Hereditary nephropathic systemic amyloidosis caused by a novel variant apolipoprotein A-I

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Hereditary nephropathic systemic amyloidosis caused by a novel variant apolipoprotein A-I. We report a family with autosomal-dominant hereditary systemic amyloidosis in three generations, presenting with renal involvement. Two members of the current generation received renal transplants for end-stage renal failure 16 and 18 years ago, and remain very well clinically despite massive visceral amyloidosis. Two other members of this generation, aged 32 and 47 years, have massive systemic amyloid but no clinical disability. Individuals known to be affected in previous generations died of renal failure in early adult life. Amyloid deposits in the proband, one of the transplanted individuals, were composed of apolipoprotein A-I (apoA-I), and among living family members there was complete concordance between amyloidosis and the presence of a novel 9 base pair in-frame deletion mutation in exon 4 of the apoA-I gene, causing a loss of residues Glu70Phe71Trp72. This predicts the acquisition of a single extra positive charge by mature apoA-I, and this variant was detected in the plasma of all carriers. All the previously reported amyloidogenic variants of apoA-I also carry an extra positive charge, indicating that this electrostatic change is likely to be relevant to the amyloidogenicity of apoA-I.

Hereditary non-neuropathic systemic amyloidosis is a very rare autosomal dominant condition that causes serious morbidity and, until recently has always been fatal [1]. There is widespread deposition of amyloid in the tissues but the major clinical problems are related to renal, cardiac and hepatic involvement. There is considerable variation in clinical phenotypic and patterns of organ damage between families and also sometimes within kindreds. Mutations encoding single residue substitutions in apoA-I [2–6], fibrinogen α-chain [7, 8] and lysozyme [9] are the cause of the condition in different kindreds, but there is no clear relationship between the amyloid fibril protein and the clinical manifestations. We recently reported the first case of hereditary amyloidosis caused by a deletion rather than a single point mutation, and this was also in the apoA-I gene [5]. Here we describe another kindred in which there is a different, previously unreported deletion mutation in apoA-I. It causes predominantly renal amyloidosis, and, despite extensive deposits elsewhere, patients whose lives have been greatly prolonged by renal transplantation have suffered remarkably little from other manifestations. Apart from their curious and interesting phenotypes, and the importance for management and counseling of the affected families, identification and characterization of these rare hereditary syndromes is of importance because of the information it may yield about the molecular pathology of amyloidosis in general.

METHODS

Proband

A 43-year-old woman (AA) developed mild hypertension, proteinuria and hepatosplenomegaly with normal liver function, at the age of 18 years. Renal function progressively declined and she commenced hemodialysis at the age of 23, undergoing successful renal transplantation from a live-related donor four years later. The renal graft continues to function satisfactorily 17 years later, although the serum creatinine has risen recently to 160 μmol/liter. A mild, persistent but non-progressive derangement of liver enzymes was noted several years after presentation, but synthetic liver function has remained normal. Routine blood biochemistry and lipid studies are otherwise normal, although the serum apoA-I is slightly low (66 mg/dl, normal range 100 to 170 mg/dl). Renal and liver biopsies at presentation had both shown amyloid, and a transplant biopsy within the first month had shown mild acute rejection but no recurrence of amyloid. The electrocardiogram has always been normal, and there has never been clinical evidence of peripheral or autonomic neuropathy. Over the last 13 years she has developed bilateral central scotomata with areas of retinal atrophy, thought to be consistent with an amyloid vasculopathy of the retinal or choroidal vessels. Visual acuity has declined to 6/30 in both eyes. Fluorescein angiography failed to demonstrate any leakage from retinal vessels.

Kindred

The mother (SJ) of the proband died from chronic renal failure in “middle age.” Autopsy demonstrated amyloid involvement of all major viscera. The maternal grandfather (TG) and his first cousin (OT) had both died young from unspecified renal disease. The sister (SP, age 47 years) of the proband has biopsy-proven amyloid of the liver and kidneys, having been investigated because...
Fig. 1. Whole body scintigraphic images after intravenous injection of $^{131}$I-human SAP. There is heavy amyloid deposition in the liver and spleen in the proband (AA), in her sister (SP) and in her two cousins (JF and BF). The faint thyroid images result from incomplete blockade of the gland with cold iodide, and the bladder, containing degradation products of the tracer, is also seen in some scans. Extravasation of injected tracer is seen in the left antecubital fossa of JF. The images of RF and KW show no amyloid and are normal.
Serum amyloid P component (SAP) binds avidly and specifically to all types of amyloid fibrils, and radiolabeled pure SAP is currently used in scintigraphy with iodinated serum amyloid P component (ECL, Amersham) for detection and monitoring of amyloidosis. Other sections were processed similarly using anti-human lysozyme as the primary antiserum. No further tissue was available for additional immunohistochemical studies.

**Detection of variant apoA-I**

Charge variant isoforms of apoA-I were sought, as previously described [3], by isoelectric focusing of delipidated whole plasma in urea-agarose gel followed by pressure blotting onto 0.45 µm nitro-cellulose membrane (Hybond ECL; Amersham International plc, Little Chalfont, Bucks, UK) for 90 minutes. Immunostaining with goat anti-human apoA-I antiserum (1:20,000, Medix Biotech) and horseradish peroxidase-labeled rabbit anti-goat IgG antibody (1:10,000, Dako Ltd) was detected by enhanced chemiluminescence (ECL, Amersham) according to the manufacturer’s Western blotting protocol.

**Plasma lipid studies**

Plasma apoA-I and apoB were estimated in fasting samples by Bayer immunoturbidimetric methods and triglyceride, cholesterol and low density lipoprotein (LDL) and high density lipoprotein (HDL) cholesterol by standard clinical chemistry methods.

**RESULTS**

**Serum amyloid P component scintigraphy**

The scans (Fig. 1) demonstrated amyloid deposits in the liver and spleen of the proband; the transplant kidney was not visualized. A similar distribution was seen in her sister (SP) and the signal from these viscera obscured any from her native kidneys. The scan of her brother (KW) was normal. Among the cousins, there were massive amyloid deposits in the liver of JF, with a lesser signal in the spleen and equivocal signal in the transplant kidney. The scan was normal in the brother RF, in accordance with the previous biopsy findings, but his clinically unaffected brother (BF) had massive visceral deposits. The family tree is shown in Figure 2.

**Histology and immunohistochemistry**

Amyloid deposits almost completely replaced normal liver tissue in the proband’s liver biopsy, and stained intensely with antiserum to apoA-I (Fig. 3). The staining was completely abolished by prior absorption of the antiserum with human high density lipoprotein (Fig. 3). ApoA-I is thus the major component of the amyloid fibrils. There was no staining with anti-lysozyme antiserum. The only amyloid present in the rectal biopsies was a tiny deposit in one (JF) and immunohistochemistry was not attempted.

**ApoA-I gene deletion mutation**

Sequencing of the apoA-I gene from the proband revealed that she was heterozygous for a mutation in exon 4 in which 9 small base pair insertions or deletions were found. These were not investigated further.

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**Fig. 2. Family tree of the affected kindred.** Solid symbols, individuals with confirmed amyloid; shaded symbols, individuals suspected of having had amyloid; open symbols, unaffected subjects.

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nucleotides had been deleted from the wild type sequence (Fig. 4). The mutation was readily detected by polyacrylamide gel analysis of the exon 4 PCR product (Fig. 5), and this test was used to screen family members. The in-frame deletion encoded loss of residues Glu70Phe71Trp72, predicting acquisition by the variant apoA-I molecule of one additional positive charge compared to the wild-type amino acid sequence. The concordance of the mutation with clinical, histological and scintigraphic evidence of amyloid is shown in Table 1.

Charge variant apoA-I in plasma

The plasma of carriers of the mutation contained the normal isoforms of wild type apoA-I and also variant apoA-I with a pI corresponding to one extra positive charge (Fig. 6, lanes 1, 2, 4 and 5). The variant was significantly less abundant than wild type apoA-I, especially in one individual (lane 2, BF). The variant was not detected in unaffected family members. Total apoA-I levels were below normal in all carriers of the mutant gene and were also slightly reduced in the two family members who did not carry the mutant gene (Table 2). ApoB and the other lipid parameters measured were generally within normal limits (Table 2).

DISCUSSION

The demonstration that the amyloid deposits in the proband were composed of apoA-I and the complete concordance between presence of the apoA-I gene mutation and development of amyloidosis indicate that the mutation is the cause of disease in this family. No tissue has become available for extraction and characterization of amyloid fibrils but, based on the previous published reports of hereditary apoA-I amyloidosis, it is likely that the fibrils consist of the N-terminal fragment of the apoA-I molecule [3, 5, 6, 12, 13].

The present, hitherto undescribed, mutation is the second amyloidogenic apoA-I gene deletion mutation to be reported. Remarkably it shares with the first one [6] and the three amyloidogenic apoA-I point mutations [5], the effect of encoding a single additional positive charge in the mature apoA-I protein sequence.

Wild type apoA-I forms senile amyloid in the pulmonary vasculature of dogs [14] and in aortic atheroma plaques in humans [15], but it is notable that all the known hereditary apoA-I amyloid variants carry an extra positive charge on the N-terminal region of the molecule. This is unlikely to be a coincidence. However, although we have recently discussed possible mechanisms elsewhere [5, 6], we still do not know the basis for the amyloidogenicity of these variants.

The phenotype of the systemic amyloidosis in the present family is interesting both for its differences from other hereditary apoA-I

Table 1. Concordance of apoA-I mutation and amyloidosis

<table>
<thead>
<tr>
<th>Subject (age)</th>
<th>ApoA-I genotype</th>
<th>Clinical phenotype</th>
<th>Histology</th>
<th>$^{131}$I-SAP scan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proband (AA)</td>
<td>del/wt</td>
<td>renal transplant</td>
<td>liver + rectum – rectum –</td>
<td>++</td>
</tr>
<tr>
<td>(43) Brother (KW)</td>
<td>wt/wt</td>
<td>retinopathy</td>
<td>rectum – rectum –</td>
<td>–</td>
</tr>
<tr>
<td>(43) Sister (SP)</td>
<td>del/wt</td>
<td>hepatomegaly</td>
<td>rectum –</td>
<td>++</td>
</tr>
<tr>
<td>(47) Cousin (JF)</td>
<td>del/wt</td>
<td>renal transplant</td>
<td>rectum +</td>
<td>–</td>
</tr>
<tr>
<td>(41) Cousin (RF)</td>
<td>wt/wt</td>
<td>retinopathy</td>
<td>rectum –</td>
<td>–</td>
</tr>
<tr>
<td>(37) Cousin (BF)</td>
<td>del/wt</td>
<td>well</td>
<td>rectum –</td>
<td>++</td>
</tr>
<tr>
<td>(32) Cousin (BF)</td>
<td>wt/wt</td>
<td>well</td>
<td>rectum –</td>
<td>–</td>
</tr>
</tbody>
</table>

Fig. 3. Liver biopsy of the proband (AA). Serial sections stained with (A) Congo red, showing birefringence (streaky white and dark areas) of the amyloid deposits in polarized light; (B) antiserum to apoA-I, showing uptake on the amyloid deposits; (C) antiserum to apoA-I absorbed before use with high density lipoprotein, showing specific abolition of the amyloid staining.

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amyloidoses and for its remarkably specific renal expression at the 
clinical level. Despite massive hepatic and splenic deposits no 
adverse clinical effects have been seen, even after greatly pro-
longed survival provided by renal transplantation. This differs 
sharply from the fatal hepatic amyloidosis with little or no 
clinical renal involvement of the Spanish apoA-I deletion/
insertion mutation [6]. Also, the heart is apparently spared in the 
present family, in contrast to some individuals with the apoA-I 
Trp50Arg variant [5], and yet the renal effects are much more 
aggressive than those seen in one of the families with the apoA-I 
Gly26Arg variant [4]. The very slow tempo of amyloid deposition 
in the transplanted kidneys is also of interest.

Overall the present family confirms and extends the general 
finding that in hereditary systemic amyloidosis there is only a 
tenuous connection between the amyloid fibril protein, the under-
lying mutation within it and the clinical expression of the pheno-
type. This is often true even within families, as demonstrated here 
where one affected individual (BF) with massive visceral deposits 
is clinically normal and still has normal renal function, and 
another has just hepatomegaly and some proteinuria, at ages 
when other affected relatives had already long been transplanted 
for end-stage renal failure. The mechanisms underlying tissue 
distribution of amyloid and those by which it damages tissue and 
organ function remain completely obscure.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Genotype</th>
<th>ApoA-I 100–170 mg/dliter</th>
<th>ApoB 60–120 mg/dliter</th>
<th>Triglyceride 0.3–2.3 mmol/liter</th>
<th>Cholesterol &lt;5.2 mmol/liter</th>
<th>HDL-Chol 0.6–2.0 mmol/liter</th>
<th>LDL-Chol &lt;3.5 mmol/liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>del/wt</td>
<td>66</td>
<td>80</td>
<td>0.7</td>
<td>4.4</td>
<td>0.7</td>
<td>3.4</td>
</tr>
<tr>
<td>BF</td>
<td>del/wt</td>
<td>39*</td>
<td>100*</td>
<td>2.3</td>
<td>6.4</td>
<td>0.6</td>
<td>4.7</td>
</tr>
<tr>
<td>SP</td>
<td>del/wt</td>
<td>76</td>
<td>70</td>
<td>9.7</td>
<td>4.0</td>
<td>1.0</td>
<td>2.7</td>
</tr>
<tr>
<td>JF</td>
<td>del/wt</td>
<td>87</td>
<td>117</td>
<td>1.1</td>
<td>4.7</td>
<td>0.8</td>
<td>3.4</td>
</tr>
<tr>
<td>KW</td>
<td>wt/wt</td>
<td>91</td>
<td>87</td>
<td>9.9</td>
<td>4.1</td>
<td>0.8</td>
<td>2.9</td>
</tr>
<tr>
<td>RF</td>
<td>wt/wt</td>
<td>84</td>
<td>75</td>
<td>1.6</td>
<td>5.3</td>
<td>1.1</td>
<td>3.5</td>
</tr>
</tbody>
</table>

Normal ranges are shown.

* turbid specimen, results unreliable

Fig. 5. Polyacrylamide gel (8%) electrophoresis of the PCR product of exon 4 of the apoA-I 
gene. The wild type allele is present in all subjects and the additional abnormal allele with 
the 9 base pair deletion is seen in individuals with amyloid (AA, JF, BF) together with a 
heteroduplex formed by the products in these patients.

Fig. 6. ApoA-I detected by immunoblotting after isoelectric focusing of delipidated plasma. 
Lane 1 (JF), 2 (BF), 4 (AA) and 5 (SP) are affected family members; lane 3 (RF) and 6 
(KW) are unaffected individuals; lane 7, unrelated normal control. The most abundant 
isoform of mature wild type apoAI migrating, as predicted from the derived amino acid sequence, 
with an extra positive charge, +1.

Table 2. Apolipoprotein and lipid analyses
Finally, the present family illustrate dramatically the power of SAP scintigraphy, which is safe and non-invasive, for diagnosis of amyloid, especially in patients in whom rectal biopsy has been negative despite the presence of massive visceral deposits.

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