Letters to the Editor

A father and his son with systemic AL amyloidosis

In systemic AL amyloidosis, fibrils are derived from a monoclonal immunoglobulin light chain produced by a plasma cell clone in the bone marrow. AL amyloidosis may be associated with multiple myeloma but more commonly the plasma cell clone is not malignant. A monoclonal gammopathy of undetermined significance (MGUS) may have preceded the development of amyloidosis. AL amyloidosis is often recognized and diagnosed at a late stage thereby giving the patients an average 10-14 months survival if they do not respond to treatment. Even though it was one of the first types of amyloidosis biochemically characterized, still little is known about how the light chains cause disease, why they are deposited in certain organs and why there is an enormous variation in patients’ clinical symptoms and outcome.

There are several types of familial amyloidoses usually inherited dominantly. The most recognized one is familial amyloidotic polyneuropathy where a mutation in the transthyretin (TTR) gene leads to an amyloidogenic protein variant. AL amyloidosis has not been considered to be hereditary and to date no two identical amyloidogenic light chains have been identified.

Nevertheless, there are a few reports of cases of systemic AL amyloidosis in the same family. In none of
these cases was a biochemical characterization of the amyloid fibril protein performed. In this paper we describe a father and his son, both dying from systemic AL amyloidosis of λ type. The study was approved by the ethical committee of the Sahlgrenska University Hospital.

Case 1. The son. A 48-year old man who suffered from fatigue and impaired general condition for two years. He had signs of peripheral sensoric polyneuropathy. Cardiac biopsy revealed amyloid depositions, which were preliminarily believed to be of TTR origin. However, neither genetic nor protein analysis could confirm this. Instead, analysis of an abdominal fat tissue biopsy with Western blot showed reaction with antibodies against protein of AL λ type. Bone marrow analysis revealed no signs of myeloma but there was a small IgG M-component in the plasma. The patient died suddenly a month later, before start of treatment aiming at autologous stem cell transplantation. Autopsy showed systemic amyloidosis involving many organs, including pronounced cardiac deposits.

Case 2. The father. A 60-year old man suffered from cardiac insufficiency. He had an IgA MGUS, but bone marrow examination revealed no overt myeloma. Two months before death he underwent surgery due to gastric retention and a suspicion of gastric rupture. Histological examination of the gastric wall and peritoneal fat tissue revealed heavy amyloid deposition. At autopsy, a pronounced systemic amyloidosis was found with deposits in the heart, kidneys, liver, pancreas, adrenal glands and stomach. From both patients, blocks of heart tissues were used for further studies (Figure 1 A and B).

Characterization of amyloid in subcutaneous fat tissue from the son was performed by Western blot analysis developed in our laboratory. From the autopsy material of the son, we obtained a formalin-fixed, paraffin-embedded heart block. Congo red staining revealed that about 50% of the material was amyloid. This material was used for the microextraction method described by Kaplan et al. The N-terminus was deblocked with pyroaminoglutamate aminopeptidase (Takara; Otsu, Shiga, Japan) according to the manufacturer’s description.

From the father, only formalin-fixed and paraffin-embedded heart material was available for extraction and characterization as described. A Congo red stained section showed that approximately 75% of the material was amyloid. Sequence analyses were performed 477A Protein Sequence Analyzer connected to 120A PTH-analyzer from Applied Biosystems and with a Procise 494 protein sequenator (Applied Biosystems, Foster City, CA).

Western blot analysis of subcutaneous fat tissue revealed that the amyloid was of immunoglobulin lambda light chain origin (data not shown). Formalin-fixed and paraffin-embedded material was extracted and electro-spray mass spectrometry of four tryptic peptides obtained by reversed phase-high performance liquid chromatography (RP-HPLC) indicated AL protein of lambda type, most likely subgroup λ 2, (data not shown). Further characterization of the material with Edman degradation was successful for 24 cycles and revealed a lambda 2a amino acid sequence. There were three substitutions compared to the germ line sequence (Figure 2).

The 21 year old formalin-fixed and paraffin-embedded material was extracted for amyloid proteins. After the RP-HPLC, material from the major protein peak was analyzed by Edman degradation. The reaction was successful for 32 cycles and revealed a lambda 3a sequence. There were 6 amino acid substitutions compared with the germ line sequence (Figure 2).

Figure 1. Section of myocardium from the father (A) and the son (B). There is pronounced amyloid infiltration in both specimens. Congo red, polarized light with partially crossed polar s.

Figure 2. N-terminal amino acid sequences of the AL proteins of the son and the father compared with the germ line sequences.
We report systemic AL amyloidosis affecting a father and his son. A possible genetic predisposition for AL amyloidosis would be complex, since all light chains have different amino acid sequences, depending both on the existence of about 50 different light chain variable domain genes and on somatic mutations. The light chain is composed of three segments, the variable (V) domain which is attached to the constant (C) chain via the joining (J) segment. Most of the variation is found in the variable region and in the area around the joining of this domain to the constant part. The literature has documented that the light chain involved in amyloidosis usually consists of the N-terminal part of a monoclonal protein. On the other hand, there have been three cases described where the amyloidogenic protein was from the C-terminal part. A missense mutation leading to AL amyloidosis is theoretically possible if it occurs in the gene for the constant or joining segment. This could lead to an amyloidogenic constant or joining region and affect the aggregation propensity of the variable domains. In this scenario, our material should consist of several different variable domains combined with one specific pair of lambda J segment and constant domain. Instead, we found two different monoclonal variable regions. Therefore, other factors are probably involved in the pathogenesis of the disease and may be responsible for the inheritance.

In summary, we report a patient who originally was believed to suffer from familial TTR amyloidosis since his father had died from systemic amyloidosis two decades earlier. However, biochemical analysis of the fibril protein of both individuals showed AL amyloidosis. These patients underline the importance of a direct determination of the amyloid type in all individuals with systemic amyloidosis.

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References