This is a revised and corrected version superseding the earlier posted version.

STANGOU et al

HEREDITARY SYSTEMIC FIBRINOGEN A- α CHAIN AMYLOIDOSIS

HEMOSTASIS, THROMBOSIS AND VASCULAR BIOLOGY

Title

Hereditary fibrinogen A α-chain amyloidosis: phenotypic characterization of a systemic disease and the role of liver transplantation.

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Abstract

Variants of fibrinogen A α-chain (AFib) cause the commonest type of hereditary renal amyloidosis in Europe, and, possibly in the USA as well. Variant fibrinogen is produced in the liver, and solitary renal allografts fail within 1-7 years with recurrent amyloidosis. We assessed 22 patients with AFib for combined liver and kidney transplantation (LKT) and report the clinical features and outcome. Twenty-one had E526V, and one the R554L variant. Coronary atherosclerosis was identified in 68% of cases, and systemic atheromatosis in 55%. Vascular atheroma excised at endarterectomy and endomyocardial biopsies contained purely variant fibrinogen amyloid. Half of cases had autonomic neuropathy. Six of the nine patients who received LKT are alive (cumulative survival 67%), with good allograft function and no amyloidosis at median 67 (33-155) months’ follow-up. Serial ⁹⁹mTc-DMSA renal scintigraphy in 2 cases of pre-emptive LKT before haemodialysis demonstrates preserved native kidney residual function at 5 years follow-up. Four explanted livers were used successfully for domino transplantation. Fibrinogen amyloidosis is a systemic amyloid disease with visceral, vascular, cardiac and neurological involvement. LKT is curative, however, cardiovascular amyloidosis may preclude this option. Our data encourage evaluation of pre-emptive solitary liver transplantation early in the course of amyloid nephropathy to prevent haemodialysis and kidney transplantation.
Introduction

Amyloidosis is a protein misfolding disorder, in which normally soluble proteins undergo conformational changes and are deposited in the extracellular space as abnormal insoluble fibrils that progressively disrupt tissue structure and function. 1 The clinical syndromes of autosomal dominant hereditary renal amyloidoses (HRA) were first described by Ostertag in 1932.2 To date over 25 different mutations in lysozyme,3 apolipoprotein ApoAI 4,5 ApoAII 6,7 and fibrinogen A α-chain genes8,9,10,11 have been identified which share in common the manifestation of amyloid nephropathy in middle age. Given the absence of overt peripheral neurological disease these forms are collectively referred to as ‘non-neuropathic hereditary renal amyloidoses’.12

Fibrinogen amyloidosis due to mutations in the fibrinogen alpha-chain gene (AFib), first described by Benson et al in 1993,8 is emerging as the commonest type of all hereditary renal amyloid diseases in the UK and Europe,11,13,14 while our data (MDB) from a tertiary USA amyloid reference centre suggest AFib is the leading cause of hereditary renal amyloidosis associated with nephrotic syndrome in the USA.8,15,16

There are currently no treatments that can lead to resolution of amyloid deposits. Management is restricted to attempting to interrupt further supply of precursor amyloidogenic proteins, in combination with supportive care of failing organs, including transplantation. The role of transplantation may be either potentially curative, as in liver replacement to eliminate the source of the variant transthyretin in familial amyloid polyneuropathy (FAP), or merely supportive to restore failing organ function.1,17,18
Fibrinogen production is exclusively hepatic. Isolated renal transplantation as a treatment for renal failure in AFib amyloidosis is of limited value. Kidney amyloid recurrence and subsequent allograft loss is almost universal, and occurs within 1-7 years. Ten-year graft survival amongst the 18 reported kidney transplants for AFib to date is 5.5%. This outcome compares poorly with the current half-life of a cadaveric renal transplant for all causes of chronic kidney disease (CKD) of at least 10-12 years, or 10-year graft and patient survival of 64 and 68% respectively.

The lack of success of isolated kidney transplantation in fibrinogen amyloidosis in the current climate of organ shortage, prompted us to evaluate hepatorenal transplantation at the Amyloidosis Treatment Centre at King’s College Hospital to manage the underlying disorder and prevent disease recurrence. Twenty-one of the cases presented here were initially evaluated at the UK National Amyloidosis Centre, Royal Free Hospital, and appeared to have exclusively renal disease.

We report major additions to the current phenotypic description of fibrinogen amyloidosis disease, previously unrecognized disease manifestations and risks, and the first systematic evaluation for liver transplantation in this series of 22 patients with fibrinogen amyloidosis managed in our centre; and we present the long-term outcome of 9 combined hepatorenal transplants and 4 sequential (domino) liver transplants utilizing the explanted livers from patients with AFib who underwent transplantation.
Patients, materials and methods

Twenty-two patients with fibrinogen amyloidosis and stage 3-5 CKD with median glomerular filtration rate (GFR) of 16 ml/min (0-52), were assessed for combined liver and kidney transplantation between 1996-2007. Three patients had been misdiagnosed as systemic acquired AL amyloidosis in association with B-cell dyscrasias, one had received inappropriate chemotherapy and 2 had each received 2 renal allografts which all failed within 58 months. Twenty patients were British and two New Zealanders, 21 had the E526V and one the R554L AFib variant. None were diabetic. Median age at presentation was 55 years (36-63), and at assessment 57 years (49-68). Patients demographics are summarized in table 1.

Four patients aged 58-63 years old with hepatitis C, cryptogenic cirrhosis or alcoholic liver cirrhosis complicated by hepatocellular carcinoma received sequential (domino) liver transplantation in which we utilized as liver grafts the functionally and morphologically normal explanted livers from AFib patients who underwent combined liver and kidney transplantation. The domino recipients received full information and comprehensive pre-transplant counseling regarding the potential risks of transmission of AFib through domino transplantation and consented to the procedure as well as the requirements for regular long term follow-up.

Patients were managed in accordance with the declaration of Helsinki. The King’s College Hospital Research Ethics Committee had sight of the study and indicated approval. Unrelated liver transplant regulatory authority (ULTRA) approval was obtained for all domino donors and recipients prior to placement on the transplant list.
Written informed consent was obtained for diagnostic investigations, for tissue transfer and for use of material in this publication.

**Clinical assessment**

Cardiovascular investigations comprised 12 lead ECG and transthoracic or transoesophageal echocardiography at baseline. Patients with ≥ 1 risk factor of ischaemic heart disease subsequently underwent coronary angiography. Endomyocardial biopsies were performed in the R554L case, and in 3 E526V patients with abnormal echocardiography. Iliac and carotid vessels were assessed with Doppler ultrasound. Cardiac autonomic function was assessed by serial measurements of heart rate variability and blood pressure monitoring at rest and after stress challenges, and 24-hour blood pressure monitoring. Peripheral neurological assessment comprised sensory topographic mapping and the modified polyneuropathy disability (PND) score for evaluation of motor function. Patients who were accepted for transplantation and placed on the liver transplant list underwent re-evaluation at 6-12 monthly intervals during waiting time.

Routine histology was carried out in all explanted livers from AFib patients undergoing combined liver and kidney transplantation before being sequentially utilized for domino transplantation, to exclude parenchymal amyloid deposition and to assess the degree of possible steatosis and graft suitability.

Post transplant follow-up comprised of 3 monthly dual allograft function, creatinine clearance and 24h urine protein levels. Neurological evaluation, allograft Doppler ultrasound scans and echocardiography were performed at least annually. Two AFib patients who had residual native kidney function with GFR of 15 and 12 mls/min.
respectively at the time of dual organ transplant, underwent dynamic and static $^{99m}$Tc-DMSA renograms annually postoperatively.

**Immunohistochemical characterization of cardiac amyloid deposits**

Immunohistochemistry was performed by standard technique. Sections were prepared and incubated sequentially in 1.5 percent goat serum for 30 minutes, rabbit antihuman fibrinogen 1:200 (Dako Cytomation, Inc, Carpinteria, CA) for 30 minutes, biotinylated goat anti-rabbit immunoglobulin G (1:200) (Vector Laboratories, Burlingame, CA) for 30 minutes, ABC reagent (Vector Laboratories) for 45 minutes and substrate for 3 to 7 minutes. Horseradish peroxidise substrate was prepared using FAST dianinobenzadine and urea H$_2$O$_2$ tablets (Sigma-Aldrich, St Louis, MO). Tissues were counterstained with haematoxylin. Representative sections were photographed on a Nikon Microphot-SA microscope with RT WE SPOT digital camera.

**Biochemical analysis of amyloid atheromatous plaque**

Fibrils were isolated from an atheroma excised at endarterectomy from the carotid artery of fibrinogen A a-chain E526V patient by hand homogenization in 2 ml of 0.1M sodium citrate, 0.15 M sodium chloride and centrifugation. The pellet was homogenized and centrifuged as above two more times. The final pellet was solubilized in 1 ml of 8 M guanidine hydrochloride, 0.5 M Tris pH 8.2 containing 10 mg dithiothreitol /ml with magnetic stirring at room temperature overnight. The sample was alkylated with iodoacetic acid (25 mg/ml) and centrifuged. The supernatant was chromatographed on a Sepharose CL6B column (0.90 X 40 cm) equilibrated and eluted with 4 M guanidine hydrochloride, 50 mM Tris pH 8.2. Fractions from approximately 25kDa to the column volume elution area were pooled.
for analysis, exhaustively dialyzed is Spectra Por 6 dialysis tubing against water, and lyophilized. The pool was digested with trypsin in 0.1 M ammonium bicarbonate overnight at room temperature, and the resulting peptides were fractionated by reverse phase HPLC on a Synchropak RP8 column (0.46 X 25 cm) equilibrated with 0.1% trifluoracetic acid in water and eluted with a 0% to 60% acetonitrile gradient over 90 minutes. HPLC fractions were dried in a Savant Speed Vac concentrator and analyzed by Edman degradation on an Applied Biosystems Model 491 cLC protein sequencer using the manufacturer’s standard cycles and methods.

**Statistical analysis**

Statistical analysis was performed using SPSS 17.0 for Windows software (SPSS Inc 17.0, UK) for estimation of median and mean values and bivariate analysis for correlation of variables. Survival was estimated by Kaplan-Meier analysis.

**Results**

Proteinuria was the commonest presenting feature, identified either on routine medical screen or diagnosed during investigations for oedema and fatigue in 55% of cases. Hypertension or complications of headaches or retinal bleed was the initial presentation in 40%, and one patient was diagnosed during family screen. All patients were subsequently placed under renal follow-up and investigated with renal biopsy for the indications of declining renal function or increasing proteinuria. Mode of diagnosis was renal biopsy revealing amyloidosis in all cases. Median GFR at diagnosis was 32.5 (0-103) ml/min, and 24h urine protein 7.2 (0-11.8) gr. Median time from initial presentation to diagnosis of fibrinogen amyloidosis was 23 (3-156) months, and time from diagnosis to end stage renal failure (GFR less than 10 ml/min)
was 8 (0-70) months. At the time of assessment in our institution median GFR was 16 (0-52) ml/min. One patient had end stage liver disease in association with extensive hepatic amyloidosis,\textsuperscript{23} and a second patient had liver amyloidosis with preserved liver function (Tables 1 and 2). Fibrinogen concentration determined by the Clauss (functional) method and thrombin clotting times (TCT) were within the normal range in all plasma samples.

Of note only 24% of patients had family history of renal disease, but 81% had family history of ischaemic heart disease (IHD) or systemic vascular disease with aortic aneurysms or cerebrovascular events. Further family screening with DNA analysis for the AFib gene, revealed that the propensitus or the family members who had cardiovascular history were indeed either the confirmed or obligatory carriers of the fibrinogen gene mutations in each evaluable case. (Table1).

**Cardiovascular findings**

Coronary atherosclerotic disease was documented in 15 patients (68%). Of those, 8 had a diagnosis of coronary artery disease or previous myocardial infarction, in half of cases pre-dating evolution of proteinuria or kidney impairment by 5-7 years. Six patients were diagnosed with asymptomatic 40-80% stenosis of the left or right coronary arteries during transplant assessment, and one had triple vessel atherosclerotic disease with limited plaque burden. (Tables 1,2).

Twelve patients (55%) had severe systemic vascular disease including mobile atheromatous plaques in the atria, aorta, splanchnic vessels, or carotids. Two patients underwent carotid endarterectomy for 80% stenosis. The excised atheroma contained amyloid purely consistent of mutant fibrinogen A α-chain. Arteries and veins of the
hilum of explanted livers contained traces of birefringent fibrinogen amyloid (Figure 1).

Echocardiography was abnormal in 11 of 21 patients (52%), demonstrating impaired relaxation pattern, increased wall thickness, and/or reduced ejection fraction, findings consistent with amyloidosis, and in one case dilated cardiomyopathy (Table 2). Three of the 4 endomyocardial biopsies revealed substantial amyloid deposition in endomyocardium, interstitium and within the walls of endocardial vessels (Figures 1, 2). The R554L patient defies nomenclature criteria for definition of cardiac amyloidosis which include restrictive physiology, \(^{27}\) and is the first AFib case diagnosed with dilated amyloid cardiomyopathy due to coronary disease and myocardial amyloidosis (Figure 2).

**Neurological manifestations**
Cardiac parasympathetic dysfunction and risk of bradycardia were identified in 12 patients (55%). Seven patients who fulfilled Mayo Clinic criteria \(^{28}\) had pacemaker insertion. Autonomic involvement of the gastrointestinal tract was a feature in 15 patients (68%) with constipation or diarrhea, early satiety and nausea or delayed gastric emptying. Polyneuropathy disability scores and sensory mapping were normal in all cases. Results are summarized in table 2.

**Morphological findings**
Kidneys: Extensive amyloid deposits were present in all renal biopsies, showing enlarged glomeruli replaced by amyloid with minimal interstitial involvement.
Spleen: Spontaneous splenic rupture occurred in one patient during haemodialysis and in further 2 cases during transplantation. The excised spleens demonstrated widespread amyloid with predominantly trabecular and subcapsular distribution.

Atheromatous plaque: the atheromatous carotid lesion exhibited Congo red positive deposits which on transmission EM examination revealed fibrillar material with a fibril diameter of approximately 10 nm compatible with amyloid. Edman analysis of tryptic peptides from the amyloid protein yielded the sequence:

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TFPGFFSPMLGEFVSETVSR
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which corresponds to the residue 509-528 peptide of mature fibrinogen A a-chain with the V526 at position 18. No residue 509-528 peptide containing the normal E526 or peptides from apolipoprotein A1 or medin were found.

Cardiac histology: endomyocardial amyloid deposits stained strongly positive on immunohistochemistry with rabbit antihuman fibrinogen antibodies (Dako) in both the E526V and R554L variants. (Figure1)

**Transplant assessment outcome**

Fourteen patients were accepted for listing for combined liver and kidney transplants. Eight patients were declined as the assessment suggested a greater than 50% five-year mortality risk due to poor ventricular function (ejection fraction less than 50%), or cardiovascular/systemic atheromatous disease not amenable to reconstructive treatment. Four patients originally listed were removed from the wait-list because of deterioration in cardiorespiratory function 11-34 months later, and one patient remains on the active waiting list. Time spent on haemodialysis correlated with frequency of identified conventional cardiovascular exclusion criteria (p<0.05).
Outcome of hepatorenal transplantation and domino liver transplantation

Nine patients received combined liver and kidney allografts between January 1996 and September 2009 in our centre. Transplant procedures were complicated by spontaneous perioperative splenic rupture in 3 and postoperative deep vein thrombosis in 2 cases. At median follow up of 67 (33-155) months, six out of 9 patients are alive and well (cumulative survival 67%) with normal liver function. Five patients have good renal function, creatinine 120 (87-159) umol/L and median estimated GFR of 54 (45-69) mls/min. The sixth patient developed renal failure after 68 months and renal biopsy demonstrated chronic allograft nephropathy. All patients who received LKT before or within 6 months from initiating renal replacement therapy are alive and well (survival 100%), but outcome of LKT was successful in only 50% of patients who were on long-term haemodialysis at the time transplant. Two fatal transplant outcomes occurred in long-term haemodialysis patients and were due to biliary dyskinesia and acute necrotizing pancreatitis complicated by fatal bradyarrhythmia in a 62 year old man, and biliary leak, sepsis, subendocardial infarct and multiorgan failure in a 55 year old woman. A further patient on peritoneal dialysis, previously misdiagnosed and inappropriately treated as AL amyloidosis in another facility, developed hepatic artery thrombosis requiring retransplantation but died 4 months after the initial transplant. Median ITU and overall hospital stay was 35 (2-130) and 40 (18-130) days respectively. Patients received standard immunosuppression with Tacrolimus and steroids and additionally mycophenolate mofetil. There were no episodes of rejection.

Twenty-three echocardiography examinations in 8 patients after LKT have documented stable cardiac indices and no evidence of progressive or de novo cardiac amyloidosis, up to 12 years follow up. Symptoms of gut dysmotility had continued in
the 2 patients who had received previous isolated kidney transplant, but resolved in all patients following hepatorenal transplantation. All patients have resumed normal everyday activities and those in non-retiring age have been able to return to full employment.

Following elimination of the source of amyloid production through hepatorenal transplantation, whole body $^{123}$I-labeled serum amyloid P component (SAP) scintigraphy demonstrated regression of systemic visceral amyloid deposits as early as at first annual follow-up scan after transplant. None of the transplanted AFib patients presented evidence of de novo or progressive amyloid deposition at up to 13 years follow-up as previously reported. In contrast, SAP scintigraphy documented progressive systemic amyloidosis and amyloid deposition in the kidney grafts in two AFib patients in this series who had previously received isolated kidney transplantation. One domino recipient had SAP scans and echocardiography examinations up to 5 years with no evidence of de novo amyloid deposition. The remaining three patients did not have SAP scans. One has normal liver and renal function at 2.5 years, and 2 were lost to follow-up after returning to their country of origin following successful LT.

Serial dynamic and static $^{99m}$Tc-DMSA renal scintigraphy following preemptive LKT in 2 cases demonstrated stable native renal function, with consistent contribution to the overall kidney function of 14 and 20 percent respectively up to 5 years follow-up (figure 3).
Discussion

Fibrinogen is a plasma protein with a crucial role in the coagulation cascade through its conversion to fibrin, and is composed of two identical sets of three polypeptide chains termed Aα, Bβ, and γ, joined by disulfide bridging. Each polypeptide is encoded by a distinct gene, FGA, FGB, and FGG. The gene for the fibrinogen Aα chain with 610 amino acid residues, is localized on chromosome 4 and has 6 exons.29 Mutations in any of the three genes encoding for fibrinogen polypeptides can cause dysfibrinogenemias, and recently identified mutations in the A α-chain gene can lead to hereditary systemic amyloidosis.12

Six amyloidogenic mutations in the fibrinogen A α-chain gene have been described to date. The first variant identified in a Peruvian-Mexican family and two unrelated African-American and French kindreds, is caused by a point mutation in the α-chain gene encoding for substitution of arginine to leucine (R554L) in the fibrinogen molecule.8,16 The E526V and E540V variants, two frame shift mutations (4904 del G and 4897 del T), and more recently an insertion/deletion (Del1636-1650 ins CA 1649-1650) variant in the fibrinogen α-chain gene were identified in kindreds of Irish, British, Portuguese, French, German, and Far Eastern origin with amyloid nephropathy due to variant fibrinogen.9,10,11,12,30

Renal amyloidosis in AFib universally progresses to complete ESRF and the focus of clinical intervention has been to provide adequate renal replacement therapy including kidney transplantation.13,22 Age of onset varies within different mutations, from childhood in the insertion deletion mutation to middle life in the commonest E526V
variant\(^8,9,10,30\). The onset of the disease is usually heralded by the development of renal amyloidosis, and penetrance is reportedly low.\(^{13,22}\)

Our data provide valuable insights into the disease phenotype and suggest that hereditary fibrinogen amyloidosis is neither solely nephropathic, nor non-neuropathic, but is a disease with a diverse and complex phenotype. All reported cases to date have been diagnosed through renal pathology, during investigation for hypertension, kidney impairment and proteinuria, frequently identified during routine medical screening. In light however of the findings in this series, corroborated by another recent report,\(^{31}\) a bias towards underdiagnosing cases with predominantly cardiovascular amyloidosis, in the absence of readily accessible tissue for histological sampling, cannot be excluded and may account for the apparent reduced penetrance.

We have identified a high incidence of cardiovascular atheromatous disease amongst our patients often predating evolution of proteinuria or renal impairment by many years. There was also a strong family history for coronary/vascular disease amongst carriers even in the absence of overt renal disease (table 1). Our findings are unlikely to be solely due to the vascular effects of renal failure,\(^{32}\) because the specific form of fibrinogen amyloid was present in the vascular walls and atheromatous plaques. Biochemical analysis indeed revealed that amyloid atheromatous plaques, cardiac and vascular amyloid deposits in AFib were composed wholly of variant fibrinogen, and excluded the presence of other amyloid precursor proteins with inherent atherogenetic properties such as senile transthyretin or apolipoprotein apoAI.\(^{33}\)

This is the first report of a link between variant fibrinogen amyloidosis and atheromatosis. A plausible explanation for the syndrome of systemic amyloid
angiopathy in AFib lies principally with direct amyloid deposition in vascular walls and myocardial vessels. The associated, but likely not necessarily prerequisite, manifestations of nephrotic syndrome, hyperlipidaemia, hypertension, and declining renal function, facilitate atheroma formation on a background of vascular amyloid deposition and impaired endothelial function, further accelerated by commencement of renal replacement therapy.\textsuperscript{32,34,35} The well described predilection for renal amyloid localisation to the glomerulus rather than interstitium largely represents a form of amyloid vascular disease within the kidney, and is in accord with our observation of vascular deposition of variant fibrinogen.\textsuperscript{13,22}

We are further investigating the mechanisms through which vascular amyloidosis may be pro-atherogenic in AFib and other types of systemic amyloidosis associated with nephrotic syndrome such as systemic AL and AA amyloidosis, as well as the possibility that coronary amyloid atheromatous lesions may \textit{per se} mimic atherosclerotic appearances on conventional angiography. The presence of amyloid deposits in the vascular walls of endomyocardial vessels as well as systemically, suggests that a specific type of global coronary amyloid cardiomyopathy may exist in fibrinogen amyloidosis. This syndrome can result in clinical presentation with dilated amyloid cardiomyopathy as in the R554L case presented here, rather than the typical features of restrictive hypertrophic cardiomyopathy universally seen in the systemic amyloidoses.\textsuperscript{27} Cautious interpretation of echocardiographic findings as well as coronary angiography imaging findings is thus required to avoid misdiagnosis or underdiagnosis of disease features.

Our observation emphasizes the importance of awareness regarding cardiovascular symptoms for those at potential risk such as AFib carriers and non-screened family
members, and counselling regarding risk factors such as smoking, obesity, hyperlipidaemia and hypertension. Conversely, screening for proteinuria is inexpensive and readily available, and we recommend its routine use in the primary care monitoring of families with history of cardiovascular events.

Neuropathic features have been observed in association with uremia, and may be reversible after successful renal transplantation. The clinical pattern of cardiac parasympathetic and systemic autonomic neuropathy in this series, corroborated by previous demonstration of cardiac denervation on MIBG scintigraphy and resolution of symptoms following hepatorenal but not after kidney transplantation, suggest this is a true amyloid related autonomic manifestation. 20,36

Splenic involvement in AFib may be clinically significant as manifested by resistant anemia, 20 in the absence of demonstrable splenomegaly. Spontaneous or intra-operative splenic rupture in 4 cases due to extensive splenic amyloid is a specific risk, and has led us to alter the transplant surgical technique to using superior mesenteric venous bypass, to avoid even transient rises in portal pressure. AFib patients receive prophylactic triple vaccination for meningitis, pneumococcal pneumonia and influenza upon placement on the waiting list.

Liver transplantation is the standard treatment for a number of genetic hepatic metabolic disorders including Wilson’s, haemochromatosis, homozygous hypercholesterolaemia, ornithine transcarboxylase deficiency, and primary hyperoxaluria type I, and over 1200 liver transplant procedures have been performed world-wide for transthyretin related familial amyloid polyneuropathy. 37,38
Fibrils isolated from amyloid deposits in AFib have consistently shown to contain exclusively variant fibrinogen. Circulating total fibrinogen in AFib consists of a mixture of wild type-variant fibrinogen in a ratio of 1:1 to 3:2, however only the variant fibrinogen molecule is incorporated in the amyloid fibrils, suggesting that unlike transthyretin, wild-type fibrinogen does not perpetuate amyloid disease. 9,20 We have previously shown by mass spectrometry (Table 2, patients 2,7,11) that following liver transplantation the variant fibrinogen is eliminated and promptly replaced by wild-type fibrinogen.19 The long-term AFib E526V hepatorenal transplant recipients have no amyloid progression up to 12 years follow-up, suggesting that liver transplantation for fibrinogen amyloidosis may be truly curative. 19,20,21,23,39 We have utilised 4 of the explanted AFib livers for sequential (domino) transplantation in patients with end stage liver disease and hepatocellular carcinoma, without evidence of AFib disease transmission up to 5 years follow-up.

The unexpected salvage of residual native kidney function in the 2 patients who received LKT pre-emptively at stage 4 CKD is of particular interest. Stable contribution of 15-20% to total (native and graft) renal function is maintained at 4 and 5 years post LKT. We suggest that the apparent long-term stabilization of residual native amyloidotic kidney function in both cases is attributed to arresting the disease through liver replacement.

In conclusion, we have shown that AFib is a systemic amyloid disease with multi-visceral and neurological involvement, and is associated with cardiac amyloid deposition and amyloid angiopathy and atheromatosis. Hepatic amyloidosis is rare but can lead to liver failure. The addition of liver transplantation to kidney transplant in AFib with ESRF is curative. We support AFib as a new indication for liver
transplantation, and suggest that low cardiovascular risk patients are a favorable group for combined liver and kidney transplantation. Long term haemodialysis patients or those cardiovascularly unsuitable for the combined approach, could receive kidney transplantation as the best form of renal replacement therapy.  

The characterization of the disease phenotype as systemic amyloid disease justifies earlier intervention to arrest amyloid disease progression, and renal outcomes of preemptive hepatorenal transplantation encourage evaluation of isolated liver transplantation early in the course of amyloid nephropathy to prevent systemic and renal progression to ESRF and requirements for dialysis and kidney transplantation. Amyloidotic kidneys may be exceptionally vulnerable to perioperative haemodynamic changes and possible nephrotoxicity of immunosuppression. It is thus recommended that AFib patients who are listed for preemptive isolated LT should be monitored monthly whilst on the waiting list, in order to ensure that GFR is maintained at levels greater than 50 mls/min at the time of a suitable liver graft being available for LT. The transplant status of patients whose GFR falls below the safety cut-off of 50 mls/min on the LT waiting list should be altered to LKT, as in case 22 in this series. The explanted liver grafts can be used in domino transplantation, thus neutralizing the impact on the supply of liver allografts.

We encourage all centers involved in the management of amyloid disease to report transplant and domino procedures for fibrinogen A α-chain amyloidosis to the Familial Amyloid Polyneuropathy World Transplant Registry (FAPWTR), to enable international centralized data collection and meaningful analysis of long term outcomes in this novel indication.
Acknowledgments

We wish to acknowledge and thank the scientists and staff in Merrill Benson’s Amyloid Research Group, Pathology and Laboratory Medicine, Indiana University School of Medicine, Indianapolis, USA, Mr Hisham Rashid, Vascular Surgery Department and Mr Benjamin Corcoran Nuclear Medicine Department at King’s College Hospital, Mr Bart Wagner in the Electron Microscopy Department, Northern General Hospital, Sheffield, Mrs Katharine Bleasdale-Barr and technicians in the Neurovascular Medicine Department, NHNN, Queen Square, Dr Margaret Burke and Dr Alex Bell, Histopathology Department, Harefield Hospital, Professors Ian Simpson and Edward Gane, Auckland City Hospital, New Zealand, Professors Mark Pepys and Philip Hawkins and Staff in the National Amyloidosis Centre, Royal Free Hospital, and the Nephrology Departments of the Queen Elizabeth, Heartlands and Wolverhampton Hospitals, Birmingham, the Glasgow Western Infirmary Hospital, the Moriston Hospital, Swansea, Wirral Hospital, the Preston and Blackpool Victoria Hospitals, the Bournemouth Hospital, the John Radcliffe Hospital and Nuffield Department of Surgery, Oxford UK.

Authorship

Contributions: AJS, JO’G, NDH and MDB are responsible for the conception, design, organization and execution of the study. AJS wrote the manuscript. JO’G and NDH contributed to editing of the manuscript. NRB contributed to the design and organization of the study, drafting and editing of the manuscript and carried out
some of the cardiovascular investigations. BMH contributed to the renal management of the patients and the editing of the manuscript. MR devised the surgical technique for liver transplantation in fibrinogen amyloidosis and performed the combined liver and kidney transplants. BP examined and typed the histological samples, and contributed in editing the manuscript. JW has been responsible for expert intensive care management and has seen and approved the manuscript. MM and PMcC contributed to the cardiovascular investigations. MB-T was responsible for the nuclear medicine investigations. CJM has been responsible for all neurological investigations and has seen and approved the manuscript. J JL and MDB carried out the amyloid fibril characterization and typing of amyloid deposits, and MDB contributed to drafting and editing the manuscript.

**Conflict-of-interest disclosure:** The authors declare no competing financial interests.

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**References**


Tables

Table 1. Patients demographics, clinical characteristics, presenting features, diagnosis and course of renal disease.

Table 2. Findings during assessment for LKT, patient selection and transplant outcome.
<table>
<thead>
<tr>
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<th>Symptoms</th>
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<th>Urinary protein (g/24h)</th>
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* indicates patients with a later age at diagnosis (after 30 years old)
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<td>E526V</td>
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Abbreviations: Pt No: Patient Number (number sequence represents the same patients in table 1 at table 2, at presentation and during evaluation for LKT respectively), Cr Cl: Creatinine Clearance, ESRF: end stage renal failure (glomerular filtration rate 15 ml/min or less), HTN: hypertension, TIA: Transient ischaemic attack, CVA: Cerebrovascular attack, IHD: ischaemic heart disease, MI: myocardial infarct, Renal bx: renal biopsy, Tx: transplant, CKD: chronic kidney disease

Symbols: * Patient number 2 who presented initially with hypertension and proteinuria, is the only AFib patient reported to date with amyloid related liver failure and she received LKT for the indication of both liver and kidney failure. She was anuric and on HD–creatinine clearance 0 mls/min- at assessment for LKT (ref 39). Patient number 3 had liver amyloid deposits with normal function.
† Patient number 9, had a strong family history of IHD and aortic aneurysms including mother, maternal uncle and grandmother, none of whom had renal disease. CT scan excluded aortic aneurysms in the patient before LKT. On subsequent family screen, a maternal aunt was diagnosed as heterozygous for the E526V variant, thus suggesting the above mentioned family members as obligatory carriers.
‡ Patient number 16 had no family history of renal disease; his father had IHD and died in his late 50s of cerebrovascular attack. Patient had extensive carotid, systemic and coronary disease at aged 55. His sister was diagnosed with IHD requiring bypass graft in her early 40s with normal renal function. She was identified as carrier of the AFib E526V variant during subsequent family screen, and has during follow-up developed renal amyloid and ESRF at aged 53 years. Two further siblings are non-carriers and remain free of cardiac events at aged 48-60.

§ Patients number 20 and 21 are related and both have paternal history of renal amyloidosis. Patient number 20 presented with IHD and acute MI at aged 52 years with normal kidney function; he noticed foaming urine and was tested positive for trace proteinuria 7 years later, while GFR was 102 mls/min, and BP normal. Nephrotic range proteinuria was documented 2 years later; renal biopsy revealed amyloid.

§ Patient number 21 presented with MI and normal renal function, as did a further sibling, requiring angioplasty/stenting for IHD 5 years earlier. Patient 21 is at stage 3 CKD; sibling is a carrier and was recently diagnosed with non-nephrotic range proteinuria and GFR 105 ml/min. A further 2 members of the same kindred have long-standing IHD and amyloid nephropathy; non-carrier siblings have no history of IHD.
Table 2. Findings during assessment for LKT, patient selection and transplant outcome

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<th>Pt</th>
<th>Duration of dialysis at assessment for LKT (months)</th>
<th>CrCl ml/min</th>
<th>Autonomic neuropathy</th>
<th>Echo findings (thickness of IVS, LVPW in mm)</th>
<th>Coronary disease</th>
<th>Systemic vascular disease</th>
<th>LKT listing decision</th>
<th>Duration of dialysis at LKT (months)</th>
<th>ITU stay (days)</th>
<th>Hospital stay (days)</th>
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§ Patients 13, 14, 15 and 16 were initially assessed as suitable candidates and were listed for LKT, but were removed from the wait-list due to deteriorating cardiac function or progressive IHD 11-34 months later.
‡ Patient 22 was initially listed for pre-emptive isolated liver transplantation, but transplant status was changed to listing for LKT when GFR fell below 50 mls/min during follow up on the waiting list.
Figure legends

Figure 1. Morphological findings in the common fibrinogen A α-chain amyloidosis E526 variant.

(A) Congo red stain in renal biopsy (X100) in fibrinogen A α-chain amyloidosis. Extensive amyloid infiltrate with glomerular enlargement and replacement of the normal glomerular architecture by amyloid deposition, with almost no amyloid in the interstitium.

(B) Previous section, panel A, shows bright apple green birefringence of the amyloid deposits viewed under cross-polarized light.

(C) Congo red stain in histological sample from a ruptured spleen (X100) in AFib E526V exhibits extensive splenic amyloid deposits with predominantly trabecular and subcapsular distribution.

(D) Endomyocardial biopsy specimen (X100) in a patient with AFib E526V and renal failure, with abnormal echocardiography (patient 19, tables). Cardiac histology stained with Congo red demonstrates extensive, diffuse amyloid deposition in the myocardium. An endomyocardial vessel is demonstrated at the centre of the section, showing complete replacement of its entire wall thickness by amyloid deposition.

(E) Bright apple green birefringence of the amyloid deposits in myocardium and endocardial vessels of section in panel D under cross-polarized light.

(F) Endomyocardial biopsy (X40) H&E stain, in a patient with AFib E526V variant (patient 16, tables), on haemodialysis for 18 months, who had severe carotid atherosclerosis, and whose echocardiography showed normal wall thickness with impaired LV relaxation.

(G) Congo red stain demonstrates amyloid deposition at the periphery of an atrophic muscle fiber from the same sample (X100).
(H) Apple green birefringence under polarized light in previous section.

(I) Arterial intima atheroma (X100) excised during endarterectomy for the indication of 80% carotid stenosis in the same patient as in panels D and E. Congo red stain demonstrates extensive amyloid deposition within the intima and atheromatous plaque (arrows). The Congo red material exhibited strong apple green birefringence. Immunostaining with antifibrinogen antibodies was not performed because normal (wild-type) fibrinogen is expected to be part of the thrombus, and therefore a positive immunohistochemistry could not be reliably positive for a diagnosis of variant fibrinogen amyloidosis.

(J) Transmission electron microscopy (X150,000) images of the atheroma in panel F, demonstrates fibrillar material with fibril diameter of approximately 10 nm, compatible with amyloid. Fibril extraction and characterization revealed the amyloid atheromatous plaque to consist wholly of variant E526V fibrinogen.

(K) Images from subsequent coronary angiography in the same patient, carried out to exclude significant asymptomatic coronary atherosclerosis as part of generalized amyloid angiopathy, in context of findings in Panels F, G, H and I. Left anterior oblique cranial angiogram of left coronary artery shows diffuse atheroma in the left anterior descending coronary artery (LAD) with heavy calcification (arrows).

(L) Left anterior oblique angiographic projection of the right coronary artery (same patient as in panel K) shows diffuse atheroma throughout its course. Leads from dual chamber pacemaker inserted for the indication of arrhythmias are also seen (arrow).

Figure 2. Dilated amyloid cardiomyopathy in fibrinogen A α-chain amyloidosis
R554L variant.

(A) Endomyocardial biopsy (X100) in a 55 year old patient with the AFib R554L variant and stage 2 CKD, whose echocardiography during evaluation for combined
LKT showed dilated cardiomyopathy with ejection fraction of only 25% (patient 18, tables). Endomyocardial histology shows bright apple green birefringence of diffuse Congo red positive endocardial and interstitial amyloid deposits. No additional pathology or potential causes for dilated cardiomyopathy other than amyloidosis were identified.

(B) Strongly positive fibrinogen immunohistochemistry using rabbit antihuman fibrinogen 1:200 (Dako Cytomation, Inc, Carpinteria, CA), in the same section of panel A.

(C) Apical 4 chamber 2 dimensional (2 D) echocardiographic view at end diastole, demonstrating a dilated and globular left ventricular cavity.

(D) Imaging from M-mode echocardiogram in the same patient, demonstrating increased left ventricular dimensions and reduced function.

(E) Parasternal long axis echocardiographic images of increased left ventricular end diastolic dimensions.

(F) Invasive angiogram of the left coronary artery (right anterior oblique projection), showing a 40% stenosis in the proximal anterior descending artery (black arrow), lumen irregularity and more minor narrowing in the intermediate vessel (dotted white arrow) and narrowing in the first septal perforator (white arrow). The patient’s right coronary had minor irregularity (not shown).

**Figure 3.** Serial 99mTc-DMSA scintigraphy shows stable renal graft: native kidneys divided function at 1 and 4 years following preemptive combined liver and kidney transplantation, in AFib E526.

Panels A and B show serial 99mTc-DMSA scintigraphy in an AFib E526V patient who received preemptive combined liver and kidney transplantation at stage 5 CKD, one month before scheduled commencement of haemodialysis.
(A) exhibits posterior view of dynamic renal scintigraphy at 1 year after combined liver and kidney function, 3 hours post injection of 80 MBq $^{99m}$Tc-DMSA. Regions of interest (ROI-circles) shown are used in the calculation of divided function. The percentage of divided function is: transplant kidney 86% and total native 14%.

(B) demonstrates DMSA scintigraphy in the same patient at 4 years after LKT. The panel shows posterior view scintigraphy 3 hours after injection of 80 MBq $^{99m}$Tc-DMSA, and same regions of interest are used in the calculation of divided function. The percentage of divided function is: transplant kidney 87% and total native kidney function 13%. We have confirmed by mass spectrometry in this patient that variant fibrinogen A $\alpha$-chain has been completely eliminated from the plasma and is replaced by normal (wild-type) fibrinogen A $\alpha$-chain following LKT.
Figure 1: Morphological findings in the common AFib E526V variant
Figure 2. Dilated amyloid cardiomyopathy in the AFib R554L variant
Figure 3. Serial $^{99m}$Tc-DMSA scintigraphy shows stable renal graft:native kidneys divided function at 1 and 4 years following preemptive combined liver and kidney transplantation, in AFib E526V
Hereditary fibrinogen A $\alpha$-chain amyloidosis: phenotypic characterization of a systemic disease and the role of liver transplantation

Arie J Stangou, Nicholas R Banner, Bruce M Hendry, Mohamed Rela, Bernard Portmann, Julia Wendon, Mark Monaghan, Philip MacCarthy, Muriel Buxton-Thomas, Christopher J Mathias, Juris J Liepnieks, John O'Grady, Nigel D Heaton and Merrill D Benson