Atherosclerosis 216 (2011) 161–165

Contents lists available at ScienceDirect

Atherosclerosis

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

Short communication

Proteinuria in early childhood due to familial LCAT deficiency caused by loss of a disulfide bond in lecithin:cholesterol acyl transferase

A.G. Holleboom[a,*], J.A. Kuivenhoven[b], C.C. van Olden[a], J. Peter[b], A.W. Schimmel[b], J.H. Levels[b], R.M. Valentijn[c], P. Vos[d], J.C. Defesche[b], J.J.P. Kastelein[a], G.K. Hovingh[a], E.S.G. Stroes[a], C.E.M. Hollak[e]

[a] Department of Vascular Medicine, Academic Medical Center, Amsterdam, The Netherlands
[b] Department of Experimental Vascular Medicine, Academic Medical Center, Amsterdam, The Netherlands
[c] Department of Nephrology, Haga Hospital, The Hague, The Netherlands
[d] Department of Pediatrics, Haaglanden Medical Center, The Hague, The Netherlands
[e] Department of Endocrinology, Academic Medical Center, Amsterdam, The Netherlands

ARTICLE INFO

Article history:
Received 20 November 2010
Received in revised form 6 January 2011
Accepted 7 January 2011
Available online 21 January 2011

Keywords:
Familial LCAT deficiency
High density lipoprotein
Renal disease
Mutation
Pediatrics

ABSTRACT

Introduction: Familial lecithin:cholesterol acyltransferase (LCAT) deficiency (FLD) is a rare recessive disorder of cholesterol metabolism characterized by the absence of high density lipoprotein (HDL) and the triad of corneal opacification, hemolytic anemia and glomerulopathy.
Patients: We here report on FLD in three siblings of a kindred of Moroccan descent with HDL deficiency. In all cases (17, 12 and 3 years of age) corneal opacification and proteinuria were observed. In the 17-year-old female proband, anemia with target cells was observed.
Results: Homozygosity for a mutation in LCAT resulted in the exchange of cysteine to tyrosine at position 337, disrupting the second disulfide bond in LCAT. LCAT protein and activity were undetectable in the patients' plasma and in media of COS7 cells transfected with an expression vector with mutant LCAT cDNA. Upon treatment with an ACE inhibitor and a thiazide diuretic, proteinuria in the proband decreased from 6 g to 2 g/24 h.
Conclusion: This is the first report that FLD can cause nephropathy at a very early age.

1. Introduction

Lecithin:cholesterol acyl transferase (LCAT) is a key enzyme in HDL metabolism. It catalyses the maturation of HDL particles by esterifying free cholesterol molecules [1]. LCAT has two disulphide bridges. The first bridge (Cys74–Cys98) allows for the formation of the lid region, which covers the catalytic center and is opened upon contact to a lipid surface [1]. The function of the second disulfide bond (Cys337–Cys380) is less clear [2].

Homozygosity or compound heterozygosity for LCAT mutations that result in complete loss of function cause familial LCAT deficiency (FLD), characterized by corneal opacification, mild hemolytic anemia, and progressive renal disease [3]. Proteinuria is on average detected between the age of thirty and forty (see Supplementary Table 2 for an inventory of mutations in LCAT described to result in FLD with reported proteinuria and age of first documentation of proteinuria) [3].

Therapeutic options for FLD are poorly defined, associated with the rareness of the disease (<100 patients described worldwide). Plasma transfusions transiently normalized plasma lipids and lipoproteins, as well as the abnormal erythrocyte membranes [4,5]. Recently, combined treatment with nicotinic acid and fenofibrate was associated with a reduction in plasma lipoprotein X levels and a concomitant reduction in proteinuria in one FLD patient [6]. Intensive renoprotective therapy with blockade of the RAS, as well as adequate treatment of hypertension, may delay renal deterioration [7]. Enzyme replacement [8] and gene therapy [9] are eagerly awaited.

Here, we describe FLD in three children of Moroccan descent due to a mutation in LCAT that causes loss of the second disulfide bond in LCAT. This is the first description of renal pathology at a very early age. Effects of ACE inhibition in the proband are described.

2. Methods

2.1. Genetic analysis

PCR amplification and sequence reactions and analysis were performed as described [10].

* Corresponding author at: F4-142, Academic Medical Center, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands. Tel.: +31 205 668 675; fax: +31 205 669 343.
E-mail addresses: a.g.holleboom@amc.uva.nl, onno.holleboom@gmail.com (A.G. Holleboom).

0021-9150/$ – see front matter © 2011 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.atherosclerosis.2011.01.025

2.2. Biochemical analysis

Plasma lipids and lipoproteins were analyzed as described [10]. Plasma LCAT levels were measured by ELISA [11]. Plasma LCAT α-activity was measured with a proteoliposome assay as described in Supplementary methods [12].

2.3. In vitro characterization of p.C337Y mutation

Wild-type LCAT and LCAT^{C337Y} were expressed in COS7 cells as described in Supplementary methods. LCAT protein and α- and ß-activity of media were measured as described in Supplementary methods [12,13].

2.4. Carotid ultrasound imaging

Ultrasound scans of the carotid arterial wall were assessed according to a standardized protocol [14].

3. Results

3.1. Patients

A 17-year-old girl was referred to our clinic for renal pathology compatible with a metabolic disorder, including FLD. At age 15, she presented with abdominal pain for which no cause could be identified. Routine urinalysis revealed excess urinary protein, subsequently quantified at 6 g/24 h, indicating severe proteinuria. Blood pressure was 115/70 mmHg, mild pitting edema was noted on both lower extremities and plasma albumin was low at 25 g/l. Complement factors were normal and anti-nuclear antibodies or anti-dsDNA could not be detected. Creatinine clearance calculated from a 24 h urine collection was elevated at 164 ml/min, indicative of hyperfiltration due to early renal damage. Kidneys were of normal size and morphology on ultrasound examination. Peripheral corneal opacification was apparent (Fig. 1a), and on slit lamp examination, central opaque dots were observed, with a clear zone separating the limbus and the lipoid arc (Fig. 1a). Her plasma HDL cholesterol level was 0.14 mmol/l, and hemoglobin was mildly decreased at 11.4 g/dl, with normal mean corpuscular volume and number of reticulocytes. Target cells were seen in the blood smear. Haptoglobin was not decreased, nor was LDH increased. Light microscopy of a renal biopsy revealed non-amyloid mesangial and pericapillary depositions in the majority of the glomeruli. Upon electron microscopic analysis, vacuoles in the glomerular basement membrane were identified, containing electron dense material (Fig. 1b). To exclude Fabry disease, alpha-galactosidase activity was measured in leucocytes of the patient, and found to be normal.

Parents of the proband were first cousins of Morroccan descent. The proband had a 12 year old sister, and two younger brothers, aged 6 and 3 years (see pedigree in Table 1). The sister and the youngest brother were asymptomatic, but had proteinuria of 0.45 g/l with a urinary protein to creatinine ratio of 34 mg/mmol and glomerular filtration rate of 196 ml/min/1.73 m^2 (eGFR, estimated with Schwartz equation), and of 0.16 g/l with a urinary protein to creatinine ratio of 15 mg/mmol and eGFR rate of 127 ml/min/1.73 m^2, respectively [15]. No edema was noted in these two siblings, and plasma albumin levels and creatinine clearance were normal. In both, slight limbal opacification of the cornea was observed.

3.2. Genetic analysis

In the proband, a homozygous mutation at position c.1010 G > A was identified (TGT > TAT), leading to substitution of cysteine by tyrosine at position 337 (p.C337Y). Her sister and youngest brother were also homozygous for this mutation while the parents and oldest brother were heterozygous (for pedigree, see Table 1).

3.3. Plasma lipids and LCAT measurements

The 3 homozygous patients presented with very low HDL cholesterol levels ranging from 0.14 to 0.38 mmol/l averaging 0.33 mmol/l. The heterozygotes had on average 50% lower levels of HDL cholesterol and apoA-I compared to reference values. In the homozygotes, levels of apoA-I were also reduced, but in the heterozygotes these were remarkably normal. LCAT protein and α-activity were undetectable in plasma of the homozygotes and reduced by 52% and 27% compared to reference values, respectively, in the heterozygotes [16] (see Table 1).

3.4. In vitro characterization of p.C337Y mutation in LCAT

In the media of COS7 cells transfected with p.C337Y-LCAT, no immunoreactive LCAT protein could be detected, compared to clearly detectable LCAT media of cells transfected with wild-type LCAT. Compared to media of cells transfected with wild-type LCATT, both α- and β-LCAT activity were severely reduced in the media of cells transfected with p.C337Y-LCAT (see Supplementary Table 1).

3.5. Carotid ultrasound imaging

Carotid intima media thickness (IMT), a surrogate endpoint for atherosclerosis, was within normal range in the four oldest family members (I:01, I:02, II:01 and II:02) [17]. The two other siblings (II:03 and II:04) were considered too young to be assessed.

3.6. Symptomatic therapy

After 1 year of treatment with an ACE inhibitor, lisinopril 20 mg once daily, and hydrochlorothiazide 25 mg once daily, the proteinuria in the proband was 3-fold decreased from 6 g/24 h to 2 g/24 h and the pitting edema had resolved. Kidney function calculated from 24 h urine was initially elevated and remained elevated. The proteinuria and the urinary protein to creatinine ratio in the 12-year old homozygous sister of the proband (II:02) had decreased from 0.45 g/l to 0.20 g/l and from 34 mg/mmol to 25 mg/mmol, respectively, after a year of treatment with another ACE inhibitor enalapril at 5 mg twice daily. The 3 year old brother was followed but has so far remained untreated. His proteinuria level and urinary protein to creatinine ratio remained stable around 0.20 g/l and 15 mg/mmol, respectively. eGFRs remained unchanged in the two younger patients.

4. Discussion

Here we describe FLD in three siblings of Moroccan descent and report that this metabolic disorder can cause renal damage in early childhood. A novel causal mutation in LCAT in this first family from Northwest Africa in which FLD is described, results in loss of a disulfide bond in the enzyme, associated with a complete loss of immunoreactive protein in plasma and a complete loss of LCAT activity.

To date 78 mutations in *LCAT* have been described [18]. The current study identifies the first naturally occurring mutation leading to disruption of a disulfide bond in LCAT, due to the substitution from cysteine to tyrosine at position 337 (p.C337Y). By definition, this causes a change in the secondary structure of the enzyme. Based on the 3D model of LCAT [19], the substitution possibly induces a shift in the stereotypic position of His377, a component of the catalytic triad [19,20], thus disrupting the structure of this triad