Molecular characterization of two patients with severe LCAT deficiency

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Introduction

Lecithin:cholesterol acyltransferase (LCAT; EC 2.3.1.43) is the major enzyme responsible for the esterification of cholesterol in circulating plasma lipoproteins. This is important in the human because unlike many other animal species, cholesterol exported into the plasma from the liver is largely unesterified. LCAT associates in plasma preferentially with the discoidal apo A-I containing high-density lipoproteins (preb₁-HDL), where it esterifies the free cholesterol (FC) using apo A-I as co-factor [1]. Through this action, LCAT plays a central role in both HDL maturation to α-migrating spherical HDL and in reverse transport of cholesterol from peripheral tissues to the liver, directly by interaction of the mature HDL with the hepatic SR-BI receptor, or indirectly by transfer of CE by cholesteryl ester transfer protein (CETP) to the VLDL and then LDL, much of which is ultimately cleared by the liver via the LDL receptor [2]. In addition, LCAT protein seems to have a scavenger effect toward LDL oxidation products, which is independent of its cholesterol-esterifying activity [3]. Mutations of LCAT (chr. 16q22.1, 6 exons) in homozygous or compound heterozygous form can cause two major phenotypes: FLD (familial LCAT deficiency) and FED (Fish Eye Disease). Patients with FLD (OMIM No, 245900) have a complete loss of α-LCAT activity (i.e. LCAT activity exerted on HDL) and β-LCAT activity (i.e. LCAT activity exerted on LDL), an increased proportion (>80%) of unesterified cholesterol in plasma. Clinical manifestations generally include corneal opacification, anaemia and renal disease with proteinuria, which progresses to terminal renal insufficiency. In FED (OMIM No. 131620), there is partial loss of α-LCAT activity with normal or slightly elevated FC in plasma and corneal opacification without renal disease [4,5]. Intermediate phenotypes have also been described. To date, more than 60 mutations of LCAT have been identified [4–6]; they involve all regions of the coding sequence and produce a variety of defects, including normal secretion of LCAT with total loss of catalytic activity, reduced secretion with a partial or total loss of catalytic activity, secretion of an unstable or rapidly catabolized enzyme, and complete degradation of the enzyme at its site of synthesis [4]. All subjects with FED or FLD have greatly reduced plasma HDL cholesterol concentrations (usually <0.3 mmol/l) and plasma levels of apo A-I below 50 mg/dl [6,7]; however, premature coronary artery disease is absent in most FLD cases, but sometimes present, for unclear reasons, in some patients with FED [4,6,8]. We investigated two unrelated patients with clinical features of LCAT deficiency. Two of the three LCAT mutations found in these patients were novel.

Case report

A 33-year-old Pakistani female (AI), whose parents were first cousins, had bilateral corneal opacification (Figure 1A) near the limbus, mild normochromic anaemia, no biochemical evidence of liver dysfunction, but a history of moderate and stable proteinuria (>3 g/day) with a normal level of plasma creatinine (71–77 μmol/l). She had normal blood pressure, but a history of arterial hypertension during...
pregnancy. Renal biopsy revealed mesangial expansion with the presence of mesangial foam cells and glomerular basement membrane thickening. Electron microscopy confirmed the presence of marked basement membrane abnormalities, with inclusions of lipid droplets in subepithelial, intramembranous, subendothelial, paramesangial and mesangial locations (Figure 1B). Laboratory analysis yielded the results shown in Table 1. HDL-C was very low, but both apo A-I and serum paraoxonase activity (PON1) were only at the lower end of the normal range; LDL cholesterol was not detectable and the percentage of FC was very high; LCAT activity was very low, but the LCAT mass was normal; CETP activity was also very low.

The second case was a 41-year-old English male (GW), who had bilateral corneal opacities with central stromal haze and more pronounced opacity near the limbus, normochromic anaemia, arterial hypertension and chronic renal failure (plasma creatinine 241 μmol/l) with nephrotic range proteinuria (9–10 g/day). A renal biopsy contained redundant and sclerosed glomeruli and advanced basement membrane changes in preserved glomeruli. There was moderate-to-severe interstitial fibrosis and tubular atrophy. Laboratory analysis yielded the results shown in Table 1 and results were very similar to those obtained for Case 1.

Both patients were apparently free of cardiovascular disease. No family members of these probands were available for the study. Both patients gave their informed consent for this case report.

Identification of mutations in the LCAT gene

The sequence of LCAT in AI revealed homozygosity for a c.295 T>C transition in exon 2, predicted to cause an arginine (CGG) for tryptophan (TGG) substitution at position 99 of LCAT protein (p.W99R) [W75R]. The mutation was confirmed by the restriction fragment analysis after Msp I digestion. GW was a compound heterozygote for the following LCAT mutations: (i) c.167 T>C transition in exon 2, predicted to cause a proline (CCG) for leucine (CTG) substitution at position 56 (p.L56P) [L32P]; and (ii) c.802 C>T transition in exon 6, predicted to cause a cysteine (TGC) for arginine (CGC) substitution at position 268 (p.R268C) [R244C]. Their mutations were confirmed by restriction fragment analysis after Sma I and Tsp RI digestion, respectively. The screening for the three LCAT mutations found in our probands was performed in 60 healthy randomly selected individuals from the general population and in 80 subjects with primary hypoalphalipoproteinaemia. No carriers of these mutations were found.

Comments

The clinical findings in AI, namely the corneal opacification without severe renal dysfunction, suggests an intermediate phenotype between FED and FLD [4]. However, the high percentage of FC in plasma, the very low LCAT activity and the histological findings on renal biopsy is more in keeping with FLD. She was homozygous for a novel mutation in exon 2 of LCAT predicted to cause an arginine (charged polar amino acid) for tryptophan (uncharged unpolar) substitution at position 99 (p.W99R) of the full-length protein, at position 75 [W75R] of the mature protein. Computational analysis using Poly-Phen (www.bork.embl-heidelberg.de/PolyPhen/), which predicts the effect of amino acid changes on protein function [9], indicates that this
sequence variation has a 'probably damaging effect', given the PSIC (Position-Specific Independent Counts) score difference of 3.995. On the other hand, the tryptophan at position 75 is conserved in LCAT of human, baboon, rabbit, mouse, rat, chicken and c.elegans [10]. This amino acid substitution is immediately adjacent to the interfacial recognition domain, involving residues 50–74, which contributes to the enzyme-substrate interaction [11]. Since the LCAT circulating mass in our patient was within the normal range, we can assume that the enzyme is normally secreted from the liver, but in a functionally inactive form, probably because of conformational changes of the peptide sequence required for substrate binding and/or interfacial recognition [11]. A novel salt bridge between R75 and some negatively charged residues may induce a conformational change, which prevents the substrate binding and so the catalytic activity.

In GW, the clinical characteristics were typical of FLD. Sequencing of LCAT showed compound heterozygosity. He carried two missense mutations, located in exon 2 of one gene and in exon 6 of the other. The former is predicted to cause a proline for leucine substitution at position 56 (p.L56P) of the full-length protein, at position 2 [L32P] of the mature protein. Computational analysis [9] of this mutation indicates a 'probably damaging effect' given the PSIC score difference of 2.710. Mutational events at this codon were previously described: (i) R244G (PSIC score difference of 2.675), in homozygous form, was found in a patient with FDL [11,15]; the LCAT mass and activity were 26% and 12% of the wild-type, respectively [5]; and (ii) R244H (PSIC score difference of 2.255), in heterozygous form, was described by our group in four related subjects with low HDL-C (0.67–0.80 mmol/l) and significantly reduced LCAT activity as compared with non-carrier family members [16].

In spite of very low HDL levels, our two probands were apparently free of cardiovascular disease, as judged by ECG stress testing and carotid ultrasound examination. This was in keeping with previous observation in patients with LCAT deficiency, especially in patients with FLD [4,6,8]. At least part of the explanation for this finding has recently been provided by kinetic studies, which demonstrated, in LCAT-deficient patients, an increased catabolism of LDL, which is abnormal in composition (poor in cholesteryl esters and rich in triglyceride and phospholipids), and an up-regulation of LDL receptor pathway [17]. This is the first report showing that serum PON1 activity, although at the lower end of the normal range in both patients and at about 50% of the average activity in healthy subjects, was not grossly decreased in LCAT deficiency. Interestingly, similarly preserved PON1 activities have been found in other HDL-deficiency states, such as Tangier disease [18] and FED [19]. This is despite the fact that physiologically, PON1 is physically strongly associated with HDL. It is, however, located on a subtraction of apo A-I containing HDL which contributes little

### Table 1. Summary of lipoprotein profiles of the LCAT-deficient patients

<table>
<thead>
<tr>
<th></th>
<th>AI</th>
<th>GW</th>
<th>(normal range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum cholesterol (mmol/l)</td>
<td>3.21</td>
<td>2.23</td>
<td>(3.53–5.91)</td>
</tr>
<tr>
<td>Free cholesterol (mmol/l)</td>
<td>3.09</td>
<td>1.93</td>
<td>(0.88–1.83)</td>
</tr>
<tr>
<td>Free: total cholesterol ratio</td>
<td>0.96</td>
<td>0.87</td>
<td>(0.19–0.30)</td>
</tr>
<tr>
<td>Serum triglycerides (mmol/l)</td>
<td>1.26</td>
<td>3.03</td>
<td>(1.15–1.63)</td>
</tr>
<tr>
<td>VLDL cholesterol (mmol/l)</td>
<td>0.25</td>
<td>0.48</td>
<td>(0.21–0.33)</td>
</tr>
<tr>
<td>HDL-C by heparin/MnCl₂ method (mmol/l)</td>
<td>0.16</td>
<td>0.11</td>
<td>(1.36–1.74)</td>
</tr>
<tr>
<td>VLDL triglycerides (mmol/l)</td>
<td>0.48</td>
<td>1.97</td>
<td>(0.45–0.69)</td>
</tr>
<tr>
<td>Direct HDL cholesterol (mmol/l)</td>
<td>0.35</td>
<td>0.18</td>
<td>(1.37–1.71)</td>
</tr>
<tr>
<td>Serum triglycerides (mmol/l)</td>
<td>1.26</td>
<td>3.03</td>
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<td>(0.88–1.83)</td>
</tr>
<tr>
<td>LCAT mass (g/ml)</td>
<td>4.8</td>
<td>3.1</td>
<td>(3.1–6.7)</td>
</tr>
<tr>
<td>LCAT activity (nmol/ml/h)</td>
<td>3.9</td>
<td>2.2</td>
<td>(24–66)</td>
</tr>
<tr>
<td>LCAT activity (nmol/ml/h)</td>
<td>&lt;1.0</td>
<td>&lt;1.0</td>
<td>(25–55)</td>
</tr>
<tr>
<td>LCAT mass (μg/ml)</td>
<td>4.8</td>
<td>3.1</td>
<td>(3.1–6.7)</td>
</tr>
<tr>
<td>CETP activity (nmol/ml/h)</td>
<td>2.90</td>
<td>0.14</td>
<td>(6–28)</td>
</tr>
<tr>
<td>APOE genotype</td>
<td>ε3ε3</td>
<td>ε3ε4</td>
<td></td>
</tr>
</tbody>
</table>

PON1, paraoxonase; LCAT, lecithin:cholesterol acyl transferase; CETP, cholesterol ester transfer protein. aNormal range based on 95% confidence interval; bEndogenous lipoprotein method; cExogenous substrate method.
to its overall concentration [20]. The overall presumption must be that even the small amount of apo A-I containing lipoproteins present in the circulation in LCAT deficiency and other genetic HDL deficiencies may be sufficient to maintain its levels. Together with the low levels of LDL cholesterol and apo B in LCAT deficiency, the maintenance of PON1 function may counteract the proatherogenic effect of low circulating levels of HDL. Furthermore, in vitro experiments showed that LCAT-deficient plasma was as efficient as control plasma in promoting the cholesterol efflux from cholesterol loaded fibroblasts [21], and that γ-LpE could be the acceptor of cholesterol from peripheral tissue in LCAT-deficient patients [22]. It remains to be established whether this mechanism operates in vivo in FLD. These two cases do, however, serve to illustrate the complexity of HDL metabolism and that it should not be assumed that decreases in HDL concentration are necessarily proatherogenic, or that increases are necessarily protective against atheroma: the mechanism by which the change occurs may also be important.

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Conflict of interest statement. None declared.

References


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