A novel apolipoprotein E mutation (p.Arg150Cys) in a Chinese patient with lipoprotein glomerulopathy

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To the Editor: Lipoprotein glomerulopathy (LPG) is an uncommon inherited renal disease characterized by lipoprotein thrombi in the markedly dilated capillary lumina of affected glomeruli, high plasma concentrations of apolipoprotein E (apoE), and proteinuria.[1][2] It mainly affects people of Japanese and Chinese origin, and approximately 117 cases have been reported to date.[3][4] Several genetic studies have demonstrated that APOE gene mutations may lead to the development of LPG,[5][6] however, conflicting published evidence has found that some patients with APOE variants were unaffected.[7] Thus, further studies are needed to examine the role of APOE mutations in the pathogenesis of LPG. This study investigated a novel point mutation in the APOE gene in a family with one LPG patient and an asymptomatic carrier of the same APOE variant.

This study was conducted in accordance with the declaration of Helsinki and was approved by the Ethics Committee of Shenzhen University. Written informed consent was obtained from all participants.

A 21-year-old Chinese woman was in good health until December 2016, when during a 3-month-antenatal examination, it was determined that she had moderate proteinuria. Throughout her pregnancy, she had marked splenomegaly. At admission, she was 162 cm tall and weighed 55 kg. Her blood pressure was 115/77 mmHg. She did not have pedal edema. Her urinary protein excretion was 4.0 g/day, her C4, C3, and C1q were not detected. Strong, segmental staining for both apoe [Figure 1D] and apoB (not shown) was observed in the capillary lumina. One glomerulus was examined by electron microscopy. Almost all of the dilated capillary lumina were occluded by numerous lipid granules and lamellate vacuoles [Figure 1C].

Other laboratory examinations revealed white blood cell count: 5.08 × 10^9/L, hemoglobin: 119 g/L, platelet count: 146 × 10^9/L, albumin: 38 g/L, blood urea nitrogen: 4.2 mmol/L, serum creatinine: 45.8 μmol/L, uric acid: 525.6 μmol/L, total cholesterol (TC): 8.53 mmol/L, low-density lipoprotein cholesterol (LDL-C): 6.44 mmol/L, high-density lipoprotein cholesterol: 1.34 mmol/L, triglycerides (TG): 1.56 mmol/L, and fasting blood glucose: 4.46 mmol/L. Hepatitis B surface antigen, anti-hepatitis C antibody, and antinuclear antibody tests were all negative. Complement levels were normal. An abdominal ultrasound showed that her renal shape and size were normal, but marked splenomegaly was observed.

A percutaneous renal biopsy was performed for definitive diagnosis. By light microscopy, a total of 21 glomeruli were evaluated, 2 of which showed global sclerosis. Glomerular volume was slightly increased and was distorted by markedly dilated diffuse capillaries containing pale-staining thrombi in a layered structure, and mesangial cells and stroma showed mild to moderate proliferation. A few of the glomeruli had small synechiae between the glomerular capillaries and Bowman capsules [Figure 1A]. Staining with oil red O showed a number of lipid droplets in the glomerular capillary lumina [Figure 1B]. Foam cells were not present in either the glomeruli or the tubulointerstitium. There was interstitial edema with focal inflammatory cellular infiltration, mild and limited interstitial fibrosis, and tubular atrophy. Routine fluorescent microscopy showed trace to 1+ linear deposits of immunoglobulin M (not shown). Other immunoglobulins, C3, C4, and C1q were not detected. Strong, segmental staining for both apoE [Figure 1D] and apoB (not shown) was observed in the capillary lumina. One glomerulus was examined by electron microscopy. Almost all of the dilated capillary lumina were occluded by numerous lipid granules and lamellate vacuoles [Figure 1C].

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In this study, exon 4 of the APOE gene was analyzed in 4 separate individuals by DNA sequencing. Genomic DNA was extracted from blood using the DNA Blood Magen Kit (Magen, Guangzhou, China) and 1 mL of the extraction was used as a template for PCR amplification. Exon 4 of the APOE gene was amplified using PCR 2xTaq Master Mix (Vazyme, Nanjing, China). The primers for exon 4 were as follows: forward 5'-TGGGATTACAGGCGTAGAG-3' and reverse 5'-GCCAGCAGATGCGTGAAACTT-3'. The PCR reactions contained 100 ng of genomic DNA and 200 nmol/L of the primers in a total volume of 50 μL. The DNA was denatured at 94°C for 30 s, annealed at 55°C for 30 s, and extended at 72°C for 45 s for a total of 40 cycles using a thermocycler (Boier, Hangzhou, China). The GenBank accession number for the human APOE gene is M10065.1. The DNA was sequenced using an ABI automated DNA sequencer (Thermo Fisher, Massachusetts, USA).

The sequences of 2 control individuals were identical to the reference APOE sequence, whereas the patient and her mother had a c.308C>T substitution in exon 4 of the APOE gene. Figure 1E shows the nucleotide sequence of part of exon 4 for both alleles of the proband (II-1). This missense mutation is a new APOE variant, encoding for the amino acid substitution p.Arg150Cys. There were no other sequence abnormalities identified in our patient. The pedigree of the patient is illustrated in Figure 1F. The mother (I-2) and her only daughter (II-1) were heterozygous carriers of the same mutation (p.Arg150Cys). However, the mother (I-2) did not show any clinical symptoms or signs of renal damage.

The laboratory characteristics of the LPG patient are shown in the attachment. She had significantly elevated levels of plasma TG, TC, LDL-C, and apoE, but a lower LDL-C level compared with non-carriers of the APOE mutation (P<0.05; not shown). In addition, the patient’s mother, a carrier of the novel APOE mutation, had plasma lipid and lipoprotein levels within a normal range. To decrease her plasma lipid and lipoprotein levels and urinary protein, the LPG patient was treated with fenofibrate and perindopril. After 2 months of treatment, her total cholesterol was 5.1 mmol/L, her triglyceride level was 1.33 mmol/L, her renal function was normal, and her 24-h urinary protein output decreased to 2.03 g.

In this study, we identified a novel APOE point mutation in codon 150 (C to T) in 2 family members, with only one of them presenting with LPG. We have named the variant APOE Shenzhen. The etiology of LPG is uncertain, but many researchers believed that mutations in the APOE gene might play an important role. Luo et al[8] found a novel variant of APOE in a Chinese family with 4 LPG patients and 1 asymptomatic family member. The variant, APOE Guangzhou (p.Arg150Pro), was reported as a cause
of LPG. This was in contrast to the findings of Chen et al,\(^7\) in which APOE gene mutations were not the only cause of LPG, and it was shown that other abnormalities might play a role in disease pathogenesis. This study suggested that the presence of mutant APOE Shenzhen (p.Arg150Cys) was related to the cause of LPG.

It is known that the basic amino acid residues (136–158) of apoE interact with the acidic residues of the ligand-binding domain of the LDL receptor.\(^9\) Thus, it has been suggested that APOE mutations result in inefficient receptor binding and increased lipid, lipoprotein, and apoE levels. However, LPG has not been associated with the extra-renal manifestations of hyperlipoproteinemia and hyperlipidemia, such as arteriosclerosis.\(^2\)

Histological examination confirmed a diagnosis of LPG in the Chinese family. The glomerular impairments in our patient were characteristic of LPG, as previously reported,\(^4\) and the presence of lipoprotein thrombi in almost all glomeruli, the absence of foam cells, and the elevated apoE levels were typical features. The abnormal lipid profile of the LPG patient in this study was characterized by increased plasma levels of TC, TG, LDL-C, and apoE. It would be interesting to investigate the features of the mutant apoE in this LPG patient, such as the binding affinity of the mutant apoE to the LDL receptor.

In this case, splenomegaly was observed. The potential causes of splenomegaly were investigated by several doctors of internal medicine, but its origin remained unknown. In general, LPG is considered a renal-limited disease and extra-renal manifestations are rare; only one report has shown an LPG patient with splenomegaly.\(^5\) If the splenomegaly in our patient was associated with the APOE mutation, it would be the key to unlock the mechanism of lipoprotein deposition.

Previous cases have shown the efficacy of fibrates over statins and perindopril in LPG treatment, as supported by the pathology observed in renal biopsies and clinical experience.\(^4\) Although fibrates were an efficient treatment in our patient, there were reports of patients developing renal dysfunction in the end. This reminded us that careful follow-up is still needed.

In conclusion, a novel APOE mutation, APOE Shenzhen (p.Arg150Cys), in 2 members of a family was described, but only the daughter presented with LPG. There might be other factors required for the development of LPG. In addition, a reduction of plasma lipids by fibrate resulted in a marked reduction of lipoprotein levels and improvement of the patient’s renal manifestations.

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### Conflicts of interest

None.

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