Exceptional Case

Hereditary renal amyloidosis caused by a heterozygous G654A gelsolin mutation: a report of two cases

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Abstract
Finnish-type familial amyloidosis (FAF) is a rare hereditary systemic amyloidosis that mainly exhibits cranial neuropathy. We describe a Japanese family with FAF manifested predominantly as renal amyloidosis. The proband was a 42-year-old woman with a 21-year history of proteinuria due to renal amyloidosis. Her mother was subsequently diagnosed with a similar disorder. After the first renal biopsy, both patients were followed up routinely for a period of 14 years. Genetic analysis of DNA samples revealed a heterozygous G654A gelsolin mutation. Severe renal involvement has not been reported previously in patients with FAF bearing a heterozygous gelsolin mutation.

Keywords: hereditary renal amyloidosis; gelsolin; Finnish-type familial amyloidosis (FAF); nephrotic syndrome

Introduction

Hereditary renal amyloidosis is a rare disease in which mutations of transthyretin, apolipoprotein AI, apolipoprotein AII, fibrinogen A α-chain and lysozyme have been identified in different pedigrees [1, 2]. More common forms of hereditary systemic amyloidosis can present as similar diseases [3]. Of these diseases, Finnish-type familial amyloidosis (FAF) is characterized by autosomal-dominant inheritance with predominant cranial involvement, particularly facial nerve involvement and corneal lattice dystrophy [4] and gelsolin-related amyloid deposition [5, 6]. Since its first description in 1969 [7], FAF has become established as an endemic disease in Finland, although a small number of individuals with this disease have also been reported in other European countries, the USA, Iran and Japan [8]. In addition, severe renal amyloidosis has been reported in a few FAF patients [9–11] homozygous for the G654A mutation of the gelsolin gene.

We report a Japanese mother and daughter with FAF who developed prominent nephropathy. Both patients were heterozygous for the G654A gelsolin mutation. We performed two renal biopsies 14 years apart and evaluated the natural history of the renal lesions.

Case report

Case 1

A 21-year-old woman exhibited proteinuria during a regular medical examination. She developed hypertension (158/92 mmHg), and a 24-h urine collection showed that proteinuria had increased to 890 mg/day. She was admitted to our hospital. A renal biopsy was performed when she was 28 years old. Light microscopy demonstrated an increase in mesangial cell number and expanded mesangial areas due to amorphous deposits. These deposits were positive for direct fast scarlet staining but negative for silver staining. Capillary basement membrane thickening was evident (Figure 1A). Small amounts of deposits were observed in the interstitium and the walls of the renal arterioles. Immunoglobulins, including λ and κ light chains and complement factors, were undetectable by immunofluorescence. Electron microscopy showed deposits comprising small non-branching fibrils 10 nm in diameter, consistent with amyloid fibril morphology. Although the capillaries and foot processes were substantially normal, we observed amyloid fibrils concentrated in the mesangial areas and along the lamina rara interna (the subendothelial layer closest to the endothelium) of the basement membrane (Figure 1B). After diagnosis, angiotensin receptor blocker therapy (losartan, 50 mg/day) was initiated to treat the proteinuria and hypertension and was continued thereafter.

At 42 years of age (14 years after the first renal biopsy), she was readmitted with massive proteinuria and oedema. She had normal skin and no evidence of bilateral ptosis, blepharochalasis, rough facial folds or droopy lower lip. Neurological examination revealed diminished movement of the orbicularis oris and a positive
ciliary sign. The other cranial nerves, limb muscular power and all tendon reflexes and nerve conduction velocities in the median and tibial nerves were normal. No autonomic dysfunction was observed. Slit lamp ophthalmological examination revealed a bilateral peripheral lattice line in the cornea without visual disturbances. Retinal examination was unremarkable with fluorescein angiography showing no evidence of peripheral retinal pigment clumping.

Serum creatinine and urea nitrogen concentrations were 0.7 mg/dL (61.9 µmol/L) and 5.9 g/dL (59 g/L), respectively. Serum total protein was 5.9 g/dL (59 g/L) and albumin concentration was 3.5 g/dL (35 g/L). The urinary sediment contained 10 white blood cells per high power field, with no red blood cells or granular casts. Her 24-h urine collection showed proteinuria of 4,400 mg/day and a creatinine clearance ($C_{Cr}$) of 109 mL/min. The selectivity index was 0.12. The second renal biopsy demonstrated that amyloid deposition, particularly along the peripheral capillaries, had increased in a distinct diffuse global manner (Figure 1C). Of note, electron microscopy revealed diffuse foot process effacement (Figure 1D). Immunohistochemical studies of amyloid deposits using antibodies against amyloid A protein and transthyretin were negative.

**Case 2**

The mother of Case 1 had a history of diabetes mellitus and acromegaly. She had been taking antihypertensive...
drugs. She was first examined at our hospital at 52 years of age and was shown subsequently to have moderate proteinuria (1700 mg in her 24-h urine collection). Renal function was almost normal [serum creatinine level 0.8 mg/dL (70.7 µmol/L)]. She had bilateral ptosis and blepharochalasis, bilateral facial weakness, rough facial folds and mild dysphagia. Limb muscular power was normal, although all the tendon reflexes were hypoactive. The light touch and vibratory sensations were impaired in the distal aspect of all limbs. Motor and sensory nerve conduction velocities in the bilateral median and tibial nerves were decreased. Slit lamp ophthalmological examination revealed bilateral corneal lattice dystrophy. The patient underwent a renal biopsy that revealed a similar pattern of amyloid deposits as observed with Case 1. Although her renal function remained stable over the following 10 years, we performed a second biopsy due to an increase in proteinuria. Many glomeruli were evidently enlarged, hyalinized and obsolescent in the specimen as a result of increased amyloid deposition.

The grandfather and younger sister of the patient also had proteinuria (Figure 2A). As hereditary renal amyloidosis was tentatively diagnosed, amyloid fibril protein identification and causative gene abnormality analysis was performed. Amyloid fibril protein was extracted from biopsied renal tissue, and the amino acid sequences were determined using liquid chromatography–ion trap mass spectrometry [12]. This showed that the partial sequences of the extracted protein were consistent with the internal sequences of gelsolin (148–161, 162–166 and 231–243) [13]. Immunohistochemical analysis of the amyloid fibril protein was conducted as follows. Deparaffinized sections were stained by the avidin–biotin peroxidase technique and a rabbit antiserum primary antibody for a purified low-molecular-weight subunit of FAF amyloid (anti-AGel) and applied (Figure 1F) [14]. All amyloid deposits in the biopsied samples from Cases 1 and 2 were immunolabelled specifically with this antibody (Figure 1E and F). DNA analysis was performed for Cases 1 and 2, indicating a heterozygous G654A mutation in the gelsolin gene.

Fig. 2. (A) Family pedigree showing an autosomal dominant inheritance. Closed ones represent affected patients. (B) Restriction fragment length polymorphism analysis of the gelsolin gene in Cases 1 and 2. A 1160-bp subsequence of the gelsolin gene was amplified using PCR, as described previously [24]. The PCR product was digested with the endonuclease Mun I (New England Biolabs, Beverly, MA, USA) and electrophoresed. After enzyme digestion, the 116-bp DNA produced two fragments of 90 and 26 bp, indicating that the mutated gene was heterozygous. (C) DNA sequencing of Cases 1 and 2 revealed a heterozygous G654A mutation in the gelsolin gene.

Few cases of FAF have been reported outside Finland. Of these, a Danish and a Czech family were shown to carry another mutation of gelsolin, G654T (D214Y) [17]. An American family carried a G580A (G194R) mutation, leading to renal amyloidosis with low eGFR and mild proteinuria [18]. In Japan, the six families with reported FAF
[19, 20] have the common G654A (D214N) mutation of the gelsolin gene. The present cases with FAF were not related to the previously identified Japanese FAF families.

Renal amyloid deposition with nephrotic syndrome has been described in four patients with FAF homozygous for the G654A gelsolin mutation (Table 1). Three of the four patients underwent renal transplantation for end-stage renal disease (ESRD) [11, 13]. In contrast, heterozygous patients frequently exhibited transient proteinuria [7, 9, 21, 22], which began at an advanced age and developed slowly. However, none of the patients had the nephrotic syndrome. Nevertheless, the heterozygous patients developed significant amyloid deposition in the renal glomeruli. Glomerular amyloid deposits were greater than those typically found in heterozygotes and similar to those found in homozygotes [10, 22].

The reason for the development of distinctive renal involvement is unknown in our heterozygous cases. It has been postulated that the difference in clinical manifestation between homozygotes and heterozygotes may depend on the ‘dosage effect’ of aberrant gelsolin fragments [9]. Furthermore, a high plasma concentration of amyloidogenic gelsolin has been shown to facilitate increased polymerization of amyloid fibrils in glomerular tissues [9]. Nephrotic syndrome with homozygous FAF usually develops by the 20 s, followed by rapid progression to ESRD in the 30 s (Table 1) [9–11]. In contrast, although proteinuria in Case 1 developed at 21 years, her renal function remained stable for >20 years. In addition, greater amyloid protein production in homozygotes causes early onset of typical symptoms such as lattice corneal dystrophy and upper facial paresis (Table 1). However, in Cases 1 and 2, these symptoms had a late onset, similar to that seen in heterozygotes reported previously [9, 23]. These observations are not consistent with the ‘dosage effect’ concept, and further studies are needed to clarify the underlying mechanisms.

Table 1. Summary of clinical, biological and histological data of familial amyloidosis Finnish-type patients

| Study | Patient no. | Age (years)/sex | Zygote/sequence | Renal proteinuria | Urin protein (g/day) | Cr (mg/dL) | Renal clinical course | Foot processes effacement |
|-------|-------------|-----------------|-----------------|-------------------|---------------------|-----------|---------------------|--------------------------|
| Meretoja et al. [7, 21] (1971) | 1 | 59/F | Hetero/G654A | 43 years | 7 | 0.9 | Normal | Glomerular + |
| Meretoja et al. [12] (1971) | 3 | 79/M | Hetero/G654A | 47 years | 7 | 0.9 | Normal | Glomerular + |
| Meretoja et al. [13] (1971) | 4 | 72/M | Hetero/G654A | 47 years | 7 | 0.9 | Normal | Glomerular + |
| Meretoja et al. [14] (1972) | 5 | 58/F | Hetero/G654A | 47 years | 7 | 0.9 | Normal | Glomerular + |
| Meretoja et al. [15] (1972) | 6 | 28/M | Homozygous | 28 years | 12 | 0.9 | Normal | Glomerular + |
| Meretoja et al. [9] (1992) | 8 | 39/F | Homo/G654A | 19 years | 13 | 0.9 | Normal | Glomerular + |
| Current Case 1a | 9 | 35/F | Homo/G654A | 13 years | 19 | 0.9 | Normal | Glomerular + |
| Current Case 2d | 10 | 28/F | Hetero/G654A | 21 years | 19 | 0.9 | Normal | Glomerular + |
| Current Case 1d | 11 | 42/F | Hetero/G654A | 21 years | 19 | 0.9 | Normal | Glomerular + |
| Current Case 2d | 12 | 62/F | Hetero/G654A | 21 years | 19 | 0.9 | Normal | Glomerular + |

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Conflict of interest statement. None declared.
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