

## Genetic epidemiology of familial amyloid polyneuropathy in the Balearic Islands (Spain)

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**Abbreviations:** *FAP, familial amyloid polyneuropathy; TTR, transthyretin*

### Abstract

Between 1976 and 2003, we diagnosed 144 patients with familial amyloid polyneuropathy (FAP) in the Balearic Islands (Spain). Analysis of genetic epidemiological data from 102 confirmed patients showed 62% were men. Parental transmission was paternal in 38, maternal in 25, and unknown in 39. No family history of FAP was found in 32 patients. TTRVal30Met associated with haplotype I was present in the individuals studied. Mean age-at-onset was 45.7 years which lies between that of Sweden and those of Portugal, Japan and Brazil. Duration of FAP was of 9.7 years. Age-at-onset, age-at-death, duration and fertility were similar between sexes. Twenty-nine intergeneration familial pairs of patients were ascertained. Raw anticipation was positive in twenty-four pairs, zero in one, and negative in four. Differences greater than 9 years between age-at-onset of the first and second member were considered relevant; positive relevant anticipation was found in 76% of the whole pairs. The frequency of positive anticipation of parent-child pairs was not significantly different than those described in the Swedish and Portuguese series. Significant positive correlation in age-at-onset was confirmed in twenty-seven types of pairs supporting the hypothesis that a genetic factor may modulate age-at-onset. The Balearic focus of FAP is expanding and constitutes a public health problem.

### Introduction

Familial amyloid polyneuropathy (FAP) is an autosomal dominant systemic amyloidosis with adult-onset, first described by Andrade [1]. It is associated with a mutation in the transthyretin (TTR) gene which codes for a TTR variant. Over 80 amyloidogenic TTR variants have been reported [2]; the most frequent is TTRVal30Met.

Prior to 1976 no case of FAP had been diagnosed in Majorca. This same year, one of us (MMQ) attended a male patient aged 26 years with an identical clinical picture to his mother. In 1952, MMQ had diagnosed the patient's mother, a sister and a female paternal first cousin as familial lumbosacral syringomyelia; the respective fathers died in 1937 and 1950 with identical clinical pictures; the diagnosis of this disease was supported by 15 cases in 2 Majorcan municipality [3] families whose clinical pictures were analogous. Based on the report of Andrade [1], we considered that the clinical picture of the young patient in 1976 was consistent

with FAP. This was later confirmed by the detection of amyloid in a gastric biopsy. For this reason the clinical history of his mother was revised. She had undergone lumbar laminectomy without demonstration of suspected syringomyelia but an enlarged posterior spinal ganglion which was biopsied and stained with hematoxylin-eosin without finding any specific pathologic lesion. Taking into account the pathology of the spinal ganglion described by Andrade [1], we suspected the existence of amyloid in the cited biopsy; it was then demonstrated by Dr J. Lamarca (Servicio de Patología del Hospital del Mar, Barcelona) on examination with polarized light and Congo Red staining. We concluded that this patient and the other four deceased relatives had been FAP patients just as the 15 aforementioned patients [3].

The possibility of the origin of FAP in the island from only one mutation (Povoá de Varzim [4] one founder hypothesis) was considered. It could have come from Portugal in the early 13th century when the Portuguese arrived in Majorca [5] with El Infante Don Pedro de Portugal. He was the main authority

in the island, the Lord of the Kingdom of Majorca, for 26 years (1231–1256) and settled in 5% of the island. The mutation could also have come at a later date from Portugal, in the 14th century, during Portuguese navigations to ship pine pitch and salt from Majorca, or, more likely still, following the immigration of 150 Portuguese Jews who arrived in 1394 to re-populate the ghetto of Palma de Mallorca (Es Call) after the genocide in 1391 [6]. Nevertheless, we also considered the possibility of the independent appearance of the mutation in several parts of the world -the multiple origin hypothesis- specially after the reports of the results of haplotype analysis in FAP families of Japanese and Portuguese origin [7], wherein it was concluded that the Val-Met mutation probably recurred to generate FAP families of independent origin. For this reason, the haplotype of some Majorcan families was investigated [8] ascertaining that it was haplotype I, the same as Portuguese families, thereby supporting the first hypothesis.

The aim of this work was to investigate several genetic epidemiologic aspects of an FAP population in the Balearic Islands to update knowledge, serve as a basis for future longitudinal and transverse studies and to compare with available epidemiologic studies of other FAP populations worldwide.

## Patients and methods

### Subjects

After identification of the first FAP patient and based on data of the aforementioned retrospective investigation, we suspected the probable existence of non-diagnosed FAP patients, alive or deceased. We therefore planned their localization through several surveys. We had the advantage of doing so on an island – a true epidemiological laboratory due to its geographic and population characteristics – which enable easier follow up of possibly affected patients. (a) First we contacted the descendants of the 15 aforementioned patients [3] to collect data about the evolution and to know the names of their physicians. (b) After the prospective or retrospective diagnosis of each patient, we constructed the pedigree starting from the index case or proband; we tried to include personal and clinical information from at least three generations. (c) We sent a clinical report to the family physician to request complementary information on the patient's deceased relatives. (d) To complete the study of deceased patients of physicians no longer alive, we made a survey through the Ecclesiastic and Civil Registries to learn the cause of death. (e) We also carried out a survey among many doctors, specially neurologists, gastroenterologists and clinicians to know if they had diagnosed patients

with a clinical picture consistent with FAP; in which case they forwarded clinical reports to us. (f) In addition, we asked pathologists for information about patients diagnosed with amyloidosis as well as retrospective investigation of amyloid by Congo Red staining on previous biopsy blocks from patients either dead or living probably affected. (g) Since 1987, when we became pioneers of molecular investigation in our country with the cooperation of Drs. P. P. Costa and M. J. M. Saraiva, we explained the possibility of specific diagnosis of FAP and detection of asymptomatic carriers to the physicians on the island; for this reason they send us patients with a clinical picture consistent with the disease. (h) Finally, one particularly relevant source of patients has been word-of-mouth; many of our patients and/or their relatives have informed others with a similar clinical picture and they have contacted us for advice.

To complement the study of the endemic focus, since 1987 we have advised the systematic investigation of the biochemical marker TTRVal30Met to the consanguineous relatives of each patient to detect the carrier state; moreover we suggested a clinical review for the asymptomatic carriers to ascertain if they had data consistent with the onset of FAP.

Between 1976 and 2003 we diagnosed in the Balearic Islands (Spain) a total of 144 patients: 102 confirmed cases and 42 probably affected cases. Confirmation of FAP was established in 19 patients by detection of amyloid in biopsies previously stained with Congo red and in 83 by the presence of the biochemical marker TTRVal30Met in serum [9] and/or the G for A substitution in the TTR gene [10]. The remaining 42 patients, deceased consanguineous relatives of confirmed patients in whom the diagnosis was supported by a clinical picture consistent with FAP, were not included in order to decrease or eliminate accidental or systematic errors (bias).

The Balearic Islands (Spain) which form the Balearic Archipelago, are located in the Western Mediterranean Sea; they range in size from the largest, Majorca (3.609 km<sup>2</sup>), to Minorca (694 km<sup>2</sup>), Ibiza (572 km<sup>2</sup>), Formentera (83 km<sup>2</sup>) and Cabrera and other islets (48.7 km<sup>2</sup>), as illustrated in relation to each other and to the Iberian Peninsula (Spain and Portugal) in the map (Figure 1).

The patients belonged to 53 unrelated families: 50 from Majorca and three from Minorca. Distribution of all patients in the municipal areas of both islands is presented in Table I (Population from the *Instituto Balear de Estadística*, 2001). The study of the 102 confirmed patients included 53 probands, 10 ascendants, 15 collaterals and 24 descendants.

In our first reports [11,12] we had only diagnosed FAP in families from Majorca and consequently concluded that the endemic focus on this island should be termed the Majorcan focus. As we have

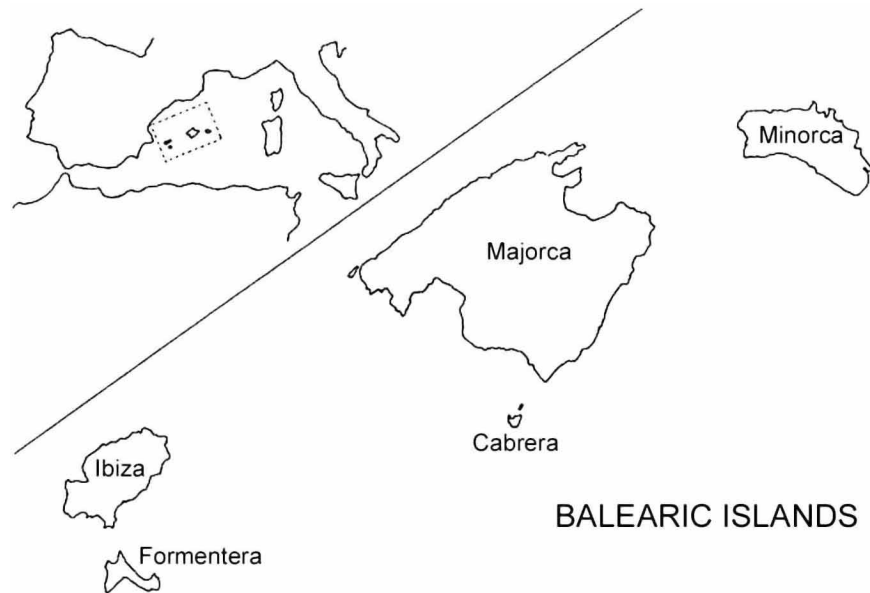


Figure 1. *Upper*: Map of the Western Mediterranean Sea with the Balearic Archipelago in dotted lines. *Lower*: Map of the Balearic Islands.

Table 1. Families and patients. Geographical distribution.

<i>Municipalities</i>	<i>Population</i>	<i>Patients</i>	<i>Confirmed</i>	<i>Non confirmed</i>
<b>Majorca</b>				
Alaró	4,121	23	13	19
Algaida	3,868	2	2	0
Campos	7,132	2	2	0
Consell	2,403	2	2	0
Inca	23,361	3	3	0
Llubí	1,931	5	4	1
Llucmajor	24,750	10	9	1
Marratxí	22,275	5	3	2
Palma	346,720	54	43	11
Pollensa	14,647	3	1	2
Porreres	4,363	3	2	1
Sa Pobla	10,736	1	1	0
Sencelles	2,214	5	4	1
San Lorenzo	6,692	2	2	0
Sta. María	4,937	7	4	3
Selva	2,975	4	4	0
<b>Minorca</b>				
Ciudadela	23,706	2	2	0
San Lluís	4,626	2	1	1
		144	102	42

now diagnosed FAP in 50 families from Majorca and 3 from Minorca, we consider the Balearic focus would be a more suitable name.

## Methods

A genetic epidemiologic study of the FAP was carried out in the two mentioned communities. We collected and analysed the following epidemiological features: sex distribution, parental transmission, age-at-onset, family history, age-at-death and duration of

FAP, incidence, prevalence, fertility, asymptomatic heterozygotes, and differences in age-at-onset in familial pairs of FAP patients.

## Statistics

Data are shown as mean, standard error (SEM) and range. To assess the significance of differences between quantitative variables we used the unpaired Student's *t*-test or, when indicated, the non-parametric Mann-Whitney test. The relationship

between age-at-onset of the members of familial pairs of FAP patients was assessed using Pearson's correlation coefficient or the non-parametric Spearman's rank correlation coefficient. Comparison of frequencies was performed using binomial distribution values:  $p < 0.05$  was regarded as significant.

#### *Ethical standards*

The ethical guidelines of the Helsinki Declaration of the World Medical Association were observed.

## Results

### *Sex*

A predominance of affected men versus affected women was observed (62% vs 38%).

### *Parental transmission*

The origin of the mutation was paternal in 38 patients, maternal in 25 and unknown in the remaining 39 cases.

### *Age-at-onset*

Mean age was  $45.7 \pm 1.6$  ranging from 20 to 84. This parameter was similar in men and women ( $46.1 \pm 2.1$  vs  $45.0 \pm 2.6$ ). According to reported criteria [13,14] we classified the patients into classical onset cases (beginning before 40 years of age) and late onset cases (presentation at 40 years or later). The second group was predominant (42% vs 58%). The existence of familial and isolated cases with very late onset age, over 50 years, has been described in heterozygote patients in Portugal [15–17] and Japan [18,19] and also in homozygotes [20]. For this reason, we include a graph (Figure 2) with the onset age in our series and note that 44% patients were in the very late onset age.

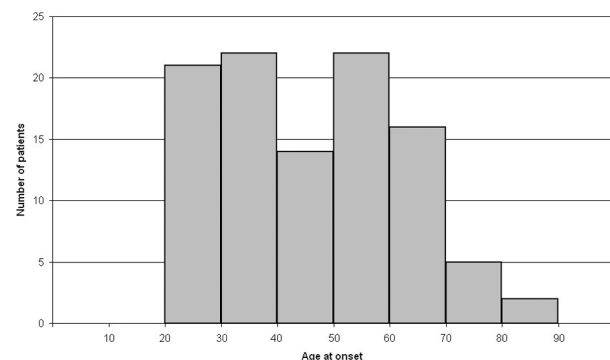


Figure 2. Age-at-onset of 102 confirmed patients (horizontal axis). Number of patients per decade (vertical axis).

### *Family history*

According to the existence of FAP in their families we classified the patients in familial and isolated or sporadic cases. Clinical history of FAP (confirmed or probably affected) were observed in the first group but not in the second; obviously each isolated case was the proband or index case of his/her family. Familial cases predominated over isolated cases (69% vs 31%). Mean age-at-onset in isolated cases was higher than in familial cases ( $50.4 \pm 2.4$  vs  $43.5 \pm 2.1$ ); the difference was prominent but not significant ( $p = 0.05$ ).

Since the pathogenesis of FAP was ascertained we have learned that isolated cases are explained by one or both parents being asymptomatic carriers transmitting the mutation to their descendants, whereas they themselves remained disease-free. In our series, there were 32 isolated cases, but we were able to study the transmitter only in 10.

Consequently, we identified 22 isolated cases of unknown origin because we were unable to investigate the carrier state as their parents were deceased. We ascertained that 64% had an onset of FAP after 50 years of age and for this reason they were patients with very late onset.

Among the remaining 10 cases, in seven we were able to investigate both parents, ascertaining that the father was the transmitter in four and the mother in three. In the three remaining cases, we were only able to investigate the mother as the father was deceased; the result was negative so we concluded that the father had probably been the transmitter (non-confirmed carrier father).

### *Age-at-death and duration of FAP*

Sixty-three patients were deceased (62%): 44 men and 19 women. Mean age at death was  $57.9 \pm 1.8$  (range 26–87) and was similar between sexes ( $58.3 \pm 2.2$  for men vs  $57.1 \pm 3.2$  for women) whereas duration of the disease was  $9.7 \pm 0.6$  (range 1–19) and also similar between men and women ( $9.8 \pm 0.7$  vs  $9.4 \pm 1.0$ ).

### *Incidence*

We investigated the incidence of new cases per year in our series (Figure 3). The number increased considerably between 1976 and 2003.

### *Prevalence*

The prevalence rate of FAP – patients who remained alive since the beginning of the study – was calculated for both islands. In 2001 the Majorcan population was 702,122 inhabitants and the Minor-

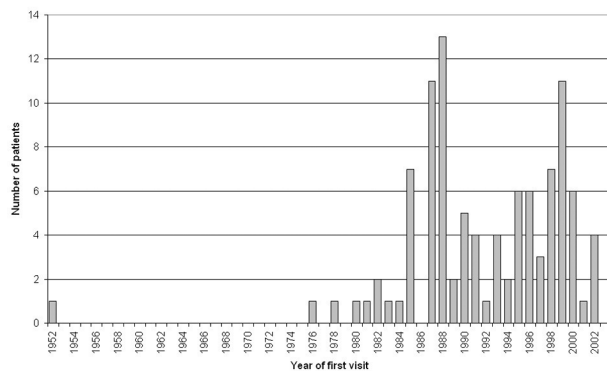


Figure 3. Year of first visit (horizontal axis). Number of new confirmed patients (vertical axis).

can population 72,296 (*Instituto Balear de Estadística*). As 38 of the Majorcan patients were alive, the prevalence rate was 5 patients per 100,000 inhabitants, and in the Minorcan patients, 1 per 100,000 inhabitants as there was only 1 survivor.

#### Fertility

The number of children from 73 patients (45 male and 28 female) was 159 (96 vs 63). The number of male and female patients who have had descendants was similar: 71% (45/63) and 72% (28/39), respectively. The ratio number of children/parent was not dependent on the sex of the parent ( $2.13 \pm 0.17$  for male parents vs  $2.25 \pm 0.19$  for female parents,  $p=0.6$ ). The 73 fertile FAP patients were all from Majorca. For this reason, we selected a control group of 73 Majorcan parents who were neither FAP patients or asymptomatic carriers, with similar age and percentage of men and women; the fertility figures of this control group were not significantly different to those from FAP patients ( $2.31 \pm 0.17$  children/parent from 49 male parents, and  $2.29 \pm 0.23$  children/parent from 24 female parents). In addition, we investigated the mean age-at-onset of FAP in the 73 patient group. This was  $48.5 \pm 1.9$  (20–84) and we observed that FAP onset was after 40 years of age in 66%. It is thus probable that the majority of patients had had their offspring before the onset of FAP. These data indicate that in our series there was no relation between FAP and patients' fertility.

#### Twin patients

We identified two kinds of twins. The first was a pair of monozygotic twins, FAP male patients, with discordant age-at-onset and clinical expression [21]. The second was a pair of dizygotic twins; one FAP female patient who died at 68 years, and her brother, a carrier of the mutation who died disease-free at age 86.

#### Asymptomatic heterozygotes

We investigated 292 consanguineous relatives and detected 116 asymptomatic carriers (40%). The genealogic distribution was 17 ancestors, 26 collaterals and 73 descendants. The proportion of men and women was similar (49% vs 51%). Mean age was  $35.3 \pm 2$  (1–87). We found only 8 deceased carriers (6%); mean age at death was  $81.4 \pm 2.8$  years (67–91).

#### Familial pairs of FAP patients

**Classification.** We ascertained 39 familial pairs belonging to 30 families; three intergeneration types (24 parent-child, 3 grandparent-grandchild, and 2 uncle/aunt-nephew), and two intrageneration (9 sib-sib and 1 first cousin-first cousin). We discarded the intrageneration group to avoid possible bias.

**Anticipation of age-at-onset.** We searched the type of raw anticipation taking into account the magnitude of the difference in age-at-onset between the first and the second member of the pairs. It can be positive (earlier onset in the second member), non-existent and negative (later onset in the second member) [22]. We followed the criteria of the various authors devoted to the study of anticipation and consequently we made a transversal analysis. Our results thereby detected the differences in age-at-onset in the familial pairs at the time of investigation.

The number of families, types of pairs and anticipation are summarized on Table II. In 83% of pairs (24/29) the anticipation was positive whereas in 14% (4/29) was negative and non-existent in 3% (1/29). The proportion of positive raw anticipation was 83% (20/24) of the parent-child pairs, 100% (3/3) of the grandparent-grandchild pairs and 50% (1/2) of the uncle/aunt-nephew pairs.

**Differences in age-at-onset.** The mean age-at-onset of the first and second member in the whole group of 29 pairs and the different types are shown in Table III. As can be appreciated the age-at-onset of the second member was lower than of the first. The difference was statistically significant for the whole pairs, but in the analysis of each type the differences only reached statistical significance in parent-child pairs. This may be due, at least in part, to the small numbers of cases in the two other types of pairs.

The average length of anticipation in each kind of pairs was: (a) In the 24 parent-child pairs,  $16.7 \pm 2.8$  years (range 4–41); the mean years of anticipation according to the sex of parents was similar ( $17.7 \pm 4.0$  male vs  $15.5 \pm 4.0$  female); (b) In the 3 grandparent-grandchild pairs,  $29 \pm 5.8$  years (range 19–

Table II. Families, pairs and anticipation of age-at-onset.

Families	Pairs	Number	Positive	None	Negative
17	Parent-child	24	20	1	3
3	Grandparent-grandchild	3	3	0	0
2	Uncle/aunt-nephew	2	1	0	1
22		29	24	1	4

Table III. Comparison of age-at-onset in familial pairs.

Pairs	First member	Second member	p
All pairs (29 pairs)	49.28 (3.22)*	32.07 (2.20)*	0.000**
Parent-child (24 pairs)	47.46 (3.62)*	30.79 (1.78)*	0.000**
Grandparent-grandchild (3 pairs)	50.67 (5.00)#	21.67 (2.00)#	0.046##
Uncle/aunt-nephew (2 pairs)	69.00 (3.00)#	63.00 (2.00)#	0.438##

\* Mean (sem); \*\* Student t test; # Mean (average of ranks); ## Mann-Whitney test.

39). (c) In the 2 uncle/aunt-nephew pairs,  $6 \pm 11$  (range 5–17).

We considered as relevant the differences in age-at-onset between the first and second member of cited pairs higher than the cut-off value of 9 years, which was their percentile 25. We found relevant positive anticipation (more than 9 years) in 76% of the whole pairs, 75% (18/24) in parent-child, 100% (3/3) in grandparent-grandchild and 50% (1/2) in uncle/aunt-nephew. On the contrary in the 4 pairs with negative anticipation (3 parent-child and 1 aunt-nephew) the absolute value of the difference in age-at-onset was small (less than 9 years, range 2–5 years); thus the negative anticipation was non-relevant.

We also analysed the relationship of age-at-onset between the first and second members in the whole group of pairs and in the first two mentioned kind of pairs (Table IV). A high positive correlation was found in the whole group (0.61), parent-child (0.68) and grandparent-grandchild (0.87).

## Discussion

The number of patients has become progressively higher in our series. In an earlier report [11] we described the main features of 44 patients (29 confirmed and 15 probably affected) in 17 Majorcan families. In the second publication [12] we presented data on 78 patients (57 confirmed and 21 probably affected) belonging to 37 Majorcan families. The number of patients is presently 144 (102 confirmed and 42 probably affected) in 50 Majorcan families and three Minorcan families; for this reason, the most accurate name for our series is the FAP Balearic focus, which still ranks among the five most important endemic areas in the FAP worldwide

Table IV. Correlation between age-at-onset in familial pairs.

Pairs	R	P
All pairs (29 pairs)	0.61*	0.000
Parent-child (24 pairs)	0.68*	0.000
Grandparent-grandchild (3 pairs)	0.87#	0.330

\*Pearson's correlation coefficient; # Spearman's rank correlation coefficient.

epidemiological map, surpassed only by Portugal, Japan, Sweden and Brazil.

The progressive number of patients in Majorca together with the diagnosis of three patients in Minorca confirm that FAP is expanding in the Balearic Islands. We consider that the expansion in the Balearic Islands is the result of greater awareness of the disease both by physicians and the general population. Many patients, both living and deceased, now are identified as FAP when they previously had been diagnosed as neurologic or extraneurologic diseases. Based on the reported fertility data, we can not consider that the expansion of FAP is attributable to these patients having higher fertility than in the general population.

FAP constitutes a public health problem in the Balearic Islands due to its incidence, its relentless course with multiple handicaps and progressive disability, fatal prognosis, autosomal mode of inheritance, familial and economic problems, and health care and social security costs. For these reasons, health measures are necessary to identify patients not yet diagnosed, so as to treat an increased number of patients when appropriate with liver transplants, and to detect asymptomatic carriers to

prevent and eventually eradicate this fearsome disease.

The age-at-onset (45.7 years) was higher (Table V) than that reported in Portugal [23], Japan [20,24] and Brazil [25], but lower than in Sweden [23]. Based on the fact that the mutation and the haplotype in the Portuguese and Balearic series are the same, the marked difference in mean age-at-onset between them must be related to acquired and/or environmental factors. We emphasize the similar age-at-onset in males and females in contrast with a Portuguese series [26] where a later onset was observed in females ( $33.7 \pm 5.8$  vs  $29 \pm 6.4$ ). Worth mentioning is the substantial number of isolated cases (31%) and the predominance of late onset cases (58%) which has been the cause of delayed diagnosis of FAP.

The age-at-death (57.1 years) was also higher in our series than that reported in the Portuguese [23] and Japanese [24] series, but lower than the Swedish figures [23], whereas duration of FAP was 9.8 years, very similar to the figures of the above-mentioned series. In our sample, duration was similar in men and women but in the aforementioned Portuguese series [26] duration was higher in women (11.3 vs 10.2).

Fertility was similar in men and women whereas in the cited Portuguese series [26] female patients had a significantly higher number of children ( $3.7 \pm 2.6$  vs  $2.7 \pm 2.1$ ). The number of parent-child pairs increased significantly (24 vs 15) since our first report [27]. Analyses of anticipation in these pairs revealed that the proportion of raw positive anticipation (83% of 24 pairs), was not significantly different than those previously described in the Swedish series (84% of 44 pairs) and the Portuguese (72% of 227 pairs) [23]; comparison with one Japanese series was not possible because selection criteria were different [28]. The mean number years of anticipation in our sample was similar in the children of paternal and maternal origin whereas in the Swedish, Portuguese and Japanese series was higher in the children of maternal origin [23,24,28]

The high positive correlations in age-at-onset found in parent-child pairs (0.68), and grandpar-

ent-grandchild (0.87) supports the hypothesis that genetic factors may modulate this age-at-onset in FAP; however the discordant onset age and clinical expression observed in a pair of monozygotic twins are suggestive of the influence of non-genetic factors that are either environmental factors or stochastic events at the molecular/cellular level occurring before or during embryogenesis [21].

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Table V. Data of main epidemiological focus of FAP.

Focus	Onset age	Age-at-death	Duration
Portugal [23]	33.5	40.8	10.6
Japan [20,24]	35.6	46.6	9.8
Sweden [23]	56.7	66.3	10.9
Brazil [25]	32.4	—	10.6
Balearic Islands	45.7	57.1	9.8

\*FAP: Familial amyloid polyneuropathy.

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