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CHARACTERIZATION AND NATURAL HISTORY OF DIFFERENT PHENOTYPES IN HEREDITARY TRANSTHYRETIN AMYLOIDOSIS: 40-YEAR EXPERIENCE AT A SINGLE ITALIAN REFERRAL CENTER

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Abstract

Aims: Hereditary Transthyretin Amyloidosis (ATTRv) is one of the leading etiologies of systemic amyloidosis with more than 135 mutations described and a broad spectrum of clinical manifestations. We aimed to provide a systematic description of a population of individuals carrying pathogenic mutations of transthyretin (TTR) gene and to investigate the major clinical events during follow up.

Methods: Observational, retrospective, cohort study including consecutive patients with mutations of TTR gene, admitted to a tertiary referral center in Bologna, Italy, between 1984 and 2022.

Results: Three hundred twenty-five patients were included: 106 asymptomatic carriers, 49 cardiac phenotype, 49 neurological phenotype and 121 mixed phenotype. Twenty-three different mutations were found, with Ile68Leu (41.8%), Val30Met (19%), and Glu89Gln (10%) being the most common. After a median follow-up of 51 months data from 290 subjects were analyzed; among them 111 (38.3%) died and 123 (42.4%) had a major clinical event (death or hospitalization for heart failure). Nine (11.5%) of the 78 asymptomatic carriers showed signs and symptoms of the disease. Carriers had a prognosis comparable to healthy population, while no significant differences were seen among the three phenotypes adjusted by age. Age at diagnosis, NYHA functional class, left ventricular ejection fraction, mPND score and disease-modifying therapy were independently associated with survival.

Conclusions: This study offers a wide and comprehensive overview of ATTRv from the point of view of a tertiary referral center in Italy. Three main phenotypes can be identified (cardiac, neurological and mixed) with specific clinical and instrumental features. Family screening programs are essential to identify paucisymptomatic affected patients or unaffected carriers of the mutation, to be followed through the years. Lastly, disease-modifying therapy represents an evolving cornerstone of the management of ATTRv, with a great impact on mortality.

INTRODUCTION

Hereditary Transthyretin Amyloidosis (ATTRv) is a systemic disease characterized by autosomal dominant inheritance with incomplete penetrance and variable expressivity (1) (2). Mutations in the transthyretin (TTR) gene, a transport protein mainly synthesized by the liver, result in instability of the TTR tetramer, leading to the dissociation of the four monomers. These monomers undergo misfolding, generating mature insoluble amyloid fibers that deposit in various organs and tissues (3). To date, more than 135 mutations of the TTR gene have been described (4). Single-base substitutions resulting in missense mutations represent the majority of genetic alterations described. The most common mutations described worldwide are (in descending order) the Val30Met, Val122Ile, and Glu89Gln (5). In Italy, in addition to the Val30Met variant, endemic foci have been identified, particularly in the Tuscan-Emilian Apennines region and Sicily, for the mutations Ile68Leu, Glu89Gln, Phe64Leu, and Thr49Ala (6)(7). The clinical presentation can vary widely, ranging from phenotypes with exclusively neurological clinical presentation (such as Val30Met "early onset") to predominantly cardiac phenotypes (mutations Thr60Ala, Leu111Met, Ile68Leu, and Val122Ile) (8).

We hereby present a monocenter retrospective study providing a systematic description of a population of individuals carrying pathogenic mutations for ATTRv, including both healthy carriers and those affected by neuropathy and/or cardiomyopathy. In particular, clinical and instrumental features of patients were analyzed considering the phenotype at the time of presentation. Also, the onset of new clinical manifestations during follow-up and the progression of the disease in terms of survival and major clinical events were investigated in this study. Finally, the prognostic role of the analyzed clinical variables and the impact of disease-modifying therapy (such as orthotopic heart or liver, or combined transplantation and pharmacological strategies) on the prognosis of patients were examined.

METHODS

Setting and study design

We conducted an observational, retrospective, longitudinal study with the aim to analyze the clinical and instrumental data collected in a cohort of consecutive patients, admitted to the amyloidosis center of the IRCCS Sant'Orsola Hospital of Bologna, Italy between 1984 and 2022. Inclusion criteria were patients ≥18 years old with a pathogenic mutation of the transthyretin gene.

Data collection was performed with the use of a centralized database, with anonymized data regarding clinical, electrocardiographic, echocardiographic and nuclear medicine parameters at the time of the first evaluation and during the follow-up. All patients received optimal medical therapy, including diuretic therapy, according to clinical judgement. Data regarding disease-modifying therapy such as orthotopic heart and/or liver transplantation and drug therapy were also collected. At presentation, all patients provided informed consent for the anonymous collection of scientific data. This study conforms to the principles of the Helsinki declaration and all authors guarantee the integrity of data from their respective institutions. The study was approved by the ethics committee of IRCCS S.Orsola Hospital - University of Bologna, study code: (478/2019/Oss/AOUBo).

Definitions and diagnostic criteria

The diagnosis of ATTRv was made by genetic analysis on a blood sample. The genetic study involved the amplification by polymerase chain reaction of exons 2,3 and 4 of the TTR gene. Subsequently, amplified DNA fragments were sequenced using the automatic sequencer ABI Prism 3130. The analysis was performed both in symptomatic subjects, affected by polyneuropathy and/or amyloid cardiomyopathy, and in first-degree relatives for screening purposes.

According to the traditional definitions shared by expert consensus on the disease (9), the phenotypes at the time of diagnosis were defined according to the following criteria:

1. Cardiac phenotype: patients with maximum wall thickness of the left ventricle in end-diastole (ventricular septum and posterior wall) greater than 12 mm in the absence of other plausible causes

of left ventricular hypertrophy, together with the exclusion of neurological involvement after assessment performed by an experienced neurologist. In addition, other echocardiographic signs suggestive of cardiac amyloidosis (CA) were considered, such as the "granular sparkling" appearance of the left ventricle, thickening of the atrioventricular valves and interatrial septum and the presence of pericardial effusion. The diagnosis of CA was either confirmed by histological proof of amyloid deposits or following non-biopsy criteria (i.e. patients with echocardiographic features of cardiac amyloidosis, associated with a grade 2-3 bone scintigraphy scan, in the absence of plasma cell dyscrasia), according to current guidelines (10)

2. Neurological phenotype: patients with walking disabilities, symptoms of neurological relevance (early satiety, nausea, vomiting, involuntary weight loss, diarrhea or faecal incontinence), as evaluated by a trained neurologist. When indicated, neurophysiological studies such as nerve conduction study, electromyography and cardiovascular reflex studies were performed. In these patients, diagnostic criteria for CA are absent. Severity of disease was defined using the modified Polyneuropathy Disability score (mPND) classification.

3. Mixed phenotype: patient presenting clinical and instrumental features of both phenotypes.

12-leads ECG analysis was based on standard definitions. Abnormal ECG was defined as the presence of one or more of the following features: rhythm abnormalities, conduction disturbances (atrioventricular block, right bundle branch block, left bundle branch block, left anterior or posterior hemiblock), low QRS voltages (QRS amplitude $\leq$0.5 mV in all limb leads or $\leq$1 mV in all precordial leads), ST and T wave abnormalities and 'pseudo-infarction' pattern.

Echocardiographic assessments and measurements were performed in keeping with the recommendations of the European Society of Cardiovascular Imaging. (11) Bone scintigraphy, performed with the aim of both confirming cardiac involvement and evaluating its appearance, consisted of the intravenous administration of 740MBq of Tc-99m-3,3-diphosphono-1,2-

propanodicarboxylicacid (DPD) with the subsequent late imaging evaluation (3 hours after injection of the radiopharmaceutical) of the degree of myocardial uptake using the Perugini score. (12)

The presence of chronic kidney disease was defined as glomerular filtration rate (GFR) < 60 mL/min./1.73.

Follow-up

Time to follow-up started at the time of diagnostic genetic analysis for transthyretin mutation and data recording was interrupted in August 2022. Timing of outpatient follow-up was based on individual clinical needs. The follow-up data derive from visits scheduled at the cardiology clinic of the IRCCS Sant'Orsola Hospital of Bologna, Italy, or through telephone contact with the patient himself and/or with the general practitioner in case of patients under treatment at other centers.

Carriers with a follow-up time of less than 12 months and patients with ATTRv with a follow-up of less than 6 months were excluded from the survival analysis. Major clinical events that occurred during the study period, such as death and worsening heart failure (HF) defined by at least one hospitalization for HF or urgent evaluation for HF requiring intravenous diuretic administration, were recorded.

Statistical analysis

Data suitable for our analysis were expressed as median and interquartile range (25th and 75th percentile) for the continuous quantitative variables. In case of qualitative and ordinal variables, they were expressed as absolute and relative frequencies.

In the contingency tables, the independence of categorical variables was tested using Fisher's test or Pearson's chi-square test (according to Cochran's rule). The independence of the continuous variables was analyzed using the Mann-Whitney U/Kruskal-Wallis test. For multiple comparisons, we calculated p-values with the Bonferroni-adjusted test. Overall survival was analyzed with Kaplan-Meier curves, whose statistical significance was evaluated using the log-rank test. Cox regression

analysis was initially performed using clinical and instrumental variables in order to explore risk factors associated with overall mortality. Then, a multivariate analysis was performed by entering the model the variables considered significant in the univariate analysis ($p < 0.05$). Statistical analyses were performed using STATA/IC 16.1 (StataCorp LP, College Station, TX, USA) and SPSS Statistics (version 27. IBM Corp., Chicago, IL). Data extraction was performed on September 1st, 2022. For all tests, a 2-sided p-value <0.05 was required for statistical significance.

RESULTS

Baseline characteristics

Three hundred twenty-five consecutive subjects carrying pathogenic mutations of the TTR gene were included in this study. Of these, 219 showed signs and symptoms of the disease and were divided into three phenotype categories based on the clinical-instrumental features recorded at first evaluation: 49 subjects presented a cardiac phenotype (15.1% of total population), 49 a neurological phenotype (15.1%) and 121 a mixed phenotype (37.2%). The enrolled population also included 106 subjects (32.6%) carrying a pathogenetic mutation of TTR gene in the absence of any clinical or instrumental manifestation of disease (Figure 1)

Table 1 shows the distribution of transthyretin mutations in the study population according to the phenotype. Our cohort includes 23 different mutations; of these, 22 are point mutations and 1 deletion (Glu83 deletion). The mutations Ile68Leu (136 subjects, 41.8%), Val30Met (62 subjects, 19%) and Glu89Gln (33 subjects, 10%) are the most represented. Of the 62 subjects with the Val30Met mutation, 47 were diagnosed with the ATTRv disease. Among the leading mutations, Ile68Leu was the one with the highest percentage of healthy carriers (46%), whilst Glu89Gln had the lowest (6%). Dominant mutations were found in patients with cardiac and neurological phenotype (Ile68Leu and Val30Met, respectively), while patients with mixed phenotype had marked genotypic variability.

Table 2 summarizes the main clinical and instrumental features found in the population study divided by phenotypes at the first evaluation. Patients with cardiac phenotype were mainly males (73%), just like the group with the mixed phenotype (69%); on the other hand, the groups with carrier subjects and neurological phenotypes are composed respectively of 54% and 51% of women.

Patients with exclusive cardiac involvement were diagnosed with ATTRv at a significantly older median age (73.2 years, IQR 66.5 - 78.1) than patients with mixed phenotype (58.4 years, IQR 47 - 66.3; $p<0.0001$) and those with only neurological involvement (44 years, IQR 33.5 – 62.9; $p<0.001$). The age difference was also significantly different between the group with mixed phenotype and with neurological phenotype ($p<0.0001$).

Carpal tunnel syndrome appears to be significantly more represented in patients with CA (cardiological phenotype 57.1%, mixed 59.1%) than in subjects with neurological phenotype or carriers (22.4% and 20%, respectively).

All carrier subjects were identified following family screening, while patients affected by the disease were identified through various diagnostic pathways. Most patients with mixed phenotype received the diagnosis of the disease because of neurological symptoms (54.5%), while 37.2% of them were diagnosed after cardiological evaluation and, to a small extent, in the context of family screening (8.3%).

In most patients with predominantly cardiological or neurological phenotypes, the diagnostic pathway matched with the clinical symptoms of the phenotype (respectively 82% and 69% of cases), although a more relevant group of subjects with a neurological phenotype received a diagnosis through familial screening (30.6%).

Notably, 6.1% of patients with cardiological phenotype were diagnosed after an incidental finding of cardiac uptake on bone scintigraphy performed for other reasons, while one patient (0.02%) after hematological evaluation for suspected AL amyloidosis.

Furthermore, patients with CA show worse exercise tolerance at baseline, with a statistically significant higher proportion (83%) of patients in NYHA functional class ≥ II, compared to patients

with mixed phenotype (60%), neurological phenotype or no signs of the disease. Similarly, patients with polyneuropathy associated or not with cardiomyopathy have greater difficulty in walking than patients with exclusively cardiologic involvement or carriers.

Regarding ECG, pseudo-infarction patterns, first degree atrioventricular block, left anterior fascicular block and atrial fibrillation are the most common findings in CA, obviously more frequent than in neurological phenotypes and healthy carriers. At last, cardiac and mixed phenotypes have comparable values of left ventricular ejection fraction (56% vs 58%), pericardial effusion (44% vs 54%), restrictive filling pattern (45% vs 39%) and slightly higher values of mean left ventricular wall thickness (17 vs 15,7 mm).

Disease-modifying therapy

During the follow-up, 40 patients received orthotopic liver transplantation: of these, 18 had a neurological phenotype at the baseline and 22 had a mixed phenotype. Furthermore, 13 patients received a combined orthotopic heart and liver transplant, including 2 patients with solely cardiac involvement and 11 with mixed phenotype.

Only one patient with a mixed phenotype received isolated heart transplantation, due to intraoperative complications that prevented combined liver transplantation.

Also, we analyzed patients who received pharmacological treatment. In our population, 29 patients were treated with Tafamidis 20 mg (7 with neurological involvement, 22 with mixed phenotype) with a median time of therapy of 36 months (IQR 14 - 55 months); 8 patients were treated with Tafamidis 61 mg (3 with cardiac phenotype and 5 with mixed phenotype; the latter were switched form Tafamidis 20 mg); 10 patients (1 with neurological involvement and 9 with mixed phenotype) were treated with Patisiran for a median of 8 months (IQR 6 – 19) and 2 patients (1 with neurological involvement and 1 with mixed phenotype) with Inotersen.

Finally, we analyzed the prognostic impact of disease-modifying therapies among patients of our cohort, regardless of the disease phenotype. Figures 2 shows that patients treated with surgical and/or

pharmacological strategies have a higher total survival ($p<0.00001$) than patients treated exclusively with supportive therapy.

Follow-up analysis based on phenotypes

The study population of 325 subjects was observed for a median follow-up of 51 months (IQR 24 – 90 months). Of these, 21 healthy carrier subjects with a follow-up time of less than 12 months and 3 patients with cardiac and 1 mixed phenotype with follow-up less than 6 months were excluded from the survival analysis. Overall, 10 patients were lost to follow-up (7 healthy carriers, 2 patients with cardiac phenotype and 1 with mixed phenotype).

During the observation period, 9 (11.5%) of the 78 healthy mutation carriers included in the analysis were diagnosed with ATTRv. Of these, 3 developed a cardiological phenotype (Thr59Lys, Arg34Thr and Ile68Leu mutations), 3 developed a neurological phenotype (2 with Ile68Leu mutation and 1 patient with Gly47Ala) and 3 people with Glu89Gln, Ala36pro and Gly47Ala mutations developed a mixed phenotype. The median age of patients at diagnosis of ATTRv was 50 years (IQR 44-60) after a median follow-up of 65 months (IQR 43-109 months) from the first clinical evaluation.

Furthermore, we analyzed the onset of new signs and symptoms of the disease: 4 patients with the Ile68Leu mutation, initially evaluated as suffering from isolated cardiac amyloidosis, then developed signs of neurological involvement after a median time of 31 months (IQR 17 – 44 months); 2 patients with exclusively neurological involvement with Val30Met and Ala36Pro mutations developed amyloid cardiomyopathy. (figure 3)

At the end of the follow-up time, 290 patients were included in the final analysis (70 healthy carriers, 43 cardiac phenotype, 50 neurological phenotype and 127 mixed phenotype). Of these, 111 (38.3%) died: 2 (2.8%) healthy carriers, 21 (48.8%) with cardiac phenotype, 19 (38%) with neurological and 69 (54.3%) with mixed phenotype. At the time of death, the patients with the mixed phenotype had a median age of 61.4 years (IQR 53.5 – 69.6 years), those with the neurological phenotype were 60 years old (IQR 50.3 – 68.1), while the patients with the cardiac phenotype were significantly older

with a median age of 77.7 years (IQR 71.3 – 81.3). No significant differences were found between the three phenotypes in the interval between symptoms onset and death. Twelve patients (4.1%) presented worsening HF (4 patients with cardiac phenotype, and 8 with mixed phenotypes).

Figure 4 shows the overall survival of the study population divided by phenotypes. Survival curves are adjusted by age and no significant differences are seen among the three phenotypes of the disease. Subjects without signs and symptoms of disease have a total and event-free survival comparable to the healthy population.

The main clinical and instrumental parameters (sex, glomerular filtration rate, NYHA functional class ≥ 2 at presentation, mPND score ≥ 2, age at diagnosis, disease-modifying therapy, left ventricular ejection fraction, phenotype and main TTR gene mutations) were initially investigated with a univariate analysis Cox regression aimed at identifying the predictors of death from any cause. A multivariate analysis model was subsequently performed with the parameters resulting statistically significant in the univariate analysis.

Age at diagnosis, exertional dyspnea (NYHA class ≥ II), gait disturbances (mPND score ≥ 2), left ventricular ejection fraction and the use of disease-modifying therapy were found to be independently associated with prognosis (Tables 3), while the presence of a specific genotype (Ile68Leu, Val30Met, other mutations) was not.

DISCUSSION

Our study includes a large series of patients affected by ATTRv or healthy carriers of a mutation of the TTR gene and provides an accurate description of the clinical and instrumental features of the three main disease phenotypes. Also, we aimed to describe the phenotype-genotype correlation and to compare outcomes according to clinical manifestations and disease-modifying therapy.

It is common knowledge that ATTRv is a multi-organ disease characterized by an extreme clinical heterogeneity (3). In our population, three main disease phenotypes could be identified at baseline evaluation, with the mixed phenotype resulting the most prevalent (55.2% of enrolled patients). This

result appears in keeping with previous literature, showing a high frequency of a multisystemic involvement in ATTRv cohorts. It is also remarkable the presence in the enrolled population of 106 (32.6%) subjects carrying the mutation in the absence of disease. All carriers were identified thanks to the family screening program, which underlines a greater sensitivity and awareness of the disease. Among patients with amyloid cardiomyopathy there is a clear predominance of the male sex, both in patients with isolated cardiac phenotype and in those with simultaneous neurological involvement; on the contrary, no gender difference emerged among patients with neurological phenotype and in carrier subjects. This data supports the hypothesis of a greater susceptibility of the male sex regarding the cardiac involvement of the disease (13).

Comparing the three phenotype groups, it emerges an important difference in the age of onset of symptoms. In fact, subjects with the cardiac phenotype develop the first symptoms of the disease 15 years later [73.2 (66.5 - 78.1) years-old] than patients with the mixed phenotype [58.4 (47-66.3) years-old] and 29 years later than patients with the neurological phenotype [44 (33.5 – 62.9) years-old]. If the difference between the cardiac and neurological phenotypes can be partly explained by the age-related penetrance of the different mutations (i.e. the high proportion of subjects with the Val30Met mutation with early onset of neurological symptoms), the reason for this time age gap appears less clear in patients with mixed phenotype. In fact, this group develops the first symptoms of the disease and are diagnosed with ATTRv at a younger age than patients with the cardiac phenotype, as the first symptom is neurological in 54.5% of cases and cardiological in only 37.2%. This differences in age observed in our cohort are in keeping with the published literature. Rapezzi et al. hypothesized that one of the reasons for this time gap is that myocardial infiltration in ATTR requires longer than the time required to develop axonal neuropathy. (7) Another possible explanation could be that the absence of neurological symptoms causes patients to seek medical care later, but this hypothesis appears less likely as in our cohort there is no significant difference between the three groups in the time interval between symptom onset and diagnosis of the disease. This finding is in keeping with the

higher prevalence of history of heart failure hospitalizations in subjects with isolated cardiac amyloidosis compared to the other phenotypes ($p<0.0001$).

Regarding instrumental data, CA is confirmed to be characterized by a symmetrical increase in the wall thicknesses of the left ventricle with an ejection fraction that is only slightly impaired and by the coexistence of diastolic dysfunction and pericardial effusion. There are no substantial differences in the instrumental features of cardiac amyloidosis associated with neurological involvement or not.

Among the mixed phenotype population, we observed a lower posterior wall thickness and lower mean thickness, together with a smaller atrial diameter: this could be related to an earlier stage of the disease or to a lower presence of comorbidities such as hypertension, diabetes mellitus and renal insufficiency. In favor of this hypothesis, there is a higher frequency of advanced NYHA class, history of hospitalization for heart failure and atrial fibrillation in patients with the cardiac phenotype compared to those with the mixed phenotype (56% vs 23%, $p < 0.0001$).

In this cohort the presence of mono- or bilateral carpal tunnel syndrome is confirmed to be a possible "red flag" of cardiac involvement (14), as it is significantly more present in patients with cardiomyopathy than in the other groups (57.1% in the group with cardiac phenotype, 59.1% in the group with the mixed phenotype vs 20% and 22.4% respectively in the healthy carriers and in the group with the neurological phenotype). Notably, in our population the association between amyloid cardiomyopathy and low voltages on 12-leads ECG does not appear a useful marker to identify subjects at greater risk of cardiac involvement.

Our study cohort includes a total of 23 different mutations of the TTR gene (22 point mutations and 1 deletion). The most represented mutations are Ile68Leu (136 subjects, 42%) followed by Val30Met (62 subjects, 19%) and Glu89Gln (33 subjects, 10%). Although the patients enrolled in this study were referred to our center from several Italian regions, the frequency analysis of the different mutations is clearly influenced by the geographical location of our clinic near the Tuscan-Emilian Apennines, which is known to be endemic for Ile68Leu mutation (15). This mutation appears to be one of the major causes of CA and accounts for 82% of cardiac phenotypes in this cohort. However,

despite being known as a “cardiogenic mutation", it has been previously demonstrated that in these patients neurological involvement is not uncommon (16), especially autonomonic dysfunction (17). As a matter of fact, 24% of the mixed phenotypes and 8% of the neurological phenotypes in our cohort are related to Ile68Leu mutations.

Val30Met mutation appears to be as one of the most frequent mutations and it is strongly associated with the neurological manifestations of the disease, as it is found in 53% of neurological phenotypes and 17% of mixed phenotypes, while Glu89Gln mutation shows a mixed expression in 79% of cases. Regarding the remaining mutations, a certain clinical variability can be observed and so it is not possible to detect a close genotype-phenotype correlation.

The high clinical heterogeneity and the frequent multisystem involvement found in all forms of ATTRv confirm the need for a multidisciplinary, cardiological and neurological evaluation, in all subjects, regardless of the mutation found.

Healthy carriers represent a relevant part of the study population, as a result of our extensive screening program for relatives of affected patients. Ile68Leu is the mutation with the highest prevalence of asymptomatic carriers (46%), probably given to the elder age of disease onset in this specific subset, contrariwise to some other mutations such as Glu89Gln (6% of asymptomatic carries), more aggressive since young adult age, as already acknowledged (18).

At the end of the follow-up of 51 months (IQR 24 - 90 months), 9 of the 78 healthy carrier subjects (11.5%) included in the analysis developed signs and symptoms of the disease, after a median time of 65 months. We did not observe neither a prevalent clinical presentation nor a prominent mutation, as 3 patients presented the onset of disease with cardiac amyloidosis, 3 with sensorimotor polyneuropathy, and 3 with both. This finding emphasizes the importance of following carriers through the years, as previously stated by experts’ consensus (10)(19). ATTRv is also confirmed to be an adult disease, as the median age of the patients at the time of disease onset was 50 years (IQR 44 - 60). In only two cases the disease began before the age of 40: at 28 years with a mixed phenotype

in a patient carrying the Ala36Pro mutation and at 38 years with a neurological phenotype in a woman with the Gly47Ala mutation.

From the survival analysis, it emerged that 111 (38.3%) of the 290 patients included, died, while 123 patients (42.4%) experienced a major clinical event between death and hospitalization for heart failure. As expected, carriers who have not developed ATTRv at the end of follow-up have a survival comparable to that of the general healthy population.

When comparing the Kaplan-Meier survival curves adjusted by age (Figures 4), no significant differences were seen among all phenotypes. In our multivariate analysis, age at diagnosis along with three easy to obtain parameters reflecting disease severity emerged as independent variables of death: NYHA functional class and left ventricular ejection fraction as measures of cardiomyopathy and heart failure severity, and a mPND classification ≥ 2 as a parameter of motor disability. Notably, the presence of a specific genotype (Ile68Leu, Val30Met, other mutations) was not associated with any of the pre-specified endpoint. In another study about ATTRv published by researchers from the National Amyloidosis Center in UK, patients with V122I-related ATTR-CA were more impaired functionally ($P<0.001$) and had worse measures of cardiac disease ($P<0.001$) at the time of diagnosis, a greater decline in quality of life, and poorer survival ($P<0.001$) in comparison with the other subgroups (20). Those differences underline the heterogeneity of the impact of various TTR mutations, which may carry a worse prognostic role even within the same phenotype category (e.g. Val122Ile versus Ile68Leu). Lastly, in the multivariate Cox regression model, starting a disease-modifying therapy (pharmacological or not) was strongly and independently associated with a better prognosis, as graphically confirmed by Kaplan-Meier survival curves in figure 2. This observation is limited by some bias mainly related to the retrospective nature of the study and to the variability of the applied treatment (surgical or pharmacological). Although, this evidence of efficacy on survival still represents an important remark, given the lack of data essentially based on single studies about liver transplantation, especially in Val30Met, (21) and Tafamidis on ATTR-CA (22).

CONCLUSION

This retrospective study offers a wide and comprehensive overview of ATTRv from the point of view of a tertiary referral center in Italy. We performed an analysis on a large cohort of patients affected by ATTRv or healthy carriers of mutation of the transthyretin gene, which has allowed us to confirm the clinical heterogeneity of the disease, splitable into three main phenotypes (cardiac, neurological and mixed) with specific clinical and instrumental features. Family screening programs are essential to identify paucisymptomatic affected patients or unaffected carriers of the mutation, to be followed through the years. Lastly, disease-modifying therapy represents an evolving cornerstone of the management of ATTRv, with a great impact on mortality.

Study limitations

This study presents a large series of patients with ATTRv, but it represents the experience of a single center, located in a geographic area which is endemic for a specific mutation, and with a relatively small absolute number of patients for each phenotype. Furthermore, the prolonged interval of enrollment made the diagnostic techniques and therapeutic strategies applied in the study not homogeneous.

Finally, the evaluation of the independent prognostic factors is limited by the absence in our series of complete data for some variables with known prognostic impact (NTproBNP, troponin) which, therefore, were not included in the multivariate analysis model.

References:

1. Sperry BW, Tang WHW. Amyloid heart disease: genetics translated into disease-modifying therapy. Heart [Internet]. 2017 Jun 1 [cited 2023 Oct 25];103(11):812–7. Available from: https://pubmed.ncbi.nlm.nih.gov/28255101/
2. Merlini G, Bellotti V. Molecular Mechanisms of Amyloidosis. N Engl J Med [Internet]. 2003 Aug 7 [cited 2023 Oct 25];349(6):583–96. Available from:

https://pubmed.ncbi.nlm.nih.gov/12904524/

3. Rapezzi C, Merlini G, Quarta CC, Riva L, Longhi S, Leone O, et al. Systemic cardiac amyloidoses: disease profiles and clinical courses of the 3 main types. Circulation [Internet]. 2009 Sep [cited 2023 Oct 25];120(13):1203–12. Available from: https://pubmed.ncbi.nlm.nih.gov/19752327/

4. Rowczenio DM, Noor I, Gillmore JD, Lachmann HJ, Whelan C, Hawkins PN, et al. Online registry for mutations in hereditary amyloidosis including nomenclature recommendations. Hum Mutat [Internet]. 2014 [cited 2023 Oct 25];35(9). Available from: https://pubmed.ncbi.nlm.nih.gov/25044787/

5. Yarlas A, Gertz MA, Dasgupta NR, Obici L, Pollock M, Ackermann EJ, et al. Burden of hereditary transthyretin amyloidosis on quality of life. Muscle and Nerve. 2019 Aug 1;60(2):169–75.

6. Mazzeo A, Russo M, Di Bella G, Minutoli F, Stancanelli C, Gentile L, et al. Transthyretin-Related Familial Amyloid Polyneuropathy (TTR-FAP): A Single-Center Experience in Sicily, an Italian Endemic Area. J Neuromuscul Dis [Internet]. 2015 [cited 2023 Oct 25];2(s2):S39–48. Available from: https://pubmed.ncbi.nlm.nih.gov/27858761/

7. Rapezzi C, Quarta CC, Obici L, Perfetto F, Longhi S, Salvi F, et al. Disease profile and differential diagnosis of hereditary transthyretin-related amyloidosis with exclusively cardiac phenotype: an Italian perspective. Eur Heart J [Internet]. 2013 Feb 14 [cited 2023 Oct 25];34(7):520–8. Available from: https://pubmed.ncbi.nlm.nih.gov/22745357/

8. Planté-Bordeneuve V, Ferreira A, Lalu T, Zaros C, Lacroix C, Adams D, et al. Diagnostic pitfalls in sporadic transthyretin familial amyloid polyneuropathy (TTR-FAP). Neurology [Internet]. 2007 Aug [cited 2023 Oct 25];69(7):693–8. Available from: https://pubmed.ncbi.nlm.nih.gov/17698792/

9. Maurer MS, Hanna M, Grogan M, Dispenzieri A, Witteles R, Drachman B, et al. Genotype and Phenotype of Transthyretin Cardiac Amyloidosis: THAOS (Transthyretin Amyloid

Outcome Survey). J Am Coll Cardiol [Internet]. 2016 Jul 12 [cited 2023 Oct 25];68(2):161–72. Available from: https://pubmed.ncbi.nlm.nih.gov/27386769/

10. Garcia-Pavia P, Rapezzi C, Adler Y, Arad M, Basso C, Brucato A, et al. Diagnosis and treatment of cardiac amyloidosis: a position statement of the ESC Working Group on Myocardial and Pericardial Diseases. Eur Heart J [Internet]. 2021 Apr 21 [cited 2023 Oct 27];42(16):1554–68. Available from: https://pubmed.ncbi.nlm.nih.gov/33825853/
11. Lang RM, Badano LP, Victor MA, Afilalo J, Armstrong A, Ernande L, et al. Recommendations for cardiac chamber quantification by echocardiography in adults: an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. J Am Soc Echocardiogr [Internet]. 2015 [cited 2023 Oct 27];28(1):1-39.e14. Available from: https://pubmed.ncbi.nlm.nih.gov/25559473/
12. Perugini E, Guidalotti PL, Salvi F, Cooke RMT, Pettinato C, Riva L, et al. Noninvasive etiologic diagnosis of cardiac amyloidosis using 99mTc-3,3-diphosphono-1,2-propanodicarboxylic acid scintigraphy. J Am Coll Cardiol [Internet]. 2005 Sep 20 [cited 2023 Oct 27];46(6):1076–84. Available from: https://pubmed.ncbi.nlm.nih.gov/16168294/
13. Rapezzi C, Emdin M, Aimo A. Unravelling the role of sex in the pathophysiology, phenotypic expression and diagnosis of cardiac amyloidosis. Eur J Heart Fail [Internet]. 2022 Dec 1 [cited 2023 Oct 27];24(12):2364–6. Available from: https://pubmed.ncbi.nlm.nih.gov/36059061/
14. Milandri A, Farioli A, Gagliardi C, Longhi S, Salvi F, Curti S, et al. Carpal tunnel syndrome in cardiac amyloidosis: implications for early diagnosis and prognostic role across the spectrum of aetiologies. Eur J Heart Fail [Internet]. 2020 Mar 1 [cited 2023 Oct 27];22(3):507–15. Available from: https://pubmed.ncbi.nlm.nih.gov/31975495/
15. Gagliardi C, Perfetto F, Lorenzini M, Ferlini A, Salvi F, Milandri A, et al. Phenotypic profile of Ile68Leu transthyretin amyloidosis: an underdiagnosed cause of heart failure. Eur J Heart Fail [Internet]. 2018 Oct 1 [cited 2023 Oct 27];20(10):1417–25. Available from:

https://pubmed.ncbi.nlm.nih.gov/30070416/

16. Pastorelli F, Fabbri G, Rapezzi C, Serenelli M, Plasmati R, Vacchiano V, et al. Neurological involvement in Ile68Leu (p.Ile88Leu) ATTR amyloidosis: not only a cardiogenic mutation. Amyloid [Internet]. 2021 [cited 2023 Oct 27];28(3):173–81. Available from: https://pubmed.ncbi.nlm.nih.gov/34047656/

17. Guaraldi P, Rocchi C, Cani I, Gagliardi C, Longhi S, Baschieri F, et al. Cardiovascular reflex tests detect autonomic dysfunction in symptomatic and pre-symptomatic subjects with hereditary transthyretin amyloidosis. Clin Auton Res [Internet]. 2023 Feb 1 [cited 2023 Oct 27];33(1):15–22. Available from: https://pubmed.ncbi.nlm.nih.gov/36625973/

18. Gentile L, Tournev I, Amass L, Chapman D, Mazzeo A, Barroso F, et al. Phenotypic Differences of Glu89Gln Genotype in ATTR Amyloidosis From Endemic Loci: Update From THAOS. Cardiol Ther [Internet]. 2021 Dec 1 [cited 2023 Oct 27];10(2):481–90. Available from: https://pubmed.ncbi.nlm.nih.gov/34148211/

19. Conceição I, Damy T, Romero M, Galán L, Attarian S, Luigetti M, et al. Early diagnosis of ATTR amyloidosis through targeted follow-up of identified carriers of TTR gene mutations. Amyloid [Internet]. 2019 Jan 2 [cited 2023 Oct 27];26(1):3–9. Available from: https://pubmed.ncbi.nlm.nih.gov/30793974/

20. Lane T, Fontana M, Martinez-Naharro A, Quarta CC, Whelan CJ, Petrie A, et al. Natural History, Quality of Life, and Outcome in Cardiac Transthyretin Amyloidosis. Circulation [Internet]. 2019 Jul 2 [cited 2023 Oct 27];140(1):16–26. Available from: https://pubmed.ncbi.nlm.nih.gov/31109193/

21. Coelho T, Ines M, Conceicaõ I, Soares M, De Carvalho M, Costa J. Natural history and survival in stage 1 Val30Met transthyretin familial amyloid polyneuropathy. Neurology [Internet]. 2018 Nov 20 [cited 2023 Oct 27];91(21):E1999–2009. Available from: https://pubmed.ncbi.nlm.nih.gov/30333157/

22. Maurer MS, Schwartz JH, Gundapaneni B, Elliott PM, Merlini G, Waddington-Cruz M, et al.

Tafamidis Treatment for Patients with Transthyretin Amyloid Cardiomyopathy. N Engl J Med [Internet]. 2018 Sep 13 [cited 2023 Oct 27];379(11):1007–16. Available from: https://pubmed.ncbi.nlm.nih.gov/30145929/

TABLES

Table 1. Phenotype-based distribution of pathogenic mutations in hereditary transthyretin amyloidosis at baseline

Mutation	Overall	Cardiac	Mixed	Neurological	Carrier
Ile68Leu	136	40	29	4	63
Val30Met	62	0	21	26	15
Glu89Gln	33	2	26	3	2
Phe64Leu	22	1	8	6	7
Thr49Ala	17	0	9	2	6
Gly47Ala	11	1	4	1	5
Arg34Thr	8	0	4	2	2
Ala36Pro	6	0	4	1	1
Glu54Lys	4	0	2	1	1
Ser50Arg	4	0	3	1	0
Ser23Asn	3	1	1	0	1
Thr59Lys	3	0	2	0	0
Gly47Arg	2	0	0	2	0
Val122Ile	2	1	1	0	0
Glu92Lys	2	0	1	0	1
Ala81Thr	2	1	0	0	1

Del. Glu83	2	1	1	0	0
Glu62Lys	1	0	1	0	0
His88Arg	1	0	1	0	0
Phe33Val	1	0	1	0	0
Val30Ala	1	0	1	0	0
Val32Arg	1	0	1	0	0
Val122Leu	0	1	0	0	0

Table 2. Clinical and instrumental characteristics according to phenotype at baseline

	Healthy carrier (106)	**Cardiac phenotype (49)**	**Neurological phenotype (49)**	**Mixed phenotype (121)**	**p-value**
Males, n (%)	49 (46) †§	36 (73) *	24 (49) °	84 (69)	**<0.0001**
Age at diagnosis, median [IQR]	NC	73.2 [66.5 – 78.1] *∇	44 [33.5 – 62.9] °	58.4 [47-66.3]	**0.0001**
Age at first contact, median [IQR]	46.4 [33.5 – 56.1] †‡§	72.7 [67 – 78.4] *∇	45.5 [34.1 – 62.4] °	57.4 [47.9 – 66.7]	**0.0001**
Age at symptoms onset, median [IQR]	NC	72.4 [65.1 – 77.1] *∇	42.2 [32.6 –59.2] °	54 [43.5 – 64.4]	**0.0001**
Disease duration, months, median [IQR]	NC	10.5 [4.8 – 27.5] ∇	19.5 [2.4 – 48.4]	28.6 [10.4 – 46]	**0.0001**
Diagnostic pathway, n (%)		0 (0) *∇ 5 (10.2) *∇	34 (69.4) ° 15 (30.6) °	66 (54.5) 10 (8.3)	**<0.0001**

Neurological Family screening Cardiological Hematological Incidental cardiac uptake at bone scintigraphy	0 (0) †‡§ 106 (100) †‡§ 0 (0) †‡§ 0 (0) †‡§ 0 (0) †‡§	40 (81.6) *∇ 1 (2) *∇ 3 (6.1) *∇	0 (0) ° 0 (0) ° 0 (0) °	45 (37.2) 0 (0) 0 (0)	
Age at first bone scintigraphy, median [IQR]	48.1 [35.6 – 57.1] †§	74.6 [67.5 – 78.3] *∇	45.7 [35.8 – 53.7] °	62.4 [51.4 – 72]	**0.0001**
CTS, n (%)	21 (20) †§	28 (57.1) *	11 (22.4) °	71 (59.1)	**<0.0001**
Bilateral CTS, n (%)	19 (18.1) †§	26 (54.2) *	9 (18.4) °	67 (55.8)	**<0.0001**
NYHA class, n (%) NHYA I NYHA II NYHA III NYHA IV	 104 (98.1) †§ 2 (1.9) †§ 0 (0) †§ 0 (0) †§	 8 (16.3) *∇ 29 (59.2) *∇ 11(22.5) *∇ 1 (2.0) *∇	 47 (95.9) ° 0 (0) ° 2 (4.1) ° 0 (0) °	 49 (40.5) 58 (47.9) 14 (11.6) 0 (0)	**<0.0001**
mPND score, n (%) Score 0 Score I Score II Score IIIa Score IIIb Score IV	 106 (100) ‡§ 0 (0) ‡§ 0 (0) ‡§ 0 (0) ‡§ 0 (0) ‡§ 0 (0) ‡§	 49 (100) *∇ 0 (0) *∇ 0 (0) *∇ 0 (0) *∇ 0 (0) *∇ 0 (0) *∇	 3 (6.1) 25 (51) 18 (36.7) 0 (0) 1 (2.1) 2 (4.1)	 6 (5) 61 (50.4) 46 (38) 1 (0.8) 3 (2.5) 4 (3.3)	**<0.0001**
History of AF, n (%)	2 (1.9) †§	14 (28.6) *∇	1 (2.9)	16 (14.0)	**<0.0001**

History of CAD, n/N (%)	1/99 (1)	3/47 (6.4)	0/20 (0)	5/93 (5.4)	0.195
PPM, n (%)	0 (0)	1 (2)	0 (0)	4 (3.5)	0.183
History of HF, n/N (%)	0/101 (0) †‡§	26/46 (56.5) *∇	1/20 (5)	23/100 (23)	**<0.0001**
Hypertension, n/N (%)	17/105 (16.2) †	28/45 (62.2) *∇	5/16 (31.3)	26/89 (29.2)	**<0.0001**
SBP, mmHg median [IQR]	120 [115 – 140]	130 [110 – 140]	120 [110- 130]	120 [110 -130]	0.79
History of syncope, n/N (%)	3/100 (3) §	3/46 (6.5) ∇	1/18 (5.6)	18/8 (20.7)	**0.001**
CKD, n/N (%)	0/92 (0) †‡§	22/47 (46.8) *∇	2/18 (11.1)	20/93 (21.5)	**<0.0001**
Diabetes mellitus type 2, n/N (%)	3/100 (3) †§	15.2/46 (15.2) ∇	0/16 (0)	2/88 (2.3)	**0.007**
eGFR, ml/min/1.73 mq median [IQR]	93.5 [77 – 100] †§	60 [45 – 79] *∇	84 [69 – 97]	77 [61 – 97]	**0.0001**
Rhythm, n/N (%) Sinus rhythm Atrial arrhythmia	 104/105 (99) †§ 1/105 (0.95) †§	 38/49 (77.6) * 11/49 (22.5) *∇	 36/37 (97.3) 1/37 (2.7)	 102/115 (88.7) 11/115 (9.6)	**0.0008**
1st-degree AV block, n/N (%)	1/105 (0.95) †‡§	19/48 (39.6) *	3/37 (8.1) °	33/113 (29.2)	**<0.0001**
Low voltages, n/N (%)	6/106 (5.7) †‡§	14/49 (28.6)	7/37 (18.9)	40/111 (36)	**<0.0001**
Pseudonecrosis, n/N (%)	5/104 (4.8) †§	30/49 (61.2) *	5/37 (13.5) °	70/111 (63.1)	**<0.0001**

LBBB, n/N (%)	0/105 (0) †§	7/49 (14.3)	1/37 (2.7)	9/111 (8.1)	**0.002**
RBBB, n/N (%)	7/99 (7.1) †§	9/43 (20.9)	1/22 (4.6)	14/82 (17.1)	**0.041**
LAFB, n/N (%)	3/105 (2.8) †§	14/49 (28.6) *	2/37 (5.4) °	38/111 (34.2)	**<0.0001**
QTc ms, median [IQR]	410 [400 – 421] †§	460 [446 – 479] *	404 [390 – 440] °	450 [420 – 475]	**0.0001**
ED-IVS thinckness mm, median [IQR]	9 [9 – 10] †§	17 [15 – 20] *	10 [10 – 11] °	16 [14 – 19]	**0.0001**
ED-PW thinckness mm, median [IQR]	9 [8 – 10] †§	16 [14.5 – 18] *∇	10 [9 – 10] °	15 [13 – 17]	**0.0001**
Mean wall thickness mm, median [IQR]	9 [8.5 – 10] †§	17 [15 – 18.7] *∇	10 [9.5 – 10] °	15.7 [13.5 – 17.5]	**0.0001**
Antero-posterior left atrial diameter mm, median [IQR]	35 [31 – 39] †§	47.5 [43.5 – 51] *∇	35 [31 – 41] °	44.5 [39 – 49]	**0.0001**
LVEF %, median [IQR]	66 [63- 69] †§	56 [44.5 – 62.5] *	61 [60 – 70] °	58 [50 – 66]	**0.0001**
Restrictive filling pattern, n (%)	1 (0.98) †§	21 (44.7) *	2 (4.1) °	44 (39.3)	**<0.0001**
Pericardial effusion, n/N (%)	1/102 (1) †§	21/48 (43.8) *	2/36 (5.6) °	57/105 (54.3)	**<0.0001**

Number at risk (n/N) are indicated when data were missing in ≥ 10% of the cohort.

AF: atrial fibrillation; AV: atrioventricular; CAD: coronary artery disease; CKD: chronic kidney disease (chronic dialysis, transplant, creatinine >2.26 mg/dL or >200 µmol/L); CTS: carpal tunnel syndrome; ED-IVS: end diastolic inter ventricular septum; ED-PW: end diastolic posterior wall; eGFR: estimated glomerular filtration rate; HF: heart failure; LAFB: left anterior fascicular block;

LBBB: left bundle brunch block; LVEF: left ventricular ejection fraction; mPND: modified polyneuropathy disability; NYHA: New York Heart Association, PPM: permanent pace maker; RBBB: right bundle brunch block; SBP: systolic blood pressure.

The p-values were corrected according to the Bonferroni method.

† $p<0.05$ at post hoc analysis: Healthy carrier *vs* Cardiac phenotype.

‡ $p<0.05$ at post hoc analysis: Healthy carrier *vs* Neurological phenotype.

§ $p<0.05$ at post hoc analysis: Healthy carrier *vs* Mixed phenotype.

* $p<0.05$ at post hoc analysis: Cardiac phenotype *vs* Neurological phenotype.

∇ $p<0.05$ at post hoc analysis: Cardiac phenotype *vs* Mixed phenotype.

° $p<0.05$ at post hoc analysis: Neurological phenotype *vs* Mixed phenotype.

Table 3. Univariate and multivariate Cox regression analysis for predictors of death in the affected population

	Univariate			**Multivariate[a]**		
	HR	**95% CI**	**p-value**	**HR**	**95% CI**	**p-value**
Sex (male vs female)	0.74	0.50 - 1.01	0.13			
eGFR ml/min/1.73 mq	0.98	0.97 – 0.99	0.012			
NYHA class ≥ II	**3.52**	**2.40 – 5.15**	**< 0.0001**	**1.67**	**1.02 – 2.71**	**0.043**
mPND score ≥ II	**2.32**	**1.59 – 3.40**	**< 0.0001**	**1.85**	**1.22 – 2.81**	**0.004**
Age at diagnosis	**1.04**	**1.03 – 1.06**	**< 0.0001**	**1.03**	**1.01 – 1.05**	**0.001**
Disease-modifying therapy	**0.26**	**0.16 – 0.40**	**<0.0001**	**0.34**	**0.20 – 0.56**	**<0.0001**

Left ventricular ejection fraction (LVEF)	**0.97**	**0.95 – 0.98**	**<0.0001**	**0.99**	**0.96 – 0.98**	**0.012**
Phenotype						
Neurological vs cardiac	0.43	0.23 – 0.80	0.008			
Mixed vs cardiac	0.80	0.49 – 1.30	0.8			
Nuerological vs mixed	0.54	0.32 – 0.89	0.17			
TTR mutation						
Val30Met vs Ile68Leu	1.25	0.73 – 2.15	0.42			
Val30Met vs others	1.39	0.86 – 2.26	0.18			

[a] Multivariate analysis included parameters with $p<0.05$ at univariate analysis

eGFR: estimated glomerular filtration rate; mPND: modified polyneuropathy disability;

NYHA: New York Heart Association, TTR: transthyretin

FIGURE LEGENDS

Figure 1. Distribution of different phenotypes at baseline in the overall population

Figure 2. Kaplan-Meier survival curves based on disease-modifying therapy (liver transplantation, heart transplantation, combined transplantation, or pharmacological therapy).

Figure 3. Flow-chart of follow-up of study population

Figure 4. Kaplan-Meier survival curves adjusted by age based on phenotypes.

Figure 1

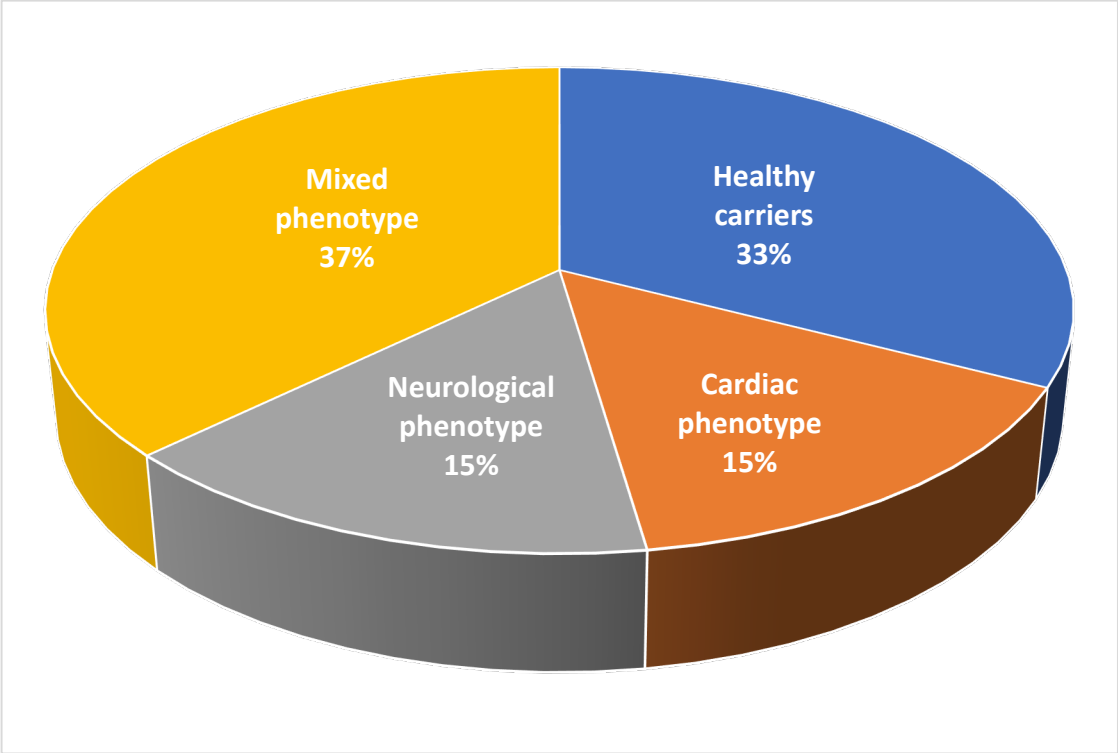
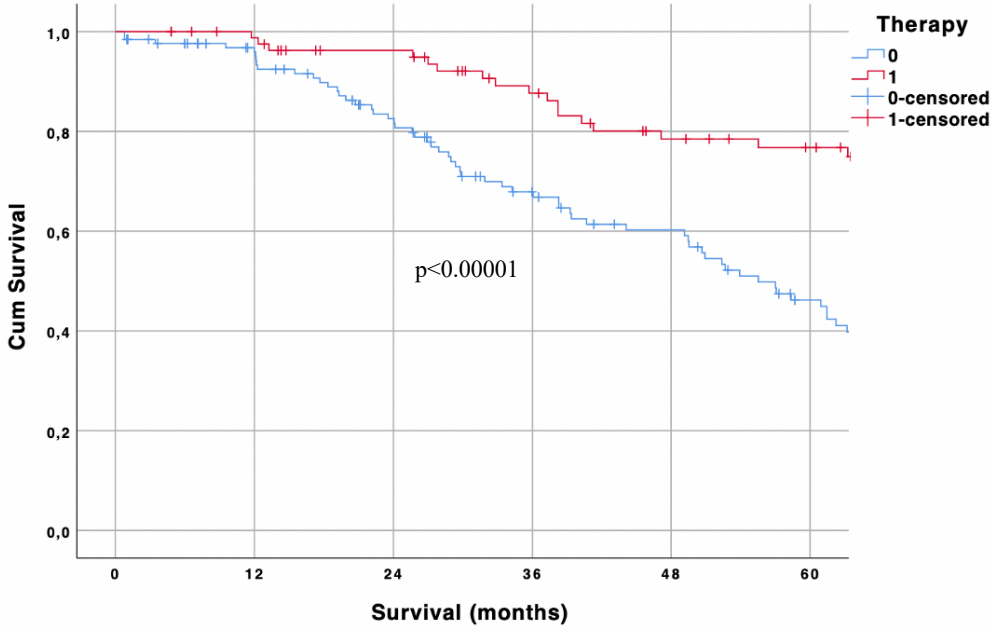


Figure 2



Number at risk						
1 (treatment)	84	79	71	59	49	44
0 (no treatment)	127	112	89	64	53	36

Figure 3

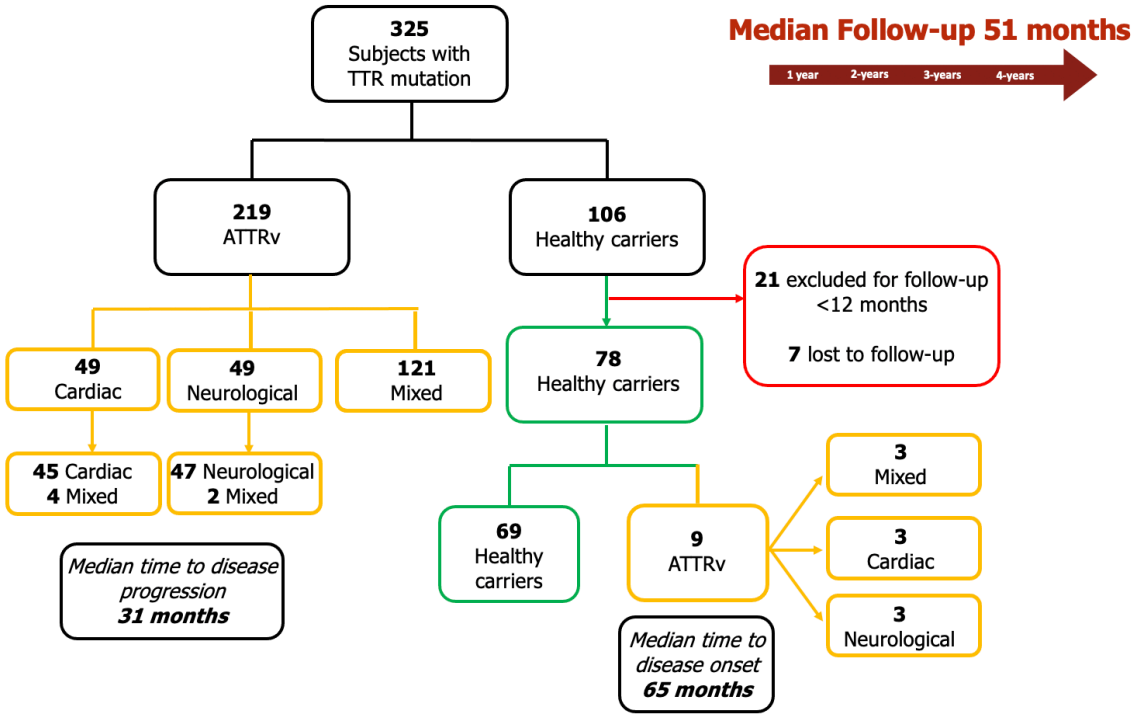
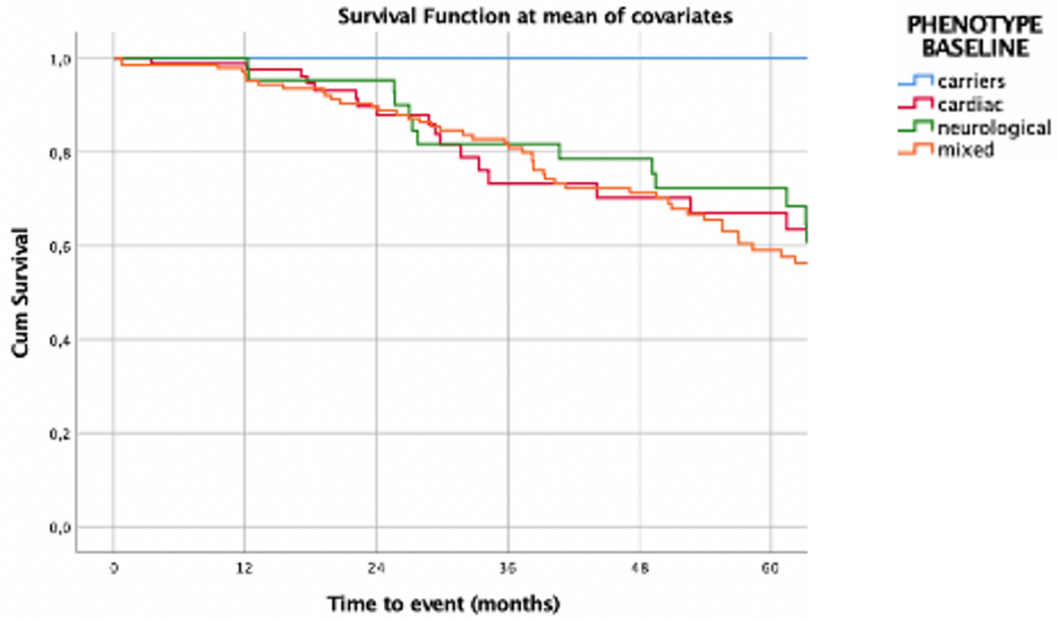


Figure 4



Number at risk						
Carriers	79	71	57	55	50	47
Cardiac	44	37	27	15	14	12
Neurological	49	46	41	32	29	24
Mixed	118	108	92	76	59	44