# Diagnosis, Pathogenesis, Treatment, and Prognosis of Hereditary Fibrinogen Aα-Chain Amyloidosis

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## ABSTRACT

Mutations in the fibrinogen Aα-chain gene are the most common cause of hereditary renal amyloidosis in the United Kingdom. Previous reports of fibrinogen Aα-chain amyloidosis have been in isolated kindreds, usually in the context of a novel amyloidogenic mutation. Here, we describe 71 patients with fibrinogen amyloidosis, who were prospectively studied at the UK National Amyloidosis Centre. Median age at presentation was 58 yr, and renal involvement led to diagnosis in all cases. Even after a median follow-up of 4 yr, clinically significant extra-renal disease was rare. Renal histology was characteristic: striking glomerular enlargement with almost complete obliteration of the normal architecture by amyloid deposition and little or no vascular or interstitial amyloid. We discovered four amyloidogenic mutations in fibrinogen (P552H, E540V, T538K, and T525fs). A family history of renal disease was frequently absent. Median time from presentation to ESRD was 4.6 yr, and the estimated median patient survival from presentation was 15.2 yr. Among 44 patients who reached ESRD, median survival was 9.3 yr. Twelve renal transplants survived for a median of 6.0 (0–12.2) yr. Seven grafts had failed after median follow up from transplantation of 5.8 yr, including three from recurrent amyloid after 5.8, 6.0, and 7.4 yr; three grafts failed immediately for surgical reasons and one failed from transplant glomerulopathy after 5.8 yr with no histological evidence of amyloid. At censor, the longest surviving graft was 12.2 yr. In summary, fibrinogen amyloidosis is predominantly a renal disease characterized by variable penetrance, distinctive histological appearance, proteinuria, and progressive renal impairment. Survival is markedly better than observed with systemic AL amyloidosis, and outcomes with renal replacement therapy are comparable to those for age-matched individuals with nondiabetic renal disease.

*J Am Soc Nephrol* 20: 444–451, 2009. doi: 10.1681/ASN.2008060614

Hereditary non-neuropathic systemic amyloidosis, first described by Ostertag in 1932,[1] is a rare autosomal dominant condition in which progressive amyloid deposition in the viscera, especially the kidneys, frequently leads to organ failure. Mutations in the genes encoding apoAI,[2–12] apoAII,[13] fibrinogen Aα-chain,[14–17] and lysozyme[18] have been identified as the cause of the disease in different kindreds. The clinical amyloidosis syndromes that accompany the various mutations in these different genes are diverse with respect to age of onset, mode of presentation, pattern of organ distribution, rate of progression, and prognosis.

Hereditary fibrinogen amyloidosis (AFib) was first characterized in 1993 in a Peruvian kindred.[14] Patients with AFib present with renal disease and

Received June 17, 2008. Accepted September 26, 2008.

Published online ahead of print. Publication date available at www.jasn.org.

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typically progress to ESRD. The natural history and clinical outcome of the disease has been little characterized, previous reports having been only of isolated kindreds, usually in the context of discovery of a novel amyloidogenic fibrinogen mutation.[15–17,19]

Here we report the clinical presentation, histologic features, molecular basis (including four novel causative fibrinogen Aα-chain gene mutations), and outcome among 71 patients with AFib who were diagnosed and prospectively studied at the U.K. National Amyloidosis Center (NAC) between 1992 and 2007.

## RESULTS AND DISCUSSION

A renal presentation with proteinuria was universal. Seventy-two percent of patients had previously been diagnosed with hypertension or were hypertensive at the time of discovery of proteinuria, and 54% of patients had impairment of renal excretory function by the time proteinuria was discovered. Median age at presentation was 58 yr (range 33 to 83 yr) and sex distribution was equal. Median delay from presentation to diagnosis of amyloidosis was 8 mo (range 0 to 164 mo).

The diagnosis of amyloidosis was made by kidney biopsy in 64 patients and by serum amyloid P component (SAP) scintigraphy in conjunction with genetic analysis in the context of renal dysfunction and a known family history of AFib in seven patients. The renal histologic appearance in every patient was characteristic and showed striking glomerular enlargement with almost complete obliteration of the normal glomerular architecture by extensive amyloid deposition. In contrast, the vessels and renal tubular interstitium of every such patient contained almost no amyloid at all (Figure 1). Definitive immunohistochemical staining of the amyloid with an antibody against fibrinogen Aα-chain was achieved in 93% of patients, whereas staining was absent in all patients with a panel of antibodies directed against serum amyloid A protein (SAA), kappa and lambda Ig light chains, and apoAI. Although immunohistochemical staining with the anti-fibrinogen antibody was not definitive in 7% of patients, every such patient had a previously reported amyloidogenic fibrinogen mutation, the same characteristic renal morphology, and an overall clinical picture and disease course that was completely typical for AFib. None of these patients had an inflammatory disease or a plasma cell dyscrasia to suggest secondary (AA) or primary (AL) amyloidosis, respectively, or a mutation in any of the other genes that encode known amyloid fibril proteins including apoAI, apoAII, and lysozyme.

Radiolabeled SAP scintigraphy was diagnostic of amyloidosis in each of 63 patients who underwent the procedure. The baseline whole-body scintigraphs, taken a median of 3 mo after the diagnosis of amyloid, showed renal amyloid in every patient who had not already reached ESRD, and asymptomatic splenic and adrenal amyloid deposits in 89 and 21% of patients, respectively (Figure 2). No patient had echocardiographic features of cardiac amyloid at the initial NAC evaluation although a regional wall motion abnormality suggesting myocardial ischemia was evident in three cases. A single patient, with a novel fibrinogen mutation encoding the T538K variant, had a biopsy proven amyloid peripheral neuropathy. No patient had clinical evidence of autonomic neuropathy.

Seven (10%) patients, all referred to the NAC with an incorrect diagnosis of systemic AL amyloidosis, had a detectable plasma cell dyscrasia that proved to be incidental. Chemotherapy comprising autologous stem cell transplantation was administered for presumed AL amyloidosis in one such patient before the correct diagnosis of AFib was achieved. Unfortunately, five other patients who did not have a detectable plasma cell dyscrasia also received cyclical chemotherapy for "presumed" AL amyloidosis before the diagnosis of AFib was made.

Direct DNA sequencing of the fibrinogen Aα-chain gene revealed that 64 patients were heterozygous for the previously reported single base substitution that altered the codon at position 526 of the mature protein from that for glutamic acid to valine.[15] All of these patients were of British Caucasian ancestry apart from six patients who belonged to a single German family. Two English patients were heterozygous for the previously reported fibrinogen mutation encoding a single base substitution that altered the codon at position 554 from arginine to

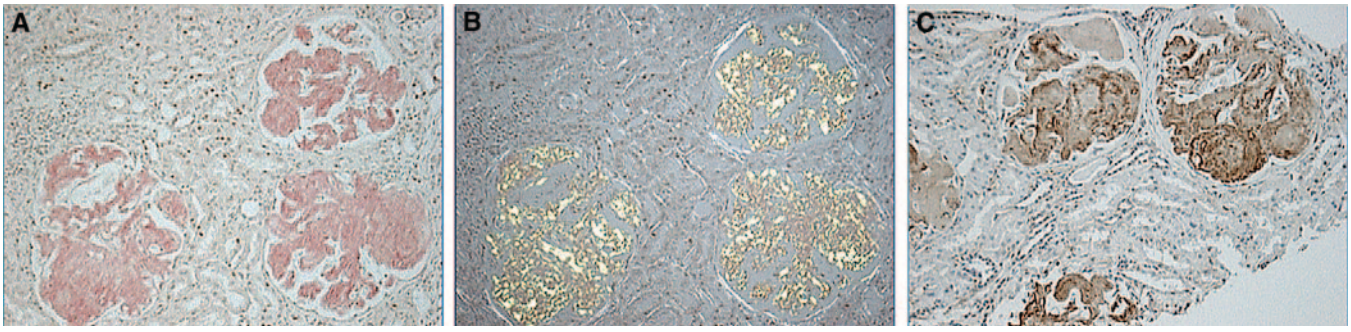


**Figure 1.** Renal biopsy in fibrinogen Aα-chain amyloidosis. Panel A shows striking glomerular enlargement and almost complete obliteration of the normal glomerular architecture by extensive amyloid deposition. The vessels and renal tubular interstitium, in contrast, contain almost no amyloid at all (Congo red stain ×100). Panel B shows red-green birefringence when the same section is viewed under cross-polarized light. Panel C shows immunohistochemical staining of the same patient's biopsy with a monoclonal sheep anti-human fibrinogen antibody (Helena Bioscience) confirming the presence of fibrinogen within the amyloid deposits.

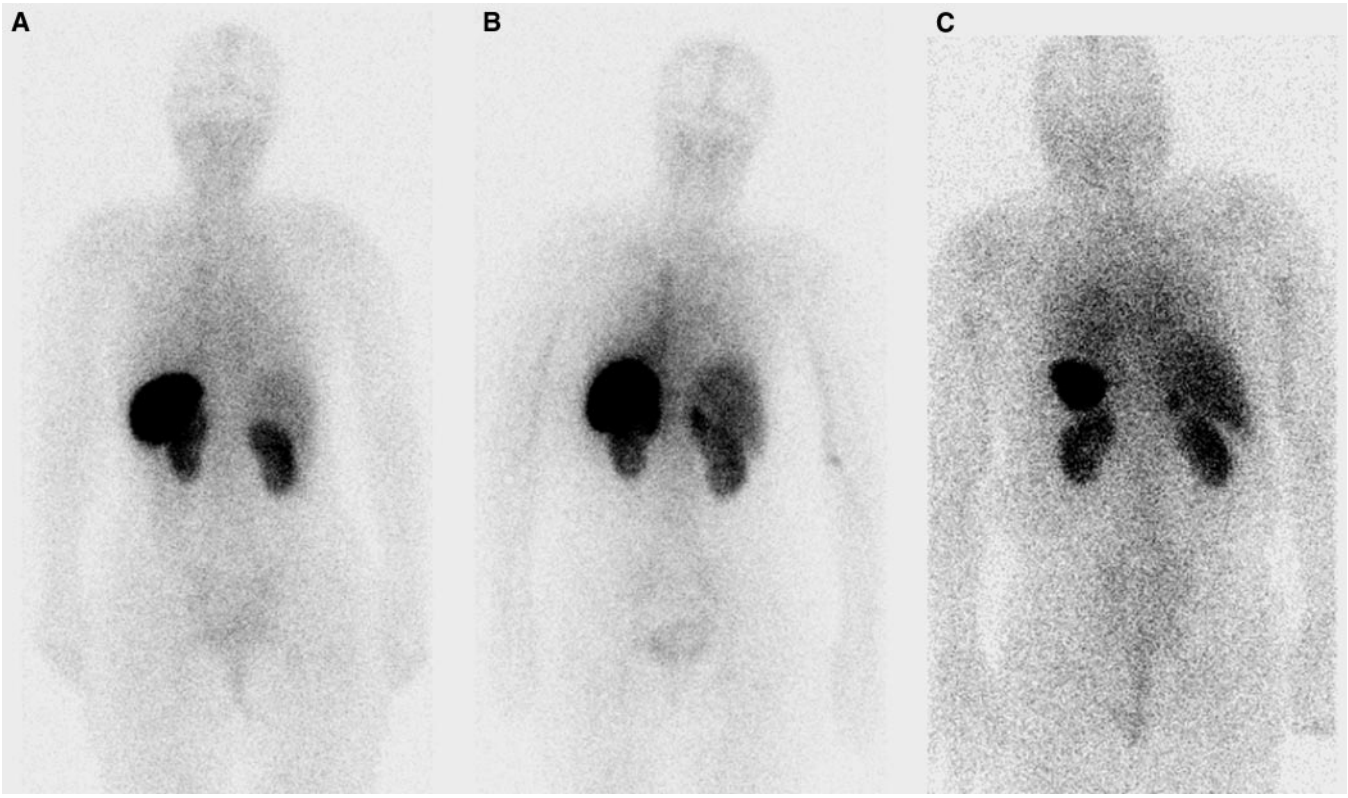


**Figure 2.** Posterior whole-body scintigraphic images after intravenous injection of $^{123}$I-labeled SAP in hereditary AFib. Panel A shows abnormal uptake into renal and splenic amyloid deposits in a 56-yr old patient with AFib associated with the previously reported fibrinogen E526V variant. Panel B shows abnormal uptake into renal, adrenal and splenic amyloid deposits in a 53-yr old German woman with AFib associated with a novel fibrinogen variant, E540V. Panel C shows abnormal uptake into renal, adrenal, and splenic amyloid deposits in a 72-yr old Afro-Caribbean man with AFib associated with a novel fibrinogen variant, P552H. The scan findings were corroborated by histologically proven renal amyloid deposits in all three patients.

leucine.[14] Four novel amyloidogenic fibrinogen mutations were discovered: one in a Chinese patient encoding a single base deletion resulting in a frameshift at codon 525 (Figure 3A), one in another Chinese patient encoding a single base substitution that altered the codon at position 538 from threonine to lysine (Figure 3B), one in two German sisters who were heterozygous for a fibrinogen mutation encoding a single base substitution that altered the codon at position 540 from glycine to valine (Figure 3C), and lastly an Afro-Caribbean patient was heterozygous for a mutation encoding a single base substitution that altered the codon at position 552 from proline to histidine (Figure 3D). The German sisters presented with proteinuria aged 47 and 50 yr, with the younger of the two progressing to ESRD within 7 yr and the other maintaining a normal GFR despite persistent proteinuria over the same period of follow up. The Afro-Caribbean man presented in his seventh decade with hypertension and proteinuria, and both Chinese patients presented in their fourth decade with proteinuria. The characteristic histologic appearance of AFib was present in the kidney biopsies of all five patients. In keeping with variable penetrance of other amyloidogenic fibrinogen mutations, only the German sisters (Figure 4A) and the Chinese patient with the frameshift mutation had a family history of amyloidosis.

Overall, a family history of renal disease or amyloidosis was absent in 46% patients with AFib, with all of the available evidence indicating that this was due to reduced penetrance rather than *de novo* mutations. Three parents who had the respective mutations were clinically healthy and 20 other first-degree family members also had an amyloidogenic fibrinogen A$\alpha$-chain mutation but did not have clinical disease at a median age of 50 yr (range 31 to 84). Six of these 23 patients were older than their oldest clinically affected relative being followed at the NAC (Figure 4B). Absence of clinical disease was corroborated by normal SAP scintigraphy in each of 12 apparently healthy gene carriers in whom the procedure was undertaken. There was no appreciable difference in phenotype between AFib patients with maternal compared with paternal inheritance.

The cohort was followed for a median of 4.0 yr (range 0 to 25.2) after diagnosis, representing 358 person-years. Data for 11 (15%) patients who were lost to follow up were censored at the time of their last assessment. A total of 17 (24%) patients died, and median age at death was 67 yr (range 57 to 85).

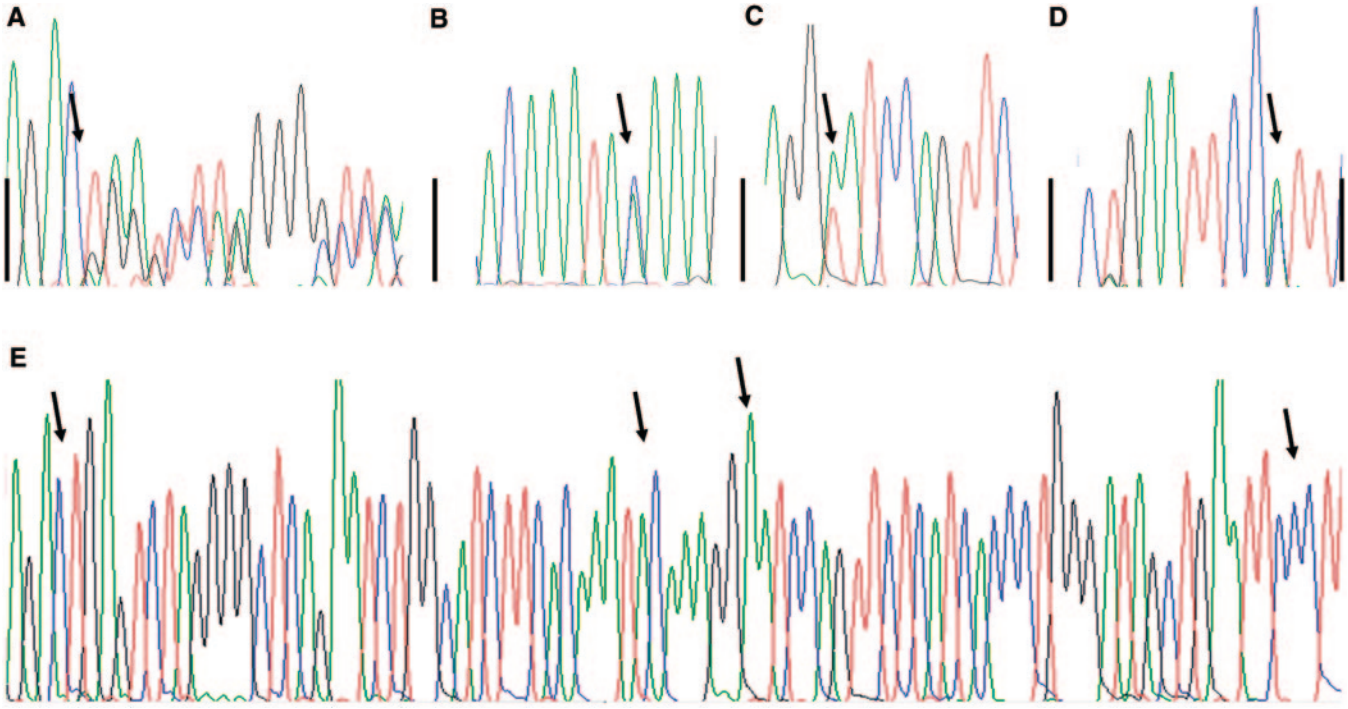


**Figure 3.** Four novel amyloidogenic mutations of the fibrinogen Aα-chain gene. Panel E shows the wild-type sequence for the portion of exon 5 of the fibrinogen Aα-chain gene that encodes the amyloid fibril subunit peptide fragment. (A) Frame-shifting mutation p.Thr525ThrfsX24 resulting from the deletion of T in the ACT codon (c.1632delT). (B) C-to-A single base substitution (c.1670C>A) encoding the p.Thr538Lys variant. (C) A-to-T single base substitution (c.1676A>T) encoding the p.Glu540Val variant. (D) C-to-A single base substitution (c.1712C>A) encoding the p.Pro552His variant. The arrows indicate the positions of the novel mutations.

Estimated median survival by Kaplan-Meier analysis was 10.9 yr (range 0 to 25.2) from diagnosis and 15.2 yr (range 0 to 25.2) from clinical presentation (Figure 5A). Cause of death was infection in six patients, metastatic malignancy in three patients, dialysis withdrawal in two patients, transplant-related mortality in two patients (combined-hepatorenal transplantation[20]), gastrointestinal blood loss in one patient, and was unknown in three patients.

At censor, a total of 44 (62%) patients had reached ESRD, having commenced renal replacement therapy at a median age of 60 yr (range 36 to 82). Median time from presentation with proteinuria to ESRD was 4.6 yr (range 0 to 10.2), and from diagnosis of amyloid to ESRD 2 yr (range 0 to 10.2) by Kaplan-Meier analysis. Among 23 patients with a baseline estimated GFR of more than 20 ml/min who were evaluated for rate of renal decline, mean rate of GFR loss was 11.5 ml/min/yr (range 0.5 to 27.7). Thirty-three patients received hemodialysis, 13 patients received peritoneal dialysis, four patients received both dialysis modalities sequentially, and two patients received preemptive kidney transplants. Thirteen of 44 ESRD patients died during follow up; estimated median survival from commencement of renal replacement therapy by Kaplan-Meier analysis was 8.2 yr (range 0.2 to 24.8), and when censored at transplantation was 9.3 yr (range 0 to 8.7).

No patient developed the typical electrocardiographic and echocardiographic features of restrictive diastolic filling, thickened ventricular walls, and reduced QRS voltages to suggest an infiltrative amyloid cardiomyopathy during follow up. Two patients underwent endomyocardial biopsies; one did not show amyloid and the other showed patchy amyloid deposits within the endocardium and interstitium in association with a dilated cardiomyopathy of undetermined etiology. Symptomatic ischemic cardiac events occurred in 11 patients and ischemic cerebral events in five patients, including three patients in whom both were present. Asymptomatic coronary artery disease was detected in seven of a further 16 patients and asymptomatic cerebrovascular disease in two of a further ten patients who underwent pretransplant cardiovascular screening studies. Hepatic amyloid deposits were detected in only two patients, both of whom had presented more than 8 yr previously. Clinically significant autonomic neuropathy was not detected in any patient during follow up or by formal screening of five patients. Apart from the amyloid peripheral neuropathy that was present at baseline in a Chinese patient with a novel fibrinogen point mutation, no patient developed a clinically significant peripheral neuropathy attributable to amyloid during follow up.

Renal transplantation was undertaken in ten patients, two of whom received two grafts. Median patient follow up from renal transplantation was 5.8 yr. At censor, five grafts were still functioning and seven had failed. Median overall graft survival was 5.9 yr (range 0 to 12.2) by Kaplan-Meier analysis. Three grafts failed immediately for technical reasons; estimated median graft survival among the remainder was 6.7 yr (range 0.9

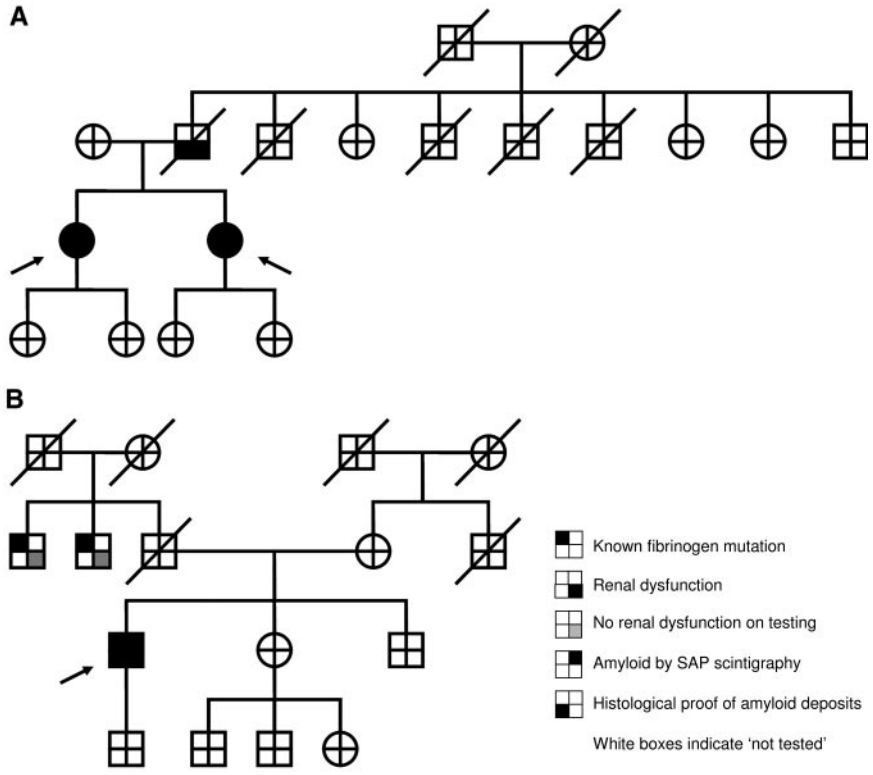


**Figure 4.** Panel A shows the family tree of two German sisters with AFib (arrows) caused by a novel amyloidogenic point mutation that encodes the E540V fibrinogen A$\alpha$-chain variant. The sisters' father had died aged 47 yr but had been dialysis-dependent with proven renal amyloid. Panel B shows the family tree of a patient with AFib who presented at the age of 55 yr (arrow). Two paternal uncles were each shown to be heterozygous for the relevant mutation encoding the E526V fibrinogen variant but did not have renal dysfunction on testing aged 76 and 81 yr, respectively.

to 12.2). Three grafts, including two sequentially in the same patient, failed because of recurrent amyloid after 5.8, 6.0, and 7.4 yr. The remaining graft failed after 5.8 yr because of transplant glomerulopathy without histologic evidence of amyloid. One graft was still functioning at censor 12.2 yr after transplantation despite evidence of recurrent amyloid demonstrated by SAP scintigraphy associated with increasing proteinuria within 7.6 yr of transplantation. The first patient to undergo combined hepatorenal transplantation for AFib was reported in 2000 and is included in the present cohort.[21] She continues to be well and completely free from amyloid 11.5 yr after the combined transplant procedure. A further six patients in this series have undergone combined hepatorenal transplantation at King's College Hospital, London, which was performed preemptively in three patients. One patient died perioperatively with acute necrotizing pancreatitis, and the remaining patients were reported to be doing well with no evidence of recurrent amyloid after a median follow up from transplantation of 24 mo.[20]

AFib is an autosomal dominant kidney disease with a characteristic renal histopathological appearance. Because immunohistochemistry fails to determine the amyloid fibril type in approximately 50 and 10% of patients with AL amyloidosis[22] and AFib, respectively, and because a family history of renal disease or amyloidosis is frequently absent in AFib due to variable penetrance, the discovery of massive glomerular amyloid, particularly in the absence of significant extraglomerular amyloid, should always prompt a search for a mutation in the fibrinogen A$\alpha$-chain gene. Importantly, the presence of a plasma cell dyscrasia in a patient with systemic amyloidosis, as was detected in 10% of the current cohort using the very sensitive techniques now available, neither excludes AFib nor proves AL-type amyloidosis, and does not alter the requirement for DNA analysis.

A proteinuric presentation followed by progression to ESRD within 5 yr was typical of AFib. The natural history of the renal decline in AFib was relatively slow compared with that in untreated systemic AL amyloidosis in which median time from diagnosis to ESRD is 7.5 to 14 mo,[23] but was substantially faster than in hereditary apoAI amyloidosis in which it is typically approximately 8 yr.[24] Despite the absence of therapy to diminish production of the amyloidogenic fibrinogen variant in most patients reported here, median patient survival from clinical presentation was more than 15 yr, contrasting markedly that of less than 2 yr and approximately 5 yr among untreated[25] and treated[26,27] patients with systemic AL amyloidosis respectively. Estimated median survival from commencement of dialysis with censoring at transplantation was 9.3 yr, substantially longer than the approximately 5-yr median survival among all nondiabetic U.K. patients aged 55 to 64 yr who commenced dialysis between 1997 and 2001 (Figure 5B).[28] Median age at death among patients with AFib was 67 yr and several patients survived into the ninth decade. The prolonged survival in AFib compared with that in systemic AL amyloidosis reflects a combination of the slower natural history of the renal disease and the lack of clinically significant extrarenal amyloid deposits that are commonly the cause of death in systemic AL amyloidosis. The current cohort was followed for a median of 4 yr, including eight patients who were followed for over a decade, and the only clinically significant extrarenal organ involvement was liver amyloid in two patients and peripheral nerve amyloid in one other patient. The significance of patchy microscopic cardiac amyloid deposits in one patient with the R554L fibrinogen variant remains unclear although is reminiscent of systemic AA amyloidosis in which amyloid deposits are frequently present upon endomyocardial biopsy but are of no clinical consequence in over 95% of patients.[29] The high prevalence of atherosclerotic cardiovascular

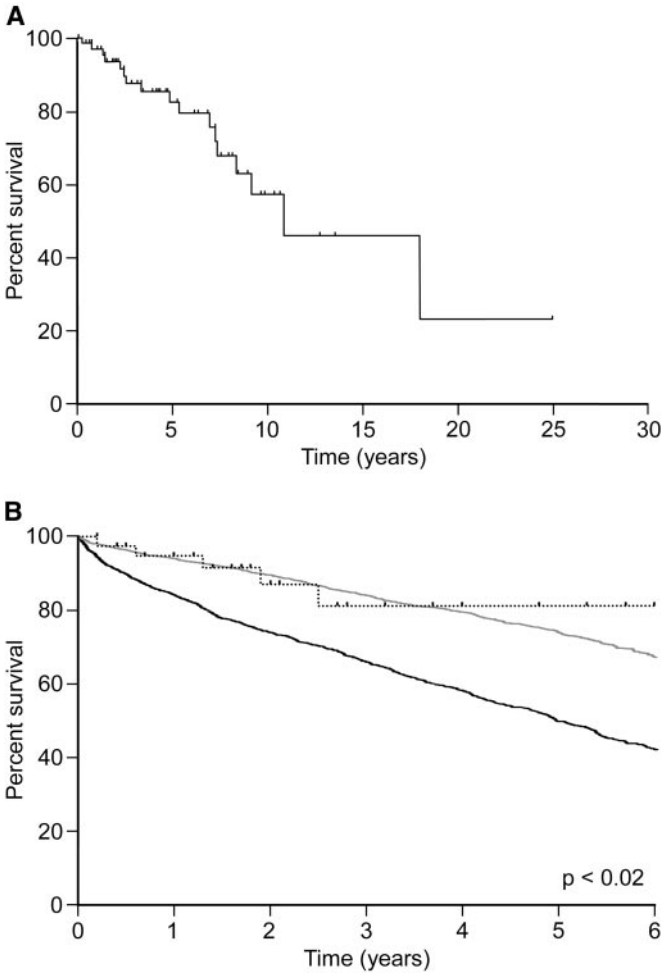


**Figure 5.** Panel A shows a Kaplan-Meier plot of patient survival from diagnosis of AFib. Panel B shows Kaplan-Meier plots of survival on dialysis to 6 yr (censored at transplantation) among patients with AFib (dotted line) compared with all U.K. nondiabetic nephropaths aged less than 55 yr (gray line) and aged 55 to 64 yr (black line). Estimated survival among the AFib cohort, whose median age at commencement of dialysis was 60 yr, was no different from U.K. patients aged less than 55 yr and was significantly better than that in patients aged 55 to 64 yr ($P < 0.02$, log-rank test[28]).

disease among the AFib patients in this cohort was comparable with that in patients with chronic kidney disease generally.[30,31]

Five amyloidogenic mutations of the fibrinogen A$\alpha$-chain gene have been previously reported.[14–17,19] The four novel amyloidogenic fibrinogen mutations reported here are all in the portion of the fibrinogen A$\alpha$-chain gene that encodes the peptide fragment that forms the fibril protein subunit in hereditary fibrinogen A$\alpha$-chain amyloidosis,[14] and all are in close proximity to the previously reported amyloidogenic mutations. The clinical phenotype and renal histologic appearance of the four patients with novel mutations were inseparable from the patients with AFib E526V. A family history of renal amyloid was present in two patients but absent in two others, similar to the situation among the AFib patients with previously reported amyloidogenic mutations.

Renal transplantation in AFib is associated with recurrence of amyloid in the graft and with resultant loss of transplant kidneys after a median of 6.7 yr, although one kidney continued to function after 12.2 yr. This contrasts with combined liver and kidney transplantation which, by removing the source of the circulating amyloidogenic fibrinogen variant,[32] prevents further amyloid deposition in the renal allograft or elsewhere,[21] but is associated with additional perioperative and subsequent risks. The outcome of isolated renal transplantation reported here suggests that the potential benefit of combined hepatorenal transplantation will not be evident for many years in most patients and thus far, it has been our practice to recommend consideration of combined liver and kidney transplantation only in younger, fitter patients with this disease.

## CONCISE METHODS

### Patients

We included in this study all 71 patients with hereditary AFib identified from the NAC database during a period of 15 yr to February 2008.

Patients attended the NAC for their initial diagnostic evaluation and were followed up at regular (usually 12 monthly) intervals for evaluation of organ function and monitoring of whole-body amyloid load by serial $^{123}$I-SAP scintigraphy. Patients attending the NAC underwent serial electrocardiography and echocardiography as well as detailed blood and urine biochemistry. Additional investigations were undertaken when clinically indicated.

All patients were managed in accordance with the declaration of Helsinki, and informed patient consent and institutional review board approval from the Royal Free Hospital Ethics committee were obtained for this study.

### Histology and Immunohistochemistry

Sections from formalin-fixed, paraffin-embedded renal biopsies were stained for amyloid with Congo red and viewed under crossed polarized light.[33] Immunohistochemical staining of the amyloid deposits was performed using monospecific antibodies reactive with SAA, kappa and lambda Ig light chains, and fibrinogen A$\alpha$-chain, as described previously.[22] Fibrinogen staining was with a monoclonal sheep anti-human fibrinogen A$\alpha$-chain antibody (CA1023, Calbiochem). Wherever indicated, additional staining with a panel of antibodies against known amyloid fibril proteins was undertaken.

### DNA Analysis

Genomic DNA was extracted from whole blood treated with EDTA as described previously.[34] A 707-bp fragment of exon 5 of the fibrinogen A$\alpha$-chain gene (c.4445 to c.5152 GenBank accession no. NW_922217) was amplified by PCR and analyzed by automated sequencing. PCR was carried out with Ready-To-Go tubes (Amersham Pharmacia Biotech) with the use of solutions and cycling conditions that have been previously described.[35] The PCR primers: 5′-GCTCTGTATCTGGTAGTACT-3′ (nucleotides 4445 to 4465) and 5′-

ATCGGCTTCACTTCCGGC-3′ (nucleotides 5135 to 5152) were designed to amplify the portion of the fibrinogen Aα-chain gene that encodes the peptide fragment that has previously been identified as the fibril subunit in AFib.[14] The PCR products were purified with a QIAquick PCR purification kit (Qiagen) according to the manufacturer's protocol and sequenced with the primer 5′-TGGGGCACATTTGAAGAG-3′ (4544 to 4561) and the Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) following the procedures recommended by the manufacturer. The sequence of the fibrinogen Aα-chain gene was analyzed on the ABI 3100 Avant Genetic Analyzer.

### Radiolabeled SAP Scintigraphy

Whole-body anterior and posterior scintigraphic imaging using an Elscint Superhelix gamma camera was undertaken 6 or 24 h after administration of $^{123}$I-labeled SAP, as described previously.[36] The labeled SAP studies were interpreted by a single physician (P.N.H) with experience of over 5000 SAP scans.

### Statistical Analysis

Patient survival, time from clinical presentation and diagnosis of amyloidosis to ESRD (renal replacement therapy) and survival on dialysis were estimated by Kaplan-Meier analyses. Rate of decline of renal function was analyzed in a subgroup of patients who had a creatinine clearance of more than 20 ml/min at baseline and was expressed in ml/min/yr.

## ACKNOWLEDGMENTS

We would like to acknowledge all of the physicians and surgeons who were involved in the clinical care of the patients reported in this study. In particular, we would like to acknowledge Dr. AJ Stangou, Dr. J O'Grady, and Professor N.D. Heaton from the Institute of Liver Studies, King's College Hospital, London, who managed the patients undergoing combined liver and kidney transplantation. We would like to thank Beth Jones for expert preparation of the manuscript. These data were presented at the 2008 British Renal Society/Renal Association meeting and will appear in abstract form.

## DISCLOSURES

None.

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