Case Report

A Novel Apolipoprotein E Mutation, ApoE Osaka (Arg158 Pro), in a Dyslipidemic Patient with Lipoprotein Glomerulopathy

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Lipoprotein glomerulopathy (LPG) is a rare disease characterized by the presence of thrombus-like deposition in markedly dilated glomerular capillaries and is often accompanied by an increased serum apolipoprotein E (apoE) level. Several gene mutations of apoE have been reported to be associated with LPG. In the current study, we report an LPG patient with a novel apoE mutation, apoE Osaka. The patient was a 45-year-old man who was hospitalized due to nephrotic syndrome. Light and electron microscopic observations of renal biopsy clearly showed characteristic findings of LPG, including lamellate thrombi in the lumen of dilated glomerular capillaries. His apoE phenotype was apoE3/2 and he had mild dyslipidemia with a mid-band on polyacrylamide gel electrophoresis. It is intriguing that the serum apoE level was within normal limits. We determined the sequence of the apoE gene using direct sequencing of the polymerase chain reaction (PCR) products. ApoE gene analysis showed a nucleotide substitution of G to C at codon 158 of exon 4. This mutation denoted an amino acid substitution of arginine residue for the proline residue at position 158 of apoE. The result of PCR associated with restriction fragment length polymorphism analysis also suggested that this mutation is heterozygous. It is possible that apoE Osaka mutation causes a conformational change of apoE protein and affects the interaction between abnormal apoE-containing lipoproteins and the endothelial cells of glomerular capillaries. The precise mechanism of LPG related with apoE Osaka, however, remains to be elucidated.


Key words; ApoE Osaka, Apolipoprotein E, Lipoprotein glomerulopathy, Mid-band

Introduction

Lipoprotein glomerulopathy (LPG) is a rare disease characterized by the presence of thrombus-like lipoprotein deposition in markedly dilated glomerular capillaries and an increased serum apolipoprotein E (apoE) level¹. Patients with LPG often suffer from renal dysfunction, including nephrotic syndrome. In most LPG patients, levels of intermediate density lipoprotein (IDL) are elevated, resembling type III hyperlipoproteinemia². Recently, genetic studies have revealed that several apoE gene mutations are associated with LPG³-¹⁰.

In this study, we report an LPG patient with a novel apoE mutation, apoE Osaka. This mutation causes an amino acid substitution of the arginine residue for the proline residue at position 158 of apoE protein. The pathology of lipoprotein glomerulopathy by this mutation will also be discussed.

Case presentation

A 45-year-old man was found to have proteinuria at a medical checkup. He was suffering from nephrotic syndrome and was hospitalized in Osaka Red Cross Hospital. His height was 173 cm and his weight
was 68.6 kg (body mass index: 22.8) on admission. The patient was prescribed probucol, which was reported to be effective against LPG. The pre-therapeutic and post-therapeutic data are shown in Table 1. The pre-therapeutic data show mild hyperlipidemia, i.e., serum triglyceride and total cholesterol levels were 234 mg/dL and 216 mg/dL, respectively. HDL-cholesterol was within the normal range. The administration of 500 mg per day of probucol for 3 months effectively normalized the lipid profile (Table 1). It is noteworthy that the serum apoE level was not elevated even in the pre-therapeutic state. Blood examination did not show the presence of anemia. Regarding renal functions, blood urea nitrogen and serum creatinine were 12.2 and 0.97 mg/dL, respectively. Urinalysis revealed moderate proteinuria (100-300 mg/dL), mild occult blood with 1-5 red cells per high power field and 1-10 hyaline casts per whole field. Probucol administration did not affect the urine findings in this treatment period. Polyacrylamide gel electrophoresis (PAGE) analysis revealed the presence of a mid-band, indicating abnormal lipoproteins in the serum (Fig. 1). The mid-band is reported to contain remnant lipoproteins and lipoprotein (a). The fact that the serum level of Lp(a) was normal implies that this mid-band possibly reflects the presence of remnant lipoproteins. The patient was subjected to renal biopsy to clarify the pathology and etiology of nephrotic syndrome (Fig. 2). Light micrograph revealed pale-stained and thrombus-like substances in the dilated capillary lumen (Fig. 2A). Electron micrograph clearly showed occlusive and lamellated thrombi with a finger-printing pattern in the glomerular capillary lumen, which are characteristic of LPG (Fig. 2B). Immunohistochemical analyses using anti-apoE and anti-apoB antibody remain to be performed to elucidate the properties of this thrombus-like deposition.

Genomic DNA was isolated from peripheral whole blood using NucleoSpin® Blood (MACHEREY-NAGEL, Bethlehem, PA). DNA segments were amplified using polymerase chain reaction (PCR). There was no deletion or insertion of large fragments in the apoE gene (data not shown).

We determined the sequence of the apoE gene by direct sequencing of the PCR products as previously described. ApoE gene analysis showed a nucleotide substitution of G to C at codon 158 of the apoE gene in exon 4. As shown in Fig. 3, this mutation denotes an amino acid substitution of the arginine residue for the proline residue normally present at position 158 of apoE. Because this patient originated from Osaka, Japan and this mutation was found in Osaka, it was named “apoE Osaka”. No other mutation was detected in the apoE gene. Next, we tried to confirm the genotype by PCR associated with restriction fragment length polymorphism (RFLP) analysis. Genomic DNA was amplified by PCR with previously designed primers. To identify the genotype of the apoE gene mutation in codon 158, PCR products were digested with the restriction enzyme HhaI. Fragments digested with HhaI were electrophoresed on 4% agarose gel at 100 V for 60 minutes. The DNA fragments were stained with SYBR Safe DNA gel stain (INVITROGEN, Carlsbad, CA). Fig. 4 depicts a representative electrophoretic pattern. The products derived from the patient possessed 91bp, 83 bp, 48 bp and 35 bp fragments but those from the control possessed 91 bp, 48 bp and 35 bp fragments only. These results indi-
cate that the mutation in this case was heterozygous. Because RFLP using HhaI, which we used in this study, is not specific to this mutation, further studies using primers specific to this mutation are necessary to confirm the genotype of this case.

**Discussion**

We report here an LPG case associated with a novel apoE mutation, apoE Osaka (Arg158 Pro). Electron microscopic analysis clearly showed thrombus-like deposition in markedly dilated glomerular capillaries in a mild dyslipidemic patient. The mid-band on PAGE indicates the accumulation of remnant lipoproteins. The DNA sequence of the apoE gene showed a G to C substitution in exon 4 at the position of codon 158. This novel mutation denotes an amino acid substitution of the proline residue for arginine residue, yielding an apoE variant (Arg158 Pro), apoE Osaka. There was no deletion or insertion of large fragments in the apoE gene. RFLP analysis confirmed that this mutation was heterozygous in this case.

![Fig. 2. Renal biopsy of the patient.](image)

Light micrograph (Panel A) shows the pale-stained accumulation of amorphous material inside the dilated capillary lumen (PAS stain, original magnification: x 200). Electron micrograph (Panel B) revealed foamy materials in markedly dilated glomerular capillaries. Lamellate thrombus-like deposition with finger-printing pattern is characteristic of lipoprotein glomerulopathy. GBM, glomerular basement membrane; FP, foot process of the podocyte; EC, endothelial cell; MC, mesangial cell; RBC, red blood cell (original magnification: x 2,000).

![Fig. 3. DNA sequence of exon 4 in apoE gene.](image)

Nucleotide substitution of G to C at codon 158 of the apoE gene in exon 4 was found in this patient.
The substitution of arginine with proline is relatively frequent in LPG cases, i.e., apoE Sendai (Arg145 Pro)\(^9\), apoE Chicago (Arg147 Pro)\(^8\), and apoE Guangzhou (Arg150 Pro)\(^9\). Since proline is reported to be a helix breaker in globular proteins\(^3\), we speculate that the substitution of proline for arginine may change the protein structure, although codon 158 is located on the \(\beta\)-sheet in apoE protein. The three-dimensional structure of apoE Osaka needs to be determined by x-ray crystallography\(^13\).

Codon 158 is the same mutated site as in apoE2 (Arg158 Cys). Most apoE2 homozygotes do not have overt hyperlipoproteinemia\(^14\). Familial type III hyperlipidemia in apoE2 homozygotes occurs only together with the second hit, such as diabetes mellitus and hypothyroidism. If there are no complications, the serum lipid profile in apoE2 homozygotes is almost normal or even hypolipidemic. ApoE2 homozygotes, however, have a mid-band on PAGE even if the lipid levels are within normal ranges. The cause of hyperlipidemia in apoE Osaka is still unclear but the disorder of lipid metabolism which is often observed in nephrotic syndrome may be partly associated. Hyperuricemia is reported to be present in up to half of the apoE2 homozygotes\(^15\) and the serum uric acid level of this patient was abnormally high (9.3 mg/dL). These findings imply that the clinical phenotype of apoE Osaka resembles that of apoE2.

On the other hand, apoE2 is generally not associated with LPG but is reported to be related to "LPG-like nephropathy", which is different from LPG in some properties\(^{16, 15}\). In LPG-like nephropathy associated with apoE2 homozygosity, mesangial foam cells are marked but lamellar formation is not observed even in large deposits or lipoprotein thrombi\(^{16}\). Light microscopic study of this case detected no foam cells in the mesangium (Fig.2A) and an electron micrograph of the current case clearly showed a layered structure of thrombus-like deposits (Fig.2B), implying that LPG-like nephropathy can be ruled out for this case\(^{17}\). Furthermore, the current case is different from “type III hyperlipoproteinemia-related nephropathy” showing foam cells and glomerulosclerosis\(^{18, 19}\).

The normal serum level of apoE may be the reason why mesangial foam cells were not observed in apoE Osaka. A crucial molecular transformation in the apoE Osaka protein different from that of apoE2 may lead to the accumulation of abnormal lipoproteins in the glomerulus.

Various mechanisms have been assumed for the pathology of LPG. Increased affinity of abnormal lipoproteins to the endothelial cells in glomerular capillaries is reported in human LPG\(^20\). Another or an additional mechanism is attributed to the impaired clearance of abnormal lipoproteins in the glomerulus, which was demonstrated in a mouse model\(^21\) and in vitro\(^22\). Regarding apoE Osaka, the former mechanism, i.e., enhancement of lipoprotein binding to the endothelium may be the main cause of LPG because the lipid profile showed only mild hyperlipidemia with a mid-band. Binding and internalization studies using hepatocytes and endothelial cells are required to clarify the pathology of the dyslipidemia and LPG in apoE Osaka.

In conclusion, we found a novel apoE mutation,
apoE Osaka (Arg158 Pro), associated with LPG in a dyslipidemic patient with remnant accumulation. Further experimental investigations remain to be performed.

References


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