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Gelsolin variant (Asn-187) in familial amyloidosis, Finnish type

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Familial amyloidosis, Finnish type (FAF), is an inherited form of systemic amyloidosis clinically characterized by cranial neuropathy and lattice corneal dystrophy. We have demonstrated that the protein subunit isolated from amyloid fibrils shows considerable sequence identity with gelsolin, an actin-binding protein. We have purified the amyloid subunit from a second case and further analysed different fractions from the previous one. Sequence analysis shows that, in both cases, the amyloid subunit starts at position 173 of the mature molecule; it has a heterogeneous *N*-terminus and contains one amino acid substitution, namely asparagine for aspartic acid, at position 15 (gelsolin residue 187), that is due to a guanine-to-adenine transversion corresponding to nucleotide-654 of human plasma gelsolin cDNA. The substitution maps in a fragment with actin-binding activity and is located in a repetitive motif highly conserved among species. Thus FAF is the first human disease known to be caused by an internal abnormal degradation of a gelsolin variant. We designate this variant of gelsolin-associated amyloidosis 'Agel Asn-187'.

INTRODUCTION

Familial amyloidosis, Finnish type (FAF) is an autosomal dominant form of systemic amyloidosis characterized by lattice corneal dystrophy, mild generalized polyneuropathy and intermittent proteinuria [1]. Although some cases have been reported from Denmark [2], The Netherlands [3] and the U.S.A. [4–6], the disease is located mainly in south-eastern Finland [7]. Histopathological studies demonstrated small deposits of congophilic material in the cornea [4,5,8] and the affected nerves [9], as well as in association with vessel walls and most basement membranes [9,10]. The amyloid fibrils are composed of polymeric forms of a fragment showing sequence identity with gelsolin, as judged by *N*-terminal amino acid sequence [11], as well as by sequence analysis of internal peptides released after enzymic cleavage [12]. Since heterogeneity was found at position 15 of the same patient [13], we isolated and partially sequenced the amyloid subunit from a second case (patient J.A.A.) and extended the *N*-terminal sequence of different amyloid fractions of the previous one (patient V.U.O.). An amino acid substitution was found at position 15 of the amyloid subunits in both cases.

MATERIALS AND METHODS

Materials

Human plasma gelsolin was kindly provided by Dr. David Kwiatkowski, Massachusetts General Hospital, Charlestown, MA, U.S.A. Sephadex G-100 was from Pharmacia. Polyvinylidene difluoride (PVDF) membranes (Immobilon P) were supplied by Millipore Corp.; anti-(human gelsolin) monoclonal antibody (MAb) and 3,3′-diaminobenzidine were from Sigma; 5-bromo-4-chloroindol-3-yl phosphate and NitroBlue Tetrazolium were from Kirkegaard and Perry Laboratories, Gaithersburg, MD, U.S.A.; pig anti-rabbit immunoglobulin and peroxidase–antiperoxidase conjugate were from Dako; uranyl acetate was from Ladd Research Industries, Burlington, VT, U.S.A.; 3-cyclohexylaminopropane-1-sulphonic acid was from Aldrich. Reagents used for gas-phase sequencing were supplied by Applied Biosystems. All other chemicals were of reagent grade or the highest purity available.

Case report

Patient J.A.A. was a 75-year-old male farmer from the Kymenlaakso district of south-eastern Finland. He had a daughter afflicted with FAF and a healthy son. Since the age of 50 years he had gradually developed sagging facial skin, and corrective plastic surgery had been performed because of blepharochalasis. On ophthalmological and neurological investigation, lattice corneal dystrophy and upper facial paresis had been found, both characteristic manifestations of FAF. The patient died unexpectedly after acute bronchopneumonia, and autopsy revealed massive pulmonary embolism. Histopathological studies showed small congophilic deposits with apple-green birefringence in polarized light occurring in most tissues, including the kidney glomeruli, skin, perineurial sheaths and blood-vessel walls.

Patient V.U.O. was described previously [13]. Immunohistochemical studies revealed that two distinct types of amyloidosis co-exist in this patient: FAF and Alzheimer's-disease-like lesions [14].

Isolation and purification of FAF amyloid protein J.A.A.

Amyloid fibrils were isolated from the kidney of patients J.A.A. and V.U.O. as described in [11]. The resulting amyloid-enriched preparation was suspended in 6 M-guanidinium chloride/0.1 M-Tris/HCl/0.34 mM-EDTA/170 mM-dithiothreitol, pH 10.4, and stirred for 24 h at room temperature. After acidifying the mixture with 2 M-guanidinium chloride/4 M-acetic acid (0.25 vol.), the material was fractionated on a Sephadex G-100 column (2 cm × 180 cm) equilibrated with 5 M-guanidinium chloride/1 M-acetic acid. The resulting fractions were extensively dialysed against distilled water and freeze-dried.

Purity was assessed by SDS/PAGE [15] on 15%-acrylamide Minigels under reducing conditions. Identification was carried out via immunoblot analysis on PVDF membranes, using human plasma gelsolin and polyclonal anti-FAF antibodies [13] and anti-(human gelsolin) MAbs, at 1:200 and 1:500 dilution respectively. Either alkaline phosphatase-labelled goat anti-rabbit IgG (1:5000) or alkaline phosphatase-labelled goat anti-mouse IgG (1:4000) was used as a second antibody. Immunoblots were developed using 5-bromo-4-chloroindol-3-yl phosphate and NitroBlue Tetrazolium.

Abbreviations used: FAF, familial amyloidosis, Finnish type; anti-FAF, rabbit antibody raised against the 12 kDa purified amyloid subunit from patient V.U.O.; PVDF, polyvinylidene difluoride; MAb, monoclonal antibody.

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Immunohistology

Rabbit anti-FAF antiserum was obtained by subcutaneous immunization with a purified amyloid subunit isolated from patient V.U.O. [13]. The animals were initially challenged with 100 μg of antigen in complete Freund's adjuvant, boosted every 15 days with a similar amount of protein without adjuvant, and bled after 60 days.

Cryostat or deparaffinated paraffin sections were incubated with either anti-FAF (1:100 dilution) or preimmune serum (1:100 dilution) for 1 h at room temperature, followed by pig anti-rabbit immunoglobulin (1:50 dilution, 30 min, room temperature) and peroxidase–antiperoxidase conjugate (1:100 dilution, 30 min, room temperature). Peroxidase activity was detected with 3,3′-diaminobenzidine and 0.015% H_2O_2. Specificity of immunoreactions was confirmed by absorption experiments [13].

Electron microscopy

Specimens from different steps of the isolation were placed on Formvar-coated nickel grids, negatively stained with 1% uranyl acetate, and examined in an electron microscope [13].

Amino acid sequence analysis

When necessary, the amyloid extract was subjected to SDS/PAGE and electroblotted on to PVDF membranes using 10 mM-3-cyclohexylaminopropane-1-sulphonic acid, pH 11.0, containing 10% (v/v) methanol. Proteins were revealed as described in [16], and the bands excised and subjected to Edman degradation.

Amino-acid-sequence analyses were performed on a 477A Protein Sequencer, and the resulting phenylthiohydantoin derivatives identified using an on-line phenylthiohydantoin analyser (Applied Biosystems, Foster City, CA, U.S.A.).

RESULTS

Anti-FAF antibody, raised against amyloid fibrils extracted from patient V.U.O. [13], did not stain normal renal tissue (Fig. 1*a*), but reacted strongly with the amyloid deposits associated with the basement membranes of J.A.A.'s kidney glomeruli (Fig. 1*b*). Preimmune serum left these structures completely unstained, and absorption of anti-FAF with the FAF amyloid subunit abolished the staining (for details, see [13]).

The distilled-water extracts containing amyloid-enriched material had the typical apple-green birefringence in polarized light after Congo Red staining. Electron-microscopic examination showed straight unbranched filaments of diameter approx. 8 nm.

After gel filtration under dissociating conditions, amyloid J.A.A. yielded two main peaks. The low-molecular-mass peak contained two bands with molecular masses of 12 and 7–9 kDa respectively, as determined by SDS/PAGE. Immunoblot experiments indicated that anti-FAF was able to recognize them (Fig. 2, lane 1), whereas anti-gelsolin MAb (directed against the *C*-terminal end of the native molecule) [17] immunoreacted only with intact gelsolin (lane 2) and not with the amyloid subunit (lane 3).

N-Terminal amino-acid-sequence analysis of protein J.A.A. yielded 28 residues, whereas extended *N*-terminal sequencing of protein V.U.O. generated 27 amino acids (Fig. 3). Both amyloid

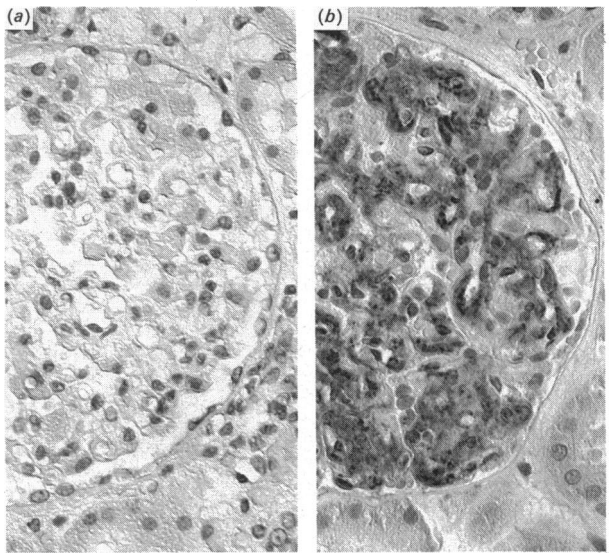

Fig. 1. Immunoperoxidase staining of renal tissue with anti-FAF antibody (dilution 1:100)

(*a*) Normal kidney; (*b*) patient J.A.A. Light Meyer's Hemalum counterstain was used to show the nuclei. Magnification ×230.

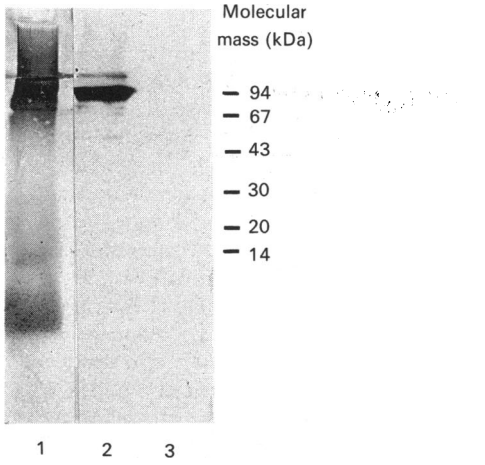

Fig. 2. Immunoblot analysis of amyloid subunit J.A.A. and human plasma gelsolin

Lanes 1 and 3, amyloid protein J.A.A.; lane 2, human plasma gelsolin. Polyclonal anti-FAF was used on lane 1 as a primary antibody, whereas lanes 2 and 3 were incubated with anti-gelsolin MAb. Molecular-mass markers were as follows: phosphorylase *b* (94 kDa), BSA (67 kDa), ovalbumin (43 kDa), carbonic anhydrase (30 kDa), soybean trypsin inhibitor (20 kDa) and α-lactalbumin (14 kDa).

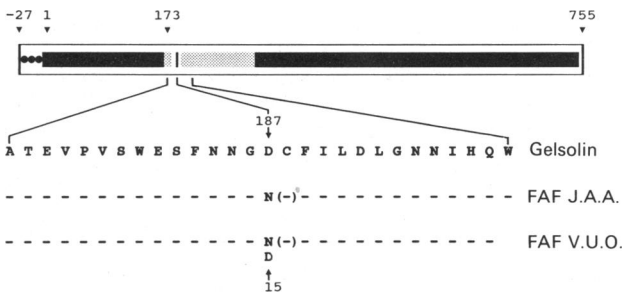

Fig. 3. *N*-Terminal sequence of amyloid proteins V.U.O. and J.A.A. in comparison with human plasma gelsolin

A schematic representation of human plasma gelsolin, as deduced from cDNA, is shown. Numbering follows that in [23]. ●, Signal sequence; ⁂, location of the amyloid protein; |, place of the amino acid substitution, position 187 (↓), corresponding to residue 15 of the amyloid subunit (↑); amino acids are expressed in the one-letter code; (-), undetermined; -, sequence identity.

proteins show sequence identity with human plasma gelsolin, starting at position 173 of the mature molecule. A certain degree of heterogeneity at the *N*-terminal end was found in both sequences: whereas, in protein J.A.A., 50% of the molecules start with alanine, 40% with threonine and 10% with glutamic acid, in patient V.U.O. approx. 70% of the molecules start with alanine, 20% with threonine and 10% with glutamic acid. One amino acid substitution, asparagine for aspartic acid, was detected at position 15 of both amyloid proteins (corresponding to residue 187 of the gelsolin molecule). Similar results were presented by Dr. C. P. J. Maury at the Sixth International Symposium on Amyloidosis held in Oslo, Norway, 5–8 August 1990. In protein J.A.A., only asparagine was found at residue 15, whereas in patient V.U.O. that position was corresponded to a mixture of 80% asparagine and 20% aspartic acid.

DISCUSSION

Gelsolin, originally called 'actin-depolymerizing factor', is a member of a class of actin-modulating proteins, found in organisms ranging from lower eukaryotes to mammals, which sever actin filaments, can nucleate actin filament growth and cap barber filament ends [18]. Gelsolin is regulated by both Ca^{2+} [19] and polyphosphoinositides [20]. A single gene located in the long arm of human chromosome 9 (bands q32–q34) [21] encodes two forms of gelsolin; one remains associated with the cytoplasm (M_r 90000), whereas the other (M_r 93000) is secreted [22]. As deduced from cDNA studies [23], mature plasma gelsolin is 755 residues long (Fig. 3), and its mean concentration in human plasma is approx. 220 μg/ml [24].

Gel-filtration experiments with amyloid protein J.A.A. basically yielded a similar elution profile when compared with amyloid protein V.U.O. [11]. However, the monomeric form of the amyloid extracted from the kidney of patient J.A.A. shows two bands on SDS/PAGE (12 and 7–9 kDa respectively). They react on immunoblots with anti-FAF antibody (a rabbit polyclonal antibody against 12 kDa amyloid V.U.O.), but do not react with anti-gelsolin, a MAb raised against the *C*-terminal end of plasma gelsolin. Moreover, anti-FAF is able to recognize not only the monomers and polymers of amyloid proteins V.U.O. and J.A.A., but also intact plasma gelsolin. *N*-Terminal sequence analysis of amyloid subunits J.A.A. and V.U.O. indicates that they are degradation products of plasma gelsolin, starting at position 173 of the intact molecule. In patient J.A.A., amino-acid-sequence analysis reveal that both bands of 12 and 7–9 kDa have the same *N*-terminus, and it is not known whether these are fragments of different length or aggregates of the 7–9 kDa subunit. The absence of reactivity with the anti-gelsolin MAb is consistent with the size of the amyloid protein and its topographical location in the gelsolin molecule.

We have detected an amino acid substitution (asparagine for aspartic acid) at position 15 of the amyloid subunit, corresponding to amino acid 187 of plasma gelsolin. In patient V.U.O. (but not in patient J.A.A.), position 15 also contains aspartic acid to the extent of approx. 20%. Whether this is due to deamination of asparagine or to the expression of both alleles in the amyloid fibril, as has been shown in hereditary cerebral haemorrhage with amyloidosis, Dutch type [25,26], remains to be elucidated. The amino acid substitution is located in a repetitive motif (**FXXXDXFIL**) of unknown function, and highly conserved among species [27]. Position 187 also maps in a 26 kDa fragment (positions 150–373), reported to have substantial binding capacity to both G- and F-actin, although with very low severing activity [28]. There is a possibility that this substitution is polymorphic in certain populations and not related to specific amyloid deposition in FAF. However, polymorphism in the gene encoding gelsolin has not been observed at this position [29], suggesting that there is a relationship between this variant and FAF. Moreover, a guanine-to-adenine transversion corresponding to nucleotide 654 of the human plasma gelsolin cDNA [23] was recently observed in five patients with FAF [30].

Amino acid substitutions due to point mutations have been observed in different autosomal dominant forms of amyloidosis, including hereditary cerebral haemorrhage with amyloidosis of Icelandic [31,32] and Dutch [25,26] origin and familial amyloidotic polyneuropathy [33], and they seem to play an important role in amyloidogenesis. Although the mechanism of amyloid-fibril formation still remains unknown, the proteolysis of the gelsolin variant to generate FAF amyloid appears to be similar to the abnormal processing of the amyloid precursor protein to yield the amyloid protein in Alzheimer's disease and hereditary cerebral haemorrhage with amyloidosis, Dutch type [34,35]. Since FAF amyloid is an internal fragment, the release of the amyloid peptide should require two enzymic cleavages, and a trypsin-like enzyme may be involved in the processing of the *N*-terminus (Arg^{172}–Ala^{173}). Further studies are necessary to understand the mechanism of amyloid formation in FAF, the first human disease known to be caused by aberrant catabolism of a gelsolin variant.

We designate this variant of gelsolin-associated amyloidosis 'Agel Asn-187'.

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