ESC European Society of Cardiology

European Journal of Heart Failure (2023) **25**, 515–524
doi:10.1002/ejhf.2776

RESEARCH ARTICLE

Prevalence, characteristics and outcomes of older patients with hereditary versus wild-type transthyretin amyloid cardiomyopathy

Aldostefano Porcari[1,2,3†], Yousuf Razvi[1†], Ambra Masi[1], Rishi Patel[1], Adam Ioannou[1], Muhammad U. Rauf[1], David F. Hutt[1], Dorota Rowczenio[1], Janet Gilbertson[1], Ana Martinez-Naharro[1], Lucia Venneri[1], Carol Whelan[1], Helen Lachmann[1], Ashutosh Wechalekar[1], Candida Cristina Quarta[1], Marco Merlo[2,3], Gianfranco Sinagra[2,3], Philip N. Hawkins[1], Marianna Fontana[1‡], and Julian D. Gillmore[1‡*]

[1]National Amyloidosis Centre, Division of Medicine, University College London, London, UK; [2]Center for Diagnosis and Treatment of Cardiomyopathies, Cardiovascular Department, Azienda Sanitaria Universitaria Giuliano-Isontina (ASUGI), University of Trieste, Trieste, Italy; and [3]European Reference Network for rare, low prevalence and complex diseases of the heart (ERN GUARD-Heart)

Received 2 November 2022; revised 28 November 2022; accepted 8 January 2023; online publish-ahead-of-print 30 January 2023

Aims Transthyretin amyloid cardiomyopathy (ATTR-CM) is often assumed to be associated with wild-type *TTR* genotype (ATTRwt) in elderly patients (aged ≥70), some of whom are not offered genetic testing. We sought to estimate the prevalence, clinical characteristics and prognostic implications of transthyretin (TTR) variants among elderly patients diagnosed with ATTR-CM.

Methods and results Data from consecutive patients over 70 years of age diagnosed with ATTR-CM at the UK National Amyloidosis Centre between January 2010 and August 2022 were retrospectively evaluated. All patients underwent clinical evaluation, biochemical tests, echocardiography and *TTR* genotyping. The study outcome was all-cause mortality. The study population consisted of 2029 patients with ATTR-CM (median age 79 years at diagnosis, 13.5% females, 80.4% Caucasian). Variant ATTR-CM (ATTRv-CM) was diagnosed in 20.7% (n = 421) of the study population of whom 327 (77.7%) carried V122I, 47 (11.2%) T60A, 16 (3.8%) V30M and 31 (7.3%) other pathogenic TTR variants. During a median (range) follow-up of 29 (12–48) months, ATTRv-CM was associated with increased all-cause mortality compared to ATTRwt-CM, with the poorest survival observed in V122I-associated ATTRv-CM ($p < 0.001$). Univariable and multivariable logistic regression analyses in those with ATTR-CM showed younger age at diagnosis (odds ratio [OR] 0.85 per year, $p < 0.001$), female sex (OR 2.73, $p < 0.001$), Afro-Caribbean ethnicity (OR 65.5, $p < 0.001$), atrial fibrillation (OR 0.65, $p = 0.015$), ischaemic heart disease (OR 0.54, $p = 0.007$), peripheral polyneuropathy (OR 5.70, $p < 0.001$) and orthostatic hypotension (OR 6.29, $p < 0.001$) to be independently associated with ATTRv-CM.

Conclusion Up to 20.7% of elderly patients with ATTR-CM have a pathogenic TTR variant. These findings support routine sequencing of the *TTR* gene in all patients with ATTR-CM regardless of age.

Keywords Amyloid cardiomyopathy • Elderly • Epidemiology • Hereditary transthyretin amyloidosis • Transthyretin genetic testing

*Corresponding author. National Amyloidosis Centre, Division of Medicine, University College London, Rowland Hill St, London NW1 2PF, UK. Tel: +44 20 74332764, Fax: +44 20 44332817, Email: j.gillmore@ucl.ac.uk
†Contributed equally as joint first authors.
‡Contributed equally as joint last authors.

Introduction

Transthyretin amyloid cardiomyopathy (ATTR-CM) is a life-threatening disease caused by progressive extracellular deposition of misfolded or cleaved transthyretin (TTR) protein.[1,2] The condition results from age-related failure of homoeostatic mechanisms in wild-type ATTR amyloidosis (ATTRwt; non-hereditary form) or destabilizing mutations in variant ATTR amyloidosis (ATTRv; hereditary form).[3] In recent years, the recognition of ATTR-CM has increased exponentially.[4,5] Major advances in imaging including echocardiography, scintigraphy with bone tracers and cardiac magnetic resonance (CMR) have significantly transformed the diagnosis of ATTR-CM such that 70% patients are now diagnosed without recourse to histological demonstration of amyloid[6–8] and coupled with development of effective treatments,[9] have shifted ATTR-CM from a rare and untreatable disease to a relatively prevalent condition that clinicians should consider on a daily basis.[2] ATTR amyloidosis is associated with a diverse clinical phenotype[10,11] which is, in part, related to geographical location; for example, the proportion of those carrying the V122I TTR variant resulting in a predominant cardiac phenotype is higher in the United States (US) than in Europe.[12]

Diagnosis of ATTRv amyloidosis has major implications for clinical management, enabling initiation of specific disease-modifying drugs in the presence of amyloid polyneuropathy, and encouraging genetic counselling and predictive testing in family members of affected individuals.[13] Sequencing of the *TTR* gene is recommended in all adult patients with ATTR amyloidosis to achieve an aetiological diagnosis, and to differentiate between ATTRwt and ATTRv amyloidosis.[14,15] In everyday clinical practice however, *TTR* sequencing is not systematically performed, particularly in elderly individuals with ATTR-CM who may be assumed to have ATTRwt amyloidosis.[16] The prevalence, clinical characteristics and possible prognostic implications of ATTRv-CM in the elderly remain largely unexplored to date.

The aim of this study was to establish the prevalence of ATTRv-CM among elderly patients (≥70 years) diagnosed with ATTR-CM in the United Kingdom (UK), to define predictors of ATTRv-CM and to evaluate the clinical implications of a diagnosis of ATTRv amyloidosis.

Methods

The National Amyloidosis Centre (NAC) in London is centrally commissioned as the single centre in the UK for diagnosis, monitoring and treatment of amyloidosis. Uniquely therefore, patients seen at the NAC are likely to represent the national caseload of patients with ATTR-CM. Patients were managed in accordance with the Declaration of Helsinki. Study approval was from the Royal Free Hospital ethics committee (ref: 06/Q0501/42).

Study population

This was a retrospective, observational study of consecutive patients referred to the NAC, between January 2010 and August 2022 in whom ATTR-CM was established on the basis of imaging or histological characteristics as detailed below.[6,14] Imaging characteristics were defined as presence of a characteristic echocardiogram and/or CMR scan and either: Perugini grade 2 or 3 cardiac uptake on 99m-technetium labelled 3,3-diphosphono-1,2-propanodicarboxylic acid (^{99m}Tc-DPD) scintigraphy in the absence of an abnormal serum free light chain ratio together with absence of a monoclonal immunoglobulin in the serum or urine by immunofixation; or direct endomyocardial biopsy proof of ATTR amyloid; or presence of ATTR amyloid in an extra-cardiac biopsy along with cardiac uptake on ^{99m}Tc-DPD scintigraphy. All patients underwent sequencing of the *TTR* gene. Age ≥70 years at diagnosis was used to define the elderly cohort as (i) it was the median age of symptom onset in the Transthyretin Amyloid Outcome Survey (THAOS) registry,[17] and (ii) nowadays, the majority of individuals <70 years are not considered 'elderly' from a clinical perspective.[18]

Echocardiography

All echocardiograms were reported by two independent operators blinded to the final diagnosis in accordance with current guidelines, and discrepancies were reviewed by a group of clinical experts in amyloid echocardiography within a multidisciplinary meeting.[19]

Nuclear scintigraphy

Cardiac scintigraphy was performed by intravenous administration of ~700 MBq of ^{99m}Tc-DPD with acquisition of whole-body planar images 3 h post-injection using low-energy high-resolution collimators followed by single photon emission computed tomography/computed tomography (SPECT/CT) imaging of the heart. Interpretation of all scans was undertaken at NAC by certified consultants in nuclear medicine who assigned a Perugini grade, as previously described.[20]

TTR gene sequencing in patients and relatives

All patients with ATTR-CM underwent sequencing of the *TTR* gene. DNA was extracted from whole blood and amplified by polymerase chain reaction assay, and the whole coding region of the *TTR* gene was sequenced, as previously described.[21]

Study outcomes

The outcome of the study was to assess the impact of ATTRv-CM on all-cause mortality. All mortality data were obtained from central National Health Service patient care records. Censor date was 15 September 2022. In order to remove any impact of disease-modifying therapy on survival, all patients receiving tafamidis (n = 84), inotersen (n = 10), diflunisal (n = 164) or patisiran (n = 27) and all patients enrolled into a clinical trial (n = 298) were censored at their start date.

Statistical analysis

Descriptive statistics between the study groups were calculated. Continuous variables were expressed as median with interquartile range (IQR) since data were not normally distributed according to the results of Kolmogorov–Smirnov test; categorical variables were expressed as absolute numbers and percentages. Differences between groups were evaluated using Mann–Whitney U test for continuous variables, while Chi square (χ^2) or Fisher's exact test were used for dichotomous variables. Univariable and multivariable binary logistic regression analysis

was used to investigate parameters associated with ATTRv-CM. Each variable was tested at binary logistic regression analysis for the presence of ATTRv-CM and those significant at $p < 0.1$ were included in a multivariable binary logistic regression analysis. Patient survival was estimated by Kaplan–Meier analysis and comparisons were by log rank test. Univariable and multivariable Cox regression analysis was used to investigate whether ATTRv-CM was independently associated with all-cause mortality. We defined a p-value <0.05 as statistically significant. All statistical analyses were performed using IBM SPSS Statistics 24.0 package (New York, NY) statistical software version 20 and R (R Foundation for Statistical Computing, Vienna, Austria; https://www.r-project.org/), packages "timeROC".

Results

During the study period, a total of 2729 consecutive patients with suspected ATTR-CM were evaluated at NAC. A total of 700 patients were aged <70 years or did not meet inclusion criteria and were thus excluded from all analyses. The study cohort consisted of 2029 patients diagnosed with ATTR-CM at ≥70 years of age. *TTR* gene sequencing, performed in the whole study cohort, revealed a pathogenic *TTR* mutation in 421 (20.7%) patients. Among the 421 patients with ATTRv-CM, the most common TTR variant was Val122Ile ($n = 327$, 77.7%), followed by Thr60Ala ($n = 47$, 11.2%) and Val30Met ($n = 16$, 3.8%). A full list of patients with ATTRv-CM associated with other TTR variants is shown in online supplementary *Table S1*. None of the patients included in the present study had genetic testing on the basis of a family history of ATTRv amyloidosis prior to being diagnosed with ATTR-CM. The proportion of ATTR-CM patients with ATTRv-CM amyloidosis or indeed V122I-associated ATTRv-CM progressively declined throughout the study period despite a progressive increase in absolute numbers of diagnoses of ATTRv-CM reflecting the huge increase in diagnoses of ATTRwt-CM across the study period (online supplementary *Figure S1*). V122I-associated ATTRv-CM accounted for 16.1% of all patients with ATTR-CM included in the study population and 26.9% in 2010–2011, 18.6% in 2012–2013, 23% in 2014–2015, 16.8% in 2016–2017, 14.8% in 2018–2019%, and 9.5% in 2020–2022. After exclusion of 383 patients from the cohort with African or Caribbean ancestry, ATTRv-CM was diagnosed in 7.2% ($n = 119/1646$) of the remaining study cohort (online supplementary *Table S2*).

Baseline characteristics of the study population are shown in *Table 1*. Compared to patients with ATTRwt-CM, those with ATTRv-CM were slightly younger at diagnosis (77 vs. 80 years, $p < 0.001$). They were more frequently female (28% vs. 9.6%, $p < 0.001$), more frequently of Afro-Caribbean ethnicity (72.4% vs. 5%, $p < 0.001$), had higher rates of polyneuropathy (29.2% vs. 7.6%, $p < 0.001$), orthostatic hypotension (6.2% vs. 2.7%, $p = 0.001$) and lower rate of atrial fibrillation (AF) (38.5% vs. 58.9%, $p < 0.001$). Echocardiography at the time of diagnosis revealed that compared to patients with ATTRwt-CM, those with ATTRv-CM had higher E/E′ values (16.8 vs. 15, $p < 0.001$), lower LVEF (46% vs. 50%, $p < 0.001$), lower stroke volume (30 vs. 37 ml, $p < 0.001$) and worse GLS (−9.7% vs. −10.8%, $p < 0.001$).

ATTRv-CM was diagnosed across all age groups, despite a progressive decrease in the relative proportion of patients diagnosed with ATTRv-CM as opposed to ATTRwt-CM with increasing age as shown in *Figure 1*: 28.9% of those aged 70–74 years at diagnosis, 24.7% of those aged 75–79 years, 16.7% of those aged 80–84 years, 12.3% of those aged 85–89 years, and 2.2% of those aged ≥90 years. Similarly, the proportion of males diagnosed with ATTRv-CM decreased with advancing age: 21.2% of those aged 70–74 years, 22.3% of those aged 75–79 years, 13.8% of those aged 80–84 years, 10.1% of those aged 85–89 years, and 2.6% of those aged ≥90 years at diagnosis.

Clinical characterization of ATTRv-CM among elderly patients

Univariable binary logistic regression analysis identified a number of parameters associated with a final diagnosis of ATTRv-CM. On multivariable analysis, age at diagnosis (odds ratio [OR] 0.85 per year, $p < 0.001$), female sex (OR 2.73, $p < 0.001$), Afro-Caribbean ancestry (OR 65.5, $p < 0.001$), ischaemic heart disease (IHD) (OR 0.54, $p = 0.007$), peripheral polyneuropathy (any degree) (OR 5.70, $p < 0.001$), orthostatic hypotension defined as >20 mmHg (OR 6.29, $p < 0.001$) and absence of AF (any type) (OR 0.65, $p = 0.015$) were independently associated with a diagnosis of ATTRv-CM in this study population. No blood biomarker or echocardiographic parameter was associated with the presence of a TTR mutation in this cohort. A full list of parameters tested at univariable and multivariable analyses is shown in *Table 2*. After exclusion of patients with Afro-Caribbean ancestry, the same clinical features were confirmed as independent predictors of ATTRv-CM on multivariable analysis with the addition of presence of a permanent pacemaker (PPM) at diagnosis (OR 3.41, $p < 0.001$) (online supplementary *Table S3*).

Implications of ATTRv-CM diagnosis for patient management and outcome

Median follow-up from diagnosis was 29 (12–48) months. Survival analysis revealed ATTRv-CM to be associated with higher rates of all-cause mortality compared to ATTRwt-CM (54.5% vs. 36.2%, $p < 0.001$) (*Figure 2*, left). Among patients with ATTRv-CM, the poorest survival was observed in V122I-associated ATTRv-CM ($p < 0.001$) (*Figure 2*, right). Survival among patients with late-onset non-V122I ATTRv-CM was comparable to that in ATTRwt-CM. Compared to ATTRwt-CM, patients with ATTRv-CM had poorer overall survival across the full spectrum of disease severity as defined by NAC Stage (online supplementary *Figure S2*).[22] On multivariable analysis, ATTRv-CM was independently associated with all-cause mortality (hazard ratio 1.68, $p < 0.001$) (*Table 3*).

Among 141 patients diagnosed with ATTRv-CM after November 2018 when inotersen and patisiran became available in the UK, 23 (16.3%) were prescribed patisiran and 7 (4.9%) inotersen on the basis of presence of amyloid polyneuropathy. Tafamidis, which is not funded in the UK, was administered to 3 (2.1%) patients with ATTRv-CM via a compassionate access programme. A total of 17% ($n = 71/421$) of patients with ATTRv-CM fulfilled inclusion criteria for and were successfully enrolled into clinical trials of

Table 1 Baseline characteristics of the study population stratified by transthyretin genotype

Parameters	Available	All (n = 2029)	ATTRwt-CM (n = 1608)	ATTRv-CM (n = 421)	p-value
Age, years	2029	79 (76–83)	80 (76–84)	77 (74–81)	**<0.001**
Female sex	2029	13.5% (273)	9.6% (155)	28% (118)	**<0.001**
BMI, kg/m^2	2029	26 (24–28)	26 (24–28)	25 (23–28)	**0.001**
Ethnicity	2029				**<0.001**
Caucasian		80.4% (140)	94.1% (1512)	25.7% (108)	
Afro-Caribbean		18.3% (397)	5% (81)	72.4% (305)	
Asian		1% (21)	0.8% (13)	1.4% (6)	
Other		0.3% (6)	0.1% (2)	0.5% (2)	
IHD	2029	22.2% (451)	24.5% (394)	13.5% (57)	**<0.001**
Diabetes mellitus	2029	17.4% (353)	15.9% (256)	23% (97)	**0.001**
Hypertension	2029	40.2% (815)	37.7% (607)	48.4% (208)	**<0.001**
Stroke/TIA	2029	11.2% (227)	11.3% (182)	10.7% (45)	0.71
Atrial fibrillation	2029	54.7% (1109)	58.9% (947)	38.5% (162)	**<0.001**
PPM	2029	11.4% (232)	11.4% (183)	11.6% (49)	0.88
Polyneuropathy, any degree	2029	12.1% (246)	7.6% (123)	29.2% (123)	**<0.001**
OH >20 mmHg	2029	70 (3.4)	2.7% (44)	6.2% (26)	**0.001**
SBP, mmHg	2029	125 (114–139)	125 (115–139)	125 (112–138)	0.107
ALP, IU/L	2009	95 (74–129)	96 (75–129)	93 (71–129)	0.21
GGT, IU/L	2016	74 (34–153)	71(34–145)	88 (37–186)	**0.003**
eGFR, ml/min/1.73 m^2	2018	58 (45–70)	58 (46–71)	56 (42–68)	**0.019**
NT-proBNP, pg/L	2021	3053 (1658–5411)	3037 (1638–5403)	2851 (1488–5511)	0.47
NAC ATTR Stage	2018				0.201
I		44.4% (897)	44.4% (710)	44.6% (187)	
II		37.2% (751)	38% (607)	34.4% (144)	
III		18.3% (370)	17.6% (282)	21% (88)	
IVS, mm	2029	17 (15–18)	17 (15–18)	16 (15–18)	0.068
RWT	1762	0.76 (0.65–0.88)	0.76 (0.65–0.88)	0.79 (0.68–0.91)	**0.015**
E/E′	2029	15 (12–20)	15 (11.6–19)	16.8 (13–21)	**<0.001**
LVEF, %	2029	49 (41–55)	50 (42–56)	46 (36–55)	**<0.001**
SV, ml	1725	34 (27–46)	37 (29–47)	30 (23–37)	**<0.001**
GLS, %	1862	−10.5 (−13.1 to −8.3)	−10.8 (−13.4 to −8.5)	−9.7 (−12.2 to −7.5)	**<0.001**

ALP, alkaline phosphatase; ATTR, transthyretin amyloidosis; ATTRv-CM, variant transthyretin amyloid cardiomyopathy; ATTRwt-CM, wild-type transthyretin amyloid cardiomyopathy; eGFR, estimated glomerular filtration rate; GGT, gamma-gutamyl transpeptidase; GLS, global longitudinal strain; IHD, ischaemic heart disease, LVEF, left ventricular ejection fraction; NAC, National Amyloidosis Centre; NT-proBNP, N-terminal pro-brain natriuretic peptide; OH, orthostatic hypotension; OR, odds ratio; PPM, permanent pacemaker; RWT, relative wall thickness, SBP, systolic blood pressure; SV, stroke volume; TIA, transient ischaemic attack.

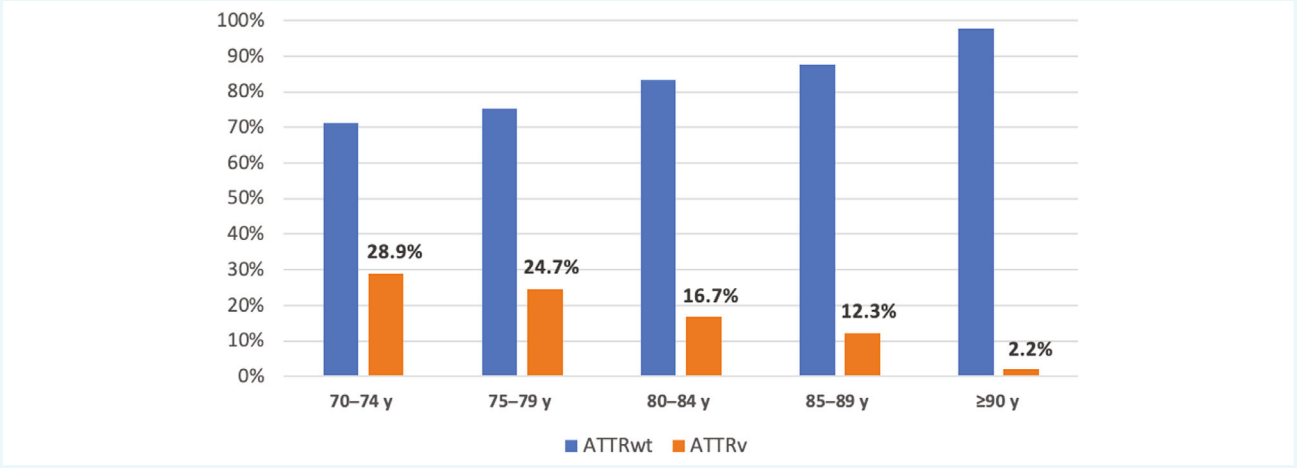

Figure 1 Trends in diagnosis of variant transthyretin amyloid cardiomyopathy (ATTRv-CM) according to age ranges at diagnosis. ATTRwt-CM, wild-type transthyretin amyloid cardiomyopathy; y, years.

Table 2 Univariable and multivariable binary logistic regression analysis for predictors of variant transthyretin amyloid cardiomyopathy in patients with a diagnosis of transthyretin amyloid cardiomyopathy

Variables	Univariable		Multivariable	
	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value
Age, per year	0.90 (0.89–0.93)	**<0.001**	0.85 (0.82–0.89)	**<0.001**
Female sex	3.66 (2.81–4.78)	**<0.001**	2.73 (1.76–4.23)	**<0.001**
Afro-Caribbean ancestries	47.35 (35.08–63.9)	**<0.001**	65.5 (43.48–98.59)	**<0.001**
SBP, per mmHg	0.99 (0.99–1.002)	0.25		
IHD	0.48 (0.35–0.64)	**<0.001**	0.54 (0.34–0.84)	**0.007**
Diabetes mellitus	1.60 (1.23–2.06)	**<0.001**	0.85 (0.55–1.33)	0.48
Hypertension	1.50 (1.21–1.85)	**<0.001**	0.84 (0.59–1.21)	0.35
Atrial fibrillation	0.44 (0.35–0.54)	**<0.001**	0.65 (0.46–0.91)	**0.015**
OH >20 mmHg	2.42 (1.51–3.88)	**<0.001**	6.29 (2.98–13.27)	**<0.001**
Polyneuropathy, any degree	4.90 (3.74–6.43)	**<0.001**	5.70 (3.71–8.74)	**<0.001**
Stroke/TIA	0.95 (0.67–1.33)	0.76		
PPM	1.03 (0.74–1.44)	0.83		
CRT	0.17 (0.04–0.72)	**0.016**	0.40 (0.09–1.80)	0.23
eGFR, per ml/min	0.99 (0.98–0.99)	**0.010**	0.99 (0.98–0.99)	0.87
NT-proBNP, per ng/L	1.00 (1.00–1.00)	0.14		
NAC ATTR stage	1.06 (0.92–1.22)	0.37		
ALP, per IU/L	1.00 (0.99–1.001)	0.62		
GGT, per IU/L	1.001 (1.00–1.002)	**0.002**	1.00 (0.99–1.001)	0.68
IVS, per mm	0.98 (0.94–1.02)	0.43		
LVEF, per %	0.96 (0.95–0.97)	**<0.001**	0.99 (0.94–1.008)	0.22
TAPSE, mm	0.98 (0.96–1.007)	0.16		
RV S′, cm/s	1.02 (0.99–1.04)	0.16		
LV-GLS, per %	1.08 (1.05–1.11)	**<0.001**	1.005 (0.94–1.06)	0.87

ALP, alkaline phosphatase; ATTR, Transthyretin amyloidosis; CI, confidence interval; eGFR, estimated glomerular filtration rate; GGT, gamma-gutamyl transpeptidase; GLS, global longitudinal strain; IHD, ischemic heart disease; IVS, interventricular septum; LV, left ventricle; LVEF, left ventricular ejection fraction; NAC, National Amyloidosis Centre; NT-proBNP, N-terminal pro-brain natriuretic peptide; OH, orthostatic hypotension; OR, odds ratio; PPM, permanent pacemaker; RV, right ventricle; SBP, systolic blood pressure; TAPSE, tricuspid annular plane systolic excursion; TIA, transient ischaemic attack.

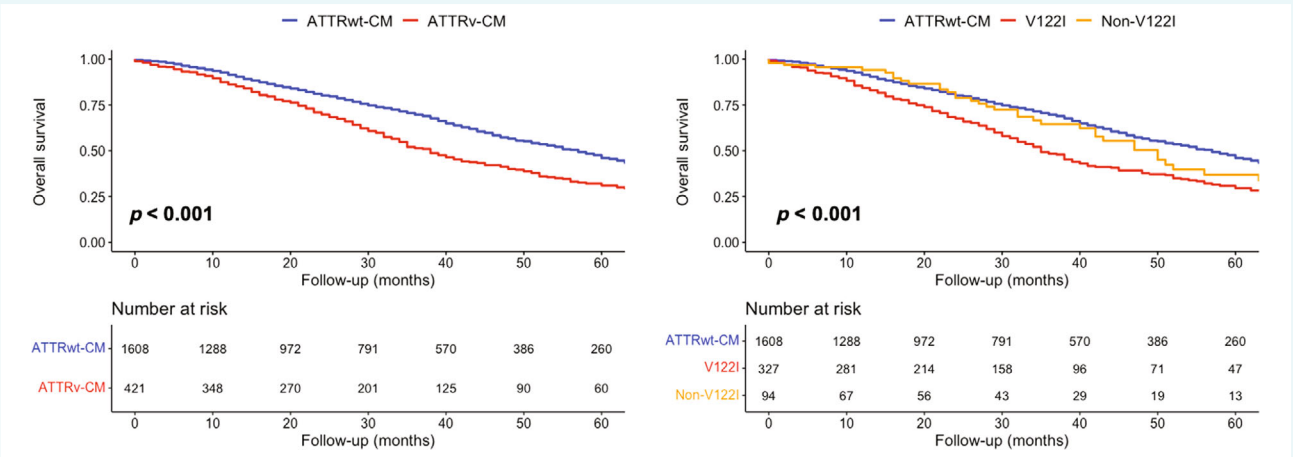

Figure 2 Prognostic impact of variant transthyretin amyloid cardiomyopathy (ATTRv-CM) and specific transthyretin variant on overall survival compared to wild-type transthyretin amyloid cardiomyopathy (ATTRwt-CM). V122I, valine-to-isoleucine substitution at position 122.

Table 3 Univariable and multivariable Cox regression analysis for predictors of all-cause mortality

Variables	Univariable		Multivariable	
	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value
Age, per year	1.018 (1.004–1.03)	**0.010**	1.016 (1.001–1.03)	**0.03**
Afro-Caribbean Ancestries	1.37 (1.18–1.60)	**<0.001**	1.16 (1.04–1.37)	0.06
Female sex	0.94 (0.77–1.14)	0.54		
ATTRv-CM	1.56 (1.34–1.81)	**<0.001**	1.68 (1.31–2.16)	**<0.001**
SBP, per mmHg	0.98 (0.98–0.99)	**<0.001**	0.99 (0.98–0.99)	**<0.001**
Hypertension	0.92 (0.81–1.06)	0.28		
OH >20 mmHg	1.08 (0.76–1.53)	0.66		
IHD	1.12 (0.96–1.31)	0.15		
Polyneuropathy	1.21 (0.99–1.48)	0.055	1.08 (0.87–1.33)	0.48
Diabetes mellitus	1.2 (1.01–1.42)	**0.036**	1.1 (0.91–1.32)	0.32
Atrial fibrillation	1.06 (0.93–1.21)	0.41		
Stroke/TIA	0.84 (0.67–1.04)	0.11		
PPM	1.18 (0.97–1.44)	0.096	1.07 (0.86–1.32)	0.53
eGFR, per ml/min	0.97 (0.97–0.98)	**<0.001**	0.99 (0.98–0.99)	**<0.001**
NT-proBNP, per ng/L	1.00 (1.00–1.00)	**<0.001**	1.00 (1.00–1.00)	**<0.001**
IVS, per mm	1.09 (1.06–1.11)	**<0.001**	1.04 (1.006–1.07)	**0.03**
LVEF, per %	0.97 (0.96–0.98)	**<0.001**	0.99 (0.98–1.001)	0.82
LV-GLS, per %	1.09 (1.07–1.11)	**<0.001**	1.03 (1.003–1.05)	**0.03**

ATTRv-CM, variant transthyretin amyloid cardiomyopathy; CI, confidence interval; eGFR, estimated glomerular filtration rate; GLS, global longitudinal strain; HR, hazard ratio; IHD, ischaemic heart disease; IVS, interventricular septum; LV, left ventricle; LVEF, left ventricular ejection fraction; NT-proBNP, N-terminal pro-brain natriuretic peptide; OH, orthostatic hypotension; PPM, permanent pacemaker; SBP, systolic blood pressure; TIA, transient ischaemic attack.

disease-modifying therapy for ATTR amyloidosis: 1 in APOLLO, 8 in APOLLO-B, 10 in ATTRibute-CM, 15 in HELIOS-A, 13 in HELIOS-B, 22 in Cardio-TTRansform, and 2 in ITL-2001-CL-001.

When categorized by genotype, the population with the greatest proportion of patients in clinical trials were ATTRwt-CM, followed by V122I, V30M and T60A (20.2% vs. 9.6% vs. 9.2% vs. 7.3%). The proportion of patients on gene silencing disease-modifying therapy according to genotype were V30M (57%), T60A (36.8%), V122I (1.8%) and ATTRwt (0%; gene silencing therapies are not licensed for ATTRwt).

Discussion

A total of 2029 UK patients were included in the present study. To the best of our knowledge, this is the first nationwide analysis to investigate the prevalence, characterize the clinical features, and determine the prognosis of ATTRv-CM in elderly patients. Our main findings can be summarized as follows: (i) up to 20.7% patients ≥70 years who are diagnosed with ATTR-CM have ATTRv-CM; (ii) among patients with ATTR-CM, younger age at diagnosis, female gender, Afro-Caribbean ethnicity, AF, IHD, peripheral polyneuropathy and orthostatic hypotension are independently associated with ATTRv-CM; (iii) among elderly patients, ATTRv-CM is associated with poorer survival than ATTRwt-CM, particularly in patients who carry the V122I TTR variant. Given that one out of five elderly patients diagnosed with ATTR-CM in our cohort carried a pathogenic TTR mutation, these data argue strongly for sequencing of the *TTR* gene to be routinely undertaken in all patients diagnosed with ATTR-CM in order to distinguish between ATTRwt-CM and ATTRv-CM, the latter having major clinical implications in terms of clinical management and family screening.

In recent years, major advances in disease awareness and cardiac imaging,[23] the latter enabling accurate non-invasive diagnosis, have identified ATTR-CM to be relatively prevalent compared to the past. In most elderly patients diagnosed with ATTR-CM, the condition is assumed to be of wild-type origin (acquired) and outside specialist referral centres for amyloidosis, patients ≥70 years are not always offered *TTR* genotyping on this basis.[18] In the present study, ATTR-CM was associated with a pathogenic *TTR* mutation in 20.7% of the population although the authors acknowledge that this proportion may fall as ATTRwt-CM is more widely diagnosed. ATTRv amyloidosis was most common in the 70–74 year old sub-group of this cohort among whom nearly one third had ATTRv-CM (*Figure 1*) although the prevalence of ATTRv-CM was >10% throughout all age groups with the exception of those ≥90 years. These data indicate that *TTR* sequencing ought to be undertaken in all patients with ATTR-CM regardless of age at diagnosis. Interestingly, the clinical characteristics at diagnosis and most frequently identified TTR variants in UK patients with ATTR-CM were similar to those reported in the US according to the THAOS registry[12] (*Figure 3*). This might reflect the high population prevalence of elderly individuals of Afro-Caribbean ethnicity in UK cities, 3–4% of whom are known to carry the V122I TTR variant.[24] Of note, the THAOS registry likely underestimated the prevalence of wild-type ATTR amyloidosis.[12] A significant proportion of non-US patients – defined as 'rest of the world' – were from Portugal (more than 1000), leading to a higher prevalence of hereditary ATTR amyloidosis, predominantly associated with the V30M

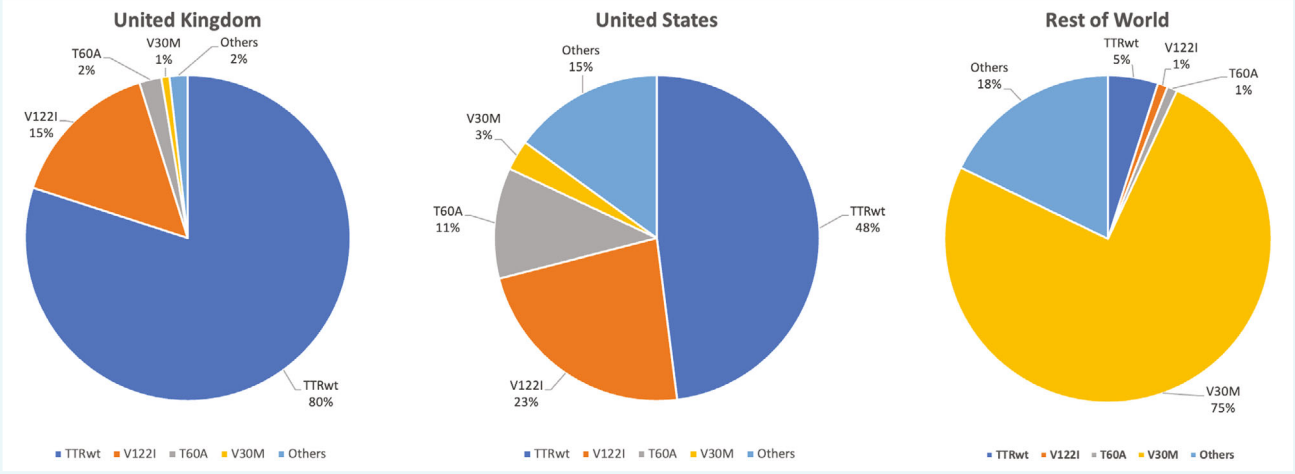

Figure 3 Distribution of transthyretin (TTR) variants in the United Kingdom, United Stated and rest of the world. ATTRv-CM, variant transthyretin amyloid cardiomyopathy; ATTRwt-CM, wild-type transthyretin amyloid cardiomyopathy; T60A, threonine-to-alanine substitution at position 60; V122I, valine-to-isoleucine substitution at position 122; V30M, valine-to-methionine substitution at position 30. Prevalence of TTR mutations in the United States and rest of the world according to the THAOS registry.[12]

(p.V50M) TTR mutation. Notably, however, even after exclusion of individuals of Afro-Caribbean ancestry, 1 in 14 patients ≥70 years diagnosed with ATTR-CM had ATTRv amyloidosis. This further highlights the need for *TTR* gene sequencing, not only in patients of all ages but also in ATTR-CM patients of all ethnicities. Importantly, absence of a family history of amyloidosis does not rule out ATTRv amyloidosis for the following reasons: the diagnosis of ATTR-CM is often missed; *TTR* gene sequencing is not routinely undertaken in patients diagnosed with ATTR-CM; the mechanisms leading to development of ATTR-CM in carriers of pathogenic TTR variants have not been fully elucidated; *TTR* mutations are associated with incomplete clinical penetrance and some *TTR* mutations are characterized by late disease onset. Thus, a significant proportion of *TTR* mutation carriers may die before manifesting a cardiac amyloid phenotype and even in those with such a phenotype, they may never be diagnosed with ATTR-CM.

In our cohort, the probability of ATTRv-CM could be estimated using easily obtainable parameters, commonly assessed during the diagnostic clinical evaluation (*Figure 4*). Firstly, advancing age was associated with progressively lower probability of ATTRv-CM. This finding could be due to the higher mortality associated with ATTRv-CM compared to ATTRwt-CM or due to the fact that ATTRwt-CM is increasingly prevalent with advancing age (*Figure 2*). Second, female gender was associated with ATTRv-CM. It is well established that ATTRwt-CM is mostly diagnosed in males,[25] although noteworthy that post-mortem series indicate presence of ATTR amyloid deposits in a higher proportion of females than that diagnosed in life suggesting that the diagnosis of ATTR-CM may be more frequently missed in females. In the present study, a diagnosis of ATTR-CM was accompanied by a pathogenic *TTR* mutation in 43.2% of females compared to 17.3% of males. Other possible explanations for the higher prevalence of TTR mutations among females diagnosed with ATTR-CM include the possibility of slower evolution of the disease phenotype in females compared to males who do not carry a TTR variant and/or that of a cardioprotective effect of oestrogens during life.[26] However, underdiagnosis of ATTRwt-CM in females is highly likely to be a significant contributor since current criteria to define cardiac hypertrophy (i.e. interventricular thickness >12 mm),[13] the usual reason for suspecting cardiac amyloidosis in the first place, do not take gender into account. Studies to establish the true prevalence of ATTR-CM, particularly ATTRwt-CM, among females are urgently required. Finally, presence of amyloid-related neuropathy, both peripheral and autonomic, was associated with ATTRv-CM. This is not surprising since most pathogenic TTR variants, including T60A and V30M (accounting for 15.5% of all patients with ATTRv-CM in the study population), are known to be associated with amyloid polyneuropathy as well as cardiomyopathy, designated a 'mixed' (cardiomyopathic and neuropathic) phenotype; ATTRwt-CM, on the other hand, is rarely associated with clinically important polyneuropathy. The T60A TTR variant is estimated to be carried by ~1% of the population from northwest Ireland. Interestingly, although the V122I variant is associated with a predominant cardiac phenotype, recent studies have demonstrated that carriers of this variant are at increased risk of polyneuropathy,[27] which frequently goes undiagnosed. Similarly, IHD is likely under-recognized in patients with ATTR-CM due to chronic elevation of serum troponin and abnormal electrocardiographic findings which may be assumed to be amyloid-related. In the present study, the higher prevalence of diabetes and hypertension found among ATTRv-CM compared to ATTRwt-CM patients was related to the increased proportion of patients of Afro-Caribbean ethnicity in the former group. Of note, IHD results from the complex interplay between risk factors for coronary artery disease and environmental factors not fully elucidated. Definition of IHD was based on clinical history of previous myocardial infarction and investigation of coronary artery disease was not systematically performed in the study population. Therefore, other factors rather than a direct protective

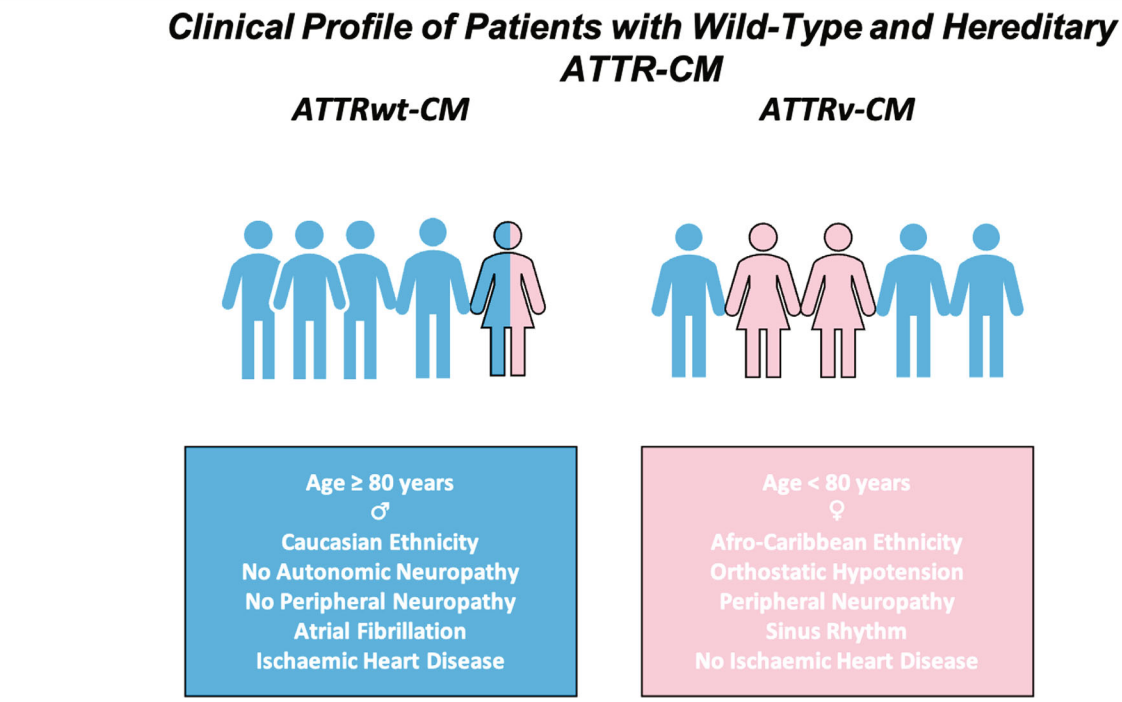

Figure 4 Profile of patients with variant transthyretin amyloid cardiomyopathy (ATTRv-CM) according to independent clinical risk factors. ATTR-CM, transthyretin amyloid cardiomyopathy; ATTRwt-CM, wild-type transthyretin amyloid cardiomyopathy.

effect of *TTR* mutations against ischaemia may have affected the association between IHD and ATTRwt-CM. Finally, AF is the most common arrhythmias in ATTR-CM and its incidence is known to increase with aging, providing an explanation for the association with ATTRwt-CM in this cohort.[13] The aforementioned independent predictors of ATTRv-CM were further confirmed in an analysis in which patients with Afro-Caribbean ancestries were excluded. The presence of a PPM at diagnosis emerged as an additional factor associated with ATTRv-CM in our cohort. PPM implantation in patients with ATTR-CM is undertaken for severe conduction disturbance (i.e. complete atrio-ventricular block), which may occur due to amyloid infiltration within the conducting system of the heart.[28] It is noteworthy, however, that the proportion of patients with V122I-associated ATTRv-CM who had PPMs was extremely low in our cohort, suggesting that the risk of conduction system disease may vary according to the specific TTR variant or that variants associated with more pronounced neuropathy, and in particular autonomic neuropathy (i.e. T60A and V30M), are associated with a higher risk of cardiac rhythm disturbance. Again, this requires further research. Awareness of the clinical features associated with presence of a TTR variant among patients diagnosed with ATTR-CM might be useful to stratify the likelihood of ATTRv amyloidosis at first clinical evaluation in the ambulatory setting, to provide more accurate information on the likelihood of diagnosing ATTRv-CM to patients who are reluctant to proceed with *TTR* gene sequencing and lastly, to enable prioritization of *TTR* gene sequencing in centres with limited resource. Our results confirm on multivariable analysis in a far larger population the findings of a single-centre study on 279 ATTR-CM patients ≥70 years[18] and further expand the range of clinical features associated with ATTRv-CM. Of note, the present study provides new evidence for the independent impact of ATTRv-CM on long-term survival along with other established independent prognostic parameters.

Identification of ATTRv-CM is associated with major implications for clinical management of patients and relatives. Among patients ≥70 years in this study, and across all NAC ATTR prognostic stages, a diagnosis of ATTRv-CM conferred a poorer survival compared to ATTRwt-CM, particularly in patients carrying the V122I TTR variant. This specific TTR variant is associated with late-onset severe ATTR-CM and has previously been shown to be associated with the poorest outcomes among all ATTRv genotypic variants.[29] Our data show that identification of the precise causative amyloid variant by *TTR* gene sequencing in ATTR-CM is prognostically relevant in elderly patients. Furthermore, it may have major implications for the management of patients given that patisiran, vutrisiran and inotersen are licensed for ATTRv amyloidosis with polyneuropathy but not ATTRwt amyloidosis. A diagnosis of ATTRv-CM ought therefore to prompt a neurology referral to assess for presence of peripheral or autonomic neuropathy, potentially enabling initiation of these 'gene silencing' disease-modifying therapies. Despite the fact that these gene silencer therapies were initially tested in trials with polyneuropathy endpoints, dedicated analyses in subgroups of patients with a mixed phenotype (in other words both polyneuropathy and cardiomyopathy), indicated a favourable impact of the therapies on echocardiographic and CMR parameters of cardiac structure and

function, such as mean left ventricular wall thickness, global longitudinal strain and extracellular volume[30,31] as well as all-cause mortality and cardiovascular hospitalizations.[31] The initial findings of the APOLLO-B trial, a placebo-controlled phase 3 clinical trial in patients with predominant ATTR-CM, including ATTRv-CM, were recently reported to show a statistically significant benefit with patisiran in comparison to placebo in the primary endpoint measure of change in exercise capacity as measured by 6-min walk test coupled with a benefit in quality of life.[32]

A diagnosis of ATTRv-CM has obvious implications for relatives of affected individuals. It enables cascade genetic screening of relatives and careful monitoring for development of amyloidosis in family members who carry the pathogenic mutation. Screening for early evidence of ATTR amyloidosis unequivocally leads to initiation of disease-modifying treatment earlier in the course of this progressive disease which has previously been shown to be associated with the most consistent benefits for patients[13] and prevents the diagnosis from being established only when patients have advanced cardiomyopathy or neuropathy which remains all too frequent among those without a known family history of ATTR amyloidosis[33] in whom signs and symptoms are often initially attributed to more common conditions.[34] Of note, there is considerable variation in the disease penetrance associated with individual pathogenic TTR variants and even within variants such that an extensive knowledge of the family history of disease along with expertise in pre-symptomatic genetic testing, means that cascade testing should be undertaken by clinical geneticists. The determinants of disease penetrance among TTR mutation carriers remain unknown and are an important area for future research.

There are a number of limitations of this study. It is a retrospective analysis conducted at a national referral centre for amyloidosis which situated in London, a large city. Whilst epidemiological data from the NAC are likely to reflect national disease epidemiology more closely than anywhere else in the world, we cannot exclude a degree of referral bias related to geographical proximity to the Centre. The geographical area of London is characterized by a multiracial and multi-ethnic population with a large Afro-Caribbean population, 3–4% of whom are likely to carry the V122I TTR variant.[24] It is likely, therefore, that the results of this study closely reflect the situation in areas of the world with multi-ethnic populations such as the US and certain parts of Europe.

In conclusion, up to 20.7% of elderly patients with ATTR-CM carry a pathogenic *TTR* mutation with a higher proportion still among specific ethnic groups. Among patients diagnosed with ATTR-CM, younger age at diagnosis, female gender, Afro-Caribbean ethnicity, AF, IHD, polyneuropathy and orthostatic hypotension are independently associated with ATTRv-CM. A diagnosis of ATTR-CM should prompt sequencing of the *TTR* gene in all patients, regardless of age, gender and ethnicity.

Supplementary Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Funding

Dr. Fontana is supported by a British Heart Foundation Intermediate Clinical Research Fellowship (FS/18/21/33447).

Conflict of interest: none declared.

References

1. Wechalekar AD, Gillmore JD, Hawkins PN. Systemic amyloidosis. *Lancet*. 2016;**387**:2641–54.
2. Martinez-Naharro A, Baksi AJ, Hawkins PN, Fontana M. Diagnostic imaging of cardiac amyloidosis. *Nat Rev Cardiol*. 2020;**17**:413–26.
3. Porcari A, Merlo M, Rapezzi C, Sinagra G. Transthyretin amyloid cardiomyopathy: an uncharted territory awaiting discovery. *Eur J Intern Med*. 2020;**82**:7–15.
4. Porcari A, Bussani R, Merlo M, Varrà GG, Pagura L, Rozze D, et al. Incidence and characterization of concealed cardiac amyloidosis among unselected elderly patients undergoing post-mortem examination. *Front Cardiovasc Med*. 2021;**8**:1680.
5. Gilstrap LG, Dominici F, Wang Y, El-Sady MS, Singh A, Di Carli MF, et al. Epidemiology of cardiac amyloidosis-associated heart failure hospitalizations among fee-for-service Medicare beneficiaries in the United States. *Circ Heart Fail*. 2019;**12**:e005407.
6. Gillmore JD, Maurer MS, Falk RH, Merlini G, Damy T, Dispenzieri A, et al. Nonbiopsy diagnosis of cardiac transthyretin amyloidosis. *Circulation*. 2016;**133**:2404–12.
7. Merlo M, Pagura L, Porcari A, Cameli M, Vergaro G, Musumeci B, et al. Unmasking the prevalence of amyloid cardiomyopathy in the real world: results from phase 2 of the AC-TIVE study, an Italian nationwide survey. *Eur J Heart Fail*. 2022;**24**:1377–86.
8. Boldrini M, Cappelli F, Chacko L, Restrepo-Cordoba MA, Lopez-Sainz A, Giannoni A, et al. Multiparametric echocardiography scores for the diagnosis of cardiac amyloidosis. *JACC Cardiovasc Imaging*. 2020;**13**:909–20.
9. Zhang KW, Stockerl-Goldstein KE, Lenihan DJ. Emerging therapeutics for the treatment of light chain and transthyretin amyloidosis. *JACC Basic Transl Sci*. 2019;**4**:438–48.
10. Cipriani A, De Michieli L, Porcari A, Licchelli L, Sinigiani G, Tini G, et al. Low QRS voltages in cardiac amyloidosis: clinical correlates and prognostic value. *JACC CardioOncol*. 2022;**4**:458–70.
11. Aimo A, Merlo M, Porcari A, Georgiopoulos G, Pagura L, Vergaro G, et al. Redefining the epidemiology of cardiac amyloidosis. A systematic review and meta-analysis of screening studies. *Eur J Heart Fail*. 2022;**24**:2342–51.
12. Maurer MS, Hanna M, Grogan M, Dispenzieri A, Witteles R, Drachman B, et al.; THAOS Investigators. Genotype and phenotype of transthyretin cardiac amyloidosis: THAOS (Transthyretin Amyloid Outcome Survey). *J Am Coll Cardiol*. 2016;**68**:161–72.
13. Porcari A, Fontana M, Gillmore JD. Transthyretin cardiac amyloidosis. *Cardiovasc Res*. 2023;**118**:3517–35.
14. Garcia-Pavia P, Rapezzi C, Adler Y, Arad M, Basso C, Brucato A, et al. Diagnosis and treatment of cardiac amyloidosis: a position statement of the ESC Working Group on Myocardial and Pericardial Diseases. *Eur Heart J*. 2021;**42**:1554–68.
15. Rapezzi C, Aimo A, Serenelli M, Barison A, Vergaro G, Passino C, et al. Critical comparison of documents from scientific societies on cardiac amyloidosis. *J Am Coll Cardiol*. 2022;**79**:1288–303.
16. Poterucha TJ, Elias P, Bokhari S, Einstein AJ, DeLuca A, Kinkhabwala M, et al. Diagnosing transthyretin cardiac amyloidosis by technetium Tc 99m pyrophosphate: a test in evolution. *JACC Cardiovasc Imaging*. 2021;**14**:1221–31.
17. Nativi-Nicolau J, Siu A, Dispenzieri A, Maurer MS, Rapezzi C, Kristen AV, et al.; THAOS Investigators. Temporal trends of wild-type transthyretin amyloid cardiomyopathy in the Transthyretin Amyloidosis Outcomes Survey. *JACC CardioOncol*. 2021;**3**:537–46.
18. Maestro-Benedicto A, Vela P, de Frutos F, Mora N, Pomares A, Gonzalez-Vioque E, et al. Frequency of hereditary transthyretin amyloidosis among elderly patients with transthyretin cardiomyopathy. *Eur J Heart Fail*. 2022;**24**:2367–73.
19. Chacko L, Karia N, Venneri L, Bandera F, Passo BD, Buonamici L, et al. Progression of echocardiographic parameters and prognosis in transthyretin cardiac amyloidosis. *Eur J Heart Fail*. 2022;**24**:1700–12.
20. Hanna M, Ruberg FL, Maurer MS, Dispenzieri A, Dorbala S, Falk RH, et al. Cardiac scintigraphy with technetium-99m-labeled bone-seeking tracers for suspected amyloidosis. *J Am Coll Cardiol*. 2020;**75**:2851–62.

21. Rowczenio DM, Noor I, Gillmore JD, Lachmann HJ, Whelan C, Hawkins PN, et al. Online registry for mutations in hereditary amyloidosis including nomenclature recommendations. *Hum Mutat*. 2014;**35**:E2403–12.
22. Gillmore JD, Damy T, Fontana M, Hutchinson M, Lachmann HJ, Martinez-Naharro A, et al. A new staging system for cardiac transthyretin amyloidosis. *Eur Heart J*. 2018;**39**:2799–806.
23. Razvi Y, Patel RK, Fontana M, Gillmore JD. Cardiac amyloidosis: a review of current imaging techniques. *Front Cardiovasc Med*. 2021;**8**:751293.
24. Quarta CC, Buxbaum JN, Shah AM, Falk RH, Claggett B, Kitzman DW, et al. The amyloidogenic V122I transthyretin variant in elderly black Americans. *N Engl J Med*. 2015;**372**:21–9.
25. Patel RK, Ioannou A, Razvi Y, Chacko L, Venneri L, Bandera F, et al. Sex differences among patients with transthyretin amyloid cardiomyopathy – from diagnosis to prognosis. *Eur J Heart Fail*. 2022;**24**:2355–63.
26. Rapezzi C, Riva L, Quarta CC, Perugini E, Salvi F, Longhi S, et al. Gender-related risk of myocardial involvement in systemic amyloidosis. *Amyloid*. 2008;**15**:40–8.
27. Parker MM, Damrauer SM, Tcheandjieu C, Erbe D, Aldinc E, Hawkins PN, et al. Association of the transthyretin variant V122I with polyneuropathy among individuals of African ancestry. *Sci Rep*. 2021;**11**:11645.
28. Porcari A, Rossi M, Cappelli F, Canepa M, Musumeci B, Cipriani A, et al. Incidence and risk factors for pacemaker implantation in light chain and transthyretin cardiac amyloidosis. *Eur J Heart Fail*. 2022;**24**:1227–36.
29. Chandrashekar P, Alhuneafat L, Mannello M, Al-Rashdan L, Kim MM, Dungu J, et al. Prevalence and outcomes of p.Val142Ile TTR amyloidosis cardiomyopathy: a systematic review. *Circ Genomic Precis Med*. 2021;**14**:e003356.
30. Fontana M, Martinez-Naharro A, Chacko L, Rowczenio D, Gilbertson JA, Whelan CJ, et al. Reduction in CMR derived extracellular volume with patisiran indicates cardiac amyloid regression. *JACC Cardiovasc Imaging*. 2021;**14**:189–99.
31. Solomon SD, Adams D, Kristen A, Grogan M, González-Duarte A, Maurer MS, et al. Effects of patisiran, an RNA interference therapeutic, on cardiac parameters in patients with hereditary transthyretin-mediated amyloidosis. *Circulation*. 2019;**139**:431–43.
32. Alnylam reports positive topline results from APOLLO-B phase 3 study of Patisiran in patients with ATTR amyloidosis with cardiomyopathy. August 3, 2022. https://www.businesswire.com/news/home/20220803005528/en/Alnylam-Reports-Positive-Topline-Results-from-APOLLO-B-Phase-3-Study-of-Patisiran-in-Patients-with-ATTR-Amyloidosis-with-Cardiomyopathy (last accessed 20 January 2023).
33. Lane T, Fontana M, Martinez-Naharro A, Quarta CC, Whelan CJ, Petrie A, et al. Natural history, quality of life, and outcome in cardiac transthyretin amyloidosis. *Circulation*. 2019;**140**:16–26.
34. Gillmore JD, Reilly MM, Coats CJ, Cooper R, Cox H, Coyne MRE, et al. Clinical and genetic evaluation of people with or at risk of hereditary ATTR amyloidosis: an expert opinion and consensus on best practice in Ireland and the UK. *Adv Ther*. 2022;**39**:2292–301.