Impact of homozygosity for an amyloidogenic transthyretin mutation on phenotype and long term outcome

G Holmgren, U Hellman, H-E Lundgren, O Sandgren, O B Suhr

Although amyloidogenic transthyretin (ATTR) mutations are common in several populations, such as black Americans, the small number of diagnosed patients homozygous for TTR amyloid and the short follow up in most studies has until now prevented an analysis of their phenotype. In Sweden, nine homozygous patients from eight families carrying the ATTR mutation Val30Met, which gives rise to fatal neuropathic amyloidosis (FAP), have been identified and have now been followed for up to 15 years. This has enabled an analysis of the phenotype of homozygous patients. Genetic testing and detection of amyloid deposits in the vitreous body or in intestinal or skin biopsies confirmed the diagnosis in all patients. The patients’ symptoms were obtained from medical records. For comparison, we used a group of 35 heterozygous non-transplanted patients with FAP (18 men and 17 women), who had been evaluated at the Department of Medicine, Umeå University Hospital before their deaths.

Vitreous amyloidosis was the most prevalent symptom in the homozygous group, and in two patients it was the only manifestation of the disease during their lifetime. The age at onset was not different from that of heterozygous patients, and their survival tended not to be shorter but actually longer than for heterozygotes. Homozygosity for the mutation associated with FAP, ATTR Val30Met, does not implicate a more severe phenotype for Swedish patients. The most common symptom was vitreous opacity, which may be the only manifestation of the disease. These findings point to the possibilities of different pathways for amyloid formation, or the presence of hitherto unknown genes operating in amyloid formation.

Hereditary transthyretin (TTR) amyloidosis is generally regarded as a rare disease. However, amyloidogenic transthyretin (ATTR) mutations appear to be more common in the population than previously suspected. In black Americans, nearly 4% carries the ATTR Val122Ile mutation, associated with a late onset cardiomyopathy that is frequently overlooked and only diagnosed at postmortem examination. Nearly 90 different ATTR mutations are reported in the literature. They are inherited as an autosomal dominant trait with variable penetrance. Among the more common mutations is that causing familial amyloidotic polyneuropathy (FAP), a neuropathic systemic amyloidosis due to the ATTR Val30Met mutation. It is endemic in areas of Japan, Sweden, Portugal, and Brazil. In Sweden, the endemic areas are the counties of Västernorrland and Norrbotten in the northern part of the country. The mean age of onset for Swedish patients is 56 years, whereas in Japan and Portugal, the onset is around the age of 36 years. The disease gives rise to a fatal, often painful, sensorimotor somatic and autonomic polyneuropathy. Gastrointestinal disturbances is another symptom of the disease, as is impaired vision due to vitreous amyloid deposits. Heart complications are common, predominantly caused by conduction disturbances that often necessitate insertion of a pacemaker. Patients in the latter stages of the disease are severely incapacitated, bedridden, or confined to a wheelchair, and often have urinary and faecal incontinence. The survival is variable but median survival is reportedly from 10 to 13 years after onset of the disease.

As TTR is predominantly synthesised by the liver, a liver transplant should replace the variant with the wild type TTR and thus halt amyloid formation. The first transplantation was carried out on one of our patients in 1990, and the progress of the disease was halted. Even though some unexpected complications have become apparent, the procedure is the only available treatment and is today carried out worldwide.

The first Swedish patient with FAP was published in 1968, and more than 600 patients with clinical manifestations of FAP have been diagnosed in northern Sweden since then. In one population study in this area, sera from 1276 healthy people aged 24–64 years were examined for the ATTR Val30Met mutation, and it was detected in 19 people, with 18 being heterozygous and 1 homozygous. The mean ATTR Val30Met carrier frequency was 1.5%, ranging from 0.0 to 8.3% in 23 subpopulations. High frequency of the trait did not correlate with large numbers of patients; the largest concentration of symptomatic patients was in the Skellefteå area, where the frequency of the trait is 2.6%, yet the onset and progress of the disease in that area, compared with Lycksele with a carrier frequency of close to 10%, was earlier and more rapid. The estimated number of ATTR Val30Met carriers in Västernorrland and Norrbotten, which have close to 500 000 inhabitants, is approximately 7500. With only about 250 currently living patients with FAP, only 5–10% of the gene carriers appears to develop the disease. The high frequency of the trait and the finding of a asymptomatic homozygous carrier made it clear that a substantial number of homozygous carriers must be present, and the first homozygous patient and his homozygous asymptomatic sister were diagnosed in 1988.

TTR is normally present as a tetramer. Tetramers of the amyloidogenic variants of TTR are unstable compared with wild type TTR, and it has been suggested that monomers assemble into amyloid after conformational changes. In the plasma of heterozygous patients, TTR consists of different

**Abbreviations:** ATTR, amyloidogenic transthyretin; FAP, fatal neuropathic amyloidosis; TTR, transthyretin
combinations of mutated and wild type TTR. The combination that is the most unstable, and therefore more amyloidogenic, has not been determined; however, amyloid from the corpus vitrum of affected patients contains approximately 80% variant TTR, whereas amyloid from heart and peripheral nerves contains 50–60%, thus a substantial amount of wild type TTR is incorporated in the amyloid. Furthermore, high concentrations of wild type TTR are found in the amyloid deposits in the heart from liver transplanted patients who died from increased cardiomyopathy after the procedure. Thus wild type TTR is amyloidogenic, as has been shown for systemic senile amyloidosis.

No systematic long term follow up of homozygous patients has been reported, so it has not been clarified if the TTR tetramer consisting of only ATTR Val30Met leads to a more aggressive disease than that originating from TTR consisting of a mixture of wild and mutant TTR.

The aim of the present investigation was to present the long term outcome of our patients who were homozygous for ATTR Val30Met with regard to survival and progression of symptoms, and to compare their outcome with that of heterozygous patients.

PATIENTS AND METHODS

Patients
The clinical data of the patients were obtained from medical records and interviews with the patients and their relatives. Testing for the ATTR Val30Met mutation and examination of surgical samples of corpus vitrum or intestinal or skin biopsies for amyloid deposits ascertained the diagnosis in all patients. The homozygous group consisted of nine Swedish patients (five men, four women; table 1). The heterozygous group consisted of all 35 deceased non-transplanted patients (18 men, 17 women), who had been evaluated at the Department of Medicine, Umeå University Hospital, and in whom genetic testing had been performed. Their onset of disease was between 1968 and 1993. Because better treatment for infections and nutritional depletion has been implemented during more recent years, a bias towards a shorter survival for this retrospective cohort compared with survival nowadays cannot be excluded.

Genetic testing
Over time, different methods have been used to detect the ATTR Val30Met mutation. The first four patients discovered to be homozygous for the mutation were analysed with Southern blotting. The remaining five patients were diagnosed by PCR based methods as previously described.

Statistics
Non-parametric statistical and descriptive analyses were used. Comparisons between groups were done with the Mann-Whitney test. Kaplan-Meier plot was used to display survival and comparison between group survival was performed by log rank test (fig 1). A p value <0.05 was considered significant. Prism 4 for Macintosh (GraphPad Software Inc., San Diego, CA, USA) was used for statistical analysis.

RESULTS
The clinical data of the nine Swedish homozygous ATTR Val30Met carriers belonging to eight families are presented in table 1. Two of the nine patients, including one liver transplanted patient, have not been previously presented. They were all detected during the routine DNA diagnostic test for FAP. The father of one patient (patient 9) had died from the disease; otherwise, the patients were sporadic without known symptomatic carriers in previous generations. However, several heterozygous siblings had developed the disease, as had a few children of some of the presented patients. Of the pair of homozygous siblings, only one developed systemic disease (patient 2, table 1).

All homozygous carriers developed vitreous amyloidosis, which was the first symptom of the disease in four patients. In two patients (patients 1 and 5), vitreous opacities were the only manifestation of FAP. One patient underwent liver transplantation (patient 9), and he developed vitreous opacities 3 years after transplantation at the age of 47 years. However, his outcome after transplantation was not different from that of patients who died from increased cardiomyopathy after the procedure.

Table 1  Clinical data of the nine homozygous ATTR Val30Met mutation patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age at onset</th>
<th>Age at death</th>
<th>Symptoms at onset</th>
<th>Additional symptoms during the course of the disease</th>
<th>Affected family members</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>64</td>
<td>78</td>
<td>VO</td>
<td>0 0 0 +</td>
<td>Brother (no. 2)</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>52</td>
<td>65</td>
<td>PN</td>
<td>+ + 0 +</td>
<td>Sister (no. 1)</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>58</td>
<td>78</td>
<td>PN</td>
<td>+ + 0 +</td>
<td>Brother, sister</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>55</td>
<td>86</td>
<td>VO</td>
<td>+ + 0 +</td>
<td>Daughter</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>56</td>
<td>73</td>
<td>VO</td>
<td>0 0 0 +</td>
<td>Daughter</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>55</td>
<td>76</td>
<td>PN</td>
<td>+ 0 0 +</td>
<td>Daughter</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>54</td>
<td>64</td>
<td>PN</td>
<td>+ 0 0 +</td>
<td>None</td>
</tr>
<tr>
<td>8</td>
<td>F</td>
<td>79</td>
<td>Alive</td>
<td>VO</td>
<td>+ 0 0 +</td>
<td>None</td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td>39</td>
<td>Alive</td>
<td>PN</td>
<td>+ 0 0 +</td>
<td>Father, sister</td>
</tr>
</tbody>
</table>

F, female; M, Male; PN, peripheral polyneuropathy; GI, gastrointestinal symptoms; H, heart conduction disturbances; VO, vitreous opacities. Liver transplanted. 0 and + refer to the absence or presence of symptoms, respectively.
of the heterozygous patients, and the progression of his neuropathy has come to a halt.

One homozygous female carrier was asymptomatic at the time of diagnosis (patient 1). She died 16 years later at the age of 78 years, with no other symptoms of amyloidosis except for vitreous deposits. Patient 5 had a similar pattern, surviving for 17 years after onset of eye symptoms. Both patients were successfully operated for amyloid opacities by vitrectomy. None of the studied patients have developed heart complications in the form of conduction disturbances nor has any received a pacemaker. The onset of disease was between 39 and 79 years of age (median 55). Seven of the patients died after a median duration of their disease of 17 years (range 10–31). After excluding the patients who never developed symptoms of systemic amyloidosis (patients 1 and 5), a similar median survival of 17 years was found for the remaining five deceased patients.

The median age at onset for heterozygous FAP patients was 56 years (range 29–74), and median survival was 12 years (range 4–23). In comparing the age at onset of disease for homozygous and heterozygous patients, no differences were noted. However, the survival was longer for homozygous patients (p<0.02; 95% confidence interval (CI) 0.26 to 1.15), and remained significant after exclusion of patients with eye symptoms only (p<0.05; 95% CI 0.21 to 0.99).

Vitreous opacities were not diagnosed in any of the heterozygous patients; however, their disease has not been followed as carefully as the homozygous, so we cannot exclude that some had developed vitreous opacities at the end stage of their disease.

**DISCUSSION**

Including the present study of 9 individuals, 19 homozygous ATTR Val30Met gene carriers have been reported in the literature. The clinical picture and age at onset of the Spanish patients were similar to that reported for Spanish-Majorcan patients, even though their sensorimotor syndrome was described as more aggressive.27 The Turkish patients were interesting because only homozygous members of the family appeared to develop symptoms.28 However, if homozygosity had been the basis for clinical expression of the trait in Swedish families, we would have expected that their heterozygous siblings should have remained unaffected, and that a homozygous sibling should have developed systemic disease. However, our data do not support this hypothesis. Considering the prevalence of the trait of 1.5%, the expected number of homozygous carriers is approximately 110 in the area studied. The penetrance is estimated to be between 5–10%, thus the nine individuals identified is close to the expected number of symptomatic patients, and indicates that the penetrance is not markedly different than that noted for heterozygous individuals.16

One of the homozygous Japanese patients reported by Yosinaga et al had unusually heavy depositions of amyloid in leptomeninges and subarachnoid vessels;29 however, the patient’s age at onset was 58 years, which is comparatively late for a Japanese patient. He died 9 years later from the disease, indicating a survival that is not different from that usually reported for Japanese patients. The same group of researchers has previously reported on an asymptomatic ATTR Val30Met carrier and also late onset homozygous patients, so they concluded that Japanese homozygous patients appear to present a clinical picture not markedly different from that of heterozygous FAP patients.30–32

A few patients homozygous for non-ATTRVal30Met have been reported. From those studies, it has not been possible to determine if homozygosity for non-ATTR Val30Met variants is more amyloidogenic than heterozygosity, even though early onset cardiomyopathy has been reported for a homozygous ATTR Phe62Leu patient.33–35 Considering that the prevalence of the genetic trait for ATTR Val122Ile in black Americans is close to 4%,1 relatively few homozygous patients have been reported, and their disease appears not to have an earlier onset than in heterozygotes.

From the available data, the presence of TTR tetramers consisting only of mutant ATTR Val30Met neither accelerates the disease progression nor makes the phenotype more malignant. Age at onset and length of survival for heterozygous patients are similar to that reported in initial studies of Swedish FAP patients.36–39 Age of onset for homozygous patients is not different from that of heterozygotes and their survival is similar or actually longer than that observed for heterozygotes. This is different from that observed for Huntington’s disease where homozygosity for the CAG mutation is associated with a more severe clinical course, even though the age at onset is similar in heterozygotes.40 It should be observed, however, that the advanced age of onset for many Swedish FAP patients implies that many patients did not succumb to their amyloid disease, but to other age related diseases. In the present study, it was not possible to obtain valid information on the cause of death in all patients. In addition, vitreous opacity was a common initial symptom of the disease in homozygotes and may not indicate the presence of systemic amyloid disease. The correlation between eye deposits and systemic deposits of amyloid has not been studied.

Vitreous opacity was also a common finding in Japanese and Spanish patients.27,30 Two of our homozygous patients died at a relatively old age, reportedly without other manifestations of the disease except for vitreous amyloid deposits. According to an earlier study, 15–20% of the Swedish FAP population including homozygous patients developed vitreous opacities.41–44 All homozygous Swedish patients have developed vitreous opacities, and even though we can not exclude that some of the homozygous patients in the control group developed this complication, it is definitively more common in homozygous. The special environment in the eye, with fewer proteins in the vitreous body than in, for example, the extracellular fluid, may facilitate amyloid formation from TTR tetramers consisting of ATTR Val30Met only. In heterozygotes, the relatively high content of variant TTR in the vitreous amyloid deposits compared with that observed in peripheral nerves and heart suggests that different fibril formation pathways may be operating. An alternative explanation may be an influence of as yet unknown genes operating in TTR amyloid diseases, which have an impact on the phenotype of the disease.

In summary, homozygous Swedish patients do not display a more aggressive or a more rapidly developing FAP disease. The clinical presentation more frequently involves vitreous opacities, which may remain as the only manifestation of the disease. Different pathways for systemic and eye amyloid formation may exist.

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