Amyloid, June 2007; 14(2): 147–152

informa
healthcare

Familial amyloid polyneuropathy associated with TTRSer50Arg mutation in two Iberian families presenting a novel single base change in the mutant gene

MIGUEL MUNAR-QUÉS[1], JAIME MASJUAN[2], TERESA COELHO[3], PAUL MOREIRA[4], CARLOS VIADER-FARRÉ[1], & MARÍA J. M. SARAIVA[4]

[1]*Grupo de Estudio de la PAF, Majorca, Spain,* [2]*Servicio de Neurología, Hospital Ramón y Cajal, Madrid, Spain,* [3]*Unidade Clínica de Paramiloidose and Serviço de Neurofisiologia, Hospital Geral Santo António, Porto, Portugal, and* [4]*Amyloid Unit, IBMC and ICBAS, Universidade do Porto, Portugal*

Keywords: *Hereditary amyloidosis, familial amyloid polyneuropathy, transthyretin variant, orthostatic hypotension, liver transplantation, vitreous amyloidosis, mutated sequence of TTR gene*

Abbreviations: *FAP = familial amyloid polyneuropathy; TTR = transthyretin; OH = orthostatic hypotension; PNP = polyneuropathy; LTX = liver transplantation*

Abstract

We present two families, from Spain and Portugal, with familial amyloid polyneuropathy (FAP) associated with the mutation TTRSer50Arg. This mutation was first described in two Japanese patients from independent families and later in a French-Italian patient and a Vietnamese family. The two families presented here, are the first to be diagnosed with this mutation in the Iberian Peninsula. In the patients of both families, FAP was very aggressive as they rapidly developed multiple symptoms with progressive deterioration; we emphasize the presence of severe orthostatic hypotension in the Spanish proband which confined him to a wheelchair. This proband was the first patient with this mutation to have undergone liver transplantation and results were encouraging. The mutation was detected in four patients and one disease-free relative by DNA sequencing of exon 3 and induced mutation restriction analysis. The most outstanding feature was the single base transversion A to C in codon 50 (CGT instead of AGT), whereas in both Japanese patients and the French-Italian patient it was T to G (AGG instead of AGT). To our knowledge only six FAP mutations with more than one single nucleotide mutation for the same codon have been reported to date.

Introduction

Familial amyloid polyneuropathy (FAP) is a late-onset inherited amyloidosis associated with a mutation on the transthyretin (TTR) gene which codes for a TTR variant. Large foci of FAP related to a particular variant TTR with a methionine for valine substitution at position 30 (TTRVal30Met) have been described in Portugal, Japan, Sweden, Brazil and the Balearic Islands. Besides these foci, isolated affected families have been described all over the world, some related to TTRVal30Met or to other TTR variants. More than 80 different pathogenic variants have been described [1].

One recently described pathogenic mutation is TTRSer50Arg. It was previously reported in two Japanese patients from independent families [2,3], in a French-Italian patient [4] and in a Vietnamese family [5].

We present the clinical and genetic data of one Spanish and two Portuguese TTRSer50Arg patients, together with short clinical reports on affected relatives of the former. We include the pedigrees in Figures 1 and 2, the symbols for which are shown in Table I.

These are the first families with this mutation in the Iberian Peninsula and the second and third in the Caucasian population.

Case reports

Spanish patients

Proband. The patient, a 42-year-old man born in Jaén, Spain, started with progressive anorexia, loss of

Dedication

This work is dedicated to Carlos Viader-Farré, deceased in November 2005, for his invaluable contribution to the genetic epidemiology in the Balearic Islands.

Correspondence: Dr. Miguel Munar-Qués. Grupo de Estudio de la PAF, Plaza Olivar 5, 07002 Palma de Mallorca, Spain. Tel: 971-713155. Fax: 971-213696. E-mail: munar-ques@ogm.jazztel.es

ISSN 1350-6129 print/ISSN 1744-2818 online © 2007 Informa UK Ltd.
DOI: 10.1080/13506120701259580

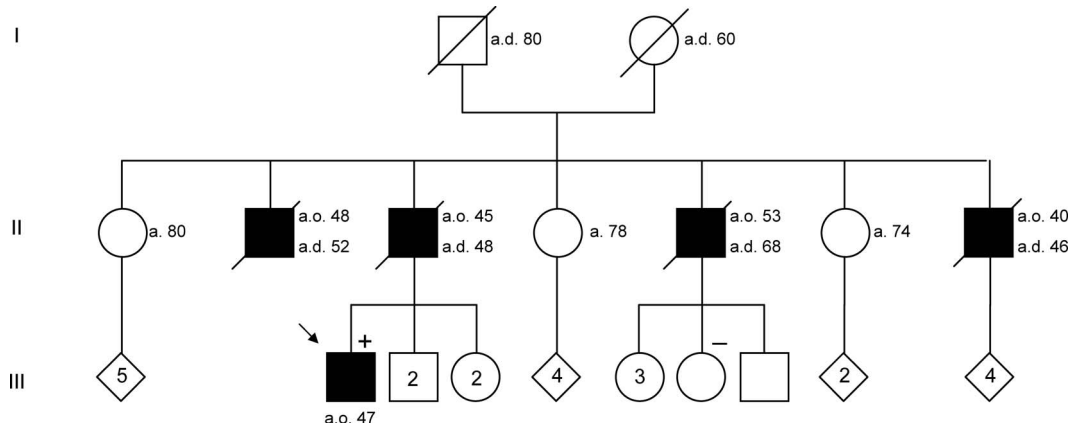

Figure 1. Pedigree of the Spanish family. See Table I for definitions of symbols.

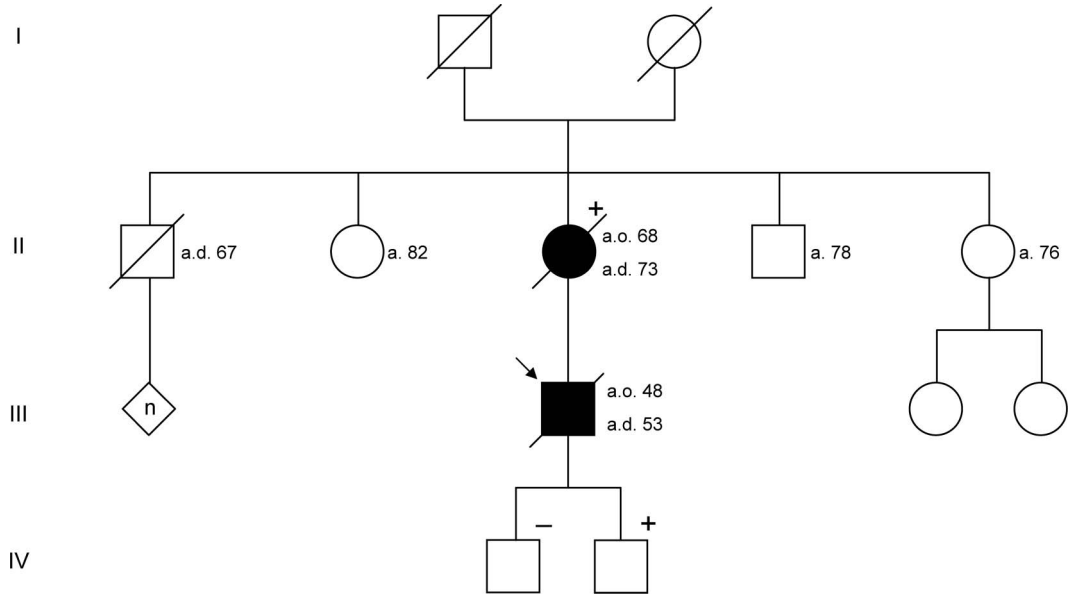

Figure 2. Pedigree of the Portuguese family. See Table I for definitions of symbols.

Table I. Symbols of pedigrees.

	Non affected
	Non-affected, unknown sex
	Confirmed patient
+ +	Patient with DNA analysis
– –	Non-carrier of the mutation
+ +	Asymptomatic carrier of the mutation
	Deceased
↘	Proband
a.o.	Age at onset
a.d.	Age at death
a.	Present age

weight and vitality, depression and muscular weakness of distal portions of lower limbs with slight difficulty in walking without steppage.

In the following 12 months his weight loss reached 40 kg. During this interval he developed: (i) progressive severe orthostatic hypotension (OH), with frequent bouts of postural syncope requiring confinement to a wheelchair; (ii) distal paresthesia in the four limbs; (iii) constipation which later alternated with progressively more frequent diarrhea; and (iv) sexual impotence.

At age 43 he was visited for the first time. An electromyography showed a chronic axonal polyneuropathy (PNP) affecting predominantly sensory nerves in the lower limbs. Rectal biopsy with prior Congo red staining (CR) revealed amyloid deposition positively stained with anti-TTR serum

confirming the diagnosis of FAP. Finally, a genetic study was performed.

To halt the progression of FAP, a liver transplantation (LTX) was indicated. It was performed in June 2000, 9 months after the first visit and 2 years from the onset of the disease. Graft function to date is excellent. A direct enzyme-linked immunosorbent assay (ELISA) with the monoclonal antibody Mab39-44 [6], showed the disappearance of the variant in plasma. Furthermore, several significant changes in the clinical picture were observed starting 1 year after LTX. First, following a slow, but progressive improvement of OH, in 2003 the patient was able to leave the wheelchair and gradually returned to work; since 2004 he has been working part-time. Second, distal paresthesias decreased. Third, amyotrophy and weakness of lower limbs was improved and he has gradually been able to increase walking time. Fourth, his appetite has improved without weight gain and, finally, since 2003 the diarrhea has disappeared. The patient is satisfied with the result of LTX as he is leading an active life with part-time work; this explains his reticence to periodic check-ups at the hospital.

The patient has four siblings aged 40, 38, 36 and 33 years, and three offspring aged 21, 17 and 12 years, all asymptomatic; they refused the genetic study.

Patient II-2. A 48-year-old man presented with progressive PNP in the lower limbs, weakness and weight loss. Rectal biopsy showed amyloid deposits detected by CR. He died aged 52 with cachexia.

Patient II-3. A 45-year-old woman, mother of the proband, presented with a clinical picture similar to her son's except for the severe OH. In a sural nerve biopsy amyloid deposits were detected by CR. After progressive deterioration she died at age 48.

Patient II-5. A 53-year-old man presented with progressive vitreous deposits in both eyes, without systemic symptoms. He underwent right vitrectomy aged 59 years; amyloid was detected by CR in these deposits. A genetic study was performed. Eight years later, still without systemic symptoms, he underwent left vitrectomy. He died of lung cancer aged 68. After his death a genetic study was performed in one offspring.

Patient II-7. A 40-year-old man presented with a clinical picture similar to his sib II-2. He was also diagnosed with FAP after detection of amyloid deposits by CR in rectal biopsy. He died aged 46 years at a very advanced stage.

Portuguese patients

Proband. At 48 years of age, this Portuguese man suffered right sciatic syndrome due to lumbar disc herniation which was relieved with surgery. A few months later he complained of neuralgic pain in both legs and feet, followed 1 year later by sexual impotence and urinary retention with vesical large residue. He was operated on again without any improvement. In addition to the previous symptoms he developed anorexia, weight loss, vomiting after meals, constipation alternating with diarrhea and difficulty in walking and using his hands. An electromyography showed a severe axonal neuropathy, affecting sensory and motor divisions of the nerves in the upper and lower limbs. In a rectal biopsy amyloid deposits were detected by CR which stained with anti-TTR serum.

At age 50 he was visited for the first time. He was a thin, wheelchair-bound man with a severe amyotrophic tetraparesis, unable to walk. He had pain and tactile anesthesia in all four limbs, and OH. He denied any family history but he informed us that his mother was also disabled due to a presumed rheumatic disease. A genetic study was performed on the patient and his three asymptomatic relatives.

During the next year he had severe episodes of syncope. An electrocardiogram showed III-degree atrioventricular block; a pacemaker was implanted and the loss of consciousness episodes were controlled. An echocardiogram was considered normal. He died at age 53, with cachexia.

Patient II-3. This was the patient's mother. At 59 years of age she also suffered a sciatic syndrome due to lumbar disc herniation, and successfully underwent surgery. At 68 years of age she noticed a loss of sensation starting in both feet, progressing to her legs and thighs; 1 year later she needed unilateral support to walk and soon required a wheelchair. She later developed anorexia, dysphagia, vomiting after meals, constipation alternating with uncontrollable diarrhea and urinary incontinence.

At age 70 the patient was visited for the first time. She remained mainly bedridden and presented severe tetraparesis and anesthesia of all sensation types in the four limbs and the front of the trunk. She had severe malnutrition and OH. A genetic study was performed. At age 73 she died due to a respiratory infection superimposed on severe cachexia.

Material and methods

Immunological studies

A direct ELISA with the monoclonal antibody Mab39-44 was performed on the Spanish patient

after LTX, to determine the presence or absence of the variant.

Molecular studies

We studied DNA and plasma from two Spanish and two Portuguese patients, one asymptomatic Spanish relative, and three asymptomatic Portuguese relatives (Figures 1 and 2).

SSCP analysis was performed on isolated genomic DNA on exons 2, 3 and 4 of the TTR gene under conditions previously described [7]. Exon 3 was amplified with primers 3A (5′-TCCTCCATGCG TAACTTAAT-3′) and 3B (5′-ACTGTGCATT TCCTGGAATG-3′) using 30 cycles of 30 s at 92°C, 1 min at 55°C and 1 min at 72°C and was cycle sequenced using ABI's Big Dye Terminators and the automatic sequencer ABI Prism 377. For restriction fragment length polymorphism (RFLP) analyses with the enzyme Bsr I, polymerase chain reaction (PCR)-amplified exon 3 of TTR was treated with the enzyme and analyzed on a 4% agarose gel stained with ethidium bromide, following procedures previously described. For mass spectrometry analyses, plasma was immunoprecipitated with anti-transthyretin antibody (DAKO) (4:1) and the immunoprecipitated proteins were resolved by SDS-PAGE and stained by Coomassie Blue; the TTR band was excised from the gel and digested with endoproteinase Lys-C. MALDI mass spectroscopic analysis was performed on a PerSeptive Voyager mass spectrometer in the linear mode [8].

DNA from the proband was first subjected to TTR-mutation scanning of exons 2, 3 and 4. The pattern obtained was normal, except for exon 3 (not shown).

Ethical standards

The ethical guidelines of the Helsinki Declaration of the World Medical Association were observed.

Results

Immunological studies

The direct ELISA with Mab39-44 showed the disappearance of the variant in plasma, which was the goal of LTX.

Molecular studies

DNA sequencing analysis of exon 3 revealed A to C transition in the first base of codon 50 normally encoding a serine (Figure 3A), giving rise to an arginine residue. The presence of this mutant arginine was confirmed by peptide mapping and mass spectrometry analysis. Thus, as shown in Figure 3B, mass spectrometry analysis of TTR peptides corresponding to residues 48–75 of the polypeptide chain from a normal subject (labeled N) shows the normal peptide of 3142 mass whereas the proband (labeled P) shows an additional peptide of 3211 mass corresponding to the mutated peptide consistent with the change in mass resulting from the serine for arginine substitution.

The mutation abolishes a cleavage site for the enzyme Bsr I and therefore RFLP analysis of the proband DNA was performed and compared with control DNA. As shown in Figure 3C, a band of 248 bps is observed in the proband (labeled P) which is absent in control DNA (labeled N). RFLP analyses were then used to identify additional carriers of the Ser50Arg mutation in the present study.

We identified the mutation in the four patients and one Portuguese relative who was an asymptomatic carrier. The other four relatives, three Portuguese and one Spanish, were non-carriers (see Figures 1 and 2).

Discussion

To our knowledge this is the second report on this mutation in Caucasian patients and the fifth described in the literature.

The first Japanese patient [2] was a man who presented with PNP at age 39 years and the most outstanding symptoms were severe weight loss and weakness; he died aged 45 and a postmortem examination was performed. The genetic study of hepatocytes showed in one allele T to G transversion in exon 3 of TTR gene which led to replacement of Ser by Arg at position 50; he was thus heterozygous for the aforementioned mutation.

The second Japanese patient [3] was a 43-year-old man who started with FAP at age 41 presenting with sexual impotence, progressive PNP in all four limbs, cardiomyopathy with atrioventricular block which required a pacemaker, and particularly severe OH, responsible for bouts of postural syncope. He died aged 46 and necropsy showed amyloid deposition in several organs which were stained with anti-TTR serum. In the DNA analysis from the patient and five asymptomatic relatives (mother, sister and three offspring) the aforementioned mutation was identified in the patient and one of the offspring, who also were heterozygous.

At 48 years of age the French-Italian woman patient [4] presented with constipation followed by progressively severe diarrhea and sensorimotor syndrome in the lower limbs later followed by weakness and loss of weight. Examination showed PNP, OH, moderately impaired renal failure and hypertrophic cardiomyopathy; she died around 54 years. In the

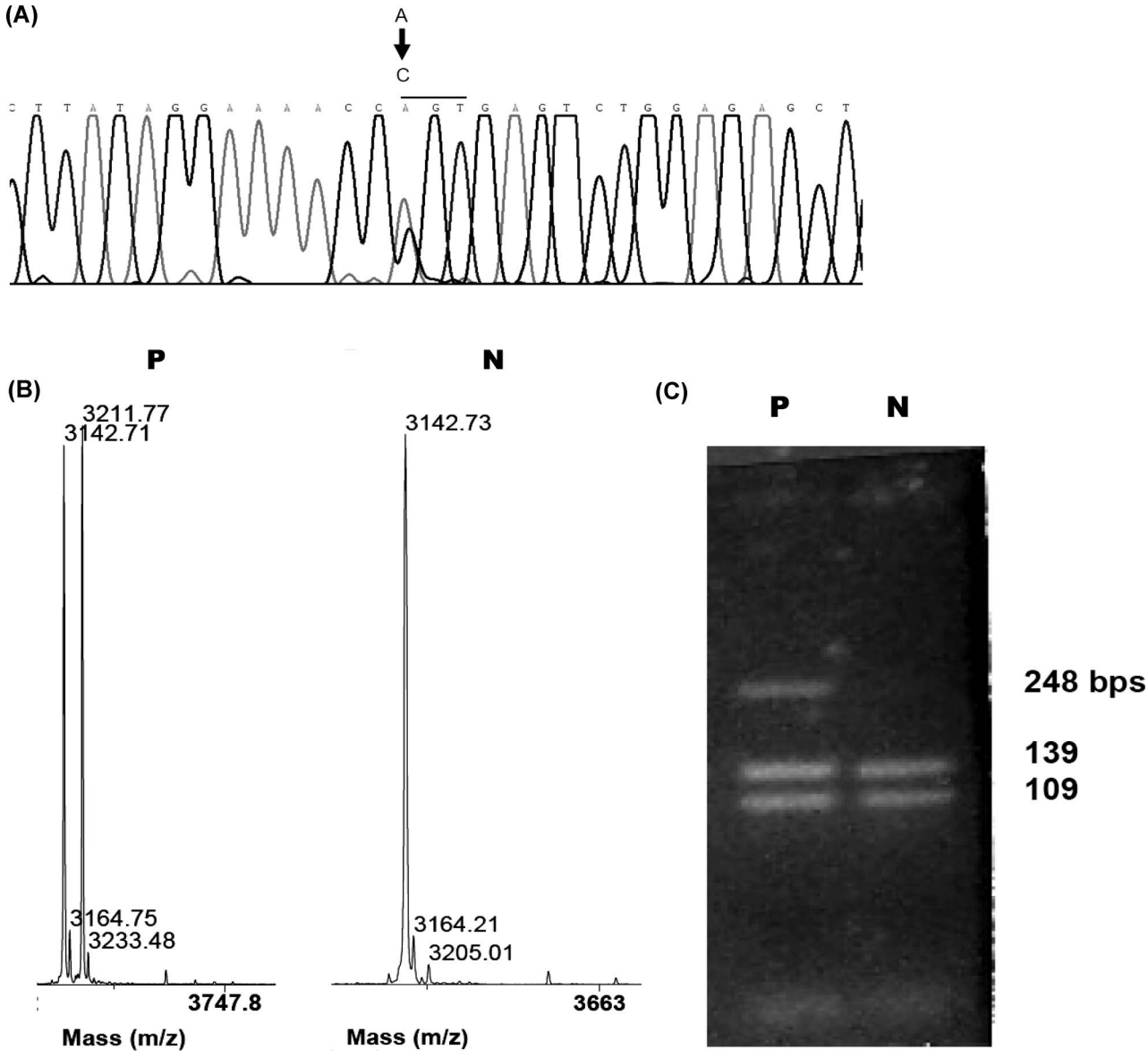

Figure 3. (A) Sequence of exon 3 of the proband showing an A to C tranversion in the first base of codon 50. (B) Mass spectrometry analysis of TTR peptides (residues 48–75) from a normal control (left) and from the proband (right). (C) RFLP analyses with BsrI on exon 3; N, normal control DNA; P, proband DNA.

DNA analysis, the same mutation was found in one allele, as in the Japanese patients.

The Vietnamese proband [5] was a 38-year-old woman who presented with fatigue, stress-induced dyspnea and chronic cough accompanied by weight loss and constipation. Restrictive cardiomyopathy due to amyloid deposition was suspected after echocardiography, ^{99}Tc-MDP scan, and cardiac catheterization; amyloid was detected in gastric and rectal biopsies and also in the left ventricle; no outcome data were reported. The mutation was identified in the proband, and three siblings (all female patients) and also in five asymptomatic cousins (three women and two men); no information was provided concerning the molecular study or the clinical picture of the three siblings.

Age at onset in both Portuguese patients (48 and 68 years) differs from the mean onset age of the patients with TTRVal30Met (33.5 years). However, onset age of the Spanish patients (40 to 53) is similar to the mean onset age of TTRVal30Met patients (45.7 years) [9].

In our patients (excepting the Spanish ancestor with only vitreous amyloidosis) and in the three previously described patients with PNP and other systemic manifestations, the course of FAP was very aggressive: multiple symptoms developed rapidly and deterioration was progressive. We emphasize the Spanish proband's confinement to a wheelchair due the OH severity, a finding not reported in FAP patients. On the contrary the patient with only vitreous amyloidosis, had slow progression of

vitreous deposits and right and later left vitrectomy were required. He died from lung cancer at 68, 15 years after the onset of FAP, without systemic symptoms.

The Spanish proband is the first with this mutation to undergo LTX. We consider that the result after 6 years is encouraging as he has shown significant improvements. Based on the aforementioned aggressiveness of this mutation, LTX must be indicated in the early first stages of the disease.

Genetic diagnosis identified the mutation in three patients and one disease-free relative. The most outstanding feature of DNA analysis was the single base tranversion A to C in codon 50 (CGT instead of AGT), whereas in both Japanese patients and the French-Italian patient it was T to G (AGG instead of AGT). To our knowledge only six FAP mutations with more than one single nucleotide mutation for the same codon have been reported to date: four in exon 2, Asp18Glu, Lys35Asn, Glu42Asp, and Gly47Arg; and two in exon 3, Ile68Leu and Lys70Asn [1].

Acknowledgements

This work was partially funded by a research grant from Fundación Eugenio Rodríguez Pascual, Madrid, Spain. Our most sincere gratitude to Professor Borja Corcóstegui and Jaume Munar-Bernat for their valuable cooperation.

References

1. Connors LH, Lim AM, Prokaeva T, Roskens VA, Costello CE. Tabulation of human transthyretin (TTR) variants, 2003. Amyloid: J Protein Folding Disord 2003;10: 160–184.
2. Ueno S, Uemichi T, Takahashi N, Soga F, Yorifugi S, Taruj S. Two novel variants of transthyretin identified in Japanese cases with familial amyloidotic polyneuropathy: transthyretin (Glu 42 to Gly) and transthyretin (Ser 50 to Arg). Biochem Biophys Res Commun 1990;169:1117–1121.
3. Takahashi N, Ueno S, Uemichi T, Fujimura H, Yorifuji S, Tarui S. Amyloid polyneuropathy with transthyretin Arg 50 in a Japanese case from Osaka. J Neurol Sci 1992;112: 58–64.
4. Reilly MM, Adams D, Booth DR, Davis MB, Said G, Laubriat-Bianchin M, Pepys MB, Thomas PK, Harding AE. Transthyretin gene analysis in European patients with suspected familial amyloid polyneuropathy. Brain 1995;118: 849–856.
5. Singer R, Schnabel A, Strasser RH. Restrictive cardiomyopathy in familial amyloidosis TTRArg50. Circulation 2003; 107:643–644.
6. Palha JA, Moreira P, Oloffson A, Lundgren E, Saraiva MJ. Antibody recognition of amyloidogenic transthyretin variants in serum of patients with familial amyloidotic polyneuropathy. J Mol Med 2001;78:703–707.
7. Torres MF, Almeida MR, Saraiva MJM. TTR exon scanning in peripheral neuropathies. Neuromuscul Disord 1995;5: 187–191.
8. Henzel WJ, Billeci TM, Stults JT, Wond SC, Grimley C, Watanabe C. Identifying proteins from two-dimensional gels by molecular mass searching of peptide fragments in protein sequence databases. Proc Natl Acad Sci USA 1993;90: 5011–5015.
9. Sousa A. A variabilidade fenotípica da polineuropatia amiloidótica familiar: um estudo de genética quantitativa em Portugal e na Suecia. Doctoral Thesis. University of Porto, 1995.