Renal ApoA-1 Amyloidosis with Glu34Lys Mutation and Intra-amyloid Lipid Accumulation

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ABSTRACT
Apolipoprotein A-1 (ApoA-1) amyloidosis occurs as a nonhereditary condition in atherosclerotic plaques, but it can also manifest as a hereditary disorder caused by mutations of the APOA1 gene. Hereditary ApoA-1 amyloidosis presents with diverse organ involvement based on the position of the mutation. We describe a case of ApoA-1 amyloidosis with a Glu34Lys mutation; testicular, conjunctival, and renal involvement; and the notable finding of lipid deposition within the amyloid deposits.

CASE REPORT
A 26-year-old man with no family history of amyloidosis initially presented with a 3-year history of infertility. Semen analysis revealed azoospermia. Given the suspicion for primary testicular failure, a testicular biopsy was performed. Histologic examination revealed extensive deposition of amyloid, confirmed by Congo red staining, diffusely involving the tubules, vessels, and interstitium. No germ cell precursors were present, and rare tubules showed residual Sertoli cells (not shown). Immunohistochemical stains for AA and AL (κ and λ) were unrevealing, results of serum and urine protein electrophoreses were negative for a monoclonal component, and serum free light chains were normal. Amyloid subtyping by liquid chromatography tandem mass spectrometry performed at the Mayo Clinic revealed a peptide profile consistent with APOA1-type amyloid with a Glu58Lys (Glu34Lys Legacy nomenclature without signal peptide) structural abnormality in the APOA1 protein corresponding to a DNA change in exon 3, 172G>A, resulting in a codon change GAA>AAA.1 The patient later developed acute-onset diplopia while operating a vehicle. A left sixth cranial nerve palsy was initially diagnosed. Examination revealed a yellow, gelatinous conjunctival mass and creamy, subretinal peripapillary lesions. Conjunctival biopsy showed amyloid deposition (not shown). Findings on imaging and laboratory evaluations—including imaging and laboratory evaluations—including magnetic resonance imaging of the brain, chest radiography, serum angiotensin-converting enzyme, rapid plasma reagin, antinuclear antibody, and thyroid-stimulating hormone—were all negative. The patient subsequently developed hypertension, with a serum creatinine level of 1.3 mg/dl, spot urine protein-to-creatinine ratio of 1.1 g/g, and urine albumin-to-creatinine ratio of 778 mg/g. Renal involvement by amyloidosis was clinically suspected; given the rarity of the disease and potential for therapeutic liver transplantation,2 a kidney biopsy was performed.

RENAI BIOPSY

Light Microscopy
Light microscopic examination revealed prominent deposits of amorphous and acellular proteinaceous material in the glomeruli. The mesangial regions were massively expanded and focally nodular with segmental involvement of the capillary loops by deposits. These deposits were silver negative (Figure 1A), were weakly positive on periodic acid–Schiff staining, and exhibited a characteristic apple-green birefringence under polarized light after Congo red staining (Figure 1B). There was mild patchy tubular atrophy and interstitial fibrosis. The arterioles were focally thickened with intramural accumulation of amorphous Congo red–positive material. Small arteries lacked substantial pathologic alterations.

Immunofluorescence
Immunofluorescent stains showed nonspecific segmental reactivity of the glomerular mesangium for IgM, C3, and C1q and were negative for IgG, IgA, κ and λ light chain, fibrinogen, and albumin; there was no notable staining of the interstitium or arterial vessels.

Electron Microscopy
Ultrastructural evaluation demonstrated extensive deposits of thin fibrils in mesangial regions and focally within glomerular

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capillary walls. The fibrils were long and unbranched, with an average thickness of 10.2 nm, and were mostly haphazardly arranged, with some in parallel arrays. These findings confirmed the diagnosis of amyloidosis at an ultrastructural level. Embedded within the mesangial fibrillary deposits were innumerable, minute, round, electron-lucent droplets most suggestive of lipid material (Figure 1, C and D). No lipid-like electron lucent droplets were observed by electron microscopy in control cases of AL amyloidosis (Figure 1E).

Oil Red O Staining
To confirm the lipid nature of the electron lucent droplets found by electron microscopy, frozen sections of tissue originally submitted for immunofluorescence were stained with oil red O, which identifies neutral lipids (cholesterol esters and triglycerides) and fatty acids. This study confirmed the presence of lipid material within the amyloid deposits at the light microscopic level (Figure 1F). To demonstrate the specificity of this unusual finding, oil red O stain was performed on three cases of AL amyloidosis. In each of these cases, oil red O failed to stain the amyloid deposits (Figure 1G).

CLINICAL FOLLOW-UP
After the renal biopsy, the patient’s serum creatinine remained in the 1.3–1.5 mg/dl range. His BP is under excellent control with lisinopril. Despite the intriguing findings of lipid droplets within amyloid deposits, his serum lipid levels are within normal range, and he has no clinical manifestations of accelerated atherosclerosis. Liver transplantation for hereditary apolipoprotein A-1 amyloidosis has been reported; thus, the patient was evaluated by the hepatology service and is currently listed for liver transplantation.

DISCUSSION
The amyloidosis case presented here is due to a rare Glu34Lys mutation of the APOA1 gene. It resulted in prominent testicular, renal, and conjunctival involvement by the amyloid deposition process and the intriguing finding of lipid accumulation within renal amyloid deposits. The ApoA-1 protein is coded on chromosome 11, is synthesized in the liver and small bowel, is the main protein component of HDL, and activates lecithin:cholesterol acetyltransferase. More than 50 natural variants of ApoA-I have been described, and a little over one third are associated with familial amyloidosis, with 19 known mutations in the APOA1 gene.\textsuperscript{2,4–9} Eriksson et al. have described different patterns of organ involvement by hereditary ApoA-1 amyloidosis based on specific mutations in hot-spot regions of the APOA1 gene: Mutations in coding regions 50–93 were more likely to cause hepatic and renal involvement, whereas mutations in regions 173–178 were more prone to cardiac, laryngeal, and cutaneous

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\caption{Kidney biopsy shows ApoA-1 amyloidosis with intra-amyloid lipid accumulation by electron microscopy and oil red O stain. (A) Jones methenamine silver stain demonstrates nodular expansion of the glomerular mesangium due to accumulation of an amorphous, acellular, and silver negative material (original magnification, ×40). (B) Nodular mesangial deposits exhibit characteristic birefringence under polarized light following Congo red stain (original magnification, ×40). (C) Electron micrograph demonstrates aggregates of round, electronlucent vesicles (arrows; original magnification, ×30,000) suggestive of lipid droplets, within deposits of amyloid fibrils. (D) Electron micrograph shows numerous electronlucent vesicles, suggestive of lipid droplets, closely associated with fibrillary amyloid deposits (arrows; original magnification, ×80,000). (E) No lipid droplets are observed by electron microscopy among the amyloid fibrils in control cases of AL amyloidosis (original magnification, ×80,000). (F) Intensely positive oil red O stain confirms the presence of lipids within a nodular amyloidotic lesion of a glomerulus involved by amyloidosis in our patient. (G) Glomerular amyloid deposits (arrows) in a glomerulus from a control patient with AL amyloidosis are negative on oil red O stain.}
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PHYSIOLOGY of the RENAL BIOPSY

involvement. In addition, the peptide comprising residues 46–59 forms amyloid-like fibrils, and researchers have suggested that this region is responsible for self-recognition, aggregation, and overall amylogenic propensity of ApoA-1 amyloid. In our case, the structural difference involves the switch from the negatively charged acid glutamate to the positively charged base lysine in coding region 34. This region is just adjacent to segment 8–33, which is characterized by random distribution of positively and negatively charged amino acids along the polar face.

Investigators have reported a variety of distributions for all amyloid subtypes within the kidney, including isolated glomerular, isolated tubulointerstitial, and primarily glomerular involvement with mild involvement of tubules and vasculature. Tissue uptake studies have shown that normal ApoA-1 readily passes through the glomerular barrier and binds to the apical membrane protein cubulin, of proximal convoluted tubule cells, to be degraded or recycled.

The finding of lipids within the ApoA1 amyloid deposits raises the questions of how ApoA-1 amyloid fibrils sequester lipids and how lipids in turn affect amyloidogenesis. This newly discovered feature of ApoA-1 amyloid in our patient may be related to the normal lipid-carrying function of the ApoA-1 protein. It is also possible that the switch from glutamate to the positively charged lysine in coding region 34 might affect hydrophilic/hydrophobic interactions and promote the accumulation of lipids within the altered ApoA-1 amyloidogenic deposits. Studies on macrophages, atherosclerosis, and amyloid formation have demonstrated that lipid and the microenvironments can influence the formation, structure, stability, and toxicity of amyloid deposits. ApoC-II amylogenic fibrils, for example, alter their structure from a twisted ribbon to rod-like with the addition of lipid. ApoA-1 is specifically deposited in age-related atherosclerotic plaques and believed to be linked to atherosclerosis progression. Patients with ApoA-1 amyloidosis may have decreased serum HDL cholesterol levels, and in some the ApoA-1 variant makes up 10% of the HDL. However, the ability of mutated ApoA-1 proteins to bind lipid and the subsequent effect on clinical manifestations of this disease remain incompletely understood. More observations are needed to establish the role of the Glu34Lys structural abnormality in the morphologic pattern and anatomic distribution of ApoA-1 amyloidosis and to investigate the ability and clinical significance of mutated ApoA-1 proteins to sequester lipid molecules in hereditary ApoA-1 amyloidosis.

REFERENCES