Case Report

A 33-year-old man with nephrotic syndrome and lecithin–cholesterol acyltransferase (LCAT) deficiency. Description of two new mutations in the LCAT gene

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Introduction

Familial lecithin–cholesterol acyltransferase (LCAT) deficiency is a rare autosomal recessive disease caused by mutation in the LCAT gene, located on chromosome 16q22 (GenBank accession nos: genomic DNA X04981, cDNA NM_000229). LCAT catalyses the formation of cholesteryl esters via the hydrolysis and transfer of sn-2 fatty acid from phosphatidylcholine to the 3-hydroxyl group of cholesterol. A deficiency of this enzyme leads to increased levels of phosphatidylcholine and unesterified cholesterol in the blood and to the formation of an abnormal lipoprotein (called 'lipoprotein-X') rich in both phosphatidylcholine and unesterified cholesterol. As a consequence, progressive lipid deposition occurs in various tissues, including the kidney [1], resulting in progressive glomerular sclerosis which becomes clinically manifest in the third to fourth decade of life and eventually leads to end-stage renal disease [2].

To date, 13 affected families have been found in Italy (including the one here described), but the disease may have been underdiagnosed.

We here report on a 33-year-old man investigated for steroid-resistant nephrotic syndrome and progressive deterioration of renal function where clinical, morphological and biochemical data led to the diagnosis of familial LCAT deficiency, confirmed by the identification of two new mutations in the LCAT gene.

Case

A 33-year-old man was admitted to our hospital for nephrotic proteinuria (4–6 g/day) and reduced renal function (serum creatinine 2.3 mg/dl; creatinine clearance 45 ml/min/1.73 m²). Proteinuria had occasionally been found 4 years earlier, when the glomerular filtration rate was within the normal range (serum creatinine 1 mg/dl), and was associated with an increase in total serum cholesterol (302 mg/dl) and tryglicerides (455 mg/dl); serum complement was within the normal range and the search for non-organ-specific autoantibodies was negative; a renal biopsy was carried out in another hospital which produced a diagnosis of ‘focal segmental glomerulosclerosis’. The patient was treated with a combination of gemfibrozil, simvastatin, enalapril and low dose acetylsalicylic acid without any significant improvement in proteinuria. Two years later, owing to the persistence of nephrotic proteinuria and an increase in serum creatinine (1.5 mg/dl), he underwent a second renal biopsy in a different hospital where the diagnosis of ‘focal segmental glomerulosclerosis’ was confirmed and a short course of steroids was undertaken without any improvement in urinary findings. In 2001, the patient was admitted to our hospital after a further increase in serum creatinine, which had now risen above 2 mg/dl.

On admission, physical examination showed moderate pedal oedema and normal blood pressure (120/80 mmHg). Corneal greyish opacities were observed.

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The family history revealed dyslipidaemia in the father, who had died at the age of 69 of myocardial infarction, and in a brother; a second brother and a sister had no sign of renal disease or lipid abnormalities. The family pedigree is reported in Figure 1.

Laboratory investigations showed: haemoglobin 10 g/dl, MCV 88 \( \mu m^3 \), WBC 4100/mm\(^3\) with a normal differential count, poikilocytosis and 'target' erythrocytes in peripheral blood film examination; haptoglobin 52 mg/dl (normal range 70–200 mg/dl); total serum proteins 5 g/dl with increased \( a_2 \) fraction at protidogram, erythrocyte sedimentation rate 99; serum complement fractions were within the normal range. Coomb's test and autoantibodies were negative. Total cholesterol was 306 mg/dl (while assuming fluvastatin 40 mg/day), high-density lipoprotein (HDL) cholesterol 16 mg/dl, triglycerides 587 mg/dl, apolipoprotein A-I 35 mg/dl (normal range 115–180), apolipoprotein A-II 5 mg/dl (normal range 26–51) and apolipoprotein B 51 mg/dl (normal range 70–160). LCAT activity was absent in the patient and in the brother with dyslipidaemia (reported as II2 in Figure 1) who had no proteinuria at urinalysis. Table 1 reports the lipid profiles of the family members investigated.

Genetic analysis showed that the proband was compound heterozygous for two novel mutations, which were designated according to the recommended nomenclature [3]. (i) A deletion of five nucleotides GCCCG (g.977–981del, c.141–145del) in exon 1. The mutation caused a frameshift after Arg23 with the introduction of 52 novel amino acids and the occurrence of a termination codon at position 76. (ii) A nucleotide transition in exon 5 (g.2439, c.614 G>A), which converted the codon 181 (AGC) for serine into a codon (AAC) for asparagine. The Ser181 residue is one of the components of the catalytic triad, which is conserved in all the animal species examined [4].

The proband’s brother carried both mutations; the other brother and the sister were heterozygous for the

A renal biopsy was carried out percutaneously. Immunofluorescence examination of renal tissue revealed diffuse granular C3 deposits in the mesangial area and on several glomerular capillary walls; focal and segmental granular deposits of IgM and fibrinogen were observed on the capillary walls.

Light microscopy showed global sclerosis involving seven out of 11 glomeruli; in the remainder, focal segmental glomerular sclerosis was observed along with mesangial expansion, a mild increase in mesangial cellularity and irregular thickening of the glomerular capillary walls, with vacuolization of the glomerular basement membrane due to intramembranous lipid deposits, resulting in a typical 'loamy' appearance. Diffuse tubular atrophy with thickening of the tubular basement membranes, along with focal interstitial fibrosis and mononuclear cells infiltrates, were also found.

Electron microscopy examination showed lipid deposits with both a vacuolar lucent appearance and electron-dense lamellar structures in the mesangial matrix, in the glomerular and tubular basement membranes, as well as in the Bowman’s capsule and endothelial cells.

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<p>| Table 1. Lipid profiles of the family |
|--------------------------------------------------|------------------|------------------|------------------|------------------|------------------|</p>
<table>
<thead>
<tr>
<th>Normal values</th>
<th>Subjects I1 (proband)</th>
<th>I2 (brother)</th>
<th>I3 (sister)</th>
<th>I4 (brother)</th>
<th>I2 (mother)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol</td>
<td>&lt;200 (mg/dl)</td>
<td>185</td>
<td>124</td>
<td>133</td>
<td>131</td>
</tr>
<tr>
<td>Free cholesterol</td>
<td>&lt;55 (mg/dl)</td>
<td>165</td>
<td>113</td>
<td>42</td>
<td>43</td>
</tr>
<tr>
<td>HDL-cholesterol</td>
<td>&lt;28 (mg/dl)</td>
<td>89</td>
<td>91</td>
<td>32</td>
<td>33</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>&lt;40 (mg/dl)</td>
<td>16</td>
<td>15</td>
<td>33</td>
<td>40</td>
</tr>
<tr>
<td>ApoA-I</td>
<td>&lt;150 (mg/dl)</td>
<td>587</td>
<td>409</td>
<td>144</td>
<td>115</td>
</tr>
<tr>
<td>ApoA-II</td>
<td>115–180 (mg/dl)</td>
<td>35</td>
<td>42</td>
<td>84</td>
<td>107</td>
</tr>
<tr>
<td>ApoB</td>
<td>26–51 (mg/dl)</td>
<td>5</td>
<td>5</td>
<td>22</td>
<td>29</td>
</tr>
<tr>
<td>LCAT activity</td>
<td>25–55 (nmol/ml/h)</td>
<td>0</td>
<td>0</td>
<td>23</td>
<td>14</td>
</tr>
</tbody>
</table>

FC/TC, free cholesterol/total cholesterol.
deletion in exon 1, and the mother was heterozygous for the missense mutation in exon 5.

Discussion

Clinical manifestations of familial LCAT deficiency include corneal opacities, haemolytic anaemia and renal involvement, initially characterized by proteinuria, with progressive deterioration of renal function [1]. End-stage renal disease, which generally occurs in the fourth decade of life, is the major cause of morbidity and mortality in these patients.

In the case reported here, corneal opacity along with a previous histological diagnosis of focal segmental glomerulosclerosis led us to consider the possibility of an LCAT deficiency. The presence of mild haemolytic anaemia (due to phospholipid abnormalities in the erythrocyte membrane), low HDL cholesterol and a family history of dyslipidaemia supported this hypothesis. The diagnosis was suggested further by the 'foamy' appearance of the glomerular basement membrane on light microscopy examination of silver-stained renal biopsy specimens and by the presence of typical lipid deposits within the glomerular basement membrane and podocytes at electron microscopic examination of renal tissue. The lipid profile and genetic analysis of the patient and his family members definitively confirmed the diagnosis by identifying mutations of the LCAT gene.

It is of interest that the brother (II2) does not show any urinary abnormality although he lacks LCAT activity and carries the same genetic mutations detected in the patient with renal failure. Obviously, we did not carry out a renal biopsy in this subject so we do not know whether some lipid deposition has occurred in his kidney, despite the absence of urinary abnormalities. It has already been observed that clinical manifestations of patients with LCAT gene mutations may vary even among members of the same family carrying identical mutations [5], so it is still unclear whether other factors besides lipid deposition in tissues may affect the clinical picture and, particularly, lead to the development of renal lesions. Immunohistochemical investigations, carried out on renal tissue obtained from LCAT-deficient subjects, has shown that oxidized phosphatidylcholine accumulates in the glomeruli of these patients, as a result of reduced or absent LCAT activity which is also able to remove oxidized lipids [6], and suggested that oxidized phosphatidylcholine may play an important role in inducing renal lesions [2]. More recently, it has been demonstrated that lipoprotein-X, which accumulates in subjects with LCAT deficiency, can stimulate monocyte chemoattractant protein-1 (MCP-1) expression in mesangial cells via nuclear factor-kB (NF-kB). This finding suggests that infiltrating monocytes can significantly contribute to renal damage by taking up large amounts of lipoprotein-derived lipids which results in foam cell formation and, on the other hand, by secreting a wide variety of cytokines and growth factors which can lead to development of glomerular sclerosis [7]. Whatever the mechanism(s) superimposed on lipid deposition leading to progressive renal damage, it is important to form a correct diagnosis not only to treat patients appropriately (and avoid useless and potentially dangerous drugs), but also to identify other affected family members and provide genetic counselling.

In conclusion, this case underlines once more the need for a careful physical examination of patients with otherwise unexplained renal disease, focusing attention on whether other organ systems are involved, and may suggest the correct diagnosis. It also stresses the importance of accurate family investigation whenever possible.

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Conflict of interest statement. None declared.

References

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