European Heart Journal (2016) **37**, 1826–1834
doi:10.1093/eurheartj/ehv583

FASTTRACK CLINICAL RESEARCH
Heart failure/cardiomyopathy

Prevalence and clinical phenotype of hereditary transthyretin amyloid cardiomyopathy in patients with increased left ventricular wall thickness

Thibaud Damy[1]*, Bruno Costes[2], Albert A. Hagège[3], Erwan Donal[4], Jean-Christophe Eicher[5], Michel Slama[6], Aziz Guellich[1], Stéphane Rappeneau[1], Jean-Pierre Gueffet[7], Damien Logeart[8], Violaine Planté-Bordeneuve[9], Hélène Bouvaist[10], Olivier Huttin[11], Geneviève Mulak[12], Jean-Luc Dubois-Randé[1], Michel Goossens[2], Florence Canoui-Poitrine[13]†, and Joel N. Buxbaum[14]†

[1]Department of Cardiology, UPEC, AP-HP Henri-Mondor Teaching Hospital, Inserm U955, IMRB GRC Mondor Amyloidosis Network, DHU ATVB and Inserm Clinical Investigation Center 1430, 51 Avenue Maréchal de Lattre de Tassigny, Créteil F-94000, France; [2]Department of Genetics and Biochemistry, UPEC, AP-HP Henri-Mondor Teaching Hospital, Inserm U955, IMRB, Créteil F-94000, France; [3]Department of Cardiology, Paris Descartes University, PRES Paris Sorbonne, AP-HP, Hôpital Européen Georges Pompidou, Inserm UMR970, Paris Cardiovascular Research Center, Paris, France; [4]Department of Cardiology, Université Rennes 1, Rennes Teaching Hospital, LTSI, INSERM 1099, Inserm Clinical Investigation Center-IT U 804, Rennes, France; [5]Department of Cardiology, Dijon Universiy, Hôpital Bocage Central, Dijon Teaching Hospital, Dijon, France; [6]Department of Cardiology, Paris-Sud University, AP-HP A. Béclère Teaching Hospital, Béclère, 92140 Clamart, France; [7]Hopital G et R Laennec, Institut du Thorax, 44093 Nantes Cedex1, France; [8]Department of Cardiology, Denis Diderot University, AP-HP, Lariboisière Hospital, 75010 Paris, France; [9]Department of Neurology, UPEC, AP-HP Henri-Mondor Teaching Hospital, Inserm U955, IMRB GRC Mondor Amyloidosis Network, Inserm Clinical Investigation Center 1430, Créteil F-94000, France; [10]Department of Cardiology, Grenoble Teaching Hospital, Grenoble, France; [11]Department of Cardiologie, Institut Lorrain du Cœur et des Vaisseaux, 54511 Vandœuvre-lès-Nancy, France; [12]Société Française de Cardiologie, Paris, France; [13]Department of Public Health, UPEC, EA 4393 CEpiA (Clinical Epidemiology And Ageing), DHU ATVB, AP-HP, Henri-Mondor Teaching Hospital, DHU ATVB, Créteil F-94000, France; and [14]Department of Molecular and Experimental Medicine, The Scripps Research Institute, La Jolla, CA, USA

Received 31 July 2015; revised 16 September 2015; accepted 7 October 2015; online publish-ahead-of-print 3 November 2015

This work has been presented at the session Heart Failure: diversity of phenotyping on Monday 31 August 2015 at the ESC London 2015.

Aims Increased left ventricular wall thickness (LVWT) is a common finding in cardiology. It is not known how often hereditary transthyretin-related familial amyloid cardiomyopathy (mTTR-FAC) is responsible for LVWT. Several therapeutic modalities for mTTR-FAC are currently in clinical trials; thus, it is important to establish the prevalence of TTR mutations (mTTR) and the clinical characteristics of the patients with mTTR-FAC.

Methods and results In a prospective multicentre, cross-sectional study, the TTR gene was sequenced in 298 consecutive patients diagnosed with increased LVWT in primary cardiology clinics in France. Among the included patients, median (25–75th percentiles) age was 62 [50;74]; 74% were men; 23% were of African origin; and 36% were in NYHA Class III–IV. Median LVWT was 18 (16–21) mm. Seventeen (5.7%; 95% confidence interval [CI]: [3.4;9.0]) patients had mTTR of whom 15 (5.0%; 95% CI [2.9;8.2]) had mTTR-FAC. The most frequent mutations were V142I ($n = 8$), V50M ($n = 2$), and I127V ($n = 2$). All mTTR-FAC patients were older than 63 years with a median age of 74 [69;79]. Of the 15 patients with mTTR-FAC, 8 were of African descent while 7 were of European descent. In the African descendants, mTTR-FAC median age was 74 [72;79] vs. 55 [46;65] years in non-mTTR-FAC ($P < 0.001$). In an adjusted multivariate model, African origin, neuropathy, carpal tunnel syndrome, electrocardiogram (ECG) low voltage, and late gadolinium enhancement (LGE) at cardiac-magnetic resonance imaging were all independently associated with mTTR-FAC.

Conclusion Five per cent of patients diagnosed with hypertrophic cardiomyopathy have mTTR-FAC. Mutated transthyretin genetic screening is warranted in elderly subjects with increased LVWT, particularly, those of African descent with neuropathy, carpal tunnel syndrome, ECG low voltage, or LGE.

Keywords Hypertrophic cardiomyopathy • Cardiac amyloidosis • Transthyretin

* Corresponding author. Tel: +33 1 49 81 22 53, Fax: +33 1 49 81 42 24, Email: thibaud.damy@hmn.aphp.fr

† These authors contributed equally to this work.

Introduction

Hypertrophic cardiomyopathy (HCM) is defined as myocardial hypertrophy in the absence of underlying conditions such as coronary artery disease, hypertension, valvular abnormalities, and congenital heart disease.[1] Hypertrophic cardiomyopathy encompasses a heterogeneous group of disorders that are associated with left ventricle hypertrophy, yet are genetically and mechanistically distinct, and includes diseases in which infiltrative processes of the extracellular matrix increase cardiac wall thickness in the absence of actual cardiomyocyte hypertrophy.[1] Most clinical genetic studies of the HCM have focussed on sarcomeric genes with >1400 mutations identified.[2]

Amyloidosis is an infiltrative disorder caused by deposition of characteristic Congophilic fibrils composed of aggregated misfolded proteins.[3] Mutated transthyretin (mTTR) amyloidosis is an autosomal dominant disorder with >100 pathogenic mutations identified thus far.[4] Mutations result in destabilization of the TTR homotetramer with release of a monomer that rapidly misfolds, aggregates, and deposits in tissues.[3] Worldwide, the most frequent mutation is V142I that occurs in 3.4% of African Americans.[5] V50M known also as the Portuguese mutation is the most common in Europe.[4] The prevalence of mTTR in patients diagnosed with HCM is unknown.

As opposed to sarcomeric HCM, mTTR familial amyloid cardiomyopathy (mTTR-FAC) carries a particularly poor prognosis since it is usually diagnosed late in the disease course with a greater risk of rapid progression of congestive heart failure and sudden death and requires specific supportive care management usually with aggressive diuresis and avoidance of digoxin, calcium channel-blocking agents, and β-blockers.[6] Several specific therapeutic modalities are currently in clinical trials; thus, it is important to establish the prevalence of mTTR and the clinical characteristics of mTTR-FAC patients.

This study was designed to determine by gene sequencing the prevalence of m-TTR in a referral population of adult patients with increased cardiac wall thickness of unknown cause, to determine whether amyloidogenic TTR alleles were present at a significant frequency, if they were associated with clinical evidence of cardiac amyloidosis and what were the characteristic features associated with mTTR-FAC.

Methods

Design

This is a national multicentre, cross-sectional study. It is currently listed as ClinicalTrials.gov-NCT01623245.

Informed consent was obtained from all participants. All investigations were in accordance with the principles of the Declaration of Helsinki. The study was approved by the IRB of Créteil, France (Comité de Protection des Personnes CPP du CHU Henri-Mondor). The collection was registered at the Commission nationale de l'informatique et des libertés-(CNIL#1111).

Inclusion criteria were age >18 years and a diagnosis of HCM with undetermined cause at the first visit. Hypertrophic cardiomyopathy was diagnosed according to the guidelines of the ESC and the WHO.[7,8] It was based on the demonstration of a maximal interventricular septum thickness (IVST) above 15 mm for sporadic forms and above 13 mm for familial forms measured at echocardiography. Exclusion criteria were aortic stenosis, cardiac hypertrophy with a previously identified mutation in the patient and/or their family or other forms of amyloidosis. Physical examination, electrocardiogram (ECG), echocardiography, cardiac-magnetic resonance imaging (MRI), and current drug therapy were recorded at time of entry into the study.

Analysis of transthyretin gene

DNA was isolated from 5 mL of whole peripheral blood (Flexi gen DNA kit Qiagen). The four exons of the TTR gene were amplified from genomic DNA. PCR products (5 μL each) were further purified by mixing them with 2 μL of Exo-SAP-IT (USB Corporation) and were sequenced using the ABI-PRISM Big Dye Terminator cycle sequencing kit (Applied Biosystems, Foster City, CA, USA) (Sequencer Applied Biosystem). Sequencing was run twice for each sample. The results were compared with the nucleotide sequence of TTR using Applied Biosystems seqscape software v2.5. Mutation numbering was based on cDNA (GenBank mRNA reference NM_000371, TTR gene, ID 7276). Traditional mutation names are also indicated in the manuscript. Exon numbering followed Human Genome Variation Society (HGVS) recommendations (exons 1–4).

Clinical evaluation

When a TTR gene mutation was identified, the investigators were informed and asked to complete the diagnostic work-up for TTR-FAC including ^{99m}-Tc-biphosphonate scintigraphy,[9] cardiac-MRI,[10] and extracardiac and/or endomyocardial biopsy.

Statistical analysis

The number of subjects to be recruited was based on a pilot study of 46 patients diagnosed with HCM.[11] Based on an expected frequency of 6% of mTTR, it was estimated that 240 patients would be needed to obtain a 5% two-tailed alpha-risk with 3% accuracy. To account for missing data or patient refusal, we planned to recruit a minimal number of 260 patients.

For continuous variables, results are expressed as median (25th–75th percentile) and categorical as absolute and relative frequencies.

Prevalence of mTTR-FAC was expressed as percentage and 95% confidence intervals (CI) assuming a binomial distribution. Data were compared between the group of patients with mTTR-FAC to those without using Pearson's or Fisher's exact test for qualitative variables and Mann–Whitney test for quantitative variables. Variables associated with mTTR-FAC at $P \leq 0.20$ were considered for the multivariate analysis. Highly correlated variables were not included simultaneously in the multivariate model. Variables with >20% of missing data were not introduced as adjustment variables in the main multivariate model. Quantitative variables were categorized into quartiles when the log-linearity assumption was not verified.

Due to the small number of events, logistic regression was initially used to estimate crude and adjusted odds ratios and 95% CI. A value of $P < 0.05$ was considered significant. All tests were two-tailed. No imputation was done for missing data. Analyses were conducted using Stata v13.0 (College Station, TX, USA).

Results

Characteristics of the overall population

Three hundred and one patients were included of whom 298 had TTR genetic testing performed (3 technical failures). The baseline

clinical data of the 298 patients are summarized in *Table 1*. The median (IQR) age at inclusion was 62 (50;74) years. Seventy-four per cent were men and 36% were in Class III–IV of the New York Heart Association (NYHA). Two hundred and eighty-eight patients had left ventricular wall thickness (LVWT) ≥15 mm. Hypertension was present in 50% of the patients.

Table 1 Clinical, electrocardiogram, echocardiography, cardiac-MRI, and biological baseline characteristics in the overall population and depending on TTR mutation and cardiac amyloidosis

Variables	All	HCM (296[a])		*P*
		With mTTR-FAC	Without mTTR	
N	298	15	281	
Demographics				
Age, years	62 (50; 74)	74 (69; 79)	61 (49; 73)	<0.001
Men, *n* (%)	219 (74)	11 (73)	206 (73)	0.99
BMI, kg m^{-2}	25 (23; 29)	24 (22; 27)	26 (23; 29)	0.16
Geographic origin				
Caucasian, *n* (%)	219 (76)	6 (40)	213 (79)	0.005
African, *n* (%)	66 (23)	9 (60)	55 (20)	
Asiatic, *n* (%)	3 (1)	0	3 (1)	
Medical history				
Carpal tunnel, *n* (%)	17 (6)	6 (46)	11 (4)	<0.001
Neuropathy, *n* (%)	15 (8)	8 (53)	7 (4)	<0.001
Family history of HCM, *n* (%)	40 (19)	5 (30)	35 (18)	0.42
Hypertension, *n* (%)	142 (50)	8 (57)	132 (49)	0.57
Atrial fibrillation or flutter, *n* (%)	97 (34)	8 (57)	89 (34)	0.07
Symptoms				
NYHA Class III–IV, *n* (%)	106 (36)	8 (53)	97 (35)	0.24
Dysautonomia, *n* (%)	5 (2)	1 (8)	4 (1)	0.076
ECG				
ECG low voltage, *n* (%)	20 (7)	5 (36)	15 (5)	<0.001
Echocardiography				
LV symmetric hypertrophy, *n* (%)	122 (46)	14 (93)	108 (43)	
Maximal LV wall thickness, mm	18 (16; 21)	18 (16; 21)	18 (16; 21)	0.86
LVEF, class				
<30%, *n* (%)	13 (5)	1 (7)	12 (4)	0.002
30–40%, *n* (%)	34 (12)	6 (40)	28 (10)	
>40%, *n* (%)	240 (81)	8 (53)	232 (83)	
LVEDD, mm	46 (41; 51)	43 (40; 53)	46 (42; 51)	0.72
E/A	1.2 (0.8; 2.1)	2.5 (1.7; 3.3)	1.2 (0.8; 2.0)	0.015
E/e′	12 (8; 16)	14 (12; 24)	11 (8; 16)	0.051
Cardiac-MRI	62 (34)	1 (100)	52 (30)	<0.001
LGE[b], *n* (%)	62 (34)	10 (100)	52 (30)	<0.001
Biology				
NT-pro-BNP[c], pg ml^{-1}	2546 (857; 5341)	5597 (2431; 9773)	2082 (814; 4637)	0.055
BNP[d], pg mL^{-1}	344 (214; 634)	368 (204; 7643)	344 (214; 634)	0.82
Creatinine, μmol l^{-1}	151 (130; 234)	172 (102; 200)	150 (130; 235)	0.90

Data are expressed as median (25th–75th percentile) or as absolute and %.
BMI, body mass index; NYHA, New York Heart Association; LVEF, left ventricular ejection fraction; LVEDD, left ventricular end-diastolic diameter; *E/A*, ratio of peak velocities of E and A waves of transmitral pulsed Doppler; *e′*, mean of Doppler lateral and septal mitral annular early diastolic peak velocity; LGE, late gadolinium enhancement; BNP, brain natriuretic peptide.
[a]Included only patients with mTTR-FAC or without mTTR. Two patients (no. 16; V42I carrier and no. 17; R123H) were excluded from the analysis.
[b]For cardiac-MRI, $n = 182$.
[c]For NT-pro-BNP, $n = 104$.
[d]For BNP, $n = 73$.

Prevalence and type of mutated transthyretin

Forty of the 298 patients with molecular DNA analysis had a *TTR* mutation. Twenty-three patients (8%) had a *TTR* nucleotide variation (non-amyloidogenic) of whom 21 had Gly26Ser polymorphism alone[12] and 2 had synonymous variations (Ser120Ser and Lys14Lys). Seventeen had potential amyloidogenic TTR mutation. The frequency of these mutations in this HCM population was 5.7%; 95% CI [3.4;9.0] (*Table 2*). The most frequent mutations were TTR c.(V142I) ($n = 8$), TTR c.(V50M), and TTR c.(I127V) with two each. One patient had TTR c.(V142I) and Gly26ser. All patients were heterozygous. One patient had the mutation TTR c.(R123H), the amyloidogenicity of which is unclear.

Prevalence of mutated transthyretin-related familial amyloid cardiomyopathy

The phenotypes of the 17 patients with mTTR are shown in *Table 2*. Of these patients, 15 (Patient 1–15 in *Table 2*) were considered to have TTR-FAC. The prevalence of mTTR-FAC was 5.0%; 95% CI [2.9; 8.2]. Fourteen of the amyloidosis diagnoses were established by biopsy and/or bisphosphonate scintigraphy. One patient (no. 10) died before having either of these exams, but his echocardiography and cardiac-MRI were consistent with cardiac amyloidosis [apical sparing and diffuse late gadolinium enhancement (LGE)].

Two patients (no. 16 and 17) had no evidence of cardiac amyloidosis. Patient no. 16 had TTR c.(V142I), but his bisphosphonate scintigraphy was negative. Two cardiac biopsies were performed consecutively which did not show amyloid infiltration. He was considered to be a carrier. Patient no. 17 had the TTR c.(R123H) mutation without evidence of amyloid deposition in biopsies of salivary gland, nerve, and kidney. His bisphosphonate scintigraphy was normal and the patient refused cardiac biopsy. Those two patients were excluded from further analyses.

Prevalence of mutated transthyretin depending on patient's origin

In the overall study, 218 (74%) patients were Caucasians, 66 (22%) were of African origin, 3 (1%) from Asia, and 10 (3%) did not wish to respond. Of the 15 with mTTR-FAC, 7 (47%) were Caucasian and 8 were of African descent. Five patients with TTR c.(V142I) were of African descent and two were Caucasian. The prevalence of TTR c.(V142I) in African descent was significantly higher than that in Caucasians ($P = 0.002$). One TTR c.(V50M) was found in a patient of African origin.

The frequencies of mTTR-FAC by age are shown in *Figure 1* and by age and ethnic origin in *Figure 2*. When considering all the patients, the prevalence of mTTR-FAC was 7.6% in patients older than 55 years. Interestingly, in Africans in the 55–64 years age group, the prevalence of mTTR-FAC was 23% and increased to 33% in subjects 65–74 years old. In Caucasians, in these age groups, the prevalences were 4.3 and 6.1%, respectively (*Figure 2*).

Phenotype of mutated transthyretin-related familial amyloid cardiomyopathy and comparison with patients without mutated transthyretin

Phenotypic comparison of mTTR-FAC patients and those with increased LVWT without mTTR are shown in *Table 1*. Mean LVWT was similar between the two groups. African origin, male gender, carpal tunnel syndrome (CTS), neuropathy, ECG low voltage, LGE, and left ventricular ejection fraction (LVEF) $< 40\%$ were more frequent in mTTR-FAC than in the non-mTTR-FAC group. Mutated transthyretin-related familial amyloid cardiomyopathy patients were also older and had higher *E/A* ratios. Intra left ventricular resting obstruction or systolic anterior motion of the mitral valve was not observed in patients with mTTR-FAC and was observed, respectively, in 25 and 27% of the patients without mTTR (data not shown).

Table 3 displays crude OR, age-adjusted OR, and full-adjusted OR (adjustment for age, ethnic origin, ECG low voltage, or CTS). Log-linearity assumption for age was not verified; therefore, age was categorized according to two classes ≥ 73 vs. < 73 years (corresponding to the highest quartile of the group without mTTR-FAC). After full adjustment, patients with the following variables: African origin, neuropathy, CTS, ECG low voltage, and LGE at cardiac-MRI were much more likely to have mTTR-FAC among referred patients with increased IVST (*Table 3*). Age ≥ 73 years (adjusted OR = 3.46; 95% CI [1.00;11.95]; $P = 0.05$) was associated with mTTR-FAC even after adjustment for African origin and ECG low voltage, but the association disappeared after subsequent adjustment for CTS.

Phenotype of Gly26Ser and comparison with patients without any mutated transthyretin

Twenty-two patients had Gly26Ser of whom 21 had no other mTTR, while 1 subject of African origin (no. 6 in *Table 2*) also had TTR c.(V142I). This patient was excluded from the Gly26ser analyses. Of the 21 subjects, 19 were Caucasians and 2 were of African origin. They all did not show any difference in age ($P = 0.07$), ethnicity ($P = 0.42$), BMI ($P = 0.57$), history of hypertension ($P = 0.10$), CTS ($P = 0.42$), neuropathy ($P = 0.65$), or NYHA class ($P = 0.69$) compared with 258 patients without mTTR. There was no difference in cardiac involvement between the two groups: LVWT ($P = 0.22$), NT-pro-BNP ($P = 0.85$), LVEF class ($P = 0.98$), and LGE at cardiac-MRI ($P = 0.45$).

Discussion

This is the first prospective study to determine the prevalence of mTTR in patients with increased LVWT of 'unknown origin' and classified as HCM according to the ESC guidelines.[8] The frequency of TTR mutations in this population was 5.7%, and the prevalence of mTTR associated with cardiac amyloidosis was 5.0%; all were older than 59 years old. After full adjustment among patients with LVWT, those of African origin, with neuropathy, CTS, ECG low voltage, and those who exhibited LGE at cardiac-MRI were more likely to have mTTR-FAC.

Table 2 **Baseline clinical, electrocardiographic, echocardiographic, cardiac-MRI, bone-scintigraphic, biological, and anatomopathological characteristics in mTTR patients**

	mTTR	Gender	Origin	Age at diagnosis	HT	NYHA	Neuropathy/ dysautonomia	CTS	PM/ICD	Sinus rhythm	Max LVWT	LVEF	*E/A*	Cardiac-MRI-LGE	BS VS	NT-pro-BNP or BNP*	EM biopsy	EC biopsy
1	V142I	Woman	Afr	86	+	III	−/−	−	−	−	16	40	NA	+	2	9646	NA	+(SG)
2[a]	V142I	Man	Cau	80	−	II	−/−	+	+	+	16	45	2.5	+	NA	na	NA	+(rectum)
3	V142I	Man	Afr	79	+	IV	−/−	−	−	+	18	43	5.5	NA	3	10 019	Refused	−(SG)
4	V142I	Woman	Afr	74	+	III	+/−	+	−	+	15	35	2.2	NA	3	5597	+	−(SG)
5	V142I	Man	Afr	72	+	II	−/−	−	−	+	21	51	3.2	+	3	2049	NA	+(SG)
6	V142I	Man	Afr	70	−	II	−/−	+	−	+	20	38	3.5	+	3	2814	+	−(SG)
7	V142I	Man	Cau	69	+	II	+/−	+	−	−	24	49	NA	NA	2	10 775	NA	−(SG/gut/nerve)
8[a]	V50M	Man	Cau	76	−	II	+/+	−	+	−	18	59	NA	+	2	3945	NA	+(SG)
9	V50M	Man	Afr	75	+	III	+/−	+	−	+	18	51	0.6	NA	2	506	NA	+(SG)
10[a]	I127V	Man	Afr	74	−	III	+/−	−	−	+	25	20	2.9	+	NA	9900	NA	+(SG and nerve)
11	I127V	Man	Afr	67	+	II	+/−	−	−	+	16	60	2.2	+	3	198*	NA	+(SG)
12	S97Y	Woman	Cau	80	−	III	+/−	+	+	+	20	45	NA	NA(PM)	2	3290	NA	+(SG)
13	I88L	Man	Cau	79	+	III	−/−	−	−	+	21	30	NA	+	NA	6210	NA	+(SG)
14[a]	A39D	Woman	Cau	68	−	III	+/−	−	−	−	18	35	NA	+	2	6016	NA	+(rectum)
15	T69I	Man	Cau	63	−	I	−/−	−	−	+	18	60	1.3	+	2	673	NA	+(SG)
16	V142I	Man	Afr	59	+	I	−/−	−	−	+	17	53	1.4	−	0	787	−	−(SG)
17	R123H	Man	Afr	69	+	III	+/−	−	−	+	16	50	0.8	+	0	3000	Refused	−(SG/nerve/skin)

Positive biopsy means positive Congo-red staining and positive TTR staining using anti-transthyretin antibodies.
mTTR, mutated transthyretine; Afr, African; Cau, Caucasian; CST, Carpal tunnel syndrome; PM, pacemaker; ICD, defibrillator; LVWT, left ventricular wall thickness; *E/A*, ratio of peak transmitral velocities; BSVS, bone scintigraphy visual score; EM, endomyocardial; EC, extra-cardiac; SG, salivary gland biopsy; NA, not available.
[a]Patients who died before the end of the study.
*= BNP, without *=NT-pro-BNP, This is very important as the values between BNP and NT-pro-BNP are different.

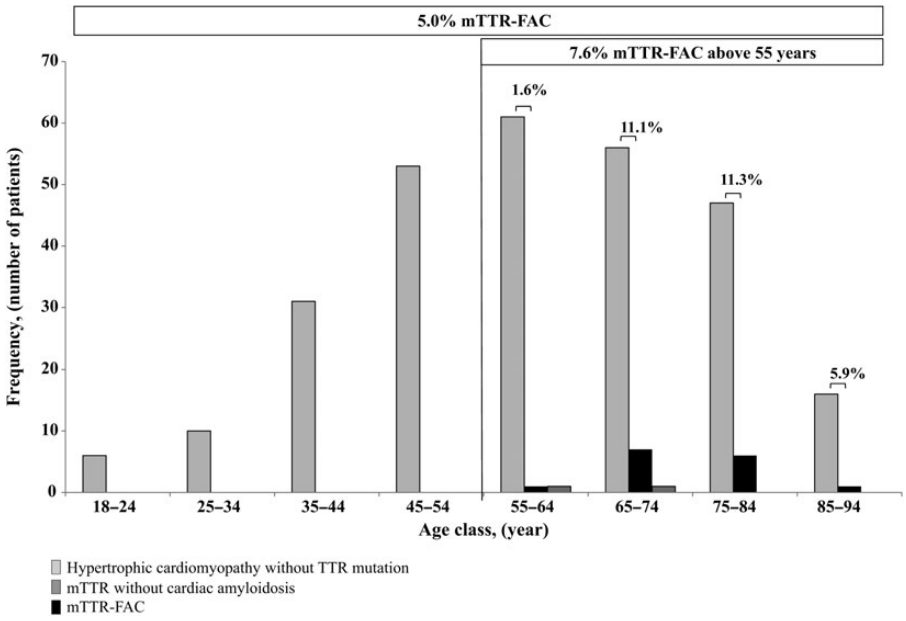


Figure 1 Prevalence of mutated transthyretin-related familial amyloid cardiomyopathy in patients with or without mutated transthyretin ($N = 296$) depending on class of age.

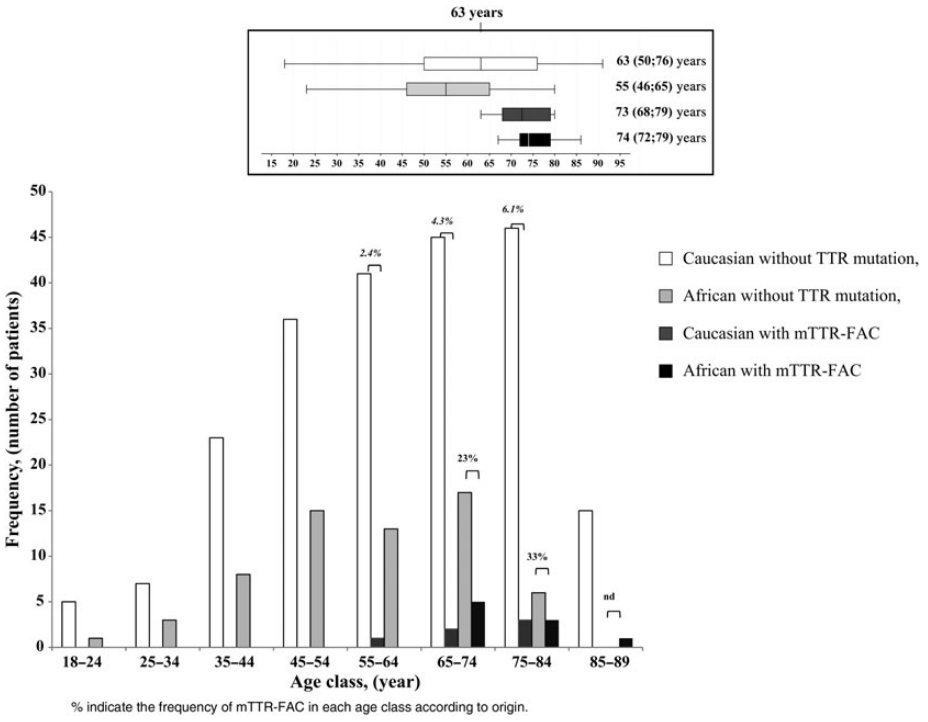


Figure 2 Prevalence of mutated transthyretin-related familial amyloid cardiomyopathy in patients with or without mutated transthyretin ($N = 283$) depending on class of age and ethnic origins. On the top, plot box of TTR genotype and FAC status distribution according to age. Percentage indicates the frequency of mutated transthyretin-related familial amyloid cardiomyopathy in each class according to origin.

The ESC guidelines[8] suggest that inherited metabolic and neuromuscular diseases, chromosome abnormalities, and genetic syndromes including hereditary TTR amyloidosis account for 5–10% of adult HCM cases. In our population, mTTR alone accounted for 5%. This means that mTTR frequency is clearly underestimated. Several factors contribute to this phenomenon. First, most of the

Table 3 Factors associated with cardiac amyloidosis in uni- and multivariate analysis in 272 patients

Variables	Crude OR	95% CI	*P*-value	Age-adjusted OR	95% CI	*P*-value	Full-adjusted OR	95% CI	*P*-value
Age ≥73 vs. <73 years	2.70	0.88; 8.29	0.08	–	–	–	2.40	0.65; 8.93	0.19
BMI (for 1 kg/m^2 increase)	0.96	0.84; 1.11	0.60	–	–	–	–	–	–
African vs. other origins	5.29	1.69; 16.6	<0.01	7.02	2.10; 23.5	0.002	5.76	1.59; 20.8	0.008
Dysautnonomia vs. without	7.98	1.14; 56.0	0.04	5.21	0.68; 40.1	0.11	3.23	0.35; 29.7	0.3
Carpal tunnel syndrome vs. without	15.91	4.56; 55.5	<0.001	13.07	3.54; 48.3	<0.001	10.49	2.65; 41.6	<0.001
Neuropathy vs. without	21.27	5.73; 55.5	<0.001	18.54	4.97; 69.1	<0.001	12.52	2.83; 55.3	<0.001
Electric LVH vs. without	0.40	0.10; 1.64	0.20	0.48	0.11; 2.01	0.31	–	–	–
ECG low voltage vs. without	9.00	2.55; 31.7	<0.001	8.17	2.29; 29.1	0.001	6.82	1.67; 27.9	0.008
AF or flutter vs. without	2.37	0.74; 7.60	0.15	1.95	0.56; 6.76	0.29	–	–	–
LA diameter (for 1 mm increase)	1.04	0.98; 1.09	0.17	1.03	0.98; 1.09	0.26	–	–	–
Symmetric vs. asymmetric LVH	10.24	1.83; 57.2	<0.01	9.17	1.63; 51.8	0.012	3.68	0.58; 23.5	0.17
LVEF (class) ≤40% vs. >40%	5.75	1.84; 18.0	<0.01	5.04	158; 16.0	0.006	2.78	0.73; 10.6	0.13
E/*A*, <1	0.77	0.08; 7.66	0.067	0.78	0.08; 7.71	0.097	0.49	0.04; 6.18	0.52
1–2	1.00 (ref)	–		1.00 (ref)	–		1.00 (ref)	–	
>2	4.91	0.77; 31.4		4.56	0.70; 29.9		4.56	0.19; 19.7	
LGE vs. without	47.4	2.70; 8.31	<0.01	46.35	2.55; 8.44[a]	0.011	27.75	1.43; 5.38[a]	0.028

Firth's logistic regression models performed with 272 patients (260 without mTTR and 12 with mTTR and cardiac amyloidosis; 24 missing data on age, ethnic origin, ECG low voltage, or carpal tunnel syndrome): first model provided crude OR, second adjusted for age, and third adjusted for age, ethnic origin, ECG low voltage, and carpal tunnel syndrome. OR, odds ratio, 95% CI, 95% confidence interval.

[a]The high value of superior threshold of 95% CI is due to the fact that there is a '0' in a box (no patient with mTTR mutation and cardiac amyloidosis had no LGE).

studies of the genetic prevalence of HCM showing a high frequency of sarcomeric genes mutations were performed in children or young adults.[13–15] In contrast to our study, all the patients identified with *TTR* mutations were older than 59 years. Second, it is possible that patients with mTTR were not referred to specialized HCM centres because of their older age. Third, most HCM prevalence studies only analysed sarcomeric gene mutations.[13,14]

We showed that several clinical variables were associated with mTTR-FAC. Patients with mTTR-FAC were all older than 63 years and frequently of African descent. It has been reported that in African Americans over age 65, TTR c.(V142I) was associated with a higher incidence of congestive heart failure, more echocardiographic features of cardiac amyloidosis, and a greater risk of death.[16] A similar increased frequency with age was also seen at autopsy.[5] In our study, all TTR c.(V142I) patients with CA were older than 69 years old. This suggests that TTR genetic testing should be systematically performed in patients of African descent older than 65 years old showing increase in LVWT.

The association of age ≥73 years with mTTR-FAC disappeared when CST was introduced in the adjustment, suggesting that CTS is a confounder in the relation between age and mTTR-FAC. This is due to the fact that CTS is another clinical manifestation of the accumulation of amyloid fibril infiltration. In our cohort, CTS occurred in 46% of the mTTR-FAC patients (vs. 4% in patients without mTTR). Similarly, neuropathy was observed in 53% of the mTTR-FAC patients. Hence, the combination of CTS and/or neuropathy in patients with increased LV thickness strongly suggests the presence of mTTR-FAC.

Interestingly, cardiac phenotypes differed between patients with mTTR-FAC and patients with no TTR mutation. Cardiac TTR deposition causes a restrictive cardiomyopathy that differs from most other HCM. ECG low voltage is relatively specific in cardiac amyloidosis. However, its presence in only one-third of the mTTR-FAC patients reflects its lack of sensitivity.[17] Its absence certainly does not rule out the diagnosis of amyloidosis. The restrictive physiology of mTTR-FAC was clearly demonstrated by a median *E*/*A* ratio above 2.5, while only one-fourth of the non-amyloid group showed *E*/*A*>2. The pattern of LV hypertrophy might be also an indicator of cardiac amyloidosis. We found only one mTTR-FAC subject with an asymmetric pattern.

Our study identified 7% of subjects carrying the Gly26Ser variant, and no differences in medical history, clinical signs, or echocardiographic variables were observed between the patients with this polymorphism and those without a TTR mutation. Gly26Ser is considered a non-pathogenic variant. However, there are few studies focusing on this polymorphism.[12,18,19] The effect of this variant and how it may affect amyloid deposition is still unclear. Three patients of African origin have this polymorphism of whom one had also the TTR c.(V142I) mutation and showed similar severity of the amyloidotic cardiopathy.

The mTTR-FAC patients also had a reduced ejection fraction (47% with LVEF < 40%) more frequently than patients without mTTR. A previous report of cardiac findings in Italian patients with mTTR (without V142I) found an LVEF < 40% in only 8% of subjects.[20] This discrepancy might reflect the fact that their population was enriched for subjects identified as relatives of known allele

carriers prior to the development of symptoms while our patients were selected for thick LVWT that suggests disease of longer standing. An analysis of TTR V142I carriers referred for ultrasonography and identified by genotyping suggested that such screening identified carriers earlier in the course of disease with ventricular walls that were not as thick and few patients with reduced LVEF.[16] Our data are in accordance with a recent study focusing on V142I allele carriers referred to an amyloid centre in which the mean ejection fraction was 25% and the patients appeared to have longer standing more severe disease as is commonly the case in such referred patients.[21] It is also likely that our patient population, being classified as HCM, hence all having LVWT, were actually selected for late-stage disease by the clinical setting in which they were identified.

Clinical implications

From a clinical and public health perspective, the knowledge that 5% of the HCM is due to mTTR suggests that molecular screening for this gene should be included in genetic screens of possible HCM subjects, particularly in the older age group. The data also suggest that the HCM phenotype appearing in subjects with mTTR is associated with the presence of late-stage disease. Indeed, the clinical management of mTTR-FAC differs from people with other HCM-causing mutations. Furthermore, survival of patients with mTTR-FAC is lower than that observed with sarcomeric mutations. Even, more importantly, specific therapies for mTTR-FAC are now in clinical trials and might be effective if administered early enough.[22,23]

Limitations

Our study design had several limitations. This was a national French study. Thus, extrapolating results to other countries could be difficult. The prevalence was established in a study population without age limitation. A higher prevalence should be expected in older patients. In addition, some patients with cardiac amyloidosis due to other causes such as WT-TTR might have been missed by our strategy limited to genetic testing. Thus, additional prevalence studies using other approaches such as bisphosphonate scintigraphy and/or systematic cardiac biopsy might be even more fruitful.

TTR c.(R123H) mutation has been identified in one patient and was suspected as pathogenic. To our knowledge, this is the first report of this mutation (http://amyloidosismutations.com/). Another mutation in the same position, TTR c.(A123S), has been reported as amyloidogenic.[24] We were not able to definitively demonstrate that this patient had mTTR-FAC; hence, we excluded him from the analysis. This may have decreased the prevalence of mTTR-FAC. Genetic analysis was limited to mTTR; therefore, we cannot exclude the possibility that a combination of mutations in genes other than *TTR* contributed to clinical phenotypes in these patients. Despite these limitations, our study provides the first estimate of the population prevalence of an HCM phenotype related to m-TTR diagnosed by genetic testing and confirmed by clinical and histopathological criteria.

Conclusion

The high detection rate of TTR gene mutations in subjects with phenotypic HCM requires that it be considered in any assessment of cardiac HCM, particularly in elderly subjects of European or African descent with unexplained increased LVWT associated with neuropathy or CTS. Mutated transthyretin screening may allow identification of patients with cardiac amyloidosis who may benefit from appropriate care and specific treatment.

Funding

This research was sponsored by the French Society of Cardiology. Institutional grant from Pfizer.

Conflict of interest: T.D., V.P.-B., and M.S. declare received research grants from Pfizer.

References

1. Elliott P, Andersson B, Arbustini E, Bilinska Z, Cecchi F, Charron P, Dubourg O, Kuhl U, Maisch B, McKenna WJ, Monserrat L, Pankuweit S, Rapezzi C, Seferovic P, Tavazzi L, Keren A. Classification of the cardiomyopathies: a position statement from the European Society Of Cardiology Working Group on Myocardial and Pericardial Diseases. *Eur Heart J* 2008;**29**:270–276.
2. Maron BJ, Maron MS, Semsarian C. Genetics of hypertrophic cardiomyopathy after 20 years: clinical perspectives. *J Am Coll Cardiol* 2012;**60**:705–715.
3. Merlini G, Bellotti V. Molecular mechanisms of amyloidosis. *N Engl J Med* 2003;**349**:583–596.
4. Damy T, Deux JF, Moutereau S, Guendouz S, Mohty D, Rappeneau S, Guellich A, Hittinger L, Loric S, Lefaucheur JP, Plante-Bordeneuve V. Role of natriuretic peptide to predict cardiac abnormalities in patients with hereditary transthyretin amyloidosis. *Amyloid* 2013;**20**:212–220.
5. Jacobson DR, Pastore RD, Yaghoubian R, Kane I, Gallo G, Buck FS, Buxbaum JN. Variant-sequence transthyretin (isoleucine 122) in late-onset cardiac amyloidosis in black Americans. *N Engl J Med* 1997;**336**:466–473.
6. Falk RH. Diagnosis and management of the cardiac amyloidoses. *Circulation* 2005;**112**:2047–2060.
7. Richardson P, McKenna W, Bristow M, Maisch B, Mautner B, O'Connell J, Olsen E, Thiene G, Goodwin J, Gyarfas I, Martin I, Nordet P. Report of the 1995 World Health Organization/International Society and Federation of Cardiology Task Force on the Definition and Classification of cardiomyopathies. *Circulation* 1996;**93**:841–842.
8. Authors/Task Force Members, Elliott PM, Anastasakis A, Borger MA, Borggrefe M, Cecchi F, Charron P, Hagege AA, Lafont A, Limongelli G, Mahrholdt H, McKenna WJ, Mogensen J, Nihoyannopoulos P, Nistri S, Pieper PG, Pieske B, Rapezzi C, Rutten FH, Tillmanns C, Watkins H. 2014 ESC guidelines on diagnosis and management of hypertrophic cardiomyopathy: the Task Force for the Diagnosis and Management of Hypertrophic Cardiomyopathy of the European Society of Cardiology (ESC). *Eur Heart J* 2014;**35**:2733–2779.
9. Galat A, Rosso J, Guellich A, Van Der Gucht A, Rappeneau S, Bodez D, Guendouz S, Tissot CM, Hittinger L, Dubois-Randé JL, Plante-Bordeneuve V, Itti E, Meignan M, Damy T. Usefulness of 99mTc-HMDP scintigraphy for the etiologic diagnosis and prognosis of cardiac amyloidosis. *Amyloid* 2015; 1–11. [Epub ahead of print].
10. Deux JF, Damy T, Rahmouni A, Mayer J, Plante-Bordeneuve V. Noninvasive detection of cardiac involvement in patients with hereditary transthyretin associated amyloidosis using cardiac magnetic resonance imaging: a prospective study. *Amyloid* 2014;**21**:246–255.
11. Morner S, Hellman U, Suhr OB, Kazzam E, Waldenstrom A. Amyloid heart disease mimicking hypertrophic cardiomyopathy. *J Intern Med* 2005;**258**:225–230.
12. Damy T, Plante-Bordeneuve V, Dogan A. Characterization of untyped cardiac amyloidosis by mass spectrometry in a patient with Gly6Ser transthyretin polymorphism in fatal cardiogenic shock. *Arch Cardiovasc Dis* 2014;**107**:706–708.
13. Morita H, Rehm HL, Menesses A, McDonough B, Roberts AE, Kucherlapati R, Towbin JA, Seidman JG, Seidman CE. Shared genetic causes of cardiac hypertrophy in children and adults. *N Engl J Med* 2008;**358**:1899–1908.
14. Bos JM, Will ML, Gersh BJ, Kruisselbrink TM, Ommen SR, Ackerman MJ. Characterization of a phenotype-based genetic test prediction score for unrelated patients with hypertrophic cardiomyopathy. *Mayo Clin Proc* 2014;**89**:727–737.
15. Lopes LR, Zekavati A, Syrris P, Hubank M, Giambartolomei C, Dalageorgou C, Jenkins S, McKenna W, Uk10k C, Plagnol V, Elliott PM. Genetic complexity in hypertrophic cardiomyopathy revealed by high-throughput sequencing. *J Med Genet* 2013;**50**:228–239.
16. Buxbaum J, Alexander A, Koziol J, Tagoe C, Fox E, Kitzman D. Significance of the amyloidogenic transthyretin Val 122 Ile allele in African Americans in the Arteriosclerosis Risk in Communities (ARIC) and Cardiovascular Health (CHS) Studies. *Am Heart J* 2010;**159**:864–870.
17. Cyrille NB, Goldsmith J, Alvarez J, Maurer MS. Prevalence and prognostic significance of low QRS voltage among the three main types of cardiac amyloidosis. *Am J Cardiol* 2014;**114**:1089–1093.

18. Jacobson DR, Alves IL, Saraiva MJ, Thibodeau SN, Buxbaum JN. Transthyretin Ser 6 gene frequency in individuals without amyloidosis. *Hum Genet* 1995;**95**:308–312.
19. Fitch NJ, Akbari MT, Ramsden DB. An inherited non-amyloidogenic transthyretin variant, [Ser6]-TTR, with increased thyroxine-binding affinity, characterized by DNA sequencing. *J Endocrinol* 1991;**129**:309–313.
20. Rapezzi C, Merlini G, Quarta CC, Riva L, Longhi S, Leone O, Salvi F, Ciliberti P, Pastorelli F, Biagini E, Coccolo F, Cooke RM, Bacchi-Reggiani L, Sangiorgi D, Ferlini A, Cavo M, Zamagni E, Fonte ML, Palladini G, Salinaro F, Musca F, Obici L, Branzi A, Perlini S. Systemic cardiac amyloidoses: disease profiles and clinical courses of the 3 main types. *Circulation* 2009;**120**:1203–1212.
21. Givens RC, Russo C, Green P, Maurer MS. Comparison of cardiac amyloidosis due to wild-type and V122I transthyretin in older adults referred to an academic medical center. *Aging Health* 2013;**9**:229–235.
22. Damy T, Judge DP, Kristen AV, Berthet K, Li H, Aarts J. Cardiac findings and events observed in an open-label clinical trial of tafamidis in patients with non-Val30Met and non-Val122Ile hereditary transthyretin amyloidosis. *J Cardiovasc Trans Res* 2015;**8**:117–127.
23. Obici L, Merlini G. An overview of drugs currently under investigation for the treatment of transthyretin-related hereditary amyloidosis. *Exp Opin Invest Drugs* 2014;**23**:1239–1251.
24. Connors LH, Lim A, Prokaeva T, Roskens VA, Costello CE. Tabulation of human transthyretin (TTR) variants. *Amyloid* 2003;**10**:160–184.

CARDIOVASCULAR FLASHLIGHT

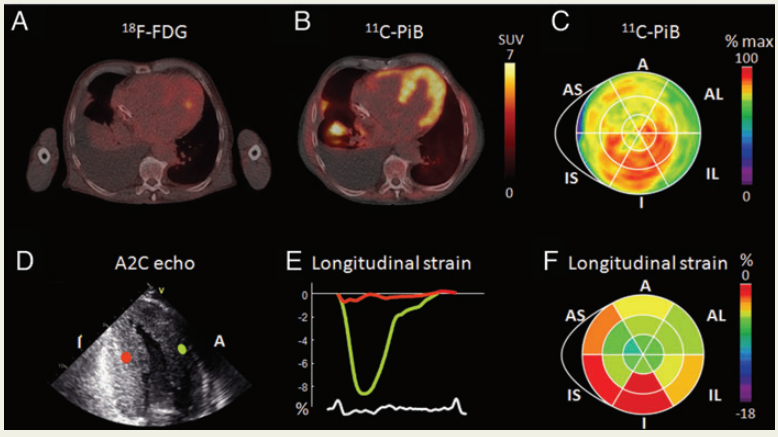
doi:10.1093/eurheartj/ehv496
Online publish-ahead-of-print 20 September 2015

Functional and molecular correlative imaging in a patient with amyloidosis

E. Pagourelias[1], M. Delforge[2], P. Claus[1], and O. Gheysens[3]*

[1]Department of Cardiovascular Diseases, University Hospital Leuven, Leuven, Belgium; [2]Department of Hematology, University Hospital Leuven, Leuven, Belgium; and [3]Department of Nuclear Medicine, University Hospital Leuven, Herestraat 49, Leuven 3000, Belgium

* Corresponding author. Tel: +32 16 343715, Fax: +32 16 343759, Email: olivier.gheysens@uzleuven.be



A 74-year-old man with a history of sarcoidosis was referred for further investigations due to relapsing heart failure symptoms. Because of a previously implanted defibrillator, cardiac work-up with echocardiography and ^{18}F-FDG PET/CT was done to evaluate cardiac function and disease activity, respectively. Echocardiography revealed left and right ventricular hypertrophy with severely impaired biventricular systolic and diastolic function, findings indicative of an infiltrative cardiomyopathy. Whole-body ^{18}F-FDG PET/CT after high-fat low-carbohydrate diet demonstrated no pathological FDG- uptake in the myocardium, except for a focal increased physiologic uptake in the papillary muscles neither evidence for extracardiac disease activity (*Panel A*).

After excluding active sarcoidosis lesions by ^{18}F-FDG PET/CT and based on the ultrasound findings suggestive for an infiltrative cardiomyopathy, endomyocardial and transbronchial biopsy were performed and diagnosis of amyloidosis was confirmed. Further analysis revealed the diagnosis of AL (kappa light chain) amyloidosis. Subsequent PET/CT imaging with ^{11}C-PiB showed a strongly increased uptake in the whole myocardium and consolidations in the right lung (*Panels B* and *C*). The ^{11}C-PiB retention index (activity concentration between 15 and 25 min after injection divided by the integral of the arterial time-activity curve between 0 and 20 min) was 0.12/min. Segmental analysis showed that segments with highest PiB-uptake corresponded well to those with the most pronounced deformation impairment, as assessed by 2D and 3D speckle tracking echocardiography (*Panels D–F*, see Supplementary material online, *movie*).

This case demonstrates that ^{11}C-PiB can be used to non-invasively assess amyloid deposits and extent of amyloid deposition correlates well with regional impairment in segmental deformation.

Supplementary material is available at *European Heart Journal* online.