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FAMILIAL AL-AMYLOIDOSIS IN THREE ITALIAN SIBLINGS

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ABSTRACT

Background and Methods. **Familial occurrence of immunoglobulin-related (AL) amyloidosis has occasionally been reported. In this work we describe the concomitance of systemic amyloidosis and monoclonal gammopathy (one case of Waldenström's macroglobulinemia and two cases without multiple myeloma or related diseases) in three Italian siblings, two males and one female.**

Results and Conclusions. **All of them showed a common pattern of polyneuropathy to different degrees; two presented a sicca syndrome and one also suffered from nephropathy. Two of them showed the same HLA typing with the same light chain type (k), but had different presenting symptoms. Polyneuropathy and a history of peptic disease in two cases was suggestive of type III familial amyloidotic polyneuropathy (FAP) occurring in the setting of a familial monoclonal component. However, immunohistochemical studies on different tissue specimens using anti-apolipoprotein A1 and anti-transthyretin antibodies were negative. Further screening of DNA samples for transthyretin (TTR) gene mutations was also negative. Clinical and laboratory investigations ruled out reactive or senile amyloidosis and immunohistochemical studies with anti-light chain antibodies on amyloidotic tissue specimens were positive. As a consequence, this family represents a new case of familial AL-amyloidosis.**

Key words: familial amyloidosis, AL-amyloidosis, Waldenström's macroglobulinemia, monoclonal gammopathies

Inherited forms of systemic amyloidosis are a heterogeneous group of clinical syndromes characterized by a varying degree of neuropathy, nephropathy and cardiomyopathy that are also known by the name of the organ predominantly affected.[1] In this group of disorders, apart from familial Mediterranean fever in which the fibrillar protein is derived from serum amyloid A (SAA), the fibrillar proteins derive from genetic variants of plasma transthyretin (TTR), apolipoprotein A-1 or gelsolin.[2-4] Recently, familial cases in which the amyloid fibrils were derived from lysozyme[5] or fibrinogen[6,7] gene mutations have also been described.

Immunoglobulin-related amyloidosis (AL) has not been recognized as having a genetic predisposition; however, familial occurrence of plasma cell dyscrasias is reported.[8-11] In fact, Gertz and coworkers[12] described three separate families with two members affected by plasma cell dyscrasias and AL. Moreover, two other families were later discovered.[13,14] In addition,[15] in our series of asymptomatic patients previously diagnosed as monoclonal gammopathies of undetermined significance (MGUS), a considerable portion (14%) showed Congo red positivity

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of periumbilical fat tissue aspirates (FTA).

In this study we describe a new familial case of AL in three Italian siblings, two of whom were affected by monoclonal gammopathy without multiple myeloma (MM) or related diseases and one by Waldenström's macroglobulinemia (WM).

Materials and Methods

All three patients underwent the routine tests for assaying serum and/or urine monoclonal component as well as bone marrow aspirate, bone marrow biopsy (cases 1 and 2), bone X-ray, abdominal and cardiac echotomography. A full eye examination (including lacrimation tests with corneal staining with fluorescein and evoked potentials) was performed together with a detailed electroneurographic study on peripheral motor and sensory nerves (ulnar, posterior tibial and peroneal for motor nerves; ulnar, median and sural for sensory nerves).

In cases 1 and 2 a diagnosis of amyloidosis was established histologically on different tissue specimens on the basis of birefringence after Congo red staining and confirmed by electron microscopy on fat tissue aspirate (FTA). In case 3 the histological diagnosis was established by FTA and confirmed by autopsy on cardiac tissue. Tissue specimens were also tested with anti-light chain antibodies and with polyclonal anti-transthyretin (DAKO, Copenhagen, Denmark) and anti-apolipoprotein A-1 (Boehringer, Mannheim, GER) antibodies using the immunohistochemical PAP method. A search for TTR gene mutations was performed by Dr. Maria J. Saraiva of *Centro de Estudos de Paramiloidose* (Porto, Portugal) according to Torres *et al.*[16] Finally, karyotype, surface marker assessment on peripheral lymphocytes, and HLA typing (this last in 5 members of the family) were carried out according to standard procedures.

Results

Case #1 (T.O.)

A 60-year-old man was first admitted to the hospital because of dyspnea. Past medical history revealed gastroresection at the age of 45 because of peptic disease. An IgMk MGUS had been diagnosed 9 years earlier. Clinical examination showed bilateral pleural effusions, generalized lymphadenopathy and hepatosplenomegaly. Serum IgMk monoclonal protein (30.3 g/L) was detected together with urinary k light chain (0.4 g/24 hours). Bone marrow biopsy was typical for WM. Lymph node biopsy showed a poorly differentiated diffuse lymphocytic lymphoma. The immunologic characteristics of pleural effusion lymphocytes were not evaluated. After 10 months of follow-up a partial remission was obtained with different sequential therapeutic schedules: BACOP, etoposide + prednisone (P), chlorambucil + P. Serum IgM decreased (14 g/L) and only slight organomegaly with right pleural effusion still remained. Bone marrow aspirate was normal. A pleural biopsy showed chronic sclerogenic pachypleuritis, but the pleura, abdominal fat tissue and previously biopsied lymph node were found to be positive for amyloidosis. An electroneurographic investigation revealed bilateral polyneuropathy, predominantly axonal, in both the upper and lower limbs. Eye examination was normal and no renal or cardiac involvement was present. In venous blood samples the total number of T cells ($CD3^+$) and CD4, CD8 and NK cell subsets was normal.

Karyotype examination showed 16% of metaphases with structural rearrangements already reported in WM.[17]

The patient was then started with chlorambucil+P and dimethyl sulphoxide (DMSO) 20% solution (0.4 g/kg/d p.o.). After 16 months of this treatment,laboratory tests and chest X-ray were almost the same and his general condition was relatively good. Three months later the patient died from bronchial pneumonia. Autopsy confirmed systemic amyloidosis.

Case #2 (T.A.)

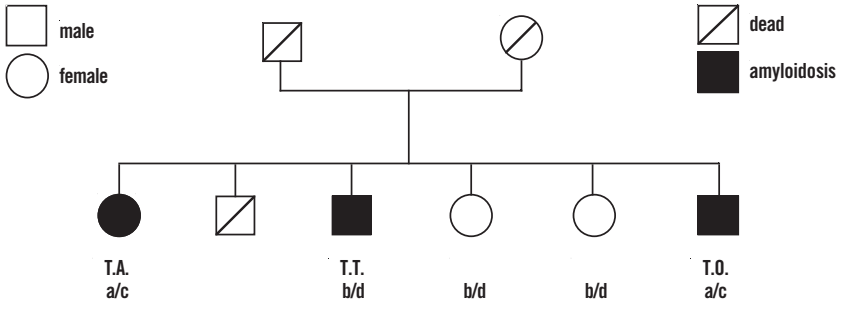
T.O.'s sister, a 74-year-old woman, was first admitted to the hospital because of a nephrotic syndrome, sicca syndrome and orthostatic hypotension. Her past medical history revealed gastroresection at the age of 28 because of a peptic ulcer.

A very small serum monoclonal component, IgGk, was present together with 0.3 g/24 hours of Bence Jones (BJ) protein excretion. Bone marrow aspirate contained 2% plasma cells. Neither osteolytic lesions nor renal or cardiac failure were present. Amyloid infiltration was demonstrated in abdominal fat, bone marrow and buccal mucosa. It is noteworthy that the serum IgG k protein had already been observed 10 years earlier. Electroneurographic study showed bilateral sensorimotor polyneuropathy in the lower limbs. A Holter registration and cardiac echotomography were normal. Urinary protein excretion ranged between 4-6 g/24 hours. In venous blood samples the total number of T cells ($CD3^+$) and CD4, CD8 and NK cell subsets was normal, as was the karyotype. After 20 months of treatment with melphalan and P, BJ excretion disappeared and the sicca syndrome, orthostatic hypotension and neurological symptoms improved. By contrast, the nephrotic syndrome remained unchanged and mild renal failure developed. Five years later the patient showed hepatomegaly with abnormalities on liver function tests, and a liver ultrasound examination revealed a dishomogeneous structure resembling hepatic cirrhosis. Markers for hepatitis were negative. Echocardiographic features were not diagnostic because of a left branch block. She is still alive seven years after the diagnosis of amyloidosis.

Case #3 (T.T.)

T.O.'s brother was a 70-year-old man who had been carrying an IgGλ/MGUS for 13 years. Since his brother and sister were already diagnosed as having systemic amyloidosis, FTA was performed and a diagnosis of amyloidosis was ascertained. The serum monoclonal component had always been stable (14 g/L) with no BJ excretion. Routine laboratory tests were within the normal range and bone marrow aspirate showed 2% plasma cells. Osteolytic lesions and renal involvement were not evident. Cardiac echotomography was normal. Unexpectedly, sicca conjunctivitis and slight sensorimotor polyneuropathy were detected during clinical investigations. In venous blood samples the total number of T cells ($CD3^+$) and CD4, CD8 and NK cell subsets were normal. Karyotype was normal, while HLA typing showed different haplotypes from the others, who shared identical HLA typing (Figure 1). The patient never received any treatment and remained subjectively asymptomatic for amyloidosis for the next 47 months, when he died of lung cancer. Autopsy demonstrated amyloid involvement of the myocardium.

To ensure that these patients did not have familial amyloidotic polyneuropathy (FAP), DNA samples of case 2 were studied for a large panel of TTR gene mutations (Ser 6, Met 30,



The reconstructed parental haplotypes are:

a) A1 Cw7 B8 Bw6 DR7 DQw2
b) A30/31 B13 Bw4 DR5 DQw3
c) A30/31 B52 Bw4 DQw1
d) A1 B35 Bw6 DR7 DQw2

Figure 1. Family pedigree, genotypes and reconstructed parental haplotypes.

ASN 90, Met 119, Ile 122). The results were negative, making the presence of TTR mutations unlikely. Furthermore, TTR staining of amyloid deposits for immunohistochemical studies (lymph node and pleura of case #1, bone marrow and abdominal fat tissue of case #2) was negative. Immunohistochemical studies with anti-apolipoprotein A-1 (pleura of case #1, abdominal fat tissue of case #2) also gave a negative result. On the other hand, immunohistochemical study with light chain antibodies yielded the expected positive result.

The patients' genealogical tree and genotypes are reported in Figure 1.

The remaining two sisters, with a history of peptic disease and breast cancer, respectively, were also studied for plasma cell dyscrasias and amyloid in FTA, with negative results. Another brother died at 50 of liver cancer, and the mother died at 74 of gastric carcinoma.

The father, who suffered from a duodenal ulcer, died at 69 from myocardial infarction. Our patients and the sister with a history of peptic disease had no progeny. Other relatives were not investigated.

Discussion

All three siblings shared a common pattern of polyneuropathy that was associated in two of them with sicca keratoconjunctivitis and with a nephrotic syndrome in the other. Differential diagnosis in these cases was centered around either AL or a type of FAP occurring on a background of familial plasma cell dyscrasia. Although renal involvement and sicca syndrome have been reported in certain forms of FAP (type I and III) and a history of peptic disease may be part of type III FAP,[18-20] the different clinical picture presented by our patients scarcely accounted for the same dominant trait.

FAP was ruled out by immunohistochemical analysis of different tissues, which failed to detect deposits of TTR or apolipoprotein A-1, and by the study of TTR gene mutations. Furthermore, none of the siblings suffered from a chronic inflammatory disease, which might account for reactive systemic amyloidosis, or from symptomatic cardiac involvement, which could suggest a senile systemic form.

On the other hand, familial cases of AL have been reported.[12] In addition, monoclonal type light chains were demonstrated in Congo red-positive tissues from our patients, but the probability of a random occurrence of AL in all our siblings affected by monoclonal gammopathy seems extremely remote.

The molecular basis for a familial occurrence of light-chain type amyloidosis is difficult to envisage. It is possible that a genetically-restricted abnormal response to a certain antigen involves amyloidogenic light chain variable regions.

No variation in the frequency of some HLA locus antigens is reported in AL[24] and tissue typing of our cases was not conclusive. Karyotype and surface marker expression on lymphoid cells were not significant, but the possibility of a genetic predisposition is not excluded.

In conclusion, these three patients represent a new case of familial AL. Routine investigations in the future for amyloidosis in patients with a familial occurrence of monoclonal gammopathy will better define the prevalence of these familial forms and possibly the underlying genetic abnormalities.

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