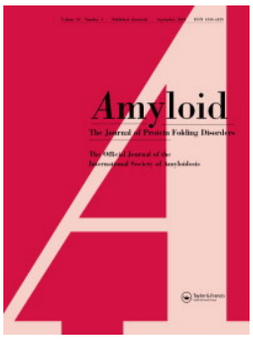

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RESEARCH ARTICLE

Heterogeneity in families with ATTRV30M amyloidosis: a historical and longitudinal Portuguese case study impact for genetic counselling

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ABSTRACT

Background: Hereditary transthyretin amyloidosis (ATTRv amyloidosis) is an inherited disease, where the study of family history holds importance. This study evaluates the changes of age-of-onset (AOO) and other age-related clinical factors within and among families affected by ATTRv amyloidosis.

Methods: We analysed information from 934 trees, focusing on family, parents, probands and siblings relationships. We focused on 1494 female and 1712 male symptomatic ATTRV30M patients. Results are presented alongside a comparison of current with historical records. Clinical and genealogical indicators identify major changes.

Results: Overall, analysis of familial data shows the existence of families with both early and late patients (1/6). It identifies long familial follow-up times since patient families tend to be diagnosed over several years. Finally, results show a large difference between parent-child and proband-patient relationships (20–30 years).

Conclusions: This study reveals that there has been a shift in patient profile, with a recent increase in male elderly cases, especially regarding probands. It shows that symptomatic patients exhibit less variability towards siblings, when compared to other family members, namely the transmitting ancestors' age of onset. This can influence genetic counselling guidelines.

Abbreviations: AOO: age-at-onset, age-of-onset; ATTRv: hereditary transthyretin amyloidosis (v stands for variant); ATTRV30M: specific genetic mutation associated with transthyretin; coef.var: coefficient of variation; DPD: diphosphono-propanodicarboxylic acid scintigraphy; ECG: electrocardiogram; GLM: generalised linear model; NMR: not most recent cases; OMR: only most recent cases; SD: standard deviation; UCA: Unidade Corino de Andrade

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ATTRv amyloidosis; epidemiological study; genealogical data; Portuguese families

Introduction

ATTRv Amyloidosis, previously known as familial amyloid polyneuropathy, was initially described by Dr. Corino de Andrade in 1952 [1,2]. It is characterised by rapidly progressing symptoms [3] and a life expectancy ranging from 2 to 10 years after onset, if left untreated [4]. Accurate diagnosis can be challenging due to disease variability, both in terms of clinical characteristics that may overlap with other disorders [5], especially when onset occurs at later ages, as well as due to the predominance of specific organ's involvement, influenced by mutations and genetic background [6].

In general, ATTRv Amyloidosis exhibits a wide variation in age-of-onset (AOO) among groups, families, and generations [7]. With over 130 genetic variations, and though rare, this disease has numerous ongoing clinical studies that increase its understanding [8–10].

Currently, Portugal remains the largest worldwide endemic region for ATTRv Amyloidosis, with ATTRV30M being its most common mutation. This variation manifests as either an early- or late-onset [11]. Previous studies indicate that women have a later AOO when compared to men, and men show greater AOO anticipation, particularly when they inherit the disease from their mothers [12]. Families recognise it due to the presence of similar symptoms and shared causes of illness. As such, family history serves as a crucial indicator for diagnosing and monitoring families with positive cases [13]. Portuguese patients usually present an early-onset diagnosis, with a predominant neuropathy. Neuropathy may show a short preclinical silent period, where disease process is in place, but symptoms are absent. Cardiomyopathy, the most common cardiac presentation, may present a larger silent preclinical period, as suggested by the cases presenting a severe carpal tunnel syndrome,

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more than ten years before cardiac failure onset. Diagnosis is based in the onset of characteristic symptoms progressing constantly, and abnormal results of non-invasive tests whose frequency depend on patient characteristics [14,15]. Neurophysiological tests, addressing small and large nerve fibres function and cardiac exams including echocardiogram, ECG and Holter records are important for the diagnosis of neuropathy and/or cardiac involvement. Previous follow-up of asymptomatic carriers allows medical professionals to pinpoint as abnormal a significant change in the results, identified as a loss of function, although still in the large range of 'normal results'. Amyloid deposition in any tissue and/or a positive DPD scintigraphy are particularly useful for the confirmation of the diagnosis as amyloid deposition (or a surrogate as scintigraphy) is recognised as a pathognomonic sign of the disease.

In this study, we retrospectively examine families affected by ATTRv Amyloidosis. Our aim is to characterise the age-related clinical and familial heterogeneity, determine the evolution of AOO over time, and describe the profile of symptomatic carriers, in northern Portuguese families.

Materials and methods

Subjects

Portugal has two national reference centres that diagnose and oversee patients with symptoms linked to ATTRv Amyloidosis. Within the northern centre, Unidade Corino de Andrade (UCA), which operates under the umbrella of Centro Hospitalar Universitário de Santo António, 934 families are enlisted, with 4611 symptomatic and asymptomatic patients (49.89% males, 50.12% females). From these, 873 families (93.5%) correspond to ATTRV30M Amyloidosis families.

In this study, we conduct a retrospective observational analysis of clinical and familial data of patients who have or are being monitored at the unit and have received a confirmed positive diagnosis. Due to the variable nature of symptom onset or the unavailability of current patient data, from the 4611 patients, a total of 644 patients (53.6% females, 46.4% males) could not be definitively classified between symptomatic or asymptomatic, or lacked matched birth dates and/or onset dates. This left us with 3967 individuals, among which there are, as symptomatic, 1494 females and 1712 males. These are the focus of this study.

Methods

To ensure a comprehensive evaluation of the entire patient cohort, and a distinct understanding of the characteristics of the most recent patients, we organised them into clusters (groups of symptomatic carriers) based on their year-of-diagnosis. After several experiments and evaluating the distribution of the AOO (Figure 1), we divided the cohort accordingly to year-of-diagnosis, to get the years that took for each 25% of patients to be diagnosed. So as not to separate patients diagnosed in the same year, and after obtaining the relevant years-of-diagnosis (i.e. 1985, 1998, and 2008), it was decided to place in Q1 patients diagnosed up to 1984, in Q2 patients diagnosed between 1985 and 1997, in Q3 patients diagnosed between 1998 and 2007, and in Q4 the rest. The groups, henceforth referred to as Q1, Q2, Q3, and Q4 are illustrated in Supplementary Figure 1, with the distribution of AOO being in Figure 2.

To align with recognised clinical categories, we further divided the Q-groups into early and late-onset. These categories determine whether a patient is classified as having early-onset, that is, if they exhibit symptoms before the age of 50, or late-onset, if symptoms occur at the age of 50 or older. We then examined these sub-groups for differences in AOO, age-of-diagnosis, time-to-diagnosis, age-at-death, and time-to-death (Tables 1 and 2). To account for overall changes, the sub-groups were analysed using Generalised Linear Models (GLM) (Table 3).

To identify and assess genealogical variability, we considered the number of patients present in the genealogical trees for each of the Q clusters. This enabled us to assess them as new and old families. We defined a family as a new family if the proband receives the diagnosis within the time period under consideration. Conversely, a family was categorised as an old family if the proband was diagnosed in a preceding period. Each assessment only considered families with current, known, and diagnosed patients. Finally, within new and old families, we further differentiated among early-onset, late-onset, and mixed-onset families.

To further evaluate genealogical variability, we selected four genealogical-based metrics (Supplementary Tables 1–4). These were chosen to capture the variability in AOO within and between families, and consist of: largest difference of AOO, largest difference AOO between siblings, largest difference AOO between parents and children, and largest difference AOO between probands and patients. For old families, we assess these metrics for most recent cases (OMR), which only considers patients that were diagnosed in current cluster, and not most recent cases (NMR), which includes patients diagnosed in previous clusters. These assessments were conducted across the full (multiple records per family) and the maximum (one record per family) variability spectrum and are depicted in Figure 3 and Supplementary Figure 3. From an empirical perspective they present factors that may influence AOO variability. Their importance was triggered by an anticipation factor which was previously studied [15,16], which suggests it is important to have the AOO of the ancestor to predict a patients' AOO. We resort to GLMs to analyse the relationship between the AOO of a patient and that of their previously symptomatic familial relations. In this case, since each patient can have a variable number of relations under each relation categories, we sampled the patients into groups of independent records. So, for each relation, we created as many groups as the highest number of relations of a patient, ending up with groups of models which allowed for average insights. We considered only viable if we had at least 10 independent patient records for each coefficient and ended up with 14 models for patients, 1 model for probands, 1 model for parent, and 3 models for sibling relations.

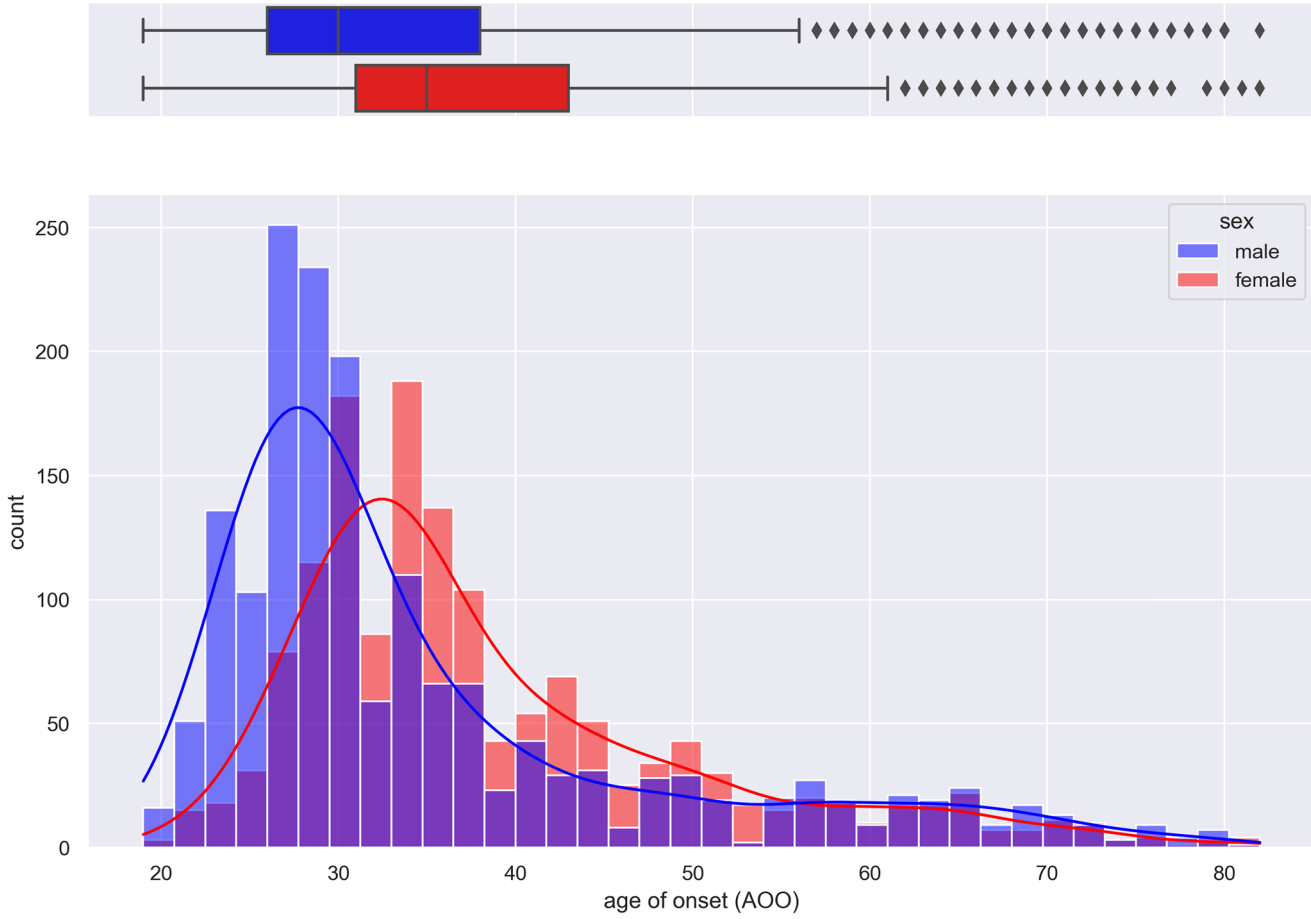

Figure 1. Age of disease onset (AOO) of all observed carriers. Results show symptomatic carriers AOO distribution. There is a difference close to five years for the peak of each distribution with male patients having a risk of presenting symptoms earlier. Mann–Whitney has a *p*-value result below .05, which shows there is a significant difference in AOO between genders.

Statistical analysis

Results are reported with a set of statistical metrics. These are average, standard deviation (SD), coefficient of variation (coef.var), median and range ([minimum, maximum]). Average and SD are presented as 'average (SD)'.

Disparities were evaluated with a two-sided Mann–Whitney *U* test, where a *p*-value of <=.05 was deemed statistically significant.

All analyses used python [v.3.9.7].

Results

The upcoming results encompass an analysis of the distribution of symptomatic carriers to show heterogeneity among patients, as well as a study of the genealogical-based indicators.

Patients

The average age-of-diagnosis for early-onset cases is 32.3 years (6.6), while for late-onset it corresponds to 64.6 years (2.7). Across Q1 to Q4, there was a 0.75 increase in early-onset cases, while late-onset exhibited an average increase of four times that (4.2 years). As for gender specific variations, results show an irregular coef.var in age-of-diagnosis, time-of-diagnosis and time-of-death, both for early and late-onset female patients. This suggests it is relevant to evaluate these subgroups, on a statistical basis (Tables 1 and 2).

Statistical results show that in early-onset group of male patients, the AOO in Q1, Q2, and Q3 has a smaller median than in Q4 (28, 29, 29, 31, respectively). Statistical tests revealed that Q1, Q2 and Q3 are statistically different from Q4 ($p \approx <.001$, $p \approx .0002$, $p \approx .0003$). These patterns are also present on the late-onset groups, where Q1 shows a median of 55, Q2 of 57, Q3 of 60 and Q4 of 64. Lastly, Q1, Q2 and Q3 are statistically different from Q4 ($p \approx .001$, $p \approx .0002$, $p \approx .0002$).

In terms of age-of-diagnosis for the early-onset group, when compared to Q4, Q3 shows a smaller median (Q4 = 32, Q3 = 30) and is statistically different ($p \approx .0008$). On the late-onset groups, the latter groups present lower medians than Q4 (Q1 = 60, Q2 = 63, Q3 = 62, Q4 = 68) and there are statistically differences between Q1, Q2 and Q3, to Q4 ($p \approx .0004$, $p \approx .004$, $p \approx .008$).

The time-of-diagnosis had a higher median in Q1 and Q2 when compared to Q4 (3, 2 and 1 respectively). Statistical tests also revealed that the latter groups are

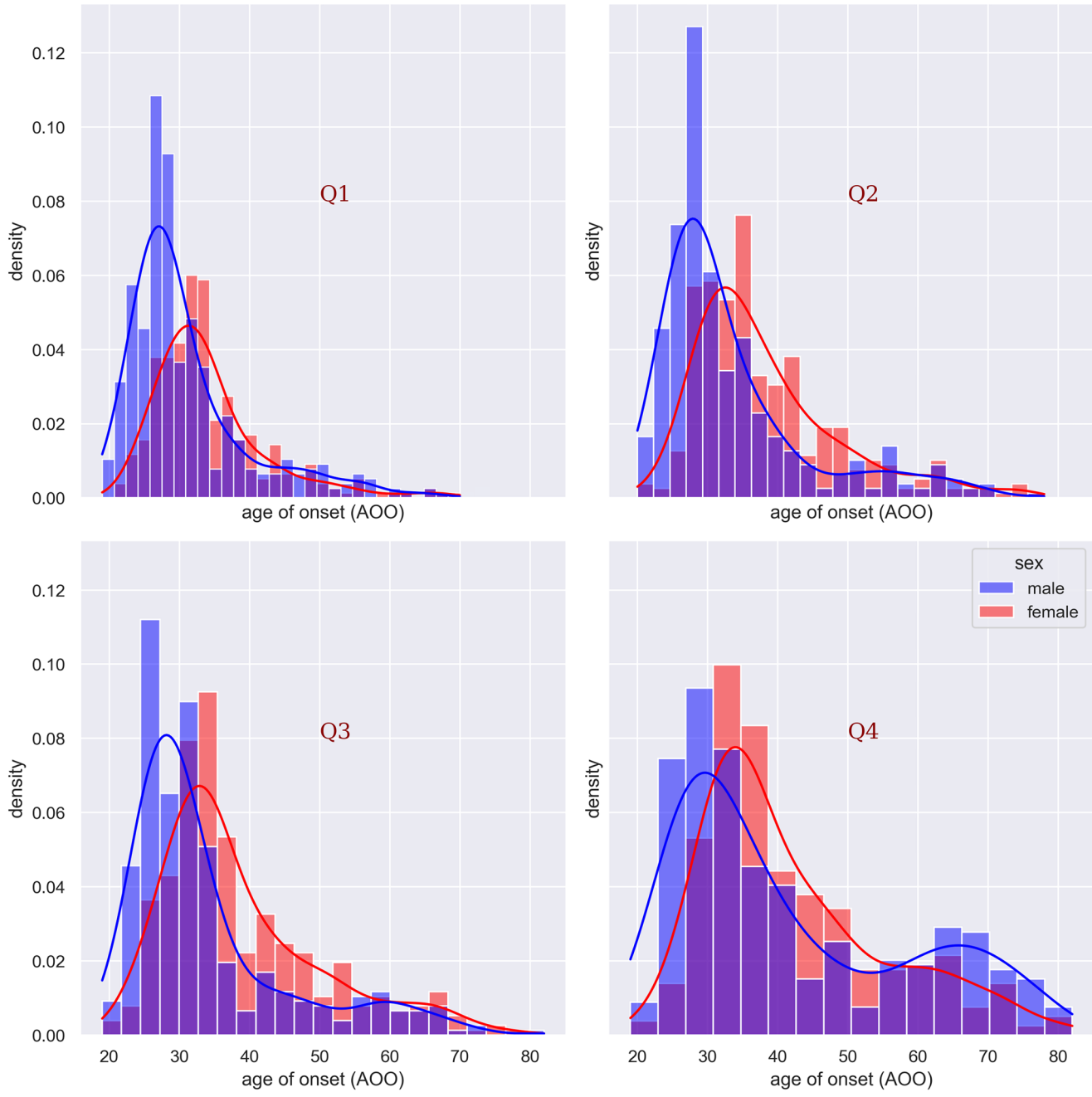

Figure 2. Distribution of age of onset of symptomatic carriers for Q1, Q2, Q3 and Q4 groups. It shows an inversion in the proportion of early as well as male onset patients, with an increase of female early-onset patients, as well as male late onset patients, in Q4.

statistically different from Q4 ($p \approx <.001$ for both) for the early-onset group. Similar findings on the late-onset groups with a more expressive values of medians of 4.5 for Q1, 3 for Q2 and 2 for Q4. Statistical tests found that Q1 and Q2 are statistically different from Q4 ($p \approx .0002$, $p \approx .007$).

As for the age-of-death on the late-onset group, the groups Q1, Q2 and Q3 are statistically different from Q4 ($p \approx .0001$, $p < .001$, $p \approx .009$), moreover the median values are lower in the older times (66, 67 and 71).

The time-of-death on the early-onset group, is higher on Q1 and Q2, when compared to the Q4 (11, 12, 9). These Q1 and Q2 groups are statistically different from group Q4 ($p \approx 0.004$, $p \approx 0.0004$). In the late-onset group, the older groups Q1 and Q3 have higher values than Q4 (9, 10, 7). These Q1 and Q3 groups are statistically different from group Q4 ($p \approx 0.0005$, $p \approx 0.0001$) (Table 1).

In the early-onset group of female patients, the AOO in Q1, Q2, and Q3 has a smaller median than the group of patients in Q4 (32, 34, 34, 35, respectively) and the statistical test revealed they are statistically different from Q4 ($p \approx <.001$, $p \approx .032$, $p \approx .07$). In terms of age-of-diagnosis, when compared to Q4, Q1 and Q3 had a smaller median (Q1 = Q3 = 35, Q4 = 36). Q1 and Q3 are statistically different from Q4 ($p \approx .045$, $p \approx .011$). The time-of-diagnosis shows a higher median in Q1 and Q2, when compared to Q4 (3, 2 and 1) and statistical tests revealed that the latter groups are statistically different from Q4 ($p \approx .001$, for both). Similar findings are also present in the time-to-death variable, where

Table 1. Statistics of clinical indicators of symptomatic male patients.

	Early onset				Late onset			
					Male			
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
n	425	368	331	268	26	45	45	99
Average	**29.4**	30.1	30.1	32.2	**55.8**	59.1	60.2	64.2
SD	6.1	5.6	5.8	6.7	5.1	6.5	6.9	7.4
coef.var	0.2	0.2	0.2	0.2	0.1	0.1	0.1	0.1
Age.onset median	28	29	29	31	55	57	60	64
Range	[19,49]	[20,49]	[19,49]	[19,49]	[50,68]	[50,78]	[50,82]	[50,80]
Statistic	42654.5	40678.0	36686.0		479.0	1354.5	1524.0	
p-Value	<0.001*	0.0002*	0.0003*		<0.001*	0.0002*	0.002*	
Average	33	33.1	**31.5**	33.4	**60.8**	62.9	63.3	66.9
SD	6.5	6.4	6.4	7.1	5.8	6.8	7.4	7.8
Coef.var	0.2	0.2	0.2	0.2	0.1	0.1	0.1	0.1
age.diag median	31	32	30	32	60	63	62	68
Range	[19,54]	[22,60]	[20,57]	[20,54]	[52,74]	[52,79]	[50,84]	[51,82]
Statistic			37281.0		701.5	1552.0	1613.0	
p-Value			0.0008*		0.0004	0.004	0.008	
Average	3.5	3	1.4	**1.3**	4.9	3.8	3.1	**2.7**
SD	2.8	2.6	1.7	1.5	2.8	2.5	2.7	2
Coef.var	0.8	0.9	1.2	1.2	0.6	0.7	0.9	0.7
Time.diag median	3	2	1	1	4.5	3	2	2
Range	[0,18]	[0,17]	[0,13]	[0,11]	[1,10]	[0,11]	[0,10]	[0,10]
Statistic	90330.5	72420.0			1897.5	2839.0		
p-Value	<0.001*	<0.001*			0.0002	0.007		
Average	**40.8**	43.7	43.4	42.8	**67**	67.1	70.7	73.8
SD	7	8	8.6	7.1	6.1	5.6	5.8	6.3
Coef.var	0.2	0.2	0.2	0.2	0.1	0.1	0.1	0.1
Age.death median	40	43	43	42	66	67	71	75
Range	[24,78]	[26,71]	[24,65]	[30,59]	[56,81]	[56,81]	[57,85]	[57,85]
Statistic					212.0	318.0	573.5	
p-Value					0.0001	< 0.001	0.009	
Average	11.3	13.2	11.2	**8.8**	10.6	8.5	10	**7.1**
SD	4.7	6.4	5.8	4.5	4.2	3.7	3.7	2.6
Coef.var	0.4	0.5	0.5	0.5	0.4	0.4	0.4	0.4
Time.death median	11	12	11	9	9	8	10	7
Range	[1,55]	[1,32]	[2,27]	[1,20]	[6,21]	[3,18]	[3,20]	[0,13]
Statistic	5748.5	4734.5			763.0		1278.5	
p-Value	0.004	0.0004			0.0005		0.0001	

AOO: age-of-onset; SD: standard deviation; coef.var: coefficient of variation.
Bold values indicate smaller variability. Only significant differences obtained by the Mann–Whitney between each Q's and Q4 are present.
Asterisk (*) indicates a significant difference with Q4 (due to Mann-Whitney having a p-value result below 0.05 which shows there is a significant difference in age of onset).

Q1 and Q2 have higher median values than Q4 (Q1 = 12, Q2 = 13, Q4 = 9) and later groups are statistically different from Q4 ($p \approx .002$, $p \approx .0004$).

In the late-onset group, time-of-diagnosis has higher median values on the older groups Q1–Q3 when compared to Q4 (Q1 = 3, Q2 = 3, Q3 = 3, Q4 = 2). Statistical tests revealed that the latter groups are statistically different from Q4 ($p \approx .002$, $p \approx .012$, $p \approx .022$). Finally, the age-of-death was lower in Q1, when compared to the Q4. These two groups are statistically different ($p \approx .017$) (Table 2). There were no statistical differences between all the Q's and Q4 for the other variables and on the different groups.

Results in Table 3 provide additional support for the descriptive assessments. They indicate that in early-onset patients, women tend to experience symptoms at a later stage of life, when compared to men. More precisely, women experienced the first symptoms approximately 4 years later than men ($p < .01$) and when receiving the diagnosis ($p < .01$). Additionally, patients diagnosed in earlier time periods tend to have significantly lower AOO (e.g. $\beta = -2.70$ and $p < .001$ for Q1, $\beta = -1.55$ and $p < .001$ for Q2, $\beta = -1.71$ and $p < 0.001$ for Q3) and lower ages-of-diagnosis (e.g. $\beta = -1.62$ and $p < .001$ for Q3), when compared to the more recent time period Q4.

Overall, time-to-diagnosis, in the early-onset group, has been decreasing consistently throughout the years. When compared with Q4, it was approximately 2 years later ($p < .001$) in Q1 and 1.6 years ($p < .001$) in Q2. This pattern is also expressed in the later-onset group, where it was at least 2 years higher in Q1 ($p < .001$) and 1 year higher in Q2 and Q3 ($p \approx .001$, $p \approx .007$).

For late-onset patients, results indicate that a male patient in Q1, Q2 and Q3 tends to develop symptoms significantly earlier than males in Q4. The ones in Q1 experienced approximately 9 years earlier ($p < .001$), the ones in Q2 approximately 6 years ($p < .001$) and the ones in Q3, 5 years earlier ($p < .001$). The women in Q1 also developed symptoms 3 years earlier than women in Q4 ($p < .013$).

Regarding the age-of-diagnosis, results show that a male patient in Q1, Q2 and Q3 tends to develop symptoms significantly earlier than males in the reference group Q4. The ones in Q1 experienced approximately 7 years earlier ($p < .001$), and the ones in Q2 and Q3 were approximately 5 years ($p < .001$ in both groups). The women in Q3 also

Table 2. Statistics of clinical indicators of symptomatic female patients.

	Early onset				Late onset			
					Female			
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
N	290	321	316	271	16	51	62	75
Average	**32.7**	34.9	34.6	36	**57.3**	59.2	59.3	61.6
SD	5.7	6.1	6.3	6.3	6.1	7.5	7.1	8.3
Coef.var	0.2	0.2	0.2	0.2	0.1	0.1	0.1	0.1
Age.onset median	32	34	34	35	55	57	58	60
Range	[22,49]	[21,49]	[21,49]	[20,49]	[51,70]	[50,76]	[50,79]	[50,82]
Statistic	27101.5	39070.0	37308.0					
p-Value	<0.001	0.032	0.007					
Average	36.4	38	**36.1**	37.5	**61.3**	62.5	63	63.6
SD	6.1	7.3	6.9	6.8	6.4	7	7.5	8.3
Coef.var	0.2	0.2	0.2	0.2	0.1	0.1	0.1	0.1
Age.diag median	35	37	35	36	59.5	61	63	64
Range	[24,56]	[22,62]	[22,59]	[21,60]	[52,75]	[51,77]	[51,83]	[51,84]
Statistic	35461.0		37603.5					
p-Value	0.045		0.011					
Average	3.6	3.1	**1.5**	1.6	3.9	3.4	3.6	**2**
SD	2.9	3.2	2	2	2.7	3	3.6	1.7
Coef.var	0.8	1	1.3	1.3	0.7	0.9	1	0.9
Time.diag median	3	2	1	1	3	3	3	2
Range	[0,15]	[0,20]	[0,16] [0,14]	[1,11]	[0,14]	[0,16]	[0,8]	
Statistic	58743.5	58429.5			888.5	2410.5	2848.0	
p-Value	<0.001	<0.001			0.002	0.012	0.022	
Average	**45.5**	48.8	47.6	47.6	**65.4**	70	71.3	73.3
SD	6.7	7.8	9	10	6.7	7.2	6.9	8.5
Coef.var	0.1	0.2	0.2	0.2	0.1	0.1	0.1	0.1
Age.death median	45	48	48	47	64	69.5	71	72
Range	[28,81]	[30,73]	[27,68]	[29,68]	[56,79]	[58,87]	[56,91]	[58,90]
Statistic					58.5			
p-Value					0.017			
Average	12.7	13.6	10.4	**9.6**	**8.1**	10.9	11.4	9.7
SD	4.9	5.7	6.3	5	2.8	5	4.9	3.4
Coef.var	0.4	0.4	0.6	0.5	0.3	0.5	0.4	0.4
Time.death median	12	13	9	9	8.5	10	11.5	9
Range	[3,35]	[1,32]	[2,29]	[2,20]	[4,13]	[4,27]	[0,21]	[4,15]
Statistic	4529.0	4915.5						
p-Value	0.002	0.0004						

AOO: age-of-onset; SD: standard deviation; coef.var: coefficient of variation.
Bold values indicate smaller variability. Only significant differences obtained by the Mann–Whitney between each Q's and Q4 are present.
Asterisk (*) indicates a significant difference with Q4 (due to Mann-Whitney having a p-value result below 0.05 which shows there is a significant difference in age of onset).

developed symptoms significantly earlier (5 years) than women in Q4 ($p < .044$).

All the models in the early-onset group had a R^2 ranging from 10% until 15% and in the late-onset group ranging from 6 until 13%. This shows that the AOO, the age-of-diagnosis and the time-of-diagnosis can only be partially explained by the Q groups and gender. The lower values of R^2 in the late-onset groups can be explained by the lower sample size.

Families

Evaluating the genealogical variability of this disease requires distinguishing between the follow-up of new and old families. So, among these, 7.45% do not have symptomatic patients, 17.41% encompass both early and late-onset patients, 58.65% consist of early-onset patients, while 16.49% exclusively comprise late-onset patients (Supplementary Tables 1–4).

Across recent decades, in percentage terms, there has been a rise in new families, primarily driven by late-onset cases. These have expanded by almost 50%. In old families, there has been a decrease in the number of patients. This, coupled with the consistent count of diagnosed families, results in a broader range of patients per family in OMR, particularly those falling within group Q4. Notably, there has also been an increase in mixed-onset families, contributing to heightened variability between familial patient clusters. Additionally, changes have emerged in the distribution of AOO between new and old families, and across each cluster (Supplementary Figure 2). Particularly noticeable is the reduced variation in old families between male and female patients, as the peaks have largely converged in Q3–Q4. In contrast, among new families, the latest landmarks have seen late male-onset carriers surpassing early male-onset carriers.

The distribution of AOO for symptomatic probands diagnosed within each cluster is depicted in Figure 4. It shows the relationship between the age and type of probands diagnosed. In Q1, early-onset patients significantly drive familial follow-up. However, this profile undergoes a shift, particularly around Q3–Q4, with male probands becoming

Table 3. Generalised linear models (GLM) for AOO, age-of-diagnosis and time-of-diagnosis, to evaluate iterations over time groups (Q1, Q2, Q3, Q4). We considered Q4 and male as the reference classes.

Model	Variables	Coef	Std.err	*p*-Value	[0.025	0.975]	R^2
Early-onset and age. onset	Intercept	32.0366	0.281	<0.01	31.486	32.587	0.13
	Q[T.Q1]	−2.7016	0.335	<0.01	−3.359	−2.045	
	Q[T.Q2]	−1.5469	0.346	<0.01	−2.225	−0.868	
	Q[T.Q3]	−1.7108	0.351	<0.01	−2.398	−1.023	
	Sex[T.women]	4.03	0.237	<0.01	3.566	4.494	
Early-onset and age. diag	Intercept	33.2869	0.308	<0.01	32.684	33.89	0.1
	Q[T.Q1]	−0.4884	0.374	0.192	−1.222	0.245	
	Q[T.Q2]	0.1387	0.379	0.714	−0.603	0.881	
	Q[T.Q3]	−1.6266	0.384	<0.01	−2.378	−0.875	
	Sex[T.women]	4.2828	0.262	<0.01	3.769	4.797	
Early-onset and time. diag	Intercept	1.3756	0.102	<0.01	1.176	1.575	0.15
	Q[T.Q1]	2.2652	0.136	<0.01	1.998	2.533	
	Q[T.Q2]	1.6777	0.138	<0.01	1.407	1.949	
	Q[T.Q3]	0.0824	0.14	0.556	−0.192	0.357	
Late-onset and age. onset	Intercept	65.416	0.676	<0.01	64.091	66.741	0.13
	Q[T.Q1]	−8.8755	1.415	<0.01	−11.649	−6.102	
	Q[T.Q2]	−6.2827	1.314	<0.01	−8.859	−3.706	
	Q[T.Q3]	−5.291	1.284	<0.01	−7.807	−2.775	
	Sex[T.women]	−3.1239	1.049	0.003	−5.179	−1.069	
	Q[T.Q1]:sex[T.women]	5.5433	2.221	0.013	1.191	9.896	
	Q[T.Q2]:sex[T.women]	3.3175	1.863	0.075	−0.333	6.968	
	Q[T.Q3]:sex[T.women]	2.7176	1.784	0.128	−0.78	6.215	
Late-onset and age. diag	Intercept	68.104	0.688	<0.01	66.755	69.453	0.09
	Q[T.Q1]	−7.3897	1.609	<0.01	−10.543	−4.237	
	Q[T.Q2]	−5.1707	1.338	<0.01	−7.792	−2.549	
	Q[T.Q3]	−4.9582	1.306	<0.01	−7.519	−2.398	
	Sex[T.women]	−3.5197	1.067	0.001	−5.611	−1.428	
	Q[T.Q1]:sex[T.women]	4.6943	2.558	0.066	−0.318	9.707	
	Q[T.Q2]:sex[T.women]	3.2595	1.895	0.085	−0.455	6.974	
	Q[T.Q3]:sex[T.women]	3.6551	1.816	0.044	0.096	7.214	
Late-onset and time. diag	Intercept	2.5234	0.177	<0.01	2.177	2.87	0.06
	Q[T.Q1]	2.0636	0.42	<0.01	1.24	2.887	
	Q[T.Q2]	1.0333	0.316	0.001	0.413	1.653	
	Q[T.Q3]	0.807	0.301	0.007	0.216	1.398	

coef: coefficient; std.err: standard error.

predominantly characterised by middle (after 40 years old) or late-onset cases (equal or greater to 50 years old).

Analysis of the genealogical family indicators (Supplementary Tables 1–4) shows a relatively low average AOO difference in new families, although these values increase in mixed-onset and late-onset families. In old families, there is a concentration on early-onset families, with OMR values showing a smaller average value, when compared to NMR, in most cases (number of records present in each group decreases). The change between NMR and OMR in unique family values shows that there is a tendency for patients of the same family to have dispersal diagnosis years. This shows that usually, patients aren't diagnosed in familial concentrations.

As for proband-patients genealogical indicator, in general terms, there is a decrease in newly diagnosed families and an increase in old, diagnosed families. Also, old families have a tendency for only having values in NMR cases. This shows there is a wide discrepancy in the diagnosis of probands and other family relatives, thus reinforcing that families tend to have a long clinical follow-up.

Regarding parent-child relations, most of the cases are present in old families, more exactly in NMR results, which suggests a difference in year-of-diagnosis. In new families, the average difference in disease-onset between parents and children has been increasing, as well as the raw number of families over the different clusters has been decreasing. This can be due to a significant difference in the time-of-diagnosis between parents and children.

As for sibling relationships, the average difference of AOO has been decreasing in early-onset families over the considered groups, while in late and mixed-onset families it has been increasing (68% of the cases exhibit a difference in the AOO below 10 years).

As for the statistical tests and when considering the extended family evolution for newly diagnosed families, the largest AOO difference of Q1 is statistically different from the values in Q4 ($p \approx .038$). It is worth mentioning that the median value of the largest AOO difference on Q1 is 8, while the same metric on Q4 is 4. No other statistical differences were found (Supplementary Table 1).

In the case of the extended family evolution for old, diagnosed families, female patients in Q2 had the largest AOO difference proband-patient significantly different from female patients in Q4 ($p < .001$). Also, female patients in Q2 are statistically different from females in Q4 in terms of the largest AOO difference siblings ($p \approx .008$) (Supplementary Table 3).

So, as for GLM models (Table 4), the family relation results indicate that women tend to develop symptoms later than other male patients. Specifically, it shows that women, in general, experience symptoms, on average, 5.35 years after male patients and, on average, 1.48 years before their male relatives. Additionally, results reveal that their AOO occurs, on average, 0.21 years later than that of their

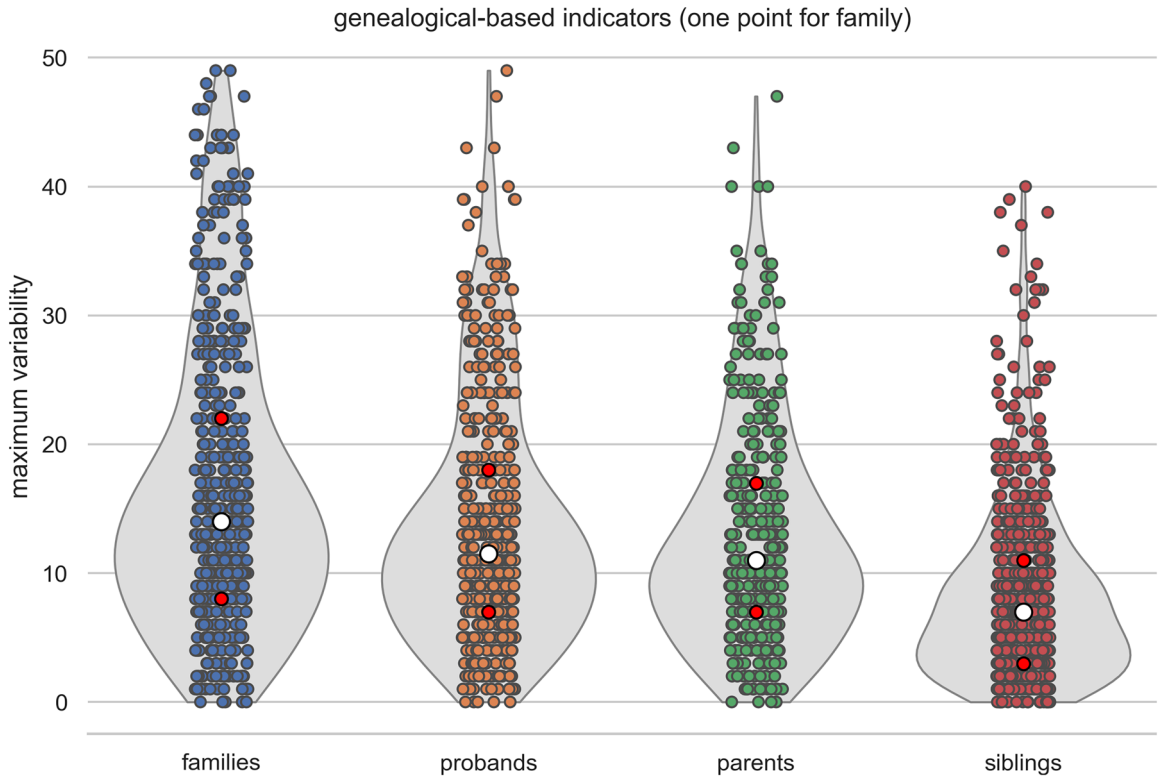

Figure 3. Violin plots depict the distribution of maximum age of onset variability across familial, proband, siblings, and parents-based relations (one record per family). The central white dot represents the 50% quantile, while the upper and lower red dots signify the 75 and 25% quantiles, respectively. These plots, along with the quantile positions, highlight that siblings exhibit the least overall variability within the studied ATTRVal30M families.

relatives. As for the proband relation, it indicates that women develop symptoms, on average, 4.84 years after other male patients, while women probands tend to experience symptoms, on average, 1.50 years before male probands. Additionally, it shows that patients, on average, tend to develop symptoms 0.49 years after their proband relations. When considering parent relation, results indicate that women, on average, tend to experience symptoms 4.45 years after males, and women parents tend to develop symptoms 2.72 years before male parents. In this case, it also shows that a patient tends to experience symptoms, on average, 0.39 years after their parents. Finally, the model that characterises patients that have siblings as their relations shows that women tend to develop symptoms, on average, 3.48 years after males, and 2.26 years before their male siblings. These final results also indicate that a patient, on average, typically shows symptoms 0.75 years after their siblings' relations. These results present an average R^2 ranging from 13% (family relation) to 66% (sibling relation), indicating an increasing explanatory power for the variability in the AOO, when considering a rank in the familial relations. It starts in the sibling's relationship (1st), proceeds to the parents (2nd), crosses to the probands (3rd), and ends with the family relationship (4th).

Discussion

To the best of our knowledge this is the first study in ATTRv Amyloidosis that analyses familial data with this degree of depth.

Overall, in clinical terms, Q4 represents the most pertinent group as it encompasses the most current and active patients. The varying time spans leading to the establishment of each 25% group (46, 13, 10, and 15 years) indicates that the peak of patient diagnoses was in the 1990s, and significant changes have transpired over recent decades. Also, worth mentioning that the cluster groups we encountered show a connection with significant disease events. As such, in 1986 – one year after the start of the second group – the introduction of the genetic test marked a pivotal moment, enabling the diagnosis of carriers even before symptoms emerged. Additionally, in 1992—six years prior to the start of the third group—a significant milestone was reached in Portugal, with the first liver transplant performed in symptomatic patients. This marked the inception of available treatments for symptomatic patients.

An analysis of the metrics between Q1 and Q4 shows an overall reduction in newly diagnosed patients, and a balance between men and women, with both currently (Q4) being diagnosed and having symptoms in later stages of life. It's worth highlighting that the reduction in patients within Q4 could be attributed to multiple factors. These include a decline in the birth rate in Portugal, and broader distribution of clinical follow-up resulting partly from a larger geographical dispersion of families due to mobility-related immigration and emigration patterns [17].

It also shows a decrease in time-of-diagnosis which suggests that across the long period of patient observations, as diagnostic methods were refined, medical teams found it easier to confirm the diagnosis. Currently, the criteria

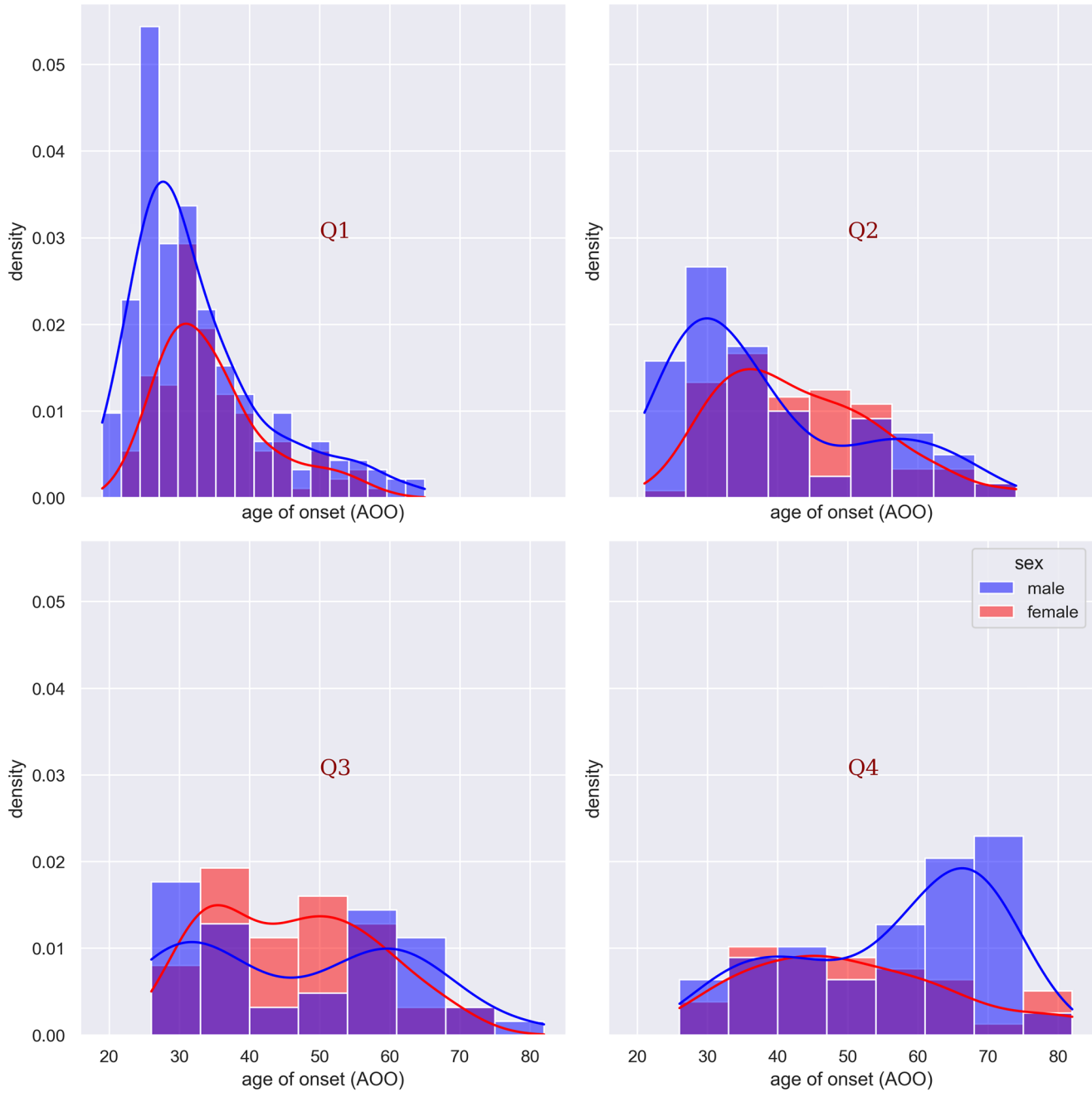

Figure 4. Distribution of the age of onset for symptomatic probands for Q1, Q2, Q3 and Q4 groups. It is clear the change in the type of probands diagnosed. If in Q1 we have clearly early-onset patients, this shifts especially around Q3 and Q4 with probands being clearly defined by middle (after 40 years old) or late onset cases for the male patients.

consider a combination of key symptoms, neurophysiological and cardiac exams, alongside a positive demonstration of amyloid deposition. This, combined with a significant number of cases of a single variant (ATTRV30M), contributes to a smaller bias in confirmed diagnosis results at our centre.

When considering the profile of proband-patient changes, since these express symptoms at ever later stages in life (Figure 4), especially when comparing Q1 to Q3–Q4, it is worth mentioning that it can be due, partly, to the interaction of neurology with other clinical specialities such as nephrology and cardiology, since a research focus on these clinical connections [18–20] lead to an improvement of the diagnosis of patients in later stages of life.

Focusing on familial variability across the four patients' clusters reveals changes. In Q1, the emergence of early-onset families is evident. In Q2, a balance is observed between newly diagnosed families and old families, the latter experiencing an uptick in diagnoses following the introduction of the genetic test. Q3 witnesses a surge in diagnoses among old family cases, possibly attributed to the introduction of the initial treatment. In Q4, a rise is noted in diagnoses of mixed and late-onset families within existing diagnosed families, potentially linked to the increased average life expectancy in recent decades. A noticeable growth in the diagnosis of new families is seen in Q4, spurred by a rise in late-onset male diagnoses.

Table 4. Genealogical AOO generalized linear models (GLM) for family, proband, parent and sibling relationships.

Model	Variables	Avg coef	ST dev.coef	R^2	ST dev.R^2	*n.* Models
Family relation	Intercept	25.26	3.39	0.2	0.09	14
	sex[T. women]	6.09	0.84			
	sex_other[T. women]	−1.75	3.18			
	ageonset_other	0.22	0.11			
Proband relation	Intercept	15.17	–	0.3	–	1
	sex[T. women]	4.84				
	sex_proband[T. women]	−1.5				
	ageonset_proband	0.49				
Parent relation	Intercept	17.15	–	0.5	–	1
	sex[T. women]	4.45				
	sex_parent[T. women]	−2.72				
	ageonset_parent	0.39				
Sibling relation	Intercept	9.13	5.09	0.7	0.12	3
	sex[T. women]	3.48	0.95			
	sex_sib[T. women]	−2.26	0.55			
	ageonset_sib	0.75	0.21			

We considered male as the reference class. avg coef: average coefficient; stdev.coef: standard deviation of the coefficient.

Overall, the genealogy-based indicators show high standard deviation (SD) values, highlighting the heterogeneity in the distribution of clinical indicators. They also show that the presence of symptomatic siblings can serve as a valuable indicator to evaluate the likelihood of a patient developing symptoms in the near future, impacting genetic counselling guidelines [21]. This is shown by the distribution of maximum AOO variability across familial, proband, siblings, and parents-based relations, and the smaller value of the 50% quantile of the siblings relation (Figure 3), besides the GLMs analysis (Table 4). Also, and family-wise, the number of new, late or mixed-onset families has shown a tendency to grow. In these cases, due to the variable nature of symptoms, trends show that the profile of future symptomatic patients will most likely continue to change.

This study reveals a consistent increase in elderly cases over the last few decades, which suggests the ever-growing need of further studies to discover approaches for diagnosis or treatment of ever-elderly symptomatic patients.

UCA's 934 families represent 33,608 individuals. These share different relations, from close ones, such as parents and siblings, to more extended ones like 2nd to 7th-degree relatives. If non-carriers individuals may be affected it, due to a caretaker role which underscores the importance of multi-disciplinary clinical teams in family follow-up.

This study has limitations. Firstly, it centres on families monitored at a single facility, although it constitutes the largest hub of the disease. Secondly, results exhibit a notable SD, underscoring the variability of the disease. Lastly, the results can be affected by various factors, like the number of patients selected by each indicator. Whenever we deemed it possible, our decisions aimed to minimise these effects.

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Ethical approval

The study was approved by the ethical and institutional review boards at Centro Hospitalar Universitário de Santo António, Porto, Portugal, prior to subject enrolment. All methods were performed in accordance with the relevant guidelines and regulations.

Disclosure statement

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Data availability statement

Patient data is not publicly available due to restrictions e.g. 'containing information that could compromise research participant privacy/consent'.

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