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High Hereditary Transthyretin-Related Amyloidosis Prevalence in Crete

Genetic Heterogeneity and Distinct Phenotypes

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Abstract

Background and Objectives

Our goal was to study hereditary transthyretin-related amyloidosis (hATTR) in Crete, Greece.

Methods

We aimed at ascertaining all hATTR cases in Crete, an island of 0.62 million people. For this, we evaluated patients with polyneuropathy, autonomic involvement, cardiomyopathy, and/or ophthalmopathy suggestive of hATTR, who presented to the physicians of this study or were referred to them by other physicians. Genetic analyses were performed on all patients suspected of suffering from hATTR. We included in our observational longitudinal cohort study all individuals, residents of Crete, who, during the study period (1993–2019), were found to carry a pathogenic *TTR* variant.

Results

Over the past 27 years, 30 individuals (15 female patients, 15 male patients), from 12 apparently unrelated families, were diagnosed with hATTR, whereas evaluation of their offspring identified 5 asymptomatic *TTR* pathogenic variant carriers. The most prevalent *TTR* variant detected was p.Val50Met, affecting 19 patients (11 female patients, 8 male patients) and causing a rather consistent phenotype characterized by predominant polyneuropathy of early adult onset (median age of symptom onset: 30 years; range: 18–37 years). Specifically, patients affected by the p.Val50Met *TTR* variant experienced progressive sensorimotor disturbances, involving mainly the lower extremities, associated with autonomic and/or gastrointestinal dysfunction. The second most frequent *TTR* variant was p.Val114Ala, found in 10 patients (4 female patients, 6 male patients) who were affected at an older age (median age of symptom onset: 70 years; range: 54–78 years). This variant caused a predominantly cardiomyopathic phenotype, manifested by congestive heart failure and associated with peripheral neuropathy, carpal tunnel syndrome, and/or autonomic involvement. In these patients, cardiac amyloid deposition was detected on 99m-technetium pyrophosphate scintigraphy and/or heart biopsy. The third *TTR* variant (p.Arg54Gly) was found in a 50-year-old male patient with ophthalmopathy due to vitreous opacities and positive family history for visual loss. As 22 patients were alive at the end of the study, we calculated the hATTR prevalence in Crete to be 35 cases per 1 million inhabitants.

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Glossary

^{99m}Tc-PYP = 99m-technetium pyrophosphate; **hATTR** = hereditary transthyretin-related amyloidosis.

Discussion

Our study revealed that the prevalence of hATTR in Crete is one of the world's highest. Three different pathogenic *TTR* variants causing distinct clinical phenotypes were identified in this relatively small population pool.

In hereditary transthyretin-related amyloidosis (hATTR), abnormal transthyretin, which is produced mainly by the liver, forms insoluble amyloid fibrils that are deposited in various tissues.[1,2] More than 150 transthyretin gene (*TTR*) pathogenic variants that cause autosomal dominant hATTR have been described. The disease is characterized by progressive sensorimotor neuropathy and autonomic dysfunction.[1,3] Diagnostic difficulties arise when the hATTR occurs sporadically and shows phenotypic variability, particularly when the disease affects other organs, such as the heart or the eye, with the nervous system involvement being a relatively minor manifestation.[4,5] Furthermore, the age at onset and the penetrance at a given age can vary among pathogenic *TTR* variants and even among the same ethnic groups.[1,6,7] Because of its phenotypic variability, hATTR remains a widely underdiagnosed condition.

hATTR was first described in Portugal[8] and subsequently reported in other countries, including Japan, Sweden, Cyprus, and Spain.[9-11] hATTR has been previously described in Greek families,[12,13] in which the disease was associated with the p.Val50Met *TTR* pathogenic variant (known as Val30Met), although a single case with the p.Ala56Pro *TTR* variant has also been reported.[14] Only a recent study has evaluated the clinical phenotypes, the variety of causative *TTR* gene variants and the prevalence of hATTR; however, this study focused in areas of Greece other than Crete.[15]

Our cohort study, spanning a 27-year period, was designed to identify and follow all cases of hATTR that occur in Crete with the goal of evaluating their epidemiologic, clinical, and genetic features and treatment approaches. The results revealed that hATTR in Crete is relatively frequent and characterized by genetic and clinical heterogeneity. In our cases with the p.Val50Met variant, orthotopic liver transplantation proved an effective treatment modality.

Methods

Participants

Participants in this project came from all 4 prefectures of Crete and were seen from 1993 to 2019 at the University Hospital of Heraklion and/or the Venizelio General Hospital, the 2 largest hospitals in Crete and the only referral centers in Crete for complex neurologic or cardiologic disorders. In addition, with the aim to achieve almost universal coverage of the island, we actively engaged local neurologists, cardiologists, and ophthalmologists in the study, inquiring about patients with clinical features suggestive of hATTR, irrespectively of the age at symptom onset. All participants in this study, who either initially sought our care or were referred to us by local physicians, were evaluated personally by at least one of the authors (M. Tzagournissakis, I.Z., A.P., C.S., E.F., D.S., M. Tsilimbaris.).

During the study period, we screened for the presence of *TTR* pathogenic variants in a total of 63 individuals with clinical features suggestive of hATTR. These hATTR-related clinical features included (1) symptoms and signs of progressive length-dependent sensorimotor and/or autonomic polyneuropathy of unknown cause (41 individuals, including 6 individuals with late disease onset), (2) cardiomyopathy with concurrent polyneuropathy and/or carpal tunnel syndrome (21 individuals, all late onset), and (3) ocular deficits with amyloid-type deposition in the eye (1 individual). In addition, we tested 28 individuals with a family history for genetically verified hATTR in a first-degree relative.

Eligible for inclusion in this observational longitudinal cohort study were all individuals, residents of Crete, found to carry a pathogenic *TTR* variant during the study period, either presenting with clinical features suggestive of hATTR or being asymptomatic carriers identified through family screening. All included patients were inquired about their family history for polyneuropathy, cardiomyopathy, or visual loss. Detailed genealogic information spanning up to 5 generations was obtained, and extended pedigrees were drawn.

This evaluation of all patients in the study included neurophysiologic studies, ECG, cardiac MRI, echocardiography, 99m-technetium pyrophosphate (^{99m}Tc-PYP) scintigraphy, ophthalmologic examination, gastroenterological evaluation, routine blood analysis, rectal or other type of biopsy, and molecular analysis to identify *TTR* gene variants. Each patient's evaluation was tailored based on the available clinical data and the clinician's judgment. In addition, patients were followed up by regular appointments with the physicians of the study.

Diagnosis of hATTR

For the diagnosis of hATTR to be established in our study, a patient should have all 3 of the following:

1. Clinical features typical of hATTR
   - symptoms and signs of progressive length-dependent sensorimotor and/or autonomic polyneuropathy
   - cardiomyopathy with concurrent polyneuropathy and/or carpal tunnel syndrome
   - ocular deficits with amyloid-type deposition in eye imaging
2. Pathogenic variant in the *TTR* gene
3. Evidence of amyloid deposition in tissue (biopsy or ^{99m}Tc-PYP scintigraphy) or genetically diagnosed hATTR in a first-degree relative

Standard Protocol Approvals, Registrations, and Patient Consents

All participants gave informed consent for inclusion in the study, as part of an extended genetic studies' protocol approved by the Institutional Review Board of the University Hospital of Heraklion, Crete, Greece.

Targeted Molecular Analysis for the p.Val50Met Variant

Exon 2 of the *TTR* gene was amplified using the following set of oligonucleotides as primers: 5′ GTTAACTTCTCACGTGTCTT 3′ (forward) and 5′ AAGTCCTGTGGGAGGGTTCT 3′ (reverse). Subsequently, PCR products were digested with the restriction enzyme NsiI (Boehringer-Mannheim), electrophoresed onto a 4% NuSieve LMP agarose gel, stained, and UV-visualized. The presence or absence of the p.Val50Met *TTR* variant was then identified. All positive results were subsequently verified by Sanger sequencing.

Molecular Analysis for the p.Val114Ala Variant

In the index p.Val114Ala patient, presenting with heart failure and amyloid deposits in a heart biopsy specimen, Sanger sequencing of exon 2 failed to identify the p.Val50Met variant (Diagenom, Rostock, Germany). After this, the remaining exons and the exon-intron splice junctions of the *TTR* gene were sequenced, and the p.Val114Ala *TTR* change was found in heterozygosity. Subsequent patients with a similar phenotypic presentation were tested for the above variant with (1) PCR amplification of either exon 4 or all exons of the *TTR* gene and Sanger sequencing of the purified PCR product (Neurogenetics Laboratory, University of Crete; Macrogen Europe, Amsterdam, the Netherlands; Diagenom, Rostock, Germany) and (2) by an amplicon-based next-generation sequencing of all *TTR* gene exons (Centogene, Rostock, Germany). After the identification of the p.Val114Ala variant in a given patient, we screened available immediate family members, either symptomatic or asymptomatic, for the same variant. This testing included sequencing of all 4 *TTR* exons, in all cases but 2, where targeted exon 4 sequencing was performed.

Molecular Analysis for the p.Arg54Gly Variant

For the patient with ocular amyloid deposits, an amplicon-based next-generation sequencing of all *TTR* gene exons (Centogene, Rostock, Germany) was performed, leading to the identification of the p.Arg54Gly *TTR* variant. Testing of the unaffected patient's sister failed to identify any pathogenic variant in the *TTR* gene.

Data Availability

Deidentified data are available on request.

Results

Thirty patients, permanent residents of Crete, were diagnosed with hATTR during the past 27 years. Genetic analyses revealed 3 different *TTR* gene pathogenic variants. Most (19/30 [63.3%]) of these patients harbored the p.Val50Met variant, whereas the second most frequent genetic defect was the p.Val114Ala variant (10/30 [33.3%]). The rare p.Arg54-Gly variant was encountered in 1 patient (3.3%). Eight of 30 patients (26.6%) with hATTR died through this 27-year period, leaving 22 patients alive at the time of the conclusion of this study. Finally, by screening the above patients' offspring, we identified 5 additional asymptomatic carriers of pathogenic *TTR* gene variants.

Patients With Predominantly Neuropathic Phenotype (Carrying the p.Val50Met Variant)

The p.Val50Met *TTR* variant was identified in 19 patients (11 female patients; 8 male patients) (Table 1). These patients were members of 6 unrelated families originating from 6 different villages located in all 4 different prefectures of the island of Crete (Figure 1). All patients had a positive family history of polyneuropathy. Three of the families originated from neighboring villages in *Messara* valley (Figure 1). Screening of the patients' offspring led to the identification of 2 additional asymptomatic p.Val50Met *TTR* variant carriers.

The median age at symptom onset was 31 years (range 18–35 years) (Table 1). Duration of symptoms prior to the diagnosis ranged from 1 to 5 years. The clinical presentation of patients with the p.Val50Met *TTR* variant was rather uniform and included paresthesias, shooting and throbbing pain, loss of temperature sensation, and progressive weakness of the lower extremities. Urinary difficulties, constipation, diarrhea, postural dizziness, and weight loss also gradually developed. The upper extremities were involved later in the disease course; 4 patients developed carpal tunnel syndrome. Although cardiac arrhythmia was common in most patients, heart failure developed only in 4 patients late in the disease course. In addition, 4 patients developed chronic kidney failure requiring hemodialysis.

Neurologic examination revealed loss of pain and temperature sensation in a glove and stocking distribution and distal weakness in all patients. All patients, except for 2, exhibited orthostatic hypotension without compensatory tachycardia.

Electromyographic evaluation showed denervation of lower limb muscles. Sensory action potentials were diminished or absent, especially in the sural nerve, as a sign of axonal injury. In less severe cases, compound muscle action potentials were

Table 1 Comparison of Patients Carrying the p.Val50Met, the p.Val114Ala, and the p.Arg54Gly *TTR* Variants, Respectively

TTR variant	p.Val50Met	p.Val114Ala	p.Arg54Gly
N (patients)	19	10	1
Families (apparently unrelated)	6	5	1
Female	11/19	4/10	0/1
Median age at symptom onset, y (range)	31 (18–35)	70 (54–78)	48
Median age at molecular diagnosis, y (range)	34 (26–42)	71 (54–79)	51
Neuropathic pain	19/19	10/10	0/1
Weight loss	19/19	4/10	0/1
Postural dizziness	17/19	3/10	0/1
Diarrhea	19/19	4/10	0/1
Constipation	19/19	2/10	0/1
Urinary difficulties	19/19	1/10	0/1
Signs of peripheral neuropathy	19/19	10/10	0/1
Signs of autonomic dysfunction	17/19	5/10	0/1
Carpal tunnel syndrome	4/19	8/10	0/1
FAP stage at diagnosis			
1	16	9	—
2	2	1	—
3	1	—	—
PND stage at diagnosis			
0	—	—	1
1	16	9	—
2	1	—	—
3a	1	1	—
4	1	—	—
Heart involvement (clinical)	4/19	9/10	0/1
Heart involvement (^{99m}Tc-PYP)	1/2	9/10	1/1
Heart involvement (US)	4/19	9/10	0/1
Heart involvement (biopsy)	NP	1/1	NP
Pacemaker insertion	7/19	4/10	0/1
Renal involvement	4/19	0/10	0/1
Ocular involvement	5/19	0/10	1/1
Positive rectal biopsy at diagnosis	4/4	3/3	0/1
Treatment: tafamidis	1/19	6/10	0/1
Treatment: orthotopic liver transplantation	14/19	0/10	0/1
Median age at liver transplantation, y (range)	34 (27–43)	NP	NP
Asymptomatic carriers	2	3	0

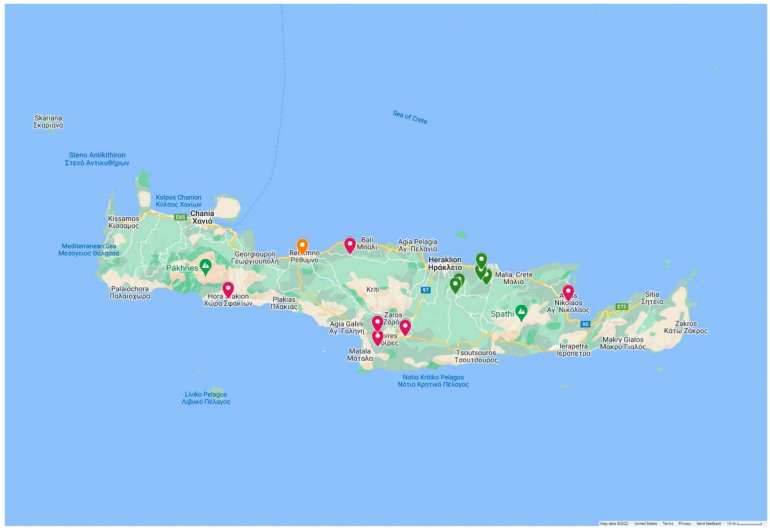
Abbreviations: ^{99m}Tc-PYP = 99m-technetium pyrophosphate; FAP = familial amyloid polyneuropathy; NP = not performed; PND = polyneuropathy disability; US = ultrasound.

normal or slightly diminished. Motor and sensory nerve conduction velocities, when able to be adequately measured, were slightly below normal range. The plantar sympathetic skin response was absent, whereas there was diminished potential and increased latency in the palms, as an additional sign of involvement of small nonmyelinated autonomic fibers.

Starting in 1993, we began treating our patients with orthotopic liver transplantation (Table 1).[16] To this day, we have treated 14 patients with the p.Val50Met *TTR* variant using this approach. Eight of the 14 patients (57%) showed remarkable improvement in their quality of life, which was associated with stabilization or even amelioration of their functional neurologic status, as shown by a score of 2 or 3 in the Clinical Global Impression-Improvement scale[17] at 1 and 3 years post-transplantation. Specifically, paresthesias and autonomic symptoms subsided, although on neurologic examination, there were no significant changes in the sensory deficits. They gained weight and muscle strength, and walking capacity gradually improved. Follow-up examinations showed no evidence for disease progression, except for 3 patients: a woman with gradual development of left ventricular hypertrophy, amyloid deposition on ^{99m}Tc-PYP heart scanning, and symptoms of heart failure; a woman with diarrhea and weight loss; and a man with recurrence of symptoms of polyneuropathy and 2 transient focal neurologic episodes. In all 3 patients, new symptoms developed more than 15 years after liver transplantation. Of the 14 patients who underwent liver transplantation, 3 have died: 1 female patient died of intracerebral hemorrhage and another female patient of multiple organ failure, while the male patient died due to unrelated cause (traffic accident).[16]

Rectal biopsies performed at our center revealed the presence of amyloid deposits in 4 patients with the p.Val50Met *TTR* variant at the time of diagnosis (Table 1). In the 14 patients with the p.Val50Met variant who underwent liver transplantation (in other centers, with most of them in the United Kingdom), positive tissue amyloid deposition testing was included in the preoperative workup, although the results

Figure 1 Origin of hATTR Patients in Crete, Greece



Origin of the families described in this work is shown with red, green, and orange signs. Red: families with the p.Val50Met *TTR* variant; green: families with the p.Val114Ala *TTR* variant; orange: family with the p.Arg54Gly *TTR* variant (Google. (n.d.). [Google Maps pinpoints of families with hATTR patients in Crete]. Retrieved November 25, 2020, from google.com/maps/d/viewer?mid=1DT6BRiN1yXYfrdGJYuMHm8R0RRzoM5nz&hl=en&usp=sharing). Map data ©2022 Google.

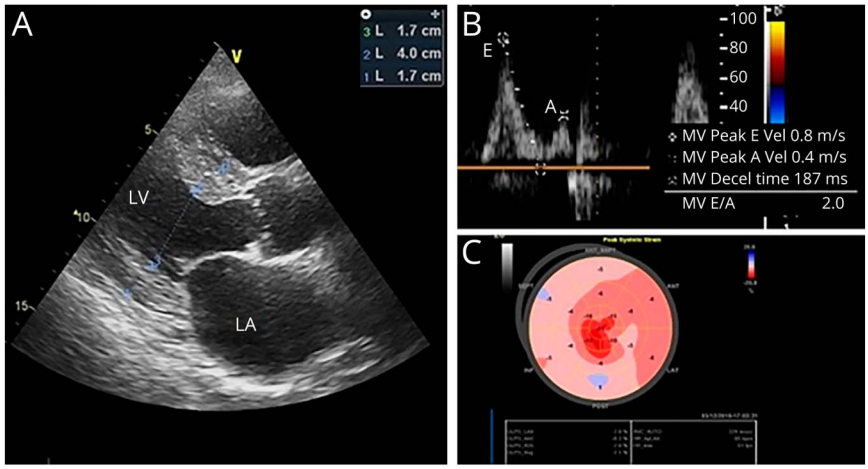
were not available for us to review. However, in the follow-up of 12 of these transplanted patients, we have performed biopsy of the upper and/or lower gastrointestinal tract that showed amyloid deposition in 11.

Patients With Predominantly Cardiopathic Phenotype (Carrying the p.Val114Ala Variant)

After identification of the p.Val114Ala *TTR* variant in a patient with cardiac amyloidosis, we systematically screened patients with heart failure and signs of peripheral nervous system involvement. We also tested their close relatives, either symptomatic or asymptomatic for the presence of the same variant. This led to the identification of 13 individuals (10 patients and 3 asymptomatic carriers) harboring the p.Val114Ala *TTR* variant, belonging to 5 different families. All originated from neighboring villages in the *Pediada* area of the prefecture of Heraklion, Crete (Figure 1).

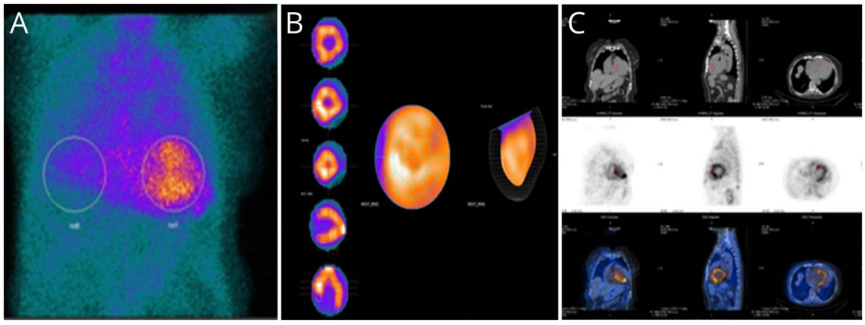
For the patients with the p.Val114Ala variant, the median age at onset was 70 years (range 54–78 years, Table 1). All, but one, of the patients had been initially evaluated by a cardiologist because of symptoms of congestive heart failure such as dyspnea on effort or orthopnea and/or ankle swelling (Table 1). Amyloid cardiac infiltration was suspected on cardiac ultrasound (Figure 2) or cardiac MRI and was revealed by grade 2–3 cardiac amyloid deposition on ^{99m}Tc-PYP scintigraphy (Table 1, Figure 3).[18] Two of these patients ended up on end-stage heart failure with severe cachexia. Only one 54-year-old male, the youngest patient with the p.Val114Ala *TTR* variant, had his first contact with a neurologist because of symptoms of

Figure 2 Cardiac Ultrasound for Amyloid Deposition



Typical findings in transthoracic echocardiogram of a patient with the p.Val114Ala *TTR* variant. (A) Long axis view shows severe concentric left ventricle (LV) hypertrophy and dilated left atrium (LA). (B) Pulsed wave Doppler of mitral inflow with E:A ratio >2 suggestive of grade III diastolic dysfunction. (C) Bullseye map of longitudinal systolic strain of LV with apical sparing pattern—the cherry-on-top sign—(red denotes normal strain at the apex and pink/blue denotes abnormal strain at the mid/basal LV).

Figure 3 Radionuclide Scintigraphy With ^{99m}Tc-PYP for Amyloid Deposition in the Heart in a Patient Harboring the p.Val114Ala *TTR* Variant



^{99m}Tc-PYP planar (A), SPECT (B), and SPECT/CT 16 slices (C) myocardial imaging, taken at 1 and 3 hours after injection. (A) Planar imaging qualitative and quantitative analysis revealed a high uptake in the region of the heart and a high H/CL ratio >1.5. (B) SPECT imaging qualitative and quantitative analysis revealed a diffuse intense ^{99m}Tc-PYP uptake in the region of the left myocardial ventricle (score 3) and a high percentage of uptake >70%. (C) SPECT/CT imaging showed a high uptake in the heart on hybrid imaging and a high standardized uptake value (suv: 3.57). These findings were diagnostic for ATTR cardiac amyloidosis. ^{99m}Tc-PYP = 99m-technetium pyrophosphate.

cognitive difficulties, peripheral neuropathy, and endocrine dysfunction. There was no evidence of cardiac amyloidosis on echocardiogram, although there was a mild uptake (grade 1) in ^{99m}Tc-PYP scintigraphy.

In 4 of our 10 patients with the p.Val114Ala *TTR* variant, the main ECG finding at the time of diagnosis was a pseudoinfarction pattern in anterior leads. No patient had low QRS voltage in the ECG, a typical ECG feature of advanced cardiac amyloidosis.[19] Conduction defects were detected in 8 of the 10 patients. Two patients had already a pacemaker implanted at the time of diagnosis, and another 2 patients required pacemaker implantation after hATTR diagnosis. Five patients had either paroxysmal or chronic atrial fibrillation treated with anticoagulant therapy.

Echocardiographic findings included symmetric left ventricular hypertrophy, diastolic dysfunction, dilatation of both atria, thickening of the right ventricle, and small pericardial effusions. Ejection fraction was preserved in all patients, except for 1 patient. Echocardiographic analyses of cardiac deformation revealed impaired global longitudinal systolic strain with apical sparing (Figure 2).

One patient had cardiac biopsy which revealed amyloidosis with Congo red staining. Three more patients had positive staining for amyloid in rectal biopsy. However, histologic analyses of abdominal fat aspirate biopsy were negative for amyloid deposition in all 4 patients tested. On the other hand, the successful use of nuclear scintigraphy with ^{99m}Tc-PYP for amyloid deposition in the heart in 1 patient[18] permitted us to use this as the primary diagnostic modality (instead of biopsy).[20] Serum-free light-chain assay and serum and urine immunofixation were negative in all patients.

The neurologic and the neurophysiologic examination revealed peripheral neuropathy with autonomic system involvement in all patients with the p.Val114Ala *TTR* variant. Eight of the patients had a history of carpal tunnel syndrome, requiring surgical decompression in 5 (Table 1). Most of the patients reported mild paresthesias and dysesthesias (burning pain) in the feet years before cardiac symptoms' onset, only when specifically questioned about them. Four patients developed progressive weakness of the lower extremities early in their disease course. Only 5 of the 10 patients had signs and symptoms of autonomic neuropathy at diagnosis (Table 1).

Treatment with tafamidis (20 mg per day) was recently given to 6 of the patients, while no other therapeutic modality had been available to them thus far.

Patient With the Predominantly Ophthalmic Phenotype (Carrying the p.Arg54Gly Variant)

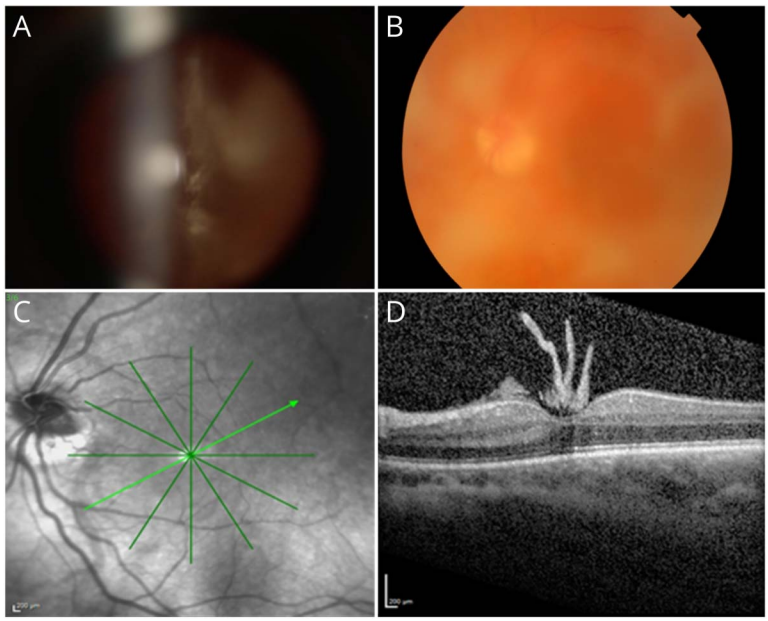
A 50-year-old male patient with predominantly ocular involvement was found to have the p.Arg54Gly *TTR* change. He was initially evaluated at the ophthalmology clinic at the age of 48 years because of floaters and progressive bilateral visual decline of 6-month duration. In addition, he had a long-term history of amblyopia in the left eye.

The patient, a permanent resident of Crete for the past 20 years, was born and raised in central Albania. None of the patient's 5 younger siblings had symptoms of ocular involvement. However, his father, who died at the age of 53 due to "stroke," had developed progressive visual loss leading to total blindness several years prior to his death. His paternal grandfather had also developed progressive visual loss and died at the age of 60 of unknown cause.

The patient's best-corrected visual acuity was 20/32 on the right eye and finger counting on the left eye. Intraocular pressures were within the normal range. Anterior vitreous was very hazy with an opaque "glass wool" appearance (Figure 4A). Fundoscopy revealed a bioscore of +3 in both eyes and 2 areas of chorioretinal atrophy in the periphery of the right retina (Figure 4B). There were no clinical signs of active or previous vasculitis nor active choroiditis. The patient underwent an extensive workup for infectious and noninfectious uveitis that yielded negative results. Fluorescein angiography was also normal.

Considering the characteristic appearance of the vitreous and the lack of obvious inflammatory process, ocular amyloidosis

Figure 4 Patient With Ocular Amyloidosis (p.Arg54Gly *TTR* Variant)



(A) Slitlamp photography of anterior vitreous demonstrating the characteristic glass wool appearance. (B) Fundus photography revealing hazy vitreous that obscures retinal details. (C, D) Same eye 1 year after vitrectomy. The media are clear permitting unimpeded imaging of retinal details in infrared photograph (C); however, in optical coherence tomography, residual amyloid deposits can be seen perpendicular to the retinal surface in the prefoveal area, leading to the characteristic needle-shaped appearance (D).

was suspected and confirmed by genetic analysis which showed the pathogenic p.Arg54Gly *TTR* change. The patient underwent vitrectomy in the right eye (mid 2017). Vitreous analysis did not identify any cells, and PCR was negative for viruses. In addition, Congo red tissue stain for amyloid was inconclusive. After vitrectomy, the patient's vision improved. Later (early 2018), a vitrectomy was also performed in the left eye (Figure 4, C and D). At that time, the patient underwent a thorough neurologic, cardiological, and gastrointestinal evaluation, including colon biopsy, which were negative for systemic amyloidosis. However, frequent neurologic follow-up evaluations were proposed, given the high possibility that this patient will develop polyneuropathy. Finally, the patient underwent cataract extraction in the left eye and repeated vitrectomy of the residual anterior vitreous for recurrent deposits. A ^{99m}Tc-PYP scintigraphy was obtained, which showed amyloid deposition in the heart of the patient.

Prevalence of hATTR (Patients With the p.Val50Met, p.Arg54Gly, and p.Val114Ala Variants)

To calculate the prevalence of hATTR in Crete, we considered all our patients with hATTR who were alive and resided on the island in August 2018. This cohort included 11 patients with the p.Val50Met change, 10 patients with the p.Val114Ala variant, and 1 patient with the p.Arg54Gly *TTR* change (22 patients in total). Given that the population of the island in the 2011 census was 623,065, this resulted in a prevalence estimate of 35.3 per million inhabitants (Table 2). The 5 asymptomatic carriers of pathogenic *TTR* variants were not taken into consideration for this prevalence estimation.

Discussion

Our study showed that hATTR occurs frequently on the island of Crete, Greece, and that the disease is due to 3 different *TTR* variants (p.Val50Met, p.Val114Ala, and, in a single patient, p.Arg54Gly) corresponding to 3 distinct phenotypic presentations. The clinical presentation of patients harboring the p.Val50Met variant was typical for early-onset neuropathic hATTR, whereas that of patients with the p.Val114Ala variant was of a later-onset cardiomyopathy, although some patients had experienced carpal tunnel neuropathy as presenting symptom. Finally, the single patient harboring the p.Arg54Gly variant had ocular amyloidosis. The combined presence of these 3 pathogenic variants gave a hATTR prevalence of 35.3 per 1 million inhabitants.

The first hATTR kindred of Greek origin was described more than 30 years ago.[12] Subsequently, in 1990, a study on 6 more Greek families of the diaspora was published.[13,14] Concerning Greece however, except for the occasional description of families with the p.Val50Met variant,[12,21] there are no systematic studies on the prevalence of hATTR, although it has been extrapolated to be 3–79 per million[9] and recently estimated to be below 1/100,000.[15] Our study systemically evaluated the prevalence of hATTR in a defined area of Greece, thereby establishing that Crete is an endemic area for this disorder.

Study of the genotypic and phenotypic features of hATTR in Crete revealed that patients with the p.Val50Met *TTR* variant experienced sensory symptoms (such as paresthesia and temperature loss) and dysautonomia in their 20s or 30s and then gradually developed weakness of the lower extremities.

Table 2 Prevalence of hATTR in Various Geographic Regions

Region	Prevalence of hATTR (per million inhabitants)	*TTR* variant(s)	References
Portugal (Póvoa de Varzim)	1,975.0	p.Val50Met (100%)	[43]
Portugal (Northern)	902.5	p.Val50Met (100%)	[44]
Sweden (Pitea)	1,040.0	p.Val50Met (100%)	[45]
Sweden (Skelleftea)	910.0	p.Val50Met (100%)	[45]
Portugal (total)	229.3 205.0 192.3	p.Val50Met (99%)	[28,43,46]
Spain (Majorca)	54.1	p.Val50Met (100%)	[47]
Cyprus	55.5 54.0 37.2	p.Val50Met (100%)	[10,28,48]
Crete, Greece	35.3	p.Val50Met (50.0%) p.Val114Ala (45.5%) p.Arg54Gly (4.5%)	This study
Sweden (total)	26.0	p.Val50Met (95%) p.His108Arg (2%)	[28]
Spain (Minorca)	13.8	p.Val50Met (100%)	[47]
Japan (Nagano)	12.2	p.Val50Met (100%)	[49]
Japan (Kumamoto)	10.1	p.Val50Met (100%)	[49]
Italy (total)	9.0/4.3	p.Val50Met (35%/23%) p.Glu109Gln (25%/13%) p.Phe84Leu (15%/22%)	[28,50]
Italy (Sicily)	8.8	p.Glu89Gln (53%) p.Phe64Leu (37%) p.Thr49Ala (10%)	[51]
France (total)	7.6	p.Val50Met (63%) p.Ser97Tyr (12%) p.Ser97Phe (6%)	[28]
Bulgaria (total)	5.6	p.Glu109Gln (81%) p.Val50Met (10%) p.Ser97Phe (7%)	[28]
Japan (Ishikawa)	4.2	p.Val50Met (93%) p.Leu78Arg (7%)	[49]
Netherlands (total)	2.7	p.Val50Met (30%) p.Val91Ala (25%) p.Tyr134Cys (20%)	[28]
Germany (total)	1.5	p.Val50Met (40%–60%) p.Val40Ile (8%–22%)	[28]
Japan (total)	1.0	p.Val50Met (99%) p.Leu78Arg (1%)	[49]
Turkey (total)	0.3	p.Glu109Gln (31%) p.Gly67Glu (25%) p.Val50Met (19%) p.Gly73Glu (19%)	[28]

Abbreviation: hATTR = hereditary transthyretin-related amyloidosis.

At the time of the genetic diagnosis, neurologic examination revealed deficits in thermal and pain sensation, as evidence for small fiber involvement. However, as the disease progressed, loss of vibration and position sense and muscle strength, indicating large fiber involvement, became more pronounced and compatible with observations made in other studies.[3]

In the literature, p.Val50Met is the most commonly described *TTR* pathogenic variant worldwide.[22] The p.Val50Met variant has been associated with 2 distinct phenotypes, 1 early onset, found mainly in Portugal and Japan, and another late onset, described in nonendemic areas and in Sweden.[23,24] The origin of the p.Val50Met *TTR* variant in Crete remains unclear. One could hypothesize that a single founder accounts for almost all p.Val50Met *TTR* variant cases found on the island or at least for the cases originating in the 3 nearby villages in the *Messara* valley (Figure 1). Given that Crete is at the crossroads of the Mediterranean and has been occupied by foreign nation armies during the last 12 centuries,[25] it is possible that the p.Val50Met *TTR* variant founder originated from outside Crete. In this regard, it has been shown that patients with the p.Val50Met variant in Brazil and Japan, but not in Sweden, are probably descendants of Portuguese founders that spread the variant following the commercial routes of the 15th and the 16th century.[26,27] Hence, the p.Val50Met may have followed an analogous route to reach Crete during the same time period, or even earlier, at the time of the Crusaders.[28]

The possibility of a Portuguese founder is suggested by the clinical phenotype of the Cretan hATTR p.Val50Met cases that is nearly identical to that occurring in Portugal. On the other hand, late-onset p.Val50Met hATTR cases, which are known to occur in Northern European populations (such as in Sweden and France) and in Japan, were not found among our population. The genetic background of a given population could play a role in the age at onset, as a recent study from France revealed.[29] Excluding the possibility of *cis*-acting or *trans*-acting DNA elements inherited from a common founder,[30] the highly consistent phenotypic expression of the disorder in Portugal and Crete may relate to a particular genetic makeup of the 2 populations, including a possible common Mediterranean ancestry. The results of a recent study revealed that the ancient bronze-age Minoan Cretans' DNA resembles that of modern inhabitants of the *Lassithi* plateau of Crete and of Neolithic and modern European populations, with this resemblance being stronger for modern Portuguese than for modern French.[31] Whether such a genetic similarity between Crete and Portugal could account for the occurrence of the Portuguese type hATTR in the population of Crete remains to be further studied.

Regarding the p.Val114Ala *TTR* variant, this has been previously described in a patient from Germany who presented with cardiac involvement at age 63 years and later developed sensorimotor polyneuropathy, autonomic dysfunction, and diarrhea.[32] Another patient has been most recently described originating from Austria.[33] The present report on the patients

harboring the p.Val114Ala variant represents a large case series of a specific *TTR* variant being associated with a late-onset predominantly cardiac phenotype.[22,34] The phenotype in our patients with the p.Val114Ala variant is characterized by cardiac symptoms and signs of heart failure at the time of diagnosis, although mild symptoms of polyneuropathy and carpal tunnel syndrome seem to have been present several years before the onset of cardiomyopathy. Notably, compared with p.Val50Met patients, patients with the p.Val114Ala variant had less prominent small fiber and autonomic involvement at diagnosis.

Regarding our case with the p.Arg54Gly *TTR* variant, this variant has been previously described also in patients with ophthalmic amyloidosis. Specifically, this variant was found in 2 members of a Chinese family with vitreous amyloidosis,[35] a patient with ophthalmic involvement from the United Kingdom,[36] and in a 57-year-old patient residing in Australia, but originating from Kosovo, with bilateral carpal tunnel syndrome and bilateral floaters.[37] On this last patient, pathognomonic bilateral vitreolenticular amyloid opacities (pseudopodia lentis) and axonal neuropathy on electrophysiologic studies were identified. One of this patient's brothers had died blind in Kosovo at age 50 years of an unidentified neurologic illness.[37] As our patient harboring the p.Arg54Gly variant was originally from Central Albania, it is possible that his disorder relates to that of the family from Kosovo. In any case, our report adds to the growing body of literature describing predominantly ocular manifestations of hATTR variants.[38]

Concerning hATTR-specific management, most of our patients with the p.Val50Met variant underwent orthotopic liver transplantation, with excellent results in most cases, as has been recently reported.[16] Our experience agrees with this of other groups suggesting that liver transplantation is an effective treatment, despite the risks associated with it. However, this is not suitable for patients with the p.Val114Ala *TTR* variant because of their severe heart involvement and advanced age. In addition, patients with the p.Arg54Gly variant are not expected to benefit from liver transplantation in their ophthalmopathic manifestations because ocular amyloid deposition is not caused by liver-derived transthyretin. In any case, the current trend is to offer other treatment alternatives, safer than OLT, to patients with hATTR, irrespective of the variant causing the disease phenotype.[39]

While *TTR*-related hereditary amyloidosis has traditionally been considered as a form of polyneuropathy, here we show that, in the same population, the disorder can present with markedly different phenotypes, including a predominately cardiomyopathic or ophthalmic phenotype with polyneuropathy being a secondary clinical feature. In addition, age at disease onset in our patients ranged widely (between ages 25 and 78 years). Thus, a neurologist should exercise great care not to miss patients with hATTR with atypical presentations or ages at onset, given that this disease is treatable and amenable to genetic counseling.

Identification of the patients with the predominantly cardiac phenotype harboring the p.Val114Ala *TTR* variant was made possible in our population because of a high index of suspicion. In fact, in hATTR, the correct diagnosis may be overlooked because of atypical presentation or the absence of family history because of reduced penetrance. In addition, some cases of hATTR could be erroneously diagnosed as wild-type *TTR* heart amyloidosis or even hypertrophic cardiomyopathy.[40] In view of the introduction of novel therapies for this devastating disease,[41,42] our findings should encourage testing for hATTR in a wide spectrum of phenotypic presentations, such as early-onset polyneuropathy, late-onset cardiomyopathy with concurrent polyneuropathy, and history of carpal tunnel syndrome and ophthalmic amyloidosis.

Although we aimed to achieve a universal coverage of the island, given that the 2 main hospitals participating in this study are the only referral centers in Crete and that we actively engaged local physicians, we cannot exclude the possibility that we have missed some hATTR cases because this was not a systematic population study. In addition, only in recent years, and after finding the second pathogenic variant (p.Val114Ala) in addition to the p.Val50Met in the island, we systematically tested the whole gene (all 4 exons) in participants with clinical features raising the possibility of hATTR. Despite these limitations of our study, the prevalence rate detected (35.3/1,000,000) makes Crete another endemic area of the disease in Europe. In fact, our hATTR prevalence is surpassed only by Portugal, Northern Sweden, Majorca, and Cyprus (Table 2), although we cannot exclude the possibility that in Crete, there could be better case ascertainment compared with other countries. This high prevalence of hATTR in Crete is due to the identification of the p.Val114Ala variant in addition to the classic p.Val50Met *TTR* variant. Similarly, there have been several other areas around the world where the p.Val50Met *TTR* variant coexists with other pathogenic *TTR* variants, adding to the hATTR burden in these populations.[28]

In summary, here we show that hATTR is frequent in Crete, Greece, and that the disorder is caused by 3 different *TTR* gene pathogenic variants (p.Val50Met, p.Arg54Gly, and p.Val114Ala, respectively) with distinct phenotypic presentations. Our results indicate that increased awareness and clinical acumen can lead to improved screening procedures, aiming at enhanced and timely patient management.

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