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Apolipoprotein CII Amyloidosis Associated With p.Lys41Thr Mutation

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Introduction: Apolipoprotein CII amyloidosis (AApoCII) is a rare form of amyloidosis. Here, we report a novel mutation associated with AApoCII amyloidosis in 5 patients and describe their clinical, renal biopsy, and mass spectrometry findings.

Methods: Five patients with renal AApoCII p.Lys41Thr amyloidosis were identified from our amyloid mass spectrometry cohort. Clinical features, kidney biopsy, and mass spectrometry findings were analyzed in this rare type of amyloidosis.

Results: The patients were older adults (mean age of 71.6 years at diagnosis), presented with nephrotic-range proteinuria, and often had declining renal function. All renal biopsy specimens showed massive mesangial nodules composed of weakly eosinophilic, periodic acid–Schiff negative, Congo red–positive amyloid deposits. There were no interstitial, vascular, or medullary deposits. In all cases, immunofluorescence studies were negative for Igs and electron microscopy showed amyloid fibrils. Proteomic analysis of Congo red–positive amyloid deposits detected large amounts of apolipoprotein CII (APOC2) protein. We also detected APOC2 p.Lys41Thr mutant protein in amyloid deposits of all patients. DNA sequencing in 1 patient confirmed the presence of the mutation. Both mutant and wild-type forms of APOC2 were detected in amyloid deposits of all patients. Molecular dynamic simulations showed the variant mediating a collapse of the native structure of APOC2, thereby destabilizing the protein.

Conclusion: We propose that AApoCII p.Lys41Thr amyloidosis is a new form of amyloidosis seen in elderly individuals, histologically exhibiting massive glomerular involvement, leading to nephrotic-range proteinuria and progressive chronic kidney disease.

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Amyloidosis is caused by extracellular deposition of misfolded proteins in an insoluble β pleated physical format. Amyloid deposits are identified by their characteristic apple green–orange birefringence under polarized light on a Congo red stain, as well as the presence of rigid, nonbranching fibrils 7.5 to 10 nm in diameter on electron microscopy.[1,2] Based on the amyloid precursor protein, various types of renal amyloidosis are recognized. Ig light chains are the precursor protein of the most common type of amyloidosis, namely, the light-chain (AL) amyloidosis.[3] Renal amyloidosis associated with other precursor proteins include serum amyloid A protein amyloidosis (AA), leukocyte chemotactic factor 2 amyloidosis (ALect2), fibrinogen α-chain amyloidosis (AFib), gelsolin amyloidosis (AGel), lysozyme amyloidosis (ALys), and apolipoproteins I, II, and IV (AApoAI, AApoAII, and AApoAIV).[4–14] Many of these are rare, and often have a genetic basis that causes instability and misfolding of the precursor proteins.

Apolipoprotein CII amyloidosis (AApoCII) is a rare form of hereditary amyloidosis that was recently described in a 61-year-old woman with an APOC2 p.Glu69Val mutation.[15] We recently found AApoCII amyloidosis associated with a novel APOC2 p.Lys41Thr mutation. This mutation is also more frequently associated with AApoCII amyloidosis than the APOC2 p.Glu69Val mutation. In this report, we present the clinical, renal biopsy, and proteomic findings of 5 patients with AApoCII p.Lys41Thr amyloidosis.

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METHODS

Proteomic Typing of Amyloid Deposits

Formalin-fixed, paraffin-embedded renal biopsy materials were sent to the Mayo Clinic amyloid typing laboratory for analysis. We used a previously established proteomics method for typing the amyloid deposits.[16,17] For each case, 6-μM-thick, formalin-fixed, paraffin-embedded sections were Congo red stained and examined to confirm the presence of amyloid. Separate 10-μM-thick sections were obtained and mounted on a special laser microdissection slide, and amyloid deposits were visualized using fluorescent light. Congo red–positive deposits were microdissected from an area of 60,000 μM^2. Resulting formalin-fixed, paraffin-embedded fragments were collected for mass spectrometry analysis. Multiple independent dissections (replicates) were performed and analyzed for each patient. Data for each patient was processed using a previously described bioinformatics pipeline, and a patient-specific amyloid proteome profile was created. For each patient, a pathologist reviewed the microdissection images to confirm that the amyloid deposits were included in the analyzed tissue. Next, the proteome profile was scrutinized for the presence of universal amyloid tissue biomarkers (apolipoprotein E, serum amyloid P component, and APOAIV).[18]

Finally, the proteome was searched for potential amyloid precursor proteins. In all 5 cases, previously described canonical amyloid precursor proteins were not present in their corresponding proteomes. However, abundant spectra corresponding to ApoCII protein were detected. An advanced bioinformatics pipeline was used to search for known or unknown mutations in proteomes of all patients. We detected APOC2 p.Lys41Thr mutation in amyloid deposits in all patients.

Immunofluorescence and Electron Microscopy

Routine immunofluorescence microscopy and electron microscopy were performed on frozen biopsy specimens at the referring institutions, and results were obtained for summarization.

Sanger Sequencing

A DNA sample from patient 1 was extracted from peripheral blood using standard procedures. Direct sequencing of the 3 coding exons and flanking intronic sequences of *APOC2* gene was conducted on an ABI 3730 DNA automated sequencer (Applied Biosystems, Frankfurt, Germany) and sequences were analyzed using Sequencher software (Gene Codes). Primer sequences for exon 1 are GGACACCGAGCTCACACAGA (forward) and GGGCTGGGAAGATGCTTGGA (reverse); for exon 2 are CCCAGGCCCTTCTTACCTCT (forward) and GGCCAGACCCCATTTCTCCA (reverse); and for exon 3 are CCCCTCCTCCCTCTAACCATCT (forward) and GGGGAGCTCAGTCTGAACCT (reverse). Conditions for polymerase chain reaction amplification are available upon request. DNA numbering of mutation was based on the *APOC2* cDNA sequence (GenBank accession number NM_000483.4).

Molecular Dynamic Simulation of ApoCII Variants

Generalized Born implicit solvent molecular dynamics simulations were carried out using NAMD and the CHARMM22 with CMAP force field.[19,20] The protein data bank structure 1SOH was used for initial conformation. Initial mutant conformations were generated using PyMOL (The PyMOL Molecular Graphics System, Version 1.5.0.3: Schrödinger, LLC).[21] We used an interaction cutoff of 15 Å with strength tapering (or switching) beginning at 12 Å, a simulation time step of 1 fs, and conformations recorded every 2 ps. Each initial conformation was used to generate 5 replicates, and each was energy minimized for 20,000 steps, heating to 300 K over 300 ps via Langevin thermostat. A further 50 ns of simulation trajectory was generated and the final 40 ns analyzed. All trajectories were first aligned to the initial WT conformation using Cα atoms. Root-mean-SD and principal component analysis were calculated using Cα atoms. We computed an alignment-free median residue−residue distance matrix within each trajectory.[22−24] The distance difference matrix quantifies the median pairwise proximity of residues to each other. Simulations were analyzed using custom scripts, the Bio3D R package and Visual Molecular Dynamics (VMD).[24,25] Differences between simulations were compared using t tests after subsampling to 100 data points from each condition. The subsampling was repeated 100 times and the median P value reported.

RESULTS

Clinical Features

Five patients with renal AApoCII p.Lys41Thr amyloidosis were identified from our amyloid mass spectrometry cohort. Four patients (80%) were female (Table 1). The mean age at the time of diagnosis was 71.6 years (range 61−86 years). Four patients were white and 1 patient was African American. All patients presented with nephrotic-range proteinuria (mean 4.4 g/24 h, range 3.2−5.9 g/24 h), and 3 of 5 patients also showed an elevated serum creatinine (mean 1.24 mg/dl, range 0.5−1.6 mg/dl). After a mean follow-up of 44.5 months, 1 patient was on dialysis, 1 patient was lost to follow-up, and 3 patients had a rise in serum creatinine level (mean 1.6 mg/dl, range 0.9−3.02 mg/dl).