European Heart Journal (2013) **34**, 520–528
doi:10.1093/eurheartj/ehs123

Disease profile and differential diagnosis of hereditary transthyretin-related amyloidosis with exclusively cardiac phenotype: an Italian perspective

Claudio Rapezzi[1]*, Candida Cristina Quarta[1], Laura Obici[2], Federico Perfetto[3], Simone Longhi[1], Fabrizio Salvi[4], Elena Biagini[1], Massimiliano Lorenzini[1], Francesco Grigioni[1], Ornella Leone[5], Francesco Cappelli[3], Giovanni Palladini[2], Paola Rimessi[6], Alessandra Ferlini[6], Giorgio Arpesella[7], Antonio Daniele Pinna[8], Giampaolo Merlini[2], and Stefano Perlini[2]

[1]Cardiovascular Department, University of Bologna and S. Orsola-Malpighi Hospital, via Massarenti 9, 40138 Bologna, Italy; [2]Center for Amyloidosis, 'Fondazione IRCCS San Matteo' and University of Pavia, Pavia, Italy; [3]Regional Amyloid Center, Azienda Ospedaliera Universitaria Careggi, Florence, Italy; [4]Department of Neurology, Bellaria Hospital, Bologna, Italy; [5]Department of Pathology, University of Bologna and S. Orsola-Malpighi Hospital, Bologna, Italy; [6]Section of Medical Genetics, Department of Diagnostic and Experimental Medicine, University of Ferrara, Ferrara, Italy; [7]Department of Cardiac Surgery, University of Bologna and S. Orsola-Malpighi Hospital, Bologna, Italy; and [8]Department of Surgery and Transplantation, Liver and Multiorgan Transplant Unit, University of Bologna and S. Orsola-Malpighi Hospital, Bologna, Italy

Received 9 January 2012; revised 4 April 2012; accepted 17 April 2012; online publish-ahead-of-print 28 June 2012

See page 483 for the editorial comment on this article (doi:10.1093/eurheartj/ehs238)

Aims Hereditary transthyretin (TTR)-related amyloidosis (ATTR) is mainly considered a neurologic disease. We assessed the phenotypic and genotypic spectra of ATTR in a Caucasian area and evaluated the prevalence, genetic background, and disease profile of cases with an exclusively cardiac phenotype, highlighting possible hints for the differential diagnosis with hypertrophic cardiomyopathy (HCM) and senile systemic amyloidosis (SSA).

Methods and results In this Italian multicentre study, 186 patients with ATTR were characterized at presentation. Thirty patients with SSA and 30 age–gender-matched HCM patients were used for comparison. Phenotype was classified as exclusively cardiac ($n = 31$, 17%), exclusively neurologic ($n = 46$, 25%), and mixed cardiac/neurologic ($n = 109$, 58%). Among the eight different mutations responsible for an exclusively cardiac phenotype, Ile68Leu was the most frequent. Five patients with an exclusively cardiac phenotype developed mild abnormalities at neurological examination, but no symptoms during a 36-month follow-up (range: 14–50). Exclusively cardiac phenotype was characterized by male gender, age >65 years, heart failure symptoms, symmetric left ventricular (LV) 'hypertrophy', and moderately depressed LV ejection fraction. This profile was similar to SSA, but relatively distinct from HCM. Compared with patients with a mixed phenotype, patients with an exclusively cardiac phenotype showed a more pronounced cardiac involvement on both echocardiogram and electrocardiogram (ECG).

Conclusion A clinically relevant subset of Caucasian ATTR patients present with an exclusively cardiac phenotype, mimicking HCM or SSA. Echocardiographic and ECG findings are useful to differentiate ATTR from HCM but not from SSA. The role of liver transplantation in these patients should be reconsidered.

Keywords Hereditary transthyretin-related amyloidosis • Cardiac phenotype • Symmetric left ventricular hypertrophy • Genotypic–phenotypic correlations • Hypertrophic cardiomyopathy • Senile systemic amyloidosis

* Corresponding author. Tel: +39 051 349 858, Fax: +39 051 344 859, Email: claudio.rapezzi@unibo.it

Introduction

Hereditary transthyretin-related amyloidosis (ATTR, also referred to as familial amyloidotic polyneuropathy) is characterized by the extracellular deposition of amyloid fibrils formed by the transport protein transthyretin (TTR), which is produced by the liver.[1] ATTR is caused by over 100 amyloidogenic TTR gene mutations and is the most common form of familial amyloidosis.[1] Its clinical spectrum varies widely from an exclusively neurological involvement to a predominantly cardiac presentation. This heterogeneity is linked to several factors including specific TTR mutations, patient and transmitting parent gender, geographical distribution, and endemic/non-endemic aggregation.[2–6] While the clinical features and natural history of ATTR associated with the Val30Met mutation have been widely studied,[2–5] the disease profile of the forms with an exclusively/predominantly cardiac involvement [also referred to as familial amyloidotic cardiomyopathy (FAC)] has been defined far less, and knowledge derives mainly from studies focusing on single mutations in different geographical contexts,[7,8] often limited to a single kindred.[9,10] In particular, at least four different mutations have been shown to be responsible for an exclusively or predominantly cardiac phenotype: Val122Ile, Thr60Ala, Leu111Met, and Ile68Leu.[7–10] FAC is probably widely underdiagnosed, mainly because the disease can mimic other causes of left ventricular (LV) hypertrophy, including hypertensive heart disease, hypertrophic cardiomyopathy (HCM), and the non-mutant form of ATTR [senile systemic amyloidosis (SSA)].[11,12]

Since our centres attract patients from a nationwide territory, the aim of this study was to assess the phenotypic and genotypic spectra of ATTR in a Caucasian area and, in particular, to evaluate the prevalence, genetic background, and disease profile at presentation of cases with an exclusively cardiac phenotype, highlighting possible hints for the differential diagnosis with HCM and SSA.

Patients and methods

Clinical setting and study design

Cross-sectional analysis

We conducted a cross-sectional, multicentre study of patients with ATTR at the time of diagnosis. The setting was the three main Italian referral centres for the diagnosis and treatment of hereditary amyloidosis (Bologna, Pavia, and Florence), all providing coordinated amyloidosis networks involving neurology, cardiology, haematology, and nephrology services, genetic research groups, and liver/heart transplantation programmes. Data on genotyping and a comprehensive set of cardiac, neurologic, and other relevant clinical/instrumental examinations were routinely collected according to an institutional protocol for all patients and stored in a dedicated database. All patients observed by our network at the time of definitive diagnosis of ATTR, between 1990 and December 2010, were eligible for the present study, including those identified through family screening who displayed clinical/instrumental evidence of disease.

Phenotype at baseline (i.e. at the time of first evaluation) was classified as: (i) 'cardiac phenotype', based on echocardiographic and/or electrocardiographic (ECG) evidence of cardiac amyloidosis in the absence of any sign or symptom of neurological involvement; (ii) 'neurologic phenotype', based on clinical/instrumental evidence of neuropathy in the absence of any echocardiographic, ECG, or clinical evidence of amyloidotic cardiomyopathy; and (iii) 'mixed (cardiac/neurologic) phenotype'.

We compared clinical and instrumental (ECG and echocardiographic) characteristics at baseline of patients with cardiac phenotype with those of patients with mixed phenotype. Baseline profiles of patients with a cardiac phenotype were also compared with those of patients diagnosed with SSA during the same time period and with those of 30 age- and gender-matched patients with genetically proven sarcomeric HCM.

Longitudinal analysis

For patients with a cardiac phenotype at the time of first evaluation, we assessed the natural history of the disease in terms of development of clinical/instrumental signs of neurological involvement. Follow-up of these patients was closed in September 2011.

All study participants provided informed consent for DNA analysis, any routine invasive procedures, and anonymous data publication. Application for formal approval by a local institutional Ethics Committee was not applicable for this retrospective observational study.

Diagnostic definitions and patients' assessment

For the diagnosis of systemic amyloidosis,[11] of familial ATTR,[13] and of SSA,[14] as well as for the definition of cardiac involvement,[15–17] of peripheral nervous system involvement,[1,18,19] and of autonomic neuropathy,[15,18] we referred to the standard diagnostic criteria. A detailed list of diagnostic definitions is found in the Supplementary material online.

In all patients, cardiac investigations included accurate search for signs and symptoms of heart failure, standard ECG,[20] and transthoracic echocardiography.[21] A description of ECG and echocardiographic definitions and analysed variables is reported in the Supplementary material online.[22,23]

Genotyping

Genomic DNA was isolated from whole peripheral blood by standard techniques. Exons 2, 3, and 4 of the TTR gene (accession number m11844) were amplified by polymerase chain reaction (Takara ExTaq polymerase) using primers described previously.[13] Amplified DNA fragments were directly sequenced using an ABI Prism 3130 automated sequencer.

Statistical analysis

Summary statistics were expressed as median (interquartile range) or numbers (percentages). In contingency tables, independence of categorical variables was tested using Fisher's exact test or Pearson's χ^2 test. Independence of continuous variables was tested using Mann–Whitney *U* test/Kruskal–Wallis test. For multiple comparisons, we calculated Bonferroni-adjusted *P*-values. Analyses were conducted using STATA 11.2 SE. All tests were two-sided; *P*-values less than 0.05 were considered significant.

Results

Study population

A total of 186 patients diagnosed with ATTR at our centres in 1990–2010 were included in the study. All patients were Caucasian and belonged to 89 distinct families originating from southern, central, and northern parts of Italy and were all of Italian descent, with the exception of one patient (carrying Phe33Val mutation)

Table 1 Transthyretin-related amyloidosis mutations in the study population according to families and patients

Mutations	Families, *n* (%)	Patients, *n* (%)
Val30Met	20 (22.5)	46 (24.8)
Glu89Gln	9 (10.2)	40 (21.5)
Ile68Leu	22 (24.7)	27 (14.6)
Phe64Leu	10 (11.2)	13 (7)
Thr49Ala	1 (1.1)	12 (6.5)
Gly47Ala	2 (2.3)	7 (3.8)
Arg34Thr	1 (1.1)	6 (3.2)
Ala36Pro	1 (1.1)	6 (3.2)
Ser50Arg	2 (2.3)	4 (2.2)
Gly47Arg	2 (2.3)	3 (1.6)
Glu54Lys	1 (1.1)	3 (1.6)
Val122Ile	3 (3.5)	3 (1.6)
Ser23Asn	2 (2.3)	2 (1.2)
Gly47Glu	1 (1.1)	2 (1.2)
Phe33Val	1 (1.1)	1 (0.5)
Hys88Arg	1 (1.1)	1 (0.5)
Val30Ala	1 (1.1)	1 (0.5)
Val14Leu	1 (1.1)	1 (0.5)
Gly53Ala	1 (1.1)	1 (0.5)
Gly57Arg	1 (1.1)	1 (0.5)
Tyr78Phe	1 (1.1)	1 (0.5)
Ile107Phe	1 (1.1)	1 (0.5)
Thr59Lys	1 (1.1)	1 (0.5)
Glu89Lys	1 (1.1)	1 (0.5)
Glu92Lys	1 (1.1)	1 (0.5)
Phe64Ile	1 (1.1)	1 (0.5)
Total	89 (100)	186 (100)

who came from the Republic of Macedonia. *Table 1* shows the complete list of the 26 different TTR mutations identified in the study population (families and patients): Val30Met was the most frequent followed by Glu89Gln and Ile68Leu. All patients were heterozygous for the identified mutation.

Baseline disease profile and genotype

At the time of diagnosis, 109 (58%) ATTR patients showed a mixed phenotype, 46 (25%) a neurologic phenotype, and 31 (17%) a cardiac phenotype. All patients with a cardiac phenotype displayed both ECG and echocardiographic abnormalities, whereas among those with a mixed phenotype, eight patients showed only ECG abnormalities (left bundle branch block, $n = 1$; right bundle branch block, $n = 2$; and left anterior hemiblock, $n = 5$) without any echocardiographic sign of amyloid infiltration: five of these eight cases carried the Val30Met mutation. Indeed, all the patients with cardiac phenotype and 101/109 (93%) of those with mixed phenotype also showed morphological findings, suggesting infiltrative myocardial disease including granular sparkling appearance of ventricular myocardium, increased thickness of atrioventricular valves or interatrial septum, and pericardial effusion.

Endomyocardial biopsy was performed in 66 patients with cardiac involvement, confirming the presence of amyloid infiltration in all cases. The main baseline clinical characteristics of the overall study population and according to the phenotype classification are summarized in *Table 2*. Regarding the distribution of the different TTR mutations in the different phenotype groups, not only each phenotype was found to be associated with more than one mutation, but also several mutations (including Val30Met, Ile68Leu, and Glu89Gln) were associated with more than one phenotype (*Table 2*).

Among the 31 patients with a cardiac phenotype, 15 (48%) had previously received a misdiagnosis (HCM, $n = 7$; dilated cardiomyopathy, $n = 2$; hypertensive heart disease, $n = 3$; chronic ischaemic heart disease, $n = 2$; and immunoglobulin light-chain (AL) amyloidosis, $n = 1$). In all these cases, the suspicion of cardiac amyloidosis emerged from a combined interpretation of clinical, echocardiographic, and ECG findings: an 'unexplained' LV hypertrophy at echocardiogram in the absence of LV hypertrophy on ECG was the main trigger for the correct suspicion.

ECG and echocardiographic characteristics of the two phenotypes with cardiac involvement are summarized in the Supplementary material online.

Follow-up of patients with a cardiac phenotype

During a median follow-up of 36 (14–50) months, four patients underwent combined heart–liver transplantation. One of these patients also underwent kidney transplantation for a coexistent non-amyloid-related chronic renal failure (previous acute glomerulonephritis). Five of the 31 cases with an exclusively cardiac phenotype developed abnormalities at neurological examination during the follow-up period, suggesting mild sensitive neuropathy (altered pain/temperature perception in the lower or upper limbs without any motor impairment). These signs were exclusively evident at neurological evaluation and were not associated with spontaneously reported symptoms.

Hereditary transthyretin-related amyloidosis vs. senile systemic amyloidosis and hypertrophic cardiomyopathy

During the study period (1990–2010), 30 patients were diagnosed with SSA. Thirty patients with genetically proven sarcomeric HCM (age- and gender-matched) were also included in the study for comparison. Sarcomere protein mutations included: MYBPC3 in 19 patients, TNNT2 in 6, and MYH7 in 4; a double MYH7/TNNI3 mutation was present in a single case. *Figure 1* and *Tables 3 and 4* report the main clinical, ECG, and echocardiographic findings of ATTR patients with cardiac phenotype and of SSA and HCM patients.

Discussion

Our study shows that, in a Caucasian population of patients originating from an ATTR non-endemic geographical area, >15% of cases at first evaluation have an exclusively cardiac phenotype.

Table 2 Patients' main clinical characteristics at baseline according to phenotype classification

	Overall	Cardiac	Mixed	Neurologic	Adjusted *P*-values
Patients, *n*	186	31	109	46	n.a.
Families, *n*	89	29	51	28	n.a.
Men, *n* (%)	130 (70)	29 (94)[a,b]	73 (67)	28 (61)	0.005*
Transthyretin mutations[c]					
Val30Met	46 (24.8)	0 (0)[a,b]	30 (27.5)	16 (34.8)	0.001*
Glu89Gln	40 (21.5)	1 (3.2)[a]	31 (28.5)	8 (17.4)	0.008*
Ile68Leu	27 (14.6)	23 (74.3)[a,b]	1 (0.9)	3 (6.5)	<0.001*
Phe64Leu	13 (7)	0 (0)	7 (6.4)	6 (13.1)	0.091
Thr49Ala	12 (6.5)	0 (0)	10 (9.2)	2 (4.3)	0.191
Gly47Ala	7 (3.8)	0 (0)	4 (3.7)	3 (6.5)	n.a.
Arg34Thr	6 (3.2)	0 (0)	3 (2.8)	3 (6.5)	
Ala36Pro	6 (3.2)	0 (0)	4 (3.7)	2 (4.3)	
Ser50Arg	4 (2.2)	0 (0)	3 (2.8)	1 (2.2)	
Glu54Lys	3 (1.6)	0 (0)	3 (2.8)	0 (0)	
Gly47Arg	3 (1.6)	0 (0)	2 (1.8)	1 (2.2)	
Val122Ile	3 (1.6)	2 (6.5)	0 (0)	1 (2.2)	
Ser23Asn	2 (1.2)	1 (3.2)	1 (0.9)	0 (0)	
Gly47Glu	2 (1.2)	0 (0)	2 (1.8)	0 (0)	
Val14Leu	1 (0.5)	1 (3.2)	0 (0)	0 (0)	
Hys88Arg	1 (0.5)	1 (3.2)	0 (0)	0 (0)	
Tyr78Phe	1 (0.5)	1 (3.2)	0 (0)	0 (0)	
Gly57Arg	1 (0.5)	0 (0)	1 (0.9)	0 (0)	
Glu89Lys	1 (0.5)	1 (3.2)	0 (0)	0 (0)	
Phe33Val	1 (0.5)	0 (0)	1 (0.9)	0 (0)	
Val30Ala	1 (0.5)	0 (0)	1 (0.9)	0 (0)	
Thr59Lys	1 (0.5)	0 (0)	1 (0.9)	0 (0)	
Ile107Phe	1 (0.5)	0 (0)	1 (0.9)	0 (0)	
Gly53Ala	1 (0.5)	0 (0)	1 (0.9)	0 (0)	
Glu92Lys	1 (0.5)	0 (0)	1 (0.9)	0 (0)	
Phe64Ile	1 (0.5)	0 (0)	1 (0.9)	0 (0)	
Age at diagnosis, years [median (IQR)]	54 (41–66)	70 (62–75)[a,b]	53 (43–62)	42 (37–61)	<0.001*
Age at onset of symptoms, years [median (IQR)]	51 (39–63)	67 (60–73)[a,b]	50 (40–59)	41 (33–58)	<0.001*
Diagnostic route, *n* (%)					
Neurologic	89 (47.8)	0 (0)	68 (62.4)	21 (45.7)	<0.001*[,a,b,d]
Cardiac	38 (20.4)	30 (96.8)	8 (7.3)	0 (0)	
Family screening	59 (31.8)	1 (3.2)	33 (30.3)	25 (54.3)	
Neurosensory involvement, *n* (%)					
0	31 (16.7)	31 (100)	0 (0)	0 (0)	n.a.
1	78 (41.9)	0 (0)	44 (43.6)	34 (63)	
2	66 (35.4)	0 (0)	50 (49.5)	16 (29.6)	
3	11 (6)	0 (0)	7 (6.9)	4 (7.4)	
Autonomic involvement, *n* (%)					
Orthostatic hypotension	55 (29.6)	0 (0)	42 (38.5)	13 (28.2)	n.a.
Urinary incontinence	42 (22.6)	0 (0)	32 (29.4)	10 (21.7)	
Gastrointestinal symptoms	79 (42.5)	0 (0)	61 (56)	18 (39.1)	
NYHA class III–IV, *n* (%)	24 (12.9)	13 (41.9)	11 (10)	0 (0)	n.a.
Vitreal involvement, *n* (%)	13 (6.9)	1 (3.2)	10 (9.2)	2 (4.3)	0.514
Carpal tunnel syndrome, *n* (%)	65 (34.9)	14 (45)[b]	45 (41.3)[d]	6 (13.1)	0.001*

n.a., not applicable.
**P*-values were adjusted according to the Bonferroni method.
[a]$P < 0.05$ at *post hoc* analysis: cardiac vs. mixed.
[b]$P < 0.05$ at *post hoc* analysis: cardiac vs. neurologic.
[c]Statistical analysis on the distribution of different mutations between the different phenotypes regards only those mutations with at least 10 cases.
[d]$P < 0.05$ at *post hoc* analysis: mixed vs. neurologic.

Although a single TTR mutation was responsible for >70% of these cases, the remaining cases showed a high genotypic heterogeneity. Although ATTR with a cardiac phenotype is frequently misdiagnosed as sarcomeric HCM, the disease profile is quite specific and could allow a non-invasive clinical and instrumental differential diagnosis.

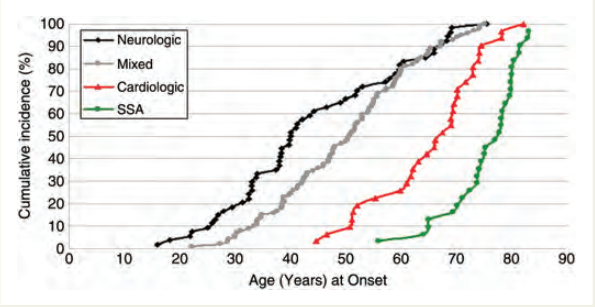

Figure 1 Cumulative age-related incidence of symptoms according to phenotype at presentation. Cases with senile systemic amyloidosis are also shown for comparison. At 50 years of age, <10% of the patients with a cardiac phenotype and none of the senile systemic amyloidosis patients have developed disease manifestations, whereas nearly 50 and 70% of the patients with a mixed and a neurologic phenotype, respectively, already show symptoms. More than 50% of the patients with senile systemic amyloidosis manifest the disease after 75 years of age, whereas all hereditary transthyretin-related amyloidosis patients with a neurologic or a mixed phenotype and most of those with a cardiac phenotype have already developed symptoms.

The study population derives from three centres that collect a relevant percentage of Italian ATTR patients through a wide spectrum of referral routes (neurological, cardiac, nephrological, haematological, etc.). The relatively low prevalence of the Val30Met mutation and the wide variety of other mutations ($n = 25$) in our cohort likely reflect a geographical scenario with a highly heterogeneous pool of genes due to historical reasons.

As expected, the characteristics of our patients were quite different from those of endemic early-onset Val30Met populations from northern Portugal, Brazil, and Japan, in whom neurologic phenotype is predominant, cardiac involvement is mainly limited to conduction disturbances, and disease onset generally occurs in the third–fourth decade of life.[2–5] In our population—as in other series of ATTR Val30Met patients including France, Sweden, and Japan[2,3,5,24]—disease onset tended to be relatively late (often during the fifth decade), frequently affecting males with overt heart involvement already at presentation.

Phenotypic heterogeneity and genotype–phenotype correlations

Systematic evaluation of the neurologic/cardiac phenotype in this extensive series of ATTR patients highlights the complexity of the relationship between the two main clinical expressions of this systemic disease. Although the vast majority (>80%) of the affected patients showed neurological involvement, cardiac

Table 3 Clinical and electrocardiographic findings of patients with hereditary transthyretin-related amyloidosis, senile systemic amyloidosis, and hypertrophic cardiomyopathy

	ATTR with cardiac phenotype ($n = 31$)	Senile systemic amyloidosis ($n = 30$)	Hypertrophic cardiomyopathy ($n = 30$)	Adjusted *P*-values
Age, years [median (IQR)]	67 (60–73)[a]	77 (72–80)[b]	64 (61–67)	<0.001*
Men, *n* (%)	29 (94)	28 (93)	26 (87)	0.65
Atrial fibrillation, *n* (%)	9 (29)	11 (37)[b]	2 (7)	0.02*
Pacemaker, *n* (%)	4 (13)	11 (37)	5 (17)	0.056
First-degree AV block, *n* (%)	4 (13)	9 (30)	6 (20)	0.243
Total QRS score, mV [median (IQR)]	123 (99–144)[c]	122 (110–155)[b]	155 (105–170)	0.023*
Low QRS voltage, *n* (%)	11 (35)[c]	10 (33)[b]	2 (7)	0.016*
Voltage/mass ratio [median (IQR)]	0.9 (0.5–1.3)	0.9 (0.5–1.4)	1.1 (0.8–1.7)	0.398
Right bundle branch block, *n* (%)	6 (19)	6 (20)	4 (13)	0.755
Left bundle branch block, *n* (%)	3 (10)	9 (30)[b]	2 (7)	0.03*
Left anterior hemiblock, *n* (%)	12 (39)	7 (23)	6 (20)	0.216
LV hypertrophy on ECG, *n* (%)	2 (7)[c]	3 (10)[b]	17 (57)	<0.001*
Presence of any infarct pattern, *n* (%)	17 (55)	10 (33)	11 (37)	0.185
'Ischaemic pattern' (negative T-waves), *n*/*N* (%)	17 (55)[c]	15 (50)[b]	24 (80)	0.037*
QTc interval, ms [median (IQR)]	486 (468–519)[c]	472 (433–499)	450 (429–469)	0.01*
Normal ECG, *n* (%)	0 (0)	0 (0)	2 (7)	0.125

IQR, interquartile range.
*P-values were adjusted according to the Bonferroni method.
[a]$P < 0.05$ at *post hoc* analysis: ATTR vs. SSA.
[b]$P < 0.05$ at *post hoc* analysis: SSA vs. HCM.
[c]$P < 0.05$ at *post hoc* analysis: ATTR vs. HCM.

Table 4 Echocardiographic findings of patients with hereditary transthyretin-related amyloidosis, senile systemic amyloidosis, and hypertrophic cardiomyopathy

	ATTR with cardiac phenotype ($n = 31$)	Senile systemic amyloidosis ($n = 30$)	Hypertrophic cardiomyopathy ($n = 30$)	Adjusted *P*-values
Diastolic interventricular septum thickness, mm [median (IQR)]	18 (16–20)	18 (16–22)	19 (17–22)	0.23
Diastolic LV posterior wall thickness, mm [median (IQR)]	16 (15–18)[a]	17 (14–19)[b]	14 (13–15)	<0.002*
Maximal LV wall thickness				
>12 mm, *n* (%)	31 (100)	30 (100)	30 (100)	n.a.
>14 mm, *n* (%)	27 (87)	25 (83)	26 (87)	0.93
Symmetric LV hypertrophy, *n* (%)	30 (97)[a]	29 (97)[b]	15 (50)	0.007*
Indexed LV mass among men, g/m^2 [median (IQR)]*	225 (193–261)[a]	247 (204–288)[b]	186 (176–200)	0.004*
Left atrial diameter, mm [median (IQR)]	47 (44–51)	51 (44–56)	51 (43–53)	0.302
LV ejection fraction, % [median (IQR)]	45 (36–51)[a]	50 (35–58)[b]	68 (67–76)	<0.001*
LV end-diastolic diameter, mm [median (IQR)]	49 (48–51)[a]	47 (43–50)	44 (40–47)	0.002*
E-wave deceleration time, ms [median (IQR)]	160 (135–175)[a]	159 (125–180)[b]	185 (180–215)	0.005*
Restrictive filling pattern, *n* (%)	12 (39)	9 (30)	4 (13)	0.079
Pericardial effusion, *n* (%)	17 (55)[a]	13 (43)[b]	1 (3)	<0.001*
Atrioventricular valve thickening, *n* (%)	19 (61)[a]	15 (50)[b]	1 (3)	<0.001*

n.a., not applicable; IQR, interquartile range.
**P*-values were adjusted according to the Bonferroni method.
[a]$P < 0.05$ at *post hoc* analysis: ATTR vs. HCM.
[b]$P < 0.05$ at *post hoc* analysis: SSA vs. HCM.

manifestations were remarkably common. As many as two-thirds of the patients had some clinical or instrumental signs of cardiac involvement, and a relevant minority (~15%) had an exclusively cardiac phenotype. For the purpose of phenotypic classification, we did not consider carpal tunnel syndrome as a specific sign of ATTR neurological involvement, since it is a common finding in the general population.[25] Although a single TTR mutation (Ile68Leu) accounted for the majority of patients with this phenotype, seven other mutations were also responsible for this presentation in our Italian patient population. This genotypic heterogeneity among our Caucasian patients with cardiac phenotype is peculiar when compared with the close relationship between single TTR mutations and cardiac phenotype found among US African-Americans (Val122Ile),[7,26] patients from the Appalachian and Irish Donegal regions (Thr60Ala),[8,27] and Denmark (Leu111Met).[9] Our findings reinforce the concept of a spectrum of genotype–phenotype correlations that ranges from largely 'neurologic' mutations (e.g. Val30Met) to purely 'cardiac' ones (e.g. Ile68Leu and Val122Ile) through mutations associated with variable, mixed phenotypes (*Figure 2*).[1–3,5,8,10,18,28–30]

The molecular basis of tissue specificity of individual TTR variants—which was outside the scope of this paper—is still largely unknown. There is no established relationship between any structural feature of the different TTR mutations and the selective amyloid deposits in cardiac, nervous, or leptomeningeal tissues.[31] However, it has been hypothesized that several factors such as protein stability, proteolytic remodelling by tissue-specific proteases, and/or interaction with extracellular components, including matrix molecules and chaperones, might be involved.[31–33] Moreover, even though the overall degree of genotype–phenotype correlation is high, a single gene mutation does not necessarily lead to the expression of a single phenotype and a relevant intra-mutation and intra-familial heterogeneity was apparent (*Table 2*).

Disease profile of patients with a cardiac phenotype and differential diagnosis

The clinical and instrumental profile of ATTR with a cardiac phenotype is relatively well defined. In fact, nearly all cases are men aged >65, with heart failure symptoms, symmetric LV 'hypertrophy' without LV dilatation, and with moderately depressed LV ejection fraction on echocardiogram. Age at diagnosis [70 (62–75) years] is on average 17 years older compared with patients with a mixed phenotype [53 (43–62) years] and 28 years higher compared with patients with a neurologic phenotype [42 (37–61) years] (*Figure 1*). These intriguing differences can only partially be attributed to the differences of age-related penetrance of the different mutations, since cardiac manifestations occur at an older age compared with neurological ones even among patients who share the same mutation. The explanation of this phenomenon is not clear. On the one hand, it is possible that the absence of neurological symptoms causes the patient to seek medical attention later. On the other, however, it is highly plausible that myocardial infiltration in ATTR (both wild-type and hereditary) requires longer than the time required to develop axonal neuropathy.

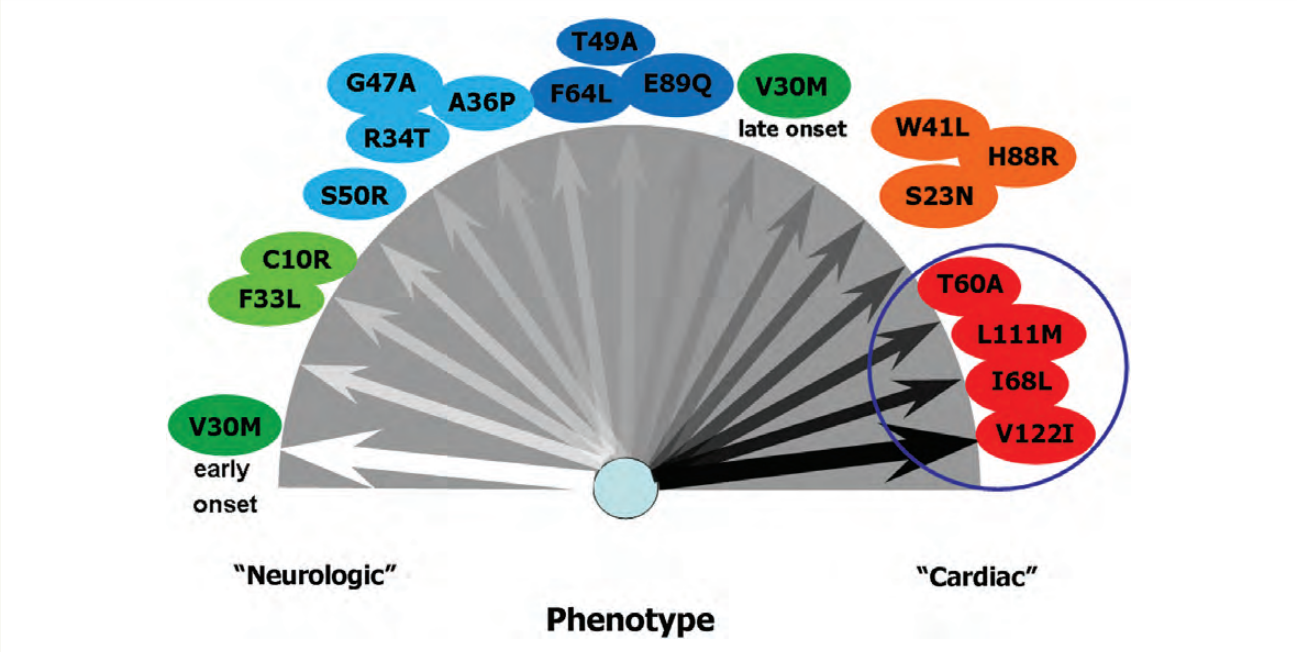

Figure 2 Possible spectrum of genotype–phenotype correlations in transthyretin-related amyloidosis. Phenotypic expression of transthyretin-related amyloidosis varies widely from an almost exclusively neurological involvement (Val30Met mutation with early-onset disease) to a predominant or exclusively cardiac involvement (Thr60Ala, Leu111Met, Ile68Leu, and Val122Ile mutations). In between, several transthyretin-related amyloidosis mutations are associated with variable degrees of neurological and cardiological involvement, including Val30Met with late-onset disease.

Our finding that most patients with cardiomyopathy were men is in line with the hypothesis that female gender may confer some degree of protection against myocardial amyloid deposition.[34]

Compared with patients with a mixed phenotype, patients with a cardiac phenotype have a more pronounced cardiac involvement as shown by both echocardiogram and ECG (Supplementary material online). Once again, it is difficult to establish whether these differences are due to a lead-time bias or, more probably, the degree of myocardial infiltration is intrinsically greater in some TTR mutations and in some ATTR patients.

When reviewing the literature, only few reports regarding ATTR patients with a cardiac phenotype are available and are mainly derived from cohorts of patients with single TTR mutations.[7–9] In the only two papers that report detailed echocardiographic data from a small number of patients with the Val122Ile mutation, both interventricular septum thickness and LV ejection fraction values are comparable with our findings.[26,35] Similarly, cardiac involvement of Irish and Scottish patients with Thr60Ala mutation is characterized by symmetric LV hypertrophy and moderate LV systolic dysfunction, in the absence of low QRS voltages in the vast majority of cases.[8]

Our data confirm that ATTR with an exclusively cardiac phenotype is frequently mistaken for HCM. The availability of an age- and gender-matched control group with genetically proven sarcomeric HCM allowed us to compare different types of cardiomyopathies with LV hypertrophy. Indeed, a careful interpretation of ECG and echocardiogram may offer many useful hints for a differential diagnosis. On echocardiogram, the major differences involve hypertrophy distribution (symmetric in ATTR and generally asymmetric in HCM), the LV systolic function (greater LV end-diastolic dimension and lower LV ejection fraction in amyloidotic cardiomyopathy), and the coexistence of pericardial effusion and increased atrioventricular valve thickness (frequent in patients with amyloidotic cardiomyopathy and almost absent in HCM).

On ECG, the main differences regard the frequency of 'ischaemic' abnormalities (much more frequent in HCM), the QTc interval (longer in amyloidotic cardiomyopathy), and low QRS voltages (higher prevalence in ATTR and SSA). Nevertheless, despite a significant difference in the average QRS score values between the two groups, only 35% of the patients with ATTR showed a low QRS voltage pattern. This finding confirms previous observations reporting a high prevalence of low QRS voltages only in AL-related cardiac amyloidosis.[17,36]

While an accurate study of the echocardiographic and ECG findings can direct towards the right diagnosis between TTR-related cardiomyopathies and HCM, the differential diagnosis between ATTR and SSA is based solely on molecular genetics. Indeed, excluding age, no other clinical or instrumental finding differed significantly between the two groups.

Therapeutic implications

Peculiar therapeutic problems arise when considering ATTR patients with an exclusively cardiac phenotype. In ATTR, orthotopic liver transplantation (OLT) has proven to be effective in halting the progression of neurological amyloid disease and currently represents the only available disease-modifying treatment.[37]

However, even after a successful OLT, a relevant number of patients with cardiac involvement show a cardiomyopathy progression.[38] In ATTR patients with an exclusively cardiac phenotype who face heart disease progression but not the development of neurological symptoms, OLT could be questionable. Our decision to submit four of our patients to combined heart–liver transplantation ensued from the need for heart replacement, while also following the current (even though generic) indications for OLT in ATTR amyloidosis. However, in patients with an exclusively cardiac phenotype, the absence of neurological manifestation could be the rationale to perform isolated heart transplantation. This strategy has already been applied in single patients with Ile122Val mutation and symptomatic amyloidotic cardiomyopathy.[39] Further larger studies would contribute to better direct therapeutic choices. Moreover, patients with a cardiac phenotype should be included in dedicated prospective protocols that investigate the effects of new TTR stabilizers (diflunisal and tafamidis) on disease progression.[40,41]

Limitations

Our study essentially provides information on the phenotypic heterogeneity of ATTR at presentation. The possible phenotypic evolution during the natural history of the disease has been only partially captured by our study design. Our data on the prevalence of different phenotypes may not necessarily apply to other geographical areas with different genetic backgrounds. Nevertheless, given the analogies between echocardiographic and ECG profiles of our patients with cardiac phenotype and those previously reported for patients carrying the Val122Ile or Thr60Ala mutations, it is plausible that our hints for differential diagnosis between ATTR and HCM could also be useful in other contexts. In this regard, despite the consistent statistical differences of many clinical and instrumental variables between TTR-related cardiomyopathy and HCM, the limited size of the subgroups did not allow us to obtain reliable specificity and sensitivity values.

Conclusions

A clinically relevant subset (~15%) of Caucasian ATTR patients from non-endemic areas has an exclusively cardiac phenotype at presentation and does not develop relevant neurological symptoms at a mid-term follow-up. This amyloidotic cardiomyopathy—which is associated with several TTR mutations—is frequently mistaken for HCM or SSA. While an accurate study of the echocardiographic and ECG findings can direct towards the right diagnosis between TTR-related cardiomyopathies and HCM, the differential diagnosis between ATTR and SSA is based solely on molecular genetics.

In patients with an exclusively cardiac phenotype, the role of liver transplantation should be reconsidered, since the absence of neurological manifestation may be the rationale to perform isolated heart transplantation.

Supplementary material

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

Funding

C.C.Q. was partially supported by a grant from the Istituto Nazionale per le Ricerche Cardiovascolari (INRC), Bologna, Italy.

Conflict of interest: none declared.

References

1. Ando Y, Nakamura M, Araki S. Transthyretin-related familial amyloidotic polyneuropathy. *Arch Neurol* 2005;**62**:1057–1062.
2. Ikeda S, Nakazato M, Ando Y, Sobue G. Familial transthyretin-type amyloid polyneuropathy in Japan: clinical and genetic heterogeneity. *Neurology* 2002;**58**: 1001–1007.
3. Suhr OB, Svendsen IH, Andersson R, Danielsson A, Holmgren G, Ranløv PJ. Hereditary transthyretin amyloidosis from a Scandinavian perspective. *J Intern Med* 2003;**254**:225–235.
4. Bittencourt PL, Couto CA, Clemente C, Farias AQ, Palácios SA, Mies S, Goldberg AC. Phenotypic expression of familial amyloid polyneuropathy in Brazil. *Eur J Neurol* 2005;**12**:289–293.
5. Conceição I, De Carvalho M. Clinical variability in type I familial amyloid polyneuropathy (Val30Met): comparison between late- and early-onset cases in Portugal. *Muscle Nerve* 2007;**35**:116–118.
6. Rapezzi C, Quarta CC, Riva L, Longhi S, Gallelli I, Lorenzini M, Ciliberti P, Biagini E, Salvi F, Branzi A. Transthyretin-related amyloidoses and the heart: a clinical overview. *Nat Rev Cardiol* 2010;**7**:398–408.
7. 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.
8. Sattianayagam PT, Hahn AF, Whelan CJ, Gibbs SD, Pinney JH, Rowczenio D, Pflugfelder PW, Fox Z, Lachmann HJ, Wechalekar AD, Hawkins PN, Gillmore JD. Cardiac phenotype and clinical outcome of familial amyloid polyneuropathy associated with transthyretin alanine 60 variant. *Eur Heart J* 2012; **33**:1120–1127.
9. Ranløv I, Alves IL, Ranløv PJ, Husby G, Costa PP, Saraiva MJ. A Danish kindred with familial amyloid cardiomyopathy revisited: identification of a mutant transthyretin-methionine111 variant in serum from patients and carriers. *Am J Med* 1992;**93**:3–8.
10. Almeida MR, Hesse A, Steinmetz A, Maisch B, Altland K, Linke RP, Gawinowicz MA, Saraiva MJ. Transthyretin Leu 68 in a form of cardiac amyloidosis. *Basic Res Cardiol* 1991;**86**:567–571.
11. Falk RH, Dubrey SW. Amyloid heart disease. *Prog Cardiovasc Dis* 2010;**52**: 347–361.
12. Seward JB, Casaclang-Verzosa G. Infiltrative cardiovascular diseases: cardiomyopathies that look alike. *J Am Coll Cardiol* 2010;**55**:1769–1779.
13. Ferlini A, Fini S, Salvi F, Patrosso MC, Vezzoni P, Forabosco A. Molecular strategies in genetic diagnosis of transthyretin-related hereditary amyloidosis. *FASEB J* 1992;**6**:2864–2866.
14. Westermark P, Sletten K, Johansson B, Cornwell GG. Fibril in senile systemic amyloidosis is derived from normal transthyretin. *Proc Natl Acad Sci USA* 1990; **87**:843–845.
15. Gertz MA, Comenzo R, Falk RH, Fermand JP, Hazenberg BP, Hawkins PN, Merlini G, Moreau P, Ronco P, Sanchorawala V, Sezer O, Solomon A, Grateau G. Definition of organ involvement and treatment response in immunoglobulin light chain amyloidosis (AL): a consensus opinion from the 10th International Symposium on Amyloid and Amyloidosis. *Am J Hematol* 2005;**79**: 319–328.
16. Suhr OB, Lindqvist P, Olofsson B-E, Waldenstroom A, Backman C. Myocardial hypertrophy and function are related to age at onset in familial amyloidotic polyneuropathy. *Amyloid* 2006;**13**:154–159.
17. 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.
18. Benson MD, Kincaid JC. The molecular biology and clinical features of amyloid neuropathy. *Muscle Nerve* 2007;**36**:411–423.
19. Coutinho P, Martins da Silva A, Lopes Lima J, Resende Barbosa A. Forty years of experience with type I amyloid neuropathy. In Glenner GG, Costa PP, Freitas AF, eds. *Amyloid and Amyloidosis*. Amsterdam: Excerpta Medica; 1980. p88–98.
20. Surawicz B, Knilans TK, eds. *Chou's Electrocardiography in Clinical Practice: Adult and Pediatric*. 5th ed. Philadelphia: W. B. Saunders; 2001.
21. American College of Cardiology/American Heart Association Task Force on Practice Guidelines (ACC/AHA/ASE Committee to Update the 1997 Guidelines

for the Clinical Application of Echocardiography). Guidelines update for the clinical application of echocardiography. *Circulation* 2003;**108**:1146–1162.
22. Devereux RB, Alonso DR, Lutas EM, Gottlieb GJ, Campo E, Sachs I, Reichek N. Echocardiographic assessment of left ventricular hypertrophy: comparison to necropsy findings. *Am J Cardiol* 1986;**57**:450–458.
23. Nagueh SF, Appleton CP, Gillebert TC, Marino PN, Oh JK, Smiseth OA, Waggoner AD, Flachskampf FA, Pellikka PA, Evangelisa A. Recommendations for the evaluation of left ventricular diastolic function by echocardiography. *Eur J Echocardiogr* 2009;**10**:165–193.
24. Plante-Bordeneuve V, Lalu T, Misrahi M, Reilly MM, Adams D, Lacroix C, Said G. Genotypic–phenotypic variations in a series of 65 patients with familial amyloid polyneuropathy. *Neurology* 1998;**51**:708–714.
25. Atroshi I, Gummesson C, Johnsson R, Ornstein E, Ranstam J, Rosén I. Prevalence of carpal tunnel syndrome in a general population. *JAMA* 1999;**282**:153–158.
26. Connors LH, Prokaeva T, Lim A, Théberge R, Falk RH, Doros G, Berg A, Costello CE, O'Hara C, Seldin DC, Skinner M. Cardiac amyloidosis in African Americans: comparison of clinical and laboratory features of transthyretin V122I amyloidosis and immunoglobulin light chain amyloidosis. *Am Heart J* 2009;**158**:607–614.
27. Staunton H, Davis MB, Guiloff RJ, Nakazato M, Miyazato N, Harding AE. Irish (Donegal) amyloidosis is associated with the transthyretinALA60 (Appalachian) variant. *Brain* 1991;**114**:2675–2679.
28. Almeida MR, Ferlini A, Forabosco A, Gawinowicz M, Costa PP, Salvi F, Plasmati R, Tassinari CA, Altland K, Saraiva MJ. Two transthyretin variants (TTR Ala-49 and TTR Gln89) in two Sicilian kindreds with hereditary amyloidosis. *Hum Mutat* 1992;**1**:211–215.
29. Solaro C, Schenone A, Di Sapio A, Pradotto L, Mancardi GL, Primavera A, Mauro A. An Italian family with Ala-47 transthyretin mutation associated with cardiomyopathy and polyneuropathy. *Neuromusc Disord* 2000;**10**:52–55.
30. Patrosso MC, Vezzoni P, Salvi F, Gobbi P, Winter P, Altland K, Ferlini A. Two new mutations in the transthyretin gene leading to polyneuropathy and cardiomyopathy in two unrelated Italian families. *Neuromuscular Disord (Suppl)* 1996;**6**:S23.
31. Sekijima Y, Wiseman RL, Matteson J, Hammarström P, Miller SR, Sawkar AR, Balch WE, Kelly JW. The biological and chemical basis for tissue-selective amyloid disease. *Cell* 2005;**121**:73–85.
32. Ihse E, Ybo A, Suhr O, Lindqvist P, Backman C, Westermark P. Amyloid fibril composition is related to the phenotype of hereditary transthyretin V30M amyloidosis. *J Pathol* 2008;**216**:253–261.
33. Buxbaum JN, Tagoe C, Gallo G, Walker JR, Kurian S, Salomon DR. Why are some amyloidoses systemic? Does hepatic 'chaperoning at a distance' prevent cardiac deposition in a transgenic model of human senile systemic (transthyretin) amyloidosis? *FASEB J* 2012;**26**:2283–2293.
34. Rapezzi C, Riva L, Quarta CC, Perugini E, Salvi F, Longhi S, Ciliberti P, Pastorelli F, Biagini E, Leone O, Cooke RM, Bacchi-Reggiani L, Ferlini A, Cavo M, Merlini G, Perlini S, Pasquali S, Branzi A. Gender-related risk of myocardial involvement in systemic amyloidosis. *Amyloid* 2008;**15**:40–68.
35. Bhuiyan T, Helmke S, Patel AR, Ruberg FL, Packman J, Cheung K, Grogan D, Maurer MS. Pressure–volume relationships in patients with transthyretin (ATTR) cardiac amyloidosis secondary to V122I mutations and wild-type transthyretin: Transthyretin Cardiac Amyloid Study (TRACS). *Circ Heart Fail* 2011;**4**:121–128.
36. Rahman JE, Helou EF, Gelzer-Bell R, Thompson RE, Kuo C, Rodriguez ER, Hare JM, Baughman KL, Kasper EK. Noninvasive diagnosis of biopsy-proven cardiac amyloidosis. *J Am Coll Cardiol* 2004;**43**:410–415.
37. Herlenius G, Wilczek HE, Larsson M, Ericzon BG, Familial Amyloidotic Polyneuropathy World Transplant Registry. Ten years of international experience with liver transplantation for familial amyloidotic polyneuropathy: results from the Familial Amyloidotic Polyneuropathy World Transplant Registry. *Transplantation* 2004;**77**:64–71.
38. Dubrey SW, Davidoff R, Skinner M, Bergethon P, Lewis D, Falk RH. Progression of ventricular wall thickening after liver transplantation for familial amyloidosis. *Transplantation* 1997;**64**:74–80.
39. Hamour IM, Lachmann HJ, Goodman HJ, Petrou M, Burke MM, Hawkins PN, Banner NR. Heart transplantation for homozygous familial transthyretin (TTR) V122I cardiac amyloidosis. *Am J Transplant* 2008;**8**:1056–1059.
40. Sekijima Y, Dendle MA, Kelly JW. Orally administered diflunisal stabilizes transthyretin against dissociation required for amyloidogenesis. *Amyloid* 2006;**13**:236–249.
41. Ratner M. Spotlight focuses on protein-misfolding therapies. *Nat Biotechnol* 2009;**27**:874.