Possible Induction of Renal Dysfunction in Patients With Lecithin:Cholesterol Acyltransferase Deficiency by Oxidized Phosphatidylcholine in Glomeruli

Shiro Jimi, Noriko Uesugi, Keijiro Saku, Hiroyuki Itabe, Bo Zhang, Kikuo Arakawa, Shigeo Takebayashi

Abstract—To clarify the causes of renal dysfunction in familial lecithin:cholesterol acyltransferase (LCAT) deficiency, kidney samples from 4 patients with LCAT deficiency (3 homozygotes and 1 heterozygote) were examined immunohistochemically. All of the patients exhibited corneal opacities, anemia, renal dysfunction, deficiencies in plasma high density lipoprotein and LCAT activity and mass, and an increase in the ratio of plasma unesterified cholesterol to esterified cholesterol. Renal lesions began with the deposition of lipidlike structures in the glomerular basement membrane, and these structures accumulated in the mesangium and capillary subendothelium. By electron microscopy, 2 types of distinctive structure were found in glomerular lesions: vacuole structures and cross-striated, membranelike structures. The plasma oxidized phosphatidylcholine (oxPC)–modified low density lipoprotein (LDL) levels in LCAT-deficient subjects were significantly (P<0.01) higher than those in controls (1.30±0.82 versus 0.42±0.32 ng/5 μg LDL, respectively), and a significant (P<0.01) difference was observed even after adjustment for confounding factors by an analysis of covariance. The patient with the highest plasma oxPC-modified LDL had the most membranelike structures in the glomeruli and showed the greatest renal deterioration from a young age. In glomerular lesions, although there was an abundance of apoB and apoE, oil red O–positive lipids, macrophages, apoA1, and malondialdehyde were scarce. OxPC was found extracellularly in glomerular lesions, and although its distribution differed from that of apolipoproteins, it was quite similar to that of phospholipids. In conclusion, these results indicate that oxPC in plasma and glomeruli is distinctive for patients with LCAT deficiency. Therefore, oxPC may be a factor in the deterioration of kidneys in patients with familial LCAT deficiency. (Arterioscler Thromb Vasc Biol. 1999;19:794-801.)

Key Words: lecithin:cholesterol acyltransferase deficiency ■ oxidized phosphatidylcholine ■ modified LDL ■ kidney

Familial lecithin:cholesterol acyltransferase (LCAT) deficiency is a rare inherited disease. The amino acid sequence of the LCAT gene has been determined. Various mutations of the LCAT gene in patients with LCAT deficiency have been reported. Mutations in the LCAT gene are responsible for the enzyme activities and symptoms found in this disease, while the clinical manifestations of such patients vary, even among members of the same family with identical mutations. Therefore, factors other than gene mutation may be involved in the clinical manifestation of patients with LCAT deficiency.

LCAT binds to HDL to catalyze the conversion of unesterified cholesterol and phosphatidylcholine (PC) to esterified cholesterol and lysophosphatidylcholine. A lack of LCAT activity causes an increase in unesterified cholesterol and phospholipids and a decrease in esterified cholesterol, which results in abnormalities of all kinds of lipoprotein particles and structures. Such lipid abnormalities are found in some organs in patients with LCAT deficiency, such as the kidney, cornea, and erythrocytes, and these changes clinically correspond to renal insufficiency, corneal opacities, and hemolytic anemia, respectively.

LCAT affects the plasma level of HDL through its role in HDL maturation. HDL is involved in the mechanism of reverse cholesterol transport by which free cholesterol is removed from peripheral tissue. Recently, Subbaiah and Liu showed that LCAT can also remove oxidized lipids. In atherogenesis, the accumulation of cholesterol derived from LDL is thought to be an important event in the progression of atherosclerotic lesions, in which oxidatively modified LDL (oxLDL) plays a central role. It has been shown that oxLDL in human atherosclerosis contains malondialdehyde...
(MDA) and oxidized PC (oxPC).28, 29 On the other hand, HDL has been shown to prevent the progression of atherosclerosis.23 Thus, patients with LCAT deficiency may have severe atherosclerosis due to extremely low levels of HDL, and mechanisms similar to atherogenesis may be involved in the progression of renal lesions in LCAT deficiency. However, lipid accumulation in atherosclerosis is not uniformly observed in patients with LCAT deficiency,1 and the mechanism of the development of renal lesions in LCAT deficiency is still unclear.

In the present study, to clarify the causes of renal dysfunction in LCAT deficiency, kidney biopsy (Bx) specimens from 4 LCAT-deficient patients with renal dysfunction were examined immunohistochemically. The plasma oxPC-modified LDL levels were also measured. Another LCAT-deficient subject with normal renal function did not undergo a renal Bx. Thus, only plasma lipid, lipoprotein, and oxPC levels were investigated in this case.

Methods

Patients

Five patients with familial LCAT deficiency from 4 different families, including a pair of brothers with the same gene mutation (case 4Y, younger brother and case 4E, elder brother), were investigated. An immunohistochemical study was conducted in 4 kidney Bx specimens from 4 of these patients with renal dysfunction, and plasma lipoproteins and oxPC were measured in all 5 patients. As shown in Table 1, all of the patients exhibited corneal opacities in childhood, and all but case 4E also showed renal manifestations; ie, proteinuria started in childhood (case 2) or adulthood (cases 1, 3, and 4Y). Renal dysfunction became apparent many years after proteinuria. Cases 2, 3, and 4Y underwent hemodialysis, and case 1 showed progressive renal failure during follow-up. Case 2 showed the most rapid renal deterioration among these patients. The respective parents of cases 1, 2, and 3 were consanguineous. The laboratory findings of all of the patients are listed in Table 1. The family histories of cases 1, 3, 4Y, and 4E have been described in detail elsewhere26, 28 and are described briefly herein. The study was approved by the Ethics Committee of Fukuoka University Hospital and informed consent was obtained from each patient.

Case 1

Case 1 underwent his first renal Bx at the age of 28 years when he was hospitalized for treatment of proteinuria (2 to 2.5 g/d) and hematuria. Although the plasma level of creatinine and creatinine clearance were normal at the time, renal Bx showed mild mesangial proliferative glomerulonephritis with segmental sclerosis.6 At the age of 33 years, he underwent a second Bx because his plasma total protein had decreased from 7.3 to 4.6 g/dL over that period, and he also exhibited nephrotic syndrome. The renal Bx showed advanced mesangial proliferative glomerulonephritis with deposition of foamy materials in glomeruli. By electron microscopy, irregularly shaped vacuoles were found within the glomerular basement membrane (GBM) and mesangial areas. Some capillary loops were distended with lipidlike, foamy material. Plasma lipid analysis revealed LCAT deficiency, and his sister also had corneal opacities and renal dysfunction. An LCAT gene analysis revealed the deletion of G at base 873 in exon 6, leading to a premature termination by frame shift.

Case 2

Proteinuria and hematuria were noted at age 7 years in a school health examination. He underwent 2 renal biopsies at ages 11 and 13 years, by which he was diagnosed with membranous glomerulonephritis. Prednisolone therapy was administered for several years but did not reduce proteinuria or hematuria. During this period, his renal function gradually deteriorated, and uncontrollable, severe hypertension and nephrotic syndrome appeared. A third Bx was performed at age 20 years; LCAT deficiency was strongly suspected from the biopsy specimen, because lipidlike, foamy material appeared to have been deposited within an expanded mesangium. His plasma and blood cells were sent to our research laboratory at Fukuoka University. LCAT gene analysis revealed a single C-to-G mutation, which converted Pro 250 (CCC) to Arg (CGC) in exon 6. His renal function deteriorated rapidly thereafter, and hemodialysis was started at age 21.30

Cases 4Y and 4E

Owing to severe decreases in the plasma levels of HDL cholesterol (HDL-C), LCAT activity, and LCAT mass, cases 4Y and 4E were diagnosed with LCAT deficiency at the ages of 38 and 46 years, respectively. They have been previously reported as homozygous cases based on their remarkable clinical features.12 Case 4Y, the younger brother, underwent a renal Bx at age 44 years owing to moderate proteinuria and moderate anemia; this Bx revealed that lipidlike, foamy material had been deposited in expanded capillary loops and mesangium. In contrast, case 4E did not have any renal manifestations and therefore did not undergo a renal Bx. Case 4Y had mild glucose intolerance at age 35 years, while case 4E was a vegetarian and consumed a low-calorie and low-fat diet (< 25 g/d). DNA sequence analysis of the LCAT gene was recently performed in

### Table 1. Clinical Manifestations and Gene Mutations in Patients With LCAT Deficiency

<table>
<thead>
<tr>
<th>Patients</th>
<th>Case 1(6)</th>
<th>Case 2(30)</th>
<th>Case 3(6,10,11)</th>
<th>Case 4Y(12,15,31)</th>
<th>Case 4E(12,15,31)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age in 1998, y</td>
<td>39</td>
<td>23</td>
<td>50</td>
<td>49</td>
<td>57</td>
</tr>
<tr>
<td>Clinical condition in 1998</td>
<td>CRF</td>
<td>HD</td>
<td>HD</td>
<td>HD</td>
<td>NRD</td>
</tr>
<tr>
<td>Gene mutation</td>
<td>G 873 Pro 250</td>
<td>G 344</td>
<td>Met 293</td>
<td>Met 293</td>
<td></td>
</tr>
<tr>
<td>Zygosity</td>
<td>Homo</td>
<td>Homo</td>
<td>Homo</td>
<td>Hetero</td>
<td>Hetero</td>
</tr>
</tbody>
</table>

HD indicates hemodialysis; CRF, chronic renal failure; and NRD, no renal disease.
TABLE 2. Laboratory Findings in Patients With LCAT Deficiency

<table>
<thead>
<tr>
<th></th>
<th>Normal Values</th>
<th>Case 1</th>
<th>Case 2</th>
<th>Case 3</th>
<th>Case 4</th>
<th>Case 4E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>(27–37)</td>
<td>20</td>
<td>35</td>
<td>41</td>
<td>45</td>
<td>38</td>
</tr>
<tr>
<td>Bx</td>
<td>Bx1</td>
<td>Bx2</td>
<td>Bx3</td>
<td>Bx1</td>
<td>Bx2</td>
<td>Bx3</td>
</tr>
<tr>
<td>Proteinuria*</td>
<td>(–)</td>
<td>2+</td>
<td>2+</td>
<td>3+</td>
<td>3+</td>
<td>3+</td>
</tr>
<tr>
<td>Hematuria†</td>
<td>(–)</td>
<td>+</td>
<td>2+</td>
<td>2+</td>
<td>3+</td>
<td>2+</td>
</tr>
<tr>
<td>Plasma-total protein, g/dL</td>
<td>(67–83)</td>
<td>7.3</td>
<td>4.6</td>
<td>4.8</td>
<td>4.0</td>
<td>4.2</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>(39–52)</td>
<td>40.2</td>
<td>32.9</td>
<td>32.5</td>
<td>23.8</td>
<td>26.4</td>
</tr>
<tr>
<td>Plasma-urea nitrogen, mg/dL</td>
<td>(6–22)</td>
<td>17</td>
<td>18</td>
<td>28</td>
<td>37</td>
<td>18</td>
</tr>
<tr>
<td>Plasma-creatinine, mg/dL</td>
<td>(0.4–1.0)</td>
<td>1.2</td>
<td>1.2</td>
<td>1.4</td>
<td>2.0</td>
<td>1.2</td>
</tr>
<tr>
<td>Creatinine-clearance, mL/min</td>
<td>(&gt;62)</td>
<td>80</td>
<td>80</td>
<td>47</td>
<td>63</td>
<td>52</td>
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</tbody>
</table>

Plasma

<table>
<thead>
<tr>
<th>Value</th>
<th>Case 1</th>
<th>Case 2</th>
<th>Case 3</th>
<th>Case 4</th>
<th>Case 4E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total chol, mg/dL</td>
<td>(130–120)</td>
<td>191</td>
<td>244</td>
<td>311</td>
<td>165</td>
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<tr>
<td>Free chol, mg/dL</td>
<td>(30–60)</td>
<td>232</td>
<td>153</td>
<td>97</td>
<td>129</td>
</tr>
<tr>
<td>Free/total chol</td>
<td>(0.14–0.46)</td>
<td>0.95</td>
<td>0.93</td>
<td>0.88</td>
<td>0.91</td>
</tr>
<tr>
<td>Triglyceride, mg/dL</td>
<td>(50–150)</td>
<td>124</td>
<td>833</td>
<td>771</td>
<td>257</td>
</tr>
<tr>
<td>Phospholipid, mg/dL</td>
<td>(160–260)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL-C, mg/dL</td>
<td>(40–96)</td>
<td>...</td>
<td>17</td>
<td>...</td>
<td>11</td>
</tr>
<tr>
<td>LCAT activity, (nmol/mL)/h at 37°C</td>
<td>(60–120)</td>
<td></td>
<td>...</td>
<td>...</td>
<td>10</td>
</tr>
<tr>
<td>(ozPC)</td>
<td>(697–749)</td>
<td></td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>LCAT mass, μg/mL</td>
<td>(4.4–8.1)</td>
<td>0.43</td>
<td>...</td>
<td>0.43</td>
<td>...</td>
</tr>
</tbody>
</table>

**Chol indicates cholesterol; *semiquantitative biuret method; †Nagasaki-Akanuma method (LCAT activity); ‡using a proteoliposome as a substrate (LCAT activity); §Bx1 was performed at the age of 44; and ... indicates that data were not measured.**

Our laboratory at Fukuoka University. We detected a heterozygous variant only at Met 294 Ile, without other defects or compound heterozygosity, which indicated that this case is heterozygous dominant.11

In the present study, we used several renal Bx specimens from sequential biopsies in each case: for morphological examination in case 1, Bx1 and Bx2; case 2, Bx3; case 3, Bx3; and case 4Y, Bx1. For immunohistochemical analysis, we used the following: in case 1, Bx2; case 2, Bx3; case 3, Bx3; and case 4Y, Bx1.

**Plasma Lipoprotein Levels and LCAT Activity and Mass**

Blood samples were obtained intravenously after an overnight fast. Total cholesterol, free cholesterol, triglyceride, and phospholipid concentrations in plasma were measured by enzymatic methods.24,25 HDL-C was measured by the heparin-calcium precipitation method.24 Plasma LCAT activity was measured using a proteoliposome as a substrate (cases 1 and 3) as previously described26 or by using dipalmitoyl PC (Nagasaki-Akanuma method; cases 2, 4Y, and 4E) as a substrate. LCAT mass (cases 1, 3, 4Y, and 4E) was measured by radioimmunoassay using a polyclonal antibody and 125I-labeled LCAT as previously described27 by Dr J.J. Albers, Washington University, Seattle.

**LCAT Gene Analysis**

LCAT gene analysis in cases 1 and 3 has been reported by Moriyama et al.6 Genomic DNA from cases 2, 4Y, and 4E was isolated from 100 μL of peripheral blood. Four DNA segments of the LCAT gene were amplified by polymerase chain reaction (PCR), as previously reported.6,7 PCR-amplified DNA was purified by ultrafiltration and directly sequenced by the dideoxynucleotide chain-termination reaction on an ABI 373 DNA sequencer.

**Pathological Examination of Renal Bx’s**

Light and electron microscopy and immunofluorescence examination were routinely performed for Bx specimens. For light microscopy, paraffin-embedded sections were stained with hematoxylin-eosin, periodic acid–Schiff, and periodic acid–methenamine silver. Frozen sections were used for direct immunofluorescence staining by using antiseras for immunoglobulins (IgG, IgM, and IgA), complement (C3 and C1q), and fibrinogen. For lipid analysis, serial frozen sections were subjected to oil red O staining for esterified cholesterol; Nile blue staining for neutral lipids (red), esterified cholesterol (pink), and acidic lipids (blue); and acid-hematin staining for phospholipids. For electron microscopy, Bx samples were fixed with 1.4% glutaraldehyde, postfixed with OsO4, and embedded in Epon resin. Ultrathin sections were double-stained with lead citrate and uranyl acetate and observed under an electron microscope (100-CX, JEOL).

**Immunohistochemical Staining**

Serial sections of frozen and paraffin-embedded samples were used for immunohistochemical staining by using an alkaline phosphatase and an anti–alkaline phosphatase (DAKO) method. We used commercial primary antibodies against human apoB (Chemicon), apo E (Chemicon), apoA1 (Chemicon), collagen types I (Chemicon) and IV (Siseido Inc), plasma fibronectin (Chemicon), cellular fibronectin (Chemicon), and human macrophages (HAM56, DAKO). We also used monoclonal antibodies for human oxLDL, which primarily recognize MDA (DLH2)28 and oxPC (DLH3).29 These antibodies can react with copper-oxidized LDL but not with native human LDL.

**Level of OxPC-Modified LDL in Plasma**

Blood samples were obtained intravenously by using EDTA as an anticoagulant from controls and 5 patients with LCAT deficiency. We measured oxPC in the sex- and age-matched controls (males, n = 31) who showed normal coronary arteries as determined by coronary angiography (controls). As for LCAT-deficient subjects, fresh plasma samples were airmailed to our laboratory from their respective physicians in charge, and then oxPC levels for the 5 patients were assayed at the same time in January 1997. For the measurement of oxPC, we used only fresh plasma samples (containing 0.25 mmol/L EDTA) and kept at 4°C. Measurement was completed within 1 week. EDTA was used as an antioxidant. The monoclonal anti–oxPC antibody (DLH3), which was originally developed to detect human oxLDL,29 was used to determine the level of oxPC-modified LDL in plasma. After the LDL fraction...
(1.019<d<1.063) had been isolated by sequential ultracentrifugation, the concentrations of oxPC-modified LDL in plasma were measured by a sandwich ELISA method and are expressed as the oxPC concentration in 5 μg LDL protein and using copper-oxidized LDL as the standard, as previously described. Measurements were performed in duplicate, and the interassay and intra-assay coefficients of variation ranged from 7% to 10%.

Statistical Analysis
Statistical analysis was performed using the SAS Software Package (version 6, Statistical Analysis System, SAS Institute Inc). Categorical variables (such as hypertension) were compared between cases and controls by a χ² analysis. The distribution of variables was examined by the Shapiro-Wilk test. Variables that were normally distributed were compared between groups by an ANOVA. Variables that were not normally distributed were compared between groups by the nonparametric Wilcoxon rank-sum test. Age, hypertension, and diabetes mellitus were adjusted for by an ANCOVA. All probability value are 2-tailed. The significance level was considered to be 5% unless indicated otherwise.

Results

LCAT Gene Mutation and Clinical and Laboratory Findings in Patients With LCAT Deficiency
The gene mutations and the clinical and laboratory findings in each patient during their follow-up periods are shown in Tables 1 and 2, respectively. Sequence analysis of the LCAT gene revealed different gene mutations in each case, except 4Y and 4E, who were brothers with an identical mutation. Cases 1, 2, and 3 showed homozygous, single-gene mutations. Cases 4E and 4Y had a heterozygous missense mutation; ie, a G-to-A transition at position 4660 that changed Met 293 (ATG) to Ile (ATA). All patients showed laboratory findings characteristic of classic LCAT deficiency, such as normocytic normochroemic anemia with the appearance of target red blood cells. An increased ratio of plasma free cholesterol to total cholesterol, a minimum level of plasma HDL-C, suppressed plasma LCAT activity, and decreased LCAT mass were observed (Table 2). Fragility of red blood cell membranes was detected in cases 1, 3, and 4Y but was not checked in case 2. Case 2, the youngest in this group, showed severe renal dysfunction with lipid abnormalities, all of which are typical of LCAT deficiency. During a 10-year follow-up, HDL-C levels were greatly decreased while the ratio of free to total cholesterol increased in cases 4Y and 4E. Although LCAT activity increased slightly in case 4Y at age 48 compared with that at age 38 [21 versus 3 (nmol/mL)/h at 37°C, respectively], LCAT mass was deficient in cases 1, 3, 4Y, and 4E.

Morphological Alterations in Renal Bx Specimens From Patients With LCAT Deficiency

Light and Immunofluorescence Microscopy
Although the extent of renal involvement by light microscopy varied between cases and the time of Bx, characteristic features of the glomerular lesions in the renal specimens used in this study were vacuolization of the GBM (Figure 1A); “ballooning” of loops filled with lipidic, foamy material (Figure 1A); and mesangial expansion with pale staining, with a “fluffy” appearance on periodic acid–Schiff staining. These glomerular lesions were found in all patients except 4E, who never underwent a renal Bx. Renal lesions began with small deposits of lipidlike structures in the GBM. Figure 1A shows diffuse vacuolization, capillary ballooning, and tuft adhesion in moderately affected glomeruli. Mesangial expansion became more apparent in advanced glomerular lesions, in which foamy cells were scarcely found in the glomeruli. As glomerular involvement progressed, interstitial damage with chronic inflammatory cell infiltration, tubular atrophy, fibrosis, and hyaline arterioles was also apparent. In the immuno-fluorescence analysis, IgM and C3 were positive in cases 1
and 4Y, whereas IgG, IgA, and C1q and fibrinogen were negative in all cases.

**Electron Microscopy**
Two distinct structures were observed in glomerular lesions, ie, variously sized electron-lucent vacuoles with or without osmiophilic particle cores (Figure 1B and 1C) and cross-striated, membranelike structures (Figure 2). The deposition of membranelike structures was found in cases 2, 3, and 4Y, while the amounts of such structures found in each case followed the order case 2 > case 3 > case 4Y, and case 2 showed a particularly large accumulation in the GBM (Figure 2B) and mesangium (Figure 2C). On the other hand, lucent vacuoles were deposited in the subendothelium, the GBM (Figures 1B and 2B), and expanded mesangium (Figure 1C) in cases 1, 2, 3, and 4Y. Some capillary loops were expanded with vacuolar structures. Thinning of the GBM, detachment of endothelial cells, fusion of epithelial foot processes, and/or tuft adhesion were frequently observed near the regions where these structures had accumulated in large amounts. In such glomeruli, no foamy cells were found.

**Immunohistochemical Analysis of Accumulated Lipids in Glomerular Lesions**
Accumulated lipids in glomerular lesions of subjects with LCAT deficiency were analyzed by special staining for lipids and immunohistochemical techniques. The lesions contained minimum amounts of oil red O–positive (Figure 3A) and Nile blue–positive lipids, whereas glomeruli were positive throughout for acid-hematin staining for phospholipids (Figure 3C). As a result, no acid-hematin–positive materials were found in normal glomeruli. Tubular epithelial cells were sometimes positive for oil red O. Immunohistochemically, distended loops and subendothelium were strongly positive for apoB (Figure 3E) and apo E (Figure 3F), whereas the
observed.1 In our study, 1 patient (case 4Y) with a heterozygous variant at Met 294 Ile, without other defects or compound heterozygosity. We think that these 2 cases (4Y and 4E) are heterozygous dominant on the basis of their clinical features, as well as on previously reported data of LCAT activities and mass.

Regarding these heterozygous brothers, case 4E was a vegetarian, and although corneal opacities were seen earlier, renal impairment had not yet appeared by the age of 56 years, whereas case 4Y was diagnosed with chronic renal failure at age 45. This difference in disease progression between brothers may be due to the difference in their diet, as described in the Patients section, but it also is likely that additional factors are involved in case 4Y. Circulating oxPC levels vary among individuals, as shown in Figure 4 and in our previous report.38 Sevanian et al41 reported a similar tendency. The level of oxPC-modified LDL in case 4E was ~68% of that in case 4Y. Therefore, we are not sure that the difference between them is significant; the data suggest that dietary habits may affect the production of oxPC, and the level of oxPC-modified LDL may reflect the severity of renal impairment in this gene mutation.

HDL has been shown to remove not only cholesterol but also oxidized lipids from peripheral tissue via reverse cholesterol transport, which is affected by LCAT activity.22 Therefore, decreased LCAT activity may severely impair the removal of oxidized lipids from peripheral tissues, resulting in the activation of scavenger receptors and subsequent lipid accumulation.26 In normal men without an LCAT gene defect, this mechanism plays a role in the progression of atherosclerosis,26 and such lesions contain large amounts of oil red O–positive lipids, esterified cholesterol, lipoproteins, and oxLDL, including both oxPC and MDA.27,28 On the other hand, oxPC and MDA are rarely, if ever, found in normal glomeruli and, when present, are accumulated in cells such as macrophages. However, in the glomerular lesions of LCAT deficiency, although there was abundant accumulation of apoB and apo E, oil red O–positive lipids, apoA1, and MDA were scarce, and only a few macrophages were detected in such glomeruli. Moreover, although the distribution of oxPC (Figure 3D) differed from that of apolipoproteins, it was similar to that of phospholipids (Figure 3C). These results suggest that oxPC in affected glomeruli in LCAT deficiency may not be carried in by lipoproteins but rather by other vehicles such as albumin, or they may be produced by glomerular cells themselves.

By electron microscopy, 2 types of distinctive structure were accumulated in glomerular lesions, ie, foamy structures and cross-striated, membranelike structures. On the basis of our experience, these 2 structures are not common in any other renal diseases. The abundant accumulation of both structures was found in glomeruli in all of the cases, and they were also found in the vessel wall and tubular basement membrane to some extent. Interestingly, 3 cases (cases 2, 3, and 4Y) with higher plasma levels of oxPC-modified LDL had membranelike structures in glomeruli, and the highest level of plasma oxPC-modified LDL was seen in case 2, who also showed the greatest renal deterioration. His glomerular lesions showed the greatest accumulation of membranelike structures (Figure 2B and 2C). These immunohistochemical and electron microscopic findings suggest that the cross-striated, membranelike structures may contain oxPC. However, the exact relationship between oxPC and these struc-

Figure 4. Levels of oxPC-modified LDL in patients with LCAT deficiency. The levels of oxPC-modified LDL were measured by a sandwich ELISA. All patients with LCAT deficiency (black bars) exhibited higher levels of oxPC-modified LDL than did healthy normal controls (open bar, mean ± SD).
tures is still unclear. An increase in PC in several different organs, including plasma, has been shown in LCAT deficiency,\textsuperscript{16} whereas an elevated level of sphingomyelin in the affected cornea is a characteristic of fish-eye disease,\textsuperscript{42} which is analogous to LCAT deficiency except that the kidney is not affected,\textsuperscript{43,44} perhaps due to some remaining LCAT activity. Stokke et al\textsuperscript{16} reported that a patient with LCAT deficiency had received a transplanted normal kidney; however, when his renal function became worse, the transplanted kidney was removed and analyzed biochemically. The removed kidney, especially the glomeruli, showed a remarkably high level of PC, suggesting that this change may have been induced by the circulating blood in this patient. In LCAT-knockout mice, the development of glomerular lesions has been noted.\textsuperscript{45} It is unclear why the kidney is a main target organ in LCAT deficiency. We found that oxPC accumulated in glomeruli in a patient with LCAT deficiency. Subbaiah and Liu\textsuperscript{24} postulated that LCAT may play a role in oxPC catabolism. Moreover, in a recent in vitro study, we found that LCAT can metabolize oxPC. After oxidized 1-palmitoyl-2-[\textsuperscript{14}C]linoleoyl PC was incubated with human plasma, nonpolar reaction products were separated by thin-layer chromatography. Several radioactive bands in addition to those for cholesteryl ester were formed in a dose- and time-dependent manner. These products were not formed from native 1-palmitoyl-2-[\textsuperscript{14}C]linoleoyl PC. Plasma from LCAT-deficient patients also failed to form additional products from oxPC. These data suggest that LCAT is capable of metabolizing a variety of oxidized products of PC and of preventing the modification of LDL (H.I., unpublished data, 1998). These findings suggest that oxPC preferentially accumulates in glomeruli by unknown mechanisms, and such oxPC cannot be removed by LCAT-deficient plasma, which may ultimately cause renal dysfunction.

Recent studies have indicated that HDL inhibits the oxidative modification of LDL,\textsuperscript{47,48} and that oxLDL inhibits LCAT activity.\textsuperscript{49,50} Sevanian et al\textsuperscript{51} reported that oxidatively modified plasma LDL is found largely among the small, dense LDL fraction, which is atherogenic. LDL particle sizes were not determined in our LCAT-deficient subjects in relation to plasma oxPC levels. However, we recently measured LDL particle size as related to the fractional esterification rate of cholesterol in VLDL- and LDL-depleted plasma, which reflects the reactivity of HDL to LCAT, in patients with coronary heart disease,\textsuperscript{52} and found that this rate was positively correlated with plasma apoB levels and negatively correlated with plasma HDL-C and LDL particle size. Therefore, decreased plasma HDL in LCAT-deficient patients accelerates the oxidation of LDL via several complicated, and still theoretical, pathways.

In this study, higher levels of oxPC-containing oxLDL were noted in all of the patients. OxLDL could suppress the remaining LCAT activity in patients with LCAT deficiency, and this may also be involved in the progression of renal impairment in LCAT deficiency. Therefore, oxPC may play an important role in renal dysfunction in familial LCAT deficiency.

Acknowledgments

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References


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