Case Report

A novel apolipoprotein E mutation, ApoE Tsukuba (Arg 114 Cys), in lipoprotein glomerulopathy

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Introduction

Lipoprotein glomerulopathy (LPG) is considered a type of renal lipidosis, characterized by the presence of lipoprotein thrombi in markedly dilated capillary lumina of the affected glomeruli [1]. LPG is a rare disease; approximately 65 cases have been reported to date [2]. Patients with LPG exhibit proteinuria and progressive renal dysfunction. In most of the LPG patients, levels of intermediate-density lipoproteins are elevated, resembling type III hyperlipoproteinaemia; moreover, apolipoprotein E (ApoE) levels are also elevated. Recent genetic studies have revealed that several ApoE gene mutations are associated with LPG [3].

In this study, we report a LPG patient with a novel ApoE mutation, without plasma lipid and lipoprotein abnormalities or elevated ApoE level.

Case report

An 18-year-old Japanese female was first found to have proteinuria at an annual school health check. For the workup of her proteinuria, she was admitted to Tsukuba University Hospital. Her past history was unremarkable. She had no known family history of renal diseases or lipidosis, but her mother had splenomegaly of unknown origin since the previous year.

The patient was 157 cm tall and weighed 53 kg. Her blood pressure was 132/70 mmHg. She did not have peripheral oedema, corneal arcus or xanthoma. Her urinary protein excretion was 0.75 g/day, creatinine clearance was 86.9 ml/min and urine sediments showed no abnormalities. Other laboratory examinations revealed white blood cell count: 7300/mm³, haemoglobin: 12.6 g/dl (126 g/l), platelet count: 142 × 10³/mm³, total serum protein: 7.4 g/dl, albumin: 4.3 g/dl, blood urea nitrogen: 14.7 mg/dl (5.25 mmol/l), serum creatinine: 0.53 mg/dl (46.9 µmol/L), uric acid: 5.3 mg/dl, total cholesterol: 127 mg/dl, triglycerides: 139 mg/dl and fasting blood glucose: 87 mg/dl. Tests for anti-nuclear antibody, hepatitis B surface antigen and anti-hepatitis C antibody were all negative. On an abdominal echogram, her renal shape and size were normal but marked splenomegaly was observed.

Percutaneous renal biopsy was performed for further investigation. By light microscopy, a total of 28 glomeruli were evaluated, 2 of which showed global sclerosis. The other glomeruli had markedly dilated diffuse capillaries containing pale-staining thrombi with a layered structure and diffuse segmental mesangial proliferation, and a small number of mesangial areas were mesangiolytic (Figure 1A). There was mild interstitial fibrosis. Foam cells were not observed. The capillary walls showed no duplication and no spike formation. By electron microscopy, the intracapillary thrombi consisted of finely vacuolated material, separated by bands of high electron density (Figure 1B). By immunofluorescence, the deposition of immunoglobulins, C3, C4 and C1q, was not detected. Strong, granular staining for ApoE was observed in the thrombi (Figure 1C).

In this case, LPG was diagnosed based on specific histology in the glomeruli at renal biopsy. Due to the inherited character of this disease, we collected 12-h fasting blood samples from the patient, her parents and her siblings for lipoprotein profiles, and genetic studies were done with informed consent.

Plasma ApoE phenotypes were analysed using isoelectric focusing (IEF) and immunoblotting. The patient and her sister and mother had alleles E2/E3, and her father and brother had alleles E3/E3 (Figure 2A). Plasma ApoE genotypes were analysed using restriction fragment length
Fig. 1. (A) Light microscopy of the renal biopsy. Capillary lumina were dilated, containing pale-staining thrombi with layer structures. PAS stain (×100). (B) Electron microscopy of the renal biopsy. The dilated capillary lumina were occluded by numerous lipid granules and concentrically laminated vacuoles (×3000). (C) For apolipoprotein E detection, rabbit anti-human apolipoprotein E antibody (DAKO A/S) was used as the primary antibody and FITC-conjugated anti-rabbit IgG antibody (ICN/Cappel) as the second antibody (×20).

Fig. 2. (A) Isoelectric focusing and immunoblot analysis of the ApoE phenotype. The three left lanes show controls with ApoE phenotype E2/E2, E3/E3 and E4/E4, respectively. Lane 1 is her father with ApoE3/E3; Lane 2 is her mother with ApoE3/E2; Lane 3 is the patient with ApoE3/E2; Lane 4 is her sister with ApoE3/E2; Lane 5 is her brother with ApoE3/3. (B) Restriction fragment length polymorphism (RFLP) analysis for ApoE genotype. PCR-amplified DNA of the ApoE gene was digested with HhaI restriction. Lane 1 is her father; Lane 2 is her mother; Lane 3 is the patient; Lane 4 is her sister; Lane 5 is her brother. The 91, 48 and 35 bp fragments showed genotype ε3/ε3 (Lanes 1–5), the 91, 72, 48 and 35 bp fragments showed genotype ε4/ε3 (left control lane).

polymorphism (RFLP) analysis, as described by Hixson et al. [4]. ApoE genotyping identified that the patient and her family had alleles ε3/ε3 (Figure 2B).

To explain the discrepancy between the ApoE phenotype (E2/E3) and genotype (ε3/ε3), we analysed a known ApoE mutation (Arg 145 Pro; ApoE Sendai) using RFLP analysis as described by Oikawa et al. [5], but no subfragment was observed after digestion by DdeI. Consequently, we further analysed the sequence of the ApoE coding region in exons 3 and 4. The regions of primer pairs 1 (5'-GCTTTCCAAGTGATTTAACCGACT-3') and 2 (5'-AGAGCTAAAGCCAGGATGTCG-3') for exon 3, 3 (5'-ATCAGGCTTTGCAGCAGCCTCGCC-3') and 4 (5'-CGTGAATTCGATGGCGCAGCGCTTCGCGCTTC-3') for exon 4 were PCR-amplified. PCR-amplified DNA was isolated by electrophoresis on 1.5% agarose gel and ligated into pT7Blue-T-vector (Novagen, Madison, WI, USA). Twelve clones were sequenced using Big Dye Terminator v3.0 cycle sequencing kit and ABI Prism 3100 genetic analyser (Applied Biosystems, Foster City, CA, USA) according to the instructions provided by the manufacturer. We identified a heterozygous mis-sense mutation (C to T) leading to an amino acid substitution Cys (TGC) for Arg (CGC) at codon 114 (Figure 3).

To identify this novel point mutation in the ApoE gene, we amplified a 138 bp segment of the ApoE gene comprising the mutation in codon 114. We used the upstream primer 5'-ACCGGGGACGGCTGTCACAGAAGA-3', and the downstream primer, 5'-TGCCGGGCCCCGCTTGAACA-3' for PCR. Amplified DNA was digested with the restriction enzyme NotI. As the use of the phenotype for the nomenclature of ApoE mutation has been established, the variant was termed ApoE (Arg 114 Cys) Tsukuba (Figure 4). Her mother and her sister were also heterozygous for ApoE Tsukuba (Figure 4).

The plasma lipoprotein profiles were measured by agarose gel electrophoresis and differential staining, according to the method of Kido et al. [6]. Plasma lipid and lipoprotein profiles of the patient and her family are summarized in Table 1. In the carriers of the novel ApoE mutation, levels of plasma lipid and lipoproteins were not elevated; furthermore, apolipoproteins including ApoE levels were also within normal ranges (Table 2).

During the follow-up at our outpatient clinic, the patient's urinary protein increased to 2–3 g/day. Her triglyceride level increased to 194–363 mg/dl, high-density lipoprotein (HDL) cholesterol: 40.8 mg/dl without other plasma lipid or lipoprotein changes. To reduce her triglyceride level, we
Table 1. Plasma lipoprotein levels of ApoE Tsukuba family

<table>
<thead>
<tr>
<th>Sex/age (years)</th>
<th>Total cholesterol (mg/dl)</th>
<th>Triglyceride (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>HDL</td>
</tr>
<tr>
<td>Carrier Patient</td>
<td>F/18</td>
<td>127.0</td>
</tr>
<tr>
<td>Mother</td>
<td>F/43</td>
<td>145.0</td>
</tr>
<tr>
<td>Sibling</td>
<td>F/16</td>
<td>168.0</td>
</tr>
<tr>
<td>Non-carrier Father</td>
<td>M/45</td>
<td>283.0</td>
</tr>
<tr>
<td>Sibling</td>
<td>M/13</td>
<td>225.0</td>
</tr>
</tbody>
</table>

Table 2. Plasma apolipoprotein levels of ApoE Tsukuba family

<table>
<thead>
<tr>
<th>ApoA-I (mg/dl)</th>
<th>ApoA-II (mg/dl)</th>
<th>ApoB (mg/dl)</th>
<th>ApoC-II (mg/dl)</th>
<th>ApoC-III (mg/dl)</th>
<th>ApoE (mg/dl)</th>
<th>Phenotype</th>
<th>Genotype</th>
</tr>
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<tbody>
<tr>
<td>(126–165)</td>
<td>(24.6–33.3)</td>
<td>(66–101)</td>
<td>(1.5–3.8)</td>
<td>(5.4–9.0)</td>
<td>(2.8–4.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carrier Patient</td>
<td>106</td>
<td>17.0</td>
<td>69</td>
<td>2.9</td>
<td>7.2</td>
<td>3.9</td>
<td>E2/E3</td>
</tr>
<tr>
<td>Mother</td>
<td>119</td>
<td>21.6</td>
<td>79</td>
<td>2.3</td>
<td>6.5</td>
<td>3.9</td>
<td>E2/E3</td>
</tr>
<tr>
<td>Sibling</td>
<td>132</td>
<td>21.0</td>
<td>74</td>
<td>2.9</td>
<td>7.9</td>
<td>3.8</td>
<td>E2/E3</td>
</tr>
<tr>
<td>Non-carrier Father</td>
<td>137</td>
<td>29.2</td>
<td>137</td>
<td>4.4</td>
<td>11.8</td>
<td>5.3</td>
<td>E3/E3</td>
</tr>
<tr>
<td>Sibling</td>
<td>125</td>
<td>20.2</td>
<td>101</td>
<td>1.5</td>
<td>5.8</td>
<td>3.7</td>
<td>E3/E3</td>
</tr>
</tbody>
</table>

Fig. 3. Sequence analysis of PCR-amplified DNA of the ApoE gene. The normal ApoE3 allele containing the sequence CGC coding for amino acid 114, arginine, is shown in the upper panel. The mutation allele containing the substitution, sequence TGC, coding for amino acid 114, cysteine, is shown in the lower panel.

Fig. 4. RFLP analysis for ApoE Tsukuba. PCR-amplified DNA of the ApoE gene was digested with NtI restriction. Lane C is the PCR sample without NtI restriction. Lane N is the control sample; Lane 1 is her father; Lane 2 is her mother; Lane 3 is the patient; Lane 4 is her sister and Lane 5 is her brother. The 243, 176 and 74 bp fragments showed ApoE Tsukuba (Lanes 2–4).

In this study, we identified a novel ApoE mutation, ApoE (Arg 114 Cys) Tsukuba, which is a point mutation in the first base of codon 114 (C to T) of the ApoE gene in a patient with LPG. In previous studies, it has been reported that ApoE mutations, ApoE Sendai (Arg 145 Pro), ApoE Kyoto (Arg 25 Cys), ApoE Tokyo/Maebashi (3 amino acid deletion, residues 141 to 143) and ApoE1 (18 amino acid deletion, residues 156 to 173) are associated with LPG [3]. ApoE has a major role in the metabolism of lipids and lipoproteins. It is found in chylomicrons, chylomicron remnants, very low-density lipoproteins (VLDLs), VLDL remnants and a subfraction of HDLs, serving as a ligand for their receptor-mediated catabolism through the LDL receptor and ApoE receptor. It is known that basic amino acid residues 136–158 of ApoE interact with acidic residues of the ligand-binding domain of the LDL receptor [7]. Thus, it was suggested that the ApoE mutations, ApoE Sendai, ApoE Tokyo and ApoE1 resulted in inefficient receptor binding and increased lipid, lipoprotein and ApoE levels. In general, however, LPG has not been associated with the extra-renal manifestation of hyperlipoproteinaemia and hyperlipidaemia, such as arteriosclerosis and xanthoma [3]. In patients with LPG, the presence of lipoprotein thrombi...
in almost all glomeruli, the absence of foam cells and elevated ApoE level were typical features [2]. In the current case, although the typical features of LPG were observed in the kidney biopsy specimen, the levels of serum lipids, lipoproteins and ApoE were within normal range.

Normolipidaemic cases of LPG, like the present case, have also been reported [8,9]. Murano et al. reported that triglyceride-rich lipoprotein possessing ApoE (Arg 25 Cys; ApoE Kyoto) showed increased binding to endothelial cells [10]. This was one of the pathogenic mechanisms for ApoE deposition in glomerular capillaries. Her mother and her sister also had ApoE Tsukuba; however, they showed no urinary abnormalities. From plasma lipoprotein analysis, the present case showed relatively higher triglyceride and lower HDL-cholesterol level (Table 1). Furthermore, during follow-up, she showed marked hypertriglyceridaemia and urinary protein increment. With the improvement of both hypertriglyceridaemia and lower HDL-cholesterol by bezafibrate, her urinary protein returned to prior level. Although the mechanisms of lipoprotein deposition of this case are uncertain, coexistence of both ApoE mutation and hyperlipidaemia may be necessary. Arai et al. also reported that bezafibrate dramatically increased HDL-cholesterol and decreased urinary protein in LPG with ApoE Kyoto [11]. The loci of both ApoE Kyoto and ApoE Tsukuba were outside the ligand-binding domain of LDL receptor and reduction of triglyceride by fibrate showed improvement of renal manifestations. Further investigation was needed to clarify the mechanism of ApoE deposition to glomerular capillaries induced by ApoE Tsukuba.

In the present case, splenomegaly was observed; the patient’s mother also had splenomegaly. The causes of splenomegaly were examined by several doctors of internal medicine, but the origin was unknown. In general, LPG is considered a renal-limited disease and extra-renal manifestation is rare, and no reported LPG patients have had splenomegaly. If the splenomegaly in our patient was associated with ApoE mutation, it would be the new key to clarify the mechanism of lipoprotein deposition due to ApoE Tsukuba.

In conclusion, we described a novel ApoE mutation, ApoE (Arg 114 Cys) Tsukuba in a patient with LPG, who showed normolipidaemia and normal ApoE level. However, further reduction of triglyceride and elevation of HDL-cholesterol by bezafibrate resulted in marked improvement of renal manifestations. In LPG patients with ApoE mutations, of outside the ligand-binding domain of LDL receptor, like ApoE Kyoto and ApoE Tsukuba, treatment of fibrate might be an effective method.

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Conflicts of interest statement. None declared.

References


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