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Case Report

Late onset cardiomyopathy as presenting sign of ATTR A45G amyloidosis caused by a novel *TTR* mutation (p.A65G)

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ABSTRACT

Objective: The clinical description of a novel *TTR* gene mutation characterized by a late onset amyloid cardiomyopathy.
Methods and Results: A 78-year-old man of Dutch origin with recent surgery for bilateral carpal tunnel syndrome (CTS) was admitted to our hospital because of heart failure with preserved ejection fraction (55%). Cardiac ultrasound showed thickened biventricular walls, and cardiac magnetic resonance imaging also showed late gadolinium enhancement. Early signs of a polyneuropathy were found by neurophysiological testing. A few months later, his 72-year-old sister was admitted to an affiliated hospital because of heart failure caused by a restrictive cardiomyopathy. In both patients, a subcutaneous abdominal fat aspirate was stained with Congo red and DNA was analyzed by direct sequencing of exons 1 to 4 of the transthyretin (*TTR*) gene. Both fat aspirates revealed transthyretin-derived (ATTR) amyloid. ^{99m}Tc-diphosphonate scintigraphy further confirmed cardiac ATTR amyloidosis in the male patient. DNA analysis of both patients showed a novel *TTR* mutation c.194C > G that encodes for the gene product *TTR* (p.A65G) ending up as the mature protein TTR A45G. The 56-year-old daughter of the male patient had the same *TTR* mutation. A full diagnostic workup did not reveal any signs of amyloidosis yet.
Conclusions: A novel amyloidogenic *TTR* mutation was found in a Dutch family. The clinical presentation of ATTR A45G amyloidosis in the affected family members was heart failure due to a late-onset cardiomyopathy. The systemic nature of this disease was reflected by bilateral CTS and by early signs of a polyneuropathy in the index patient.

1. Introduction

The etiology of hereditary transthyretin-derived (ATTR) amyloidosis is the deposition of amyloid fibrils in the extracellular matrix due to mutations of the gene encoding transthyretin (*TTR*). More than 110 *TTR* mutations have been associated with hereditary ATTR amyloidosis [1,2]. Disease presentation, severity, and clinical course of ATTR amyloidosis are variable. Usually the disease starts with the clinical picture of a polyneuropathy accompanied by autonomic neuropathy, but sometimes, a cardiomyopathy is the presenting manifestation. In addition, albeit less frequent, other organs can be affected leading to carpal tunnel syndrome (CTS), vitreous opacities, or loss of renal function [1–3]. Clinical course and disease severity vary among different mutations [4]. Despite an initial monosymptomatic presentation, other organ involvement usually develops during disease progression. In the current report, we describe a Dutch family with a novel *TTR* mutation with late-onset cardiomyopathy, that is, not symptomatic before the sixth decade [5]. Although cardiac presentation was the prominent feature, a recent history of bilateral CTS and preclinical involvement of the peripheral nervous system were recognized in one of the two cases reflecting the systemic nature of the disease.

2. Patients and methods

A 78-year-old man and his 72-year-old sister, both known with atrial fibrillation (AF), presented with symptoms of heart failure at a hospital a couple of months after each other. Amyloidosis was considered a possible cause; consequently, a subcutaneous abdominal fat tissue aspirate was obtained that showed the presence of amyloid in both cases,

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and subsequently, both cases were referred to our Amyloidosis Center of Expertise. A 56-year-old daughter of the male patient was referred later for clinical investigation.

Ample (more than 100 mg) subcutaneous abdominal fat tissue was aspirated using a 16-Gauge needle. Amyloid was extracted in 6-M guanidine hydrochloride and further analyzed as described [6]. The TTR concentration in fat tissue of the extracted amyloid was assessed immunochemically using an indirect enzyme-linked immunosorbent assay [7] and also chemically by proteomics using a selected reaction-monitoring-mass spectrometry assay (SRM-MS; kindly provided by Paula Picotti and Paul Boersema, Zürich, Switzerland). The amount of Congo red-stained birefringent material was assessed semiquantitatively [8].

DNA was analyzed in all cases by direct sequencing of exons 1 to 4 of the *TTR* gene. The primary translation product is 20 amino acids longer than the mature TTR protein because the signal peptide and propeptides are included. This leads to amino acid position numbers of the translation product that are 20 higher than those of the corresponding mature protein [2].

A full clinical investigation to detect other organs affected by amyloid was offered to all patients. Due to the observational nature of this study, according to Dutch law, informed consent and permission of the local institutional review board were not required.

3. Results

3.1. Case 1

A 78-year-old man, known with AF for the last 2 years, presented with symptoms of heart failure at our hospital. Plasma levels of NT-proBNP and hs-Troponin T were 1877 ng/l (N<125) and 48 ng/l (N<14), respectively. One year before, he suffered from CTS in both hands, which was successfully treated by surgery. His electrocardiogram showed AF, low-voltage QRS complexes, and a left anterior fascicular block. A 24-h rhythm monitoring did not reveal other arrhythmias. Echocardiography showed concentric left ventricular wall hypertrophy and a left ventricular ejection fraction (EF) of 55%. Hypertrophy of the right ventricular wall (7 mm) was also observed. A multigated acquisition scan showed a left ventricular EF>65% and a right ventricular EF of 44%. Cardiac magnetic resonance (CMR) imaging showed late gadolinium enhancement and a thickened interventricular septum (18 mm). Based on these clinical findings, amyloidosis was considered to be present. Easily accessible subcutaneous abdominal fat tissue was aspirated as screening biopsy for amyloidosis. The tissue was stained with Congo red, which indeed showed the presence (abundant, visual grade 4+) of amyloid (Fig. 1) [8]. No signs of immunoglobulin free light chain overproduction (serum kappa free light chain 13.1 mg/l (N<20) and lambda free light chain 13.0 mg/l (N<32)) nor other signs of a plasma cell dyscrasia were found in serum, urine, or bone marrow. These findings made light chain-derived systemic AL amyloidosis very unlikely. The creatinine clearance was 74 ml/min, and no proteinuria was observed. ^{123}I–Serum Amyloid P component (SAP) scintigraphy showed some splenic uptake, whereas ^{99m}Tc-diphosphonate bone scintigraphy showed strongly increased (3+) cardiac uptake (Fig. 2) [9]. ^{123}I–metaiodobenzylguanidine (^{123}I–MIBG) scintigraphy showed an increased washout (34%) and low heart–mediastinum ratio (late uptake 1.16) indicative of cardiac denervation [10]. A fine needle subcutaneous abdominal fat aspirate yielded a TTR concentration of amyloid in fat of 658-ng/mg fat tissue (N<7.5 ng/mg) [6,7]. These findings indicated ATTR amyloidosis to be present. Wild-type ATTR amyloidosis was excluded because subsequent *TTR* gene analysis showed a novel mutation c.194C > G that encodes the primary translation product (p.A65G) and the mature protein TTR A45G [11]. SRM-MS proteomics confirmed the specific TTR mutation in the amyloid extracted from fat tissue. The patient was therefore diagnosed with hereditary ATTR A45G amyloidosis with prominent cardiac involvement and CTS. He had not noticed any symptoms of peripheral or autonomic neuropathy. Neurophysiological testing, however, revealed an early-stage length-dependent axonal sensory polyneuropathy in the feet of the patient. Quantitative sensory testing of the ankle also showed thin fiber neuropathy. He was treated with the TTR tetramer-stabilizing drug tafamidis for his polyneuropathy [12], and the clinical condition of both his heart and nerves seemed to stabilize during the next 2 years of follow-up.

3.2. Case 2

Family history of Case 1 revealed that the 72-year-old sister of the patient suffered from paroxysmal AF. She was admitted to an affiliated hospital because of heart failure caused by restrictive cardiomyopathy a few months after presentation of her brother. Upon further clinical investigation, she had no clear signs of CTS, polyneuropathy, or autonomic dysfunction. However, fat tissue analysis also showed amyloid (moderate, 3+) [8]. The TTR concentration of amyloid in fat was 122-ng/mg fat tissue (N<7.5 ng/mg) [6]. *TTR* gene analysis identified the same *TTR* mutation. Therefore, the sister was also diagnosed with hereditary ATTR A45G amyloidosis. She was not interested in further clinical investigations nor in disease monitoring during follow-up.

3.3. Case 3

The daughter of Case 1 presented at the age of 56 at the outpatient clinic with nonspecific complaints. *TTR* gene analysis showed the same genotype as her father and aunt. A full diagnostic work-up for amyloidosis was initiated: symptoms and signs of polyneuropathy and autonomic neuropathy were lacking, NT-proBNP was marginally elevated (145 ng/L) with a normal hs-Troponin T (10 ng/l), no cardiac uptake was visible on the ^{99m}Tc-diphosphonate bone scintigraphy, and fat

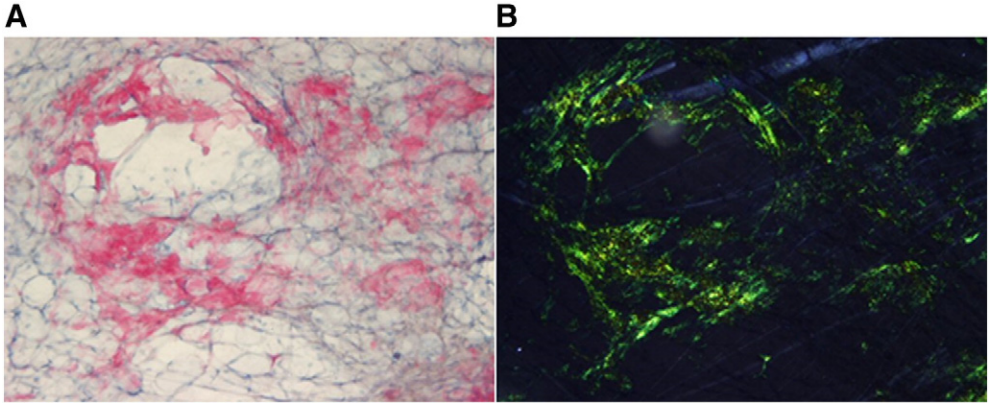

Fig. 1. Congo red-stained fine needle aspiration of abdominal fat tissue of Case 1. (A) Amyloid (4+) is red in bright light. (B) Amyloid is green in polarized light.