A new apolipoprotein E mutation, apoE Las Vegas, in a European-American with lipoprotein glomerulopathy

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Abstract
Lipoprotein glomerulopathy is a rare disease diagnosed by unique histopathologic findings of glomerular capillary dilatation by lipoprotein thrombi. The disease is caused by mutations in apoE, the gene that encodes apolipoprotein E. To date, <80 cases have been reported in the medical literature, nearly all of which are from Japan or China. Only five cases from the United States have previously been reported, of which three patients were of European ancestry. Here, we present the fourth case of lipoprotein glomerulopathy in a European-American man. Whereas prior European-American patients with lipoprotein glomerulopathy were found to have the previously reported apoE Kyoto genotype, the patient presented here was found to have a novel mutation that we have named apoE Las Vegas.

Keywords: dyslipidaemia; genetics; glomerulonephritis

Introduction
Lipoprotein glomerulopathy is a rare kidney disease characterized by moderate to severe proteinuria, progressive renal failure and distinct histopathologic findings of glomerular capillary dilatation by lipoprotein thrombi [1]. To date, <80 cases have been reported in the medical literature, nearly all of which are from East Asian countries (predominantly Japan and China).

Lipoprotein glomerulopathy is caused by mutations in apoE, the gene that encodes apolipoprotein E. Several apoE variants have been reported from the Asian patient...
population, the most common being apoE Sendai (Arg145Pro, a missense mutation substituting proline for arginine at position 145) [2], apoE Kyoto (Cys25Arg, a missense mutation substituting arginine for cysteine at position 25) [3] and apoE Tokyo (an in-frame deletion of Leu141 to Lys143del) [4].

Only five cases from the United States have previously been reported, of which one patient was Chinese-American [5], one patient was Mexican-American [6] and three patients were of European ancestry [7,8]. Whereas the previous European-American patients with lipoprotein glomerulopathy were found to have the recognized apoE Kyoto genotype [7] or no detectable mutation [8], we present here a case of lipoprotein glomerulopathy in a European-American man with a novel mutation of apoE.

We have named this mutation apoE Las Vegas, following the tradition of naming new genotypes after the city in which the patients live. Due to the rarity of this glomerulopathy in Caucasians, it is likely that other cases may be easily misdiagnosed.

Case report

A 36-year-old white male with several years of hypertension and obesity was referred to a nephrologist for 3+ proteinuria elicited on routine laboratory tests. The patient at the time was entirely asymptomatic, including no oedema. A 24-h urine collection revealed proteinuria of 12 g/day. His serum creatinine was 1.3 mg/dL and serum albumin
was 4.7 g/dL. Fasting lipid panel revealed the following: triglycerides 221 mg/dL, low-density lipoprotein (LDL) cholesterol 115 mg/dL and high-density lipoprotein (HDL) cholesterol 32 mg/dL. After laboratory work-up revealed negative or normal ANA, anti-DNA antibody, ANCA, hepatitis B and C serologies, rheumatoid factor, C3 and C4, a kidney biopsy was performed.

Among the 10 glomeruli sampled for light microscopy, 1 was globally sclerotic. The remainder were diffusely enlarged with mild to moderate increase in mesangial cell number and matrix, accompanied by segmental mesangial interposition and duplication of glomerular basement membranes. Over half the glomerular capillaries were distended by large, frequently occlusive, intraluminal masses of foamy, whorled, mesh-like material that appeared to be extracellular. The material stained pale pink with the H&E, PAS, and JMS stains and appeared white with trichrome stain, suggesting lipids (Figure 1A and B). No extraglomerular lipid deposits were detected. Tubular atrophy and interstitial fibrosis affected <10% of the cortex sampled. Immunofluorescence showed focal glomerular tuft staining for IgM (1+), C3 (3+) and C1q (1+). By electron microscopy, the glomerular capillaries were engorged by large, intraluminal, extracellular masses of finely vacuolated material on a background of mesh-like, loosely granular to amorphous material. The vacuoles were predominantly electron lucent, suggesting lipid ‘thrombi’ (Figure 1C and D). The subendothelial zone was focally expanded by electron-lucent material, with intact endothelial cells. Some mesangial zones showed detachment of mesangial cell processes from the glomerular basement membrane reflection, consistent with mesangiolysis. No endothelial tubuloreticular inclusions or immune-type electron-dense deposits were seen. There was irregular effacement of foot processes. No tubulointerstitial or arterial lipid deposits were identified.

A diagnosis of lipoprotein glomerulopathy was made based on the characteristic histologic and ultrastructural findings of glomerular intracapillary lipid ‘thrombi’ without evidence of immune-type deposits. The patient was started on angiotensin-converting enzyme inhibitor and angiotensin receptor blocker combination therapy, as well as dual statin and fibrate therapy.

To verify lipoprotein glomerulopathy, the coding region of the apoE gene was sequenced (see Appendix for detailed methods). We assumed that one of the typical apoE variants associated with lipoprotein glomerulopathy would be found. Peripheral blood leukocyte DNA for sequencing was obtained from the patient, his brother, mother and two children. None of the patient’s relatives had evidence of kidney dysfunction, proteinuria or abnormal lipid profiles. Three of the samples—the patient, one child (#2) and the patient’s mother—were found to carry a ‘C’ to ‘A’ nucleotide change for the amino acid at position 152 (in exon 4), which substitutes aspartic acid for alanine (Ala152Asp) (Figure 2). They were all heterozygous for this novel A/C mutation. None of the previously described mutations associated with lipoprotein glomerulopathy were found. The patient’s other child (#1) and brother showed a C/C wild-type genotype.

Thirty-two months after his biopsy, the patient’s most recent labs show a creatinine of 1.5 mg/dL, albumin 4.8 g/dL and a urine protein:creatinine ratio of 0.04 g/g creatinine. His lipid panel now shows triglycerides 272 mg/dL, LDL cholesterol 66 mg/dL and HDL cholesterol 42 mg/dL. He continues on dual renin–angiotensin system (RAS) blockade and dual lipid-lowering therapy, and he remains asymptomatic.

**Discussion**

Lipoprotein glomerulopathy is a rare kidney disease, with <80 cases reported in the literature to date [1]. The vast majority of cases have come from East Asian countries, principally Japan and China. Affected patients typically present with nephrotic-range proteinuria, hypertriglyceridaemia and elevated plasma apoE levels. Extra-renal
manifestations, such as atherosclerosis, skin eruptions or liver dysfunction, are absent or mild in most cases. On kidney biopsy, lipoprotein thrombi distending glomerular capillaries is a distinct finding for this disease, which often causes progressive kidney failure. Treatment with intense lipid-lowering agents, including fibrates, has been reported to lead to clinical remission along with histological resolution of lipoprotein thrombi in serial biopsies [9]. Nonetheless, approximately half of the reported cases in the literature developed renal failure 1–27 years after onset of disease.

The pathology is considered the sequelae of mutations in apoE, the gene that encodes apolipoprotein E. Human apoE is composed of 299 amino acids and mediates tissue uptake of triglyceride-rich lipoproteins through both LDL receptor and LDL receptor-related protein pathways. The wild-type allele is apoE3; apoE2 (Arg158Cys) and apoE4 (Cys112Arg) are less common. Whereas apoE3 and apoE4 are thought to bind equally to the lipoprotein receptors, apoE2 is defective in its lipoprotein receptor binding (homozygosity for apoE2 results in the development of type III hyperlipoproteinaemia). Virus-mediated transduction of an apoE mutation in apoE-knockout mice has been shown to induce lipoprotein glomerulopathy [10].

Genetic testing for the typical variants in apolipoprotein E may be confirmatory for the disease. Inheritance is typically autosomal dominant. The most common apoE variants reported to date are apoE Sendai (Arg145Pro), a missense mutation substituting proline for arginine at position 145) [2], apoE Kyoto (Cys25Arg, a missense mutation substituting arginine for cysteine at position 25) [3] and apoE Tokyo (an in-frame deletion of Leu141 to Lys143del) [4]. Single cases of novel apoE mutations have also been reported. Ando et al. reported an 18-amino-acid deletion in apoE (Gln156 to Gly173del) [11], and Miyata et al. reported a substitution of lysine for glutamine at position 3 (Gln3Lys) in a patient after kidney transplantation [12]. Sam et al. reported a Mexican-American patient with lipoprotein glomerulopathy found to have a mutation with substitution of proline for arginine at position 147 (Arg147Pro), termed apoE Chicago [6]. More recently, Kinomura et al. reported the novel variant apoE Okayama, the result of a G to C point mutation at position 150, replacing arginine with glycine (Arg150Gly) [13], while Luo et al. reported apoE Guangzhou, a substitution of proline for arginine at position 150 (Arg150Pro) [14].

Our patient similarly presented with a heretofore undiscovered mutation, a substitution of aspartic acid for alanine at position 152 (Ala152Asp) in exon 4, which is a coding region and the binding site for interaction with the LDL receptor (Figure 2). Following the tradition of naming newly discovered apoE genotypes after the cities in which the patients live, we have dubbed this mutation apoE Las Vegas. To our knowledge, this is only the 10th reported case of lipoprotein glomerulopathy from a Western country.

Two of the four family members of our patient tested were found to have the same apoE Las Vegas genotype, yet no clinical evidence of lipoprotein glomerulopathy with normal serum creatinine, urinary protein and serum lipid levels. A number of family members of previously reported cases of lipoprotein glomerulopathy in European Americans were also clinically unaffected, heterozygous carriers of a detected apoE mutation (in those families, the genotype was apoE Kyoto) [7]. The identification of such unaffected carriers—along with substantial differences in the extent of lipid abnormalities, renal dysfunction and, on histopathology, glomerular injury among patients with lipoprotein glomerulopathy—suggests that a second, third or fourth defect may be necessary for abnormal deposition of lipoprotein, development of lipoprotein thrombi in glomerular capillaries and subsequent clinical manifestation of this rare disease.

Treatment of lipoprotein glomerulopathy has consisted of intense lipid lowering with fibrates and other agents. In our patient, a combination of lipid lowering and blockade of the RAS has led to normalization of the lipid profile and return of proteinuria to normal levels. It is unclear whether one or both of these methods of therapy have played a greater role in preserving renal function to date. Regardless, recognition of this entity can lead to prompt initiation of appropriate therapy, which in turn should help preserve renal function.

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Appendix: Detailed methods

Genomic DNA was isolated from leukocytes using Puregene Blood kits (Qiagen, Valencia, CA). Oligonucleotide primers (IDT, Coralville, IA) were designed based on published apoE DNA sequence (accession number AF261279). The primer sequences were:

<table>
<thead>
<tr>
<th>Exon</th>
<th>Primer 1</th>
<th>Primer 2</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>5’AGC CCT ATA ATG GAA CAA GTC TG 3’</td>
<td>972 bp</td>
</tr>
<tr>
<td>2</td>
<td>25’ AGG GTC TGC CTT GAT GGG GCC A 3’</td>
<td>439 bp</td>
</tr>
<tr>
<td>3</td>
<td>35’ AAG CAT TTG TGG AGC ACC TTC TGT G 3’</td>
<td>45’ TAA AGC GAG TGG CAA GAA AGA GG G 3’</td>
</tr>
<tr>
<td>4</td>
<td>Exon 3</td>
<td>Exon 4</td>
</tr>
<tr>
<td>5</td>
<td>55’ TCG CCC GCC CCA TCC CAG CCC TTC 3’</td>
<td>1013 bp</td>
</tr>
<tr>
<td>6</td>
<td>65’ TGA GAA TTG TGT GGC CAG TAT GT 3’</td>
<td>Internal primers</td>
</tr>
<tr>
<td>7</td>
<td>75’ ATG CCG ATG ACC TGC AGA AGC 3’</td>
<td>674 bp</td>
</tr>
<tr>
<td>8</td>
<td>85’ TGC CCA TCT CTT CCA TCC GC 3’</td>
<td>555 bp</td>
</tr>
</tbody>
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Primers 7 and 8 were internal sequencing primers for exon 4 in order to confirm the sequences by both directions.

DNA amplification was carried out by polymerase chain reaction (PCR). Five hundred nanomolars of DNA were amplified by PCR using Mastercycler Gradient (Eppendorf). Amplification reactions were carried out in a reaction mixture of 50 μL with 50 mM KCl, 20 mM Tris–HCl (pH 8.4), 1.5 mM MgCl2, 200 μM each dATP, dCTP, dGTP, dTTP and 1X PCR enhancer (Invitrogen) with 1 unit PlatinumTaq DNA polymerase (Invitrogen). The PCR enhancer was added to help the amplification of high GC content areas. The cycling condition included an initial 94°C for 2 min to activate the Taq DNA polymerase followed by a denaturation at 94°C for 50 s, annealing at 60°C for 50 s
Thrombotic thrombocytopenic purpura associated with anti-glomerular basement membrane disease

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Abstract

Goodpasture’s disease is associated with circulating anti-glomerular basement membrane (anti-GBM) antibodies. Thrombotic thrombocytopenic purpura (TTP) is a disease related to platelet clumping and microthrombosis in the circulation. We report an unusual case where both entities coexist in the same patient. The patient was a 43-year-old Caucasian male, with a recent history of inhalational hydrocarbon exposure for ~10 weeks. He initially presented with confusion, persistent fever and acute oliguric renal failure. In addition, he was found to be thrombocytopenic and had concurrent microangiopathic haemolytic anaemia. All presenting signs, symptoms and laboratory findings had a temporal relationship within 3 weeks. In addition, he was also found to have active pulmonary hemorrhage and positive anti-GBM antibody. During his stay, the patient underwent treatment with plasmapheresis, and an open lung biopsy, which confirmed the diagnosis of Goodpasture’s disease and TTP. In addition, it adds to our current understanding of the pathophysiology of autoimmune diseases in general and supports the theory of an autoimmune

References


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