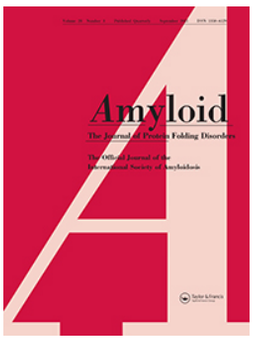

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Neurological involvement in Ile68Leu (p.Ile88Leu) ATTR amyloidosis: not only a cardiogenic mutation

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ABSTRACT

Background: Ile68Leu transthyretin-related amyloidosis (ATTR) is known as a mainly or exclusively cardiogenic variant. We hypothesized that an accurate specialized neurological evaluation could reveal a consistent frequency of mixed phenotypes.

Methods: Forty-six consecutive subjects with transthyretin (TTR) Ile68Leu (p.Ile88Leu) mutation (29 patients and 17 unaffected carriers) underwent an in-depth cardiac and neurologic evaluation at a single center.

Results: All 29 patients showed cardiac involvement. In 20 (69%) cases, it was associated with neurological abnormalities (i.e. a mixed phenotype): 10 (35% of the total) had signs and symptoms of neuropathy, 5 (17%) had abnormalities at the neurologic specialist examination but without symptoms, and 5 (17%) had abnormal nerve conduction study only. None of the asymptomatic carriers showed neurological abnormalities or cardiac involvement. The Neuropathy Impairment Score was > 5 in seven patients at baseline, and became >5 in six more patients during follow-up. The probability of experiencing a major adverse cardiac event (MACE) during follow-up was higher in the mixed than cardiologic phenotype ($p = 0.026$). Age and phenotype were independent prognostic predictors of MACE.

Conclusion: At least two-thirds of patients with Ile68Leu ATTR and amyloidotic cardiomyopathy show an associated – definite or probable – neurologic impairment of variable degree if accurately evaluated in a neurologic setting. This proportion can rise during follow-up. The mixed phenotype carries a worse prognosis compared to the exclusively cardiologic one. These observations show that more patients could be eligible for treatment with gene silencers than currently indicated and highlight the need for an in-depth and continuous multidisciplinary evaluation of Ile68Leu ATTR patients.

Abbreviations: ATTR: transthyretin-related amyloid protein; ATTRwt: wild-type transthyretin-related amyloid protein; ATTRv: hereditary transthyretin-related amyloid protein; AV: atrio-ventricular; CA: cardiac amyloidosis; CMAP: compound motor action potential; CTS: carpal tunnel syndrome; CV: conduction velocities; ECG: electrocardiography; ECHO: echocardiography; HRV: heart rate variability; IQR: interquartile range; LV: left ventricule; LVEF: left ventricular ejection fraction; MACE: major adverse cardiac event; MCV: motor conduction velocity; NCS: nerve conduction studies; NIS: Neuropathy Impairment Score; RLS: restless leg syndrome; SCV: sensory conduction velocity; SD: standard deviation; SNAP: sensory nerve action potential; SSR: sympathetic skin response; TTR: transthyretin

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Amyloidosis; transthyretin; cardiomyopathy; Ile88Leu; polyneuropathy

Introduction

During the last decade, the number of diagnosed cases of ATTR amyloidosis has progressively increased worldwide, for both the wild type (ATTRwt) and hereditary ATTR (ATTRv) [1,2]. The reasons are many but mainly relate to three factors: the availability of a non-invasive probe ('bone scintigraphy') that makes biopsy unnecessary in many cases with cardiac involvement, and the growing of the culture of this disease that has favored the exchange of experiences between different specialists, namely cardiologists and neurologists [3,4]. Another important reason, especially in the neurology field, is the recognition of the presence of late-onset cases from areas other than conventional endemic foci [5].

Genotype is one of the main determinants of phenotypic expression of ATTRv and a strong genotype–phenotype

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correlation has been identified among the 130 pathogenic mutations already identified [6–8]. Indeed some mutations – typically early onset Val30Met (p.Val50Met) – are associated with a mainly or exclusively neurologic phenotype, others with a mixed (neurologic and cardiac) phenotypic expression, and still others with a predominantly or even exclusively cardiac phenotype [7,9]. Among the latter, Val122Ile (p.Val142Ile), typical of black Americans, and Ile68Leu (p.Ile88Leu), seen especially in Italy and Southern Europe, have been studied in detail and are generally considered as typical examples of 'cardiogenic mutations' with a phenotype very close to that of ATTRwt amyloidosis [6,10–16].

However, a precise classification of ATTRv patients into phenotypes may not be so simple. Neurologic signs and symptoms, for instance, can be eclipsed by the dominant clinical picture of congestive heart failure and emerge only at an accurate neurologic specialist visit. Vice versa, a dominant neurologic profile can hide the cardiac involvement (or prevent noting its absence) at the time of diagnosis [17]. However, not all centers that evaluate patients with amyloidosis can provide the patient with both specialized cardiac and specialized neurologic skills.

The identification of a neurologic involvement hidden within a mainly cardiac phenotype is now becoming of paramount importance to have access to new gene silencer treatments like Patisiran and Inotersen, that regulatory authorities specifically indicate (and reimburse) only for patients with neurologic involvement [18,19].

In previous years, we had the opportunity to diagnose and follow a relatively large number of cases with Ile68Leu ATTRv amyloidosis considered as a prototype of cardiogenic mutation and endemic in our country [9]. Taking advantages of the simultaneous presence of cardiologists and neurologists at our clinic, we aimed in this study to characterize in detail both the cardiologic and neurologic components of the phenotype expression of Ile68Leu mutation – including outcomes and prognosis – in a wide cohort of asymptomatic and symptomatic patients.

Materials and methods

Study patients

We studied all patients with Ile68Leu (p.Ile88Leu) ATTRv seen at a single Italian Center (Bologna University) between January 1993 and December 2019. Data were extracted from a dedicated prospective local database that included both cardiological and neurologic baseline characteristics and follow-up data.

The main clinical/instrumental baseline characteristics – including symptoms at disease onset, main laboratory tests, cardiac and neurological assessments, electrocardiographic (ECG), echocardiographic (ECHO) and nerve conduction study (NCS) measurements – were analyzed. The study was approved by the local Ethics Committee.

Diagnostic criteria, definitions, and classifications

ATTR cardiomyopathy was defined as follows: interventricular septum thickness $\geq$12 mm at echocardiography in the absence of other causes of ventricular hypertrophy and associated with at least one of the following:

1. immunohistochemical evidence of TTR deposits in a tissue biopsy;
2. non-invasive documentation of intense cardiac uptake (visual score 2 or 3) on bone-tracer scintigraphy (99mTc-DPD or 99mTc-HMDP) [20];
3. exclusion of monoclonal protein in serum and urine samples [21].

Phenotype was defined as 'cardiac' if only cardiomyopathy (with/without cardiac symptoms) was present and 'neurologic' if signs and/or symptoms of sensory/motor peripheral nervous system involvement were detected at neurologic examination or if abnormal findings were detected at NCS. Phenotype was considered 'mixed' in the case of cardiac amyloidosis (CA) coexistent with sensory/motor neurologic involvement.

Autonomic and carpal tunnel syndrome (CTS) signs or symptoms were not considered as an expression of neurologic involvement.

Electrocardiographic evaluation

Standard definitions were used for the interpretation of 12-lead ECGs [22]. 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 [23]. QT prolongation was defined as QTc >450 ms in males and >470 ms in females.

Echocardiographic evaluation

Echocardiographic images were obtained from the standard parasternal long-axis, parasternal short-axis, apical and subcostal views. Chamber volumes and left ventricular ejection fraction (LVEF) quantification was performed according to the recommendations of the American Society of Echocardiography [24]. Left ventricular (LV) mass, diameters, and wall thickness were assessed by M-mode. Patterns of hypertrophy were defined as previously described [25]. A restrictive filling pattern was defined as E wave deceleration time <150 ms and E/A ratio >2.5 on trans-mitral pulsed Doppler.

Neurologic evaluation

All patients underwent a complete neurological examination, paying special attention to any signs and symptoms of peripheral nervous system involvement or dysautonomia. The neurological examination was always performed by the same specialist (F. S.).

For clinical assessment the Neuropathy Impairment Score (NIS) was used in accordance with recent international

therapeutic trials [18,19]. The NIS score ranges 0–244, with higher scores indicating greater impairment and a 2-point change considered the minimal clinically important difference [26–28]. The Andrade's classical score was also considered [29].

Autonomic involvement was defined by the presence of orthostatic hypotension (decline in systolic blood pressure >20 mmHg or >10 mmHg in diastolic blood pressure upon standing), impotence, anhidrosis, urinary incontinence, or gastrointestinal symptoms (diarrhea, constipation, fecal incontinence). CTS history was considered present when typical symptoms or previous surgery for median nerve decompression were reported.

The neurophysiological examination, including nerve conduction and autonomic function study, was performed as follows. Nerve conduction velocities (CV) were investigated by surface electrodes using standardized techniques. Antidromic sensory nerve action potential (SNAP) peak to peak amplitude and sensory conduction velocity (SCV) of the median, ulnar and sural nerves were recorded bilaterally. Compound motor action potential (CMAP) peak to peak amplitude, motor conduction velocity (MCV) and F waves of the median, ulnar, posterior tibial and peroneal nerves were recorded bilaterally as described elsewhere [30]. Values obtained for each nerve from each patient, were compared with our own normative laboratory values (mean ± 2 standard deviations), based on an age- and sex-matched population (Supplementary Table 1). F waves minimal latencies are assessed by previously published height-latency normative data and normalized for age (> 0,5 msec per decade, after 40 years) [31]. For diagnosing 'peripheral neuropathic involvement', we considered the ulnar nerve at upper limbs, the posterior tibial and peroneal nerves and the sural nerves. A study of autonomic function, including sympathetic skin response (SSR), heart rate variability (HRV), Valsalva maneuver and stand up test was performed using standard techniques [32].

Genotyping

TTR gene analysis was carried out in all subjects. 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, Takara Sake USA Inc., Berkeley, CA) using primers previously described [33]. Amplified DNA fragments were directly sequenced using the ABI Prism 3130 automated sequence.

Follow-up

Follow-up terminated on 30 May 2020. Data were obtained from the last visit or by telephone interview in those who had not made any contact in the previous 6 months.

All major adverse events were recorded and the first occurrence of any one was identified as follows:

- major adverse cardiac event (MACE), defined as: death, new onset of atrial fibrillation, pacemaker implantation, or hospitalization for heart failure;
- neurologic event, defined as a change of at least one 'stage' in Andrade's score [29].

Statistical analysis

Continuous variables were expressed as mean ± standard deviation (SD) or median and interquartile range (IQR) and categorical variables as number of patients and percentage. The clinical and instrumental differences between the subtypes of cardiac amyloidosis were analyzed using the Student *T*-test for continuous variables normally distributed, Wilcoxon rank-sum test for continuous variables non-normally distributed, and χ^2-test for categorical variables. Kaplan–Meier curves were reported to graphically analyze the overall occurrence of MACE; the Log-rank test was used to compare freedom from events between subgroups. Univariate logistic regression analysis tested the association between baseline variables and the outcome of interest (MACE). Non-correlated variables with a p value <0.1 at univariate analysis were considered in the multivariate analysis. A multivariable logistic regression analysis was carried out to test the independence of the outcome determinants previously identified. A p-value <0.05 (2-sided) was considered significant. Results were reported as odds ratio (95% confidence interval and p-values). Risk differences between patients' phenotypes were estimated and reported together with 95% confidence interval and p-values.

All analyses were carried out by MM, GF and MS using R 3.6 (R Foundation for Statistical Computing, Vienna, Austria) and Stata/SE 16 for Windows (StataCorp LLC, College Station, TX).

Results

Baseline clinical characteristics

We identified a total of 46 Caucasian individuals from thirty unrelated families with heterozygote Ile68Leu (p.Ile88Leu) mutation of the TTR gene, enrolled from 24 November 2004 to 26 November 2019. Twenty-nine were affected patients and 17 were unaffected carriers.

Baseline characteristics, clinical, ECG, and echocardiographic findings of the two groups are presented in Table 1. The overall cardiac profile (demographic, clinical and instrumental) of the affected patients was as previously reported [9] and typical of ATTR cardiomyopathy.

Neurologic clinical findings

None of the asymptomatic carriers showed any neurological involvement. Of the 29 affected patients, few patients spontaneously declared having neurologic problems, but when specifically interviewed, 10 reported symptoms suggesting peripheral nerve involvement: tingling paresthesia and numbness in the hands and feet ($n = 5$), burning paresthesia of lower limbs ($n = 2$), muscle cramp ($n = 1$). Restless Leg Syndrome (RLS) was reported by 6 patients. Eight patients reported symptoms related to dysautonomia: anhidrosis of

Table 1. Baseline characteristic of the population according to the presence of signs or symptoms of ATTRm.

	Affected population $n = 29$	Unaffected carriers $n = 17$
Age at diagnosis – years, mean ± sd	63.95 ± 11.53	50.89 ± 12.65
Males, *n* (%)	20 (69)	5 (29)
BMI – kg/mq, mean ± sd	25.83 ± 2.99	26.20 ± 6.31
HR – bpm, mean ± sd	73.21 ± 10.87	72.79 ± 11.81
SBP – mmHg, mean ± sd	124.17 ± 17.11	137.31 ± 15.76
DBP – mmHg, mean ± sd	76.04 ± 10.00	84.62 ± 8.03
NYHA at diagnosis		
I, *n* (%)	15 (52)	17 (100)
II, *n* (%)	12 (41)	0 (0)
III, *n* (%)	2 (7)	0 (0)
eGFR (CKD-EPI), mean ± sd	65.70 ± 17.47	86.75 ± 11.35
CTS history, *n* (%)	13 (45)	2 (12)
Baseline EKG findings		
Abnormal EKG, *n* (%)	20 (69)	4 (24)
Atrial fibrillation/Flutter, *n* (%)	7 (24)	1 (6)
First degree AV block, *n* (%)	6 (21)	0 (0)
Echocardiographic findings		
LVEF – %, mean ± sd	55.92 ± 12.83	68.31 ± 6.03
IVS wall thickness – mm, mean ± sd	17.04 ± 3.36	9.31 ± 1.55
LV indexed mass – g/mq, mean ± sd	209.68 ± 70.40	95.87 ± 32.02
LA volume – ml/mq, mean ± sd	45.04 ± 7.29	38.18 ± 6.21
Restrictive pattern, *n* (%)	7 (29)	0 (0)
Pericardial effusion, *n* (%)	10 (42)	0 (0)
Perugini score 2–3, *n* (%)	21/21 (100)	0/8 (0)
NT-proBNP – pg/ml, mean ± sd	2,243.67 ± 985.30	–
Follow-Up		
Follow up, months, mean ± sd	30.25 ± 25.52	25.83 ± 26.97
Patients with MACE, *n* (%)	8 (28)	0 (0)
Death, *n* (%)	3 (10)	0 (0)
HF hospitalization, n (%)	5 (17)	0 (0)
Advanced AV block, *n* (%)	2 (7)	0 (0)
AF onset, *n* (%)	5 (17)	0 (0)

BMI: body mass index; HR: heart rate. SBP: systolic blood pressure; DBP: diastolic blood pressure; NYHA: New York Heart Association; eGFR: estimated glomerular filtration rate; CTS: carpal tunnel syndrome; EKG: electrocardiogram; AV: atrioventricular; EDLV: end-diastolic left ventricular; BSA: body surface area; LVEF: left ventricular ejection fraction; IVS: interventricular septum; PW: posterior wall; LV: left ventricular; LA: left atrial; BNP: brain natriuretic peptide; MACE: major adverse cardiac events.

palms and soles ($n = 5$), impotence ($n = 5$), orthostatic hypotension ($n = 2$).

The neurological examination revealed signs of peripheral nerve involvement in 15 patients, including reduced vibratory sensation ($n = 14$), superficial sensory loss in the distal portion of the limbs affecting light touch and pinprick ($n = 9$), and reduced deep tendon reflexes ($n = 8$). The Romberg test was positive in eight patients, while only one patient presented ataxic gait. A mild reduction of strength in the dorsiflexion of the feet was present only in three patients.

The baseline NIS score for the 20 patients with abnormal neurologic signs or symptoms was calculated (Figure 1). Notably, the overall mean score was 5.50 ± 7.84 and seven patients exceeded 5, i.e. the criterion for neurologic involvement in the APOLLO study [17].

Alternative causes of neurologic abnormalities (including diabetes and chronic inflammatory disease) were ruled out on a clinical basis in each patient. Spinal cord MRI is not part of the routine assessment of our patients. Anyway, MRI was performed in selected cases where the suspicion of spinal lumbar stenosis was particularly high.

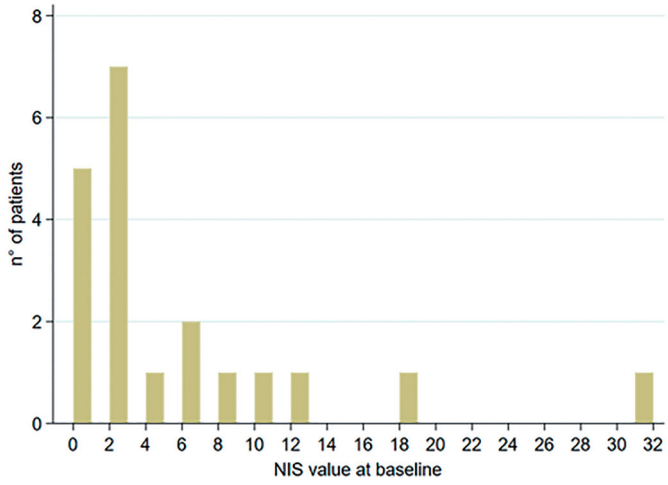

Figure 1. NIS distribution at baseline in patients with neurological involvement. NIS: Neuropathy Impairment Score.

Neurophysiological evaluation

Of the 46 participants, 33 (19 males, mean age 64 years, range 38–81; 14 females, mean age 60 years, range 39–86)

underwent a baseline neurophysiological evaluation (Supplementary Table 2); 16 repeated the neurophysiological study two to four times during follow-up. Thirteen individuals were not available for neurophysiological assessment, due to clinical conditions and/or logistic issues (heart failure in two patients, geographical issues in the others).

In 14 of 33 cases evaluated, signs of monolateral or bilateral CTS according to international diagnostic guidelines were present [33].

NCS signs of peripheral neuropathic involvement were identified in 10/33 cases, consisting of reduced CMAP amplitudes of the peroneal and tibial nerves (from 25% to 80% of the reference values) and a mild (less than 25% of the reference values) but diffused increment of F wave latencies at upper and lower limbs. F waves were absent in the lower limbs in three cases. Distal motor latencies were within the norm in all individuals. SNAP amplitudes of the sural nerve were reduced (by up to 50% with respect to reference values) in only in three cases.

Of the 10 cases with abnormal NCS data, 5 were asymptomatic (NIS = 0). The other 5 cases had NIS values between 2 and 32 (mean 4.4).

The autonomic tests were abnormal in 8/33 individuals. SSR was absent at palms and soles in five cases. HRV and Valsalva maneuver were reduced in five cases. Three individuals presented orthostatic hypotension, asymptomatic in one case.

Phenotypic classification and characterization

All 29 patients showed cardiac involvement. In 20 (69%) patients, it was associated with neurological abnormalities: 10 (35%) had signs and symptoms of neuropathy, 5 (17%) showed abnormalities at the specialist neurologic examination but were asymptomatic, and 5 (17%) had abnormal NCS only (Figure 2). On the basis of clinical and NCS data and their evolution in time, we considered the diagnosis of neuropathy as 'definite' (clear neurologic abnormalities at baseline) in 18 patients and 'probable' in 2. These two cases had only reduced vibratory sensation at baseline but showed abnormalities at NCS during follow-up (Supplementary Table 3).

We included all the 20 patients in the 'mixed phenotype' subgroup. Hence, the phenotype was deemed 'exclusively cardiac' in 9 (31%) patients and "mixed" in 20 (69%). No patient showed an isolated neurological involvement.

Clinical and neurophysiological characteristics of mixed phenotype patients are summarized in Supplementary Table 2.

Baseline clinical, demographic, and cardiac characteristics of the two phenotypes are reported and compared in Table 2. Notably only a few ECG abnormalities and NT-proBNP plasmatic concentration were, or tended to be, significantly different between the two phenotypes.

Outcomes and prognostic stratification

Mean follow-up for affected patients was 30.25 ± 25.52 months. During this period, eight patients (28%) experienced a MACE: five (17%) were hospitalized at least once for heart failure, two (7%) developed advanced atrio-ventricular (AV) block, five (17%) had at least an episode of atrial fibrillation that required medical attention, and three (10%) patients died. No major neurologic event or progression to a more advanced Andrade's score stage occurred.

The probability of experiencing a MACE during follow-up was higher in the mixed than cardiac-only phenotype ($p = 0.026$) (Supplementary Table 4) and it could occur throughout the entire follow-up period. In fact, MACE occurred in 8 (40%) patients with mixed phenotype versus 0 (0%) patients with cardiac-only phenotype (Supplementary Figure 1).

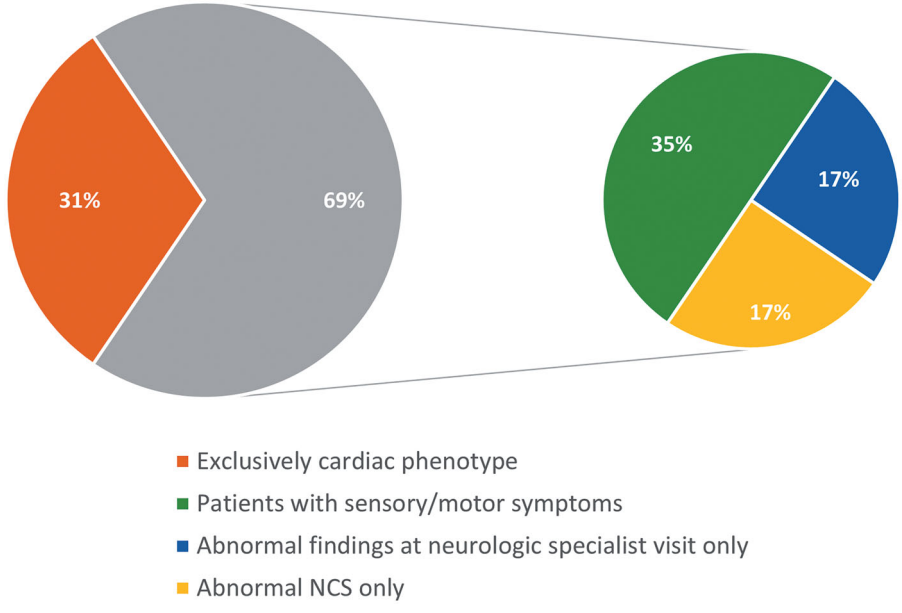

Figure 2. Distribution of patients by phenotype. NCS: nerve conduction studies.

Table 2. Baseline characteristic of patients according to phenotypic classification.

	Exclusively cardiologic phenotype $n = (9)$	Neurologic and cardiologic phenotype $n = (20)$	p value*
Age at diagnosis – years, mean ± sd	58.99 ± 14.26	66.18 ± 9.66	0.12
Males, – (%)	4 (44)	16 (80)	**0.056**
BMI – kg/mq, mean ± sd	25.86 ± 3.46	25.83 ± 2.99	0.98
HR – bpm, mean ± sd	72.60 ± 9.76	73.37 ± 11.39	0.89
SBP – mmHg, mean ± sd	134.00 ± 19.49	121.58 ± 15.99	0.15
DBP – mmHg, mean ± sd	81.00 ± 5.48	74.74 ± 10.60	0.22
NYHA at diagnosis			0.62
I, *n* (%)	5 (56)	10 (50)	
II, *n* (%)	4 (44)	8 (40)	
III, *n* (%)	0 (0)	2 (10)	
eGFR (CKD-EPI), mean ± sd	54.67 ± 17.21	67.65 ± 17.28	0.25
CTS history, *n* (%)	2 (22)	11 (55)	0.10
Baseline EKG findings	*n* = 7	*n* = 19	
Abnormal EKG, *n* (%)	4 (44)	16 (80)	**0.056**
Atrial fibrillation/Flutter, *n* (%)	0 (0)	7 (35)	**0.042**
First degree AV block, *n* (%)	1 (11)	5 (25)	0.39
QTc – ms, mean ± sd)	413.83 ± 8.23	461.12 ± 35.81	**0.005**
Total QRS score – mV, median (IQR)	87.5 (79–98)	126 (106–138)	**0.013**
Low voltages criteria, *n* (%)	1 (14)	3 (16)	0.92
Pseudonecrosis pattern, *n* (%)	1 (14)	12 (63)	**0.027**
LBBB, *n* (%)	1 (14)	2 (11)	0.79
RBBB, *n* (%)	0 (0)	2 (11)	0.37
Echocardiographic findings	*n* = 5	*n* = 19	
EDLV/BSA – ml/mq, mean ± sd	44.60 ± 13.96	50.93 ± 14.78	0.44
ESLV/BSA – ml/mq, mean ± sd	15.90 ± 5.85	23.05 ± 9.93	0.18
LVEF – %, mean ± sd	63.00 ± 5.40	53.80 ± 13.73	0.13
IVS wall thickness – mm, mean ± sd	16.00 ± 2.55	17.30 ± 3.54	0.45
PW wall thickness – mm, mean ± sd	13.80 ± 1.79	15.60 ± 3.87	0.33
LV indexed mass – g/mq, mean ± sd	182.32 ± 60.04	215.15 ± 72.40	0.41
LA volume – ml/mq, mean ± sd	44.33 ± 10.98	45.25 ± 6.15	0.79
Valve thickening, *n* (%)	2 (40)	13 (68)	0.24
Restrictive pattern, *n* (%)	1 (20)	6 (32)	0.61
Pericardial effusion, *n* (%)	1 (20)	9 (47)	0.27
Perugini score – 2–3, *n* (%)	4/4 (100)	17/17 (100)	0.31
NT-proBNP – pg/ml, mean ± sd	3146.00 ± 689.59	1942.89 ± 899.38	0.063
Follow-up			
Follow up, months, mean ± sd	22.53 ± 29.76	33.72 ± 23.37	0.28
Patients with MACE, *n* (%)	0 (0)	8 (40)	**0.026**
Death, *n* (%)	0 (0)	3 (15)	0.22
HF hospitalization, *n* (%)	0 (0)	5 (25)	0.099
Advanced AV block, *n* (%)	0 (0)	2 (10)	0.33
AF onset, *n* (%)	0 (0)	5 (25)	0.099

BMI: body mass index; HR: heart rate; SBP: systolic blood pressure; DBP: diastolic blood pressure; NYHA: New York Heart Association; eGFR: estimated glomerular filtration rate; CTS: carpal tunnel syndrome; EKG: electrocardiogram; AV: atrio-ventricular; LBBB: left bundle branch block; RBBB: right bundle branch block; EDLV: end-diastolic left ventricular; ESLV: end-systolic left ventricular; BSA: body surface area; LVEF: left ventricular ejection fraction; IVS: interventricular septum; PW: posterior wall; LV: left ventricular; LA: left atrial; BNP: brain natriuretic peptide; MACE: major adverse cardiac events.
Bold identifies p values < 0.05.

Changes in NIS score of patients with neurologic abnormalities are reported in Figure 3. Six patients exceeded the threshold value of 5 during follow-up.

Univariate and multivariable logistic regression analyses are shown in Table 3. Notably, both older age and mixed phenotype (cardiac + neurological involvement) were independent predictors of MACE.

Discussion

Our study is the first to analyze in detail the neurological profile of patients with an Ile68Leu (p.Ile88Leu) mutation, and two main findings emerged: (1) within a TTR mutation, traditionally considered as the source of a (mainly or exclusively) cardiac phenotype, at least two-thirds of the affected patients showed an associated neurologic impairment when accurately evaluated in a neurologic setting and this proportion increased during follow-up; (2) this mixed phenotype carried a worse prognosis, at least in terms of MACE.

Neurologic involvement encompasses a broad spectrum of clinical and instrumental manifestations ranging from self-reported symptoms to symptoms evoked through a specifically conducted patient interview, to clinical signs that emerge only at the neurologist visit (including specific tests to explore both sensory and motor integrity), to findings revealed by the NCS (Figure 1). It should be noted that this high prevalence of neurologic involvement does not include autonomic alterations [which were present in 8 (27.5 %) of the affected patients and in particular in 2 patients (22%) of those with only cardiac phenotype]. Among our patients, 7 at baseline and a further 6 after 34.3 ± 23.6 months of follow-up had an NIS score $\geq$ 5 (the criterion for neurologic involvement used in the Apollo Study [18]).

These observations indicate the need for a different approach to Ile68Leu patients and highlight the importance of multidisciplinary evaluation including a detailed specialistic neurologic workup, even if the visit takes place in a cardiologic setting.

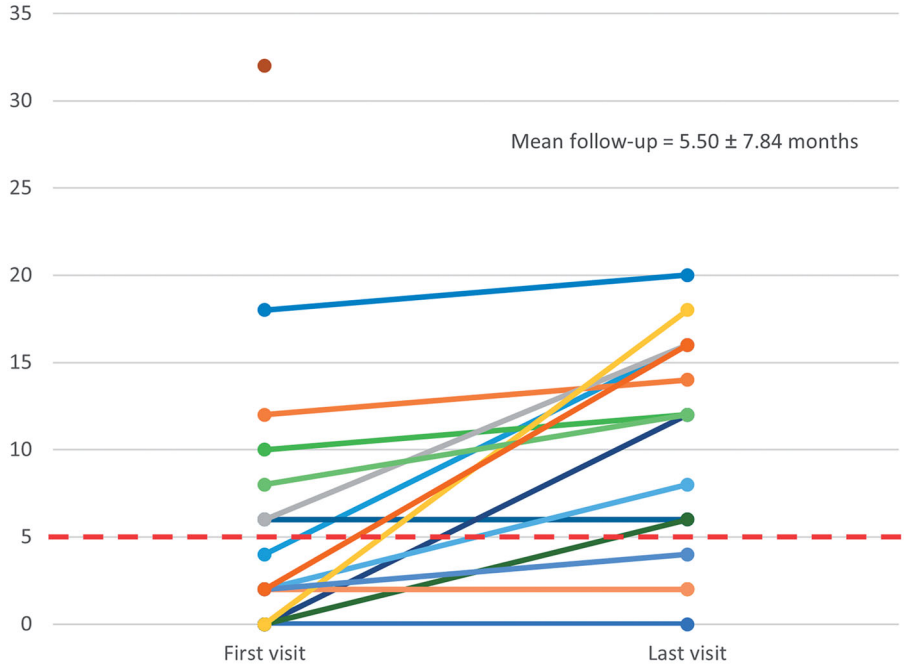

Figure 3. NIS value changes from first to last visit in patients with neurological involvement. NIS: Neuropathy Impairment Score.

Table 3. Univariate and multivariate analysis of the risk of MACE.

	Univariate			Multivariate		
	OR	95% CI	*p* value	OR	95% CI	*p* Value
Age at disease diagnosis	1.127	1.082–1.174	<0.001	1.132	1.074–1.192	<0.001
NYHA class II–III at diagnosis	2.215	1.284–3.82	0.004	1.09	0.544–2.182	0.808
QTc	1.023	1.014–1.032	<0.001	1.011	0.998–1.024	0.092
LVEF	0.959	0.938–0.981	<0.001	1.005	0.976–1.036	0.723
Mixed phenotype	12.779	4.709–34.677	<0.001	9.918	3.338–29.468	<0.001

NYHA: New York Heart Association; LVEF: left ventricular ejection fraction.

Few patients spontaneously report neurologic symptoms, and there is a risk that the neurologic involvement could escape notice as patients are generally referred to the cardiologist for severe heart failure or arrhythmias. Our patients do not show the typical small fiber painful neuropathy and the typical 'sensory dissociation' of Val30Met (p.Val50Met) patients [35]. The phenotypic characterization of the neurologic involvement (considering both baseline and follow-up) is a mild, non-painful, mainly sensory neuropathy. Impairment of vibratory sensation, superficial sensory loss in the distal portion of the limbs affecting light touch and pinprick, and loss of deep tendon reflexes are frequent. Motor signs are rare. Autonomic involvement leading to symptomatic orthostatic hypotension is present in a minority of patients and it is never the only neurologic manifestation.

The polyneuropathy shows a slow progression. In our cohort, at the end of follow-up, no patient was wheelchair-bound. As the polyneuropathy is sensorimotor axonal, a progressive reduction of SNAP and CMAP amplitudes can be observed, particularly when the nerve conduction study is repeated regularly during the follow-up. One of the first neurophysiologic signs of neuropathic involvement is a mild and diffuse increment of F wave latencies with normal motor distal latencies and normal conduction velocities: the association rules out the typical features of demyelination. On the other hand, prolongation of F wave minimal latency is indicated in the literature as a sensitive predictor of polyneuropathy [35].

It is difficult to compare our data with other studies. The only available similar report is in a small series of 12 Caucasian subjects with Val122Ile (p.Val142Ile) ATTR, where 4 out of the 9 affected patients had polyneuropathy associated with amyloidotic cardiomyopathy [36]. Also in ATTRwt amyloidosis, neuropathy appears to be more common than is usually appreciated [37]. The high prevalence of neurologic abnormalities associated with amyloidotic cardiomyopathy found in both the above report [36] and our study underlines the rarity of an exclusively cardiac phenotype, and prompts a critical reappraisal of registry-based data in which the phenotypic classification of patients is based on a single specialist observation (cardiac or neurologic) without consulting the other specialist [15,16].

In our study, cardiac patients with a neurological involvement tended to be older, more frequently male and more frequently had ECG abnormalities (Table 2), suggesting a more advanced disease status. This could indicate that the neurological involvement is an age-dependent phenomenon,

but we cannot exclude environmental or epigenetic influences.

On the other hand, the polyneuropathy that we found in our cases should not be considered simply as a somatic nerve fiber involvement related to old age. In fact, axonal neuropathic involvement is confirmed in our patients by comparing NCS data with our own laboratory reference norms obtained from an age-matched population and the clinical progression of the neurologic disease is well present, even if slow.

A coexistent neurologic involvement had a clearly negative prognostic significance in our study population (Table 2), where the occurrence of MACE during follow-up was exclusively limited to the mixed phenotype. It is true that patients with neurologic involvement have a more advanced disease and are basically older and, therefore, more frail. On the other hand, at multivariate analysis, the mixed phenotype resulted as an independent predictor of MACE, so it cannot be ruled out that other factors – environment or genetic/epigenetic factors – may influence the prognosis.

Study limitations

Although this is the largest study to date investigating the clinical and prognostic details of Ile68Leu (p.Ile88Leu) mutations, our findings are clearly limited by the small study population.

Conclusions

Although Ile68Leu (p.Ile88Leu) has always been considered a mutation determining principally or exclusively a cardiac phenotype, at least two-thirds of the affected patients in our study showed an associated neurologic impairment when they underwent an in-depth neurological assessment, and the proportion further increased during follow-up. The mixed cardiac–neurologic phenotype carried a worse prognosis at least in terms of MACE. These observations show that more patients could be eligible for treatment with gene silencers than currently indicated, and highlight the need for an in-depth and continuous multidisciplinary evaluation of these patients.

Disclosure statement

The authors report no conflicts of interest.

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