Nephrotic Syndrome Due to an Amyloidogenic Mutation in Fibrinogen A α Chain

Maria M. Picken* and Reinhold P. Linke†

*Department of Pathology, Loyola University Medical Center, Maywood, Illinois; and †Reference Center of Amyloid Diseases, Martinsried, Germany

ABSTRACT

We identified amyloid derived from a mutant fibrinogen A α chain associated with one of the hereditary amyloidoses by kidney biopsy. The recognition of molecular and etiologic diversity among amyloidoses has revolutionized the management of systemic amyloidosis and necessitates precision in amyloid typing. Pitfalls and recommendations for the differential diagnosis of renal amyloid and current standards of amyloid typing are briefly discussed. Diagnosis of the amyloidosis type must be based on identification of the chemical composition of the amyloid protein in deposits and not on clinical suspicion, laboratory tests, or genetic testing. A clinical correlation is required to support but not make a diagnosis of amyloid type. If a hereditary form is detected by amyloid protein typing, then molecular studies are indicated. Conversely, in cases in which DNA sequence indicates a mutant amyloid precursor protein, protein analysis of the deposits must provide the definitive evidence. Negative or inconclusive results must be investigated further by a reference laboratory with the capability of applying more sophisticated methods.

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A 55-yr-old white woman presented with transient periorbital and ankle edema and weight gain. Her medical history was unremarkable. She was in good overall health with urinary tract infections in her 20s and 30s. She had no recent routine medical care and no history of travels, pets, or insect bites. She denied smoking tobacco or drinking alcohol, even socially. There was no frequency, urgency, or pain on urination, and she had no fever, chills, nausea, vomiting, joint pains, rash, or sun sensitivity. She noted that her urine had become foamy but did not observe blood. At the time of presentation, she was not taking any medications. Both of her parents were deceased: Her mother died of breast carcinoma in her 40s, and her father, who was a smoker, died of lung cancer in his 60s. She has one younger sister and two children, who all were alive and well. There is no known family history of kidney disease in her Northern European ancestry.

The patient's weight on admission was 155 lb, increased from her usual weight of 140 lb. Her height was 5′2″. BP was 165/95 mmHg, and her heart rate was 72 bpm regular in sinus rhythm with no cardiac murmurs detected. She had periorbital and ankle edema. Her laboratory values were as follows: Serum creatinine level of 2.1 mg/dl, glucose level of 109 mg/dl, serum albumin level of 2.4 g/dl, cholesterol level of 260 mg/dl, triglyceride level of 240 mg/dl, and hemoglobin level of 10.4 g/dl. Urinalysis was positive for protein (4+); there were also five to 10 red blood cells but no red blood cell clasts or cellular casts; 24-h urine protein was 5.3 g, and creatinine clearance was 62.0 ml/min per 1.73 m^2. C3 and C4 levels were normal; ANA, ANCA, anti–glomerular basement membrane, liver function tests, and hepatitis screen were negative. Serum immunoelectrophoresis showed IgG-κ band. Echocardiogram showed mild to moderate left ventricular hypertrophy. Ultrasound of the kidneys showed normal size, and kidney biopsy was performed. There were >12 glomeruli on kidney biopsy, two of which showed global sclerosis. All glomeruli had hypocellular tufts with mesangial areas expanded by deposits of homogeneous material, which was weakly periodic acid Schiff positive and negative in silver stain (data not shown). These deposits were Congo red positive (salmon-pink; Figure 1A) and displayed apple-green birefringence under polarized light (Figure 1B). No extraglomerular congophilic deposits were seen. Renal amyloidosis was diagnosed on the basis of Congo red positivity with birefringence under polarized light. Immunofluorescence studies were negative or nondiagnostic for IgG, IgA, IgM, κ, λ, C3, C1q, fibrinogen, and albumin; however, the frozen section contained no amyloid and showed medulla only, so amyloid typing was performed in paraffin sec-

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Correspondence: Dr. Maria M. Picken, Department of Pathology, Building 110, Room 2242, Loyola University Medical Center, 2160 South First Avenue, Maywood, IL 60153. Phone: 708-327-2607; Fax: 708-327-2620; E-mail: mpicken@lumc.edu, mmpicken@aol.com

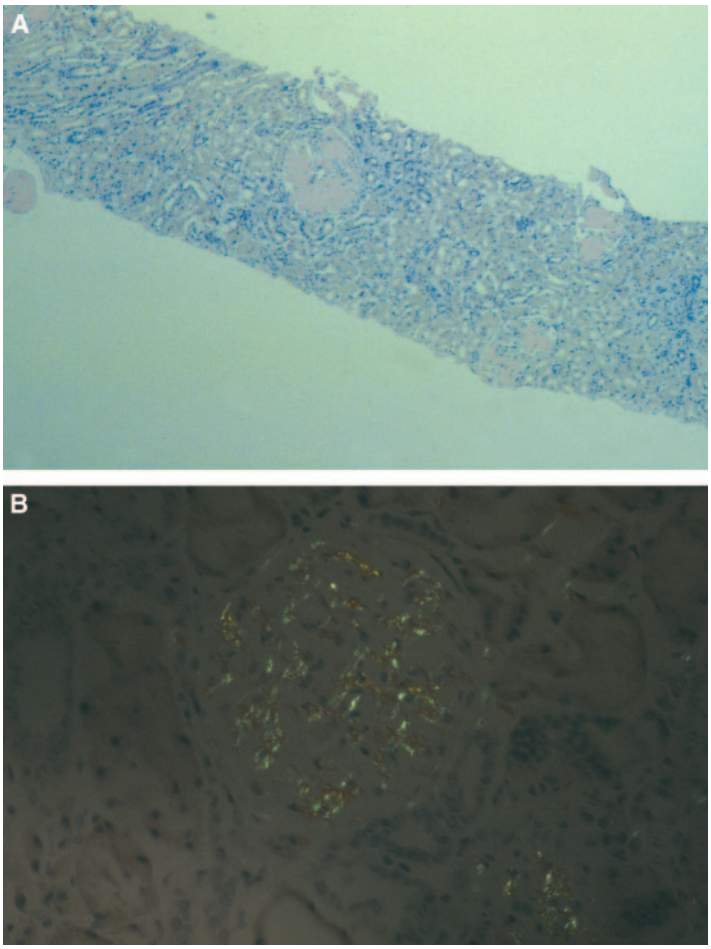

Figure 1. (A) Section of kidney showing abundant intraglomerular deposits of Congo red–positive material (salmon-pink). Paraffin section, Congo red stain, bright field. (B) Congo red stained slide viewed under polarized light showing apple-green birefringence diagnostic of amyloid deposits; no similar deposits were seen in extraglomerular locations. Paraffin sections, Congo red stain, polarized light. Magnifications: ×60 in A; ×250 in B.

tions using the immunoperoxidase method as described previously.[1] Initial stains for amyloid A protein (AA) and λ light chain amyloid were negative (Figure 2, A and B, respectively), whereas the stain for κ light chain amyloid showed (1+) positivity (Figure 2C). In contrast, the stain for amyloid P component (AP), which is present in all types of amyloid, showed strong (3+) positivity (Figure 2D); therefore, additional antibodies were tested: A stain for transthyretin (TTR) was negative (Figure 2E), whereas a stain for fibrinogen was positive (3+; Figure 2F). As expected, electron microscopy demonstrated deposits composed of rigid, nonbranching fibrils measuring between 8 and 12 nm in thickness (Figure 3). No other deposits were present. The final diagnosis was renal amyloidosis derived from fibrinogen – AFib.

Serum fibrinogen levels and coagulation studies all were normal. DNA studies on the patient's peripheral blood demonstrated a mutation in the fibrinogen A α chain. The same mutation was detected in one daughter, whereas the patient's sister and another daughter carried the wild-type fibrinogen. The daughter carrying the mutation is currently clinically asymptomatic. She was offered counseling regarding her own prognosis and that of possible children. Two years after kidney biopsy, the patient developed renal failure and underwent a combined kidney and liver transplant. Two years after transplantation, she has normal renal function.

RENAL AMYLOID: BEYOND AL AND AA

Among various organs involved in systemic amyloidosis, kidneys are the most frequently affected. This involvement leads to proteinuria and/or renal failure, which prompts a kidney biopsy.[2,3] Thus, nephrologists and renal pathologists are in a prime position to detect systemic amyloidosis, and renal amyloidosis should be routinely included in the differential diagnosis of proteinuria/nephrotic syndrome in adult patients.

Amyloidoses are a group of protein-folding disorders in which deposits of abnormally folded proteins share unique affinity for Congo red stain with birefringence under polarized light and a fibrillar ultrastructural appearance.[2,3] Diagnosis of amyloidosis is based on the detection of amyloid deposits in tissues. Although morphologically similar, amyloidoses are a very heterogeneous group of disorders. In the past, typically only two types of systemic amyloidosis were considered: AL or AA. The recognition of molecular and etiologic diversity among amyloidoses and recent developments in the management of systemic amyloidoses in particular, however, indicate that such an approach is no longer justified. Currently, >25 different proteins (and many more variants) are known to be sources of amyloid proteins. Thus, three major categories of systemic amyloidoses are recognized: AL, AA, and hereditary amyloidoses. In patients on dialysis, dialysis-related amyloidosis must be considered as well.[1–5]

The central concept of amyloidosis management is elimination of the supply of amyloidogenic protein. Thus, in AL, the most frequent type of systemic amyloidosis, in which deposits are derived from immunoglobulin (Ig) light chain, eradication of the plasma cell clonal proliferation can be achieved with aggressive chemotherapy and stem cell rescue.[2,3] In the second most common type of systemic amyloidosis

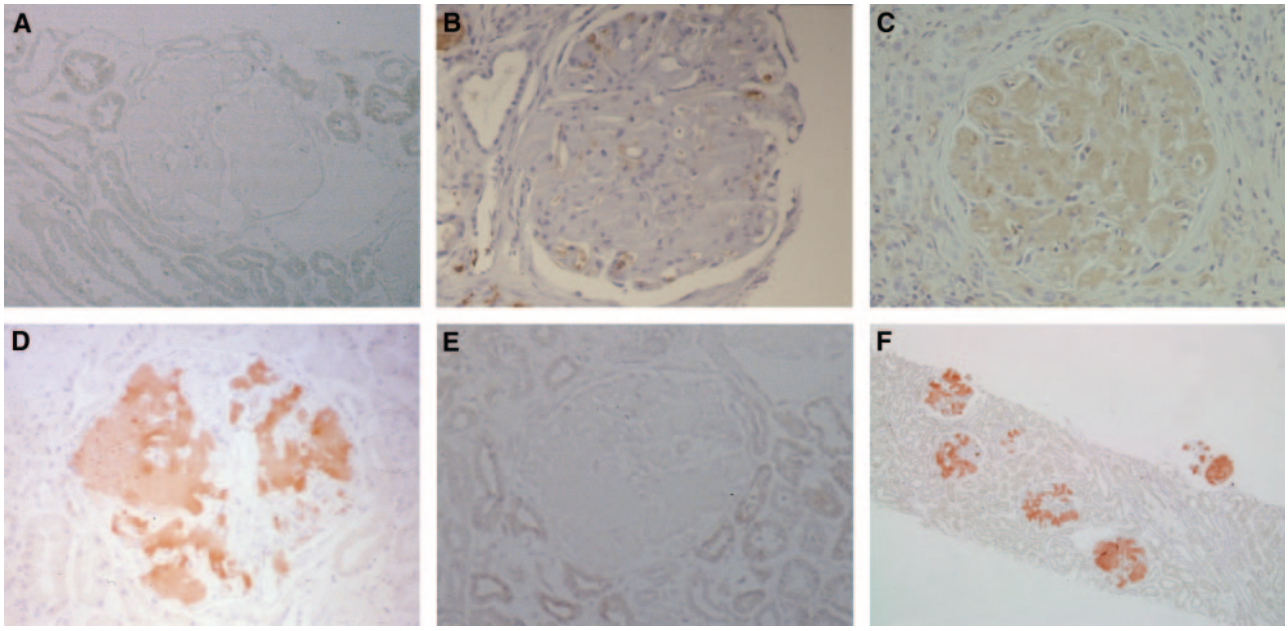

Figure 2. (A) Negative stain for amyloid A protein (paraffin section, immunoperoxidase stain, no counterstain). (B) Deposits of amyloid are negative for λ light chain; positivity for this antibody is focally seen in the lumen of glomerular vessels (paraffin section, immunoperoxidase stain, hematoxylin counterstain). (C) Stain for κ light chain showing weak (1+) positivity (paraffin section, immunoperoxidase stain, hematoxylin counterstain). (D) Stain for amyloid P component showing strong positivity (3+; paraffin section, immunoperoxidase stain, hematoxylin counterstain). (E) Negative stain for TTR (paraffin section, immunoperoxidase stain, no counterstain). (F) Paraffin sections of kidney showing abundant deposits that are strongly immunoreactive for fibrinogen (3+) and are limited to glomeruli (paraffin section, immunoperoxidase stain, hematoxylin counterstain). Magnifications: ×280 in A through E; ×60 in F.

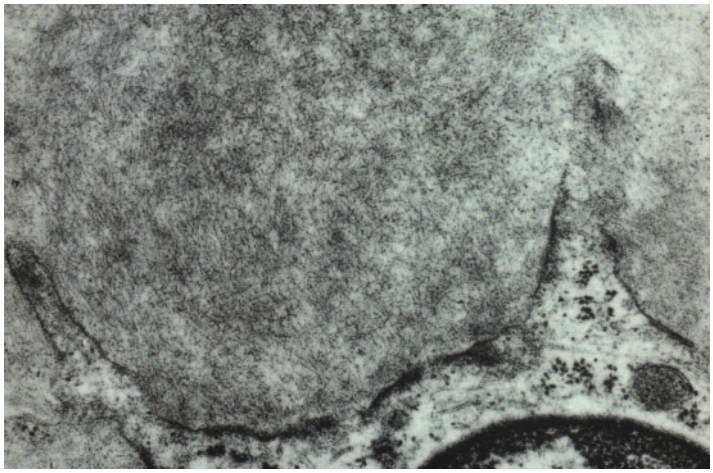

Figure 3. Ultrastructural appearance of amyloid composed of nonbranching fibrils measuring 8 to 12 nm in width (electron microscopy). Magnification, ×25,000.

worldwide, AA, reduction of the circulating fibril precursor protein is achieved by targeting the underlying inflammatory disease.[2,3] In recent years, a third category of systemic amyloidoses, hereditary amyloidoses, is gaining in recognition and clinical importance.[4,5] In hereditary amyloidoses, fibrils are derived from various serum proteins, which are rendered amyloidogenic by mutation. Several proteins harbor potentially amyloidogenic mutations: TTR, apolipoprotein AI, apolipoprotein AII, fibrinogen A α chain, lysozyme, gelsolin, and cystatin C[1–11] (Table 1).

ATTR amyloidosis, derived from mutated TTR, is the most common hereditary amyloidosis in the United States and worldwide. Interestingly, the liver produces several of these proteins exclusively or predominantly; therefore, in these patients, liver transplantation has been tried as a form of treatment targeting the supply of abnormal protein.[6,7] A new class of anti-amyloid agents is also in clinical trials for AA and ATTR.[2,3] In view of the dramatic differences in treatment, the precise diagnosis of the amyloid type is critical.[1–5]

AFib amyloidosis, derived from mutated fibrinogen, has emerged as the most common hereditary amyloidosis in Northern Europe but also with worldwide distribution.[4–6,11] In AFib amyloidosis, there is a mutation in the fibrinogen A α chain, and several variants have been reported.[5,7,8,10] The typical presentation is with nephrotic syndrome and hypertension, and the median age at presentation is 55 yr. Kidney involvement is associated with massive and exclusively glomerular amyloid with essentially no extraglomerular deposits. There is some phenotypic variability, depending on the

Table 1. Hereditary amyloidoses following mutation in the amyloid protein[a]

Serum Protein Variants	Kidney	Heart	Polyneuropathy	Other
TTR	+/+++ (depending on mutation)	+++	+++	Gut, eye, lungs, systemic vessels, subcutaneous fat
Fibrinogen	+++	+ (clinically silent)	+ (some mutants)	Liver, adrenals (clinically silent), spleen: anemia, rupture
ApoAI	+++ (deep medulla)	+++	++ (some mutants)	Liver, spleen, gastric mucosa, larynx, cutaneous, testis: infertility
ApoAII	+++	+ (some mutants)	++	
Lysozyme	+++			GI tract, spleen, liver: rupture, petechial rashes, ocular/oral sicca
Gelsolin	+++ (homozygotes)		+++ (cranial nerves)	Cutis laxa
Cystatin C	+			Cerebral vasculature with hemorrhage, systemic: clinically silent?

[a]Several proteins have multiple amyloidogenic mutations and phenotypes may vary depending on mutation. Apo, apolipoprotein; GI, gastrointestinal.

type of mutation, with involvement of other organs, but renal failure seems to dominate the clinical picture. Liver and adrenal gland involvement, if present, is clinically silent. Spleen involvement may lead to anemia and rupture, with life-threatening hemorrhage. Renal failure develops within 1 to 5 yr. Kidney transplantation alone is associated with rapid recurrence of renal amyloid with renal failure. Better results are seen in patients treated with a combination of kidney and liver transplantation, whereby elimination of the source of the abnormal protein prevents the recurrence of amyloid deposition.

Hereditary amyloidoses are underdiagnosed, although, with increased awareness, they are diagnosed more frequently. Thus, in the United States, from 1977 to 1986, only 2% of systemic amyloidoses were diagnosed as hereditary, whereas, in 2005, five times more (10%) cases were found.[12] In the United Kingdom, in 2005, 16% of systemic amyloidoses were diagnosed as hereditary.[3,4] Although hereditary amyloidoses have an autosomal dominant inheritance pattern, they display variable penetrance; hence, a family history of amyloidosis is often missing.[4] Moreover, hereditary amyloidoses frequently have late onset and clinically may mimic AL.

The reasons for variable penetrance and late onset of these hereditary amyloidoses are unknown. Interestingly, some wild-type serum proteins, most notably TTR, can also undergo fibrillogenesis in older patients, known as "senile cardiac amyloidosis."[3] Although some hereditary amyloidoses associate with polyneuropathy and others with polyneuropathy and cardiomyopathy, virtually all can involve the kidneys[4,5,7–11] (Table 1); however, the degree of renal involvement may vary, with some types affecting predominantly glomeruli, others with glomeruli and extraglomerular vasculature, and still others only in the interstitium with some limited to deep medulla.[4,5,7–11] Moreover, within a given hereditary amyloidosis type, phenotypes may vary depending on the mutation[7,8,10] (Table 1). There may also be a difference between homo- and heterozygotes.

Immunohistochemistry, involving immunofluorescence stains in frozen sections or immunoperoxidase stains in paraffin sections, is currently the mainstay of amyloid typing.[1–4,12–15] Although well-performed immunohistochemistry is fast and reliable in a significant number of patients, it can have important limitations and pitfalls.[1,3,12–15] Thus, whereas AA typing is relatively reliable in both frozen and paraffin sections, typing of AL can pose a challenge; the main danger lies in misdiagnosis as a result of confusing AL and hereditary amyloidosis.[1,3,4,15,16]

Amyloid fibrils in AL derive from intact or truncated Ig light chains. In the latter case, amyloid fibrils contain only the amino terminal end of the light chain molecule with the V region and little or no C region and therefore may be nonreactive with commercial antibodies. The reported incidence of such nonreactivity ranges from 13.6 to 35.3% in frozen sections and up to 50% in paraffin sections.[3,12–14] In the past, patients with amyloid negative for AA and light chains received a diagnosis by default of "presumed" AL, in particular when there was also clinical evidence of underlying plasma cell dyscrasia; however, such an approach has pitfalls, because approximately 25% of patients with hereditary amyloidosis may have monoclonal gammopathy of undetermined significance.[3] There are reports that several such patients were erroneously treated with chemotherapy for presumed AL.[4] Thus, differential diagnosis of the renal amyloid type must reach beyond AL and AA, and an appropriate antibody panel as well as adequate controls and interpretation must be used.[16] The quality of tissue matters: There is clearly a lower sensitivity of amyloid typing in general and AL in particular in paraffin as opposed to frozen sections.[1,3,11–14] Moreover, in paraffin sections, the typing of AL is further hampered by a higher background stain from serum proteins "locked in" during fixation.[3,16] Distinction between background stain and a true positive result can be facilitated by the inclusion of a built-in positive control such as amyloid

P component. The stain for this glycoprotein, which is present in all types of amyloid, provides a reference as to the expected intensity of a truly positive reaction (Figure 2D).[3] The quality of antibodies also matters; using antibodies directed against amyloid proteins renders immunohistochemistry more reliable.[1]

Interestingly, molecular studies alone are also insufficient for diagnosis of the amyloid type, because patients can have a potentially amyloidogenic mutation and AL.[17,18] Thus, it is currently recommended that diagnosis of amyloidosis type be based on unequivocal identification of the chemical nature of the amyloid protein in deposits and not solely on clinical suspicion or on genetic testing. Clinical correlation is required to support the diagnosis of amyloid type but not to make it. If the hereditary form is detected by amyloid protein typing, then molecular studies are indicated. Conversely, in cases in which the DNA sequence indicates a mutant amyloid precursor protein, protein analysis of the deposits must provide the definitive evidence. Negative or inconclusive results must be investigated further by a reference laboratory with the capability of applying more sophisticated methods.[18] Molecular identification of amyloid protein using proteomics methods such as microextraction and sequencing or tandem mass spectrometry are currently being tested.[19–21] Finally, it must be remembered that the full spectrum of amyloidoses continues to expand; thus, recently, yet another entity, amyloidosis from leukocyte chemotactic factor 2, has been added, and, quite possibly, additional amyloid types will emerge in the future.[22]

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DISCLOSURES

R.P.L. is the owner of Amymed, a reference laboratory for Amyloidosis in Martinsried, Germany.

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