A study of 159 Portuguese patients with familial amyloidotic polyneuropathy (FAP) whose parents were both unaffected

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Abstract
We reviewed 1233 cases of familial amyloidotic polyneuropathy (FAP) from 489 Portuguese families registered at the Centro de Estudos de Paramiloidose, Porto, Portugal. It was found that in 159 cases, neither parent had shown symptoms of this hereditary dominant form of peripheral neuropathy. These cases appear to form a distinct group, with a later age at onset (mean 45.1 years, SD 12.0) than the group of patients with one affected parent (mean 31.2 years, SD 6.9) and a geographical origin not quite in the areas where the disease is most prevalent. Thought this group is not significantly different from the general group of patients in clinical presentation at onset and severity of the disease, the average interval between onset and diagnosis (mean 4.5 years, SD 3.2) reflects the difficulties in diagnosing these patients in the absence of a positive family history.

From the analysis of pedigrees and in spite of a large number of isolated cases, the occurrence of new mutations could not be proven, and it seems more likely that, in some families, the FAP gene may result in a milder expression or even remain “silent” for several generations.

Further investigation of this discrepancy may prove to be important in elucidating the mechanisms involved in the pathogenetic process.

(F J Med Genet 1994;31:293-299)

Familial amyloidotic polyneuropathy (FAP) is a sensory, motor, and autonomic late onset neuropathy inherited in an autosomal dominant mode, first described by Andrade in Portugal. The observation in 1939 of a patient with "a peculiar form of neuropathy" from the coastal area of Póvoa do Varzim (some 30 km north of Porto) was followed in subsequent years by the ascertainment of similar cases which provided the basis for Andrade's recognition of this disease as a new clinical entity. Since then, 1233 cases from 489 pedigrees, so far unrelated, have been observed and diagnosed at Centro de Estudos de Paramiloidose (CEP) in Porto. While 60% of these patients had onset between 25 and 35 years of age and 87% developed symptoms before they were 40 years old, the age of onset in the Portuguese cases has been shown to have a much wider range (17 to 78 years) than was previously thought.

In Portugal the gene has been assumed to be fully penetrant in the large majority of families and a positive family history for the disease has always been considered an important criterion for diagnosis of FAP. However, families where the disease affects only a small number of its members have also been known for a long time. In his original description, Andrade refers to 13 cases out of 64 who reported a total absence of similar symptoms in any other member of their respective families, in particular in either parent.

In the first genetic study of FAP in Portugal nearly 30 years ago, Becker et al suggested that these so called "sporadic" cases (with no affected parent) could be the result of new mutations, but he argued in favour of the mutation being already present, but "silent", in one of the parents.

In 1978 it was shown that in FAP the major component of amyloid fibrils was related to transthyretin (TTR, formerly prealbumin). A few years later a variant TTR with a methionine for valine substitution (TTR Met30) was found to circulate in the plasma of patients and nearly half their offspring as a direct result of a point mutation in the TTR gene. Methods for the detection of this direct "biochemical marker" were developed after 1984; thus it became possible: (1) to establish that late onset (after 51 years) and classical onset cases (before 40 years) showed the same mutation, thus excluding the possibility of genetic heterogeneity between earlier and later onset cases; (2) to show that in the Portuguese population, carriers of the TTR Met30 mutation could remain disease free up to 90 years of age, and finding particularly relevant in parents of late onset cases; (3) to confirm that the same mutation is present in Japan and in Sweden (the second and third largest foci of FAP worldwide), which was particularly relevant in the case of Sweden owing to the later onset and lower penetrance in this population. Therefore, the discovery of the biochemical marker and gene analysis have answered some of the early questions regarding the possibility of incomplete penetrance in some families and have confirmed a wider phenotypic variability.

Recently, FAP type I has been associated with other TTR mutations. However, in Portugal, Brazil, and Sweden no pathogenetic TTR mutations other than TTR Met30 have been described so far.

Cases where both parents were unaffected have been identified at our centre at an increasing rate, particularly in some areas. This may be the result of improvement in medical care.
and diagnosis of FAP in particular. Since traditionally, in Portugal, the absence of a family history of FAP has made its diagnosis more difficult, this study may help in diagnosing these particular cases and may also provide some further understanding of the mechanisms underlying the variation in clinical expression and age of onset.

Patients
From 1939 to December 1992, 1233 patients from 489 unrelated pedigrees have been registered at the Centro de Estudos de Paramiloidose (CEP), Porto. In 947 patients, one of the parents was known to be affected and in 112 the information was doubtful or contradictory. However, in this population we also found 174 patients whose parents were described by their offspring as disease free until death, or at the time of their child’s diagnosis if they were still alive. All these patients had a well established family history, which means first hand information given by a proband about the parents’ age at death, cause of death, absence of suggestive FAP symptoms, and, in some cases, clinical observation of the parents if they were still alive and willing to cooperate. Of these 174 patients, we rejected 15 whose parents were disease free at death as they had died of other causes (tuberculosis, cancer, child birth, accident, etc) before the average onset age of FAP but belonged to families with several cases of FAP including in the grandparents’ generation.

Therefore, in 159 cases, FAP was not present in the parents or in the grandparents. We considered only patients with a confirmed diagnosis including typical clinical presentation, plus presence of TTR\textsuperscript{Meio} in the serum (detected by immunoblotting\textsuperscript{a}) or amyloid detected in any biopsied tissue (skin, nerve, digestive tract). In the absence of any laboratory confirmation, we accepted the description of other cases in the family or the identification of the mutation in the serum of other relatives as a confirmation of diagnosis. These 159 patients belonged to 122 unrelated families. In 14 families, the presence of TTR\textsuperscript{Meio} could not be confirmed, as they were all cases diagnosed before 1983.

Methods
From the personal registers of these 159 cases we collected information on sex, age of onset, clinical presentation of the disease, methods used for the confirmation of diagnosis and the problems with their interpretation, and time between onset of FAP and diagnosis. Whenever possible we compared the data with the subsample of patients with one affected parent.

From family registers we gathered data concerning the number of sibs, the age of the parents at the time of diagnosis of their children (if they were alive), or their ages at death and the cause of death.

We also collected available information concerning the identification of a transmitting parent: some were found to be asymptomatic carriers of the mutation, some could be deduced by familial links, and in some cases the only progenitor alive was found not to be a carrier of TTR\textsuperscript{Meio} (which made us accept the other parent as the probable transmitter).

The proband of each family was any patient who, at the time of diagnosis, denied the existence of the disease in any other member of the family; therefore we accepted multiple probands in some kindreds.

Cases were defined as isolated if there were no other affected members in the family, either first or second degree relatives.

The origin of each family was established by the place of birth (district) of its earliest affected member, or the place of birth of the most distant ancestors if they had a common origin.

Statistical Methods
The unpaired \( t \) test was used to compare means in two different groups (age of onset in male and female patients, interval between onset and diagnosis in the proband and the non-proband group of patients). The medians of the age of onset in the group with no affected parent and in the group with one affected parent were compared by the Mann–Whitney test owing to non-normality in the latter group.

Results
General Sample Description
Our sample consists of 159 cases (95 men and 64 women, a sex ratio of 1.5, which is not significantly different from 1:2, the sex ratio in the sample of 947 patients with one affected parent), diagnosed at the Centro de Estudos de Paramiloidose between 1943 and 1992. In December 1992, 64 cases were isolated, 56 had affected sibs, and 39 had some other affected relative; 117 were probands and four families had multiple probands. The geographical area of origin of these cases is quite distinct from that of the group with one affected parent (fig 1). Their distribution is more dispersed and they rarely come from the districts with a higher concentration of cases belonging to the latter group.

DISTRIBUTION OF AGE OF ONSET
Among these cases, age of onset varied from 20 to 78 years of age, while the range was 17 to 66 years for the group with one affected parent; the median was 40 years, significantly higher (\( p < 0.001 \) \( z = 14.14 \), for the Mann–Whitney test) than in the group with one affected parent (30 years). As in previous studies in Portuguese FAP patients, \( 90\% \) of women were found to have a later onset (mean 47.0, SD 11.1) than men (mean 43.9, SD 12.4).

The differences between the distribution of age at onset of these 159 cases and the cases with one affected parent are apparent in fig 2 and have concrete implications in terms of risks. It means, for instance, that while 90% of patients with one affected parent are known to develop symptoms before they are 40 years
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Presentation of the disease, mode of diagnosis, and time interval between onset and diagnosis

The symptoms at presentation of the disease were similar to previously published descriptions and also to the general population registered at CEP: most patients presented with sensory symptoms of the lower limbs with a large number also having autonomic problems (Table). A few patients (7%) had motor symptoms from the beginning of the disease and one case had only had vitreous opacities for 10 years when he was examined at CEP. All patients except the latter have developed a mixed sensory, autonomic, and motor neuropathy.

The diagnosis (always made by a neurologist) was confirmed either by detection of serum TTR<sup>mut</sup> (42 cases), by the finding of amyloid deposits in pathological material (46), or both (62). Nine patients had no laboratory examination but had a typical picture of FAP and confirmed cases in a first degree relative. The finding of amyloid in some biopsies (nerve, three cases; digestive tract, four cases) was interpreted as primary or secondary amyloidosis in seven patients and the diagnosis of FAP was delayed until later detection of TTR<sup>mut</sup>, the search for which was prompted by a clinical course considered to be abnormally long for any of these types of amyloidosis. Three patients had biopsies without showing amyloid (one sural nerve, one skin, and one rectal biopsy) and the diagnosis was considered only because of typical clinical presentation in two cases, and, in one case, by simultaneous detection of TTR<sup>mut</sup> in serum in the course of the investigation of a poly-neuropathy. This shows that a diagnosis based only on pathological findings may be obscured by the patchy distribution of amyloid and emphasises the importance of the detection of the mutation.

The mean interval between onset and diagnosis was, as expected, significantly higher in probands (mean 4.8 years, SD 2.9) than in non-probands (mean 3.6 years, SD 3.9), reflecting the difficulties in diagnosing this group of patients (p < 0.05).

Pedigree analysis

Six pedigrees will be described in detail (fig 3). They illustrate different situations in which

Symptoms at presentation of the disease

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>% of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensory symptoms of lower limbs</td>
<td>80</td>
</tr>
<tr>
<td>Vomiting</td>
<td>3</td>
</tr>
<tr>
<td>Constipation</td>
<td>21</td>
</tr>
<tr>
<td>Constipation alternating with diarrhoea</td>
<td>12</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>17</td>
</tr>
<tr>
<td>Loss of weight</td>
<td>31</td>
</tr>
<tr>
<td>Impotence</td>
<td>24</td>
</tr>
<tr>
<td>Motor symptoms</td>
<td>7</td>
</tr>
</tbody>
</table>

Old, the equivalent risk for the offspring of a non-manifesting carrier is only 35%.
Figure 3  Family pedigrees.
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These cases may occur concerning the possible origin of the mutation, the number of affected members in each family, and age of death of the parents.

FAMILY A
Both parents of case II.3 died (of other causes) before FAP was known as a distinct clinical entity. They might have manifested symptoms if they had lived longer, though they were unaffected by FAP at an age beyond the average age of onset for Portuguese patients.

Case II.3 had first symptoms of FAP (vitreous opacities) at 61 years of age, but the diagnosis was delayed until 10 years later (1973) when his own son (born 1930) showed typical polyneuropathy. At that time, at 71 years of age, vitreous opacities were his only symptom. Two elderly sibs died in their ninth decade. His younger brother was examined at our centre in 1990 and the neurological examination was normal. He was shown to be a proven carrier of the TTR\textsuperscript{Met30} mutation but he remains asymptomatic at 89 years of age, while his son’s disease started at 52 years of age.

FAMILY B
The only affected member of this pedigree was the only child of II.6. Her mother died in child birth at 19 years of age and her father died of lung cancer. Her (only) maternal uncle migrated to Mozambique and she has lost contact with that branch of the family. No similar case from her father’s side is known to her.

FAMILY C
The proband in this pedigree is the only offspring of the couple in the first generation. His father was tested last year and found to be a non-carrier of the TTR\textsuperscript{Met30} gene. Either our case is a new mutation, or the mother was the carrier (dead of other causes before manifesting FAP symptoms), or the tested father is not the biological father.

FAMILY D
The proband of this family is a man born in 1936. Both his parents belonged to very small sibships. His father died at 82 years of age of lung disease (he worked in a windmill) and his mother was alive and healthy at 82 years of age, when her son was diagnosed. In spite of a large sibship, none of his sibs has yet developed symptoms of FAP, and therefore the possibility remains that he is the result of a new mutation.

FAMILY E
Case II.4 developed symptoms of FAP at 68 years of age, with motor symptoms from the beginning. His onset age was the latest known to us. Both his parents had died at a very old age with no signs of the disease, so he might have represented a new mutation. However, some years later, his elderly brother also presented symptoms of FAP at 78 years of age, being the oldest case ever observed in our centre. Since it is unlikely that they could both be new mutations, one of the parents must have been a carrier of the mutant gene. They died in the ninth decade without any signs of the disease.

FAMILY F
This family comes from the area of Serra da Estrela, where the disease has been found for quite a long time. The mother of our case is the youngest of a sibship of seven and all her sibs died at an old age. At the time of her daughter’s diagnosis, she was found to be a carrier of the mutation. She died at 91 years of age, still asymptomatic.

THE TRANSMITTING PARENT
In 94 cases it was impossible to identify the transmitting parent: either both parents had died before the diagnosis of their offspring, or they were not tested, or the disease did not manifest in other branches of the family.

However, in other families (33 cases), family links enabled us to detect the most probable pathway of gene transmission. In 22 cases corresponding to 17 sibships, one of the parents (11 mothers, six fathers) was found to be a carrier of the TTR\textsuperscript{Met30} mutation: the oldest was a woman, alive and asymptomatic at 92 years of age. In other families, one of the parents had died and the one who was alive was tested and found to be a carrier of the wild type mutation (10 cases); we thus assumed the dead parent to be the probable carrier. These cases included four mothers (corresponding to six patients) and two fathers (one from family C and another with three affected children). In only these two families could non-paternity be a problem; very unlikely, however, in the latter case. Therefore, in 65 patients, the transmitting parent could be identified: 37 had inherited the disease from their mother and 28 from their father.

Parents who were found to be asymptomatic carriers were aged between 58 and 92 years at the time of their offspring’s diagnosis or at the time of their last observation at our centre. Among the transmitters identified through familial links, only three were alive (aged between 47 and 60) when their children were diagnosed and 27 had already died. When considering the ages at death in the latter group, we found that only six died before 48 years (corresponding to the 75th centile of the age of death distribution in the general FAP group\textsuperscript{[20]}). Also, in the group with an unknown transmitter, we found 64 patients (in 58 sibships) both of whose parents had died after 48 years.

These data on the identified transmitter, together with cases with an unknown transmitter where the hypothesis of a new mutation is very unlikely, clearly show that a subpopula-
tion of carriers is protected from manifestation of the disease.

Discussion
This study has reviewed all cases with no affected parent registered at the Centro de Estudos de Paramiloidose (Porto, Portugal) and diagnosed between 1943 and 1992. They represent 13-0% of all cases and form a distinct group, with an age at onset higher than average, and belonging to families with a geographical origin not quite in the areas where FAP is most prevalent.

The distinct distributions of age of onset in this group of patients and in the group with one affected parent show that two realities coexist in Portugal: families with affected patients in successive generations and families with reduced penetrance, where only a few members (if any) become affected at later ages and where some members may remain asymptomatic throughout their life.

In spite of the fact that “sporadic” patients have been reported since the early descriptions of FAP,1,2 until recently their importance has mostly been underestimated in Portugal, in both clinical and genetic practice. Even in the country with the highest prevalence of FAP, the diagnosis of these cases was difficult, as shown by the longer delay in diagnosis of probands. Some difficulties were not because of an atypical clinical presentation but rather an onset age above average, the absence of a positive family history, and the fact that some of these patients came from areas where FAP was hardly known.

Therefore, the awareness of such cases in the Portuguese population may improve their ascertainment and diagnosis. A second question concerns genetic counselling, which poses some specific problems in these families: along with a lower risk for patients in the first affected generation, we have found, occasionally, large anticipation of age of onset in their offspring.11,12

The genetics of these cases is of great interest in understanding and discussing gene expression in autosomal dominant diseases. There were two main circumstances in which neither parent of these cases was affected: when the transmitting parent died of other causes before “the age at risk” (in several cases in our sample, one of the parents died young in child birth or of tuberculosis, accident, etc) or when at least one of the parents (sometimes both) have “outlived their risk” without developing symptoms. The latter situation is not uncommon in this study, raising interesting questions from a genetic point of view.

The variable penetrance, as expressed by the existence of late onset, isolated cases and asymptomatic carriers of an old age, makes it more difficult to evaluate the rate of mutation of the FAP gene. It remains an open question whether some of our isolated cases are the result of a new mutation or whether they reflect the lack of expression of the FAP gene in one of the parents: in some families, migration, geographical dispersion, or a reduced family size could explain the occurrence of isolated cases, while in others it seems strange that the proband remains the only affected member, after many years of regular follow up and in spite of a large sibship.

So far, in Portugal, neither haplotype analysis nor genealogical investigation have been able to prove or disprove the occurrence of multiple mutations or the hypothesis of a single gene source, which has been claimed for a long time.24-26

The association between a later onset, a different geographical origin of these cases, and the incomplete penetrance of the gene is a challenge in the study of the interaction of genetic/environmental factors in the expression of the FAP gene, whose variability may be the result of some modifying genes in some families, of different genetic backgrounds in each of the two groups, and also of differences in environment.

It seems clear that there is some kind of protection in some Portuguese FAP families, resulting in a later onset and fewer (if any) affected members. A significant proportion of gene carriers may go undetected (because of lack of symptoms) throughout their life, which is why the real incidence of the mutations is higher than detected. In this respect, there are strong similarities with some families from the two other large foci of FAP in Europe: Sweden, where a large proportion of cases are isolated,27,28 and Majorca, where 13-6% of the reported patients lack family antecedents.29

We believe that the understanding of the pathogenetic process could also gain from a thorough study of factors involved in the non-expression of the FAP gene in aged asymptomatic carriers.

This research was made possible by grant number 17/91 from Comissão de Fomento da Investigação em Saúde from the Portuguese Ministry of Health (November 1991–December 1992). We thank Mr Anselmo Carraca for his technical assistance, particularly in drawing the pedigrees.

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