

# Phenotypic profile of Ile68Leu transthyretin amyloidosis: an underdiagnosed cause of heart failure

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## Aims

Cardiac amyloidosis remains a great challenge for the cardiologist. One of the three main aetiological forms, transthyretin-related hereditary amyloidosis (ATTRm), can present with several phenotypes, depending mainly on the specific mutation. We aimed to characterize the phenotype of patients with ATTRm due to Ile68Leu mutation, comparing them to patients with wild-type transthyretin amyloidosis (ATTRwt).

## Methods and results

Data of 67 Ile68Leu ATTRm patients from two Italian referral centres (Bologna and Florence) were retrospectively analysed and compared to those of 82 ATTRwt patients. Fifty-five unaffected mutation carriers were also analysed. Cumulative disease onset was 50% at age 71. A total of 56/67 (84%) patients had a predominantly cardiac phenotype at presentation with concentric increase in left ventricular wall thickness [median 17 mm], and normal or near normal left ventricular ejection fraction (79% of patients). Low QRS voltages were present only in 29% of patients but voltage/mass ratio was low (0.5). Carpal tunnel syndrome was noted in 43%. The overall phenotypic profile was similar to ATTRwt but Ile68Leu ATTRm patients typically presented younger (median 71 vs. 78 years) and were more likely to have (mild) symptomatic neurological involvement (19% vs. 2%). Male prevalence was 44% in unaffected mutation carriers and 78% in affected patients. Age-adjusted survival was comparable between groups.

## Conclusions

Ile68Leu ATTRm is a cause of familial amyloidotic cardiomyopathy endemic in central-northern Italy and presents as hypertrophic/restrictive cardiomyopathy quite similar to ATTRwt. Male preponderance is present in affected patients but not in unaffected mutation carriers. Age-adjusted survival is similar to ATTRwt.

## Keywords

Amyloidosis • Transthyretin • Cardiomyopathy • Heart failure

## Introduction

Hereditary transthyretin (TTR) amyloidosis (ATTRm) is an autosomal dominant disorder caused by the extracellular deposition of amyloid fibrils derived from TTR, a transport protein mainly

synthesized by the liver.<sup>1,2</sup> ATTRm is characterized by a high genotypic heterogeneity, with over 120 amyloidogenic point mutations described to date.<sup>3</sup> The clinical spectrum can vary widely from an exclusively neurological to a predominantly cardiac phenotype.<sup>4</sup> Only a small number of TTR variants are associated with a mainly

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cardiac involvement [also known as familial amyloidotic cardiomyopathy (FAC)] and have been identified in well-defined populations, such as Val122Ile in USA patients of African descent,<sup>5–7</sup> Leu111Met in Danish kindred<sup>8</sup> and Thr60Ala in north-western Ireland.<sup>9</sup> Non-mutant protein (i.e. wild-type TTR) can also cause amyloidotic cardiomyopathy (ATTRwt) and is frequently associated with carpal tunnel syndrome (CTS)<sup>10</sup> with very little or no neuropathy.

We previously reported that >15% of Italian ATTRm patients referred to specialized Amyloidosis Centres have a late-onset cardiac phenotype, which is almost indistinguishable from ATTRwt, and that a single TTR variant (Ile68Leu) accounts for many of these cases.<sup>11</sup> In the present study, we carry out the first comprehensive description of the clinical profile and outcome of patients affected by Ile68Leu ATTRm and compare them to a cohort of patients diagnosed as ATTRwt who presented over the same period and in the same geographic areas.

## Methods

### Clinical setting and study population

Data regarding patients with Ile68Leu ATTRm and ATTRwt evaluated in two Italian referral Centres (Bologna and Florence) between January 1993 and December 2017 were extracted from dedicated prospective local databases that include baseline and follow-up data. The main clinical/instrumental variables at presentation—including diagnostic route, symptoms at disease onset, previous (mis)diagnoses, main laboratory tests, cardiac and neurological assessments, electrocardiographic and echocardiographic details—were analysed. Follow-up data were obtained from the last visit or by telephone interview in those without any contact in the last 6 months. The study was approved by the local ethics committee.

We also compared data from our study population with published data on patients with Val122Ile ATTRm from the THAOS registry.<sup>12</sup>

### Definitions and classifications

A diagnosis of TTR-related amyloidotic cardiomyopathy (AC) was made when diastolic interventricular septum thickness was >12 mm on echocardiography in the absence of other causes of ventricular hypertrophy and in association with at least one of the following: (i) evidence of TTR deposits by immunohistochemistry in a tissue biopsy; (ii) non-invasive documentation of intense cardiac uptake (visual score 2 or 3) on bone-tracer scintigraphy (<sup>99m</sup>Tc-DPD or <sup>99m</sup>Tc-HMDP) with exclusion of monoclonal gammopathy on serum and urine samples.

The phenotype at presentation was defined as: (i) 'predominantly cardiac', based on echocardiographic evidence of AC with no signs or symptoms of neurological involvement (erectile dysfunction in men aged >50 and CTS were not considered signs of neurological involvement *per se* as they are not infrequent in the general population); (ii) 'predominantly neurological', if neurological involvement was detected clinically in absence of signs of cardiac amyloidosis; and (iii) 'mixed' (cardiac/neurologic) for all other cases.

Neurological symptoms were staged according Coutinho *et al.*<sup>13</sup>: 0, no symptoms; 1, unimpaired ambulation, mostly mild sensory, motor, and autonomic neuropathy in the lower limbs; 2, assistance with ambulation required; mostly moderate impairment progression to lower

limbs, upper limbs, and trunk; 3, wheelchair-bound or bedridden; severe sensory, motor, and autonomic involvement of all limbs. Autonomic involvement was defined by presence of orthostatic hypotension (decline >20 mmHg in systolic blood pressure, or > 10 mmHg in diastolic blood pressure upon standing), urinary incontinence or gastrointestinal symptoms (diarrhoea, constipation, fecal incontinence). CTS history was considered positive in the presence of typical symptoms or of previous surgery for median nerve decompression. 'Vitreal involvement' refers to visual impairment leading to ophthalmologic detection of vitreous opacities.

### Electrocardiogram

Standard definitions were used for the interpretation of 12-lead electrocardiograms (ECGs). Low QRS voltages were defined as QRS amplitude  $\leq 0.5$  mV in all limb leads, or  $\leq 1$  mV amplitude in all precordial leads.<sup>14</sup> The voltage/mass ratio was defined as total QRS score divided by left ventricular mass measured on echocardiogram indexed to body surface area. QT prolongation was defined as QTc  $\geq 450$  ms in males and  $\geq 470$  ms in females.

### Echocardiography

Echocardiographic images were obtained from the standard parasternal long-axis, parasternal short-axis, apical and subcostal views. Chamber and left ventricular ejection fraction (LVEF) quantification was performed according to the recommendations of the American Society of Echocardiography.<sup>15</sup> Left ventricular mass, diameters and wall thickness were evaluated by M-mode. Patterns of hypertrophy were defined as previously described.<sup>16</sup> A restrictive filling pattern was defined as E wave deceleration time < 150 ms and E/A ratio > 2.5 on transmitral pulsed Doppler. Myocardial contraction fraction was calculated according to the formula described by Maurer and colleagues.<sup>17</sup>

### Genotyping

Transthyretin gene analysis was carried out in all patients. 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 previously described.<sup>18</sup> Amplified DNA fragments were directly sequenced using ABI Prism 3130 automated sequence.

### Statistical analysis

Continuous variables are expressed as median and interquartile range (IQR) and categorical variables as number of patients and frequencies/percentage. The clinical and instrumental differences between the subtypes of cardiac amyloidosis were analysed using the Wilcoxon rank-sum test for continuous variables and  $\chi^2$  test for categorical variables. Bonferroni correction for multiple comparisons was applied to control the family-wise error rate. Kaplan–Meier curves were reported to graphically analyse overall survival; the log-rank test was used to compare freedom from overall death between subgroups. Clinical, laboratory, electrocardiographic and echocardiographic variables were evaluated with a Cox regression model to identify independent predictors of survival in Ile68Leu patients. Non-correlated variables with *P*-values  $\leq 0.2$  at univariate analysis were considered in the multivariate analysis. Model building followed a backward-stepwise

**Table 1** Baseline characteristics of the study population

	Ile68Leu (n = 67)	ATTRwt (n = 82)	P-value
Families, n	63	–	
Males, n (%)	52 (78)	73 (89)	0.059
Age at diagnosis, years, median (IQR)	71 (65–78)	78 (72–81)	<0.001*
Disease duration, months, median (IQR)	10 (3–24)	10 (3–21)	0.872
BMI, kg/m <sup>2</sup> , median (IQR)	25 (23–27)	25 (23–28)	0.905
Heart rate, b.p.m., median (IQR)	75 (65–80)	75 (67–83)	0.833
SBP, mmHg, median (IQR)	120 (110–132)	120 (110–130)	0.368
DBP, mmHg, median (IQR)	80 (70–80)	70 (60–80)	0.007
eGFR, mL/min, median (IQR)	54 (45–74)	52 (43–65)	0.276
Diagnostic route, n (%)			0.093
Incidental	4 (6)	10 (12)	
Neurological	1 (1)	0 (0)	
Cardiac	59 (88)	72 (88)	
Family screening	3 (5)	0 (0)	
Neurological symptoms according to Coutinho classification, n (%)			0.001*
0	54 (81)	80 (98)	
1	13 (19)	2 (2)	
2	0 (0)	0 (0)	
3	0 (0)	0 (0)	
Autonomic involvement, n (%)			N/A
Orthostatic hypotension	3 (4)	0 (0)	
Urinary incontinence	1 (1)	0 (0)	
Gastrointestinal symptoms	1 (1)	0 (0)	
Carpal tunnel syndrome, n (%)	29 (43)	30 (37)	0.406
Vitreous involvement, n (%)	0 (0)	0 (0)	N/A
Amyloidotic cardiomyopathy, n (%)	64 (96)	82 (100)	0.089
NYHA class III/IV, n (%)	18 (27)	27 (33)	0.423
NT-proBNP, pg/mL, median (IQR) <sup>†</sup>	3287 (1745–5658)	4800 (1706–12376)	0.229
Phenotype, n (%)			0.062
Predominantly cardiac	56 (84)	77 (94)	
Predominantly neurological	3 (4)	0 (0)	
Mixed	8 (12)	5 (6)	

ATTRwt, wild-type transthyretin amyloidosis; BMI, body mass index; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; IQR, interquartile range; N/A, not applicable; NT-proBNP, N-terminal pro brain natriuretic peptide; NYHA, New York Heart Association; SBP, systolic blood pressure.

\*P-values with statistical significance persisting after Bonferroni correction.

<sup>†</sup>Data available in 34 Ile68Leu and in 61 ATTRwt patients.

approach, term of significance was tested with the Wald  $\chi^2$  test with cut-off P-value of 0.1 for removal and 0.05 for addition. Harrell's C-statistic was used to assess the goodness of fit of Cox regression models. A P-value <0.05 (2-sided) was considered significant. All analyses were carried out with Stata/SE 14.2 for Windows (StataCorp LLC, College Station, TX, USA).

## Results

### Clinical profile

The study cohort consisted of 67 subjects with heterozygous Ile68Leu ATTRm from 63 unrelated families (Table 1), mainly from Emilia-Romagna and Tuscany regions (94%), with the exception of two patients from Lombardy and two from Marche. Sixty-four patients were probands and three were diagnosed by

family screening. Family screening, which included genetic testing, clinical evaluation, ECG and echocardiography, also identified 55 unaffected mutation carriers with no signs of amyloidosis, including a 48-year-old homozygous man. In detail, 24/55 (44%) of the unaffected carriers were males, with a median age of 49 (43–56), and a mean age of  $51 \pm 11$  years. Echocardiogram showed normal wall thickness in all cases with the exception of a single patient with hypertension and obesity in whom diastolic interventricular septum thickness was 12 mm. ECG was within normal limits in 52/55 cases with non-specific repolarization abnormalities in two patients and left anterior hemiblock in a single case. The comparator group consisted of 82 ATTRwt patients. In 4 Ile68Leu ATTRm and in 10 ATTRwt patients the diagnosis of AC was incidental following bone-tracer scintigraphy performed for oncological or rheumatological reasons. In all but one of the remaining probands the diagnostic workup was initiated by cardiac symptoms.

**Table 2** Electrocardiographic findings

	Ile68Leu (n = 67)	ATTRwt (n = 82)	P-value
Atrial fibrillation, n (%)	20 (30)	37 (45)	0.056
Permanent pacemaker, n (%)	6 (9)	12 (15)	0.324
First degree AV block*, n (%)	13 (21)	15 (21)	0.844
Total QRS score, mV, median (IQR)	114 (98–135)	118 (101–141)	0.548
Low QRS voltages, n (%)	18 (29)	19 (25)	0.603
Voltage/mass, mV/g/m <sup>2</sup> BSA, median (IQR)	0.5 (0.4–0.7)	0.5 (0.5–0.7)	0.823
Right bundle branch block*, n (%)	12 (20) <sup>†</sup>	14 (20) <sup>‡</sup>	0.963
Left bundle branch block*, n (%)	8 (13)	8 (11)	0.742
Left anterior hemiblock*, n (%)	27 (44) <sup>†</sup>	29 (41) <sup>‡</sup>	0.744
Any infarct pattern*, n (%)	38 (63)	44 (63)	0.955
Ischaemic pattern* (negative T waves), n (%)	28 (47)	32 (46)	0.914
QTc interval*, ms, median (IQR)	474 (447–495)	454 (433–495)	0.010
Prolonged QTc interval*, n (%)	41 (67)	32 (46)	0.013
Normal ECG, n (%)	5 (8)	3 (4)	0.295

ATTRwt, wild-type transthyretin amyloidosis; AV, atrioventricular; BSA, body surface area; ECG, electrocardiogram; IQR, interquartile range.

\* Referred to patients without pacemaker.

<sup>†</sup> Including 5 patients with left anterior hemiblock + right bundle branch block.

<sup>‡</sup> Including 6 cases with left anterior hemiblock + right bundle branch block.

No comparison is associated with statistical significance after Bonferroni correction.

In the Ile68Leu group, the diagnosis of AC was reached invasively by endomyocardial biopsy in 25/67 patients (37%) and non-invasively in 42 (63%). In the ATTRwt group, the diagnosis was reached invasively by endomyocardial biopsy in 59/84 patients (70%) and non-invasively in the other 25 (30%).

The main clinical findings are reported in Table 1. Median age at diagnosis was 7 years younger in Ile68Leu patients [71 (65–78) vs. 78 (72–81);  $P < 0.001$ ]. At baseline evaluation, 84% of Ile68Leu patients showed a predominantly cardiac phenotype, 12% had a mixed phenotype with a predominant cardiac involvement and 4% had a predominantly neurologic phenotype. On specific questioning, 13 Ile68Leu patients (19%) reported mild neurological motor symptoms on walking (vs. 2 patients, 2% of ATTRwt;  $P = 0.001$ ). A history of CTS was reported by 43% of Ile68Leu ATTRm patients and by 37% of ATTRwt ( $P = 0.406$ ). Ninety-six percent of Ile68Leu ATTRm group and 100% of ATTRwt had a cardiomyopathy diagnosed on echocardiography. Heart failure was the reason that most frequently brought Ile68Leu ATTRm patients to medical attention (53/67 patients, 79%). At presentation, the same number of patients had signs or symptoms of heart failure and 18/67 of these (27%) were in New York Heart Association (NYHA) class III/IV. Sixteen/67 patients (24%) reported a reduced exercise tolerance, while in 37/67 patients (55%) dyspnoea was associated with right heart failure. All patients with heart failure were treated with variable doses of diuretics.

Nine Ile68Leu ATTRm patients with no neurological symptoms underwent a thorough neurological examination, with detection of a mild lower limb sensory impairment in five patients and mild mixed lower limbs sensory-motor impairment in two. Of the 18 unaffected mutation carriers examined by the neurologists, two were found to have mild lower limb sensory impairment and three had possible initial manifestations of CTS.

Of the three Ile68Leu ATTRm patients diagnosed by family screening, one (an 80-year-old male) had signs of cardiac involvement with previous heart failure hospitalization, the second was a 63-year-old female with mild symptomatic peripheral neuropathy, and the third was a 58-year-old male with mild symptomatic peripheral neuropathy, borderline echocardiographic findings and mild cardiac uptake (visual score = 1) on <sup>99m</sup>Tc-DPD scintigraphy.

Electrocardiographic findings are reported in Table 2. Twelve-lead ECG was normal in only 8% of Ile68Leu ATTRm patients. The ECG profile of ATTRwt patients was similar with the only exception of a slightly higher prevalence of atrial fibrillation (45% vs. 30%,  $P = 0.056$ ). In both groups, prevalence of low QRS voltages was observed in less than 30% of patients.

The main echocardiographic findings are reported in Table 3. No significant differences were present between Ile68Leu ATTRm and ATTRwt patients. Specifically, the mean value of left ventricular wall thickness ranged 15–19 mm (median 17 mm) and LVEF was  $\geq 40\%$  in 79% of Ile68Leu ATTRm patients.

Male and female Ile68Leu patients had a similar profile (online supplementary Tables S1–S3) with the only exception of a higher prevalence of CTS in males (50% vs. 20%,  $P = 0.039$ ) and a mildly older age at diagnosis in females (median 75 vs. 71 years).

The comparison between Ile68Leu ATTRm and Val122Ile ATTRm is summarized in Table 4.

## Estimation of penetrance

During the study period, 55 unaffected Ile68Leu mutation carriers were identified through family screening. Median age at first evaluation was 49 (43–56) years and 44% were males. The cumulative onset of symptomatic disease according to age is reported in Figure 1 and is consistent with a late and incomplete penetrance (50% at age 71).

**Table 3** Echocardiographic findings

	Ile68leu (n = 67)	ATTRwt (n = 82)	P-value
Diastolic IVS, mm, median (IQR)	17 (15–20)	17 (15–19)	0.850
Diastolic LV PW, mm, median (IQR)	16 (14–18)	15 (14–18)	0.744
Mean diastolic LV wall thickness, mm, median (IQR)	17 (15–19)	17 (15–19)	0.858
Mean diastolic LV wall thickness/BSA, median (IQR)	9 (8–10)	9 (8–10)	0.779
Left atrial diameter, mm, median (IQR)	47 (44–51)	47 (44–52)	0.940
LVEF, %, median (IQR)	51 (43–60)	50 (43–62)	0.777
LVEF <40%, n (%)	14 (21)	17 (21)	0.999
LVEF 40–49%, n (%)	14 (21)	17 (21)	
LVEF ≥50%, n (%)	39 (58)	48 (58)	
LVEDD, mm, median (IQR)	48 (45–51)	46 (42–50)	0.038
LVESD, mm, median (IQR)	35 (30–38)	32 (28–37)	0.109
LVEDD/BSA, median (IQR)	26 (24–27)	25 (23–27)	0.062
LVESD/BSA, median (IQR)	19 (16–21)	17 (16–21)	0.307
E-wave DT, ms, median (IQR)	165 (140–204)	168 (145–195)	0.900
Restrictive filling pattern, n (%)	23 (37)	26 (34)	0.683
Pericardial effusion, n (%)	32 (50)	39 (49)	0.940
LV mass, g/m <sup>2</sup> BSA, median (IQR)	208 (173–247)	208 (167–252)	0.923
AV valve thickening, n (%)	44 (69)	47 (59)	0.253
MCF, median (IQR)	13 (10–16)	13 (10–18)	0.847

ATTRwt, wild-type transthyretin amyloidosis; AV, atrioventricular; BSA, body surface area; DT, deceleration time; IQR, interquartile range; IVS, interventricular septum; LV, left ventricular; LVEDD, left ventricular end-diastolic diameter; LVEF, left ventricular ejection fraction; LVESD, left ventricular end-systolic diameter; MCF, myocardial contraction fraction; PW, posterior wall.

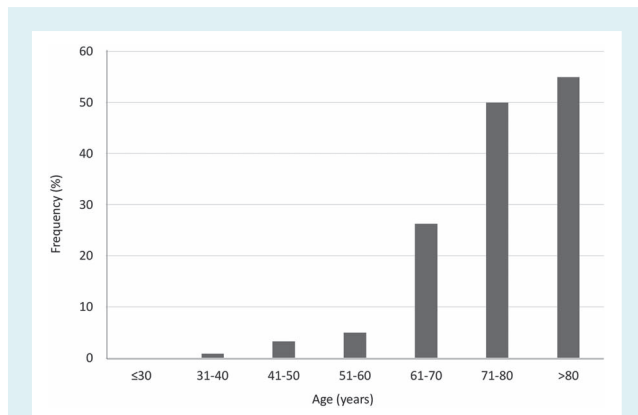
No comparison is associated with statistical significance after Bonferroni correction.

**Table 4** Comparison between Ile68Leu TTR patients from our study vs. Val122Ile TTR patients from the THAOS registry<sup>12</sup>

	Ile68Leu (n = 67)	Val122Ile (n = 91)
Men, n (%)	52 (78)	69 (76)
Ethnic descent	Caucasian 100%	African 87%
Age at diagnosis, years, median (IQR) or mean ± SD	71 (65–78)	69 ± 10
BMI, kg/m <sup>2</sup> , median (IQR) or mean ± SD	25 (23–27)	28 ± 6
Heart rate, b.p.m., median (IQR) or mean ± SD	75 (65–80)	80 ± 14
SBP, mmHg, median (IQR) or mean ± SD	120 (110–132)	112 ± 17
DBP, mmHg, median (IQR) or mean ± SD	80 (70–80)	69 ± 11
NYHA class III–IV, n (%)	18 (27)	42/76 (55)*
NT-proBNP, pg/mL, median (IQR)	3287 (1745–5658)	2734 (2307–4467)
Carpal tunnel syndrome, n (%)	29 (43)	16/55 (29)*
Atrial fibrillation, n (%)	20 (30)	14/27 (52)*
Low QRS voltages, n (%)	18 (29)	22/48 (46)*
Any infarct pattern, n (%)	38 (60)	NA
Ischaemic pattern (negative T waves), n (%)	28 (44)	NA
Normal ECG, n (%)	5 (8)	NA
Diastolic IVS, mm, median (IQR) or mean ± SD	17 (15–20)	17 ± 4
Diastolic LV PW, mm, median (IQR) or mean ± SD	16 (14–18)	17 ± 4
LVEF, %, median (IQR) or mean ± SD	51 (43–60)	51 ± 11
Restrictive filling pattern, n (%)	23 (37)	NA
Pericardial effusion, n (%)	32 (50)	NA
AV valve thickening, n (%)	44 (69)	NA
MCF, median (IQR) or mean ± SD	13 (10–16)	16 ± 11

AV, atrioventricular; BMI, body mass index; DBP, diastolic blood pressure; ECG, electrocardiogram; eGFR, estimated glomerular filtration rate; IQR, interquartile range; IVS, interventricular septum; LV, left ventricular; LVEF, left ventricular ejection fraction; MCF, myocardial contraction fraction; NA, not available; NT-proBNP, N-terminal pro brain natriuretic peptide; NYHA, New York Heart Association; PW, posterior wall; SBP, systolic blood pressure; SD, standard deviation; TTR, transthyretin.

\*Percentage related to the subgroup of patients with available data.



**Figure 1** Cumulative onset of symptomatic disease according to age in Ile68Leu carriers.

## Outcome

Follow-up data were available for all patients. Median and mean follow-up were 34 (18–60) and  $43 \pm 8.3$  months, respectively. Survival estimated by Cox analysis for Ile68Leu ATTRm patients was 92% at 1 year, 59% at 3 years, and 37% at 5 years. Although unadjusted estimated survival was poorer in ATTRwt patients (91% at 1 year, 54% at 3 years, and 31% at 5 years; *Figure 2A*), no difference was present after adjustment for age (*Figure 2B*). At multivariate analysis, the only variables associated with death were older age at diagnosis and NYHA class III/IV at presentation (*Table 5*). To explore the prognostic role of the Ile68Leu mutation itself, a stepwise model was constructed using the same covariates in addition to a forced entry covariate (presence of Ile68Leu). The presence of Ile68Leu mutation was not significantly associated with survival (hazard ratio 0.90, 95% confidence interval 0.59–1.38;  $P = 0.641$ ).

During follow-up, 33 Ile68Leu ATTRm patients were hospitalized for heart failure, 12 developed atrial fibrillation, and 7 developed advanced atrioventricular block requiring a permanent pacemaker. All patients with atrial fibrillation were treated with anticoagulation. No patients received treatment with disease-modifying drugs or underwent cardiac and/or liver transplant.

No patients with a cardiac phenotype developed neurological symptoms. Of the three patients with a neurological phenotype, one died due to causes unrelated to amyloidosis and the other two were alive at the end of follow-up with no changes in phenotype. None of the unaffected mutation carriers developed signs and/or symptoms of the disease.

## Discussion

Our study provides the first detailed characterization of the phenotype and outcome of patients with Ile68Leu ATTRm and expands our knowledge on so-called FAC. The mutation is essentially ‘cardiogenic’, leading to an exclusively or mainly cardiac phenotype almost in all patients. Although Ile68Leu appears to be endemic in central-northern Italy, particularly in the Apennines of Tuscany and Romagna (an area of approximately 15 000 km<sup>2</sup>; online supplementary *Figure S1*), its phenotype is very similar to both ATTRwt and

Val122Ile ATTRm, that has been previously described in patients of African descent.<sup>12</sup>

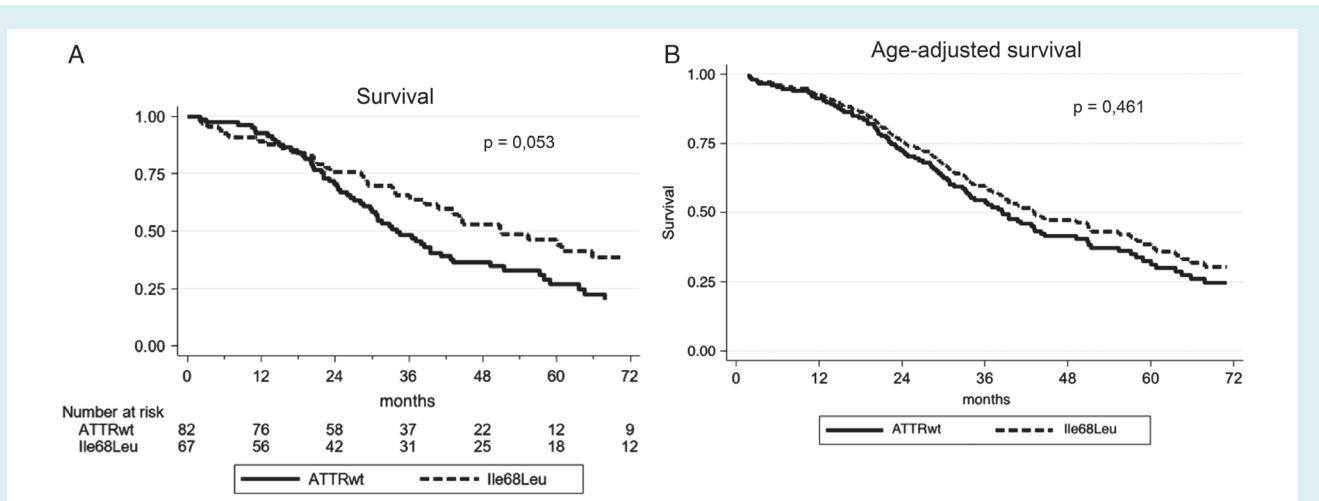
The typical Ile68Leu ATTRm patient is Caucasian, older than 70 years, seeks medical attention with heart failure symptoms, and receives the correct diagnosis of AC almost 1 year after the onset of these symptoms. In almost 45% of cases, a diagnosis of CTS precedes the diagnosis of AC by 5–7 years. At presentation, about 20% of patients admitted (mild) neurological symptoms that caused some walking difficulty. However, in the subset of neurologically asymptomatic patients who underwent a comprehensive neurological examination ( $n = 9$ ), seven were found to have mild lower limb sensory-motor abnormalities. The overall clinical picture mimics that of ATTRwt, with the only exceptions of a slightly younger age at diagnosis (median age 71 vs. 78 years) and a higher frequency of (mild) neurological symptoms alongside heart failure.

Although not all the necessary data to calculate mutation penetrance were available, cumulative disease onset according to age (*Figure 1*) reaches a plateau (around 50%) only after the seventh decade of life. This can partially explain why patients only rarely perceive (and therefore report) a family history of cardiomyopathy and/or heart failure.

A high frequency of pseudo-infarct pattern and intraventricular conduction delay, including left anterior hemiblock and bundle branch block (*Table 2*), characterized the 12-lead ECG of Ile68Leu patients. It should be noted that low QRS voltages were present only in 29% of cases, but the voltage/mass ratio was consistently low and only 8% of patients had a normal ECG. We confirm that the imbalanced voltage/mass ratio is a distinctive element of TTR-related AC and is a potentially useful tool for differential diagnosis with hypertrophic cardiomyopathy and hypertensive heart disease. No significant differences emerged from the comparison with ATTRwt patients, with the exception of a higher prevalence of atrial fibrillation at diagnosis, which is probably related to older age.

The echocardiographic profile was homogeneous including a non-dilated left ventricle, symmetric increase in left ventricular wall thickness, normal or near normal LVEF (*Table 3*). Left ventricular hypertrophy was asymmetric (septum/posterior wall thickness ratio > 1.5) only in 3/67 patients, and LVEF was often at the lower end of the normal spectrum. Interestingly, the prevalence of a mild pericardial effusion and atrioventricular valve thickening (potentially useful red-flags for the differential diagnosis with hypertrophic cardiomyopathy) was high (50% and 69%, respectively). Only one third of patients had Doppler signs of a restrictive pathophysiology. As for the clinical and electrocardiographic profile, the echocardiographic phenotype of Ile68Leu ATTRm was indistinguishable from that of ATTRwt.

Our data confirm the gender imbalance in patients with TTR-related AC that has already been observed in previous studies and led to the hypothesis of a protective or delaying effect of female sex for myocardial involvement in these patients<sup>19–21</sup> (online supplementary *Tables S1–S3*). Notably, male prevalence was higher amongst affected patients (78%) than in unaffected mutation carriers (44%). Median age of affected females was 4 years older than males at presentation. The severity of AC tended to be milder in females with a lower left ventricular wall thickness and a lower prevalence of restrictive filling pattern. It is



**Figure 2** Kaplan–Meier survival curve (A) and Cox age-adjusted survival (B) in Ile68Leu ATTRm and ATTRwt patients. ATTRm, transthyretin-related hereditary amyloidosis; ATTRwt, wild-type transthyretin amyloidosis.

**Table 5** Univariate and multivariate analysis for survival in Ile68Leu ATTRm patients

	Univariate		Multivariate	
	Hazard ratio (95% CI)	P-value	Hazard ratio (95% CI)	P-value
Age at diagnosis (for each 1-year increase)	1.07 (1.02–1.12)	<b>0.002</b>	1.07 (1.03–1.11)	<b>0.002</b>
Male gender	0.89 (0.39–2.07)	0.793		
Autonomic involvement	0.59 (0.14–2.46)	0.467		
Atrial fibrillation	1.77 (0.89–3.52)	<b>0.104</b>		
LVEF	0.97 (0.94–1.0)	<b>0.030</b>		
MCF	0.97 (0.92–1.03)	0.316		
NYHA class III/IV	3.71 (1.9–7.24)	<b>&lt;0.001</b>	4.01 (1.98–8.16)	<b>0.0001</b>
Heart rate	1.00 (0.98–1.02)	0.824		
IVS thickness	1.07 (0.97–1.18)	<b>0.156</b>		
eGFR	0.97 (0.95–0.99)	0.001*		
NT-proBNP	1.00 (1.00–1.00)	0.426		
Systolic blood pressure	0.98 (0.96–1.00)	<b>0.067</b>		
Restrictive filling pattern	1.35 (0.68–2.70)	0.386		
Low QRS voltage	1.02 (0.51–2.06)	0.954		

ATTRm, transthyretin-related hereditary amyloidosis; CI, confidence interval; eGFR, estimated glomerular filtration rate; IVS, interventricular septum; LVEF, left ventricular ejection fraction; MCF, myocardial contraction fraction; NT-proBNP, N-terminal pro brain natriuretic peptide; NYHA, New York Heart Association.

In bold, variables considered in the multivariate model.

\*Not considered in the multivariate model for missing data.

also worth noting that initial symptoms were neurological in 2/15 females but only in 1/52 males.

An analogy similar to the one between Ile68Leu ATTRm and ATTRwt found in this study emerges when comparing Ile68Leu ATTRm to Val122Ile ATTRm patients reported in the THAOS registry.<sup>12</sup> Specifically, they share the advanced age at presentation, a high prevalence of CTS, and an echocardiographic phenotype with symmetric hypertrophy and a normal or near normal LVEF.

More in general, our findings confirm the existence of a close genotype–phenotype correlation in TTR-related amyloidosis, with some forms (due to Ile68Leu and Val122Ile in particular) associated

with a predominantly cardiac involvement. The exact mechanism that starting from different mutations leads to the infiltration of specific organs, however, remains unclear and it cannot be excluded that local tissue factors are also involved, along with a central commitment represented by the mutation.

The natural history of Ile68Leu ATTRm is characterized by high mortality (41% at 3 years and 63% at 5 years) with no significant difference from ATTRwt after age adjustment of the Kaplan–Meier curves (Figure 2). Notably, Val122Ile ATTRm patients have been shown to have a worse outcome than ATTRwt patients<sup>22</sup>; this reinforces the hypothesis<sup>23</sup> that both biological and social factors

(including a worse access to care) can explain outcome of Val122Ile African American patients in the USA.

Age at diagnosis and advanced heart failure symptoms at presentation were the only factors independently associated with mortality in Ile68Leu ATTRm. However, our search for risk factors was limited by missing laboratory data and due to this limitation it was not possible to stratify patients' survival according to the scores proposed by Grogan *et al.*<sup>24</sup> and Gillmore *et al.*<sup>25</sup>

## Limitations

Although this is the largest study describing the Ile68Leu TTR mutation, the absolute number of patients is limited, as is that of the family members who were evaluated. Genetic analysis was limited to the TTR gene, while haplotype analysis (that could have demonstrated a common founder effect) was not carried out. Only standard echocardiographic data were analysed in each patient, whereas tissue Doppler, strain and right ventricular function were not systematically recorded due to the long recruitment period. The same limitations apply to N-terminal pro brain natriuretic peptide, that was collected only in the last 13 years of the study, as was troponin. The possible phenotypic evolution during the natural history of the disease has been only partially captured due to the study design. Finally, while survival data were accurately collected for all patients, the exact cause of death was not systematically recorded.

## Clinical implications

The Ile68Leu TTR mutation is a cause of FAC that has not been systematically described to date and leads to a phenotype similar to ATTRwt. Awareness of these findings is essential for a differential diagnosis with late-onset hypertrophic cardiomyopathy and can appropriately guide the choice of medical treatment.

## Supplementary Information

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

**Figure S1.** Geographic origin of Ile68Leu patients.

**Table S1.** Baseline characteristics of Ile68Leu ATTRm patients according to gender.

**Table S2.** Electrocardiographic findings among Ile68Leu ATTRm patients according to gender.

**Table S3.** Echocardiographic findings among Ile68Leu ATTRm patients according to gender.

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