Original Article

Hereditary renal amyloidosis associated with variant lysozyme in a large English family

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Abstract

Background. Two kindreds with hereditary systemic amyloidosis caused by the first two mutations to be described in the human lysozyme gene were discovered recently and study of the variant lysozyme has been powerfully informative about mechanisms of amyloid fibrillogenesis. However, the clinical manifestations in these families, additional members of which have lately been identified, have not previously been reported in detail.

Methods. The proband presented with proteinuria aged 50 and a family history of amyloidosis, and underwent renal biopsy, whole-body serum amyloid P component (SAP) scintigraphy, and sequencing of the lysozyme gene. Her family history and the phenotype of hereditary lysozyme amyloidosis were thoroughly documented and compared with the presentation and natural history of all other known patients with this condition.

Results. The proband belonged to an extended English family other members of which were known to have hereditary lysozyme amyloidosis. Those with amyloid in previous generations presented with renal involvement, frequently developed complications due to gastrointestinal amyloid, and died before age 60. All amyloid deposits were composed of lysozyme and complete concordance was established between amyloid and heterozygosity for a point mutation in the lysozyme gene, encoding the previously reported Asp67His substitution in the mature protein.

Conclusion. The phenotype, reported for the first time in this extended kindred, contrasts with that of an apparently unrelated family carrying the same mutation who presented with spontaneous hepatic haemorrhage and rupture, and with the manifestations in a family with the lysozyme Ile56Thr variant who presented with dermal petechiae before proceeding to fatal visceral amyloidosis. A remarkably wide spectrum of disease can be caused by the same amyloid fibril protein, although renal involvement predominates in all cases except those dying of hepatic rupture.

Key words: amyloid; amyloidosis; hereditary lysozyme amyloidosis; lysozyme

Introduction

Hereditary non-neuropathic systemic amyloidosis is a very rare autosomal dominant condition that causes serious morbidity and is usually fatal [1]. There is widespread deposition of amyloid in the tissues but the major clinical manifestations are related to renal, cardiac, and hepatic involvement. In different kindreds, the condition is caused by mutations in the genes encoding apolipoprotein A-I (apoA-I) [2–5], fibrinogen α-chain [6,7] and lysozyme [8].

Variant lysozyme was first discovered to be an amyloid fibril protein associated with hereditary systemic amyloidosis in 1993 [8]. Since the structure of human lysozyme was known to atomic resolution [9], and the folding has been extensively studied, this discovery provided a powerful model for understanding fibrillogenesis [10]. However, the clinical manifestations of hereditary lysozyme amyloidosis have not previously been reported in detail. We report here a kindred with hereditary lysozyme amyloidosis found to be part of a previously reported family, detailing the clinical manifestations for the first time. In addition, we report the correct amyloid type and phenotype in a family originally reported to have apoA-I amyloidosis [11] and review the clinical manifestations of hereditary lysozyme amyloidosis in all of three kindreds known to have this condition.

Subjects and methods

Proband and kindred

A 51-year-old Caucasian English woman with a family history of renal disease was found to have proteinuria during a routine examination when aged 50 years. She underwent extensive investigation to elucidate the cause of her proteinuria. The family history was reviewed in detail and several members of the kindred were similarly investigated.
Scintigraphy with iodinated serum amyloid P component

Serum amyloid P component (SAP) binds avidly and specifically to all types of amyloid fibrils and radiolabelled pure SAP is a quantitative in vivo probe for detecting and monitoring amyloid deposits [12]. SAP was labelled with 123I. Each of eight patients investigated received an intravenous injection of 100 MBq of 123I-SAP and underwent anterior and posterior whole-body gamma-camera scanning 24 h later.

Histology and immunohistochemistry

Formalin-fixed paraffin-embedded renal biopsy specimens were available from the proband (III8), her non-identical twin sister (III5), and relatives III7, III3, III4 and III6. Lung and gastrointestinal tissue from III7 were also available. No tissues were available to us from the proband’s older brother or mother but amyloid had previously been identified in their renal biopsies at other UK centres. Amyloid was identified by its pathognomonic green birefringence in 6-μm sections stained with Congo red and viewed in crossed polarized light [13]. Immunohistochemical staining for lysozyme in 6-μm tissue sections was performed exactly as previously described, using monospecific anti-lysozyme antiserum [8]. Specificity of staining was established by its complete abolition following absorption of the antibody with pure human lysozyme (Sigma). Sections were also stained with monospecific anti-serum to human apoA-I.

Direct DNA sequencing

DNA was extracted from the blood of the proband, her sister (III5), and individuals III7, IV1, IV2, III3, III4 and III8. Exon 2 of the lysozyme gene was amplified by polymerase chain reaction (PCR) and the nucleotide sequence determined as previously reported [8].

Results

Proband

The proband was clinically well at presentation, with blood pressure 140/80 and no other physical abnormality. Serum creatinine was 104 μmol/l, albumin 35 g/l, and 24-h urine protein 0.5 g; liver function tests were normal. A renal biopsy at presentation revealed amyloid. Electrocardiogram, echocardiogram, and nerve conduction studies were normal, and there was no evidence of peripheral or autonomic neuropathy. Proteinuria and renal function have remained stable in the year since presentation.

Kindred

A family tree of the kindred is shown in Figure 1. The proband has two daughters (IV6, IV7) aged 26 and 25, respectively, who are both clinically well. The proband’s elder brother (II3) developed renal failure, received a transplant aged 49, and died of congestive cardiac failure aged 57. Her non-identical twin sister (III6) presented aged 48 with anaemia for which no cause was found, but 2 years later her creatinine was 160 μmol/l and a renal biopsy revealed amyloid. Renal function has remained stable in the 1 year since then, but she developed hypochromic, microcytic anaemia associated with melaena. She unfortunately refused endoscopic investigation following a normal barium meal. She has six children between the ages of 22 and 36 years, all of whom are clinically well but have not been investigated further. The proband’s younger brother (III10) is clinically well at 48 years of age, but has not been investigated further. The proband’s mother (II11) presented with anaemia aged 56 and died within a year with end-stage renal failure due to biopsy-proven amyloidosis.

The proband’s maternal grandfather (I1), who died aged 38, was one of 12 siblings and had a cousin of the same name (I2) who was known to our Unit. She (I2) was married twice and children from both marriages were found to have amyloidosis. Although she herself was well until her sudden death from unknown causes aged 60 years, she must have been heterozygous for the lysozyme mutation. Of the affected children from the first marriage, II2 died aged 43 years with end-stage renal failure and II1 died with ‘uncharacterized carcinoma’ in his 40s. He had three children, all of whom had biopsy-proven amyloidosis. Patient III3 presented at the age of 33 years with hypertension and deteriorating renal function. He became dialysis dependent aged 41 years at which time there was miliary mottling on the chest radiograph and lung biopsy revealed amyloidosis. At the age of 49 years he received a renal transplant but died post-operatively of extensive bleeding from gastric amyloid. A cholecystectomy specimen from III1 contained amyloid; however, he and his three sons (IV1, IV2, IV3) are clinically well.

Patient III6 presented with hypertension and renal impairment aged 45 years [14]. She became dialysis dependent and died at the age of 48 years of sepsis. Of the affected children from I1’s second marriage, II1 died in renal failure aged 38 years, and II2 presented with hypertension and renal impairment aged 33 years and died 3 months later following rapid progression of his renal failure. His daughter, aged 43 years, remains clinically well, but his son (III6) presented to our Unit in 1981 aged 23 years, with renal impairment (serum creatinine 136 μmol/l) and dry, gritty eyes. We diagnosed hereditary non-neuropathic amyloidosis and reported the case, although we had not then identified the amyloid fibril protein [15]. His renal impairment has deteriorated remarkably slowly in a non-linear fashion and he remains dialysis independent 18 years later with a serum creatinine of 218 μmol/l, but has intermittent rectal bleeding, and retrosternal burning pain relieved by omeprazole.

Scintigraphy with iodinated serum amyloid P component

The scans (Fig. 2A) demonstrated extensive amyloid deposits in the kidneys, spleen, and liver of the proband. A similar distribution was seen in her non-identical twin sister (III6), III8 and III3. Interestingly,
III₆ has now been followed up for 10 years with serial SAP scintigraphs, during which time there has been no evidence of accumulation of amyloid deposits (Figure 2B). SAP scintigraphy in III₅, IV₄, IV₅ and III₁ was normal.

**Histology and immunohistochemistry**

Extensive amyloid deposits were found in the renal biopsy specimens of the proband, her sister (III₈), III₇, III₅, III₄ and III₆, and all stained specifically with antibodies to lysozyme although with variable intensity, possibly reflecting different fixation methods. The staining was completely abolished by prior absorption of the antiserum with an excess of pure human lysozyme (Sigma). Lysozyme is thus the major component of the amyloid fibrils. There was no staining with antibodies to apoA-I.

**Lysozyme gene mutation**

Sequencing of exon 2 of the lysozyme gene from the proband, III₈, III₉, and III₁ revealed that they were heterozygous for a single base substitution that altered the codon at position 67 of the mature protein from that for Asp to His (Fig. 3). The remainder of the gene in those individuals was identical to the published sequence of the human lysozyme gene [16]. Amplification of exon 2 of the lysozyme gene of III₉, IV₄, IV₅ and III₁ revealed wild-type sequence. The concordance of the mutation with clinical, histological and scintigraphic evidence of amyloid is shown in Table 1.

**Discussion**

The demonstration that the amyloid deposits in affected members of this kindred were composed of lysozyme and the complete concordance between presence of the lysozyme gene mutation and development of amyloidosis indicate that the mutation is the cause of disease in this family. We have previously reported part of this kindred [8,15], and it is one of only three known families with hereditary lysozyme amyloidosis. The others are an English family, which we have been unable to link to our present kindred, even though they carry the identical Asp67His lysozyme mutation [17], and a kindred with the Ile56Thr lysozyme mutation [8], previously incorrectly reported by another group to have apoA-I amyloidosis [11]. This was based upon immunohistochemical staining but omitting the critical specific antigen absorption control.

A renal presentation was almost universal among affected members of the present kindred although the age at which it was detected varied from 23 to 50 years. The renal impairment progressed at rates differing widely between members of the kindred. The serum creatinine in III₆, the proband in our original report [15], has only increased from 136 to 218 μmol/l over 18 years, whilst his father (II₆) had rapidly progressive renal failure and died within 3 months of presentation. Two members of the kindred have received renal transplants, with one post-operative death due to gastric bleeding and with the other graft functioning successfully for 8 years.

Despite massive hepatic and splenic amyloid deposits in all affected members of the kindred, none of them exhibited adverse clinical effects related to this organ.
Fig. 3. (A) Partial DNA sequence of the lysozyme gene of the proband. A substitution of guanidine by cytosine results in a change at codon 67 from GAT (aspartic acid) to CAT (histidine) (mutation arrowed). (B) This substitution introduces an NdeIII restriction enzyme site. Lane 1, uncut lysozyme exon 2 PCR product, lanes 2 and 3 are the normal and His67 variant PCR products respectively, digested with NdeIII.

subsequently developed gastrointestinal bleeding associated with macroscopic amyloid deposits throughout the gastrointestinal tract. It is noteworthy that the proband’s sister (III9) is currently undergoing investigation for gastrointestinal blood loss, that III2 died of bleeding from extensive gastric amyloid, and that III6 has had intermittent blood loss per rectum and retrosternal burning relieved by omeprazole. Indeed, all upper gastrointestinal biopsies have shown very extensive amyloid, and gross mucosal lesions have been evident at endoscopy in most symptomatic individuals.

The peripheral and autonomic nervous systems were apparently spared in all affected members of the present kindred. The proband, III9, III6, and III3 were carefully screened by electrocardiography and echocardiography, but showed no evidence of cardiac amyloid deposition. Similarly, the surviving member of the other kindred with Asp67His lysozyme amyloidosis does not have cardiac involvement or peripheral neuropathy. This contrasts with hereditary systemic amyloidosis due to variant transthyretin in which the heart and nerves are invariably involved, and renal apoA-I amyloidosis in which this occurs in some kindreds [5,18].

The only other known lysozyme variant, Ile56Thr, also caused systemic amyloidosis, but presented differently [11], with dermal petechiae in all affected subjects as well as major visceral amyloid. The proband of the single reported family had multiple amyloid nodules in a resected section of small bowel, in addition to amyloidotic hepatosplenomegaly and proteinuria. The

involvement. This differs sharply from the other family with Asp67His lysozyme amyloidosis, in whom the three affected members in different generation all presented with massive, and usually fatal, hepatic haemorrhage between the ages of 15 and 50 [17]. The reason for the contrasting phenotype in these kindreds is unknown. The single affected survivor from this family underwent emergency liver transplantation but has
Hereditary lysozyme amyloidosis

Table 1. Concordance of lysozyme mutation and amyloidosis

<table>
<thead>
<tr>
<th>Subject</th>
<th>Lysozyme genotype</th>
<th>Clinical phenotype</th>
<th>Histology</th>
<th>$^{12}$I-SAP scintigraphy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proband (IIIa)</td>
<td>His67/wt</td>
<td>Proteinuria</td>
<td>Renal+</td>
<td>+ +</td>
</tr>
<tr>
<td>Sister (IIIb)</td>
<td>His67/wt</td>
<td>Renal impairment</td>
<td>Renal+</td>
<td>+ +</td>
</tr>
<tr>
<td>Cousin (IIIc)</td>
<td>His67/wt</td>
<td>Renal impairment</td>
<td>Renal+</td>
<td>+ +</td>
</tr>
<tr>
<td>Cousin (IIId)</td>
<td>wt/wt</td>
<td>Well</td>
<td>No tissue</td>
<td>—</td>
</tr>
<tr>
<td>Cousin (IVa)</td>
<td>wt/wt</td>
<td>Well</td>
<td>No tissue</td>
<td>—</td>
</tr>
<tr>
<td>Cousin (IVb)</td>
<td>wt/wt</td>
<td>Well</td>
<td>No tissue</td>
<td>—</td>
</tr>
<tr>
<td>Cousin (IVc)</td>
<td>wt/wt</td>
<td>Well</td>
<td>No tissue</td>
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</tbody>
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His67, codon 67 CAT encoding histidine; wild-type codon 67 GAT encoding aspartate.

proband’s mother died of renal failure aged 52, 1 year after the discovery of proteinuria. The proband’s sister
developed melena and renal impairment aged 24, and
over 10 years progressed very gradually to end-stage
renal failure, from which she died. At autopsy the liver,
spleen and abdominal lymph nodes were all
enlarged with heavy amyloid deposition. An affected
cousin of the proband presented aged 27 with hepato-
megaly and left-sided abdominal pain requiring sple-
nectomy. He subsequently developed renal impairment and a
chronic cough, and the chest radiograph showed pro-
minent miliary mottling, similar to that in subject
IIIc of the present Asp67His kindred.

Lysozyme is the major secreted product of macro-
phages and is produced by several cell types in the
gastrointestinal tract (possibly accounting for the gas-
trointestinal amyloid involvement) as well as by hepa-
tocytes. Elimination of the supply of variant lysozyme
by orthotopic liver transplantation, analogous to that
used to treat hereditary transthyretin amyloidosis [19,20], is therefore not possible. The only therapy
available for lysozyme amyloidosis at present is sup-
portive management. It is interesting, however, that
our patient IIIc, who has had very slowly progressive
renal impairment and remains clinically well, has had
no evidence of accumulation of amyloid deposits in
10 years. Our extensive experience with serial SAP scin-
tigraphy in over 1000 patients with amyloidosis suggests
that many individuals with hereditary systemic amy-
loidosis attain a steady-state equilibrium between
deposition and mobilization. This mechanism may
explain the lack of progression of amyloid in our case
and several other cases of hereditary apolipoprotein A-I
amyloidosis in which the total body amyloid load was also
large [21].

In conclusion, the phenotype of hereditary lysozyme
amyloidosis is very variable, both within and between
families. It is generally associated with renal dysfunc-
tion, which in the present family is the usual mode of
presentation, but the age of onset and rate of progres-
sion of renal failure are highly variable. Marked hepatic
and splenic amyloidosis are universal but may have
few clinical effects, although all cases in one family
with the Asp67His variant presented with rupture of the
liver. Amyloid deposition in the gastrointestinal tract is common and may cause haemorrhage. In
marked contrast to hereditary amyloidosis caused by
other amyloidogetic variant proteins, the heart and
peripheral nerves are spared. Pulmonary amyloid, indi-
cated by radiographic miliary mottling, occurs occa-
sionally but has not been clinically important. The
prognosis is variable with some patients surviving for
20 years from diagnosis and others dying within a
year. To date, no known affected patient has survived
beyond the age of 60 years (see Note added in proof).

The mutation is penetrant in all subjects studied so
far in the present Asp67His lysozyme family. It is
therefore likely that affected descendants of this large
kindred may have presented with renal amyloidosis
and the diagnosis of amyloid been missed, or else its
type mis-identified, probably as AL (formerly known
as primary amyloidosis), possibly leading to inap-
propriate treatment with cytotoxic chemotherapy.
Thorough investigation of the family history is there-
fore essential in all patients presenting with renal
amyloid disease.

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**Note added in proof**

We have lately discovered subclinical lysozyme Asp67His amyloidosis in a 74-year-old lady. Her relationship to the present kindred is at present unknown.