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# Electrocardiographic heterogeneity of patients with variant transthyretin amyloid cardiomyopathy: Genotype-phenotype correlations

Domitilla Russo [a], Francesco Cappelli [b], Gianluca Di Bella [c], Giacomo Tini [a], Aldostefano Porcari [d], Alberto Cipriani [e], Marco Canepa [f,g], Marco Merlo [d], Roberto Licordari [c], Pier Filippo Vianello [f], Mattia Zampieri [b], Laura De Michieli [e], Riccardo Scirpa [a], Federico Perfetto [b], Gianfranco Sinagra [d], Camillo Autore [a], Claudio Rapezzi [h,i], Maria Beatrice Musumeci [a,*]

[a] Cardiology Department, Clinical and Molecular Medicine Department, Sapienza University of Rome, Rome, Italy
[b] Tuscan Regional Amyloidosis Centre, Careggi University Hospital, Florence, Italy
[c] Clinical and Experimental Department of Medicine and Pharmacology, University of Messina, Italy
[d] Center for Diagnosis and Treatment of Cardiomyopathies, Cardiovascular Department, Azienda Sanitaria Universitaria Giuliano-Isontina (ASUGI), University of Trieste, European Reference Network for Rare, Low Prevalence and Complex Diseases of the Heart-ERN GUARD-Heart, Trieste, Italy
[e] Department of Cardiac, Thoracic and Vascular Sciences and Public Health, University of Padua, Italy
[f] Cardiovascular Disease Unit, IRCCS Ospedale Policlinico San Martino, Genova, Italy
[g] IRCCS Italian Cardiovascular Network & Department of Internal Medicine, University of Genova, Genova, Italy
[h] Centro Cardiologico Universitario di Ferrara, University of Ferrara, Italy
[i] Maria Cecilia Hospital, GVM Care & Research, Cotignola (RA), Italy

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ABSTRACT

*Backgorund:* Hereditary transthyretin(vATTR) cardiac amyloidosis has extremely different features according to the type of transthyretin(TTR) mutation. Data about electrocardiographic findings(ECG) in vATTR are limited and not informative of genotype correlation. Aim of this study is to analyze ECG characteristics and their correlation to clinical and echocardiographic aspects in patients with vATTR, focusing on different TTR mutations.
*Methods and results:* This is a multicentric, retrospective, observational study performed in six Italian referral centres. We divided patients in two groups, according to the previously described phenotypic manifestations of the TTR mutation. Of 64 patients with vATTR, 23(36%) had prevalent cardiac(PC) TTR mutations and 41(64%) patients had a prevalent neurological(PN) TTR mutations. Patients with PC mutations were more frequently males and older, with advanced NAC staging. At baseline ECG, atrial fibrillation was more common in patients with PC, while pacemaker induced rhythm in PN mutations. PQ and QRS durations were longer and voltage to mass ratio was lower in PC mutations. Different TTR mutations tend to have distinctive ECG features.
*Conclusions:* ECG in vATTR is extremely heterogeneous and the specific mutations are associated with distinct instrumental and clinical features. The differences between PN and PC vATTR are only partially explained by the different degree of cardiac infiltration.

## 1. Introduction

Transthyretin (TTR) amyloid cardiomyopathy is characterized by a pseudohypertrophic and stiff heart due to amyloidotic fibrils infiltration of misfolded TTR proteins [1]. This is caused by a single point mutation on the TTR gene (vATTR), or by a degenerative process associated with aging (wtATTR). In vATTR, the frequency and type of organ involvement is mainly related to the specific TTR mutation, with a prevalent

*Abbreviations:* AF, atrial fibrillation; AV, atrioventricular; ECG, electrocardiogram; LAH, left anterior hemiblock; LBBB, left bundle branch block; LV, left ventricular; LVEF, left ventricular ejection fraction; NAC, National Amyloidosis Centre; NYHA, New York Heart Association; PC, prevalent cardiac; PMK, pacemaker; PN, prevalent neurological; RBBB, right bundle branch block; TAPSE, tricuspid annular plane systolic excursion; TTR, transthyretin; vATTR, cardiomyopathy due to a mutation on TTR gene; wtATTR, cardiomyopathy due to a degenerative process aging related.

* Corresponding author at: Cardiology Department, Clinical and Molecular Medicine Department, Sapienza University of Rome, Rome, Italy.
*E-mail address:* beatrice.musumeci@uniroma1.it (M.B. Musumeci).

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cardiac phenotype or a prevalent neurological one [2].

Cardiac phenotypic expression varies from a mild to an extensive infiltration similar to the senile disease. Indeed, some mutations such as the Ile68Leu and Val122Ile present a phenotypic spectrum close to wtATTR amyloidosis, with prevalent cardiac involvement and neurological features occurring only in very late disease. Conversely, other TTR mutations have less evident cardiac infiltration and prevalent neurological symptoms, these latter being responsible for bringing patients to clinical attention [3].

Electrocardiogram (ECG) is considered a simple and remarkable tool for the diagnosis of cardiac amyloidosis, showing common alterations such as low QRS voltages or disproportion between left ventricular (LV) mass and QRS amplitude, pseudonecrosis and T waves changes [4–6]. Moreover, the occurrence of atrioventricular (AV) or intraventricular blocks may be related to disease progression [7]. Notably, most information on standard ECG in cardiac amyloidosis originate mainly from studies describing AL and wtATTR cohorts [5–8]. Data about vATTR are limited and not informative of genotype correlation because, despite the extreme heterogeneity, the vATTR cohort is often defined as a single study population group, regardless of the peculiar features of neurologic and cardiologic phenotypes [9]. In the present era of precision medicine, an accurate assessment of genotype-phenotype correlations in cardiac amyloidosis due to the different TTR mutations is needed to understand clinical phenotype and natural history, and eventually optimize treatments.

In the present study, we analyzed ECG characteristics and their correlation to clinical and echocardiographic aspects at baseline evaluation and during follow-up in patients with vATTR cardiomyopathy, focusing on the differences among the diverse mutations.

## 2. Materials and methods

This is a multicentric, retrospective, observational study performed in six Italian referral centres for cardiac amyloidosis: Trieste (Cattinara Hospital), Florence (Careggi Hospital), Genoa (San Martino Hospital), Padua (Padua University Hospital), Rome (Sant'Andrea Hospital) and Messina (Messina University Hospital). Rome acted as coordinating centre of the study. The local regional institutional review board approved the study and the participating centres obtained local institutional review board approvals for the collection of anonymous data. The study was conducted according to the Declaration of Helsinki and informed consent was obtained under the institutional review board policies of the hospital administrations.

### 2.1. Study population

We analyzed clinical, ECG and echocardiographic data of 64 patients with an established diagnosis of vATTR cardiac amyloidosis, consecutively evaluated between January 1, 2013 and December 31, 2020. The end of follow-up was set at December 31, 2021. Patients with TTR mutation without cardiac involvement (unaffected carriers) were not considered in the study. We divided patients into two groups according to the phenotypic manifestations of the TTR mutation [3]. Ile68Leu and Val122Ile were considered as mutations with *prevalent cardiac phenotype (PC)*. Other TTR variants were considered as mutations with *prevalent neurological phenotype (PN)*.

The first clinical evaluation at each participating center was considered as the baseline evaluation. Baseline data were retrieved from electronic medical records, including: clinical examination, ECG, echocardiography, and blood tests. Clinical, ECG and echocardiographic findings were collected in all individuals also at follow up. New York Heart Association (NYHA) class was evaluated and UK National Amyloidosis Centre (NAC) stage was calculated for all patients at baseline [10]. Mean follow up duration was 24 ± 12 months (range 9–40 months) for patients with PC mutations and 25 ± 15 months (range 5–90 months) for patients with PN mutations ($p = 0.88$).

#### 2.1.1. Electrocardiography

Standard definitions were used for the interpretation of 12-lead ECGs. Low QRS voltages were defined as QRS amplitude <0.5 mV in all limb leads or < 1 mV amplitude in all precordial leads [5,6]. Total QRS score was derived from the sum of voltage amplitude in each lead; peripheral and precordial QRS score were also calculated. The voltage/mass ratio was defined as the total QRS score divided by LV mass measured on echocardiogram indexed to body surface area. AV conduction blocks, defined as first, second or third degree AV blocks, and intraventricular conduction blocks, defined as left bundle branch block (LBBB), right bundle branch block (RBBB) or left anterior hemiblock (LAH), were also evaluated. A pseudonecrosis pattern was defined as pathological Q waves (1/4 R amplitude) or QS waves on 2 consecutive leads in the absence of known ischemic heart disease [8]. Abnormal ECG was defined in the presence of one or more of the following features: conduction disturbances (AV block, RBBB, LBBB, LAH), low QRS voltages, ST and T wave abnormalities and pseudonecrosis pattern.

Two experienced cardiologists (B. M. and D. R.) independently reviewed all ECGs.

#### 2.1.2. Echocardiography

Echocardiographic images were obtained from the standard parasternal long-axis, parasternal short-axis, apical, and subcostal views with commercially available vendor machines. Left ventricular ejection fraction (LVEF) quantification and diastolic function were evaluated according to the recommendations of the European Association of Cardiovascular Imaging [11]. Systolic pulmonary artery pressure, tricuspid annular plane systolic excursion (TAPSE) and E/e' ratio were also collected according to the same recommendations [11]. Left ventricular mass, diameters, and wall thickness were evaluated by M-mode according to the Deveraux formula [11].

#### 2.1.3. Diagnostic criteria

Cardiac involvement was defined in presence of an end-diastolic interventricular septum thickness > 12 mm at echocardiographic evaluation, in the absence of other causes of left ventricular hypertrophy, i.e. arterial hypertension or valvulopathies, with exclusion of monoclonal gammopathy on serum and urine samples [12]. The diagnosis of ATTR cardiac amyloidosis was confirmed by tissue biopsy or through established non-invasive criteria, according to the latest recommendations from the European Society of Cardiology [13,14]. In particular bone scintigraphy was performed either with 99mTc-DPD, 99 m PYP or 99mTc-HMDP, depending site standard and experience. Genetic testing was performed in all patients to identify mutations in the TTR gene. Genomic DNA was isolated from whole peripheral blood by standard techniques and genetic testing was performed at all sites using the different platforms available over time. In case of TTR mutations in which bone scintigraphy has been previously demonstrated to be less sensitive, a previously proposed alternative algorithm to detect cardiac involvement was followed [15].

### 2.2. Statistical analysis

Data were analyzed with SPSS software version 28 (SPSS Inc., Chicago, Illinois). Continuous variables are reported as median and interquartile range (IQR) (not normal distributions at the Kolmogorov-Smirnov Test) and were compared with nonparametric tests, as appropriate. Post-hoc analyses for continuous variables were adjusted with the Bonferroni correction. Categorical variables, reported as absolute numbers and percentages, were compared between groups with the Chi-squared test (or a Fisher exact test when any expected cell count was <5). Kaplan – Meier curve was performed to graphically analyze overall survival; the log-rank test was used to compare freedom from overall death between subgroups. A *p*-value less than or equal to 0.05 was considered as statistically significant.

**Table 1**
Distribution of TTR mutations in study population.

| Mutations | Number of patients |
|---|---|
| Prevalent cardiac | |
| Ile68Leu | 14 |
| Val122Ile | 9 |
| Prevalent neurological | |
| Glu89Gln | 19 |
| Val30Met | 7 |
| Phe64Leu | 6 |
| Tyr78Phe | 3 |
| Thr49Ala | 2 |
| Gly67Glu | 1 |
| Gly77Arg | 1 |
| Ala120Ser | 1 |

**Table 2**
Clinical characteristics of patients regarding the prevalent phenotype at baseline and follow up. Abbreviations: body mass index (BMI); atrial fibrillation (AF); pacemaker (PMK); implantable cardiac device (ICD); National Amyloidosis Centre (NAC).

| | Prevalent cardiac (23 patients) | Prevalent neurological (41 patients) | p-value |
|---|---|---|---|
| Males, n (%) | 21 (91.3) | 27 (65.9) | 0.03 |
| BMI, m ± sd | 25 ± 4 | 24 ± 4 | 0.29 |
| Age at diagnosis, years, m ± sd | 75 ± 7 | 58 ± 1 | <0.001 |
| Age at first visit, years, m ± sd | 75 ± 7 | 61 ± 1 | <0.001 |
| Time from diagnosis to first visit, year, median (range) | 0.3 (0–3) | 2.5 (0–14) | 0.01 |
| History of AF, n (%) | 8 (34.7) | 7 (17.1) | 0.04 |
| History of PMK, n (%) | 1 (4.3) | 4 (9.8) | 0.64 |
| History of ICD, n (%) | 0 | 2 (4.9) | 0.40 |
| NYHA functional class, n (%) | | | 0.32 |
| -I/II | 20 (86.9) | 36 (87.8) | |
| -III/IV | 3 (13.0) | 5 (12.2) | |
| NAC stage, n (%) | | | 0.009 |
| -I | 9 (39.1) | 36 (87.8) | |
| -II | 12 (52.1) | 4 (9.7) | |
| -III | 2 (8.6) | 1 (2.4) | |
| Follow up duration, months, m ± sd | 24 ± 1 | 25 ± 1 | 0.88 |
| Progression to NYHA functional class III-IV, n (%) | 10 (43.4) | 7 (17.1) | 0.04 |
| De novo atrial fibrillation, n (%) | 4 (17.4) | 3 (7.3) | 0.24 |
| PMK implantation, n (%) | 1 (4.3) | 2 (4.9) | 1 |
| ICD implantation, n (%) | 1 (4.3) | 1 (2.4) | 1 |
| Ventricular arrhythmias, n (%) | 0 | 0 | 1 |
| Stroke, n (%) | 2 (8.7) | 0 | 0.12 |
| Heart transplantation, n (%) | 0 | 3 (7.3) | 0.54 |
| Death, n (%) | 3 (13.0) | 3 (7.3) | 0.66 |

## 3. Results

### 3.1. Study population

Of 64 patients, 23 (36%) had PC TTR mutations including 14 with Ile68Leu and 9 with Val122Ile; the other 41 (64%) patients had PN TTR mutations: 19 Glu89Gln, 7 Val30Met, 6 Phe64Leu, 3 Tyr78Phe, 2 Thr49Ala, 1 Ala190Ser, 1 Gly67Glu, 1 Gly77Arg, 1 Ala120Ser. Distributions of TTR mutations in study population is shown in Table 1.

Patients with PC mutations were more frequently males (91.3 vs 65.9%, $p = 0.03$), older at vATTR diagnosis (75 ± 7 vs 58 ± 1 years, $p < 0.001$) and had a shorter time from diagnosis to baseline visit ($p = 0.01$) compared to those with PN mutations (Table 2). At first visit, most of patients in both groups were in NYHA functional class I or II, but those with PC mutations had more frequently a history of atrial fibrillation (AF) (34.7 vs 17.1%, $p = 0.04$), and were more commonly in NAC stage 2 or 3 (60.7 vs 12.1%, $p = 0.009$). During follow up (Table 2), patients with PC mutations progressed more frequently to NYHA functional class III or IV (43.4% vs 17.1%, p = 0.04) and showed a trend toward a higher risk of AF and stroke. Mortality was similar in the two groups (log rank: $p = 0.78$).

**Table 3**
Echocardiographic and electrocardiographic features of patients regarding the prevalent phenotype at baseline. Abbreviations: left ventricular (LV); intraventricular septum (IVS); left atrial (LA); tricuspid anular plane excursion (TAPSE); sinus rhythm (SR); atrial fibrillation (AF); pacemaker (PMK); atrioventricular (AV); left bundle branch block (LBBB); right bundle branch block (RBBB); left anterior hemiblock (LAH);.

| | Prevalent cardiac (23 patients) | Prevalent neurological (41 patients) | p-value |
|---|---|---|---|
| *Echocardiogram* | | | |
| LV Mass, gr, m ± sd | 361 ± 9 | 291 ± 1 | 0.05 |
| IVS thickness, mm, m ± sd | 17 ± 3 | 16 ± 4 | 0.13 |
| End-diastolic diameter, mm, m ± sd | 47 ± 7 | 45 ± 5 | 0.27 |
| Posterior wall thickness, mm, m ± sd | 16 ± 2 | 14 ± 3 | 0.04 |
| LA dimension, mm, m ± sd | 44 ± 4 | 40 ± 6 | 0.03 |
| E/e' ratio, m ± sd | 17 ± 6 | 12 ± 6 | 0.005 |
| Systolic pulmonary artery pressure, mmHg, m ± sd | 40 ± 9 | 29 ± 7 | 0.004 |
| LV ejection fraction, %, m ± sd | 53 ± 1 | 57 ± 8 | 0.08 |
| TAPSE, mm, m ± sd | 16 ± 5 | 19 ± 3 | 0.03 |
| Pericardial effusion, n (%) | 2 (8.7) | 12 (29.3) | 0.18 |
| Restrictive diastolic filling, n (%) | 9 (39.1) | 11 (26.8) | 0.24 |
| *Electrocardiogram* | | | |
| Rhythm | | | 0.03 |
| -SR | 18 (78.3) | 35 (85.3) | |
| -AF | 5 (21.7) | 2 (4.9) | |
| -PMK | 0 | 4 (9.8) | |
| Heart rate, bpm, m ± sd | 73 ± 9 | 73 ± 11 | 0.79 |
| P wave duration, msec, m ± sd | 106 ± 22 | 102 ± 23 | 0.63 |
| PQ duration, msec, m ± sd | 203 ± 47 | 174 ± 30 | 0.01 |
| QRS duration, msec, m ± sd | 109 ± 23 | 98 ± 21 | 0.06 |
| Total QRS score, mm, m ± sd | 101 ± 35 | 97 ± 37 | 0.41 |
| Peripheric QRS score, mm, m ± sd | 35 ± 17 | 32 ± 12 | 0.35 |
| Precordial QRS score, mm, m ± sd | 72 ± 21 | 67 ± 27 | 0.39 |
| AV block, n (%) | 8 (34.8) | 8 (19.5) | 0.20 |
| LBBB, n (%) | 3 (13) | 3 (7.3) | 0.66 |
| RBBB, n (%) | 5 (21.7) | 5 (12.2) | 0.47 |
| LAH, n (%) | 7 (30.4) | 7 (17.1) | 0.19 |
| Low voltages, n (%) | 7 (30.4) | 13 (31.7) | 1 |
| Pseudonecrosis pattern, n (%) | | | 0.26 |
| -QS | 0 | 3 (7.3) | |
| -low R wave progression | 9 (42.8) | 11 (26.8) | |
| -No pseudonecrosis | 12 (57.2) | 26 (63.4) | |
| Anterior pseudonecrosis, n (%) | 5 (21.7) | 11 (26.8) | 0.76 |
| Inferior pseudonecrosis, n (%) | 3 (13) | 4 (9.8) | 0.67 |
| Lateral pseudonecrosis, n (%) | 9 (39.1) | 12 (29.3) | 0.39 |
| High lateral pseudonecrosis, n (%) | 5 (21.7) | 3 (7.3) | 0.11 |
| T waves inversion, n (%) | 3 (13) | 6 (14.6) | 1 |
| Voltage to mass ratio | 0.17 ± 0.1 | 0.22 ± 0.1 | 0.02 |

### 3.2. ECG and echocardiogram characteristics according to the type of mutation

Baseline ECG and echocardiogram characteristics of both groups are listed in Table 3.

Patients with PC mutations had greater LV mass ($p = 0.05$) and left atrial dimension ($p = 0.03$), higher LV end-diastolic pressure ($p = 0.005$) and pulmonary artery pressure ($p = 0.004$), lower LVEF ($p = 0.08$) and TAPSE (p = 0.03), as compared to patients with PN.

At baseline ECG, AF was more common in patients with PC, while pacemaker (PMK) induced rhythm in those with PN mutations (p = 0.03). Patients with PC mutations had non statistical trend for longer PQ and QRS duration ($p = 0.06$) but prevalence of AV and intraventricular

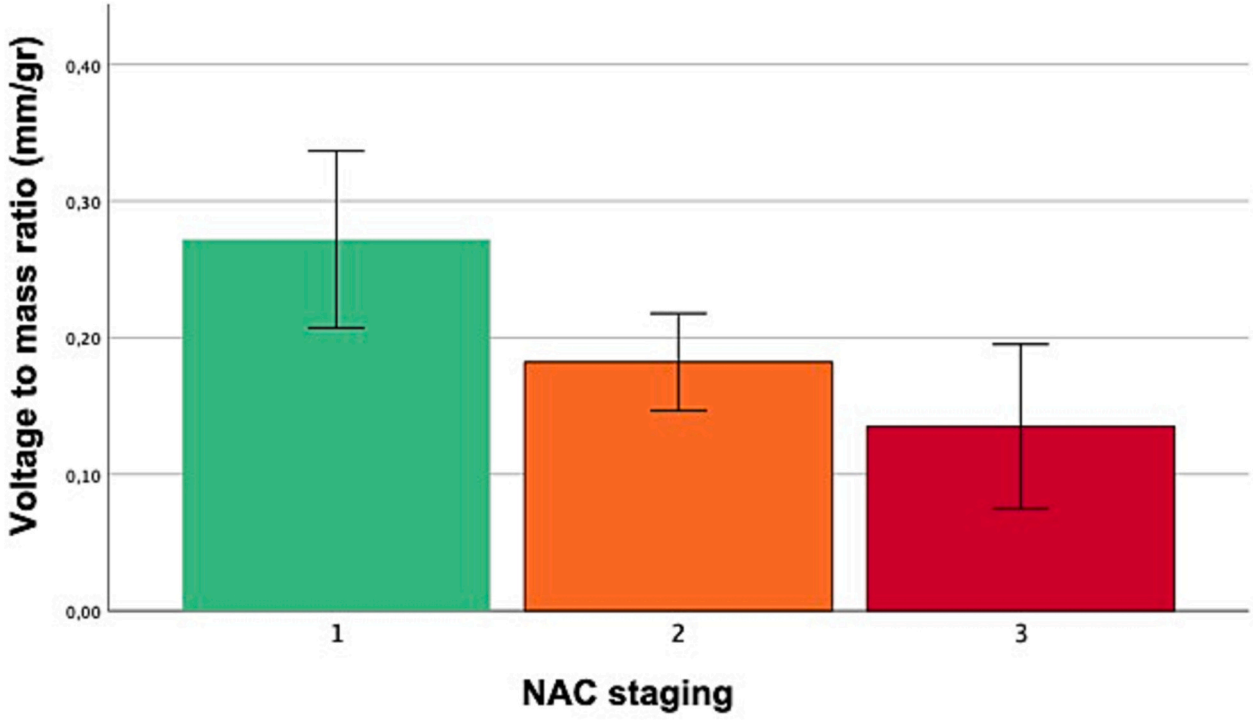


**Fig. 1.** Voltage to mass ratio was inversely related to NAC staging ($p = 0.004$).

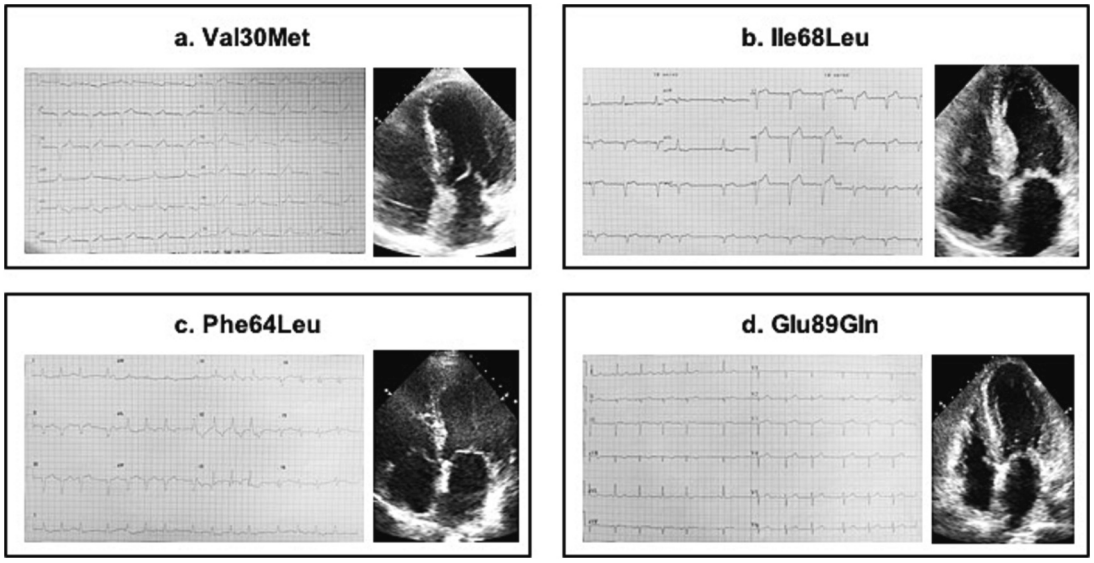


**Fig. 2.** Electrocardiographic and echocardiographic features in vATTR mutations:
a. In Val30Met mutation, the need of PMK is very frequent and often already present in the first visit, associated with evident echocardiographic signs of infiltration;
b. Ile68Leu mutation is characterized by lengthening of PR interval and QRS duration, related to a marked myocardial infiltration;
c. In Phe64Leu mutation the presence of RBBB and LAH, together with less thick LV ventricular wall, is typical;
d. Glu89Gln mutation is marked by a normal or near normal ECG, despite a clear myocardial infiltration;

blocks were not significantly different in the two groups. Presence of low voltages and pseudonecrosis patterns were similar.

Voltage to mass ratio was lower in PC mutations than in PN ones ($p = 0.02$), and was inversely related to NAC staging ($p = 0.004$, Fig. 1).

During a follow up of 2 years, the relative increase of PQ (24 vs 12 msec, $p = 0.45$) and QRS intervals (3 vs 13 msec, $p = 0.50$) were not different in PC vs. PN mutations. The relative variation of voltage to mass ratio was similar in the two groups (0.05 vs 0.04, $p = 1$).

Peculiar features of different mutations are reported in Fig. 2 and in Table 4. Although the low number of cases did not allow statistical comparison, some mutations showed specific characteristics. Patients with Glu89Gln mutation were more frequently female (57.9%) and diagnosed at younger age. Their ECG appeared substantially normal despite a high LV mass. In about 50% of cases with Phe64Leu a characteristic ECG with RBBB and LAH with low voltages was present, although LV mass was lower in Phe64Leu than in other mutations. Patients with Val30Met had more frequently a history of PMK implantation and AV block associated with LAH at basal ECG.

**Table 4**
Clinical and instrumental features regarding type of mutation at first visit. Abbreviations: atrial fibrillation (AF); pacemaker (PMK); sinus rhythm (SR); atrioventricular (AV); left bundle branch block (LBBB); left anterior hemiblock (LAH); right bundle branch block (RBBB); left ventricular (LV).

| | Val122Ile<br>*N* = 9 | Ile68Leu<br>*N* = 14 | Glu89Gln<br>*N* = 19 | Phe64Leu<br>*N* = 6 | Val30Met<br>*N* = 7 |
|---|---|---|---|---|---|
| Mutation type | Prevalent cardiac | Prevalent cardiac | Prevalent neurological | Prevalent neurological | Prevalent neurological |
| Males, n(%) | 7 (77.8) | 14 (100) | 8 (42.1) | 6 (100) | 6 (85,7) |
| Age at diagnosis | 74 + 5 | 75 + 8 | 51 + 6 | 64+ 6 | 69 + 5 |
| History of AF, n (%) | 4 (44.4) | 4 (28.5) | 1 (5.3) | 1 (16.7) | 2 (28.6) |
| History of PMK, n (%) | 1 (11.1) | 0 | 1 (5.3) | 0 | 3 (42.9) |
| NAC stage, n(%) | | | | | |
| I | 6 (67) | 5 (33) | 16 (87) | 6 (100) | 7 (100) |
| II | 3 (33) | 8 (56) | 3 (13) | 0 (0) | 0 (0) |
| III | 0 (0) | 1 (11) | 0 (0) | 0 (0) | 0 (0) |
| *Electrocardiogram* | | | | | |
| Rhythm | | | | | |
| -SR | 7 (77.8) | 11 (78.6) | 17 (89.5) | 6 (100) | 3 (42.9) |
| -AF | 2 (22.2) | 6 (21.4) | 0 | 0 | 1 (14.3) |
| -PMK | 0 | 0 | 2 (10.5) | 0 | 3 (42.9) |
| AV block | 2 (22.2) | 6 (42.9) | 3 (15.8) | 1 (16.7) | 3 (42.9) |
| LBBB | 3 (33.3) | 0 | 3 (15.8) | 1 (16.7) | 1 (14.3) |
| LAH | 2 (22) | 5 (36) | 1 (5.2) | 2 (33) | 3 (50) |
| RBBB | 2 (22.2) | 3 (21.4) | 2 (11.1) | 3 (50) | 1 (14.3) |
| RBBB + LAH | 0(0) | 1 (7) | 0 (0) | 2 (33) | 0 (0) |
| Low voltage | 2 (22.2) | 5 (35.7) | 4 (21.1) | 5 (83.3) | 1 (14.3) |
| Pseudonecrosis pattern | 1 (11) | 4 (29) | 6 (33) | 0 (0) | 2 (33) |
| Pathological electrocardiogram | 9 (100) | 13 (92.9) | 11 (57.9) | 6 (100) | 5 (71.4) |
| LV mass | 381 ± 128 | 348 ± 78 | 324 ± 174 | 227 ± 102 | 313 ± 46 |
| Voltage to mass ratio | 0.16 ± 0.1 | 0.17 ± 0.05 | 0.20 ± 0.11 | 0.22 ± 0.1 | 0.25 ± 0.1 |

## 4. Discussion

In this multicentric retrospective study, conducted in cardiological amyloidosis referral centres and analyzing ECG characteristics of the various TTR mutations, we found a large heterogeneity of ECG features among vATTR patients, with a number of specific genotype-phenotype correlations.

We observed and described that cardiac involvement in vATTR may have distinct clinical and echocardiographic features according to the type of TTR mutation. In literature, patients with PN and with PC variants are generally considered as a single vATTR group. However, PN and PC patients show specific epidemiological, clinical and ECG features. In our cohort, patients with PN mutations were generally 18 years younger than PC ones, and received their first cardiological evaluation after a longer time interval since the diagnosis of vATTR (30 months vs. 4 months). They had lower NAC stage and progressed less frequently to advanced NYHA functional class than patients with PC mutations (Table 2). At baseline echocardiogram, although the interventricular septal thickness was not significantly different, patients with PN mutations had a lower LV mass, a smaller left atrium, and a less impaired biventricular systolic and diastolic function (Table 2). Unexpectedly, these subjects had a higher frequency of pericardial effusion.

To our knowledge, an analysis comparing PC and PN TTR mutations within a single cohort of patients with definite amyloidotic cardiomyopathy has not yet been done. Within the international THAOS Registry, symptomatic subjects with exclusively cardiac or cardiac and neurologic phenotype have been compared but the analysis included all the subjects enrolled regardless of the presence of cardiomyopathy [16]. Results similar to ours (milder cardiomyopathy in the mixed phenotype compared with the cardiac one despite a common definition of cardiac involvement) may be extrapolated by an indirect comparison between vATTR patients enrolled in the ATTR-ACT trial (all with a PC phenotype) [17] and those from the cardiac subgroup of the APOLLO trial (all with a PN phenotype) [18]. This relevant concept deserves consideration when comparing the results of disease-modifying treatments trials.

In our study, although classical ECG amyloidosis-related features (i.e., low voltages, conduction disturbances, pseudonecrosis pattern) were similarly distributed between PC and PN mutations, some differences were present, including the higher prevalence of AF, the trend toward longer PQ and QRS intervals, and a lower mass-to-voltage ratio in the PC group. These electrocardiographic differences can probably be explained with the differences in LV structure and function between the two groups described above.

Moreover, our study focused on the ECG differences within the distinct TTR mutations. Some variants tend to have peculiar ECG features (Table 4, Fig. 2). Val30Met is characterized by a higher prevalence of subjects with PMK at first observation, despite their relatively young age. In cases with a non PMK-induced rhythm, the prevalence of a first grade A-V block and LAH is the highest of all mutations. In contrast, bifascicular blocks (RBBB and LAH) with or without associated low QRS voltage are particularly frequent in patients with Phe64Leu. Notably, as shown in a recent study from our group, PR > 200 msec and QRS > 120 msec are two independent predictors of future PMK implantation [19]. Glu89Gln is the mutation with the lowest mean age and the lowest frequency of abnormal ECG, despite a great LV mass. The reasons for this ECG heterogeneity are unclear. The echocardiographic differences represented by LV wall thickness measure and LV mass cannot fully explain the ECG features. Probably, the peculiar ECG patterns in different TTR mutations may be due to properties related to differences in protein sequences and in amyloid fibril composition [20,21]. The type of fibrils (A or B) generating the amyloid deposits are the results of complex and unknown genetic and environmental factors [21–23]. The monomer concentration, the monomer proteolysis rate, structural stability and glycosylation, previously described as key variables determining the rate of development of amyloidosis, may also influence the individual phenotypic expression [15,21–23]. This variability, for example, influences other mutation-related specificities including the different myocardial affinity for bone tracers, as previously described [15].

Further studies with larger sample size are needed to better define and characterize the differences between PC and PN mutations, so as to enlarge our knowledge about the physiopathology of this heterogeneous disease and to identify accurate and repeatable markers for the individualization of therapy and monitoring of treatment response.

### 4.1. Limitations

Some shortcoming of our work should be acknowledged. Firstly, our considerations are limited by the low number of analyzed patients and need to be confirmed in larger population studies. In particular, the small number of cases mostly allowed only a description of patterns or the identification of trends.

However, it should be considered that vATTR in non-endemic zones is a very rare disease; and our study collected data from several Italian amyloidosis tertiary referral centers. Due to the retrospective nature of this study, cardiac magnetic resonance data were not systematically available. As well, troponin values were not considered for the analysis, because of the lack of standardization between centers and high variability in assays and biomarkers use over time.

## 5. Conclusion

In conclusion, vATTR amyloid cardiomyopathy is substantially different among patients with PC and PN mutations despite the common diagnostic criteria (end-diastolic interventricular septum thickness > 12 mm at echocardiographic evaluation). Standard ECG is extremely heterogeneous in vATTR and the different TTR mutations are associated with distinct findings only partially explainable by the different degree of cardiac infiltration. Awareness of ECG heterogeneity in vATTR is essential for a better understanding of the disease and for its recognition in clinical practice.

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## Declaration of Competing Interest

The authors report there are no competing interests to declare.

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