosclerosis Research Group, Clinical Research Institute of Montreal (IRCM), 110 Pine Avenue West, Montreal, QC, Canada, H2W1R7

ARTICLE INFO

Article history:
Received 4 March 2009
Received in revised form 16 April 2009
Accepted 7 May 2009
Available online 21 May 2009

Keywords:
Familial LCAT deficiency (FLD)
HDL-C
Apo E
Lipoprotein X

ABSTRACT

Familial LCAT deficiency (FLD) is a disease characterized by a defect in the enzyme lecithin:cholesterol acyltransferase (LCAT) resulting in low HDL-C, premature corneal opacities, anemia as well as proteinuria and renal failure. We have identified the first French Canadian kindred with familial LCAT deficiency. Two brothers, presenting classical signs of FLD, were shown to be homozygous for a novel *LCAT* mutation. This c.102delG mutation occurs at the codon for His35 and causes a frameshift that stops transcription at codon 61 abolishing LCAT enzymatic activity both *in vivo* and *in vitro*. It has a dramatic effect on the lipoprotein profile, with an important reduction of HDL-C in both heterozygotes (22%) and homozygotes (88%) and a significant decrease in LDL-C in heterozygotes (35%) as well as homozygotes (58%). Furthermore, the lipoprotein profile differs markedly between the two affected brothers who had different *APOE* genotypes. We propose that *APOE* could be an important modifier gene explaining heterogeneity in lipoprotein profiles observed among FLD patients. Our results suggest that a *LCAT*−/− genotype associated with an *APOE* ε2 allele could be a novel mechanism leading to dysbetalipoproteinemia.

1. Introduction

LCAT (lecithin:cholesterol acyltransferase; EC 2.3.1.43) is a 63 kDa enzyme essential for the esterification of plasma cholesterol. This enzyme catalyzes the transfer of the sn-2 acyl group of phosphatidyl choline (PC) to the 3β-OH group of cholesterol. LCAT is produced by the liver and secreted in the plasma where it is mainly found associated with HDL, but also with apo B containing lipoproteins such as LDL and VLDL [1,2]. Apo A-I is the major co-factor of LCAT, whose activity is key in the maturation of discoidal pre-β HDL to spherical α-HDL particles [3]. Once esterified, cholesterol present on the surface of HDL will migrate to form the lipophilic core of the mature HDL particles [4]. These HDL particles will transport cholesterol from the peripheral tissues to the liver, where it will be taken up via the SR-BI receptor (scavenger receptor class B type I). Cholesterol present in HDL can also be exchanged with triglyceride of VLDL via the action of the cholesterol ester transfer protein (CETP). These mechanisms explain how LCAT plays an important role in the metabolism of HDL particles as well as in reverse cholesterol transport [4].

The *LCAT* gene located on chromosome 16q22.1, spans 4.2 kb and comprises six exons and five introns. Over 70 mutations in the LCAT gene have been reported and span the whole length of the cDNA (Human Gene Mutation Database http://www.hgmd.cf.ac.uk/ac/index.php). These mutations can be classified into two major classes: familial LCAT deficiency (FLD, OMIM# 245900) and Fish-Eye disease (FED, OMIM# 136120) [1,2]. Both diseases are inherited via an autosomal recessive pattern and are extremely rare. The true prevalence of both the heterozygous and homozygous states is unknown. Familial LCAT deficiency was initially described by Norum and Gjone [5]. In FLD, LCAT activity is completely suppressed either by the production of a truncated non-functional protein or a point mutation in a key amino acid. Both the α-LCAT activity (LCAT activity on HDL particles) and β-LCAT activity (LCAT activity on LDL particles) are lost leading to low plasma HDL cholesterol (<5th percentile) and LDL cholesterol.

In addition lamellar HDL particles forming rouleaux on electron microscopy as well as lipoprotein X (LpX), may also be present in the plasma of patients with FLD [6]. LpX are very large lipoproteins which have a vesicular structure and are composed of phospholipids, free cholesterol, small amounts of apo A-I, apo E and apo C as well as large amounts of albumin [1].

The classical symptoms associated with FLD are premature corneal opacifications, anemia, progressive proteinuria and renal failure [2]. Bilateral corneal opacities progress from childhood and

Abbreviations: HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; VLDL, very low density lipoprotein; IDL, intermediate density lipoprotein; LpX, lipoprotein X; Apo, apolipoprotein; SR-BI, scavenger receptor class B type I; CETP, cholesterol ester transfer protein; LCAT, lecithin:cholesterol acyltransferase; LPL, lipoprotein lipase; LDLR, LDL receptor.

* Corresponding author. Tel.: +1 514 987 5500x3326; fax: +1 514 987 5732.
E-mail address: a.baass@umontreal.ca (A. Baass).

0021-9150/$ – see front matter © 2009 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.atherosclerosis.2009.05.014

seem to be caused by the accumulation of free cholesterol as well as amyloid deposits in both the Bowman layer and the anterior stroma of the cornea [7]. Mild normochromic-normocytotic anemia is frequently observed resulting from reduced erythropoiesis as well as hemolysis secondary to the abnormal lipid composition of the red blood cell membrane. This gives rise to the presence of codocytes (target cells) and reticulocytes on the peripheral blood smear [8]. FLD is also characterized by a progressive proteinuria which leads to terminal renal insufficiency by the 4th or 5th decade. Histological studies of renal biopsies revealed free cholesterol deposits in the mesangium as well as foam cell accumulation in the glomeruli [9]. The proposed mechanism of renal injury suggests that LpX could cause damage to the renal capillary endothelium [10]. Renal impairment is probably the leading cause of morbidity and mortality in FLD patients.

It has been observed that members of a same family, homozygous for the same LCAT mutation, can often present very different lipid profiles. Gjone et al. observed that plasmas of certain FLD patients presented LpX whereas others did not [10]. Furthermore, some patients have low plasma triglycerides, whereas others have overt hypertriglyceridemia [11–14]. We hypothesized that *APOE* may be a modifier gene explaining variations in lipoprotein phenotypes observed in FLD patients, and that it can contribute to the level of triglycerides and cholesterol.

2. Materials and methods

2.1. Subjects

The index case, a 24-year-old man (IV-5 on Fig. 1), was referred to our lipid clinic by the patient's ophthalmologist on the basis of corneal anomalies and low HDL-C. The patient presented progressive corneal opacities which were first identified at the age of 12 years. Slit lamp examination showed an inhomogeneous opacity of the corneal stroma which was prominent in the limbus. The patient had no other known prior medical history. Further investigation revealed a normochromic-normocytotic anemia with the presence of codocytes on the peripheral blood smear. Renal function was unaltered as the calculated estimated glomerular filtration rate (eGFR) was normal, but a slight proteinuria was detected (0.27 g/day). Detailed questioning of the patient and a thorough physical examination failed to reveal any clinical evidence of atherosclerosis. Doppler ultrasound of the carotid arteries did not reveal any atherosclerotic plaques and the intima-media thickness (IMT) was normal for both the left (0.6 mm) and right (0.5 mm) carotid arteries.

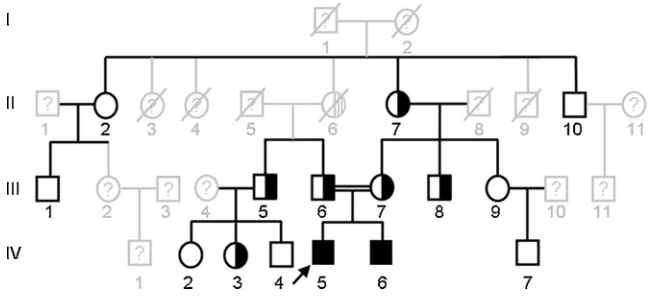

Fig. 1. Pedigree of a French Canadian family with familial LCAT deficiency. Males are represented by squares and females by circles. Non affected family members are presented in white, heterozygote carriers are presented by a half white and half black diagram and homozygous patients are represented in black. The proband is indicated by an arrow. Both the proband and his affected brother were born from a consanguineous union since their parents were first cousins. Family members who underwent biochemical and genetic analysis are presented in full lines whereas members who did not participate are presented in a subdued grey tone. Family member II-6 was deceased at the time of the study but it is very likely that she was a heterozygote carrier.

The index case's brother, a 26-year-old man (IV-6 on Fig. 1), had a childhood history of a slight normochromic-normocytotic anemia of unknown origin and had been treated with oral iron supplements and folic acid for several years. Furthermore his pediatrician had noted an enlarged spleen of unknown cause. Investigations revealed the presence of slight bilateral corneal opacifications as well as a stable anemia. This patient did not present any renal dysfunction as the calculated eGFR was normal and there was an absence of proteinuria. There was no clinical evidence of cardiovascular disease. Doppler ultrasound of the carotid arteries did not reveal any atherosclerotic plaques. Left carotid IMT (0.6 mm) and right carotid IMT (0.7 mm) were both within normal limits.

We assessed the patients' kindred (Fig. 1). All other members examined were apparently healthy and did not present any ophthalmic, hematologic, renal or cardiovascular disease. Only one patient (II 10) used a drug which could interfere with the lipid profile (omega 3 supplements 1 g/day). The study was approved by the Ethics Review Board of the Clinical Research Institute of Montreal. Written informed consent was obtained from all participants.

2.2. Genetic analysis

The patients' genomic DNA was isolated from peripheral blood leukocytes using commercial kits produced by Qiagen (Qiagen, Missisauga, ON). Genomic DNA was amplified on an automated PCR thermocycler (PerkinElmer, Norwalk, CT). Sequences of the primers and amplification conditions for each exon were obtained from NCBI (http://www.ncbi.nlm.nih.gov/genome/probe/variantseqr). All primers contained an M13 tail used for sequencing. The amplified fragments were purified from agarose gels using QIAquick Gel Extraction kit (Qiagen, Missisauga, ON) and sequenced on a CEQ automatic sequencer from Beckman (Beckman Instruments, Palo Alto CA). Sequences were analyzed with a 3130 XL Genetic analyser from ABI (Applied Biosystem, Foster City, CA). Mutation detection was done using Sequencher^{TM} v.4.8 software (Gene Code Corp). All six exons of the *LCAT* gene were sequenced for patients (III-6, III-7, IV-5 and IV-6). For all other patients, only the first exon was sequenced. All exons of both the *LPL* and the *LIPC* genes were sequenced for patients IV-5 and IV-6. *APOE* genotype was determined in all kindred by restriction enzyme analysis as previously described by Hixson and Vernier [15].

2.3. Biochemical analysis

Fasting blood samples were centrifuged at 3000 × *g* for 15 min and the plasma was aliquoted and stored at −70 °C. Total cholesterol, HDL-C and triglycerides were measured by enzymatic assay on an Advia 1650 automated analyzer (Siemens Diagnostics, Toronto, ON). LDL-C was calculated using the Friedewald formula [16]. Free cholesterol was assayed using a kit produced by Wako (Wako Pure Chemical, Richmond, VA) and optical densities were measured on a Beckman DU-64 spectrophotometer (Beckman Coulter, Mississauga, ON). Apo A-I and apo B were measured by nephelometry on a Dade-Behring BN Prospect automated muti-analyzer (Siemens Diagnostics, Toronto, ON). Total plasma apo E was measured by noncompetitive polyclonal enzyme-linked immunoassay as previously described [17].

2.4. Measurement of LCAT activity

LCAT activity was measured using a commercial fluorescent LCAT assay kit (Calbiochem,USA) on a SpectraMax Gemini EM