# Post-transplantation Recurrence of Fibrinogen A-α Amyloidosis

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## INTRODUCTION

Amyloidosis is a disease caused by extracellular deposits of amyloid, an insoluble, fibrillary material derived from protein precursors that assumes an α-sheet conformation, developing the capacity of auto-aggregation, deposition, and tissue toxicity.[1,2] The nature of amyloid deposits is highly variable, including localized or systemic deposition and expression as a quickly lethal disorder or as an incidental finding. A total of 36 amyloidogenic proteins have been identified so far in human beings, leading to clinical manifestations that depend on the affected organs as well as on genetic and environmental factors.[3,4]

As one of the most affected organs, the kidney is involved in 50% to 80% of the cases with systemic amyloidosis.[5] Significant renal involvement occurs most often in light-chain amyloidosis (AL amyloidosis) and in amyloidosis secondary to chronic inflammatory disorders (AA amyloidosis). In a series of 474 patients with biopsy-proven renal amyloidosis from Mayo Clinic, 86% were associated with Igs, 7% were classified as AA, and the remaining ones as hereditary renal amyloidosis.[6]

Hereditary amyloidosis comprises rare, underdiagnosed forms of systemic amyloidosis that manifest mainly as a nephropathy.[7] These disorders are caused by genetic variants in several loci that lead their protein products to acquire amyloidogenic properties, such as fibrinogen A α-chain (AFib), apolipoproteins (AI, AII, and AIV), lysozyme (ALys), transthyretin (ATTR), and leukocyte chemotactic factor 2 (ALECT2).[8] Together, the inherited forms respond for approximately 10% of the cases of amyloidosis.[3]

AFib amyloidosis is caused by pathogenic mutations in *FGA*, the gene that encodes the A α-chain of fibrinogen. This gene was originally described in 1993.[9] AFib is currently recognized as the most common form of hereditary amyloidosis in Europe,[S1] accounting for nearly 5% of the patients with renal amyloidosis.[S2] Kidney biopsy findings reveal massive deposition of amyloid material in glomeruli, with architectural loss and obliteration of capillary loops.[3,7] Its clinical presentation includes proteinuria, systemic hypertension, and gradual progression to end-stage kidney disease (ESKD) in about 5 years after the diagnosis.[S3,S4]

Indication for isolated kidney transplantation is controversial in patients with AFib amyloidosis, as the disease may relapse in the graft and cause its loss.[S3,S4] In this report, we present the clinical case, renal histological findings, and molecular genetic investigation of a woman with AFib amyloidosis who had been undergone kidney transplantation and developed disease relapse in her graft.

## CASE PRESENTATION

A 41-year-old woman with history of hypertension for the past 8 months sought medical attention for lower-limb edema and foamy urine. Her physical examination did not show pethechia, macroglossy,

hepatosplenomegaly, or peripheral neuropathy. She reported a sister who started hemodialysis at the age of 44 years with no established diagnosis. Her sister never underwent a renal biopsy and died at 52 years of age. The patient's laboratory tests revealed the following: blood urea nitrogen (BUN) 67 mg/dl, serum creatinine 7.7 mg/dl, estimated glomerular filtration rate (eGFR) by Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation 5.9 ml/min per 1.73 m$^2$, proteinuria 11.4 g/24 h, no hematuria or leukocyturia, total protein 4.3 g/dl, albumin 2.5 g/dl, globulins 1.8 g/dl, total cholesterol 173 mg/dl, low-density lipoprotein (LDL) 98 mg/dl, high-density lipoprotein (HDL) 38 mg/dl, triglycerides 227 mg/dl, uric acid 2.1 mg/dl, Na 142 mEq/l, K 4.9 mEq/l, ionic Ca 1.13 mmol/l, P 7.9 mg/dl, hemoglobin 6.7 g/dl, white blood cell count 4800/mm$^3$, and platelet count 210,000/mm$^3$. The investigation for secondary causes of glomerulopathies was negative, serologies for hepatits B and C and for HIV were negative; no evidence of monoclonal protein was detected in blood and urine samples; and serum complement was normal. Echography showed a 9-cm right kidney and a 9.5-cm left kidney at the long axis, with increased echogenicity and loss of cortico-medullary differentiation, revealing signs of chronic parenchymal disease. Once diagnosed with nephrotic syndrome, the patient underwent a kidney biopsy. The biopsy specimen showed diffuse deposition of an amorphous and eosinophilic material in glomeruli and arterioles, positive for Congo red staining, and with positive birefringence under polarized light. Diffuse interstitial fibrosis and tubular atrophy were also observed. Immunofluorescence did not show IgG, IgA, IgM, κ, λ, C3, or C1q deposits, and immunohistochemistry did not reveal positive staining for amyloid A, establishing the diagnosis of renal amyloidosis. Myelography and bone marrow biopsy ruled out plasma cell dyscrasia. The patient was promptly initiated on dialytic renal replacement therapy and, 2 years later, received a living—related donor kidney transplant from another sister. Her follow-up showed an appropriately functional graft while she was maintained on sodium mycophenolate 360 mg twice daily, tacrolimus 2 mg twice daily, and prednisone 5 mg daily.

Despite maintaining an eGFR of 54.9 ml/min per 1.73 m$^2$ (CKD-EPI), 5 years after transplantation the patient presented with nephrotic-range proteinuria (4.55 g/g creatinine) without hypoalbuminemia, hypertension, or lower-limb edema. A graft biopsy specimen revealed deposits of amorphous eosinophilic material in the mesangium and subendothelial space with global glomerular involvement, weakly stained for periodic acid—Schiff (PAS) and negative for periodic acid silver methamine stain (PAMS) and Masson's trichrome

(Figure 1a and b); PAMS showed coarse spikes with a segmental pattern (Figure 1c). The amorphous deposits when exposed to Congo red staining revealed salmon-pink deposits (Figure 1d) showing apple-green birefringence under polarized light (Figure 1e). Immunofluorescence analysis revealed nonspecific amyloid material in glomeruli with IgA (+2/+3), IgM (+1/+3), IgG (+1/+2), C1q (+2/+3), kappa (+2/+3), lambda (+2/+3), and C4d (+2/+3), as well as positive glomerular staining of amyloid material with fibrinogen (+3/+3) (Figure 1f). C4d deposits were not detected in peritubular capillaries. Mild interstitial fibrosis and tubular atrophy were observed and were negative for C4d. Based on these findings, the patient was diagnosed with relapsed AFib amyloidosis.

The biopsy findings associated with her family history of kidney disease led to the suspicion of hereditary amyloidosis. In this setting, direct sequencing of *FGA* was performed, revealing the c.1634A>T: p.Glu545Val variant in heterozygosity (Figure 2). The identified variant, also referred to as E545V, has been shown to be pathogenic in human kidney tissue and is classified as pathogenic according to the American College of Medical Genetics criteria.[S5] Proteinuria persisted for the following 6 months at approximately 3.5 g/d, whereas eGFR gradually declined to 24.6 mL/min per 1.73 m$^2$. At this point, a new graft biopsy was performed, with the specimen showing no signs of acute rejection or acute glomerular disease. Renal impairment was therefore attributed to recurrence of the disease. Diagnostic investigation was expanded to family members, who were invited to undergo genetic testing. The patient's 37-year-old brother, diagnosed with renal amyloidosis and on dialysis, had the same *FGA* variant. The patient's sister, the donor for the aforementioned kidney transplant, declined to be tested. Other family members are currently being contacted and offered genetic testing.

## DISCUSSION

AFib amyloidosis is an autosomal dominant disease that is often associated with renal involvement.[S2] It is, in fact, the most common type of hereditary renal amyloidosis in the United Kingdom, Ireland, and the United States.[S4] Clinical manifestations include hypertension, proteinuria, and impairment of renal function, translated into fast progression to end-stage kidney disease (ESKD). Although amyloid deposition has been described in the heart, liver, and spleen in advanced stages of the disease, the clinical relevance of such findings remains unclear.[S3,S6]

*FGA* maps to chromosome 4, comprises 6 exons, and encodes the A-α subunit, which, with the B-β and γ subunits, composes fibrinogen. The A-α subunit
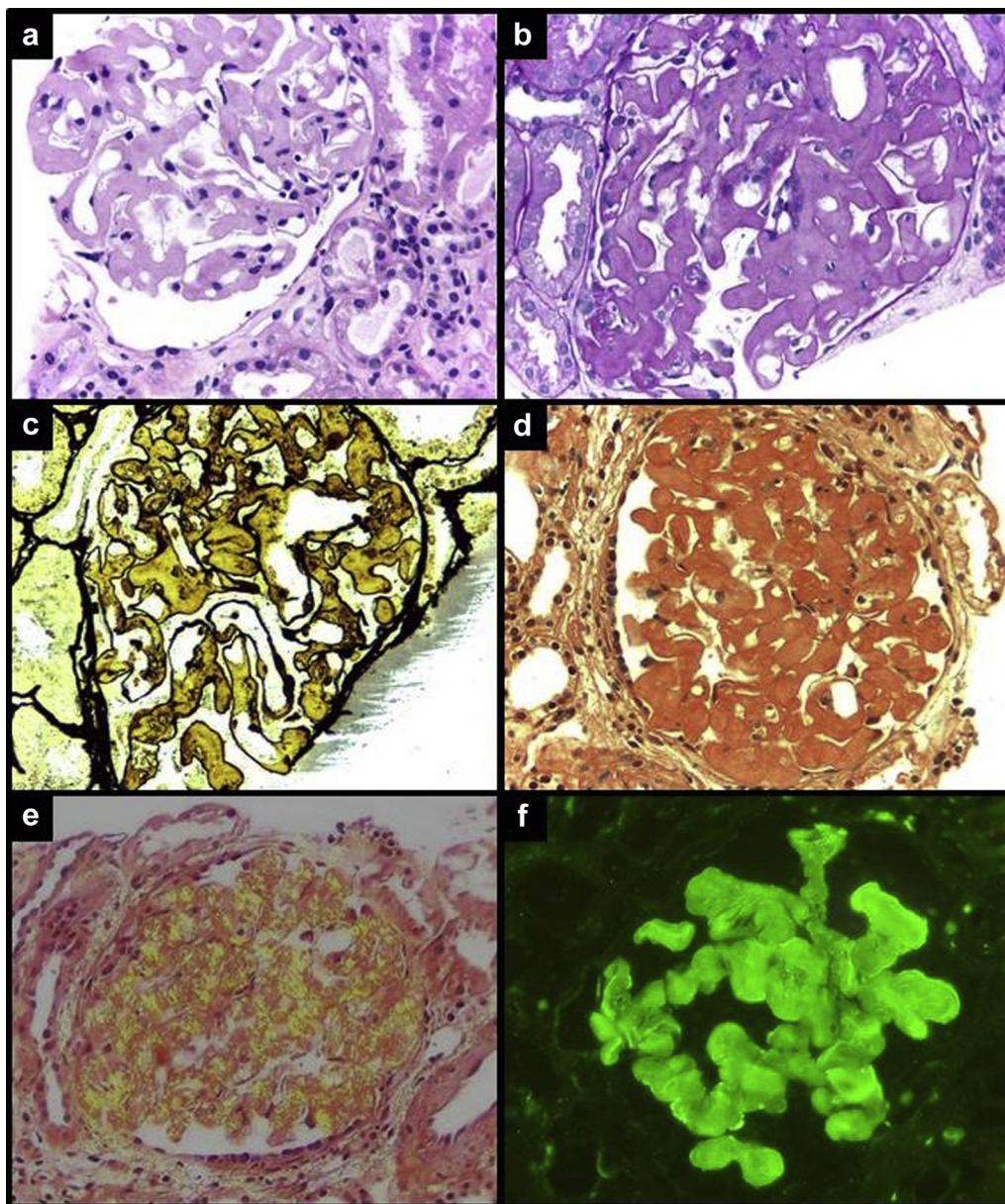
**Figure 1.** Renal graft biopsy. Glomerular architecture reveals (a,b) amorphous, eosinophilic deposits (hematoxylin and eosin, original magnification ×400), located in the mesangium and subendothelial space, with (c) rough spikes in the glomeruli capillary (periodic acid silver methamine stain [PAMS], original magnification ×400), (d) stained by Congo red (original magnification ×400), which exhibited (e) positive birefringence under polarized light (original magnification ×400). On immunofluorescence microscopy, the amorphous deposits revealed (f) strong signal for fibrinogen in the entire glomerulus (original magnification ×400).

contains 886 amino acids (aa), including a 19-aa signal peptide. Interestingly, extracts of amyloid protein aggregates display residues 519 to 599 of A α-chain. It is thought that increased sensitivity of the C-terminal region to proteases may facilitate progression to amyloid deposition. Accelerated catabolism of the mutant chains, in turn, may explain their absence in the plasma of patients with genotype and phenotype of AFib amyloidosis. Three regions of the mentioned aa segment have shown to display propensity to intrinsic aggregation: 518 to 531, 551 to 557, and 580 to 598. It is not a surprise, therefore, that all 15 *FGA* variants associated with AFib renal amyloidosis and nephrotic syndrome identified up to

now are positioned within the residue interval of 536 to 574, affecting the C-terminus of fibrinogen A α-chain.[S7–S9] (http://www.amyloidosismutations.com) (Figure 2). Of note, the valine to glutamic acid substitution in position 545 (or 526 according to a different annotation), harbored by our patient, is the most frequent variant associated with AFib amyloidosis.[S10] Curiously, the A α-chain segment affected by pathogenic mutations is poorly conserved among species.

Performance of isolated kidney transplantation in individuals with systemic amyloidosis who reach ESKD is a controversial issue, given the risk of disease relapse and early graft loss determined by continuing
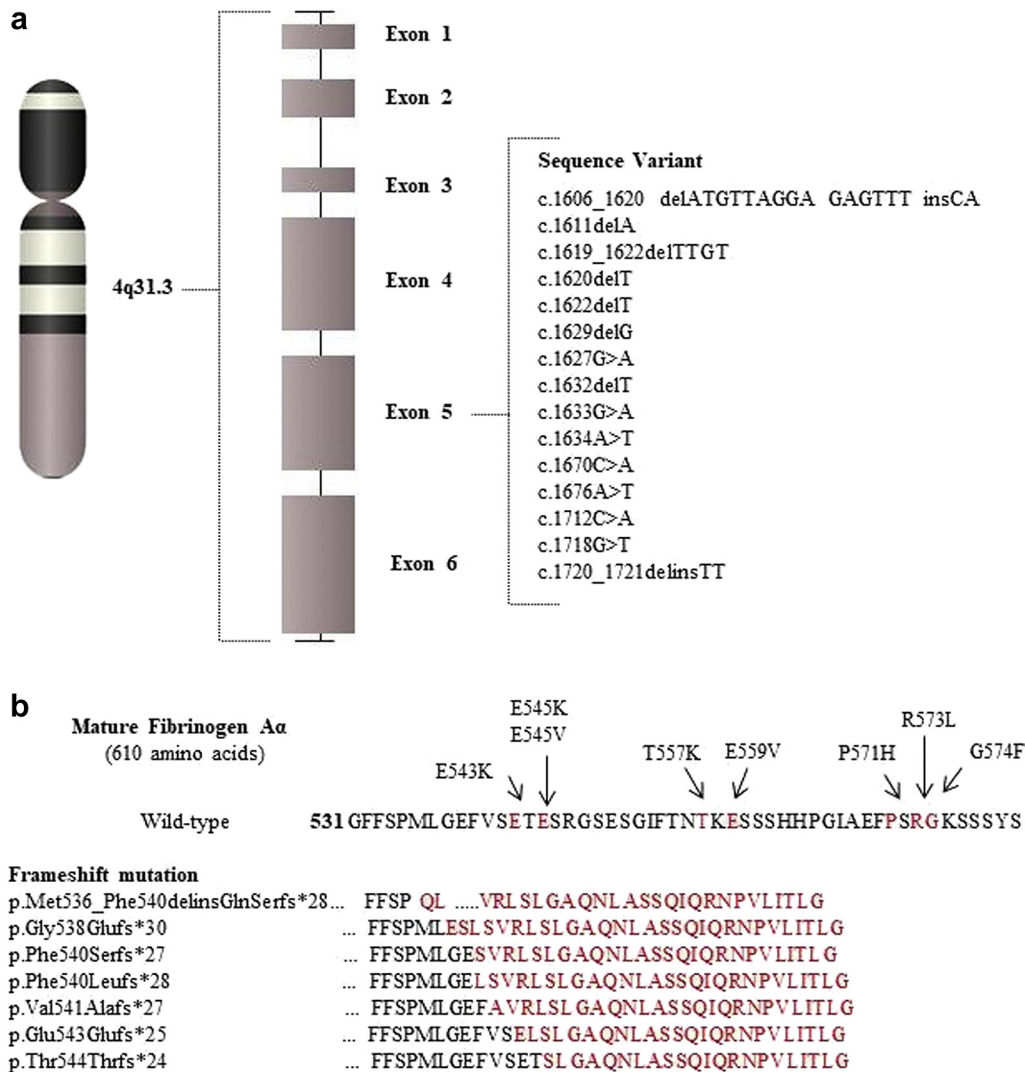
**Figure 2.** (a) Previously reported *FGA* variants in their respective exons. (b) Location of 15 amyloidogenic *FGA* variants (7 missense, 6 deletions, and 2 delins) and the C-terminal sequences resulted from frameshift variants (red letters).

deposition of amyloid in the transplanted organ.[S4,S11,S12] Recurrence of AFib amyloidosis and subsequent graft loss occur on average 7.3 years after transplantation, whereas 85% of the transplanted kidneys remain functional 5 years after the procedure.[S11] Grafts have been described to function for as long as 12 to 16 years, but renal failure ultimately is inevitable.[S3,S7] Only 30% of the grafts survive for ten years or longer in patients with AFib amyloidosis,[S3,S11,S13,S14] whereas more than 60% of individuals who have undergone transplantation due to other etiologies of renal failure have functioning renal grafts 10 years after the procedure.[S8] A study comprising 104 patients with systemic amyloidosis who underwent kidney transplantation found that the disease progressed more slowly in patients with ALys and AApoAI amyloidosis, revealing good graft survival and low rates of disease relapse. These findings contrast with descriptions in AL, AA, and AFib amyloidosis, which progress more aggressively and whose recurrence is more prevalent.

Our patient presented with hypertension, nephrotic-range proteinuria, and rapid deterioration of renal function 5 years after kidney transplantation. Biopsy findings evinced disease recurrence, with glomerular deposition of amyloid material and absence of histological findings suggestive of graft rejection. Our case

**Table 1.** Teaching points

- AFib amyloidosis is caused by pathogenic mutations in FGA, the gene that encodes the A α-chain of fibrinogen.
- Clinical presentation includes proteinuria, systemic hypertension and gradual progression to ESKD.
- The diagnosis of AFib amyloidosis can be made by kidney biopsy, genetic test and/or proteomic analysis of amyloid deposits.
- The only treatment capable of preventing progression of AFib amyloidosis is liver transplantation.
- Several cases of disease recurrence in the graft following isolated kidney transplantation have been reported.

AFIB, fibrinogen A α-chain; ESKD, end-stage kidney disease.

is in line with previous reports, supporting the current concept that recurrence of AFib amyloidosis in the graft is very common.

Because fibrinogen is produced primarily in the liver, the only treatment capable of hindering disease progression is liver transplantation, by replacing the generation of anomalous fibrinogen with the production of wild-type fibrinogen, a non-amyloidogenic molecule.[S3,S4] A series enrolling AFib patients who underwent combined liver and kidney transplantation showed no amyloid deposition in the graft or relapse of AFib amyloidosis after 12 years of follow-up, indicating a role for liver transplantation in the cure of patients with this disease.[S13] It must be noted, however, that combined liver–kidney transplantation has been associated with significant risk of early perioperative mortality, as revealed by 3 patients who died shortly after surgery out of a group of 9 individuals who underwent this procedure.[S11,S13] The number of patients offered liver–kidney transplantation and the length of follow-up are still insufficient to support a survival benefit in individuals with renal amyloidosis. Moreover, this is an extensive procedure performed in patients at significant cardiovascular risk.[S4,S12,S13] In this scenario, some centers consider liver–kidney transplantation for AFIb patients carrying frameshift mutations, as such variants result in aggressive, early-onset disease (mean age 30 years) and are associated with significant risk of recurrence compared to individuals with missense mutations, in whom the mean age of presentation is 59 years.[S6,S15]

Hereditary renal amyloidosis is an underdiagnosed cause of renal disease.[S16] Our patient faced this situation when undergoing a living–related donor renal transplant. Because hereditary amyloidosis is an autosomal dominant disease with varying levels of penetrance, molecular genetic testing is required to diagnose individuals with this condition even when there is no family history of renal disease. Indeed, up to half of the patients with AFib amyloidosis do not report a family history of renal disorder at the time of diagnosis.[S3] Molecular genetic testing is required not only to confirm or to establish the diagnosis of AFib amyloidosis but also to screen family members with the aims of genetic counseling and selecting potential donors, as variant carriers may be asymptomatic.[S3]

Despite the high recurrence rate of amyloid disease, kidney transplantation alone must be considered as an important component in the treatment. Novel therapies acting on amyloidogenesis are being developed to inhibit the formation and/or to help the release and clearance of amyloid deposits, thereby significantly improving the therapeutic options for patients with the disease.[S9,S17,S18]

The case currently reported raises several points potentially facing an AFib patient, and highlights the key actions to be taken (Table 1): (i) to consider AFib amyloidosis in the differential diagnosis of renal amyloidosis; (ii) to appropriately establish the diagnosis of AFib amyloidosis, including genetic testing; (iii) to establish potential disease severity; (iv) to consider and assess the risk of disease relapse following transplantation; (v) to weigh the risks and benefits of isolated kidney and combined liver–kidney transplantation; and (vi) to extend genetic testing to family members for genetic counseling and kidney donor assessment.

## DISCLOSURE

All the authors declared no competing interests.

## SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

**Supplementary References.**

## REFERENCES

1. Benson MD, Buxbaum JN, Eisenberg DS, et al. Amyloid nomenclature 2018: recommendations by the International Society of Amyloidosis (ISA) nomenclature committee. *Amyloid*. 2018;25:215–219.

2. Chiti F, Dobson CM. Protein misfolding, amyloid formation, and human disease: a summary of progress over the last decade. *Annu Rev Biochem*. 2017;86:27–68.

3. Picken MM. The pathology of amyloidosis in classification: a review. *Acta Haematol*. 2020;143:322–334.

4. Merlini G, Bellotti V. Molecular mechanisms of amyloidosis. *N Engl J Med*. 2003;349:583–596.

5. Dember LM. Amyloidosis-associated kidney disease. *J Am Soc Nephrol*. 2006;17:3458–3471.

6. Said SM, Sethi S, Valeri AM, et al. Renal amyloidosis: origin and clinicopathologic correlations of 474 recent cases. *Clin J Am Soc Nephrol*. 2013;8:1515–1523.

7. Sethi S, Theis JD. Pathology and diagnosis of renal non-AL amyloidosis. *J Nephrol*. 2018;31:343–350.

8. Sipe JD, Benson MD, Buxbaum JN, et al. Nomenclature 2014: amyloid fibril proteins and clinical classification of the amyloidosis. *Amyloid*. 2014;21:221–224.

9. Benson MD, Liepnieks J, Uemichi T, et al. Hereditary renal amyloidosis associated with a mutant fibrinogen alpha-chain. *Nat Genet*. 1993;3:252–255.