Case Report

Point mutation (C to T) of the LCAT gene resulting in A140C substitution

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Familial lecithin: cholesterol acyltransferase (LCAT) deficiency is an autosomal recessive disorder characterized by corneal opacity, hemolytic anemia, proteinuria, and a low serum level of high-density lipoprotein cholesterol (HDL-C). Also, LCAT activity is remarkably decreased or absent.

A 57-year-old Japanese man presented with corneal opacity, proteinuria, and a very low serum level of HDL-C. His LCAT activity was too low to measure. From clinical observations and results of examinations, we suspected LCAT deficiency. We performed a kidney biopsy and gene analysis.

Light microscopy revealed the vacuolation of glomerular capillary tufts. Electron microscopy revealed small deposits in the glomerular basement membrane (GBM), extracellular matrix, and vascular endothelial cells.

We identified a homozygous C to T point mutation at nucleotide 501 (g.501 C>T) of exon 4 at codon 140, resulting in an arginine (Arg) to cysteine (Cys) amino acid substitution (A140C) in the patient.

These findings were characteristic of LCAT deficiency, which was confirmed to be due to a mutation that has only been reported in Japan.


Key words; LCAT deficiency, gene mutation, A140C

Introduction

Familial lecithin: cholesterol acyltransferase (LCAT) deficiency is a rare autosomal recessive disorder in which LCAT activity is decreased or absent1. The characteristic clinical features include bilateral corneal opacity, hemolytic anemia (normocytic), and progressive renal dysfunction2.

To date, 45 mutations of the human LCAT gene have been reported (http://www.hgmd.cf.ac.uk/ac/gene.php?gene=LCAT), with a total of 13 mutations being reported in Japanese patients3. In patients with this disease, there are variations in enzymatic activity and symptoms depending on the mutation responsible4.

Dyslipidemia also occurs, with a marked decrease in the level of high-density lipoprotein cholesterol (HDL-C), a decrease in the concentration of apolipoprotein (apo) A-I and apo A-II, and an increase of apo E. Although the total level of cholesterol (TC) in serum is low in homozygotes, serum TC and triglyceride (TG) concentrations tend to be high in heterozygotes. Histological examination reveals deposits of TCs and phospholipids in the kidney, liver, spleen, and cornea5. Electron microscopy shows small depo-
its in the GBM and extracellular matrix. Similar deposits can be detected in vascular endothelial cells and the renal tubular epithelium.

There is no specific treatment available for LCAT deficiency, so standard management is focused on treating renal failure with either dialysis or transplantation.

In a patient who presented with corneal opacity and proteinuria, we diagnosed LCAT deficiency based on the detection of lipidosis and renal histopathological findings. Genetic analysis confirmed an LCAT mutation that has only been reported in the congress proceeding in Japan.

Methods

Case report

The patient was a 57-year-old Japanese male who had suffered from corneal opacity and abnormal results of urinalysis since childhood. His family history was unclear because his parents had divorced when he was a child. The man was single, and had no children. He was brought up separately from his older brother with whom he had no contact since childhood. He was diagnosed with hypertension when about 30 years old.

Physical examination showed severe corneal opacity (Fig. 1). His blood pressure was 182/113 mmHg, height was 171 cm, and weight was 51.1 kg. His intellect was normal. Chest radiography, electrocardiography, and echocardiography gave essentially normal findings.

Laboratory findings were as follows. Urinalysis revealed microscopic hematuria and proteinuria. Daily protein excretion was 0.71 g/24 hr. Serum urea nitrogen (UN) and creatinine (Cre) were within normal limits (UN 22 mg/dL, Cre 0.66 mg/dL), but he had mild normocytic anemia (red blood cells 412 \( \times \) 10^6/µL, Hemoglobin 12.9 g/dL, Hematocrit 39.9%).

Lipids Analysis

Blood was collected after an overnight fast. TC was quantified by the Cholesterol oxidase-Peroxidase (COD-POD) method (Kyowa Medex Co., Ltd., Tokyo, Japan) with a Majesty JCA-BM8600 analyzer (Japan Electron Optics Laboratory Co., Ltd., Tokyo, Japan). Plasma triglycerides were quantified by the Glycerol kinase - Pyruvate kinase-Lactate dehydrogenase (GK-PK-LD) method (Kyowa Medex), again with the same analyzer. HDL-C and low-density lipoprotein cholesterol (LDL-C) were quantified by the homogeneous method (Kyowa Medex) with the same analyzer. Levels of apolipoprotein (apo) A-I, A-II, B, C-II, C-III, and E were measured by turbidimetric immunoenzymoassay (Sekisui Medical Co., Ltd., Tokyo, Japan) with an H-7600 analyzer (Hitachi High-Technologies Co., Ltd., Tokyo, Japan).

Renal Biopsy

We performed a renal biopsy with a 16G (22 mm needle and obtained tissue from the lower pole of the left kidney.

Serum LCAT activity

Serum LCAT activity was measured by a sensitive method using proteoliposomes (Sekisui Medical Co., Ltd., Tokyo, Japan) containing apo A-I as the substrate. LCAT mass was determined by radioimmunoassay using a rabbit anti-human LCAT antibody (Daiichi Pure Chemicals Co., Ltd., Tokyo, Japan).

Genetic analysis

Written informed consent was obtained from the patient.

Genomic DNA was isolated from peripheral white blood cells according to the standard procedure using a DNA Extractor WB Kit (Wako, http://www.wako-chem.co.jp/, Japan). We performed polymerase chain reactions (PCRs) to amplify all the exons and exon-intron boundaries of the human LCAT gene by using an aliquot (0.4 mg) of genomic DNA together with pairs of forward and reverse primers and Taq DNA polymerase (Takara Bio, http://www.takara-bio.co.jp/, Japan). The PCR products were run on 1% NuSieve GTG agarose gels (Takara Bio, Japan) and purified by phenol/chloroform extraction after excision from the gels. The purified products were added to a Big Dye Terminator ver. 1.1 Ready Reaction mix

Fig. 1. Appearance of the cornea
Severe corneal opacity prevented funduscopy.
Results

Serum lipid levels are summarized in Table 1. HDL-C and LDL-C concentrations were low. The cholesteryl ester ratio was very low, suggesting a deficiency of LCAT. Although apo A-I, A-II, C-II and C-III levels were low, the apo E level tended to be high, and the apoB level was normal. Serum LCAT activity and mass were not detectable, being below the limit of sensitivity of the assays.

The renal biopsy specimen contained 31 glomeruli, three of which showed global sclerosis. Irregular thickening and vacuolation of the GBM were recognized by Periodic acid-methenamine-silver (PAM) staining. Vacuoles were also seen in mesangial areas. Electron microscopy revealed characteristic high-density deposits in the GBM and mesangial areas (Fig. 2a). Electron microscopy revealed, characteristic high-density deposits in the GBM and mesangial areas (Fig. 2b). There were few tubulo-interstitial changes, but deposits were detected in some arteries (Fig. 3). From these characteristic findings, a diagnosis of LCAT deficiency was made.

Genetic analysis confirmed a homozygous C to T
A point mutation at nucleotide 501 (g.501C>T) of exon 4 at codon 140, resulting in an arginine (Arg) to cysteine (Cys) amino acid substitution (A140C) in the patient. The sequence is shown in Fig. 4. This one base change is not found in the general population.

We performed treatment with an angiotensin II receptor blocker (ARB), but the patient’s compliance was poor and his renal function gradually declined while his proteinuria increased.

**Discussion**

The patient had had corneal opacity since childhood, and blood tests revealed an extremely low HDL-C level. These two findings are typical of LCAT deficiency. An absence of LCAT activity means a lack of esterification of free cholesterol in plasma to produce cholesteryl esters. As a result, free cholesterol and phosphatide accumulate in various organs, so this disease can present with multiple symptoms. Because LCAT is an enzyme involved in the esterification of free cholesterol, the formation of HDL-C is inhibited in this disease, resulting in changes in the structure and levels of all lipoproteins. If LCAT activity is low, free cholesterol cannot be processed and accumulates in blood. Because free cholesterol or phospholipids are deposited in the cornea, corneal opacity occurs from childhood. It has been reported that the levels of phospholipids and free cholesterol are increased in erythrocyte membranes, leading to dysfunction and anemia.

In severe cases, LCAT deficiency leads to kidney failure and a major factor determining the prognosis of these patients is renal impairment that requires di-
ysis). The cause of renal insufficiency is the deposition of surplus free cholesterol and phospholipids in the kidneys. As with other kidney diseases, a control of blood pressure and proteinuria is important to prevent the progression of renal impairment. In recent years, high-dose angiotensin receptor blocker therapy has been used for LCAT deficiency. In addition, a low-fat diet is necessary to prevent lipid deposition in tissues. To avoid nephropathy, a low-fat and low caloric diet is considered important and it may be useful to become a vegetarian.

We performed a genetic analysis in the present study. The LCAT gene is 4.2 kilobases in size and located in the q21-22 region of chromosome 16. Our patient had a homozygous C to T point mutation at nucleotide 501 (g.501C>T) of exon 4 at codon 140, resulting in an arginine (Arg) to cysteine (Cys) amino acid substitution (A140C). This mutation has been reported in another patient in Japan (conference proceeding), though the two individuals are not related. This is the first report about the gene mutation in English. A patient from Austria was reported to have a single G to A nucleotide transition in exon 4, resulting in a A140H substitution.

The substitution of Cys for Arg is a more important mutation than that of His because of the marked change in the amino acid configuration. This difference in mutation may have caused the lower enzymatic activity.

Because we did not perform an animal experiment, the relation of the amino acid mutation to the influence on enzymatic activity is not clear. Regarding the case from Austria, the Arg to His substitution is a minor change, because Arg and His are the amino acids with a positive electric charge and a similar structure. However, the change from Arg to Cys markedly alters the configuration of the amino acid as well as the electric charge. As a result, the enzymatic albuminous structure changes greatly, and it is thought that this has a marked influence on the enzymatic activity.

There have been no reports of a mutation like that of our patient outside Japan, suggesting this mutation to be specific to Japanese.

In conclusion, we performed a genetic analysis in a patient with LCAT deficiency and confirmed the existence of a mutation that has only been reported in Japan.

References