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

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Running title Homozygosity for transthyretin Val30Met mutation

Key words: amyloidosis – inherited – neuropathic – vitreous; mutation – transthyretin; homozygosity.
Abstract

Background. Even though amyloidogenic transthyretin (TTR) mutations are common in several populations, such as black Americans, the small number of diagnosed homozygous TTR amyloid cases and short follow-up has hitherto not permitted an analysis of their phenotype. In Sweden 9 homozygous patients from 8 families carrying the amyloidogenic TTR mutation: ATTR Val30Met that gives rise to a fatal neuropathic amyloidosis have now been identified and followed for up to 15 years. This has enabled an analysis of homozygous patients’ phenotype.

Methods. 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. A group of 35 heterozygous deceased non-transplanted FAP-patients (18 males and 17 females), who had been evaluated at the Department of Medicine, Umeå University Hospital was used for comparison.

Results. 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, and their survival tended not to be shorter but actually longer than that observed for heterozygous.

Conclusion. Homozygosity for the mutation associated with FAP, ATTR Val30Met, does not implicate a more severe phenotype for Swedish patients. The most common symptom is vitreous opacities that may prevail as the only manifestation of the disease. The findings point to the possibilities of different pathways for amyloid formation, or the presence of hitherto unknown genes operating in amyloid formation.
Introduction

Hereditary transthyretin (TTR) amyloidosis is generally regarded as a rare disease. However, amyloidogenic transthyretin mutations (ATTR) appears to be more common in the population that previously suspected. In black Americans, nearly 4% carries the ATTR Val122Ile mutations associated with a late onset cardiomyopathy that often is overlooked and only diagnosed at post mortem examination [1]. Nearly 90 different ATTR’s are reported in the literature [2]. They are inherited as an autosomal dominant trait with variable penetrance. Among the more common mutations is Familial amyloidotic polyneuropathy (FAP). It is a neuropathic systemic amyloidosis that is due to the ATTR Val30Met mutation [3]. Endemic areas are found predominantly in Japan, Sweden, Portugal and Brazil [4-7]. In Sweden, the endemic areas are the counties of Västerbotten and Norrbotten in the northern part of the country [4]. 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 [4, 5, 8].

The disease gives rise to a fatal, often painful sensory-motor somatic and autonomic polyneuropathy. Gastrointestinal disturbances are other symptoms of the disease as well as impaired vision due to vitreous amyloid deposits [4, 5, 8-10]. Heart complications are common, predominantly caused by conduction disturbances that often necessitating insertion of a pace maker [11, 12]. The patients in the later stages of the disease are severely incapacitated, bedridden or confined to a wheelchair and are often suffering from urinary and faecal incontinence. The survival is variable but median survival is reportedly from 10 to 13 years after onset of the disease [4, 13]. Death is often a result of pronounced malnutrition and infections [9, 13].

Since TTR predominantly is synthesised by the liver, a liver transplantation should replace variant with the wild type TTR and halt amyloid formation. The first transplantation was carried out on one of our patients in 1990, and the progress of the disease was halted [14]. Even though some unexpected complications have become apparent, the procedure is the only available treatment and is today carried out worldwide [15].

The first Swedish case of FAP was published in 1968 [10], and more than 600 patients with clinical manifestations of FAP have been diagnosed in Northern Sweden since then. In a population study in Northern Sweden, sera from 1276 healthy persons aged 24 - 64 years were examined for the ATTR Val30Met mutation, and it was detected in 19 persons with 18 being heterozygous and one homozygous [16]. The mean ATTR Val30Met carrier frequency was 1.5%, ranging from 0.0 to 8.3% in 23 subpopulations. Areas with a high frequency of the trait were not those with a high number of patients. The largest concentration of symptomatic patients was in the Skellefteå area, where the frequency of the trait is 2.6 %, and the onset and progress of the disease in that area, compared to Lycksele with a carrier frequency of close to 10 %, was earlier and more rapid. The estimated number of ATTR Val30Met carriers in Västerbotten and Norrbotten with close to 500,000 inhabitants is approximately 7500. With only about 250 currently living FAP patients, only 5-10 % of the gene carriers appears to develop the disease [16]. 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 was diagnosed in 1988 [17].

TTR is normally present as a tetramer. Tetramers of amyloidogenic variants of TTR are unstable compared with wild type TTR, and it has been suggested that monomers after
conformational changes assemble into amyloid [18, 19]. In the plasma of heterozygous patients, TTR consists of different 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 vitrium of affected patients contains approximately 80 % variant TTR [20], whereas amyloid from the heart and peripheral nerves contains 50 - 60 %, thus a substantial amount of wild type TTR is incorporated in the amyloid [21, 22]. Furthermore, high concentrations of wild type TTR are found in the amyloid deposits in the heart from liver transplanted patients whom succumbed from an increased cardiomyopathy after the procedure [22]. Thus wild type TTR are amyloidogenic, as has indeed been shown for systemic senile amyloidosis [23].

No systematic long-term follow-up of homozygous patients has been presented, 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 homozygous ATTR Val30Met-patients with regard to survival and progression of symptoms and compare their outcome to 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 as described below and examination of surgical samples of corpus vitrium or intestinal or skin biopsies for amyloid deposits ascertained the diagnosis in all cases. The homozygous group consisted of nine Swedish homozygous patients that included 5 males and 4 females (Table 1). The heterozygous group consisted of all 35 deceased non-transplanted patients, 18 males and 17 females, 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. Since better care for infections and nutritional depletion has been implicated during the years, a bias towards a shorter survival for this retrospective cohort compared with today’s survival can not be excluded.

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

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Gender</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>Brother</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= gastro intestinal symptoms; H= heart conduction disturbances; VO= vitreous opacities. * = liver transplanted. 0 and + refer to the absence or presence of symptoms, respectively.
**Genetic testing**

Over time different methods have been used to detect the ATTR Val30Met mutation. The first 4 patients discovered to be homozygote for the mutation were analysed with Southern blot. The remaining 5 patients were diagnosed by polymerase chain reaction (PCR) based methods as previously described [24, 25].

**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 logrank test. A P-value below 0.05 was considered significant. Prism 4 for Macintosh (GraphPad Software, Inc, San Diego, CA., USA.) was used for the 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 had not been previously presented. They were all detected during the routine DNA diagnostic test for FAP [17, 24, 26]. The father of one patient (case no 9) had died from the disease. Otherwise the cases were sporadic without known symptomatic carriers in previous generations. However, several heterozygous siblings has developed the disease, as well as some of the presented cases’ children. In the pair of homozygous siblings, only one developed systemic disease (case no 2, Table 1).

All homozygous carriers developed vitreous amyloidosis, and in four patients it was the first symptom of the disease. In two patients (cases no 1 and 5), vitreous opacities were the only manifestation of FAP. One patient was liver transplanted (no. 9) and he developed vitreous opacities 3 years after transplantation at an age of 47. However, his outcome after transplantation was not different from that of heterozygous patients, and the progression of his neuropathy has come to a halt.

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

The median age at onset for heterozygous FAP-patients was 56 (range 29 – 74) years. Their median survival was 12 (range 4 – 23) years. 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%CI: 0.26 – 1.15), and was also the case after exclusion of patients with eye symptoms only (P< 0.05; 95% CI: 0.21 – 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 can not exclude that some had developed vitreous opacities at the end stage of their disease.
Discussion
Including the present material of 9 individuals, 19 homozygous ATTR Val30Met gene carriers have been reported in the literature (Table 2). The Spanish cases’ clinical picture and age at onset were similar to that reported for Spanish-Majorcan cases, even though their sensory-motor syndrome was described as more aggressive[27]). The Turkish cases were interesting because only homozygous members of the family appeared to developed 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 systematic 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 9 individuals identified is close to the expected number of symptomatic cases, and indicates that the penetrance is not markedly different than that noted for heterozygous[16]).

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

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

From the available data, the presence of TTR tetramers consisting of mutant ATTR Val30Met only, neither accelerates the disease progression or it makes the phenotype more malignant. The age at onset and survival for the heterozygous patients are similar to that reported in initial studies of Swedish FAP patients [4, 13]. Homozygous patients’ age of onset is not different from that of heterozygous and their survival is similar or actually tends to be longer than that observed for heterozygous. This is different from that observed for Huntington disease where homozygosity for the CAG mutation is associated with a more severe clinical course, even though the age at onset was similar to that of heterozygous [36]. It should be observed, however, that the advanced age of onset for many Swedish FAP-cases implies that many patients did not succumb to their amyloid disease, but to other age-related diseases. In the present material, it was not possible to obtain valid information on the cause of death in all cases. In addition, vitreous opacities was a common initial symptom of the disease in homozygous, 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 opacities were a common finding in Japanese and Spanish patients [27, 30]. Two of
our homozygous patients died at a relatively high 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 [37, 38]. All homozygous Swedish patients have developed vitreous opacities, and even though we can not exclude that some of the heterozygous patients in the control group developed this complication, it is definitively more common in homozygous. The special environment in the eye, with fewer other proteins in the vitreous body than in, for example, the extracellular fluid, may facilitate amyloid formation from TTR-tetramers consisting of ATTR Val30Met only. The relative high content of variant TTR in the heterozygous’ vitreous’ amyloid deposits compared to those observed in peripheral nerves and heart suggests that different fibril formation pathways may be operating. An alternative explanation may be an influence of hereto-unknown genes operating in TTR-amyloid diseases, which have an impact on the phenotype of the disease.

In summary, homozygous Swedish patients does not display a more aggressive and definitively not a more rapid developing FAP-disease. The clinical presentation more often 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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Ethics.
The study was conducted according to the principles of the Helsinki declaration.

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References


Legend to figure

Fig. 1. Kaplan-Meier plot of survival after onset of disease for 8 homozygous (7 deceased and one living) and 35 heterozygous (deceased) ATTR Val30Met patients.
Survival of heterozygous versus homozygous patients

P < 0.02
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