

Familial Amyloidotic Polyneuropathy: how transthyretin associated amyloidosis involves the kidney

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ABSTRACT

Amyloidoses are diseases in which soluble proteins aggregate and form insoluble proteins that accumulate in extra-cellular spaces. The tissue deposits disrupt organ architecture and function and accompany or cause a wide range of systemic diseases. Transthyretin (TTR) is a beta-sheet-rich homotetrameric protein that must undergo rate-limiting tetramer dissociation and partial monomer unfolding to misassemble into amyloid and other aggregates. In familial amyloidotic polyneuropathy (FAP) of Portuguese-type, an amino acid substitution of methionine (M) for valine (V) at position 30 of TTR molecule (ATTR V30M) is present. FAP nephropathy has special epidemiological features with characteristic risk factors. The most important are age of onset of neuropathy, gender (female has higher risk) and presence of family history of renal disease. Low-penetrance of FAP has been found in the families of probands with end-stage renal disease (ESRD).

In TTR V30M gene carriers a stage of microalbuminuria can precede the onset of symptomatic neuropathy. The progression to overt renal disease develops in one half of patients with MA, usually 2 years after MA detection. Overall, one third of patients develop clinical nephropathy and 10% progress to ESRD. All patients have amyloid deposits in the kidney, even without renal expression of nephropathy. A more extensive glomerular and vascular involvement is present in patients with nephropathy. A

peripheral sensitive neuropathy is the commonest first manifestation in nephropatic patients, unlikely, cardiac conduction disturbances have been observed as initial picture. Anaemia is present in one fourth of patients, even without renal failure, and erythropoietin levels are significantly lower than expected for the degree of anaemia. In our centre haemodialysis has been the option to treat ESRD (109 patients) but six patients underwent simultaneous kidney-liver transplantation. We do not register rejection episodes or clinical evidence of recurrence of kidney amyloidosis after the transplant. Finally, in this review we define a nephropatic type of FAP. Structural changes in TTR V30M variant, genetic or environmental factors may influence this particular phenotype.

Key-Words:

Amyloid; epidemiology; kidney; proteinuria; transplantation; transthyretin.

INTRODUCTION

The term amyloid is used to describe several type of diseases in which protein molecules aggregate into an ordered structure to make fibrils with 75 to 100 Å in cross and indeterminate length. X-ray diffraction analysis revealed that fibrils share the feature of being ordered in a β -pleated sheet conformation¹. The fibrils accumulate in extra-cellular spaces to form deposits which have selective affinities for Congo red

dye and apple-birefringence under polarised light, a property considered diagnostic for amyloid. Each type of amyloid is defined by the protein constituent of the fibrils and the classification is based on the chemical composition of the fibrils. To date, 25 structurally unrelated proteins are known to cause amyloidosis². The initial step in amyloid fibril formation is a misfolding event. Whatever the variants of the precursor proteins, they are highly prone to self aggregation and under appropriate conditions will form amyloid fibrils or plaques.

The tissue deposits disrupt organ architecture and function and accompany or cause a wide range of systemic diseases. These include Ig light chain (AL), hereditary (*e.g.*, transthyretin [TTR], fibrinogen A α , lysozyme, apolipoprotein AI, apolipoprotein AII, gelsolin, cystatin), inflammatory conditions (AA) and chronic renal dialysis (β 2-microglobulin). Localised forms are relatively frequent (such as Alzheimer's disease, spongiform encephalopathies and type II diabetes), but completely different from each other and from systemic forms³. The three most important types of systemic amyloidosis are AL, AA and TTR associated (ATTR).

■ AMYLOID AND TRANSTHYRETIN

ATTR (MIM 176300) is the most frequent form of hereditary systemic amyloidosis and was described by Andrade³⁻⁵. TTR is a transport protein for retinol-binding protein and thyroxine and works as a rapid turnover protein. It has been used as a nutrition assessment protein in the appraisal of the acute phase nutritional status in various diseases because it contains four tryptophans in the tetramer of the protein and its half life is 1.9 days. TTR is a beta-sheet-rich homotetrameric protein that must undergo rate-limiting tetramer dissociation and partial monomer unfolding to misassemble into amyloid and other aggregates.

The single gene for TTR is located on human chromosome 18 (18q11.2-q12.1). The human gene has four exons³. Exon 1 codes for a 20-residue signal peptide and the first three amino acids of the mature protein, exon 2 for residues 4 to 47, exon 3 residues 48 to 92, and exon 4 residues 93 to 127. The liver is the source of the precursor protein for amyloid

deposits in the vascular tree, in the heart and the kidney. This source was the base for proposal of orthotopic liver transplantation (OLT) as a treatment option for familial amyloidotic polyneuropathy (FAP). TTR concentration in plasma ranged from 20-40 mg/dl.

In ATTR the misfolding results from an amino acid substitution of the protein, a cause for instability of the tetrameric structure. A mutated protein is not necessary, however, for amyloid deposits, as wild-type is found in senile amyloidosis, meaning that TTR has an intrinsic instability⁷⁻⁹.

More than 90 point mutations in TTR have been reported and the most of them are amyloidogenic⁵. In kindreds with FAP of Portuguese-type an amino acid substitution of methionine (M) for valine (V) at position 30 (ATTR V30M) is present. The disease is autosomal dominant and most patients with ATTR are heterozygotes, with a normal allele and one variant allele⁵. Homozygous patients for the V30M allele have been found in Sweden but the onset and clinical progression of amyloidosis does not seem different from the heterozygous patients. In Portugal 3 homozygous patients have been registered during the last 20 years (personal observations). Compound heterozygous (TTR V30M/T119M) have been described but, curiously, these individuals have a protective effect of the second mutation on the TTR gene, and do not develop symptoms or at least the disease progression is slower¹⁰.

■ CLINICAL IMPACT OF TTR MUTATIONS

Transthyretin amyloidosis presents a wide spectrum of clinical pictures. While this variability could be based on a specific mutation of TTR, the same mutation causes different phenotypes or clinical heterogeneity^{11,12}. Neuropathic forms are the most frequent but cardiac predominant involvement is associated to other mutations such as L111M. In Portugal, the S52P, S50R, V28M¹³ and S23N¹⁴ mutations have been identified in addition to V30M. In the last three renal diseases is absent. The S52P courses with an aggressive form of kidney disease and cardiomyopathy; renal failure predominates over proteinuria¹⁵. Unequivocal kidney disease is also described in G47¹⁶ and S77Y¹⁷. A few non-amyloidogenic variant forms have been identified, with G6S, Y90N and T119M the most common.

■ THE WORLDWIDE ATTR V30M

The north of Portugal (Póvoa de Varzim, Vila do Conde, Barcelos, Braga, Esposende, Matosinhos) is still the place in the world with higher prevalence rates of the disease¹¹. Other foci in the country are the Figueira da Foz, Beiras and Estremadura areas. New families are emerging from the unsuspected area of Douro. The penetrance of disease in Portugal is high, especially in the north. In this country, the expression of the trait varies between distinct families and areas. In what are known as “non-endemic” districts, penetrance, age of onset and organ involvement may lead to this diagnosis being missed¹⁸.

At Portugal's *Centro de Estudos de Paramiloidose and Unidade Clínica de Paramiloidose*, over 2000 patients belonging to 500 unrelated families have been registered.

The second largest focus of TTR V30M variant is Sweden (around Skellefteå and Piteå), where in some communities, 3 to 5 percent of the population may be heterozygous for the trait. The penetrance of disease is higher in Portugal than in Sweden and its age of onset is considerably different. In Portugal the mean age of onset is 33.5 ± 9.5 years and in Sweden 56.6 ± 12.1 years. 81% of Portuguese patients develop symptoms before age 40, but the probability of a Swedish patient manifesting disease before that age is only 13%¹¹.

FAP associated to this variant has been found in Japan¹⁹, with age of onset similar to Portuguese patients. Portuguese immigration carried the mutation to Brazil and Argentina²⁰. Other foci of ATTR V30M have been identified in Majorca²¹, England, Turkey, Italy, Cyprus, and Greece²²⁻²⁴.

■ FAP AS A NEURODEGENERATIVE DISORDER

TTR fibrils are diffusely distributed in the peripheral nervous system of FAP patients. Amyloid deposits are prominent in the endoneurium, Schwann cells, near the vessels and collagen fibrils. There is axonal fibre degeneration, beginning in the unmyelinated and low diameter myelinated fibres. The presence of toxic non-fibrillar TTR aggregates is connected to

their ability to induce the expression of oxidative stress and inflammation in neuronal cells and consequently apoptotic pathways²⁴.

Typically FAP is a small fibre disorder and in a symptomatic TTR gene carrier with normal electromyography more detailed neurophysiologic tests are recommended²⁵.

The clinical picture of FAP ATTR V30M is a symmetric peripheral neuropathy. It usually starts with painful sensory symptoms in the lower extremities but a carpal tunnel syndrome can be found at presentation⁴. Weight loss and motor or autonomic neuropathy usually develop later. Sphincter dysfunction makes bladder drainage difficult. Cardiac conduction disturbances frequently require pacemaker implantation. Ocular involvement with vitreous opacities, dry eye and glaucoma are also common manifestations²⁶.

Although neurological features are the most common initial symptoms, variations exist, and proteinuria, cardiac dysfunction or ocular involvement may herald the disease.

■ FAP AS A RENAL DISORDER

■ Epidemiology

It is important to regard FAP nephropathy as an involvement having special epidemiological features with characteristic risk factors. The most important are age at onset of neuropathy, gender and family history of renal disease.

The age of onset of FAP (neurological onset) in patients who have progressed to end-stage renal disease (ESRD) is, on mean, 41 years, 8 years later than the global sample. Onset after 40 years²⁷ gives a 3.5 risk of developing nephropathy. The gender ratio in ESRD subjects is 1 male to 1.6 females, and in the global sample there are 1.3 males to 1 female. Low-penetrance of FAP has been found in the families of probands with ESRD¹⁸.

The study of symptomatic relatives (affected by neuropathy) belonging to families where there is at least one ESRD patient showed a higher frequency of renal disease in these subjects, particularly in sibs

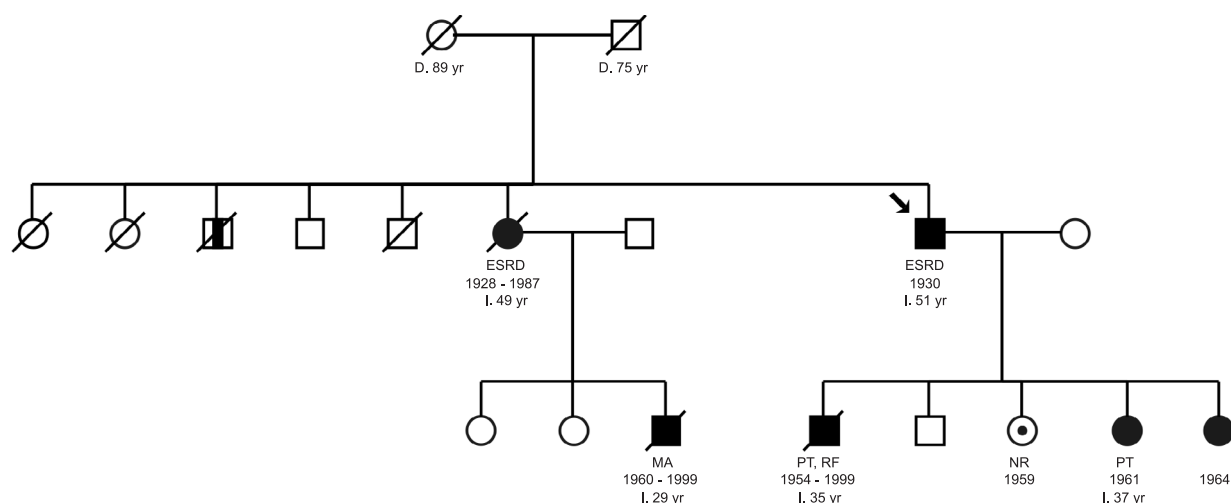


Figure 1

Familial aggregation of nephropathy and later onset. Two sibs had end-stage renal disease (ESRD) and age of onset later than 40 years; their parents died after the eighth decade of life without symptoms and affected descendents developed microalbuminuria (MA), proteinuria (PT), or renal failure (RF); I: age of onset. D: age at death.

(Figure 1). Sibs of ESRD probands have a 38% proportion of ESRD. Overall, the risk for nephropathy is twice as high when there is a family history of ESRD.

In Portugal, FAP is a currently growing disease. New patients and new families from different geographic areas are diagnosed every year; families without previous history of FAP, presence of asymptomatic gene carriers or onset at old age. A nationwide prospective study of new families diagnosed between January 1994 and December 2004 showed that districts of origin now include Beiras, Douro, Aveiro instead of the traditional fishing communities in the north. In the new families, one third of the probands develop ESRD, 3 times more than in FAP overall. These probands had an onset 11 years later than ESRD-free probands.

In the presence of a sensitive neuropathy and renal disease, FAP should represent a differential diagnosis, even in the absence of family history or an origin from typical districts.

■ Progression of nephropathy

In TTR V30M gene carriers a stage of microalbuminuria (urinary albumin excretion $\geq 20 \mu\text{g}/\text{min}$ and $< 200 \mu\text{g}/\text{min}$) can precede the onset of symptomatic neuropathy. Microalbuminuria (MA) has been found

in 75% of the patients with neuropathy, and commonly appears within the third and fiftieth year of neurological disease. The progression to overt renal disease (proteinuria) develops in one half of patients with MA, usually 2 years after MA detection. After a course of microalbuminuria, about 20% of the patients show some degree of renal insufficiency. Overall, one third of patients develop clinical nephropathy and 10% progress to ESRD²⁸.

Renal function is currently evaluated by serum cystatin C, a sensitive method for the detection of a slight decrease in glomerular filtration rate, instead of creatinine clearance based on collection of 24 hours urine, particularly in patients with neurogenic bladder.

A regular evaluation of FAP patients with low levels of proteinuria and in early phases of renal failure, especially those with epidemiological risk factors, prevents the deterioration of renal function associated to pyelonephritis, anaemia and cardiac disease. The oedema, associated to hypotension, and an easy dehydration (vomiting) makes the management of renal disease difficult. A small percentage of patients develop hypertension, usually when renal failure begins. The main indications for dialysis are fluid overload and metabolic acidosis. An early and systematic evaluation of serum bicarbonate is recommended during follow-up.

In our experience of 109 consecutive patients on dialysis, we advise the construction of vascular access for dialysis when serum creatinine attains 3 mg/dL as bacterial infections may precipitate rapid deterioration of renal function. The choice of contra-lateral side to pacemaker implantation for construction of arteriovenous fistulas, grafts or tunnelled cuffed catheters avoids a future venous hypertension or even venal cave syndrome associated to vascular access²⁹.

■ Nephropathy distant or close to neuropathy

The old idea that renal disease was a late event in FAP progression does not fit in the group of patients with poorer prognosis in kidney involvement. In our 17 year survey and in the patients in whom first renal features (proteinuria, renal failure) was documented, nephropathy began, on average, 4 years after neuropathy. The most precocious form of overt nephropathy was massive proteinuria, which was observed about 3 years after the disease began. We verified that in 11% of patients with evolution towards ESRD the first feature of amyloidosis was nephropathy, not neuropathy. Massive proteinuria at presentation is a characteristic typical of women who later progressed to ESRD, not of men. ESRD happened, on average, 10 years after neuropathy appeared²⁹.

■ Features associated to nephropathy

A peripheral sensitive neuropathy is the most common first manifestation of FAP in nephropathic patients. It is unlikely that cardiac conduction disturbances are

observed as initial picture. Isolated symptomatic bradycardia (atrioventricular block or sinus node dysfunction) with few or no neuropathic complaints is a typical first feature in nephropathic patients. Rhythm disturbances pre-dialysis motivated a pacemaker implantation in 80% of the patients. ESRD women need a pacemaker earlier than men.

■ Renal pathology

All patients had amyloid deposits in the kidney, even without renal expression of nephropathy. Our routine evaluation of 80 renal biopsies before liver transplantation confirmed these results. Kidney deposits can be present even in asymptomatic gene carriers, more than five years before expression of neuropathy and without renal features.

Large amyloid deposition in medullary zone and tubules is characteristic of ATTR derived from V30M variant; tubular amyloid deposits mainly in loops of Henle and distal segments are present, but cortical interstitium and proximal convoluted tubules are spared. A more extensive glomerular (in the mesangium and vascular pole) and vascular amyloid involvement is present in patients with microalbuminuria or proteinuria³⁰. In cases of nephrotic range proteinuria associated or not to renal insufficiency, enlarged glomeruli are almost obliterated by amyloid deposition and vascular deposits are found, as in other types of amyloidosis (Figure 2). Immunohistochemistry with monoclonal anti-TTR antibodies demonstrates that the amyloid deposits are due to transthyretin. The severity of renal amyloid deposition did not consistently parallel that of myelinated

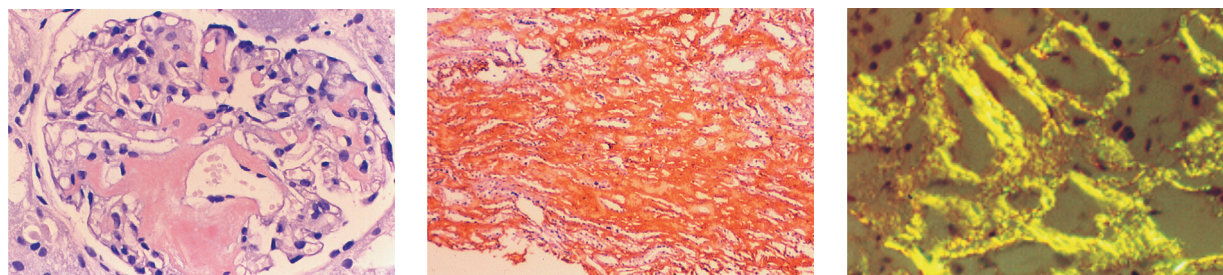


Figure 2

Renal biopsy of ATTR. **A:** mesangial and vascular pole deposition (Congo red x400); **B:** anti-TTR fixation in medullary (immunohistochemistry, x200); **C:** deposition in tubular basement membranes (Congo red under polarization light x400).

nerve fibre loss in the sural nerve³¹, which corroborates our experience that neuropathy and nephropathy are dissociated in some patients.

■ SPECIFIC DISORDERS AND NEPHROPATHY

■ Anaemia

The evaluation of 165 FAP patients showed that anaemia is present in one fourth of the patients and is similar in both genders³². Erythropoietin (EPO) levels are significantly lower than expected for the degree of anaemia and in 17.5% of them EPO was undetectable. Iron stores, B12 vitamin and serum folate were normal. Interestingly, low EPO levels were observed independently of the presence of renal failure or anaemia, and sometimes preceded clinical disease. Concomitant infections are more common in anaemic than in nonanaemic patients, although the prevalence of chronic infection (>1 month) is similar. In a study of EPO levels before and after OLT an increased rate of anaemia and maintenance of low EPO were evident³³. The observed to expected (O/E) EPO level ratio decreased even further after OLT. Despite the decrease in creatinine clearance, a similar median O/E EPO level was observed, independently of renal failure. The increase of anaemia and maintenance of low EPO after liver transplantation seems to exclude an inhibitory effect of the circulating mutated protein on the EPO-producing cells. The localisation of EPO producing cells in the kidney of FAP patients is under investigation.

■ Vitamin D deficiency

While vitamin D (25-OHD) deficiency is common in FAP, PTH levels do not rise. A study involving 47 nondialysed patients and 14 asymptomatic gene carriers was performed to evaluate 25-OHD levels. In 24 patients and in 2 asymptomatic gene carriers 25-OHD was < 20 ng/mL (deficiency), in 10 patients < 5 ng/mL. Although 25-OHD was significantly lower in patients than in asymptomatic subjects, no differences were found in PTH and calcium; 25-OHD was significantly and positively correlated with serum albumin, transferrin, vitamin B12 and glomerular filtration rate estimated by serum cystatin C (not with Cockcroft-Gault formula or serum creatinine); significantly and

negatively with duration of symptoms. The biological actions of vitamin D reinforce the early treatment of this deficit.

■ TREATMENT STRATEGIES FOR NEPHROPATHY

Haemodialysis has been the option to treat ESRD²⁹. Peritoneal dialysis is not implemented because the disability and gastrointestinal disturbances make this treatment difficult. Survival on dialysis is, on mean, 21 months. Current ESRD treatment is dialysis or simultaneous liver-kidney transplantation. The majority of patients do not fit the global neurological criteria for transplantation. The waiting list is too long for a prompt liver transplant in an early phase of renal disease (subnephrotic-range proteinuria) and before renal failure develops. The haemoglobin levels must be regularly evaluated. Anaemia is promptly corrected with low doses of recombinant erythropoietin.

■ Prognostic factors for survival after dialysis

The under-nutrition and subsequent low albumin values were identified as prognostic factors in the outcome. An early intervention and nutritional support in nephropathic patients is essential. Parenteral nutrition may be instituted during dialysis to good tolerance at a later stage and the metabolic rate increased, in spite of decreases in energy intake.

■ The expectancy of liver-kidney transplantation

As TTR is produced mainly by the liver, with a liver transplant, the mutant TTR will be replaced by the normal protein. The long-term problems after liver transplantation are related to a disease that was not cured, associated to medical morbidity and mortality due to infections. The susceptibility for this kind of complications is intrinsic to FAP as a multi-organ disease.

Combined liver-kidney transplantation is an option for FAP patients with ESRD, but autonomic neuropathy causes urinary retention or incontinence, and represents an additional problem in kidney transplantation.

With combined liver-kidney transplantation we can expect no recurrence of amyloidosis in kidney graft and the deterioration of neuropathy will be avoided. In the FAP international registry the survival for liver and kidney transplantation at one year is 65% (see www.fapwtr.org). In our centre, 6 patients received combined liver-kidney transplantation, one pre-emptive. The post operative period was uneventful except in one patient who died of sepsis after a liver re-transplantation (one month after the first transplant). A suprapubic cystostomy during transplant surgery was an elective procedure in another patient. The follow-up ranged between 94 and 15 months, both organs had good functions and there were neither rejection episodes nor clinical evidence of recurrence of kidney amyloidosis. Serum creatinine at the end of follow-up ranged from 0.7-1.8 mg/dL. The major problems after transplantation are associated to urinary and skin infections. Prophylaxis of bacteriuria with antibiotics is advisable.

■ THE PREFERENTIAL RENAL INVOLVEMENT

Biological differences in tissue selectivity for amyloid or TTR precursor proteins may explain distinct clinical and pathological patterns for the same mutation. TTR interactions with matrix proteins, promotion of glomerulosclerosis and fibrosis certainly play a pathogenic role in nephropathy and anaemia. It is likely that sub-clinical renal disease is early in susceptible patients. Structural changes in TTR V30M variant, genetic or environmental factors may influence the particular renal phenotype.

Conflict of interest statement. None declared.

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