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CARDIOLOGY
JOURNAL

ISSN: 1897-5593

e-ISSN: 1898-018X

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Authors: Monika Gawor, Katarzyna Holcman, Maria Franaszczyk, Marta Lipowska, Piotr Michałek, Anna Teresińska, Zofia T. Bilińska, Paweł Rubiś, Magdalena Kostkiewicz, Wojciech Szot, Piotr Podolec, Jacek Grzybowski

DOI: 10.5603/CJ.a2020.0104

Article type: Original articles

Submitted: 2019-12-19

Accepted: 2020-07-13

Published online: 2020-08-11

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Spectrum of transthyretin gene mutations and clinical characteristics of Polish patients with cardiac transthyretin amyloidosis

Short title: **Polish transthyretin mutations**

Monika Gawor¹, Katarzyna Holcman², Maria Franaszczyk³, Marta Lipowska⁴, Piotr Michalek⁵, Anna Teresińska⁶, Zofia T. Bilińska⁷, Paweł Rubiś², Magdalena Kostkiewicz^{2, 8}, Wojciech Szot⁸, Piotr Podolec², Jacek Grzybowski¹

¹Department of Cardiomyopathy, Institute of Cardiology, Warsaw, Poland

²Department of Cardiac and Vascular Diseases, John Paul II Hospital, Jagiellonian University Medical College, Krakow, Poland

³Department of Medical Biology, Institute of Cardiology, Warsaw, Poland

⁴Department of Neurology, Medical University of Warsaw, Poland

⁵Rapid Diagnosis Department, Emergency Room, Institute of Cardiology, Warsaw, Poland

⁶Department of Nuclear Medicine, Institute of Cardiology, Warsaw, Poland

⁷Unit for Screening Studies in Inherited Cardiovascular Diseases, The Cardinal Stefan Wyszyński Institute of Cardiology, Warsaw, Poland

⁸Department of Nuclear Medicine, John Paul II Hospital, Jagiellonian University Medical College, Krakow, Poland

Address for correspondence: Monika Gawor, MD, PhD, Department of Cardiomyopathy, Institute of Cardiology, ul. Alpejska 42, 04–628 Warszawa, Poland, tel: +48 22 343 46 71, e-mail: mgawor@ikard.pl

Abstract

Background: Transthyretin amyloidosis (ATTR) is a rare, life-threatening systemic disorder. We present first findings on the cardiac hereditary ATTR in Poland.

Methods: Sixty-eight consecutive patients with suspected or known cardiac amyloidosis were evaluated, including blood tests, standard 12-lead electrocardiography (ECG) and transthoracic echocardiography. ATTR was confirmed histologically or non-invasively using ^{99m}Tc-DPD scintigraphy. Transthyretin (*TTR*) gene sequencing was performed.

Results: In 2017–2019, 10 unrelated male patients were diagnosed with hereditary ATTR. All patients had very uncommon *TTR* gene mutations: 7 patients had p.Phe53Leu mutation, 2 patients

had p.Glu109Lys mutation and 1 patient had p.Ala101Val mutation. The age of onset ranged from 49 to 67 years (mean [SD] age, 58.7 [6.4] years). On ECG, most patients (70%) had pseudoinfarct pattern and/or low QRS voltage. The maximal wall thickness (MWT) on echocardiography varied considerably among the patients from moderate (16 mm) to massively increased (30 mm). Most patients (90%) had decreased LV ejection fraction (mean [SD], 43 [11] %). On follow-up, we observed progressive heart failure in almost all cases. The first patient with p.Phe53Leu mutation died of heart failure, the second died suddenly, the third successfully underwent combined heart and liver transplant with 15 months survival from the surgery. The patient with p.Ala101Val mutation died of stroke.

Conclusions: According to available research, this is the first time types of *TTR* mutations and clinical characteristics of Polish patients with cardiac hereditary ATTR have been reported. Previous literature data about Polish background in families with p.Phe53Leu mutation and the present results, suggest that this *TTR* mutation might be endemic in the Polish population.

Key words: cardiac amyloidosis, hereditary transthyretin amyloidosis, transthyretin amyloidosis, transthyretin cardiomyopathy, transthyretin mutation

INTRODUCTION

Transthyretin amyloidosis (ATTR) is a rare, life-threatening systemic disorder. It results from extracellular deposition of transthyretin (TTR)-derived amyloid fibrils leading to dysfunction of affected organs. It occurs in both a wild-type form (wtATTR) and a hereditary form (hATTR) transmitted in an autosomal dominant inheritance with a variable penetrance [1]. Without treatment the disease progresses gradually. According to recent reports, median survival of patients with cardiac ATTR is approximately 25 months to 41 months from onset of the first symptoms. The prognosis is worse in cases of hATTR [2].

More than 140 different disease-causing mutations of the *TTR* gene have been reported [3]. The prevalence of different mutations varies according to ethnicity and geographic region. Although there are endemic regions of ATTR such as Portugal, Sweden and Japan with certain high frequency mutations, the disease has been identified worldwide [1, 4–6].

Transthyretin amyloidosis is known for its heterogeneity. Some *TTR* mutations induce cardiomyopathy as a main presentation while others are associated primarily with familial amyloid polyneuropathy. Other manifestations of hATTR often include gastrointestinal, renal and ocular symptoms as well as carpal tunnel syndrome [2, 7, 8]. The phenotypic expression can vary not only

among mutations but within mutations. The geographical origin and ethnic background of the patient can have implications for disease onset and initial symptoms for a given mutation [1]. Surprisingly, cases of monozygotic twins, in which one twin develops the disease and the other does not, have also been reported, proving that there are factors modulating amyloid fibrils formation [9, 10].

Screening of the *TTR* gene and differentiation hATTR from wtATTR have serious clinical implications. In contrast to wtATTR, patients with hATTR may benefit from liver transplant or combined heart and liver transplant. Information on the type of *TTR* mutation also provides prognostic value not only for the proband but also for their family members. The spectrum of *TTR* gene mutations in Polish patients with cardiac ATTR and their clinical characteristics has never been described. Therefore, herein presented for the first time are the results of genetic testing and a description of clinical heterogeneity of patients with cardiac ATTR diagnosed in the documented centers within the prior 3 years.

METHODS

The study complies with the principles outlined in the Declaration of Helsinki and was approved by the Institutional Ethics Committee.

Study population

The study included consecutive patients diagnosed with hereditary cardiac ATTR in 2017–2019. 68 consecutive patients examined with suspected or known cardiac amyloidosis were referred to two cardiology centers for further evaluation. All patients underwent routine assessment including medical history, physical examination, blood tests with N-terminal pro-B-type natriuretic peptide (NT-proBNP) and high-sensitive cardiac troponin T (hs-cTnT) measurements, as well as standard 12-lead electrocardiography (ECG) and transthoracic echocardiography. ATTR was confirmed histologically with immunohistochemistry and/or non-invasively (intensive cardiac uptake at ^{99m}Tc-DPD bone scintigraphy) [11]. Light chain amyloidosis was excluded by free light chain testing: conventional electrophoresis and immunofixation of serum or urine. Patients underwent *TTR* gene sequencing as part of their routine workup. Neurological assessment was performed in all patients with hATTR. Informed written consent was obtained from each participant included in the study.

Analysis of *TTR* gene

Patients assessed at the Institute of Cardiology, Warsaw, Poland underwent genetic testing in a local laboratory. Genomic DNA in all patients was extracted from whole blood leukocytes with the salting-out method. The entire coding regions of the *TTR* gene together with splice sites were sequenced with the Sanger method using a 3130xL Genetic Analyzer (Life Technologies, Foster City, California, United States) and the Big Dye Terminator v1.1 Cycle Sequencing Kit (Life Technologies) according to manufacturer instructions. For chromatograms analysis Variant Reporter 1.1 (Life Technologies) was used. All patients from both centers also underwent commercially available *TTR* genetic testing developed and performed by Centogene AG, Rostock, Germany.

RESULTS

Of the 68 patients, 10 (14.7%) male patients had cardiac hATTR, 19 (27.9%) had wtATTR, 9 (13.2%) were diagnosed with cardiac light chain amyloidosis and 30 (44.1%) had no evidence of amyloid deposition. All patients with hATTR had very uncommon *TTR* gene mutations: p.Phe53Leu (NM_000371.3:c.157T>C, rs121918068), p.Glu109Lys (NM_000371.3:c.325G>A) and p.Ala101Val (NM_000371.3:c.302C>T). The most prevalent was p.Phe53Leu mutation identified in 7 out of 10 patients with hATTR. The baseline clinical data of the patients with hATTR was shown in Table 1.

The age of onset ranged from 49 to 67 years (mean [SD] age, 58.7 [6.4] years) and differed considerably even among patients with the same mutation. Family history suggestive of cardiac amyloidosis was positive in 6 probands. On the ECG, most patients (70%) had a pseudoinfarct pattern in precordial leads. Similarly, most patients (70%) had low QRS voltage in limb leads. Echocardiography revealed concentrically increased thickness of the left ventricular (LV) wall in all patients. The maximal wall thickness (MWT) varied markedly among the patients from moderate (16 mm) to massively increased (30 mm) (mean [SD] MWT, 22 [4] mm). Most patients (90%) had decreased LV ejection fraction (LVEF) (mean [SD] LVEF, 43 [11] %). Baseline laboratory examination revealed increased levels of hs-cTnT and NT-proBNP in all patients. Even individuals with initially mild symptoms of heart failure (HF) had elevated serum concentrations of examined biomarkers (Fig. 1).

Seven patients presented with mixed-phenotype and suffered also from polyneuropathy. Only 2 patients primarily presented with polyneuropathy (patients with p.Phe53Leu mutation) and cardiac assessment was performed after familial amyloid polyneuropathy was confirmed. Five other patients were diagnosed with polyneuropathy during follow-up. Of the 10 patients, 7 suffered from gastrointestinal disturbances. However, it was difficult for the patients to determine at what stage of

the disease the first symptoms from the gastrointestinal tract appeared and whether they preceded the appearance of cardiac manifestations. Five patients presented with carpal tunnel syndrome, the so-called 'red flag' symptom for ATTR, which evidently preceded the appearance of cardiac symptoms. One of the patients had ocular symptoms, which seem to occur frequently in patients with p.Glu109Lys mutation. Orthostatic hypotension was present in 4 patients with advanced symptoms of HF regardless of the presence of polyneuropathy.

Clinical characteristics of patients with p.Phe53Leu *TTR* mutation

Although the patients with p.Phe53Leu *TTR* mutation had heterogeneous clinical presentation and were diagnosed at various stages of the disease, ranging from accidentally discovered disease in asymptomatic patient to the advanced stages of biventricular HF, all of them had significant abnormalities on ECG and echocardiography from the first assessment. It is noteworthy that all patients except for proband 6, who was diagnosed at a very early stage of the disease, had reduced LV systolic function. Interestingly, symptoms of HF assessed in accordance to the New York Heart Association (NYHA) classification, did not reflect the impairment of LV systolic function, patients with only mild symptoms of HF had already significantly impaired LVEF (Fig. 2). Most of the patients with p.Phe53Leu mutation (71.4%), except for 2 cases, presented with mixed phenotype and had also polyneuropathy at various stages of advancement.

The case of proband 3 is particularly interesting as a male patient was diagnosed with hATTR at a time when he was completely asymptomatic with good exercise tolerance confirmed. He was referred for cardiological assessment due to ECG abnormalities found during annual medical check-up (sinus rhythm with low QRS voltage in the limb leads and nonspecific ST-T wave changes). Physical examination and chest X-ray were normal. However, baseline levels of NT-proBNP and hs-cTnT were significantly increased from the beginning. Echocardiography revealed moderately increased LV wall thickness (MWT 16 mm), mildly enlarged left atrium and significantly reduced LV systolic function without segmental hypokinesis (LVEF 40%). Moreover, coronary computed tomography angiography, performed to reveal the reasons for the impaired LVEF, showed right coronary artery (RCA) chronic total occlusion as well as stenosis in the left main coronary artery, in the left anterior descending artery (LAD) and in the left marginal artery. The patient was diagnosed with occult multivessel coronary artery disease. Invasive coronary angiography with fractional flow reserve demonstrated that only stenosis in the LAD was hemodynamically relevant. Percutaneous coronary intervention using drug eluting stents was performed for occlusion in the RCA and for stenosis in LAD. Although at 20-month follow-up, the patient remained asymptomatic, with good exercise tolerance confirmed on ECG exercise testing,

further deterioration of LV function and a significant increase of cardiac biomarkers were observed. HF symptoms progressed rapidly, however signs of polyneuropathy never appeared in this patient. Ultimately, the patient underwent successful combined heart and liver transplantation.

Interestingly, proband 1, a 64-year-old male with advanced biventricular HF, also had ischemic heart disease and was treated with coronary artery bypass graft surgery. The patient had many comorbidities, including atrial fibrillation (AF) with third-degree atrioventricular block requiring pacemaker implantation, subsequently upgraded to cardiac resynchronization therapy, polyneuropathy grade 1 (he was able to walk without aid), recurrent pleural effusion and bilateral carpal tunnel syndrome.

Clinical characteristics of patients with p.Glu109Lys *TTR* mutation

Two patients with features of restrictive cardiomyopathy and carpal tunnel syndrome were identified with the p.Glu109Lys *TTR* mutation. Proband 8, a 49-year-old male, was diagnosed at a very early stage of the disease (NYHA class I), without overt signs of HF, with only mildly increased levels of cardiac serum biomarkers. However, echocardiography and ECG demonstrated significant abnormalities, including mildly reduced LV systolic function, restrictive LV filling pattern, small pericardial effusion as well as pseudoinfarct pattern and low QRS voltage in limb leads. The patient had a family history suggestive of cardiac amyloidosis.

A second patient with p.Glu109Lys *TTR* mutation, proband 9, was referred to the documented institution due to a 3 month history of progressive exertional dyspnea (NYHA class III at initial presentation) and massive lower-limbs edema. He also suffered from periorbital purpura, weight loss, mild symptoms of polyneuropathy (predominantly paresthesias) and ocular symptoms including vitreous opacities. His family history was unremarkable. ECG demonstrated occult AF of unknown duration, pseudo-infarct pattern, nonspecific ST segment and T-wave abnormalities. QRS voltage was in the lower range of the norm. The chest X-ray showed pulmonary congestion and small left sided pleural effusion. Echocardiography revealed increased LV wall thickness (max. 23 mm at interventricular septum), sparkling echoes, restrictive LV filling pattern and decreased LVEF 45%. Right ventricle (RV) was enlarged with increased wall thickness. Both atria were augmented and small pericardial effusion was present. Diagnosis of ATTR was confirmed with bone scintigraphy with ^{99m}-DPD tracer (Fig. 3A–D). Two months after initial presentation the patient was stable but with a further increase in NT-proBNP and hs-cTnT levels (3513 pg/mL and 68 ng/L, respectively). He was referred for compassionate treatment with tafamidis as a bridge to combined heart and liver transplantation.

Clinical characteristics of patients with p.Ala101Val *TTR* mutation

Only 1 patient, 67-year-old male with progressive HF (NYHA class II/III at initial presentation), permanent AF, significantly increased levels of cardiac biomarkers was identified with p.Ala101Val *TTR* mutation. Echocardiography of this patient showed massively increased RV and LV wall thickness (max. 27 mm at interventricular septum), significantly decreased LV systolic function (LVEF 35%) and small pericardial effusion. The patient also suffered from orthostatic hypotension, bilateral carpal tunnel syndrome mild symptoms of polyneuropathy and monoclonal gammopathy of unknown significance. Due to the presence of only microdeposits of amyloid in the tissue biopsy and no access to ^{99m}Tc-DPD scintigraphy at that time, diagnosis of ATTR in the patient was established using mass spectrometry.

Follow-up

Information about the outcome of patients with hATTR are collected in Table 1. In almost all cases progressive HF was observed. One patient with p.Phe53Leu *TTR* mutation (proband 1) died of biventricular HF, proband 2 died due to sudden cardiac death, proband 3 successfully underwent combined heart and liver transplantation with 15 a month survival from the surgery. One patient with p.Glu109Lys *TTR* mutation, proband 9, is currently waiting for combined heart and liver transplantation. Patient with p.Ala101Val *TTR* mutation (proband 10) died of stroke.

DISCUSSION

Herein, reported for the first time, types of *TTR* mutations and clinical characteristics of Polish patients with cardiac hATTR. Currently, literature data on Polish patients with cardiac amyloidosis concerns mainly light chain amyloidosis and consists of only a few case reports and two original papers [11–15]. The presented study, although based on the small cohort of patients from two cardiology centers, fills an important gap in diagnosis and clinical management of cardiac ATTR in Poland. This research highlights that improvements in diagnostics, in particular introducing genetic analysis of the *TTR* gene and ^{99m}Tc-DPD scintigraphy into a routine diagnostic path of patients with suspicion of ATTR, hugely facilitates diagnostics. Moreover, it allows establishment of a non-invasive diagnosis [8, 16]. It is crucial to identify patients with ATTR, especially now, when the targeted treatment of ATTR is possible.

In the present study, patients were reported with unexpectedly rare *TTR* mutations: p.Phe53Leu, p.Ala101Val and p.Glu109Lys. Surprisingly, none of the most prevalent *TTR* variants: p.Val50Met, p.Val142Ile or p.Thr80Ala [4, 17, 18], were identified. Moreover, a high incidence of p.Phe53Leu *TTR* mutation were observed. This occurred in the majority of the patients (70%).

The phenylalanine 53 is highly conserved amino acid of the TTR protein across species [19]. Position 53 of TTR protein is not directly involved in the formation of the transthyretin tetramer, however, loss of an aromatic ring associated with phenylalanine, may change the molecule conformation and lead to fibril formation [20]. The p.Phe53Leu *TTR* mutation was described previously only in 7 unrelated families including 2 American families of Polish descent and single Swedish, Taiwanese, Chinese, Hungarian and Israeli families [19–25]. This mutation was associated mostly with late-onset, progressive polyneuropathy and simultaneous presentation of severe cardiomyopathy. Previous data in the literature about a Polish background in families with p.Phe53Leu mutation and present findings (another 7 unrelated Polish families identified with p.Phe53Leu mutation) imply that this *TTR* mutation might be very frequent in the Polish population [20, 21]. Of note, almost all of the patients with p.Phe53Leu mutation presented in this study (85.7%) were of south Poland origin, suggesting that this might be an endemic region, where p.Phe53Leu mutation is found with high frequency. It is also possible that this rare mutation was introduced into the population by a common ancestor. However, based on available data it is not possible to prove so-called “founder effect”. The heterogeneous clinical presentation of the patients with p.Phe53Leu *TTR* mutation (different age of onset, concomitant polyneuropathy but not in all cases) suggests also that other genetic or environmental factors may be involved in the disease development and progression.

p.Glu109Lys *TTR* mutation was reported previously only in three families and is characterized by early onset, concomitant ocular manifestation and severe phenotype with early heart dysfunction leading to heart and liver transplantation [26–28]. A similar course of the disease was observed in the case of proband 9, including the rapid progression of HF and ocular manifestations.

p.Ala101Val *TTR* mutation is an unusually rare mutation. So far, only 2 patients were previously reported with this mutation, including 1 patient from Institute of Cardiology who was diagnosed genetically in the National Amyloidosis Center in London in 2008 [17]. The patient suffered from severe biventricular HF with massive leg edema, persistent pericardial and pleural effusion requiring recurrent hospitalizations. ECG demonstrated sinus rhythm, first degree atrioventricular block (PR 320 ms), low QRS voltage in limb leads and regression of r waves in lateral (V5–V6) leads. Echocardiography demonstrated features of restrictive cardiomyopathy with concentrically increased LV wall thickness (max. 15 mm), moderated LA enlargement and mildly decreased LV systolic function (LVEF 50%). During follow-up, further deterioration of LV function was observed (LVEF 38%) as well as features of moderate pulmonary hypertension (PASP 40

mmHg), thickening of RV wall and dilatation of both atria. The patient died of advanced HF while awaiting heart transplantation.

In the present study, in line with the previous literature data, cardiac biomarkers are very sensitive for diagnostic and prognostic purposes were observed [29]. Even patients with only mild symptoms of HF (NYHA I) or asymptomatic patients, had already elevated serum levels of cardiac biomarkers (Fig. 1).

CONCLUSIONS

The high prevalence of p.Phe53Leu *TTR* mutation in the study group and in previous literature data suggest that a possible endemic mutation has been found among Polish patients with cardiac hATTR. Considering the fact that the diagnosis of cardiac hATTR has clinical implications and information on the presence of *TTR* mutation is useful for affected patients, pre-symptomatic carriers and their relatives, patients should be identified with this disorder to better define this disease. The emergence of novel treatments in cardiac ATTR, which may improve patient prognosis is another argument for the intensification of epidemiological and cross-sectional studies like this.

Acknowledgments

We would like to acknowledge Centogene AG, Rostock, Germany for support in genetic testing.

Conflict of interest: None declared

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Table 1. Baseline clinical characteristics and outcome of patients with hereditary transthyretin (TTR) amyloid cardiomyopathy.

Patient	1	2	3	4	5	6	7	8	9	10	
<i>TTR</i> mutation	p.Phe53Leu	p.Phe53Leu	p.Phe53Leu	p.Phe53Leu	p.Phe53Leu	p.Phe53Leu	p.Phe53Leu	p.Phe53Leu	p.Glu109Lys	p.Glu109Lys	p.Ala101Val
Age of onset	64	57	49	58	64	55	66	49	57	67	
NYHA class	III/IV	I	I	II	IV	I	III	II	III	II/III	
Positive family history	+	+	–	–	+	+	+	+	–	–	
ECG	Ventricular pacing, AF	SR, SVT, nsVT	SR	SR	SR	SR	AF, nsVT	SR	AF	AF	
Low QRS voltage	NA	+	+	+	+	+	–	+	–	+	
Pseudoinfarct pattern	NA	+	–	–	+	+	+	+	+	+	
NT-proBNP [pg/mL]	10954	1199	925	1049	10780	191	13541	394	2340	6070	
hs-cTnT [ng/L]	103	98	28	51	122	26	136	19	50	78	
MWT [mm]	30	20	16	18	25	20	20	24	23	27	
Increased RV wall thickness	+	–	–	+	–	–	–	–	+	+	
LVEF [%]	30	40	40	55	40	62	29	55	45	35	
Restrictive LV filling pattern	–	–	+	–	+	–	–	+	+	–	
Pericardial effusion	+	–	–	–	+	–	+	+	+	+	
Orthostatic hypotension	+	–	–	–	+	–	+	–	–	+	
Polyneuropathy	+	+	–	+	–	+	+	–	+	+	
CNS manifestations	+	–	–	–	–	+	+	–	–	+	
Small fiber neuropathy	+	+	–	+	–	+	+	–	+	+	
Gastrointestinal	+	+	–	–	+	+	–	+	+	+	

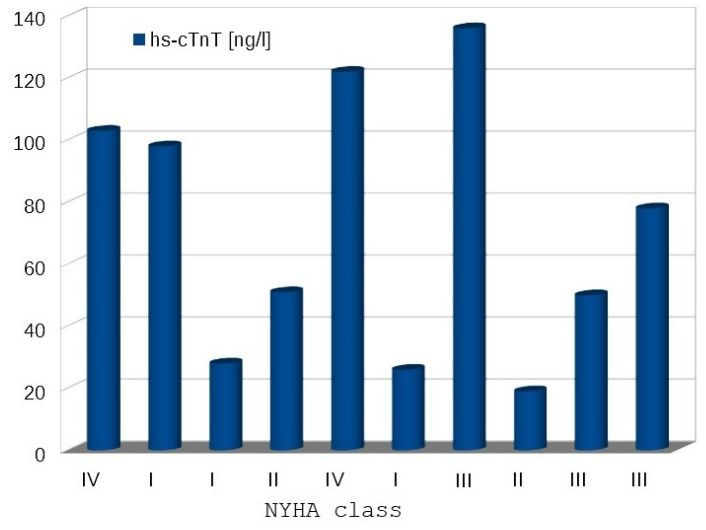
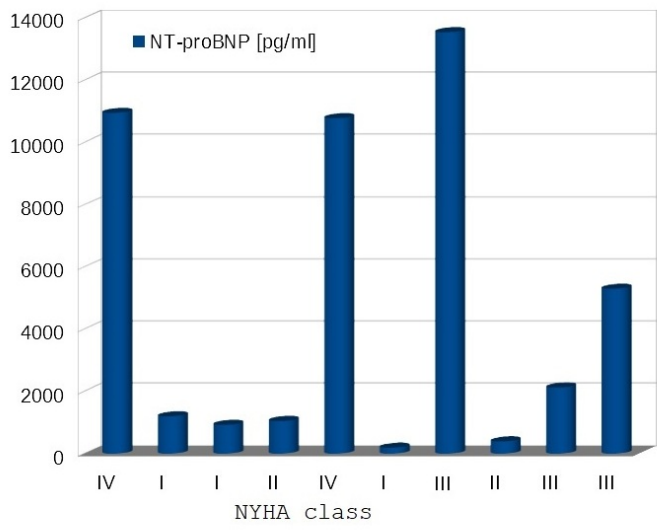
manifestations										
Ocular manifestations	–	–	–	–	–	–	–	–	–	+
Biceps tendon rupture	–	–	–	–	+	–	–	–	–	–
Chronic kidney disease	–	+	–	–	+	–	+	–	–	–
Carpal tunnel syndrome	+	+	–	–	–	–	–	+	+	+
Outcome	Death at age of 67 due to HF	SCD at age of 59	Successful combined heart and liver transplantati on	Alive	Alive, progressive HF	Alive, progressive neuropathy	Alive	Alive	Awaiting combined heart and liver transplantati on	Death at age of 71 due to stroke

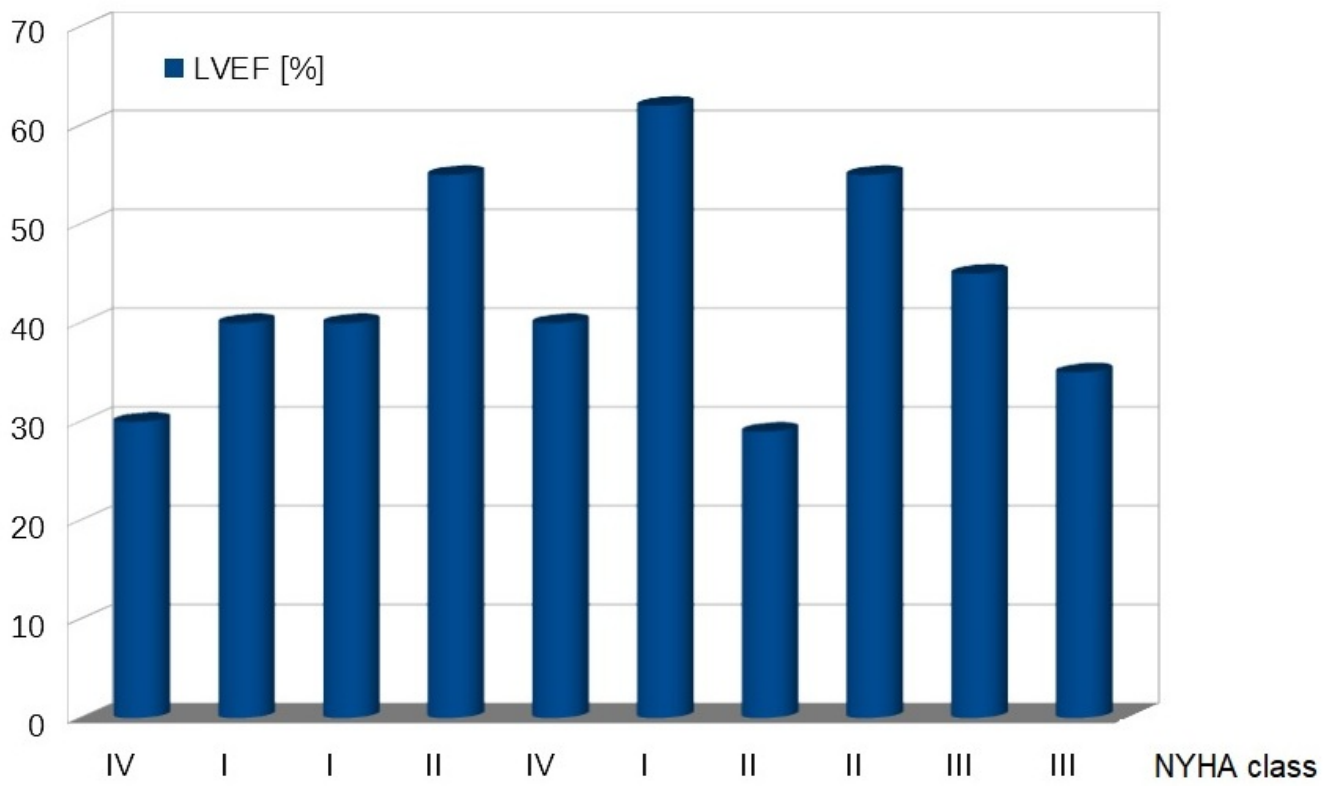
AF — atrial fibrillation; CNS — central nervous system; ECG — electrocardiogram; HF — heart failure; hs-cTnT — high-sensitivity cardiac troponin T; LV — left ventricular; LVEF — left ventricular ejection fraction; MWT — maximal wall thickness; NA — not applicable; nsVT — nonsustained ventricular tachycardia; NT-proBNP — N-terminal pro-B-type natriuretic peptide; NYHA — New York Heart Association classification of heart failure; RV — right ventricular; SCD — sudden cardiac death; SR — sinus rhythm; SVT — supraventricular tachycardia

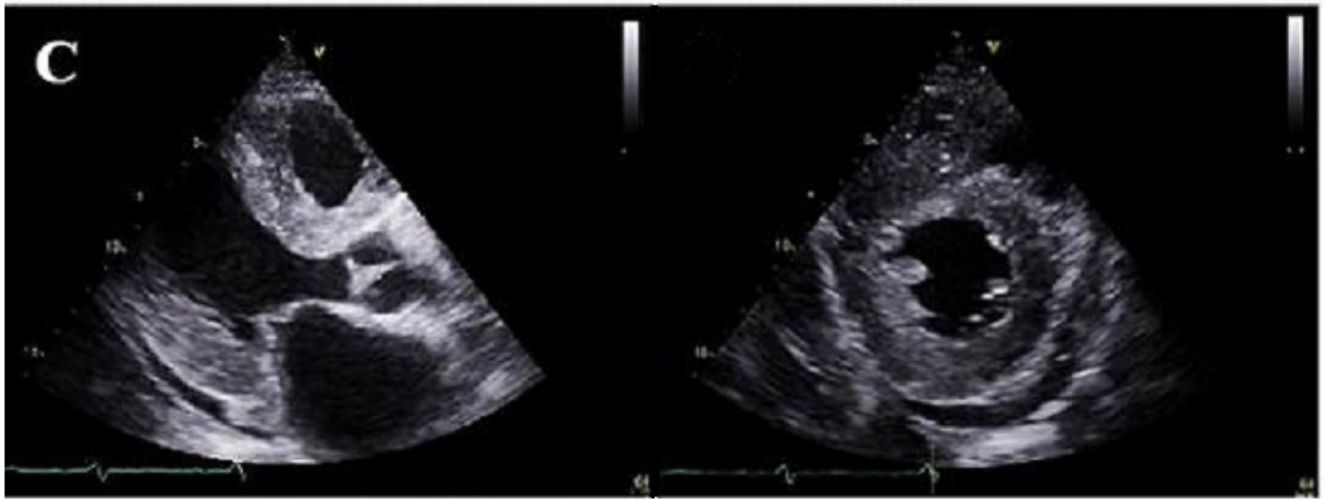
Figure 1. Baseline N-terminal pro-B-type natriuretic peptide (NT-proBNP; normal range: 0–125 pg/mL) and high-sensitive cardiac troponin T (hs-cTnT; normal range: 0–14 ng/L) levels in the patients classified according to the New York Heart Association classification of heart failure.

Figure 2. Left ventricular ejection fraction in the patients classified according to the New York Heart Association classification of heart failure.

Figure 3. Clinical data of proband 3; **A.** Standard 12-lead electrocardiogram; **B.** Chest X-ray; **C.** Transthoracic echocardiography; **D.** Bone scintigraphy with ^{99m}Tc-DPD tracer confirming transthyretin amyloid cardiomyopathy in anterior (left) and posterior (right) whole body scan.







D

