Cardiac amyloidosis: a review and report of a new transthyretin (prealbumin) variant

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Summary

Cardiac amyloidosis is caused by amyloid deposits derived from different human plasma proteins. It can lead to cardiac conduction disturbances, restrictive cardiomyopathy, and low output heart failure. The heart is variably involved during the development of systemic amyloidosis and seems to be more frequently affected in immunoglobulin (primary) than in reactive (secondary) amyloidosis. Amyloid is common in the elderly. Isolated atrial amyloid, for which a major subunit is the atrial natriuretic peptide, seems to be three times more frequent than senile cardiac amyloid, which is derived from normal prealbumin (transthyretin). Like polynuropathy, cardiac amyloidosis is a prominent clinical feature of hereditary amyloidosis, namely of the autosomal dominant transthyretin (TTR) type. All 28 cases of TTR amyloidoses reported so far were heterozygotes for a single nucleotide change in the gene for TTR that resulted in amino acid substitutions in the mature protein. A new TTR genetic variant is reported in a German family where the index patient presented at the age of 63 with anginal pain and arrhythmia. Electrocardiography was suggestive of a pseudoinfarction pattern, and echocardiography and cardiac catheterisation showed signs of hypertrophic non-obstructive cardiomyopathy with increased ventricular filling pressures and a prominent "a" wave. Amyloid of the TTR type was identified by immunohistochemistry in the endomyocardial biopsy specimen. Hybrid isoelectric focusing established heterozygosity by showing normal TTR protein and an electrically neutral TTR variant differing from all known TTR variants so far. The patient died in an accident before investigations were complete. Electrophoretic analysis of the plasma from his first degree relatives (son, daughter, brother, and mother) identified the asymptomatic 22 year old son as an apparently heterozygous carrier of the mutant TTR protein. Comparative tryptic peptide mapping and sequencing showed that isoleucine at position 68 of the amino acid sequence was replaced by leucine.

Amyloidosis is the term used for a group of diseases characterised by tissue deposition of fibrillar proteins. These proteins bind Congo red and fluoresce in polarised light. Despite their common morphology these extracellularly accumulated proteins are chemically different and they can be classified according to the deposited protein that is identified by immunohistochemistry or biochemical analysis. The clinical symptoms are generally related to the chemical composition of the deposited proteins.

Immunoglobulin-type (primary) amyloidosis, which is also seen in plasma cell dyscrasia, is caused by the deposition of fibrils derived from immunoglobulin light chains. In individuals with chronic inflammatory disease reactive (or secondary) amyloidosis can develop whereas fibrils formed by a fragment of the acute phase reactant serum amyloid A are deposited. Several other proteins have been identified in amyloid deposits, the most recently reported being gelsolin. Genetic variants of the plasma protein prealbumin, now named transthyretin (TTR), cause autosomal dominant inherited forms of amyloidosis. This protein transports thyroxine and retinol and circulates in plasma as a tetramer of identical polypeptide chains of 127 amino acid residues. Most patients with autosomal dominant amyloidosis of the TTR-type present with peripheral ascending neuropathy (familial amyloidotic polyneuropathy) and cardiac amyloidosis.

Cardiac involvement is a major problem of immunoglobulin amyloidosis. Most commonly it leads to the ventricular arrhythmias and congestive heart failure that are common causes of death in those patients. Reactive amyloidosis results mainly in disorders of kidney, intestine, liver, and spleen: cardiac muscle is less likely to be affected. In contrast in elderly patients infiltration of the heart by amyloid is very common. Two senile forms of infiltration have been identified: (a) amyloid limited to the atria and mainly consisting of atrial natriuretic peptides which was found in nearly 95% of octogenarians and (b) TTR, which is deposited in both the atria and the ventricles.

Neither of the senile forms is usually recognised in life, though in rare cases they can cause cardiac death. Hereditary amyloidosis, caused by heterozygous TTR variants, can affect the heart as well as the nervous system. Table 1 (adapted from information supplied by Saraiva et al 7) lists the 28 TTR variants...
known so far and their clinical manifestations. We report on the clinical and pathological findings in a 63 year old man with cardiac amyloidosis and a hitherto unknown TTR variant.

Methods
As well as taking a history and performing a physical examination, we examined the propositus, his son, and his daughter by echocardiography, Holter ECG recording, chest x ray and laboratory tests. The propositus was also examined by gastroscopy, rectal biopsy, and cardiac catheterisation with cineangiography and left ventricle endomyocardial biopsy. Paraffin embedded sections of the heart muscle and rectum were examined histochemically with a panel of anti-amyloid antibodies directed against various amyloid fibril proteins (AA, A-kappa, A-lambda, AF, AB).13 14

Plasma from the patient and his relatives was examined by double one-dimensional electrophoresis in polyacrylamide gels followed by hybrid isoelectric focusing as described by Altland et al.15 modified to study the folding-unfolding reactions of TTR in the presence of urea. TTR concentrations were measured by the Behring nephelometric analysis.

Results
Patient
The propositus, a dentist, was admitted to hospital in 1988 at the age of 61 with arrhythmia and dyspnoea after bronchitis two months before. He also complained of stomach pain and dysaesthesia in both hands that became worse when he worked. There was a pansystolic murmur at the left sternal edge. The electrocardiogram showed left anterior bundle branch block with small R waves in leads II, III, aVF, and V1—V4. Echocardiography showed an increase in left atrium diameter (46 mm) and septal hypertrophy (27 mm). The Holter electrocardiogram showed arrhythmias of Lown class IVa. The exercise test, chest x ray, laboratory tests, and gastroscopy were normal. Examination did not show any neurological abnormality. No further diagnostic tests were performed and the patient was treated with propafenone.

A year later he was admitted to hospital with signs of congestive heart failure (exercise-induced dyspnoea and mild pretilial oedema). The size of the liver and spleen was normal and there was no ascites. Blood pressure was 100/70 mm Hg. Ultrasound examination of the abdomen was normal. No proteinuria was found. Laboratory investigations showed no evidence of haematological abnormality or infection. Chest x ray showed general enlargement of the heart. The electrocardiogram showed Q waves in leads II, III,
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Figure 2. TM echocardiogram of the propositus with cardiac amyloidosis showing a thickened interventricular septum (IVS) and left posterior wall (LVPW) compatible with hypertrophy and non-obstructive cardiomyopathy (LV, left ventricle).

avF, and V1—V3 (fig 1). Echocardiography showed considerable left ventricular hypertrophy with a normal left ventricular end diastolic diameter (43 mm), a dilated left atrium (diameter 50 mm), and a thickened interventricular septum (28 mm) and left posterior wall (31 mm), which were compatible with hypertrophic non-obstructive cardiomyopathy (fig 2). Doppler echocardiography showed mild mitral and tricuspid regurgitation. Scintigraphy showed evidence of ischaemia in the inferior, antero-septal, and antero-lateral areas of the myocardium but no scars. Cardiac catheterisation excluded coronary macroangiopathy, and cineangiography showed no focal dyskinesia. An ejection fraction of 69% confirmed that systolic left ventricular function was normal. The cardiac index (2-8 l/min/m²) was reduced by an increase in both right (12 mm Hg) and left ventricular end diastolic pressures (35 mm Hg). Pressure in the pulmonary artery was also increased (20 mm Hg). In addition, there was a prominent “a” wave, caused by atrial contraction. An endomyocardial biopsy specimen taken from the left ventricle contained amyloid stained by Congo red. Biopsy specimens of the rectal mucosa and submucosa did not stain positively for amyloid deposits. Polyneuropathy was diagnosed on the basis of the patient’s complaints of dysaesthesia in both hands and gastric pain. Further studies were planned but shortly afterwards the patient died in an accident abroad. No necropsy material could be obtained.

HISTOLOGICAL EXAMINATIONS
Examination of the endomyocardial biopsy specimen of the left ventricle showed extracellular amyloid stained by Congo red that fluoresced under polarised light. Immunoperoxidase staining with anti-AF was strongly positive for the presence of TTR-derived amyloid. The control reactions with AA, A-lambda, A-kappa, and AB were all negative.

PROTEIN ANALYSIS
Electrophoretic analysis showed that both normal TTR and a variant TTR were present in plasma from the propositus and his son. Plasma samples from all the available first degree relatives and the wife of the propositus were normal. The isoelectric point of the variant was indistinguishable from that of the normal TTR monomer, indicating an electrically neutral amino acid substitution in the variant. Other data, which will be published elsewhere, indicated that the conformational stability of the variant TTR monomer was reduced—as it was in other cases of amyloidosis associated with TTR variants (K Altland, unpublished observation).

FAMILY INVESTIGATION
The patient’s father died at the age of 56 in an accident. His mother, aged 87, is healthy. Electrophoresis of serum from the mother, from the patient’s 60 year old brother, and from the patient’s wife and daughter showed normal TTR patterns. The 22 year old son showed normal TTR and an altered TTR protein. Figure 3 shows the pedigree. All the TTR concentrations in serum samples from the family, including the son, (0-28—0-37 g/l) were within the normal range (table 2).

History, electrocardiogram at rest and exercise, chest x ray, echocardiography, laboratory tests, ophthalmological and neurological examination by electromyography and nerve conduction studies were normal in the patient’s daughter, who was examined as a control. The son who was a carrier of the mutant protein, had slightly reduced sensory nerve conduction velocity. He will be followed up prospectively to see whether polyneuropathy develops.

Discussion
Cardiac involvement in amyloid disease can result in four types of clinical signs. The most common is systolic dysfunction leading to

Table 2. Age and serum TTR in family members

<table>
<thead>
<tr>
<th>Family number</th>
<th>Date of birth</th>
<th>TTR in serum (g/l)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>I 1</td>
<td>15 March 1903</td>
<td>0.37</td>
</tr>
<tr>
<td>II 1</td>
<td>11 April 1930</td>
<td>0.28</td>
</tr>
<tr>
<td>II 2</td>
<td>7 Aug 1927</td>
<td>0.33</td>
</tr>
<tr>
<td>II 3</td>
<td>30 Dec 1930</td>
<td>0.33</td>
</tr>
<tr>
<td>III 1</td>
<td>21 March 1968</td>
<td>0.34</td>
</tr>
<tr>
<td>III 2</td>
<td>17 Nov 1969</td>
<td>0.34</td>
</tr>
</tbody>
</table>

*Control 0-1–0.4 g/l.
congestive heart failure. Secondly, signs of a restrictive cardiomyopathy can be prominent in our patient, and orthostatic hypotension can occur. The least common feature is abnormal cardiac impulse formation and conduction. All these signs may be variably expressed in one patient. The most striking signs in our patient were those of hypertrophic non-obstructive cardiomyopathy and arrhythmia, leading to exercise-induced dyspnoea. The following findings were typical of cardiac amyloidosis: the pseudoinfarction pattern with prominent Q waves in leads II, III, aVF, and V1–V3 and with normal contraction shown by echocardiography. Roberts and Waller reported this electrocardiographical finding in 83% of 54 patients with cardiac amyloidosis, but low voltage was not often seen. Amyloidosis does not necessarily cause the heart to become enlarged. Typically, echocardiography shows abnormal diastolic function owing to rigid walls. Both atria were dilated because of reduced ventricular filling. Hypertrophy of the interventricular septum was confirmed in our patient. Haemodynamic patterns in amyloidosis are not pathognomonic. Increased left and right ventricular filling pressure caused a prominent "a" wave (atrial wave) and reduced the cardiac index. Echocardiographic evidence of thickening of the left ventricle without adequate electrocardiographic signs of hypertrophy strongly suggests the diagnosis cardiac amyloidosis. The diagnosis in our patient was confirmed by endomyocardial biopsy. We excluded reactive AA amyloidosis because there were no signs of chronic infection or evidence of a haematological disorder in a bone marrow aspirate. Because we did not find amyloid deposits in other organs (rectal biopsy specimens) we suspected that our patient had focal senile cardiac amyloidosis characterised by ASC1 (amyloid senile cardiac I), and the immunohistochemical cross reactions, known to occur with ASC1 and AF, seemed to confirm this hypothesis. But dysaesthesia and gastric pain reported by the patient were compatible with an inherited form of amyloidosis, first described as familial amyloidotic polynearopathy. Subsequent, electrophoretic analysis of serum from the propositus and his relatives detected a TTR variant in both the patient and his son. Amino acid sequencing of the abnormal peptide in the variant showed that isoleucine was replaced by leucine at position 68. Sequencing of PCR amplified DNA from exon 3 of the TTR gene showed a thymine for adenine substitution in the first base of codon for isoleucine 68.

The 1990 guidelines for nomenclature and classification of amyloid and amyloidosis indicate the amino acid substitutions that account for the inherited amyloid protein (A for amyloid) variants by a three letter code of the replacement protein followed by the position of the substitution (for example, ATTR Met 30). The ATTR Leu 65 variant we report has not been described before. Reported ATTR variants are associated with various degrees of polyneuropathy, cardiomyopathy, kidney infiltration, ocular alterations, and age of onset. Three cases have been reported (ATTR Thr 45, Met 111, and Ile 122) with cardiomyopathy as the only organ manifestation (table 1). In 20 of the variants polyneuropathy was the leading feature (for example ATTR Met 30, Ile 33). The remaining eight variants were associated with cardiomyopathy causing death by congestive heart failure (for example ATTR Met 30, Ile 33). The remaining eight variants were associated with cardiomyopathy causing death by congestive heart failure (for example ATTR Met 30, Ile 33). The remaining eight variants were associated with cardiomyopathy causing death by congestive heart failure (for example ATTR Met 30, Ile 33). The remaining eight variants were associated with cardiomyopathy causing death by congestive heart failure (for example ATTR Met 30, Ile 33). The remaining eight variants were associated with cardiomyopathy causing death by congestive heart failure (for example ATTR Met 30, Ile 33). The remaining eight variants were associated with cardiomyopathy causing death by congestive heart failure (for example ATTR Met 30, Ile 33). The remaining eight variants were associated with cardiomyopathy causing death by congestive heart failure (for example ATTR Met 30, Ile 33). The remaining eight variants were associated with cardiomyopathy causing death by congestive heart failure (for example ATTR Met 30, Ile 33). The remaining eight variants were associated with cardiomyopathy causing death by congestive heart failure (for example ATTR Met 30, Ile 33). The remaining eight variants were associated with cardiomyopathy causing death by congestive heart failure (for example ATTR Met 30, Ile 33). The remaining eight variants were associated with cardiomyopathy causing death by congestive heart failure (for example ATTR Met 30, Ile 33). The remaining eight variants were associated with cardiomyopathy causing death by congestive heart failure (for example ATTR Met 30, Ile 33). The remaining eight variants were associated with cardiomyopathy causing death by congestive heart failure (for example ATTR Met 30, Ile 33). The remaining eight variants were associated with cardiomyopathy causing death by congestive heart failure (for example ATTR Met 30, Ile 33). The remaining eight variants were associated with cardiomyopathy causing death by congestive heart failure (for example ATTR Met 30, Ile 33). The remaining eight variants were associated with cardiomyopathy causing death by congestive heart failure (for example ATTR Met 30, Ile 33). The remaining eight variants were associated with cardiomyopathy causing death by congestive heart failure (for example ATTR Met 30, Ile 33). The remaining eight variants were associated with cardiomyopathy causing death by congestive heart failure (for example ATTR Met 30, Ile 33). The remaining eight variants were associated with cardiomyopathy causing death by congestive heart failure (for example ATTR Met 30, Ile 33). The remaining eight variants were associated with cardiomyopathy causing death by congestive heart failure (for example ATTR Met 30, Ile 33). The remaining eight variants were associated with cardiomyopathy causing death by congestive heart failure (for example ATTR Met 30, Ile 33). The remaining eight variants were associated with cardiomyopathy causing death by congestive heart failure (for example ATTR Met 30, Ile 33). The remaining eight variants were associated with cardiomyopathy causing death by congestive heart failure (for example ATTR Met 30, Ile 33). The remaining eight variants were associated with cardiomyopathy causing death by congestive heart failure (for example ATTR Met 30, Ile 33). The remaining eight variants were associated with cardiomyopathy causing death by congestive heart failure (for example ATTR Met 30, Ile 33). The remaining eight variants were associated with cardiomyopathy causing death by congestive heart failure (for example ATTR Met 30, Ile 33).
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