A clinical, echocardiographic and genetic characterization of a Danish kindred with familial amyloid transthyretin methionine 111 linked cardiomyopathy

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Aims To identify carriers and non-carriers of the mutant transthyretin methionine 111 linked familial amyloid disease, to detect early signs of the restrictive cardiomyopathy and other clinical manifestations characteristic of this inheritable disease

Methods and Results Out of 125 living family members 99 were available for clinical, echocardiographic and genetic examination. Twenty-five family members were heterozygous carriers of the mutant transthyretin methionine 111 genotype, while 74 were non-carriers. Among the 25 carriers, none had overt clinical signs of heart disease. Eight carriers, all above the age of 35, showed echocardiographic abnormalities suggestive of developing or manifest restrictive cardiomyopathy. Three had biopsy verified transthyretin-related amyloid cardiomyopathy. None of the 15 carriers in the younger age group exhibited aberrant echocardiographic patterns. Nine carriers had carpal tunnel syndrome as opposed to none of the non-carriers.

Conclusion For early detection of familial amyloid cardiomyopathy, echocardiography is the investigation of choice. The first sign is diastolic dysfunction detected as an abnormal relaxation pattern. The appearance of echocardiographic aberrations solely in the older age group suggests that the cardiomyopathy is a late onset disease. Carpal tunnel syndrome appears to be the earliest presenting clinical symptom. A curative treatment seems to be an early liver transplantation.

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Key Words: Amyloid, cardiomyopathy, hereditary, transthyretin, echocardiography.

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Introduction

Amyloidosis is a heterogeneous disease characterized by deposition of proteinaceous fibrils in various tissues. The disease can roughly be classified into hereditary and non-hereditary types. Among the non-hereditary forms the primary type (AL amyloidosis) is the most common. The amyloid fibrils are composed of monoclonal immunoglobulin light chains in this variant of the disease. Renal, cardiac and neuropathic symptoms dominate the clinical picture. Secondary (AA amyloidosis) develops as a complication of chronic inflammatory diseases such as rheumatic arthritis. In this case the fibrils contain the protein serum amyloid A. Renal symptoms most often dominate.

The inherited amyloid syndromes are most often associated with a mutation in the serum protein transthyretin. At present, nearly 50 different amyloidogenic mutations have been described in the transthyretin gene. The most common clinical type is familial amyloidotic polyneuropathy, which is characterized by progressive peripheral and autonomic neuropathy. It is a well known disease in Portugal, Sweden and Japan[1].

In Denmark, a family with inherited amyloid cardiomyopathy has been described[2]. The family members die at the age of 40–60 years from cardiac failure due to severe restrictive cardiomyopathy. Their amyloid fibrils have been shown to contain a mutant transthyretin with a methionine-for-leucine substitution at amino acid number 111[3,4], also verified at the DNA level[5].
The mode of inheritance of this amyloid disease is autosomal dominant and linked with the occurrence in serum of the specific mutant transthyretin. Individuals carrying the specific genotype can be detected by DNA analysis, based on the mutational induced loss of a Dde I restriction enzyme site in exon 4 of the gene.

The family was first examined in 1959–1960. At that time the family numbered 40 living members, of whom 37 were examined. Eight out of the 10 siblings of the first generation had died or died eventually from rapidly developing restrictive cardiomyopathy, usually before the age of 50. Autopsies revealed massive amyloid deposits in their myocardium. The therapeutic options were limited to digitalodiuretic treatment which apparently had little effect on the course of the disease. The patients died within a few years due to cardiac failure. Since then, three additional family members (of the second generation) have died from cardiac amyloidosis.

In recent years methods have been developed which makes pre-clinical diagnosis of the disease possible. Based on the analysis of frozen serum samples and formalin-fixed, paraffin embedded tissues, information on the carrier status of 33 living and 12 dead members of the family were available before the start of the present study.

Doppler echocardiography has been established as a suitable method to characterize and monitor the development of left ventricular diastolic dysfunction connected with amyloid cardiomyopathy. As novel therapeutic options, heart and liver transplantation can now be offered to patients with transthyretin related familial amyloid disease.

The diagnostic and therapeutic options provided seemed to justify a new approach to the previously described Danish family in order to pursue the following aims: (1) to identify carriers and non-carriers of the transthyretin methionine 111 mutation among the family members; (2) to compare clinical and echocardiographic findings in carriers and non-carriers in order to identify possible early stages of amyloid cardiomyopathy or to support the theory of a ‘late onset disease;’ (3) to permit the early identification of possible candidates for transplantation.

Subjects and methods

Subjects

This study was approved by the Regional Ethics Committee and confirmed approval was obtained from the Danish Central Ethics Committee. The study complies with the Declaration of Helsinki. At the end of 1992 the family numbered 125 living persons. Informed consent for inclusion was obtained from 73 adults and 26 children (from their parents). Six adults refused participation or were unavailable for investigation; 20 children were denied participation by their parents.

The clinical examination

Ninety-nine family members were examined clinically and genetically. Ninety-eight persons underwent echocardiographic examination. Medical history was taken and ordinary physical examination was made on all participants. Additionally, 12-lead ECG, chest X-ray in antero-posterior and lateral projections, and routine blood and urine analysis was taken. The blood analyses included haemoglobin, sodium, potassium and creatinine. Urine was tested for protein, blood and glucose.

The echocardiographic techniques

All of these echocardiographic examinations were performed by the same experienced echocardiographer, who was unaware of the subjects’ carrier status.

2-dimensional and M-mode echocardiography

Two-dimensional echocardiographic examinations comprised parasternal long-axis and multiple short-axis views and apical long-axis, four- and two-chamber views. Using 2-D guided M-mode echocardiography, standard measurements including left ventricular end-diastolic and end-systolic diameters, and ventricular septal and posterior wall thickness, were made according to the recommendations of the American Society of Echocardiography.

Doppler echocardiography

In pulsed wave recordings the lowest available wall filter was used. In both pulsed wave and continuous wave recordings the gain was reduced as much as possible in order to improve the definition of the velocity recording and thereby facilitate the assessment and calculation of the parameters used.

All Doppler measurements were performed averaging two end-expiratory sequences each consisting of two beats (i.e. a total of four beats). The end-expiratory phase of the respiration cycle was determined visually by inspection of the chest. During the recordings the horizontal sweep velocity was 100 mm/s.

Left ventricular inflow velocities

Left ventricular inflow velocities were obtained from an apical view using pulsed wave Doppler. Sample volume was positioned between the tips of the mitral leaflets. Isovolumetric relaxation time was obtained using continuous wave Doppler with the beam directed across the left ventricular outflow tract and the mitral valve. The beam was adjusted so that aortic valve closure and onset of forward transmitral flow were recorded simultaneously as described by Klein et al.

Doppler measurements (Fig. 1)

Peak flow velocities in early diastole (E) and in late (with atrial contraction) diastole (A) were measured.
from baseline to maximal velocity. In addition, the early to late peak velocity ratio (E/A) was calculated. The deceleration time was measured by extrapolating the deceleration of the mitral flow velocity curve from early peak flow velocity (E) to baseline. The isovolumetric relaxation time was measured as the time interval from aortic valve closure (AVC) to onset of forward transmitral flow (OFTF).

Definition of abnormal left ventricular filling pattern
An abnormal relaxation pattern was defined as a decreased E/A ratio (<1) and a prolonged isovolumetric relaxation time (>100 ms). At the same time one would expect a prolonged acceleration time, a prolonged deceleration time, as well as a decrease of E and an increase of A. A restrictive filling pattern was defined as a shortened deceleration time (<150 ms) and a mid-diastolic reversal of the transmitral flow. Simultaneously one would expect to find shortened isovolumetric relaxation time, deceleration time and acceleration time, combined with increased E and decreased A. These definitions are basically in agreement with those used by Klein et al.[13], although our criteria are more strict.

The genetic examination
For molecular detection of the transthyretin genotype, exon 4 of the transthyretin gene was amplified directly from washed blood cells of samples obtained from the family members, using the polymerase chain reaction[22]. The amplified DNA fragments were subjected to restriction enzyme digestion, and the digested fragments separated by agarose gel electrophoresis and visualised in UV-light[22].

Data analysis
Data were expressed as mean (1 SD). Differences between the two groups (carriers vs non-carriers) were compared by an unpaired t-test. Discrete variables were compared by confidence intervals. Values of $P \leq 0.05$ were considered statistically significant.

Results
Genetic results
Following the DNA analysis, 25 of the family members could be characterized as having heterozygous transthyretin methionine 111 genotype (Fig. 2). This group will now be referred to as ‘carriers’. The other 74 family members were not affected by the point mutation and this group is referred to as ‘non-carriers’.

The genetic status for all living or dead family members examined is given in Fig. 3. Carriers and non-carriers identified in previous studies are also included. The results of the genetic examination are consistent with earlier findings[6,9].
Clinical findings

The mean age of the carriers was 31.3 years (range 5–55) compared to 26.3 years for the non-carriers (range 7 months–64 years). None were found to have cardiomegaly or pulmonary congestion on chest X-ray and their medical histories were inconsistent with significant clinical heart disease. In addition, none of the carriers was found to have low voltage ECG or conduction disturbances. Two of the non-carriers were suffering from mild congestive heart failure secondary to ischaemic heart disease and hypertension, respectively.

Seven of the 25 carriers (aged 38–55 years) had undergone surgery for carpal tunnel syndrome 1–4 years prior to the investigation. In only one case had a synovial biopsy specimen been examined, and this was found to stain positive for Congo red indicating amyloid deposition. Two carriers aged 54 and 28, presenting with symptoms compatible to carpal tunnel syndrome, had not yet undergone surgery. None of the non-carriers in the corresponding age group showed any signs of carpal tunnel syndrome. Accordingly, nine of the carriers had carpal tunnel syndrome (36%, 99% confidence interval: 14.0–63.4%) in contrast to none of the non-carriers (0%, 99% confidence interval: 0–6.9%) (P<0.01).

Furthermore, five of the carriers, aged 45–55 years, had earlier been diagnosed as having various rheumatic conditions: three with familial chondrocalcinosis, one

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Table 1  Echocardiographic dimensions

<table>
<thead>
<tr>
<th>Age Group</th>
<th>LVDD (mm)</th>
<th>LVDD (mm·m⁻²)</th>
<th>IVS (mm)</th>
<th>IVS (mm·m⁻²)</th>
<th>PWT (mm)</th>
<th>PWT (mm·m⁻²)</th>
<th>LVMI (g·m⁻²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carriers aged ≤12 (n=2)</td>
<td>39.0 (5.7)</td>
<td>39.9 (1.5)*</td>
<td>7.0 (1.4)</td>
<td>7.1 (0.7)</td>
<td>6.0 (1.4)</td>
<td>6.1 (0.8)</td>
<td>74.7 (38.0)</td>
</tr>
<tr>
<td>Non carriers aged ≤12 (n=14)</td>
<td>36.1 (5.3)</td>
<td>54.5 (24.0)</td>
<td>6.4 (0.9)</td>
<td>9.5 (3.1)</td>
<td>5.9 (0.8)</td>
<td>8.8 (3.0)</td>
<td>78.9 (29.9)</td>
</tr>
<tr>
<td>Carriers aged &gt;12 (n=23)</td>
<td>48.4 (4.8)</td>
<td>27.5 (2.1)</td>
<td>9.6 (1.8)</td>
<td>5.5 (1.2)*</td>
<td>8.8 (1.4)</td>
<td>5.0 (0.9)*</td>
<td>100.8 (20.9)</td>
</tr>
<tr>
<td>Non carriers aged &gt;12 (n=58)</td>
<td>48.2 (4.1)</td>
<td>27.8 (2.8)</td>
<td>9.0 (1.6)</td>
<td>5.2 (0.9)</td>
<td>8.2 (1.2)</td>
<td>4.7 (0.7)</td>
<td>93.8 (27.6)</td>
</tr>
<tr>
<td>All subjects aged ≤12 (n=16)</td>
<td>36.5 (5.5)</td>
<td>52.7 (22.9)</td>
<td>6.5 (0.9)</td>
<td>9.2 (3.0)</td>
<td>5.9 (0.9)</td>
<td>8.5 (2.9)</td>
<td>78.4 (29.6)</td>
</tr>
<tr>
<td>All subjects aged &gt;12 (n=81)</td>
<td>48.2 (4.3)</td>
<td>27.7 (2.6)</td>
<td>9.1 (1.7)</td>
<td>5.2 (1.0)</td>
<td>8.4 (1.3)</td>
<td>4.8 (0.8)</td>
<td>78.4 (29.6)</td>
</tr>
</tbody>
</table>

LVDD=internal dimension of the left ventricle during diastole (mm)
LVDDI=LVDD index (mm·m⁻²)
IVS=interventricular septum (mm)
IVSI-IVS index (mm·m⁻²)
PWT=posterior wall thickness (mm)
PWTI=PWT index (mm·m⁻²)
LVMI=left ventricular mass index (g·m⁻²)
Values are presented as mean (1 SD), *P<0.05.

Table 2  Echocardiographic systolic and diastolic function variables

<table>
<thead>
<tr>
<th>Age Group</th>
<th>FS (%)</th>
<th>E (cm·s⁻¹)</th>
<th>A (cm·s⁻¹)</th>
<th>E/A</th>
<th>DT (ms)</th>
<th>IRT (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carriers aged ≤12 (n=2)</td>
<td>39.5 (3.3)</td>
<td>80.0 (7.1)</td>
<td>47.5 (24.7)*</td>
<td>1.9 (0.8)</td>
<td>130.0 (14.1)</td>
<td>65.0 (7.1)</td>
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<tr>
<td>Non carriers aged ≤12 (n=14)</td>
<td>40.1 (4.5)</td>
<td>90.2 (11.1)</td>
<td>42.4 (8.7)</td>
<td>2.2 (0.7)</td>
<td>128.6 (7.7)</td>
<td>52.9 (10.7)</td>
</tr>
<tr>
<td>Carriers aged &gt;12 (n=23)</td>
<td>36.6 (3.6)</td>
<td>80.2 (12.9)</td>
<td>50.8 (16.6)*</td>
<td>1.8 (0.7)</td>
<td>160.9 (37.8)</td>
<td>74.8 (20.0)</td>
</tr>
<tr>
<td>Non carriers aged &gt;12 (n=58)</td>
<td>37.8 (3.5)</td>
<td>76.3 (13.8)</td>
<td>44.0 (12.1)</td>
<td>1.9 (0.8)</td>
<td>146.2 (32.2)</td>
<td>67.6 (17.0)</td>
</tr>
<tr>
<td>All subjects aged ≤12 (n=16)</td>
<td>40.0 (4.3)</td>
<td>88.9 (11.0)</td>
<td>43.1 (10.5)</td>
<td>2.2 (0.7)</td>
<td>128.8 (8.1)</td>
<td>54.4 (10.9)</td>
</tr>
<tr>
<td>All subjects aged &gt;12 (n=81)</td>
<td>37.5 (3.6)</td>
<td>77.4 (13.6)</td>
<td>45.9 (13.7)</td>
<td>1.9 (0.8)</td>
<td>150.3 (34.2)</td>
<td>69.3 (18.0)</td>
</tr>
</tbody>
</table>

FS=shortening fraction of the left ventricle (%)
E=peak velocity of early rapid filling rate of the left ventricle (cm·s⁻¹)
A=peak velocity of late filling rate of the left ventricle (cm·s⁻¹)
E/A=no dimension
DT=deceleration time (ms)
IRT=ivsomular relaxation time (ms)
Values are presented as mean values (1 SD), *P<0.05.
with scleroderma and one with rheumatoid arthritis. One of the non-carriers had Reiters syndrome. No significant differences were found between the mean values for the results of the blood and urine sample analyses performed on carriers and non-carriers.

**Echocardiographic findings**

The results of the echocardiographic examination are shown in Tables 1, 2 and 3. From Table 1 it can be seen that carriers, with an age above 12 years (mean age 33·4 years) had significantly higher posterior wall thickness index \( P<0·05 \) and interventricular septum index \( P<0·05 \) compared with the non-carrier group of the same age (mean age 31·6 years). This finding is in accordance with the increasing wall thickness occurring during the course of the amyloid deposition. Also, carriers have significantly higher values for \( A \) than non-carriers \( P<0·05 \) (Table 2).

Of the 10 carriers above the age of 35, a total of eight persons presented with echocardiographic features compatible with an early to a more developed stage of cardiac amyloidosis (Table 3). Six of the individuals (age 35–55 years) were found to have a pattern of abnormal relaxation. Of these six persons, two had concomitantly increased wall thickness. One of these, a women aged 45 had a history of mild hypertension. Two persons (46–48 years) showed a fully developed restrictive pattern (Fig. 4(a), Table 3). They also displayed concomitantly increased wall thickness. Both were later referred to invasive cardiac investigation together with a female carrier aged 55, also showing an abnormal echocardiographic relaxation pattern (Fig. 4(b)). The invasive investigation confirmed the finding of abnormal left ventricular filling patterns and myocardial biopsies revealed the presence of amyloid by staining positive for anti-transthyretin. All these eight carriers had normal systolic function. In the remaining two carriers (aged 38 and 39 years) and in all carriers from the younger age group (<35 years), no echocardiographic abnormalities were found. Of the non-carriers, one individual (aged 58) had a reduced ejection fraction due to an earlier myocardial infarction located to the anterior wall. Two other 60-year-old persons with well-known hypertension showed increased wall thickness and abnormal relaxation, as would be expected when left ventricular hypertrophy is present.

The echocardiographic mean values for all non-carriers were within normal ranges\(^{23,24}\).

**Discussion**

Out of 99 family members the DNA analysis showed 25 to be carriers of the transthyretin methionine 111 mutation. None of the non-carriers had affected offspring. These findings confirmed the autosomal dominant mode of inheritance of the disease in this family. The penetrance is assumed to be 100%.

In spite of the fact that several of the carriers were more than 50 years old, none were found to exhibit significant clinical signs of congestive heart failure or arrhythmias at the time of the examination. However, the family data indicates that the expectancy for surviving after the age of 60 is low.

From Table 3 it appears that the earliest echocardiographic sign indicating amyloid cardiomyopathy is diastolic dysfunction appearing as an abnormal relaxation pattern. Comparison of the echocardiographic data revealed a significant increase in wall thickness and late peak flow velocity \( (A) \) in the carriers compared with the non-carriers.
In the original report on the Danish family, Fredericksen et al. [2] noticed the frequent occurrence of acroparaesthesias, however without employing the term ‘carpal tunnel syndrome’. The present study confirms the previous observations: out of the 10 carriers older than 35, eight had carpal tunnel syndrome. That this syndrome is part of the familial amyloid disease is evident from the finding of amyloid in a synovial tissue biopsy from one of the carriers. The two carriers over 35 years of age with normal echocardiographic findings had both been operated on for carpal tunnel syndrome. This suggests that carpal tunnel syndrome is the earliest clinical manifestation of amyloid disease associated to the Transthyretin Methionine 111 variant. Also, early amyloid deposition in the joints and skin may account for the presence of ‘chondrocalcinosis’ and other musculo-skeletal and dermal manifestations in five of the carriers.

The present result is consistent with the hypothesis that the inherited amyloid cardiomyopathy present in this Danish family is a late-onset disease with a rapid course when it first develops [20]. However, apparently the amyloid disease starts earlier in life than hitherto expected.

It is to be expected that future echocardiographic monitoring of the carriers now showing abnormal relaxation will reveal a gradually changing echocardiographic pattern towards restrictive cardiomyopathy, most likely through an intermediate pattern of pseudo-normalization, as described in cases with light chain amyloidosis [10]. This will probably happen before a deterioration in their clinical condition sets in.

During recent years several patients with other types of transthyretin-related amyloid syndromes have been treated with heart- as well as liver transplantation [6,14-16,18,19,25]. The latter is theoretically a
curative treatment as the majority (98%) of transthyretin is produced by the liver. Heart transplantation is a symptomatic treatment, and it is as yet unclear for how long it may prolong or improve the life of the patients. The present experience suggests that every carrier of the Danish family will eventually develop amyloid cardiomyopathy. On the other hand, our present knowledge does not allow us to rule out the possibility of a more systemic development of the amyloid disease in a later stage, thus involving other organs. The presence of manifest restrictive cardiomyopathy in a transthyretin methionine 111 carrier will most certainly merit a cardiac allotransplantation, but an additional liver graft will then be necessary, to prevent future development of the disease.

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