A Novel Mutation ApoE2 Kurashiki (R158P) in a Patient with Lipoprotein Glomerulopathy

Takehiko Tokura¹, Seiji Itano¹, Shinya Kobayashi¹, Atsunori Kuwabara¹, Sohachi Fujimoto¹, Hideyuki Horike¹, Minoru Satoh¹, Norio Komai¹, Naruya Tomita¹, Akira Matsunaga², Takao Saito³, Tamaki Sasaki¹, and Naoki Kashihara¹

¹Division of Nephrology and Hypertension, Department of Internal Medicine, Kawasaki Medical School, Okayama, Japan
²Department of Laboratory Medicine, Fukuoka University School of Medicine, Fukuoka, Japan
³Division of Nephrology and Rheumatology, Department of Internal Medicine, Fukuoka University School of Medicine, Fukuoka, Japan

Lipoprotein glomerulopathy (LPG) is a rare glomerulopathy caused by lipoprotein thrombi. In almost all cases of LPG, several apolipoprotein (apo) E mutations were reported. Here, we present a case of LPG caused by a novel mutation that we named ApoE2 Kurashiki, which substitutes arginine with proline at apoE codon 158. ApoE2 polymorphism is well known for its relationship to type III hyperlipoproteinemia, and the common apoE2 isoform is encoded by the R158C allele. ApoE2 Kurashiki substitutes at the same codon and cannot be distinguished from common apoE2 by standard apoE genotyping or phenotyping.

Key words; Lipoprotein glomerulopathy, ApoE2, Mutation

Introduction
Lipoprotein glomerulopathy (LPG) is a rare glomerular disease caused by lipoprotein thrombi and is known to exhibit several apolipoprotein (apo) E mutations. LPG has been considered as a lipid disorder. Most cases of LPG occur in East Asia, and about half of these fall into the category of end-stage renal disease. Here we report a case of LPG with a novel mutation at the same codon as common apoE2 but with a different amino acid substitution. In the present case, phenotype and genotype were shown as heterozygous E2/3 and ε2/3 by isoelectric focusing (IEF) and regular restriction fragment length polymorphism (RFLP) using HhaI, respectively. The restriction site at amino acid 158 in one of the apoE alleles was confirmed by RFLP using BsaJI. Finally, DNA sequencing revealed a point mutation at codon 158 from the change of the CGC sequence of arginine to the CCC sequence of proline.

Case Presentation
A 26-year-old man presented with mild proteinuria at his company's annual health checkup. In December 2009, he was admitted to our hospital for evaluation of his proteinuria. He had no family history of kidney disease or hereditary disease, and none of his family members had hyperlipidemia. On admission, his blood pressure was 128/70 mmHg. There was no pitting edema. He showed no symptoms of extra-renal manifestations of hyperlipoproteinemia or hyperlipidemia, such as arteriosclerosis and xanthoma. Urinalysis revealed mild proteinuria but hematuria was not detected. The patient's 24-h urinary protein excretion was 0.51 g. In addition, blood examination revealed no anemia, and the red blood cell count was 5.20 million cells/µL with a hemoglobin level of 15.1 g/dL, hematocrit 44.6%, MCV 85.8 fl, MCH 29.0 pg, MCHC 33.9%.

Laboratory findings were as follows: blood urea
nitrogen, 8 mg/dL; serum creatinine, 0.67 mg/dL; 24-h creatinine clearance, 62.3 mL/min; albumin, 4.2 g/dL; total cholesterol, 164 mg/dL; and triglycerides, 261 mg/dL. Plasma lipoproteins revealed by polyacrylamide gel electrophoresis were as follows: high-density lipoprotein cholesterol, 35%; low-density lipoprotein cholesterol, 27%; midband, 9%; and very low-density lipoprotein cholesterol, 29%. Apolipoprotein profiles before treatment are shown in Table 1. Both antinuclear antibody and anti-DNA autoantibody tests were negative. C3 level was 108.3 mg/dL and C4 level was 18.0 mg/dL. Hepatitis B surface antigen and hepatitis C antibody tests were also negative. We suspected IgA nephropathy, and a kidney biopsy was subsequently performed.

Pathological findings: Renal biopsy specimens were processed using standard methods and were found to consist of 2 fragments of renal cortex containing 11 glomeruli, but there was no indication of global sclerosis. One glomerulus showed segmental sclerosis, which was not a characteristic finding of any glomerular disease, while all glomeruli showed that the glomerular capillary walls were dilated by amorphous thrombi when stained slightly with periodic acid-Schiff (Fig. 1A). Mild mesangial cell proliferation and mesangial matrix accumulation were also observed. No foam cells were present in the glomeruli or the tubulointerstitium. There was no interstitial fibrosis or tubular atrophy in this tissue. Routine fluorescent microscopy showed less than a 1+ linear deposit of immunoglobulin A (not shown).

Two glomeruli were subsequently examined by electron microscopy. Almost all of the glomerular capillaries were dilated by numerous lipid granules and lamellate vacuoles in the capillary lumen. A myelin-body like structure was partly observed in the capillary lumen (Fig. 1B). Immunohistochemical study was performed using an antibody against Apo E (Millipore, Temecula, CA, USA), which indicated that ApoE protein could be mainly present at capillary walls (Fig. 1C).

DNA amplification by polymerase chain reaction and restriction fragment length polymorphism analysis: Genomic DNA was amplified by polymerase chain reaction (PCR) using oligonucleotide primers. For apoE genotyping, each amplification reaction was performed as described by Hixon et al. Exon 4 was PCR amplified using sense 5'-ACGCGGGCACA-TGGTCAAGAGGAGTA-3' and antisense 5'-TGGCTCCGCCGCCGCCCTGATACAC-3' for apoE genotyping by HhaI (Takara Shuzo Co., Kyoto, Japan), and 5'-GAGTGCCGCCGCGGAGGTGTCAGG-3' and 5'-TCGCGGGCCGCCGCCCTGATACAC-3' for digestion by BsaJI (New England BioLabs, Beverly, MA). The PCR products were digested with the restriction enzymes HhaI for 3 h at 37°C or BsaJI for 4 h at 60°C according to the manufacturers’ instructions. Digested fragments were then electrophoresed on 8% polyacrylamide gel at 100 V for 40 min and the DNA was later stained with ethidium bromide.

DNA sequence analysis: Three fragments of genomic DNA containing all coding sequences of mature apoE were amplified by PCR with the following primers for the apoE gene: 5'-GCTTTCCAAGTGATTAAAACCGACT-3' and 5'-AGAGCTAAAGCCTAGAGGCTAG-3' for exon3; 5'-CCTTGGGCTTCTCTGCTGCT-3' and 5'-CGATCTCTTTCCACCTCTGTCCA-3', and 5'-GCCGATGGAGGATGGAGGATGCA-3' and 5'-GATCGTGCCACTGCACCTCTA-3' for exon 4. The amplified DNA fragments were purified by a PCR Purification Kit (Qiagen, Düsseldorf, Germany) and directly sequenced with a 310 DNA sequencer (Applied Biosystems, Foster, CA) using a BigDye Terminator Cycle Sequencing Kit (Applied Biosystems). The study protocol was approved by the institutional clinical investigation committee of Kawasaki Medical School Hospital.

**Table 1.** Lipid profile of this patient after fibrate therapy

<table>
<thead>
<tr>
<th></th>
<th>Before</th>
<th>After</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-Chol</td>
<td>136</td>
<td>200</td>
</tr>
<tr>
<td>TG</td>
<td>326</td>
<td>129</td>
</tr>
<tr>
<td>HDL-C</td>
<td>57</td>
<td>124</td>
</tr>
<tr>
<td>LDL-C</td>
<td>123</td>
<td>152</td>
</tr>
<tr>
<td>Apo-I</td>
<td>29.5</td>
<td>39.6</td>
</tr>
<tr>
<td>Apo-II</td>
<td>57</td>
<td>100</td>
</tr>
<tr>
<td>Apo-C-II</td>
<td>3.4</td>
<td>3.2</td>
</tr>
<tr>
<td>Apo-C-III</td>
<td>11.0</td>
<td>8.6</td>
</tr>
<tr>
<td>ApoE</td>
<td>3.8</td>
<td>3.1</td>
</tr>
<tr>
<td>Lipoprotein (a)</td>
<td>6</td>
<td>6</td>
</tr>
</tbody>
</table>

All data are expressed in mg/dL. T-Chol, total cholesterol; TG, triglyceride; HDL-C, HDL-cholesterol; LDL-C, LDL-cholesterol; Apo-I, apolipoprotein A-I; Apo-II, apolipoprotein A-II; ApoB, apolipoprotein B; Apo-C-II, apolipoprotein C-II; Apo-C-III, apolipoprotein C-III; ApoE, apolipoprotein E.

Results

The phenotype and genotype of apoE in this case were revealed as E2/3 and e2/3 by IEF, performed by polyacrylamide gels as described previously, and RFLP using HhaI, respectively (Fig. 2A, B). Moreover, the restriction site at codon 158 in one of the
apoE alleles was confirmed by RFLP using BsaJI (Fig. 2C). Direct DNA sequence analysis of the apoE allele demonstrated that the CCC sequence of proline substituted the CGC sequence of arginine at codon 158 (Fig. 3). Accordingly, we detected the novel apoE variant, apoE2 (Arg158Pro), and named it ApoE2 Kurashiki.

Clinical course: The patient was first given angiotensin receptor blocker; however, it was not very effective in reducing the proteinuria. After diagnosis of LPG, we added 400 mg/day bezafibrate as fibrate therapy, because immunosuppressive therapy is not very effective for this disease\(^2\). After 6 months, urinalysis showed complete remission.

**Discussion**

We performed RFLP using the enzymes HhaI and BsaJI to confirm that genotype \(\epsilon2/3\) had a restriction site at amino acid 158 (Fig. 2B, C); however, DNA sequencing showed that the substitution of arginine at codon 158 was not by cysteine, as is common for apoE2, but by proline.

These findings are very informative because ApoE2 Kurashiki is a novel mutation with a different amino acid substitution at the apoE2 polymorphism site. Furthermore, the polymorphism site of apoE2 is well known to mediate type III hyperlipoproteinemia\(^9\); however, there are no reports of LPG associated with common apoE2, except for 2 patients with similar pathological indications of LPG\(^10, 11\). On the other hand, our patient clearly showed that the substitution of arginine by proline at codon158 was an important cause of morphological change in the glomeruli, which led to LPG.

Other reports of LPG have also shown the substitution of arginine by proline at apoE; for example, ApoE-Sendai\(^3\), ApoE-Chicago\(^12\) and ApoE-Guangzhou\(^13\). These reports hypothesize that the structural changes of apoE, induced by substitution with proline, may reduce the binding ability of LDL receptors because proline acts as a structure breaker in the mid-
Recently, experimental LPG was induced by administering a virus vector with ApoE-Sendai to apoE knockout mice \(^{14,15}\). The authors concluded that apoE mutation, such as ApoE-Sendai, plays a crucial role in the pathogenesis of LPG \(^{15}\).

In this case, serum apoE was not increased, but apoE showed in the glomerular capillary walls (Fig. 1C). This result suggested that mutated apoE is important even when serum apoE is within the normal range. This case showed a small mid-band (Fig. 2D), but lipoprotein (a) was also not increased. This lipoprotein profile is not much changed after fibrate therapy; however, after treatment with fibrate for 1 year, the patient’s urinalysis showed complete remission. A recent report suggested that early diagno-
sis and starting fibrate therapy at that stage of LPG is very effective\textsuperscript{5,16}.

As discussed, LPG is a genetic kidney disease, but with a lipoprotein profile\textsuperscript{17} similar to that of chronic kidney disease (CKD), and shows increases of serum triglycerides, VLDL and IDL\textsuperscript{18}; therefore, LPG analysis might be important for identifying the relationship between dyslipidemia and various other kidney diseases. We expect that additional cases of LPG will contribute to revealing the pathophysiology of the relationship between CKD and lipoproteins as well as of LPG itself.

**Conclusion**

Here we confirmed a new apoE mutation in a patient with LPG. This case suggests that the substitution of arginine with proline is an important event for the development of LPG.

**Acknowledgment**

This study was supported in part by a Grant-in-Aid for Progressive Renal Diseases Research from the Ministry of Health, Labour and Welfare of Japan and a Grant-in-Aid (#21591049) from the Ministry of Education, Culture, Sports, Science and Technology.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure3.png}
\caption{Direct sequence of exon 4 of ApoE2 Kurashiki propositus. The normal apoE3 allele contains the sequence CGC coding for the amino acid arginine at codon 158. The substitution of the apoE2 Kurashiki allele, sequence CCC coding for proline, is indicated by an asterisk.}
\end{figure}

\begin{thebibliography}{9}
\end{thebibliography}