Clinical and Genetic Findings in Eight Israeli Patients with Transthyretin-Associated Familial Amyloid Polyneuropathy

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ABSTRACT: **Background:** Transthyretin (TTR)-associated familial amyloid polyneuropathy (FAP) is an autosomal dominant multisystem disease with neurological and extra-neurological manifestations. It is caused by various mutations in the *TTR* gene leading to the formation of insoluble amyloid.

Objectives: To describe the clinical and genetic findings in patients with *TTR*-associated FAP in Israel.

Methods: We evaluated eight patients clinically and genetically during the years 2006 to 2011.

Results: At onset, all the patients exhibited sensory loss of the lower and upper limbs, five patients experienced muscle pain, and one patient had lower limb weakness. Five patients had autonomic nervous system manifestations, and four demonstrated evidence of amyloid cardiomyopathy. Nerve conduction studies showed sensorimotor axonal neuropathy in all patients. Sural nerve biopsies were obtained in five patients; only three biopsies revealed amyloid deposit. In four patients of Yemenite descent, genetic analysis of the *TTR* gene demonstrated ser77tyr mutation. One patient of Tunisian descent and one Ashkenazi patient harbored the val30met mutation. One patient of Iranian descent showed val32ala mutation, and another Ashkenazi patient showed phe33leu mutation.

Conclusions: *TTR*-associated FAP is a progressive and fatal disease that exists in the Israeli population and is unproportionally common among Yemenite Jews. This disease may be under-diagnosed and should be considered in the differential diagnosis of any patient with rapidly progressive neuropathy, especially with autonomic involvement or extra-neural features. The absence of amyloid in nerve biopsy should not rule out the diagnosis.

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KEY WORDS: neuropathy, transthyretin gene, familial amyloid polyneuropathy, nerve biopsy, nerve conduction studies

Transthyretin-associated familial amyloid polyneuropathy is an autosomal dominant multisystem disease with variable penentrance among geographic and ethnic groups. Patients with FAP demonstrate sensory motor deficit associated with autonomic disturbances and various extra-neurological manifestations [1]. The disease was first described in Portugal [2], and subsequently in Japan [3] and Sweden [4]. A val30met mutation in the *TTR* gene was revealed in most patients. The disease was believed to be limited mainly to these endemic areas; however, it has emerged gradually around the world, with numerous mutations other than the common val30met mutation [5].

Only a few patients with FAP have been described in Israel. The first patient was reported in 1981 [6]. This patient, of Jewish Ashkenazi descent, had severe, early-onset, dominantly inherited familial amyloidosis. Initially, a thr49gly mutation in the *TTR* gene was reported, but a phe33ile substitution was subsequently found. Later, an additional mutation (gly6ser) was described [7]. Thus, this was the first FAP patient reported to have a double variant allele. The second family in Israel was described in 2005 by Lossos et al. [8] in a third-generation Jewish-Yemenite family. A previously described substitution of tyrosine for serine at position 77 was found as the pathogenic mutation. The third Israeli patient who was reported soon after the previous case was of Jewish-Iranian ancestry [9]. The pathogenic mutation was a substitution of valine to alanine at codon 32 of the *TTR* gene.

We report the clinical and genetic findings in eight new Israeli patients of various ethnic origins with *TTR*-associated FAP. Two of them belong to the families that had already been described.

PATIENTS AND METHODS

We retrospectively evaluated all patients with *TTR*-associated FAP. We included only those with genetic confirmation of *TTR* mutation. We identified eight patients who were diagnosed during the years 2006–2011. All had undergone neurological

FAP = familial amyloid polyneuropathy
TTR = transthyretin

assessment and nerve conduction studies. Motor and sensory conduction was measured using the Keypoint machine (Dantec, Denmark) and surface electrodes by standard methods. Sural nerve biopsies were obtained from five patients. The specimens were fixed in 10% formaldehyde, paraffin-embedded, and after cutting into 5 µm sections were examined with the following staining techniques: hematoxylin-eosin, Luxol fast blue, Masson's trichrome, periodic acid-Schiff, Congo red, and immunocytological stains for myelin basic protein and panaxonal protein PGP 9.5. Congo red-positive deposits were observed under polarized light, and the characteristic yellow-green birefringence confirmed the amyloid nature of the deposits. Rectal biopsy was obtained from one patient. Echocardiogram was performed using standard methods.

Blood for DNA extraction was drawn after the patients signed an informed consent. Sequencing of the coding parts of the *TTR* gene was performed in three patients by Dr. V. Planté-Bordeneuve (Department of Neurology, Centre Hospitalier Universitaire, Henri-Mondor, Creteil, France), and in one patient by Dr. Y. Shinar (Laboratory for investigation of familial Mediterranean fever and autoimmune diseases, Sheba Medical Center, Tel Hashomer). In four patients a point mutation was investigated by Dr. H. Rosenmann (Neurological Genetic Laboratory, Hadassah Medical Center, Jerusalem).

RESULTS

FAP PATIENTS AND FAMILIES

We identified eight FAP patients (six males and two females). All are Jewish Israelis; four are of Yemenite descent, two are Ashkenazi, one is Iranian and one emigrated from Tunisia. Their age at onset ranged from 50 to 70 years (mean 56.4 years). The time to diagnosis was 1 to 5 years (mean 2 years) [Table 1]. One patient of Yemenite origin belongs to the family reported by Lossos et al. [8] and the sibling of the Jewish Iranian woman was described by Kaplan et al. [9]. All the other patients are unrelated and had no relatives with known FAP.

INITIAL SYMPTOMS

In all patients the first symptoms were those of sensory neuropathy. These included numbness, paresthesias and neuropathic pain. The lower limbs were the first to be affected in all. The upper limbs were affected within 0.5 to 2 years. Weakness of the lower limbs developed within 0.5 to 3 years [Table 2].

DISTRIBUTION OF SENSORY LOSS AND MUSCLE WEAKNESS

All patients described symmetric sensory loss of distal lower and upper limbs. During disease progression all modalities were affected including touch, temperature, vibration sense and proprioception. Worsening of pain with advanced disease was noted by most patients. Weakness started in the dorsiflexors of the feet and progressed to the distal upper limbs.

Table 1. Patient's characteristics and mutations

Patient/Gender	Age at onset	Descent	Family	Initial presentation	Other features	Mutation	Age at death
K.V. (f)	56	Ashkenazi	–	Neuropathy	Autonomic malabsorbtion	phe33leu	60
S.D. (m)	55	Tunisian	–	Neuropathy	Autonomic cardiomyopthy	val30met	62
W.L. (m)	70	Ashkeazi	–	Neuropathy		val30met	–
C.A. (f)	59	Iranian	Ref. 9	Neuropathy		val32ala	–
K.S. (m)	50	Yemenite	–	Neuropathy	Autonomic malabsorbtion, cardiomyopathy	ser77tyr	56
K.Z. (m)	60	Yemenite	–	Neuropathy		ser77tyr	–
F.M. (m)	52	Yemenite	–	Neuropathy	Autonomic	ser77tyr	–
Z.A. (m)	60	Yemenite	Ref. 8	Neuropathy	autonomic	ser77tyr	–

Table 2. Background and clinical features

	No.
Men/women	6/2
Age of onset (yrs)	58 (50–70)
Time to diagnosis (yrs)	2 (1–5)
Initial symptoms	
Sensory loss	8/8
Lower limb weakness	1/8
Autonomic symptoms	5/8
Cardiomyopathy	4/8
Pain	5/8
Carpal tunnel syndrome	4/8
Amyloid in nerve biopsy	3/5
Cause of death	
Malabsorbtion	2/3
Cardiomyopathy	1/3

Eventually the proximal lower limbs were affected; the patients had to use canes and finally were confined to a wheelchair.

AUTONOMIC MANIFESTATIONS

Erectile dysfunction was the most common complaint, reported by four male patients. Other autonomic symptoms were constipation, urinary incontinence, dry mouth and dry eyes, and orthostatic hypotension with recurrent syncope. In two patients the gastrointestinal impairment became the main problem. They suffered severe weight loss with intestinal malabsorbtion that eventually led to their death.

CARDIOMYOPATHY

Echocardiogram was obtained in all. Four patients demonstrated evidence of amyloid cardiomyopathy. Two had granular sparkling echo findings, and two showed left ventricular hypertrophy.

Figure 1. Congo red stain of sural nerve biopsy viewed with polarized light demonstrates apple green birefringence of amyloid

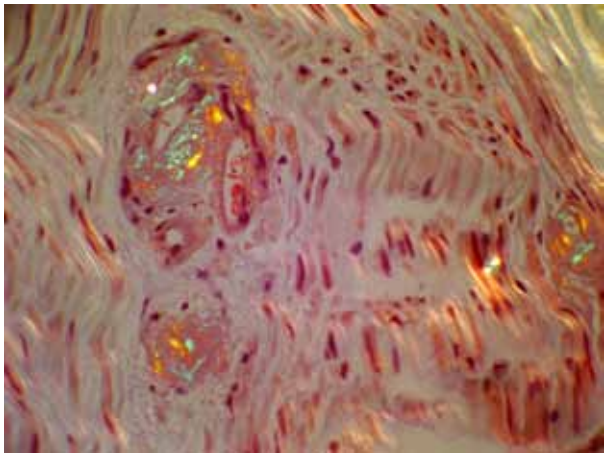
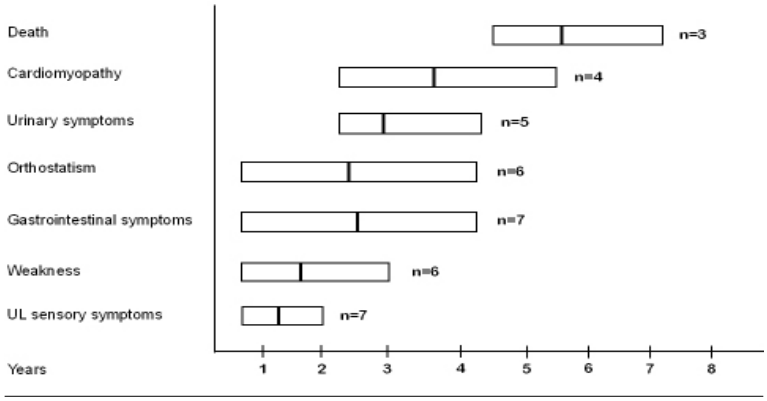


Figure 2. Time course of the main symptoms presentation after disease onset. The line inside the bars denotes the mean for this group of patients. n = number of patients. UL = upper limbs



NERVE CONDUCTION STUDIES

Nerve conduction studies were performed in all the patients. In one patient the first study was interpreted as normal but, later, sensorimotor axonal neuropathy was evident. In all other patients, sensorimotor axonal neuropathy was found, more pronouncedly in the lower limbs.

NERVE BIOPSY

Sural nerve biopsy was obtained in five patients. All had pathological findings of axonal neuropathy with reduced number of thick and thin myelinated and small unmyelinated fibers. In two biopsies no amyloid deposits were observable, while another two biopsies revealed amyloid [Figure 1]. One patient underwent two nerve biopsies, and amyloid was seen only in the second one.

RECTAL BIOPSY

Rectal biopsy was obtained from one patient. The specimen showed amyloid deposit within the muscularis mucosa.

GENETIC STUDIES

Genetic analysis of the *TTR* gene in the four patients of Yemenite descent demonstrated substitution of serine for tyrosine at position 77. One Ashkenazi patient (born in Russia) had substitution of phenylalanine for leucine at position 33. The other Ashkenazi patient (born in Germany) and a patient of Tunisian descent had the common val30met mutation. The patient of Iranian ancestry had a val32ala mutation.

DISEASE COURSE

In the patients with sufficient length of follow-up the disease progressed rapidly [Figure 2]. Three patients died within 4 to 7 years of the onset of symptoms. The cause of death was intestinal malabsorbtion and cachexia in two and cardiomyopathy in the third.

DISCUSSION

TTR is a 127 amino acid protein, encoded by a small gene (7.6 kilobases) containing four exons, located on the long arm of chromosome 18. It is produced mainly by the liver, although a small quantity is synthesized by the choroid plexus and retina. The role of TTR is to transport thyroxin and retinol. To date 113 amyloidogenic mutations in the *TTR* gene have been identified [5]. These mutations decrease the stability of *TTR* tetramers, leading to monomer aggregation in the extracellular space and, through a complex process, creation of insoluble amyloid [10].

TTR-associated FAP is a progressive and fatal disease with variability in age of onset, course, and pattern of clinical deterioration. The disease has been classified as late or early onset. Patients with late onset of symptoms are typically diagnosed beyond the fifth decade of life [11], while patients with early onset are usually diagnosed and die before the fourth decade [12]. Several mutations have been associated with late-onset disease and others with early-onset disease [11-13].

In the first report of the disease [2] the duration of disease among Portuguese patients ranged from 7 to 10 years. However, in subsequent reports from Portugal and Sweden the mean disease duration was 10.8 and 10.7 years respectively [14,15]. On the other hand, Japanese patients with late-onset FAP due to Val30Met mutation progressed rapidly with mean disease duration of 7.3 years [1].

The first reported Israeli patient [6] had early-onset disease with a rapid course, probably due to the presence of two mutations. In all the other described patients, as well as in our series, disease onset was late with a relatively rapid course of deterioration.

Until recently the only available treatment was liver transplantation. The rationale for liver transplantation is to prevent the formation of additional amyloid deposits by removing the main source of mutated *TTR*. Biochemical studies have confirmed the considerable and persistent reduction of mutated

TTR in the serum. Livers from FAP patients can be used as donor grafts in domino transplantation. If the recipients of the liver are old enough they will not be affected since the time for disease development is several decades.

Liver transplantation must be performed early in the course of FAP. If performed shortly after onset of symptoms most of the patients remain stable. It may increase the median survival to more than 20 years [16]. In Sweden, for example, the 5 year survival rate is 92% [17]. However, liver transplantation does not prevent the development of heart arrhythmia, and it has no effect on ocular and central nervous system complications of amyloidosis since the mutated *TTR* continues to be secreted from the retina cells and choroid plexus.

Recently, medical treatment for *TTR*-associated FAP was approved by the European Community. The medication, tafamidis meglumine, binds to the two thyroxine-binding sites on the native tetrameric form of *TTR* and prevents dissociation into monomers and formation of amyloid. A recent randomized double-blind placebo-controlled trial showed a disease- modifying effect on the neuropathy, neurophysiological function and especially on quality of life and body mass index [18]. As in liver transplantation, it is reasonable to start the treatment as soon as possible. Whether treatment should be given to healthy carriers of the mutated gene is unknown and has yet to be decided.

In conclusion, *TTR*-associated FAP exists in the Israeli population and is probably underdiagnosed. There is a focus among Yemenite Jews. All harbor the ser77tyr mutation. Thus, in a Yemenite Jew with unexplained neuropathy, particularly if associated with autonomic signs, the ser77tyr mutation in the *TTR* gene should be sought. In fact, *TTR*-associated FAP should be in the differential diagnosis of any patient with rapidly progressive neuropathy, especially with autonomic involvement or extra-neural features. The absence of amyloid in a nerve biopsy does not rule out the diagnosis since it may be a sampling error, as occurred in two of our patients. The val30met mutation is not common in the Israeli population. This may explain the relatively rapid course of the disease in our series.

The first domino liver transplantation in Israel was recently performed successfully in a patient with a ser77tyr mutation. We hope that *TTR*-associated FAP will be prioritized for liver transplantation in the available units in Israel. These patients deteriorate rapidly and late transplantation is therefore dangerous and inefficient. It is our hope that the new medical treatment for this devastating disease will soon be approved in Israel.

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References

1. Koike H, Tanaka F, Hashimoto R, et al. Natural history of transthyretin Val30Met familial amyloid polyneuropathy: analysis of late-onset cases from non-endemic areas. *J Neurol Neurosurg Psychiatry* 2012; 83: 152-8.
2. Andrade C. A peculiar form of peripheral neuropathy: familial atypical generalized amyloidosis with special involvement of the peripheral nerves. *Brain* 1952; 75: 408-27.
3. Araki S, Mawatari S, Ohta M, et al. Polyneuritic amyloidosis in a Japanese family. *Arch Neurol* 1968; 18: 593-602.
4. Andersson R. Familial amyloidosis with polyneuropathy. A clinical study based on patients living in northern Sweden. *Acta Med Scand (Suppl)* 1976; 590: 1-64.
5. Plante-Bordeneuve V, Said G. Familial amyloid polyneuropathy. *Lancet Neurol* 2011; 10: 1086-97.
6. Pras M, Prelli F, Franklin EC, Frangione B. Primary structure of an amyloid prealbumin variant in familial polyneuropathy of Jewish origin. *Proc Natl Acad Sci USA* 1983; 80: 539-42.
7. Jacobson DR, Buxbaum JN. A double-variant transthyretin allele (Ser 6, Ile 33) in the Israeli patient 'SKO' with familial amyloidotic polyneuropathy. *Hum Mutat* 1994; 3: 254-60.
8. Lossos A, Soffer D, Steiner-Birmanns B, et al. Extended phenotype in the transthyretin Tyr77 familial amyloid polyneuropathy. *Eur Neurol* 2005; 53: 55-9.
9. Kaplan B, Shinar Y, Avisar Ch, Livneh A. Transthyretin amyloidosis in a patient of Iranian-Jewish extraction: a second Israeli-Jewish case. *Clin Chem Lab Med* 2007; 45 (5): 625-8.
10. Merlini G, Bellotti V. Molecular mechanisms of amyloidosis. *N Engl J Med* 2003; 349: 583-96.
11. Yasuda T, Sobue G, Doyu M, et al. Familial amyloidotic polyneuropathy with late-onset and well-preserved autonomic function: a Japanese kindred with novel mutant transthyretin (Ala97 to Gly). *J Neurol Sci* 1994; 121: 97-102.
12. Yamamoto K, Hsu SP, Yoshida K, et al. Familial amyloid polyneuropathy in Taiwan: identification of transthyretin variant (Leu55-Pro). *Muscle Nerve* 1994; 17: 637-41.
13. Blanco-Jerez CR. Jimenez-Escrig A, Gobernado JM, et al. Transthyretin Tyr77 familial amyloid polyneuropathy: a clinicopathological study of a large kindred. *Muscle Nerve* 1998; 21: 1478-85.
14. Coutinho P, Martins da Silva A, Lopes Lima J, et al. Forty years of experience with type I amyloid neuropathy. Review of 483 cases. In: Glenner GG, Pinho e Costa P, Falcao de Freitas A, eds. Amyloid and Amyloidosis. Amsterdam: Excerpta Medica, 1980: 88-98.
15. Ikeda S, Hanyu N, Hongo M, et al. Hereditary generalized amyloidosis with polyneuropathy: clinicopathological study of 65 Japanese patients. *Brain* 1987; 110: 315e37.
16. Okamoto S, Wixner J, Obayashi K, et al. Liver transplantation for familial amyloidotic polyneuropathy: impact on Swedish patients' survival. *Liver Transpl* 2009; 15: 1229-35.
17. Yamamoto S, Wilczek HE, Nowak G, et al. Liver transplantation for familial amyloidotic polyneuropathy (FAP): a single-center experience over 16 years. *Am J Transplant* 2007; 7: 2597-604.
18. Coelho T, Maia L, Martins da Silva A, et al. Tafamidis (Fx-1006A): a first-in-class disease-modifying therapy for transthyretin familial amyloid polyneuropathy. *Neurology* 2010; 74: A286.

The worst kind of people are those who confuse kindness for weakness

Werner Makowski (b. 1929), German banker