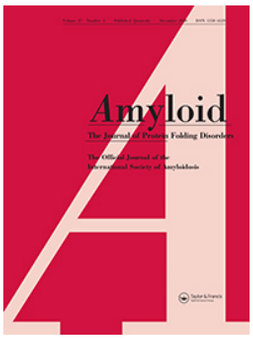

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ARTICLE

Anticipation on age at onset in kindreds with hereditary ATTRV30M amyloidosis from the Majorcan cluster

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ABSTRACT

Background: Hereditary transthyretin amyloidosis (ATTRV30M) is a rare disease caused by amyloid deposition and characterized by a heterogeneous presentation. Anticipation (AC) is described as the decrease in age at onset (AO) within each generation. Our aim was to study AC in a large number of ATTRV30M kindred from Majorca (Spain), and gain further insight into parent-of-origin effects.

Methods: In a cohort of 262 subjects with ATTRV30M amyloidosis belonging to 51 families, we found 37 affected pairs. AO is defined as the age at the first symptom and AC (parent's age at disease onset minus that of the offspring) were calculated. Chi-square test, independent *t*-test and paired *t*-test were used for comparisons between groups. Association between AO of parents and offsprings were assessed by Pearson's correlation coefficient.

Results: Offspring mean AO was 16 years lower than that of the parents ($p < .001$) regardless of the sex of the parents and the offspring. AC occurred in 31 out of the 37 pairs, with no differences related to the sex of parents or offspring. There was a moderate correlation ($r = 0.49$; $p < .001$) between AO of the parents and that of the offsprings.

Conclusion: AC was no uncommon in our cohort, and AO tended to decrease in successive generations.

Abbreviations: AC: anticipation; AO: age at onset; ATTRV30M: amyloid V30M transthyretin; FAP: familial amyloid polyneuropathy; mtDNA: mitochondrial DNA; PADO: predicted age at onset; PST: presymptomatic testing; TTR: transthyretin; UM-TTR: Multidisciplinary Unit of Transthyretin Amyloidosis

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Introduction

Hereditary transthyretin amyloidosis (ATTRV30M), also known as familial amyloid polyneuropathy (FAP) or Andrade's disease, is characterized by deposits of amyloid fibrils derived from the accumulation of unstable conformations of the transthyretin protein (TTR) [1]. To date, more than 140 mutations in the *TTR* gene (chr18q12.1) have been described [2]. Val30Met (p.Val50Met) is the most common one in general and in particular in the island of Majorca (Spain) where ATTRV30M amyloidosis is considered endemic [3,4].

ATTRV30M amyloidosis can affect multiple organs and systems, including the nervous and gastrointestinal systems, the heart, kidneys and eyes [1]. It presents in many different forms and with considerable variation in signs and symptoms across individuals and geographic locations [1]. The clinical features of ATTRV30M are mainly neuropathic, with a heterogeneous presentation of peripheral (sensory and motor) and autonomic neuropathy. Gastrointestinal impairment, cardiomyopathy, ocular manifestations and nephropathy are other frequent manifestations of the disease [1].

Diagnosis in the early stages of the disease is crucial for timely treatment to prevent progression. However, early recognition remains a challenge, with a significant delay in diagnosis still occurring often due to misdiagnosis [5–10], especially among those without a family history of the disease. This could be explained by the age-dependent penetrance of the gene, the infrequent occurrence of *de novo* mutations or the misdiagnosis of parents [5–7,10,11].

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Genetic counseling poses another important challenge for the management of ATTRV30M amyloidosis. Although there are no standard guidelines for providing comprehensive genetic counseling, performing presymptomatic testing (PST) for the *TTR* gene in kindred, or initiating clinical management of asymptomatic carriers of the *TTR* gene variant following the identification of an index patient in a family [12], some expert recommendations have been published arguing in favor of PST according to the growing evidence of asymptomatic carriers detection and early diagnosis [13].

Andrade first described ATTRV30M, formerly FAP, in Northern Portugal as a disease occurring between the third and fourth decade of life [14]. Variation in AO between clusters and within the same focus has already been described [15–17]. Additionally, mean age at onset (AO) varies between geographical areas such as Portugal, Japan, Sweden, Majorca and Brazil, although the range is similar: 19–82 in Portugal [18], 23–79 in Majorca [19]. Among Portuguese families, an increasing number of late-onset cases (≥50 years) have been observed and asymptomatic carriers aged up to 95 years have been identified [16].

Variation in AO between generations has also been observed: late-onset parents often have early-onset offspring (≤40 years) – an evidence for anticipation (AC) – whereas the opposite has never been described for this disease. AC is defined as the occurrence of a significantly earlier AO of the disease in younger generations [20] and has been observed among ATTRV30M patients worldwide [18,21,22]. Coelho et al. reported that AC is a true biological phenomenon in ATTRV30M amyloidosis [18].

Thus, ATTRV30M amyloidosis is highly heterogeneous not only in terms of AO but also in terms of clinical manifestations, which are unlikely to be similar between individuals carrying the same mutation even within the same family. As new genetic modulators of AO in this disease are being discovered, there is increasing evidence suggesting that this variability is influenced by genetic [22,23], epigenetic and even environmental factors in addition to the single point mutation in the *TTR* gene. Furthermore, incomplete penetrance [24,25], together with AC, makes prediction of AO and determination of the timely moment to start monitoring asymptomatic *TTR* mutation carriers [26] extremely difficult.

The aim of our study was to investigate the occurrence of AC in a cohort of V30M carriers and ATTRV30M patients from Majorca.

Methods

Study sample

The Multidisciplinary Unit of Transthyretin Amyloidosis (UM-TTR) at the Hospital Universitario Son Llàtzer (HUSLL) has the largest data set of ATTRV30M patients in Majorca. Since the beginning of the UM-TTR, a total of 262 subjects from 51 families were registered (in November 2018). All the subjects included in this study were carriers of the V30M variant. We extracted from this register all pairs which parent and offspring had been clinically evaluated and for whom a complete medical record was available at our hospital. We also took into account pairs formed with more than one offspring (for example, a father with 2 affected children were considered as two pairs). Patients were classified as early-onset (<50 years) or late-onset (>50 years) according to the age of first symptoms. Ethical approval was granted by the Ethics Committee of the Balearic Islands and the Research Commission of HUSL (Decision number: IB 3858/19 PI).

Definitions

AO was defined as the age of occurrence of the first amyloidosis-related symptom as previously defined [26]. Basically, when clinical features appears, such as autonomic neuropathy, including sweating abnormalities, sexual dysfunction and orthostatic hypotension; gastrointestinal manifestations, such as diarrhea, unintentional weight loss or constipation; and polyneuropathy symptoms, such as loss of pain and temperature sensation, paresthesia and hyperalgesia, confirmed by an expert in the disease, and subsequently verified by an abnormal neurological or neurophysiological evaluation [26]. AC was defined, in accordance with Drugge et al., as the parent's age at AO minus that of the offspring (in years) [21].

Data collection

Since the establishment of the UM-TTR, patients and carriers have been included in a local data set and their family trees routinely collected. Index cases have sometimes been identified exclusively on the basis of subjective symptom descriptions by family members. However, for the purpose of this study, only pairs with complete data for disease onset as defined above were included.

Statistical methods

Differences in mean AO and AC were calculated. For parents with more than one offspring, we took into account all kindred, and age of the parents was considered only once when calculating means. In addition, some offsprings were also considered as parents if applicable. The chi-square test was used to evaluate associations between the sex of parents and offspring. Independent samples *t*-test or paired *t*-test were used for comparisons between groups after assessing data normality. Pearson's correlation coefficient was used to investigate the relationship between AO of parents and that of their offspring. Interclass correlation coefficient was used to ascertain the percentage of variability in AO that can be explained by the fact that patients are grouped in sibships. $p < .05$ was considered as statistically significant. Statistics and graphs were performed using GraphPad Prism software.

Results

As of November 2018, the HUSLL UM-TTR cohort contained 262 individuals: 79 patients alive, 108 asymptomatic V30M carriers, 66 deceased patients and 9 deceased carriers.

We were able to identify 37 pairs of affected parent-offspring with a recorded AO. Therefore, 64 individuals were included in the study, 27 parents and 37 offsprings. Mean (SD) AO was 46 (17) years. Our sample cohort included 37 (44%) females. Of the 37 pairs studied, 19 parents (41%) and 16 offsprings (43%) were females. No differences were found in AO between males and females. The comparison between AO of parents and offspring and sex showed that mean AO of the offsprings was 16 years lower than that of the parents (Table 1), meaning that AO was significantly lower in the offspring ($p < .01$) This difference was consistent regardless of the sex of the parents or the offsprings.

Of the 37 parent-offspring pairs, 19 patients descended from an affected mother and 18 from an affected father. AC was found in 31 of the 37 parent-offspring pairs studied.

Mean (SD) AC for these pairs was 20 (19) years (range: 1–52). Differences in AC according to the sex of the offspring and that of the parent are shown in Table 2.

In the remaining 6 pairs, the offsprings developed the disease at an older age than the parents, with a mean (SD) delay of 4 (3) years (range 1–8). There was one man who descended from an affected father; one man who descended from an affected mother; two women who descended from an affected father and two women who descended from an affected mother.

Five parents and their offspring (3 females and 2 males) had an early onset of the disease whereas the other pair had a late onset. The latter was the only same-sex parent–offspring pair (male–male) in which AC was not identified. AC according to the concept of early or late onset is shown in Table 3. AC increased with a later AO of the parents. Differences in AC according to disease onset in parent-offspring pairs are shown in Table 4.

The mean (SD) difference in AO in parent-offspring pairs was 33 (12) years for the 13 pairs in the late-onset parent group but was only 9 (6) years for the 13 pairs in the early-onset parent group, indicating that although AC occurred in both groups, the difference in AO in the parent-offspring pairs was higher for late-onset parents. Pearson's correlation coefficient, calculated to assess the association between AO of parents and offsprings, was 0.49 ($p < .001$) for the 31 parent-offspring pairs. This means that AO in offsprings is moderately related to AO of the affected parent. The later the age of presentation of the disease is in the parent, the later it is in the offspring.

The interclass correlation coefficient was calculated for nine sibships with more than one affected member. The mean number of patients per sibship was 2.2 and the mean interclass correlation coefficient was 0.35, showing a weak degree of similarity among sibs.

Table 1. Comparison of age at onset and sex in the study cohort. Results are expressed as means (SD) unless otherwise stated.

	Parents ($n = 27$)	Offsprings ($n = 37$)	p Value
AO	55 (18)	39 (12)	<.001
Females (%)	41.3	43.2	NS
AO (females)	59 (19)	40 (15)	.006
AO (males)	52 (16)	38 (11)	.003

AO: age at onset; NS: not significant ($p > .05$).

Table 2. Parent-of-origin effect in anticipation age.

	Sex parent	
	Men	Female
Sex offspring		
Men	$n = 12$ (11) AC = 17 (13)	$n = 9$ (8) AC = 23 (15)
Female	$n = 6$ (4) AC = 7 (5)	$n = 10$ (8) AC = 26 (16)

Total samples size (sample size of pairs with anticipation) are reported. Results are expressed in mean (SD) anticipation age. AC: Anticipation.

Table 3. Anticipation according to age at onset of parents and offsprings.

AO	Number of pairs	AC mean (SD)	AC range	p Value
Parent early	12	9 (6)	1–25	
Parent late	19	26 (15)	3–53	<.01
Offspring early	26	21 (15)	1–53	
Offspring late	5	13 (9)	4–27	NS

AC: anticipation; AO: age at onset; NS: not significant; SD: standard deviation. Early: <50 years. Late: >50 years.

Discussion

AC was common in our cohort and more pronounced among offsprings of affected mothers, reaching up to 52 years. There was also a trend towards an earlier AO in successive generations. In addition, we found a moderate correlation between offspring AO and AO of the affected parent.

In our cohort, there were only six pairs that did not present AC. Those six pairs are: three from the same mother, whose AO was 31 years. Other two parents whose AO was 25 and 30 years, respectively, and the only late-onset, a 55 years-old man whose son had an AO of 56 years. The difference in the ages at onset of parents and offspring for those six pairs was between 1 and 8 years.

Previous research on AC in ATTRV30M has focused primarily on potential mechanisms of this phenomenon [18,21,22,27]. Lemos et al. previously reported that the sex of the transmitting parent influences disease AC, with a more pronounced AC among offsprings of affected mothers, especially among mother-son pairs [18]. These differences have also been identified in Swedish [21] and Japanese patients [28]. The small size of our cohort could explain the lack of statistical difference based on the sex of affected

Table 4. Differences in anticipation according to disease onset in parent-offspring pairs.

Parent AO–Offspring AO	Number of pairs	AC mean (SD)	AC range	p Value
Early–Early	13	9 (6)	1–14	
Late–Early	13	33 (12)	16–53	<.01
Early–Late	0	NA	NA	NA
Late–Late	5	13 (9)	4–27	NA

AC: anticipation; AO: age at onset; NA: not applicable; SD: standard deviation; Early: <50 years. Late: >50 years.

parents. Nonetheless, we found a trend towards an increased AC among offsprings of affected mothers.

It has also been suggested that the sex of affected parents plays a role in disease penetrance, with a higher penetrance in case of maternal inheritance of the disease, partially explained by mitochondrial DNA (mtDNA) polymorphism [29]. mtDNA has also been shown to be associated with AO in a Portuguese cohort. A higher mtDNA copy number was found in early-onset offspring, suggesting a risk effect of mtDNA inherited from affected mothers [30]. This influence of the sex of the transmitting parent has also been reported in other diseases [31].

Although it seems clear that female sex has been associated with higher penetrance and increased AC, other factors, genetic and epigenetic, are also likely to account for AC variability. Environmental factors have been suggested to be related to amyloid deposition in mice [32]. However, as all the patients included in this study were from the same focus, the potential influence of environmental factors on AC was minimized.

No correlation has been observed between AO and clinical features and TTR plasma levels [33], so initial reports suggested that *TTR* gene regulation expression did not play a major role in disease onset. However, it has been suggested that distinct haplotypes could have a modulatory effect on the expression/activity of TTR gene and its functional consequences [34]. Other genetic factors such as length of other gene alleles (i.e. ATXN2) [35] or complement C1Q polymorphisms [36,37] could modulate AO.

A Japanese study found that AC was present (with a mean [SD] of 11.5 [10.9] years) only in the early-onset subgroup [38]. In contrast, we found AC in both early- and late-onset subgroups, in line with other authors [18]. Like these authors, we found no cases of late-onset offspring from early-onset parents. This observation deserves further investigation as it is possible that other disease phenotypes (e.g. isolated cardiac amyloidosis) could be misdiagnosed in predominantly neurologic disease foci such as ours. AC not only entails an earlier onset of the disease but also different disease expressions between the same family members.

It has been proposed that amyloid fibril composition exerts a more profound impact on disease phenotype than age [39]. Early-onset patients usually have amyloid fibrils with only full-length TTR and a glittering kind of birefringence after Congo red staining (type B fibrils), while amyloid fibrils of late-onset patients contain C-terminal fragments and show a non-glittering kind of birefringence (type A fibrils) [40]. It follows from these observations that AC could also be associated with a change in fibril deposition pattern between parents and offspring. However, this has not been confirmed.

In a recently published international consensus on ATTRV30M amyloidosis focusing on early diagnosis [26], the authors recommended to start monitoring asymptomatic *TTR* mutation carriers 10 years before the predicted age at onset (PADO). However, their suggested PADO depends on the particular mutation, the typical AO for that mutation and the AO in relatives with ATTRV30M amyloidosis. Our findings and those published elsewhere suggest this recommendation should be considered with caution, especially in endemic areas where AC is common. Although thorough routine monitoring of asymptomatic carriers is not required, annual comprehensive visits are recommended as some early clinical manifestations may go unnoticed by these carriers.

Our data provide further evidence for the phenomenon of true AC in ATTRV30M amyloidosis and, therefore, for the importance of genetic counseling and asymptomatic carriers follow-up as well as for the need to improve both. Our study also shows that AC is present in the Majorcan cohort and reinforces the support for early follow-up of asymptomatic carriers, a finding that could be also important for the follow-up of undiagnosed parents, namely with cardiac symptoms. To our knowledge, this is the first study confirming AC in a Spanish cohort of ATTRV30M patients.

Our study has some limitations. First, the number of pairs analyzed was relatively small. Nonetheless, they were representative of our global cohort as they amounted to almost half of the registered pairs. Moreover, we are located in the largest endemic focus of ATTRV30M amyloidosis in Spain where AC has not been studied. Second, AO was defined as age at the occurrence of the first disease-related symptom. Even though this could represent a bias given that recent diagnostic tests are more sensitive for the diagnosis of ATTRV30M amyloidosis and therefore allow for an earlier diagnosis, the fact that in our symptom onset based definition diagnosis was always confirmed by a physician from the same group of experts at a referral ATTRV30M center minimized the risk of bias. Third, the retrospective nature of the study did not allow us to analyze other potential factors, such as other gene mutations or polymorphisms, associated with AC. Finally, with a larger sample size, we could have performed linear regression models, such as generalized estimation equation, to adjust for other potential confounders and to take into account relatedness, adjusting for intrafamilial correlations. With our limited sample size, due to the low prevalence of the disease, we cannot rule out that relatedness may interfere with our results.

In conclusion, AC was not uncommon in our cohort and AO tended to decrease in successive generations. This observation has important consequences for the management of asymptomatic carriers.

Ethics approval

Ethical approval was granted by the Ethics Committee of the Balearic Islands and the Research Commission of Hospital Universitario Son Llàtzer. Decision number: IB 3858/19 PI.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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