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TITOLO TESI

ROLE OF GENDER IN TRANSTHYRETIN-RELATED AMYLOIDOSIS: AN INTERNATIONAL PERSPECTIVE BASED ON THE TRANSTHYRETIN AMYLOID OUTCOMES SURVEY (THAOS)

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ABSTRACT

Background. Transthyretin amyloidosis (ATTR) is an underdiagnosed disease caused by destabilization of transthyretin (TTR) due to pathogenic mutations (ATTRm) or aging (ATTRwt). We explored the role of gender in determining clinical picture using the largest available database on ATTR, the ongoing Transthyretin Amyloid Outcomes Survey (THAOS) international registry.

Methods. Data through 1st April 2019 were explored. Symptomatic ATTRm (n=3737), asymptomatic ATTRm (n=644) and ATTRwt (n=874) patients were studied.

Results. Male prevalence was 61% in the entire registry, 53% in ATTRm and 95% in ATTRwt. In the overall cohort, cardiac phenotype was more frequent in males (30.7% vs 10.5%, p<0.001). Among ATTRm, 72.3% of patients with amyloidotic cardiomyopathy (ATTR-CM) were males (p<0.001) but echocardiographic features showed no substantial gender differences. Sensory abnormalities (70.1% vs 64.1%, p<0.001), autonomic abnormalities (60% vs 48.5%, p<0.001) and walking disabilities were more frequent among ATTRm males. Carpal tunnel syndrome was more frequent in ATTRm males (18.6% vs 15.5%, p=0.014). In ATTRwt cohort, females had a more pronounced (but anyhow mild) walking disability. Male-to-female ratio varied within genotype, from 0.61 in Val30Met to 11.11 in ATTRwt; furthermore, males' imbalance was more evident among symptomatic patients rather than in asymptomatic ones. Male gender, age at presentation and specific genotype were independently associated with the presence of ATTR-CM.

Conclusions. In ATTR, cardiac involvement is more frequent in men, supporting the hypothesis that some biologic characteristics may "protect" from myocardial amyloid infiltration in women. Further investigations are needed to identify possible underlying protective mechanism and orient the research for innovative, gender-tailored therapeutic approaches.

INTRODUCTION

Transthyretin amyloidosis (ATTR) is a progressive, fatal disease in which deposition of amyloid fibrils derive from either mutant (hereditary ATTR, ATTRm) or non-mutant (wild-type ATTR, ATTRwt) transthyretin (TTR), causing progressive, life-threatening organ damage.[1,2] The liver is the primary source of circulating tetrameric transthyretin protein. Clinically, morbidity and mortality in ATTR are mainly determined by heart (amyloidotic cardiomyopathy, ATTR-CM) and/or of nerves (amyloidotic polyneuropathy, ATTR-PN) involvement.[1,3]

ATTR-CM is an infiltrative, restrictive cardiomyopathy characterized by right and left heart failure, usually with preserved left ventricular ejection fraction, causing heart failure, arrhythmias or sudden death due to severe conduction disorders.[4,5] ATTR-PN typically presents with peripheral sensorimotor disturbances ascending centripetally with some degree of autonomic impairment determining orthostatic hypotension, diarrhea, impotence, and bladder disturbances.[6]

ATTRm is caused by autosomal-dominant pathogenic point mutations of TTR gene and displays tissue tropism according to genotype and, for some forms, for patient's country of origin.[7] To date, more than 100 TTR point mutations have been described.[8] Phenotype varies from predominant neurologic (e.g. Val30Met "early onset" in Portugal),[9,10] through "mixed" cardio-neuro (e.g. Val30Met "late onset" in other European country),[11,12] to predominant cardiac forms (Val122Ile in Afro-Americans and Ile68Leu in center-northern Italy).[13,14] ATTRm is an inexorably progressive disease: survival is 2 to 15 years after the neuropathy symptoms onset[15–17] but only 2 to 5 years among patients presenting with cardiomyopathy.[18,19]

ATTRwt usually presents as ATTR-CM associated with carpal tunnel syndrome (CTS) without self-reported sensory-motor or neurovegetative symptoms.[20] Survival is 54-74% at 3 years.[14,20]

Previously, small clinical studies speculated on gender differences in ATTRm, assuming a kind of protective or delaying effect of female sex on cardiac involvement.[14,21–23] The most important limitations of these studies were the small number of patients and of TTR mutations described.

Our aims were to investigate the role of gender in determining clinical profile in ATTRwt and ATTRm among a wide spectrum of mutations using the Transthyretin Amyloid Outcomes Survey (THAOS) international registry.

METHODS

Source data

THAOS was established in 2007 and is the largest ongoing, worldwide, longitudinal, observational registry created to collect demographic, clinical and instrumental data of ATTR subjects (both ATTRm and ATTRwt) from 62 active sites in 18 country (ClinicalTrials.gov: NCT00628745). All study sites received ethical and institutional review board approval prior to subject enrolment, and each subject provided written informed consent. The registry follows the International Conference on Harmonisation Good Clinical Practice guidelines and the principles of the Declaration of Helsinki. Data are collected during routine clinical evaluation and include medical history, cardiac and neurologic findings, laboratory tests and quality of life assessments. THAOS is sponsored by Pfizer, which provides financial support for the development and maintenance of the database and compensation to the survey sites for data collection. For this study, have been analyzed data through 1st April 2019.

Study population and data collection

All subjects with confirmed ATTRwt or ATTRm enrolled in the THAOS registry were included in the analysis, both symptomatic and asymptomatic patients.
Clinical, electrocardiographic (ECG), echocardiographic and laboratory data were collected from THAOS. Baseline characteristics are referred to time of enrolment and "duration of symptoms" refers to time between symptoms onset and enrollment in THAOS.

Definitions and classifications

Subjects were grouped both by genotype and phenotype. Genotype categories were: ATTRwt; Val30Met ATTRm (the most-described in literature and the most frequent mutation in the registry); mutations non-Val30Met associated with cardiac disease (i.e. Val122Ile,[13] Thr60Ala[24], Ile68Leu[14] and Leu111Met[25]); mutations non-Val30Met and not associated with cardiac disease. "Early onset" and "late onset" are referred to age of symptoms onset (<50 and ≥50 years, respectively). "Asymptomatic patients" referred to ATTRm patients with signs of disease (cardiac and/or neurological) without any symptoms.
Phenotype categories, based on clinical presentation at the time of inclusion in THAOS, were: (i) "cardiac", in presence of ATTR-CM (defined by interventricular septum [IVS] thickness >1.2 cm on echocardiography) plus at least one among abnormal ECG or signs or symptoms of heart failure, in absence of more than mild signs or symptoms of neurological involvement (erectile dysfunction, constipation and

CTS were not considered signs of neurological involvement *per se* as they are not infrequent in the general population); (ii) "neurologic", in presence of walking disability or other neurologic symptoms, including sensory, gastrointestinal symptoms (i.e. early satiety, nausea, vomiting, unintentional loss of weight, diarrhea, constipation, or fecal incontinence) or other autonomic symptoms (i.e. orthostatic hypotension [decline >20 mmHg in systolic blood pressure, or > 10 mmHg in diastolic blood pressure upon standing] or urinary incontinence) in absence of echocardiographic signs of ATTR-CM, abnormal ECG and signs or symptoms of heart failure; (iii) "mixed" (cardiac-neurologic) for all other cases.

Neuropathic symptoms have been assessed by the polyneuropathy disability (PND) score[11] as follows: stage 0: no impairment; stage I: sensory disturbances but preserved walking capability; stage II: impaired walking capability but ability to walk without a stick or crutches; stage IIIb: walking only with the help of one stick or crutch; stage IIIb: walking with the help of two sticks or crutches; stage IV: confined to a wheelchair or bedridden.

Kidney involvement was considered present in presence of a protein/creatinine value greater than 45 mg/mmol or an albumin/creatinine value greater than 30 mg/mmol.

Modified body mass index (mBMI) was defined as [weight in kilograms divided by square of height in meters] × albumin level in grams per liter.

The Karnofsky Performance Status Scale was defined by clinicians, according to authors.[26]

CTS history was considered positive in the presence of typical symptoms or of previous surgery for median nerve decompression. "Vitreal involvement" refers to visual impairment leading to ophthalmologic detection of vitreous opacities.

Statistical analysis

Simple binary and continuous variables are shown using descriptive statistics. Differences in mean outcomes between genotype and phenotype groups were assessed for statistical significance by: analysis of variance, to calculate p-values by comparing means between groups for continuous variables; the Pearson $\chi 2$ test to calculate p-values for variables with cell counts >5; the Kruskal–Wallis test to calculate p-values by comparing medians between groups for continuous variables; and the Fisher's exact test to calculate p-values for non-ordinal variables with cell counts ≤5.

Follow-up data were obtained from the periodical, scheduled visits (usually, with 6-months intervals). In cases in which no visits had been made after one year from the previous one, follow-up on vital status was performed by telephone.

Independent variables related to the presence of ATTR-CM were assessed by multivariable regression analysis.

RESULTS

Subject enrollment profile

As of April 2019, 4.648 (61% males) subjects worldwide were enrolled in THAOS (table 1a). Of these, 3.093 were ATTRm symptomatic patients (55% males), 644 ATTRm asymptomatic patients (46% males) and 874 ATTRwt (95% males). Data of 37 TTR mutation carriers (without any symptoms and signs of amyloidotic disease) were not analyzed. Age at enrollment and at symptoms onset were both significantly higher among men (respectively, 58.3 vs 47 and 53.9 vs 43.6, $p<0.001$). Figure 1 displays phenotype categories at presentation related to site of enrollment.

A clear prevalence of cardiac and mixed disease in men compared to females was observed in both overall population (table 1a, figure 2) and in ATTRm cohort (table 1b). Males ATTRm were more probably symptomatic at enrollment compared to females but these last were younger at symptoms onset (46.2 vs 51.0 years, $p<0.001$, table 1b). In ATTRm cohort, ATTR-CM and ECG abnormalities were more frequent among men. Females ATTRm had more probably a mainly neurologic disease compared to males, but their symptoms were usually milder (sensory abnormalities, autonomic impairment and walking ability were significatively less pronounced in females, table 1b). CTS was more frequent among men in both the entire cohort and in ATTRm-only patients (respectively, 25.4% vs 16.5% and 18.6% vs 15.5%, table 1a and 1b).

Gender-related genotype is shown in figure 3. ATTRwt was more frequent among males (male-to-female ratio 11.11). The most frequent TTR mutation was Val30Met (male-to-female ratio 0.61).

Figure 4 reports male prevalence according to main genotype categories: excluding early onset Val30Met, males were more numerous in all other groups, in particular among ATTRwt and cardiac non-Val30Met patients; furthermore, male prevalence was higher among symptomatic patients of each group (figure 5) than the asymptomatic ones.

In the sub-analysis of ATTRwt subjects (table 1c), females presented with a slightly more advanced neurologic disease, related to a more pronounced walking disability (68.1% of males were in PND score 0 compared to 45.5% of females, $p<0.001$). NT-pro BNP values were higher among females (4641 vs 9007.3 pg/ml, $p=0.044$). Figure 6 shows main non-cardiac abnormalities among ATTRwt according to gender: the most frequent pathological finding was CTS, that was present in 52.2% of females and in 42% of males.

In the overall ATTR-CM population (ATTRm plus ATTRwt, table 2a), both neurosensorial disease and autonomic neuropathy were more frequent in females but

neurologic profile appeared similar between genders in mutated-only ATTR-CM (table 2b). In ATTR-CM cohort (table 2a) and in mutated ATTR-CM subgroup (table 2b) LV wall thicknesses were higher in males but, once indexed to height, no differences were noted. LV EF and indexed LV mass were the only echocardiographic variables that showed gender imbalance (table 2a and 2b), being respectively higher and lower in females.

Analysis of different clinical and instrumental variables among ATTRm symptomatic male patients are shown in figure 7 and 8. Males become gradually more prevalent at worsening of Karnofsky Performance Status Scale and PND score, while percentage of males increases at increasing values of mBMI (figure 7). Males' prevalence increases too for worsening of indexed-to-height LV mean wall thickness, indexed-to-height LV mass, LV EF ($p<0.05$) and NT-pro BNP ($p=0.015$) but not for worsening of NYHA class ($p= 0.74$) (figure 8).

Table 3a and 3b shows univariate and multivariate analysis for risk of developing ATTR-CM in ATTRm patients. Male gender, late onset disease (i.e. ≥50 years) and presence of cardiac non-Val30Met and of non-cardiac non-Val30Met vs Val30Met genotype were all significantly associated with the presence of ATTR-CM in multivariate analysis (table 3b).

DISCUSSION

To the best of our knowledge, our study provides the wider and most detailed characterization by gender of ATTR patients, both ATTRwt and ATTRm. The THAOS registry is a unique tool that offers the opportunity to better characterize TTR amyloidosis on a global perspective and represents a model for the study of rare diseases. This analysis of THAOS registry demonstrates that ATTR is a very heterogeneous disease, responsible of different clinical manifestations that can vary from mainly neurologic to mainly cardiac, through different spectra of mixed cardio-neurologic forms. It is supposed that not only the specific TTR mutation but also environmental factors (first of all, the country of origin) and gender could influence the clinical manifestations.

In our study, females ATTRm appeared to be somehow protected against myocardial infiltration because of lower probability of developing ATTR-CM. Age at symptoms onset was lower and duration of symptoms was higher in females ATTRm (table 1b), but disease manifestations seem to proceed "slower" over time compared to men: indeed, despite indexed wall thickness was similar among sexes, females had higher LV EF, lower indexed LV mass and lower NT-pro BNP (even if the latter did not reach statistical significance, table 1b and 2b). Neurologic involvement was less evident among females ATTRm, both for sensorimotor and autonomic impairment, although females more probably suffered of a mainly neurologic disease at diagnosis. Interesting, CTS was more frequent among men ATTRm (tab 1b), while in literature a net female prevalence in general population has been described.[27]

This "protective effect" of female gender could be the reason why ATTRwt is an almost-exclusively men's disease.[20] In our study, as expected, male prevalence was very high (95%, table 1c) and no differences have been noted about age of symptoms onset and duration of symptoms. NT-pro BNP values were higher in females (table 1c), but this result could influenced by the very limited data available. Females ATTRwt had a more pronounced (but anyhow mild) walking disability compared to males (table 1c), while there were no differences in sensory abnormalities and in autonomic impairment. It is important to highlight how, in both gender, sensory abnormalities and autonomic impairment were present in a non-negligible percentage of ATTRwt patients (table 1c and figure 6).

It is likely that some factors (sexual hormones?) protect women in premenopausal age by amyloidotic organs infiltration but, once signs and symptoms of ATTRwt are present, phenotypic and functional advantage of being female appears less evident.

In ATTR, a possible role in determining the clinical picture could be also related to complex gender-genotype interactions. Male-to-female ratio varies among genotype

groups (figure 4), with higher prevalence among cardiac non-Val30Met and ATTRwt. This trend was evident for both asymptomatic and symptomatic subjects (figure 5) but was more evident in the latter ones.
The higher prevalence of males among ATTRm symptomatic was confirmed by Karnofsky Performance Status Scale and PND scores (figure 7) but not, apparently, by mBMI: it is likely that the higher percentage of females among lower mBMI values are related to physiologic sex imbalance of this variable.[28] Also echocardiographic variables and NT-pro BNP levels showed more severe values among males (figure 8) but without important functional impact, in view of the lack of NYHA class worsening.
Susceptibility to develop cardiac amyloidosis in ATTRm was related to male sex, late onset disease and specific TTR mutations, in particular cardiac non-Val30Met (Val122Ile, Thr60Ala, Ile68Leu and Leu111Met TTR mutations) and non-cardiac non-Val30Met compared to Val30Met patients (table 3a and 3b).
Further investigations could identify possible underlying protective mechanisms in ATTR females, unveil the complex interactions between genotype and phenotype and orient the research for innovative, gender and genetic-tailored therapeutic approaches.

Conclusion

Female gender appears to somehow protect against ATTR-related cardiac disease, especially in cardiac non-Val30Met and in ATTRwt. Male sex, late onset (i.e. > 50 years) and specific genotype (e.g. TTR mutations Val122Ile, Thr60Ala, Ile68Leu and Leu111Met) confer higher probability of develop cardiac amyloidosis in familiar TTR amyloidosis.

Limitations

Analysis of gender-related echocardiographic variables of ATTRwt was not possible due to limited availability of females' data.

TABLES

Characteristic	Overall	Males	Females	p-value
Study Population, n	4,648	2,849	1,799	
ATTRm, n (%)	3,774 (81.2%)	2,022 (71.0%)	1,752 (97.4%)	
symptomatic	3,093 / 3,737 (82.8%)	1,698 / 1,994 (85.2%)	1,395 / 1,743 (80.0%)	**<0.001**
asymptomatic	644 / 3,737 (17.2%)	296 / 1,994 (14.8%)	348 / 1,743 (20.0%)	
ATTRwt, n (%)	874 (18.8%)	827 (29.0%)	47 (2.6%)	**<0.001**
Age at enrollment (yrs)				
n	4,648	2,849	1,799	**<0.001**
mean (SD)	53.9 (18.9)	58.3 (18.8)	47.0 (16.9)	
Age at symptoms onset (yr)				
n	3,939	2,500	1,439	**<0.001**
mean (SD)	50.2 (17.8)	53.9 (17.8)	43.6 (15.7)	
Duration of symptoms (yrs)				
n	3,661	2,400	1,261	0.34
median (Q1,Q3)	4.4 (1.8, 9.8)	4.4 (1.8, 9.8)	4.4 (1.9, 9.9)	
Phenotype, n (%)				
cardiac	852 (23.9%)	725 (30.7%)	127 (10.5%)	**<0.001**
neurologic	1,699 (47.6%)	925 (39.2%)	774 (64.1%)	
mixed	1,017 (28.5%)	711 (30.1%)	306 (25.4%)	
Abnormal ECG, n/N (%)	1,734 / 2,819 (61.5%)	1,297 / 1,814 (71.5%)	437 / 1,005 (43.5%)	**<0.001**
Complete AV block or PM, n/N (%)	445 / 1,100 (40.5%)	351 / 868 (40.4%)	94 / 232 (40.5%)	0.98
LAHB, n/N (%)	299 / 1,089 (27.5%)	220 / 857 (25.7%)	79 / 232 (34.1%)	**0.011**
LPHB, n/N (%)	34 / 1,085 (3.1%)	30 / 855 (3.5%)	4 / 230 (1.7%)	0.17
LBBB, n/N (%)	129 / 1,086 (11.9%)	109 / 856 (12.7%)	20 / 230 (8.7%)	0.093
RBBB, n/N (%)	209 / 1,092 (19.1%)	171 / 862 (19.8%)	38 / 230 (16.5%)	0.26
ATTR-CM, n/N (%)	1,140 / 1,711 (66.6%)	951 / 1,258 (75.6%)	189 / 453 (41.7%)	**<0.001**
Kidney involvement, n/N (%)	780 / 1,315 (59.3%)	356 / 621 (57.3%)	424 / 694 (61.1%)	0.16
Neurological involvement:				
Sensory abnormalities, n/N (%)	2,820 / 4,605 (61.2%)	1,682 / 2,814 (59.8%)	1,138 / 1,791 (63.5%)	**0.011**
Autonomic impairment, n/N (%)	2,225 / 4,605 (48.3%)	1,371 / 2,814 (48.7%)	854 / 1,791 (47.7%)	0.49
PND score, n/N (%)				
0	1,442 / 3,487 (41.4%)	816 / 2,023 (40.3%)	626 / 1,464 (42.8%)	**<0.001**
I	1,375 / 3,487 (39.4%)	751 / 2,023 (37.1%)	624 / 1,464 (42.6%)	
II	369 / 3,487 (10.6%)	258 / 2,023 (12.8%)	111 / 1,464 (7.6%)	
IIIa	135 / 3,487 (3.9%)	94 / 2,023 (4.6%)	41 / 1,464 (2.8%)	
IIIb	101 / 3,487 (2.9%)	59 / 2,023 (2.9%)	42 / 1,464 (2.9%)	
IV	65 / 3,487 (1.9%)	45 / 2,023 (2.2%)	20 / 1,464 (1.4%)	
Carpal tunnel syndrome, n/N (%)	978 / 4,471 (21.9%)	688 / 2,711 (25.4%)	290 / 1,760 (16.5%)	**<0.001**
Vitreal involvement, n/N (%)	131 / 4,554 (2.9%)	75 / 2,772 (2.7%)	56 / 1,782 (3.1%)	0.39

Table 1a. Enrollment characteristics by gender.

ATTRm: hereditary transthyretin-related amyloidosis; ATTRwt: wild-type transthyretin-related amyloidosis; AV: atrio-ventricular; PM: pacemaker; LAHB: left anterior hemiblock; LPHB: left posterior hemiblock; LBBB: left bundle branch block; RBBB: right bundle branch block; ATTR-CM: amyloidotic cardiomyopathy; PND: polyneuropathy disability

Characteristic	Overall	Males	Females	p-value
Study Population, n	3,774	2,022	1,752	
ATTRm, n (%)	3,774 (100.0%)	2,022 (100.0%)	1,752 (100.0%)	
symptomatic	3,093 / 3,737 (82.8%)	1,698 / 1,994 (85.2%)	1,395 / 1,743 (80.0%)	**<0.001**
asymptomatic	644 / 3,737 (17.2%)	296 / 1,994 (14.8%)	348 / 1,743 (20.0%)	
Age at enrollment (yrs)				
n	3,774	2,022	1,752	**<0.001**
mean (SD)	48.8 (16.9)	51.0 (17.1)	46.2 (16.2)	
Age at symptoms onset (yr)				
n	3,089	1,696	1,393	**<0.001**
Mean (SD)	45.1 (15.8)	47.1 (16.2)	42.7 (15.0)	
Duration of symptoms (yrs)				
n	2,817	1,601	1,216	**0.004**
median (Q1,Q3)	4.3 (1.9, 9.3)	4.2 (1.9, 8.9)	4.5 (1.9, 9.9)	
Phenotype, n (%)				
cardiac	344 (12.6%)	238 (15.2%)	106 (9.1%)	**<0.001**
neurologic	1,660 (60.8%)	888 (56.7%)	772 (66.4%)	
mixed	725 (26.6%)	440 (28.1%)	285 (24.5%)	
Abnormal ECG, n/N (%)	1,059 / 2,125 (49.8%)	658 / 1,157 (56.9%)	401 / 968 (41.4%)	**<0.001**
Complete AV block or PM, n/N (%)	272 / 627 (43.4%)	185 / 422 (43.8%)	87 / 205 (42.4%)	0.74
LAHB, n/N (%)	191 / 619 (30.9%)	122 / 414 (29.5%)	69 / 205 (33.7%)	0.29
LPHB, n/N (%)	24 / 615 (3.9%)	20 / 412 (4.9%)	4 / 203 (2.0%)	0.082
LBBB, n/N (%)	61 / 617 (9.9%)	44 / 414 (10.6%)	17 / 203 (8.4%)	0.38
RBBB, n/N (%)	87 / 618 (14.1%)	58 / 415 (14.0%)	29 / 203 (14.3%)	0.92
ATTR-CM, n/N (%)	596 / 1,131 (52.7%)	431 / 704 (61.2%)	165 / 427 (38.6%)	**<0.001**
Kidney involvement, n/N (%)	778 / 1,307 (59.5%)	354 / 614 (57.7%)	424 / 693 (61.2%)	0.19
Neurological involvement:				
Sensory abnormalities, n/N (%)	2,519 / 3,742 (67.3%)	1,400 / 1,997 (70.1%)	1,119 / 1,745 (64.1%)	**<0.001**
Autonomic impairment, n/N (%)	2,046 / 3,742 (54.7%)	1,199 / 1,997 (60.0%)	847 / 1,745 (48.5%)	**<0.001**
PND score, n/N (%)				
0	1,139 / 3,035 (37.5%)	523 / 1,593 (32.8%)	616 / 1,442 (42.7%)	**<0.001**
I	1,259 / 3,035 (41.5%)	644 / 1,593 (40.4%)	615 / 1,442 (42.6%)	
II	352 / 3,035 (11.6%)	242 / 1,593 (15.2%)	110 / 1,442 (7.6%)	
IIIa	125 / 3,035 (4.1%)	84 / 1,593 (5.3%)	41 / 1,442 (2.8%)	
IIIb	97 / 3,035 (3.2%)	57 / 1,593 (3.6%)	40 / 1,442 (2.8%)	
IV	63 / 3,035 (2.1%)	43 / 1,593 (2.7%)	20 / 1,442 (1.4%)	
Carpal tunnel syndrome, n/N (%)	624 / 3,640 (17.1%)	358 / 1,926 (18.6%)	266 / 1,714 (15.5%)	**0.014**
Vitreal involvement, n/N (%)	123 / 3,705 (3.3%)	67 / 1,969 (3.4%)	56 / 1,736 (3.2%)	0.76
NT pro-BNP (pg/mL)				
n	927	536	391	0.060
mean (SD)	1748.8 (8115.0)	2177.0 (10290.0)	1161.7 (3241.7)	

Table 1b. Enrollment characteristics by gender in ATTRm (excluding ATTRwt).

ATTRm: hereditary transthyretin-related amyloidosis; ATTRwt: wild-type transthyretin-related amyloidosis; AV: atrio-ventricular; PM: pacemaker; LAHB: left anterior hemiblock; LPHB: left posterior hemiblock; LBBB: left bundle branch block; RBBB: right bundle branch block; ATTR-CM: amyloidotic cardiomyopathy; PND: polyneuropathy disability; NT pro-BNP: N-terminal pro–brain natriuretic peptide

Characteristic	Overall	Males	Females	p-value
Study Population, n	874	827	47	
Age at enrollment (yrs)				
n	874	827	47	0.053
mean (SD)	76.4 (7.2)	76.3 (7.1)	78.3 (8.3)	
Age at symptoms onset (yrs)				
n	850	804	46	0.38
mean (SD)	68.3 (11.5)	68.3 (11.5)	69.8 (12.4)	
Duration of symptoms (yrs)				
n	844	799	45	0.72
median (Q1,Q3)	5.1 (1.8, 11.5)	5.1 (1.8, 11.4)	4.3 (2.1, 11.9)	
Phenotype, n (%)				
cardiac	508 (60.5%)	487 (61.3%)	21 (47.7%)	0.17
neurologic	39 (4.6%)	37 (4.7%)	2 (4.5%)	
mixed	292 (34.8%)	271 (34.1%)	21 (47.7%)	
Abnormal ECG, n/N (%)	675 / 694 (97.3%)	639 / 657 (97.3%)	36 / 37 (97.3%)	0.99
Complete AV block or PM, n/N (%)	173 / 473 (36.6%)	166 / 446 (37.2%)	7 / 27 (25.9%)	0.24
LAHB, n/N (%)	108 / 470 (23.0%)	98 / 443 (22.1%)	10 / 27 (37.0%)	0.074
LPHB, n/N (%)	10 / 470 (2.1%)	10 / 443 (2.3%)	0 / 27 (0.0%)	0.43
LBBB, n/N (%)	68 / 469 (14.5%)	65 / 442 (14.7%)	3 / 27 (11.1%)	0.61
RBBB, n/N (%)	122 / 474 (25.7%)	113 / 447 (25.3%)	9 / 27 (33.3%)	0.35
ATTR-CM, n/N (%)	544 / 580 (93.8%)	520 / 554 (93.9%)	24 / 26 (92.3%)	0.75
Kidney involvement, n/N (%)	2 / 8 (25.0%)	2 / 7 (28.6%)	0 / 1 (0.0%)	0.54
Neurological involvement:				
sensory abnormalities, n/N (%)	301 / 863 (34.9%)	282 / 817 (34.5%)	19 / 46 (41.3%)	0.35
autonomic impairment, n/N (%)	179 / 863 (20.7%)	172 / 817 (21.1%)	7 / 46 (15.2%)	0.34
PND score, n/N (%)				
0	303 / 452 (67.0%)	293 / 430 (68.1%)	10 / 22 (45.5%)	**<0.001**
I	116 / 452 (25.7%)	107 / 430 (24.9%)	9 / 22 (40.9%)	
II	17 / 452 (3.8%)	16 / 430 (3.7%)	1 / 22 (4.5%)	
IIIa	10 / 452 (2.2%)	10 / 430 (2.3%)	0 / 22 (0.0%)	
IIIb	4 / 452 (0.9%)	2 / 430 (0.5%)	2 / 22 (9.1%)	
IV	2 / 452 (0.4%)	2 / 430 (0.5%)	0 / 22 (0.0%)	
Carpal tunnel syndrome, n/N (%)	354 / 831 (42.6%)	330 / 785 (42.0%)	24 / 46 (52.2%)	0.18
Vitreal involvement, n/N (%)	8 / 849 (0.9%)	8 / 803 (1.0%)	0 / 46 (0.0%)	0.50
NT pro-BNP (pg/mL)				
n	469	450	19	**0.044**
Mean (SD)	4817.9 (9243.4)	4641.0 (9032.8)	9007.3 (12918.5)	

Table 1c. Enrollment characteristics by gender in ATTRwt (excluding ATTRm).

ATTRwt: wild-type transthyretin-related amyloidosis; ATTRm: hereditary transthyretin-related amyloidosis; AV: atrio-ventricular; PM: pacemaker; LAHB: left anterior hemiblock; LPHB: left posterior hemiblock; LBBB: left bundle branch block; RBBB: right bundle branch block; ATTR-CM: amyloidotic cardiomyopathy; PND: polyneuropathy disability; NT pro-BNP: N-terminal pro–brain natriuretic peptide

Characteristic	Overall (n=1.140)	Males (n=951)	Females (n=189)	p-value
Age at enrollment (yrs)				
n	1,140	951	189	**<0.001**
mean (SD)	69.7 (10.9)	70.5 (10.7)	66.0 (11.5)	
Duration of symptoms (yrs)				
n	1,118	933	185	0.20
median (Q1,Q3)	5.1 (2.2, 11.0)	5.1 (2.1, 10.8)	5.4 (2.8, 11.9)	
NYHA class				
n	876	743	133	0.70
mean (SD)	2.5 (0.7)	2.5 (0.7)	2.5 (0.8)	
NYHA class >, n (%)	409 (46.7%)	344 (46.3%)	65 (48.9%)	0.58
Neurosensorial disease				
n	779	638	141	**<0.001**
mean (SD)	2.0 (1.3)	2.0 (1.2)	2.4 (1.4)	
Neurosensorial disease stage > 2				
n	779	638	141	**<0.001**
mean (SD)	0.1 (0.3)	0.1 (0.3)	0.2 (0.4)	
Autonomic neuropathy, n/N (%)	497 / 1,134 (43.8%)	394 / 945 (41.7%)	103 / 189 (54.5%)	**0.001**
E wave deceleration time (msec)				
n	542	449	93	0.19
mean (SD)	182.5 (50.7)	183.8 (51.2)	176.3 (47.7)	
E wave/A wave ratio				
n	390	309	81	0.16
median (Q1,Q3)	1.6 (0.9, 2.7)	1.7 (1.0, 2.9)	1.4 (0.8, 2.2)	
LV EF (%)				
n	960	798	162	**<0.001**
mean (SD)	56.4 (12.4)	55.6 (12.4)	60.1 (12.1)	
Diastolic IVS thickness (mm)				
n	1,140	951	189	**<0.001**
mean (SD)	17.5 (3.2)	17.7 (3.2)	16.5 (3.1)	
Diastolic IVS thickness (mm)/height (m)				
n	1,093	912	181	0.79
mean (SD)	10.2 (2.0)	10.2 (2.0)	10.2 (2.0)	
Diastolic PW thickness (mm)				
n	1,097	917	180	**<0.001**
mean (SD)	15.5 (3.2)	15.7 (3.2)	14.5 (3.0)	
Diastolic PW thickness (mm)/height (m)				
n	1,056	884	172	0.98
mean (SD)	9.1 (2.0)	9.1 (2.0)	9.1 (1.9)	
LV mean wall thickness (mm)				
n	1,097	917	180	**<0.001**
mean (SD)	16.5 (2.9)	16.7 (2.9)	15.5 (2.7)	
LV mean wall thickness (mm)/height (m)				
n	1,056	884	172	0.93
mean (SD)	9.6 (1.8)	9.6 (1.8)	9.6 (1.7)	
LV mass index (g/m2)				
n	1,017	848	169	**0.004**
mean (SD)	170.8 (53.5)	172.9 (54.0)	160.0 (49.8)	

Table 2a: Enrollment characteristics by gender in patients with ATTR-CM.
ATTR-CM: amyloidotic cardiomyopathy; NYHA: New York Class Association; LV: left ventricular; EF: ejection fraction; IVS: interventricular septum; PW: posterior wall

Characteristic	Overall (N=596)	Males (N=431)	Females (N=165)	p-value
Age at enrollment (yrs)				
n	596	431	165	0.72
mean (SD)	64.0 (10.8)	63.9 (10.7)	64.3 (11.0)	
Duration of ATTR symptoms (yrs)				
n	587	425	162	**0.044**
median (Q1,Q3)	5.0 (2.3, 10.5)	4.9 (2.2, 10.3)	5.3 (2.8, 11.3)	
NYHA class				
n	386	276	110	0.95
mean (SD)	2.5 (0.8)	2.5 (0.8)	2.5 (0.8)	
NYHA class > II, n (%)	191 (49.5%)	137 (49.6%)	54 (49.1%)	0.92
Neurosensorial disease				
n	472	344	128	0.44
mean (SD)	2.4 (1.3)	2.4 (1.3)	2.5 (1.4)	
Neurosensorial disease stage > 2				
n	472	344	128	0.18
mean (SD)	0.2 (0.4)	0.2 (0.4)	0.2 (0.4)	
Autonomic neuropathy, n/N (%)	387 / 594 (65.2%)	287 / 429 (66.9%)	100 / 165 (60.6%)	0.15
E wave deceleration time (msec)				
n	267	184	83	0.25
mean (SD)	182.6 (53.7)	185.1 (55.9)	176.9 (48.1)	
E wave/A wave ratio				
n	232	157	75	0.98
median (Q1,Q3)	1.3 (0.8, 2.4)	1.3 (0.8, 2.5)	1.2 (0.8, 2.2)	
LV EF (%)				
n	489	347	142	**0.030**
mean (SD)	58.7 (13.3)	57.8 (13.7)	60.7 (12.0)	
Diastolic IVS thickness (mm)				
n	596	431	165	**<0.001**
mean (SD)	17.2 (3.3)	17.5 (3.3)	16.3 (3.0)	
Diastolic IVS thickness (mm)/height (m)				
n	559	401	158	0.67
mean (SD)	10.1 (1.9)	10.1 (2.0)	10.1 (1.9)	
Diastolic PW thickness (mm)				
n	562	406	156	**0.003**
mean (SD)	15.1 (3.4)	15.4 (3.5)	14.4 (3.1)	
Diastolic PW thickness (mm)/height (m)				
n	531	382	149	0.35
mean (SD)	8.9 (2.0)	8.8 (2.0)	9.0 (2.0)	
LV mean wall thickness (mm)				
n	562	406	156	**<0.001**
mean (SD)	16.1 (3.0)	16.4 (3.1)	15.3 (2.7)	
LV mean wall thickness (mm)/height (m)				
n	531	382	149	0.48
mean (SD)	9.5 (1.8)	9.4 (1.8)	9.6 (1.7)	
LV mass index (g/m2)				
n	516	370	146	**0.023**
mean (SD)	165.7 (49.6)	168.8 (49.5)	157.8 (49.1)	

Table 2b: Enrollment characteristics by gender in ATTRm with amyloidotic cardiomyopathy.
ATTRm: hereditary transthyretin-related amyloidosis; NYHA: New York Class Association; LV: left ventricular; EF: ejection fraction; IVS: interventricular septum; PW: posterior wall

Independent variable	N	Odds Ratio (CI)	P-value
Sex (male vs. female)	1,131	2.51 (1.96, 3.21)	**<0.001**
Age at onset (>=50 vs. <50)	1,022	6.46 (4.91, 8.51)	**<0.001**
Genotype: Val30Met vs Cardiac non-Val30Met	723	0.23 (0.17, 0.33)	**<0.001**
Genotype: Val30Met vs non-Cardiac non-Val30Met	893	0.34 (0.26, 0.45)	**<0.001**
Genotype: Cardiac non-Val30Met vs non-Cardiac non-Val30Met	646	1.46 (1.03, 2.05)	**0.032**

Table 3a: Univariate analyses of dependent variable: amyloidotic cardiomyopathy (only ATTRm)

Parameter	Type III SS p-value	Comparisons	Estimate	Standard Error	Lower	Upper	p-value
Gender	<.0001	Male vs Female	2.0947	0.2526	1.5996	2.5898	**<0.0001**
Onset	<.0001	Late vs Early	3.1853	0.2528	2.6898	3.6808	**<0.0001**
Genotype	<.0001	Cardiac non-Val30Met vs Val30Met	2.6988	0.3385	2.0347	3.3630	**<0.0001**
		Non-cardiac non-Val30Met vs Val30Met	2.4005	0.2707	1.8693	2.9316	**<0.0001**
		Cardiac non-Val30Met vs non-cardiac non-Val30Met	0.2984	0.3441	-0.3769	0.9736	0.3861

Table 3b: Multivariate regression analysis of dependent variable: amyloidotic cardiomyopathy (only ATTRm)

FIGURES

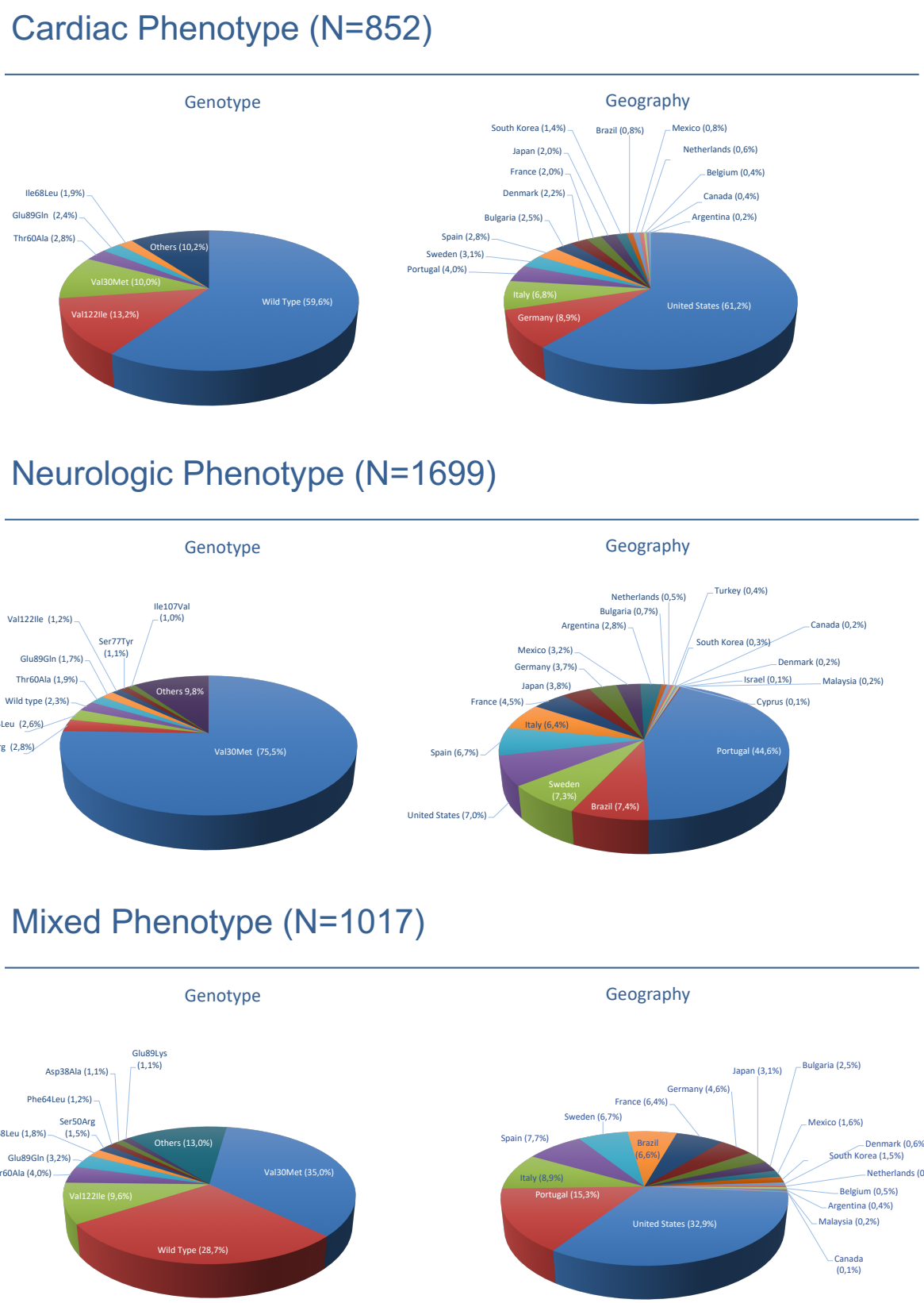

Figure 1: Phenotype at presentation related to site of enrollment

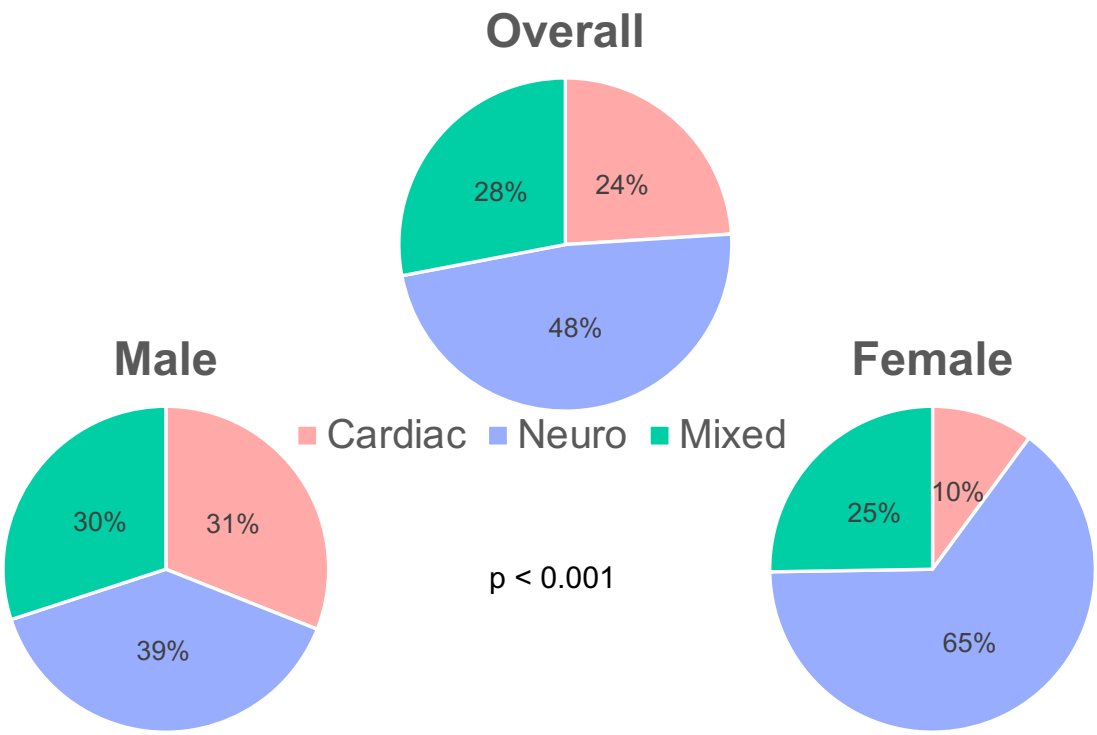

Figure 2: Phenotypic profile at enrollment by gender.

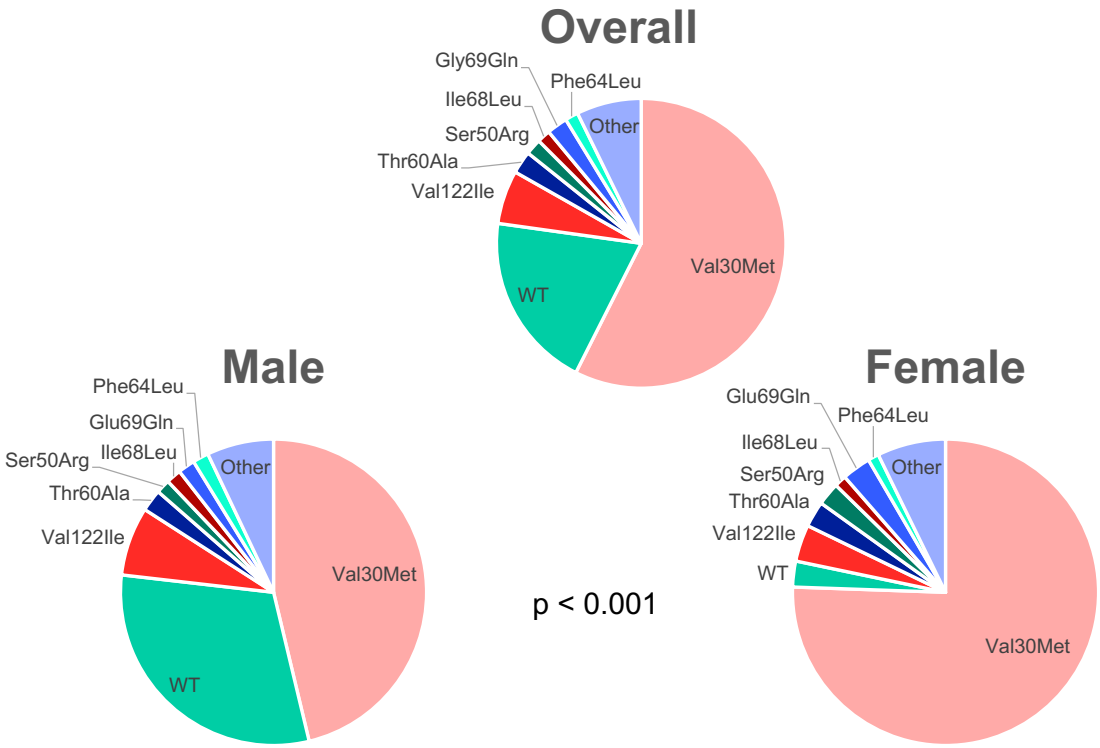

Figure 3: Genotype by gender.

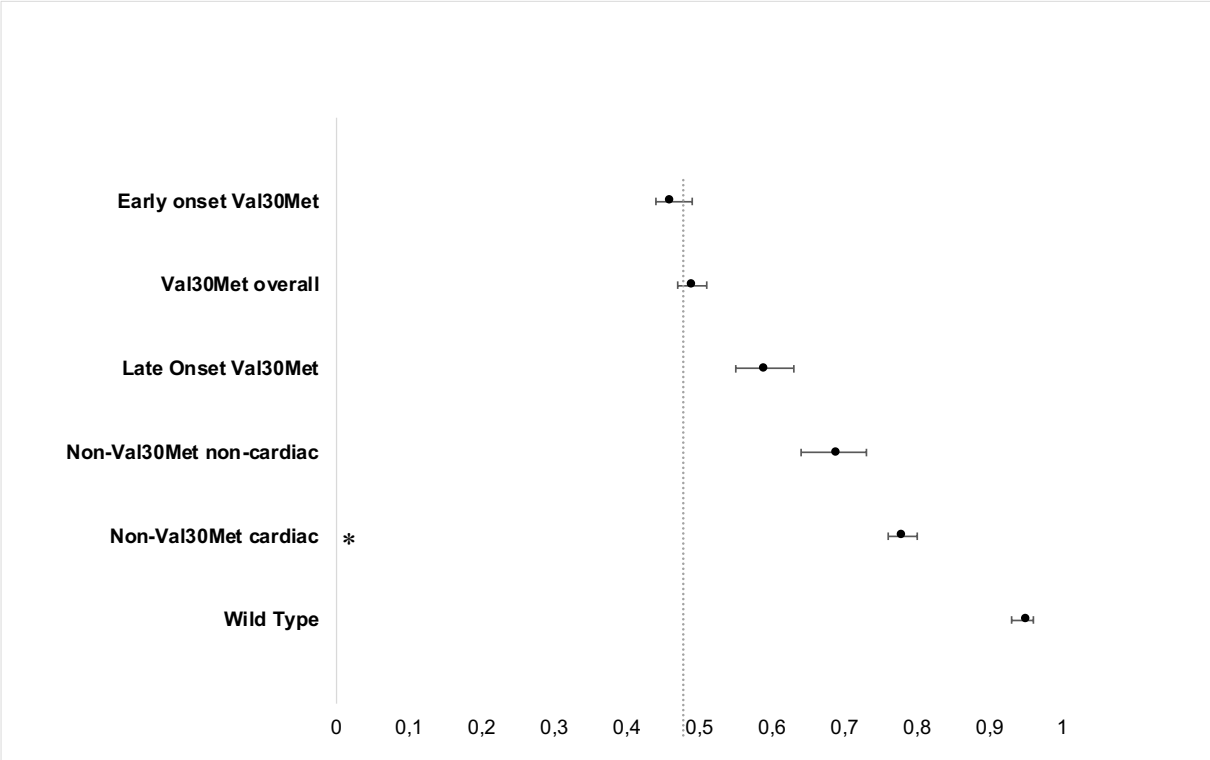

Figure 4: Male prevalence according to main genotype categories.
* Val122Ile, Thr60Ala, Ile68Leu an Leu111Met TTR mutations

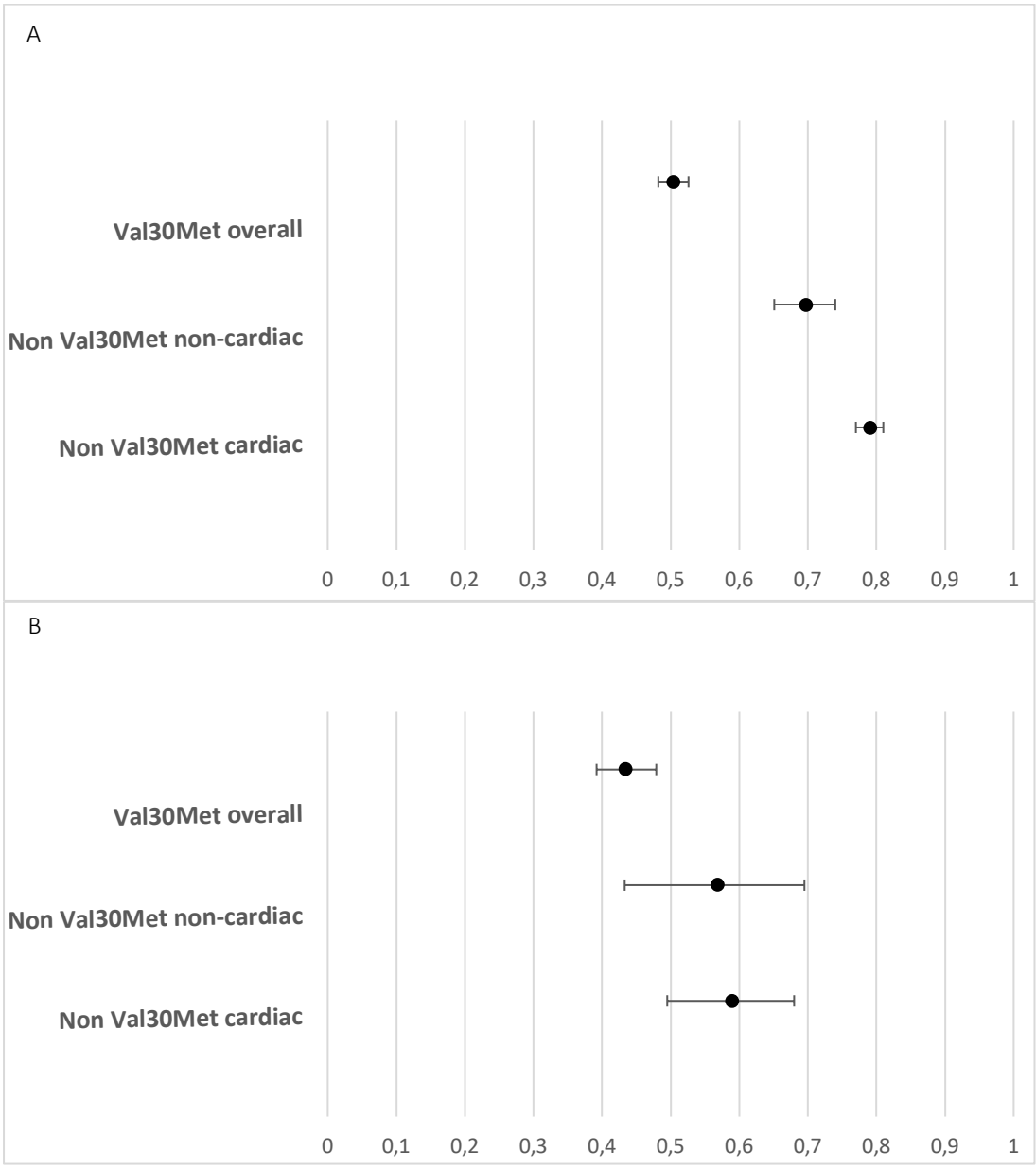

Figure 5: Male prevalence according to disease symptoms at enrollment (A: symptomatic patients; B: asymptomatic subjects).

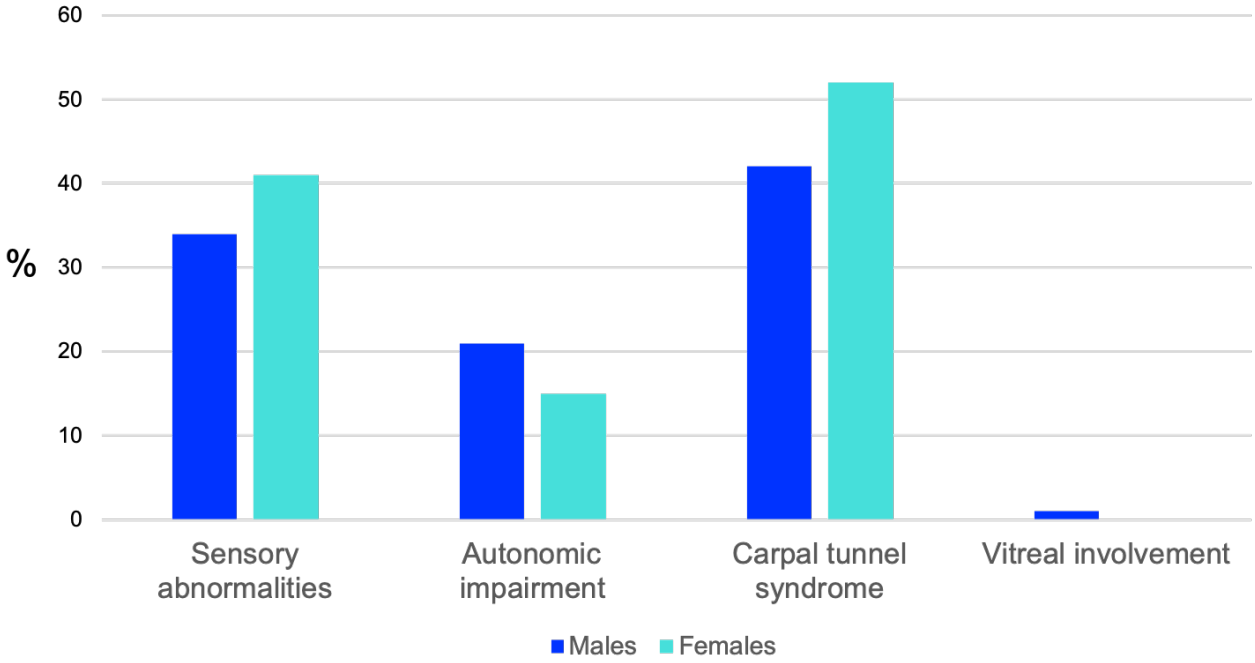

Figure 6: Main non-cardiac abnormalities in ATTRwt patients according to gender.

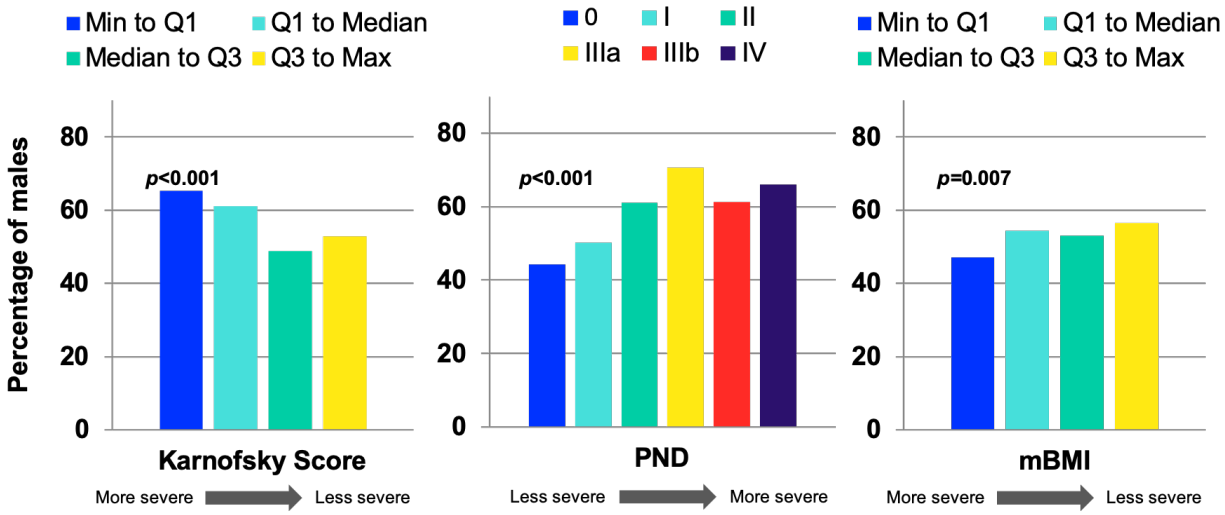

Figure 7: Percentage of males referred to different quartiles or stages of Karnofsky Score, PND score and mBMI in ATTRm symptomatic patients.
PND: polyneuropathy disability; mBMI: modified body mass index

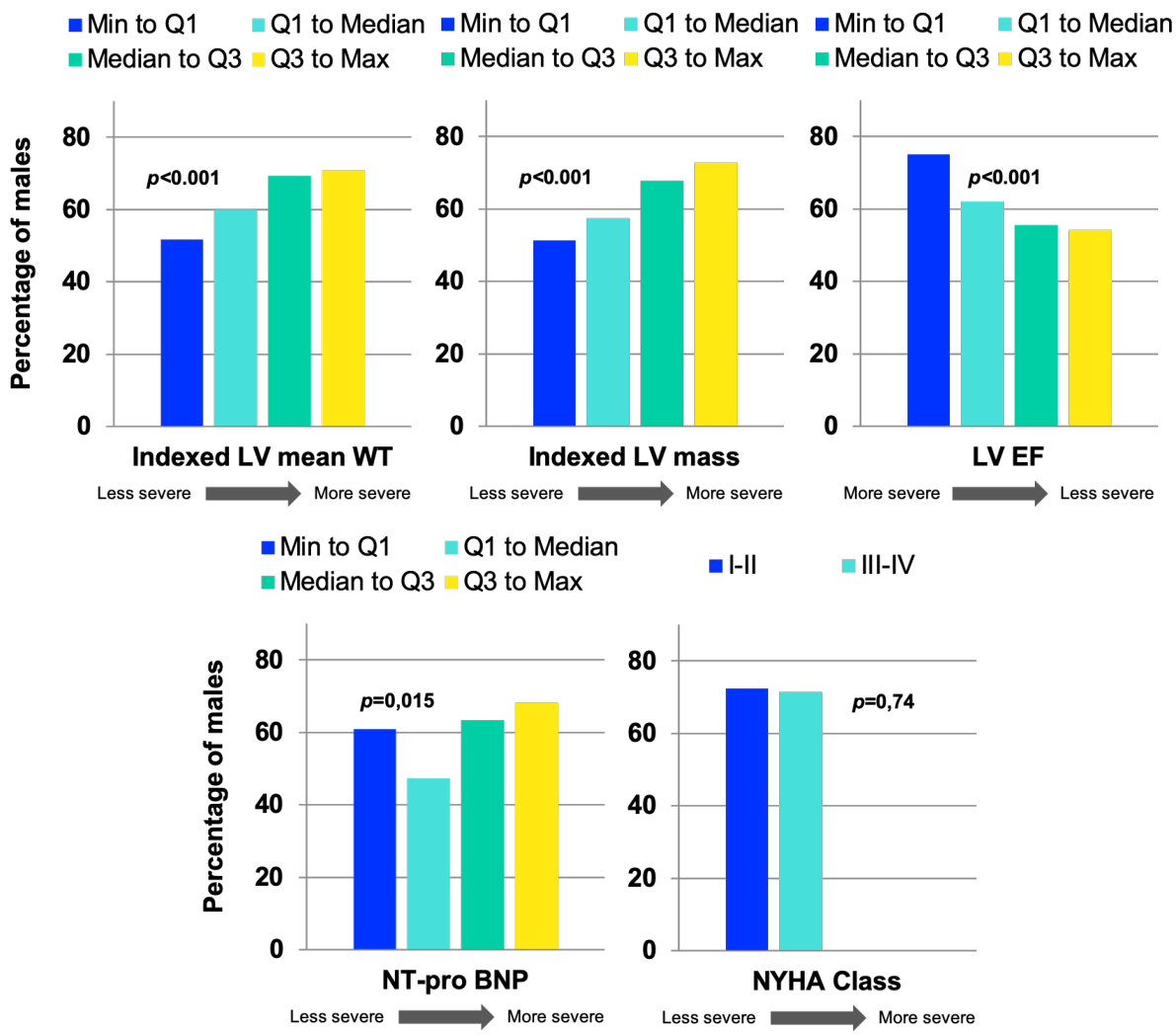

Figure 8: Percentage of males referred to different quartiles or stages of indexed LV mean WT, indexed LV mass, LV EF, NT-pro BNP and NYHA class in ATTRm symptomatic patients. LV: left ventricle; WT: wall thickness; EF: ejection fraction; NT-pro BNP: N-terminal pro–brain natriuretic peptide; NYHA: New York Heart Association.

BIBLIOGRAPHY

1. Hawkins PN, Ando Y, Dispenzeri A, Gonzalez-Duarte A, Adams D, Suhr OB. Evolving landscape in the management of transthyretin amyloidosis. Ann Med 2015;47(8):625–38.
2. Ruberg FL, Berk JL. Transthyretin (TTR) cardiac amyloidosis. Circulation 2012;126(10):1286–300.
3. Rapezzi C, Lorenzini M, Longhi S, et al. Cardiac amyloidosis: the great pretender. Heart Fail Rev 2015;20(2):117–24.
4. Dungu JN, Anderson LJ, Whelan CJ, Hawkins PN. Cardiac transthyretin amyloidosis. Heart Br Card Soc 2012;98(21):1546–54.
5. Maurer MS, Elliott P, Comenzo R, Semigran M, Rapezzi C. Addressing Common Questions Encountered in the Diagnosis and Management of Cardiac Amyloidosis. Circulation 2017;135(14):1357–77.
6. Parman Y, Adams D, Obici L, et al. Sixty years of transthyretin familial amyloid polyneuropathy (TTR-FAP) in Europe: where are we now? A European network approach to defining the epidemiology and management patterns for TTR-FAP. Curr Opin Neurol 2016;29 Suppl 1:S3–13.
7. Damy T, Kristen AV, Suhr OB, et al. Transthyretin cardiac amyloidosis in continental Western Europe: an insight through the Transthyretin Amyloidosis Outcomes Survey (THAOS). Eur Heart J 2019;ehz173.
8. Rowczenio DM, Noor I, Gillmore JD, et al. Online registry for mutations in hereditary amyloidosis including nomenclature recommendations. Hum Mutat 2014;35(9):E2403-2412.
9. Sousa A, Coelho T, Barros J, Sequeiros J. Genetic epidemiology of familial amyloidotic polyneuropathy (FAP)-type I in Póvoa do Varzim and Vila do Conde (north of Portugal). Am J Med Genet 1995;60(6):512–21.
10. Conceição I, De Carvalho M. Clinical variability in type I familial amyloid polyneuropathy (Val30Met): Comparison between late- and early-onset cases in Portugal. Muscle Nerve 2007;35(1):116–8.
11. Ando Y, Coelho T, Berk JL, et al. Guideline of transthyretin-related hereditary amyloidosis for clinicians. Orphanet J Rare Dis 2013;8(1):31.
12. Rapezzi C, Quarta CC, Riva L, et al. Transthyretin-related amyloidoses and the heart: a clinical overview. Nat Rev Cardiol 2010;7(7):398–408.
13. Quarta CC, Buxbaum JN, Shah AM, et al. The amyloidogenic V122I transthyretin variant in elderly black Americans. N Engl J Med 2015;372(1):21–9.
14. Gagliardi C, Perfetto F, Lorenzini M, et al. Phenotypic profile of Ile68Leu transthyretin amyloidosis: an underdiagnosed cause of heart failure: Ile68Leu transthyretin amyloidosis. Eur J Heart Fail 2018;20(10):1417–25.
15. Adams D, Coelho T, Obici L, et al. Rapid progression of familial amyloidotic polyneuropathy: a multinational natural history study. Neurology 2015;85(8):675–82.
16. Koike H, Tanaka F, Hashimoto R, et al. Natural history of transthyretin Val30Met familial amyloid polyneuropathy: analysis of late-onset cases from non-endemic areas. J Neurol Neurosurg Psychiatry 2012;83(2):152–8.
17. Mariani L-L, Lozeron P, Théaudin M, et al. Genotype-phenotype correlation and course of transthyretin familial amyloid polyneuropathies in France. Ann Neurol 2015;78(6):901–16.
18. Ruberg FL, Maurer MS, Judge DP, et al. Prospective evaluation of the morbidity and mortality of wild-type and V122I mutant transthyretin amyloid cardiomyopathy: the Transthyretin Amyloidosis Cardiac Study (TRACS). Am Heart J 2012;164(2):222-228.e1.
19. Castaño A, Drachman BM, Judge D, Maurer MS. Natural history and therapy of TTR-cardiac amyloidosis: emerging disease-modifying therapies from organ transplantation to stabilizer and silencer drugs. Heart Fail Rev 2015;20(2):163–78.
20. González-López E, Gagliardi C, Dominguez F, et al. Clinical characteristics of wild-type

transthyretin cardiac amyloidosis: disproving myths. Eur Heart J 2017;38(24):1895–904.
21. Rapezzi C, Perugini E, Salvi F. Female Sex as a Possible Protective Factor From Myocardial Involvement in Transthyretin-Related Amyloidoses. Arch Intern Med 2005;165(20):2429.
22. Hörnsten R, Pennlert J, Wiklund U, Lindqvist P, Jensen SM, Suhr OB. Heart complications in familial transthyretin amyloidosis: impact of age and gender. Amyloid Int J Exp Clin Investig Off J Int Soc Amyloidosis 2010;17(2):63–8.
23. Arvidsson S, Pilebro B, Westermark P, Lindqvist P, Suhr OB. Amyloid Cardiomyopathy in Hereditary Transthyretin V30M Amyloidosis - Impact of Sex and Amyloid Fibril Composition. PloS One 2015;10(11):e0143456.
24. Sattianayagam PT, Hahn AF, Whelan CJ, et al. Cardiac phenotype and clinical outcome of familial amyloid polyneuropathy associated with transthyretin alanine 60 variant. Eur Heart J 2012;33(9):1120–7.
25. Ranløv I, Alves IL, Ranløv PJ, Husby G, Costa PP, Saraiva MJ. A Danish kindred with familial amyloid cardiomyopathy revisited: identification of a mutant transthyretin-methionine111 variant in serum from patients and carriers. Am J Med 1992;93(1):3–8.
26. Karnofsky DA, Burchenal JH. The Clinical Evaluation of Chemotherapeutic Agents in Cancer. C. M. MacLeod, Ed, Evaluation of Chemotherapeutic Agents, Columbia University Press, New York, 1949, P. 196.
27. Mondelli M, Giannini F, Giacchi M. Carpal tunnel syndrome incidence in a general population. Neurology 2002;58(2):289–94.
28. Flegal KM. Body mass index of healthy men compared with healthy women in the United States. Int J Obes 2005 2006;30(2):374–9.