Progression of transthyretin amyloid neuropathy after liver transplantation

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ABSTRACT

Objective: To biochemically characterize peripheral nerve amyloid in subjects with transthyretin (TTR) amyloidosis and assess effect of orthotopic liver transplantation (OLT) on progression of neuropathy.

Methods: Amyloid fibrils were isolated from peripheral nerve tissues of 6 patients with TTR amyloidosis who were heterozygous for an amyloid-associated TTR mutation. Ratio of variant to wild-type TTR in the fibrils was determined by amino acid sequencing of tryptic peptides containing either the variant amino acid residue or the corresponding normal amino acid.

Results: Amyloid fibrils from 3 subjects who died without having received a liver transplant were composed of 60%–65% variant TTR and 35%–40% wild-type. Amyloid fibrils from a subject who died 5 years after liver transplantation contained 25% variant and 75% wild-type TTR.

Conclusion: Ratios of variant to wild-type TTR in amyloid patients heterozygous for an amyloid-associated TTR mutation are similar to published ratios for amyloid fibrils in cardiac tissue. Survival after liver transplantation for TTR amyloidosis may be associated with progression of neuropathy due to continued deposition of amyloid derived from wild-type TTR. ***Neurology*® 2010;75:324–327**

GLOSSARY

OLT = orthotopic liver transplantation; **SDS-PAGE** = sodium dodecyl sulfate–polyacrylamide gel electrophoresis; **TTR** = transthyretin.

The most common clinical manifestations of hereditary transthyretin (TTR) amyloidosis are peripheral neuropathy and cardiomyopathy.[1] TTR amyloidosis is an autosomal dominant inherited form of amyloidosis that may present in any decade of adult life and usually leads to death within 5 to 15 years. There are more than 100 TTR mutations that are associated with development of amyloidosis.[2] Plasma TTR is almost exclusively the product of liver synthesis, and thus orthotopic liver transplantation (OLT) has been utilized as a therapy for TTR amyloidosis to stop synthesis of plasma variant TTR.[3,4] Many patients with the valine to methionine mutation at amino acid position 30 of TTR (Val30Met) have benefited from liver transplant; however, clinical reports have shown progression of cardiomyopathy and neuropathy in many patients with TTR mutations other than Val30Met as well as some Val30Met patients after OLT.[5-8] It has been hypothesized that continued cardiac deposition of TTR can occur from wild-type TTR.[9] Biochemical analysis of cardiac amyloid deposits in patients heterozygous for a TTR mutation has shown a predominance of variant over wild-type TTR.[10-13] In contrast, amyloid isolated from fibrils in patients after liver transplant has shown a predominance of wild-type over variant TTR.[11,12] These data indicate that wild-type TTR produced by the liver transplant can continue to deposit as cardiac amyloid after OLT. The present study was initiated to address whether progression of peripheral nerve amyloid deposition from wild-type TTR continues after OLT.

METHODS

Patients. Six subjects with TTR amyloid neuropathy were studied; 3 died without liver transplantation, 1 died 1 month after liver transplantation, 1 died 15 years after receiving a liver transplant, and 1 died 5 years after receiving a liver transplant (table). All nerve preparations were obtained from sciatic nerve at time of postmortem except for the subject who died 15 years after

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Disclosure: Author disclosures are provided at the end of the article.

Table Transthyretin composition of nerve amyloid

Patient	TTR mutation	Age, y	Years post-OLT[a]	Variant TTR, %	Wild-type TTR, %
1	Val30Met	63	—	65	35
2	Thr49Ala	79	—	64	36
3	Thr49Ala	82	—	60	40
4[13]	Ala25Ser	58	0	60	40
5	Val30Met	55	9	57	43
6	Thr60Ala	61	5	25	75

Abbreviations: OLT = orthotopic liver transplantation; TTR = transthyretin.
[a] Time of nerve harvest.

OLT. He had an infected neuropathic (Charcot) knee joint which required amputation of the lower extremity 9 years after OLT. In that case, amyloid fibrils were obtained from the common peroneal nerve.

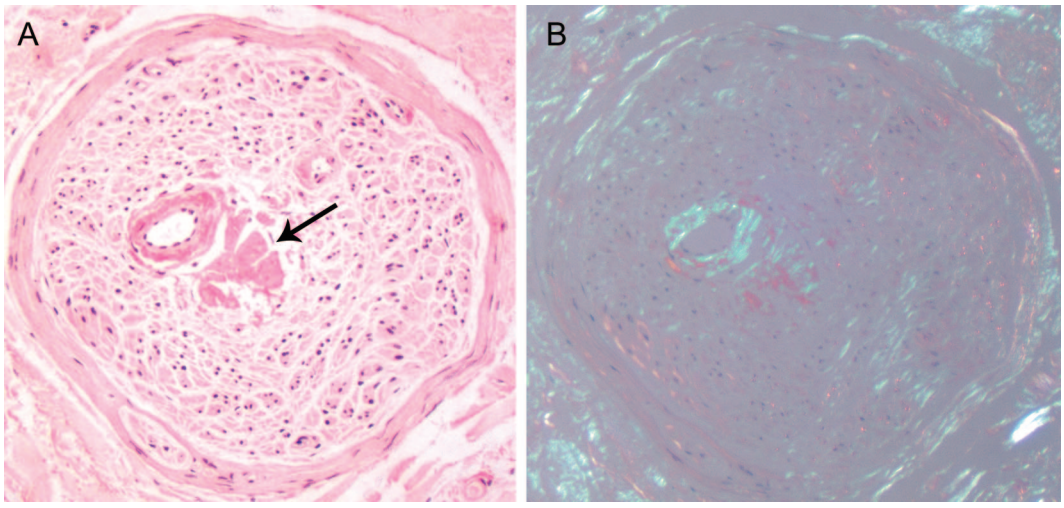
Isolation of amyloid protein. Nerve tissue stored at −80°C was pulverized in a mortar and pestle, homogenized in citrate/saline, and centrifuged repeatedly to remove soluble proteins.[13] Isolated fibrils from each specimen were solubilized in 8 M guanidine hydrochloride, reduced with dithiothreitol, alkylated with iodoacetic acid, and centrifuged. The supernatant was fractionated over a Sepharose CL6B column (0.9 × 60 cm) to isolate the amyloid protein. Pooled fractions were exhaustively dialyzed against water and lyophilized.

Characterization of amyloid protein. Protein samples were analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) on 16.5% Tris-Tricine Ready Gels (Bio Rad). Sepharose CL6B pools were digested with trypsin in 0.1 M ammonium bicarbonate and the digests were fractionated by reverse-phase high-pressure liquid chromatography on a Synchropak RP-8 column (250 × 4.6 mm) eluted with an acetonitrile gradient. Samples were analyzed by Edman degradation on an Applied Biosystems Procise 491 cLC protein sequencer using the manufacturer's standard cycles. The relative amounts of wild-type and variant TTR in nerve amyloid were estimated from the recovered amounts of tryptic peptides containing the wild-type or variant amino acid residue.[10]

RESULTS Amyloid protein was isolated from nerve tissue of 3 patients who had not undergone OLT (1 Val30Met and 2 Thr49Ala TTR patients), and 3 patients who had OLT (1 each with Ala25Ser, Val30Met, and Thr60Ala). All had extensive nerve amyloid deposits (figure). Analysis by SDS-PAGE of the Sepharose CL6B pools from solubilized nerve tissues showed similar patterns in all 6 patients. All contained major bands in the 8–11 kDa region and only very weak bands at 15 kDa and 30 kDa, the positions of intact TTR monomer and dimer, indicating the amyloid TTR was highly proteolyzed. Edman N-terminal sequence analysis of the Sepharose CL6B pools from patients 3, 4, and 6 in the table showed low amounts of sequence starting with residues 49 and 52 of TTR, while the other 3 patients gave no discernable sequence.

Relative amounts of normal and variant TTR in nerve amyloid protein were estimated from the recovered amounts of tryptic peptides containing the normal or variant amino acid in residues 22–34 of TTR for the Ala25Ser and Val30Met patients, and in residues 49–70 of TTR for Thr49Ala and Thr60Ala patients. In the 3 non-OLT patients, variant TTR comprised 60%–65% of the amyloid TTR and wild-

Figure Cross-section of a fascicle from sciatic nerve of a patient with TTR Thr60Ala amyloidosis who died 5 years after liver and heart transplantation



Amyloid is present throughout the fascicle with denser deposits around endoneurial vessels (arrow). Severe depletion of myelinated nerve fibers in this fascicle is representative of the histology of the entire nerve at this anatomic level. (A) Congo red stain, original magnification 100×. (B) Viewed between crossed polars showing typical green birefringence of Congo red–stained amyloid.

type TTR 35%–40% of the amyloid protein (table, patients 1–3). We have previously characterized the nerve amyloid in the Ala25Ser patient who died 1 month after OLT and found 60% variant and 40% wild-type TTR (table, patient 4).[13] This would best be considered as essentially a nontransplant patient.

Analysis of amyloid protein in nerve tissue from the Val30Met patient who had lower extremity amputation 9 years post OLT showed 57% variant and 43% wild-type TTR (table, patient 5). Nerve conduction studies in this patient over a 3-year period (2 years prior to and 1 year after OLT) showed a 75% decrease in ulnar compound action potential amplitude but no change in conduction velocity. The median sensory action potential amplitude dropped from 50% of normal to nondetectable in the same time.

Nerve amyloid from the Thr60Ala TTR patient who died 5 years after combined heart and liver transplant contained 75% wild-type Thr60 and 25% variant Ala60 TTR (table, patient 6). This result is consistent with continued amyloid deposition in nerve from wild-type TTR.

DISCUSSION More than 1,500 patients have had OLT for TTR amyloidosis. Many patients have benefited. Data collected by the Familial Amyloidotic Polyneuropathy World Transplant Register, however, show that, while patients with the Val30Met mutation have a 5-year survival of 80%, patients with other TTR mutations as a whole have a survival of only 50%–60%.[14] It has now been demonstrated that these latter patients often have clinical progression of systemic amyloidosis, and we have previously demonstrated by biochemical analysis that cardiac deposition of amyloid derived from wild-type TTR continues after OLT.[12] If a patient has combined heart and liver transplantation, amyloid deposition in the heart graft does not occur; however, progression of amyloid deposition in other organ systems has been observed. The finding of an increased ratio of wild-type TTR in peripheral nerve of a patient with the Thr60Ala mutation who died 5 years after OLT is consistent with the same finding for patients who have progressive TTR cardiomyopathy after OLT. This patient who had combined heart and liver transplant had no amyloid deposition in the heart transplant at the time of death. After the transplants, clinical progression of neuropathy was indicated by need for crutches to ambulate and eventual confinement to a wheelchair during the last year of the patient's life.

Biochemical analysis of nerve amyloid for the other 2 OLT patients did not show reversal of the variant/normal TTR ratio that was so pronounced for the Thr60Ala patient. The Ala25Ser patient (patient 4, table) died 1 month after OLT. Variant/normal TTR ratio would be expected to be comparable to the patients who had not had OLT. The Val30Met patient (patient 5, table) whose nerve amyloid was studied 9 years after OLT also had more variable than normal TTR in the amyloid fibrils. A possible explanation for the disparate results for the Thr60Ala and Val30Met patients could be related to the differences in phenotype for these 2 TTR mutations. Val30Met patients with disease onset before age 50 usually present with early onset and rapidly progressive peripheral neuropathy. Only later do they develop significant cardiac or renal disease. The OLT Val30Met patient in this study had fairly advanced neuropathy at the time of OLT and probably extensive nerve amyloid deposits which did not change significantly after OLT. This rationalization is supported by data which show better survival statistics for Val30Met OLT patients than for those with other TTR mutations.[14] Thr60Ala patients typically present with cardiomyopathy and little or no clinical evidence of peripheral neuropathy. They usually die as a result of the restrictive cardiomyopathy unless they have a heart transplant. Prolongation of life by heart transplant can then allow time for amyloid progression in other organs, such as peripheral nerve. The continued amyloid deposition can only be from normal TTR if a liver transplant has been done at the same time as the heart transplant. This could explain the predominant amount of normal TTR found in nerve of patient 6 and is consistent with the findings we have previously reported for cardiac TTR deposits in patients who died 3 or more years after OLT.[12]

In the case of peripheral nerve amyloid deposition, it has been suggested that TTR fibril precursor protein may be from the choroid plexus, which produces TTR found in CSF.[15] This would require transmission of TTR from the CSF through channels to the peripheral nerves. If this was the explanation for progression of neuropathy after liver transplantation, it would be expected that the ratio of variant to wild-type TTR in nerve deposits of patients who have had liver transplantation would be similar to the ratio observed in nontransplanted patients. This is not the case for the Thr60Ala OLT patient presented here. The increased ratio of wild-type to variant TTR in nerve amyloid deposits is consistent with hepatic production of only wild-type, which perpetuates the neuropathy by continued deposition of amyloid.

It is hypothesized that patients who have combined heart and liver transplantation do not develop amyloid in the heart graft because it does not have preformed amyloid deposits (nidus) to facilitate ad-

dition of wild-type to the deposits. In most patients with TTR amyloidosis, some degree of peripheral nerve deposits are already present at the time of liver transplantation and, therefore, can serve to perpetuate deposition of fibrils from wild-type TTR. While numerous patients who have had both heart and liver transplants have fared well, it is obvious that progression of neuropathy is a limiting factor in prognosis and emphasizes the need for medical treatments to prevent TTR amyloid formation.

DISCLOSURE

Dr. Liepnieks serves on the editorial board of *Amyloid: The Journal of Protein Folding Disorders* and receives research support from the NIH (PHS AG10133 [Co-I], DK42111 [Co-I], and RR-00750 [Co-I]), RLR Veterans Affairs Medical Research, the Marion E. Jacobson Fund, and the Machado Family Research Fund. L.Q. Zhang reports no disclosures. Prof. Benson serves on the editorial board of *Amyloid: The Journal of Protein Folding Disorders*; receives royalty payments (to Indiana University) for a patent re: Transgenic Mouse Expressing V717F; has served as a consultant for Isis Pharmaceuticals, Inc. and Alnylam Pharmaceuticals; and receives research support from the NIH (PHS AG10133 [PI], DK42111 [PI], and RR-00750 [PI]), RLR Veterans Affairs Medical Research, the Marion E. Jacobson Fund, and the Machado Family Research Fund.

Received January 4, 2010. Accepted in final form April 1, 2010.

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