Amyloid Fibril Protein in Familial Amyloidosis with Cranial Neuropathy and Corneal Lattice Dystrophy (FAP Type IV) is Related to Transthyretin

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Immunocytochemical methods were used to study the nature of the amyloid deposits in the Finnish type-familial amyloid polyneuropathy (FAP) type IV, which is characterized by cranial neuropathy and corneal lattice dystrophy. Commercial antisera to human plasma transthyretin (prealbumin) did not stain the amyloid deposits, but in every case a positive staining was obtained with antibodies raised against transthyretin-related amyloid fibril whole protein isolated from the myocardium of a patient with familial amyloid polyneuropathy from the state of New York. The FAP type IV amyloid deposits stained also with antiserum to serum amyloid P component, but did not stain with antisera to retinol-binding protein, amyloid A protein, γ-trace protein, β2-microglobulin, or immunoglobulin light chains. The serum level of serum transthyretin was significantly decreased in FAP type IV patients (256 ± 75 (SD) mg/L, n = 15) as compared with Finnish control subjects (360 ± 56 mg/L, n = 30, P < 0.001), whereas the level of retinol-binding protein was within the normal range. The results of this study strongly suggest that the amyloid fibril protein in FAP type IV amyloidosis is related to transthyretin. (Key words: Familial amyloidosis; Finnish hereditary amyloid neuropathy; Familial amyloid polyneuropathy type IV; Amyloid corneal lattice dystrophy; Transthyretin; Prealbumin; Retinol-binding protein; Amyloid P component) Am J Clin Pathol 1988; 89: 359–364

FAMILIAL AMYLOID POLYNEUROPATHY (FAP) syndromes are autosomal dominant disorders characterized by amyloid deposits in tissues and polyneuropathy.1 The FAP syndromes differ from each other with respect to distribution and degree of involvement of affected nerves and organs, age of onset, and ethnic origin. The Finnish type of familial amyloidosis (FAP type IV) is a systemic disease characterized by slowly progressive cranial neuropathy, corneal lattice dystrophy, and distal sensimotor neuropathy without autonomic dysfunction.13–15 The nature of the amyloid fibril protein in FAP type IV has remained unresolved, in contrast to the findings in FAP type I and FAP type II syndromes which have been shown to be caused by specific variant transthyretin* (prealbumin) molecules that selectively deposit in tissues as amyloid.5,7,8,21,23,26,27 A recent immunohistochemical study of FAP type IV suggested that the amyloid fibril protein is not related to transthyretin or to any other protein known to constitute amyloid fibril protein of systemic amyloidosis.10 In this study, we investigated FAP type IV amyloid deposits, using an antiserum raised against transthyretin-related FAP amyloid fibril whole protein11 and by measuring the serum concentrations of transthyretin in patients with FAP type IV. Our results show that FAP type IV amyloid is specifically stained by the transthyretin-related amyloid fibril whole protein antiserum and that the serum concentrations of transthyretin are significantly decreased in FAP type IV patients. These findings strongly suggest that the amyloid fibril protein in FAP type IV is related to transthyretin.

Materials and Methods

Tissue Samples

Skin (four patients) and rectum (one patient) biopsy specimens from Finnish patients with familial amyloidosis with cranial neuropathy and corneal lattice dystrophy (FAP type IV) were studied. Control tissues included skin biopsy specimens from two normal individuals, two patients with lichen amyloidosis and one patient with nodular amyloidosis, two renal biopsy specimens and one thyroid biopsy specimen from patients with secondary (amyloid A) amyloidosis, and an autopsy and a biopsy liver specimen from two patients with amyloid light chain amyloidosis.

* Nomenclature according to the suggestion by the Nomenclature Committee of the IUB and IUB-IUPAC Joint Commission on Biochemical Nomenclature. (J Biol Chem 1981;256:12–14).
Antisera

Antiserum against FAP transthyretin-related amyloid fibril whole protein was raised in rabbits,\(^1\) and the IgG fraction was prepared as described.\(^12\) This antiserum gives reactions of identity with commercial antiserum on standard Ouchterlony plates when examined with amyloid fibril whole protein, commercial tetrameric transthyretin or human plasma. The antiserum does not differ from commercial antitransthyretin in double immunodiffusion at right angles or in tandem-rocket immunodiffusion. On immunoblots the antiamyloid fibril whole protein reveals the same monomeric, dimeric, trimeric, and tetrameric bands of transthyretin as do commercial antisera. In contrast to commercial antisera, however, the staining of the transthyretin bands obtained from amyloid fibril whole protein is partially blocked by absorption of the antiserum with intact amyloid fibril or human plasma. The antiserum does not differ from commercial antitransthyretin in double immunodiffusion at right angles or in tandem-rocket immunodiffusion. On immunoblots the antiamyloid fibril whole protein reveals the same monomeric, dimeric, trimeric, and tetrameric bands of transthyretin as do commercial antisera. In contrast to commercial antisera, however, the staining of the transthyretin bands obtained from amyloid fibril whole protein is partially blocked by absorption of the antiserum with intact or monomeric normal human plasma transthyretin. Antiserum to amyloid A protein was raised in rabbits as described.\(^9\) Commercial antisera to human plasma transthyretin were from Behringwerke AG (Marburg, FRG), Dako (Copenhagen, Denmark), and Orion Diagnostica (Espoo, Finland). Antiserum to amyloid P component, retinol-binding protein, and immunoglobulin light chains were from Dako, antiserum to post-gamma protein (\(\gamma\)-trace) was from Behringwerke, and antiserum to \(\beta_2\)-microglobulin was from Bio-Yeda (Rehovot, Israel).

Immunoperoxidase Staining

Histologic sections, 4-\(\mu\)m thick, were deparaffinized in xylene and washed in phosphate-buffered saline (PBS). Endogenous peroxidase activity was destroyed by incubating the sections with 0.3% hydrogen peroxide in methanol at room temperature for 30 minutes and treated with 1% pepsin in 0.01 mol/L HCl at 37 °C for 1 hour, after which the sections were washed in PBS, containing 2% normal rabbit or normal sheep serum. The test sections were incubated in moist chambers with rabbit antiserum diluted 1:100 to 1:800 at +4 °C for 3 days. As a second layer, peroxidase-conjugated swine immunoglobulins to rabbit IgG (diluted 1:200) (Dako) were used for 30 minutes. The sections were then incubated for 10 minutes with 3',3'-diamino-benzidine tetrahydrochloride in \(H_2O_2\) (DAB 0.5 mg/mL, 0.003% \(H_2O_2\)) (Sigma Chemical Co., St. Louis, MO). Control sections were treated similarly, but the first antiserum was replaced with normal rabbit serum. The adjacent sections were stained with Congo Red. No counterstain was added to the immunoperoxidase-treated sections.

Assay of Serum Transthyretin, Retinol-binding Protein and C-reactive Protein

The concentrations of these proteins were measured by radial immunodiffusion using specific antisera (Dako and Orion Diagnostica). Control human plasma (Behringwerke AG) was used as standard.

Results

Congo-red staining of the skin biopsy specimens from FAP type IV patients revealed amyloid deposition most prominently around the eccrine sweat glands, hair follicles, and sebaceous glands as well as along the collagen fibers. The antiserum to FAP fibril whole protein and to amyloid P component stained the amyloid in all specimens (Table 1, Fig. 1), whereas none of the commercial antisera to tetrameric serum transthyretin (prealbumin) stained the amyloid deposits. The antiserum to immunoglobulin light chains, amyloid A, retinol-binding protein, \(\gamma\)-trace protein, and \(\beta_2\)-microglobulin also did not stain the amyloid deposits (Table 1). Absorption of the FAP whole fibril protein antiserum with commercial transthyretin did not abolish the positive staining (Fig. 2). FAP whole fibril antiserum did not stain the amyloid in nodular or light chain (AL)-amyloidosis. In one biopsy specimen from a patient with lichen amyloidosis and in both renal biopsy specimens from patients with secondary amyloidosis, a very weak staining with FAP antiserum was seen (Fig. 1, Table 1). Table 2 shows the serum levels of transthyretin (TTR) and retinol-binding protein (RBP) in subjects with FAP type IV. Transthyretin levels were found to be significantly depressed in FAP type IV; this was not due to a negative acute phase reaction, since C-reactive protein (CRP) levels were not raised in these patients (Table 1).

Discussion

FAP type IV has been described primarily in the Finnish population; by the end of the 1970s, more than 300 cases had been recorded.\(^{14,16}\) Occasional cases have also been reported outside Finland, including cases from the United States,\(^6,22,23\) and the Netherlands.\(^3,29\) The disease is inherited in autosomal dominant mode and, in heterozygous patients, the disease has a late-onset and is slowly progressive. By the age of 20 years, corneal lattice dystrophy is usually manifested, and by the age of 40 years, most patients have developed cranial neuropathy.
Skin, renal, and cardiac manifestations may also occur.\textsuperscript{13-15} Clinically FAP type IV patients differ from the FAP type I patients who present with polyneuropathy of the lower limbs combined with severe autonomic dysfunction, and from the FAP type II patients who present with cardiomyopathy.

In FAP type IV, the distribution of amyloid is systemic; amyloid is found in the intima and media of arteries and in capillary walls and in most organs also in the basement membrane. Of the nerves, cranial nerves are the most affected, and in the skin, amyloid is found most abundantly around the cutaneous appendages and along collagen fibers.\textsuperscript{15} On electron microscopy, the deposits show typical characteristics of amyloid: microfibrils of approximately 10 nm in diameter are found.\textsuperscript{15} Polyacrylamide gel electrophoresis of the isolated amyloid proteins has revealed two major bands corresponding to Mr of 15,000 and 17,000.\textsuperscript{16} The fractions have, however, been characterized only incompletely; none of the fractions reacted with antiamyloid A antiserum.\textsuperscript{16}

Previous immunohistochemical studies of the FAP type IV amyloid deposits have yielded inconclusive and partially conflicting results. Purcell and associates,\textsuperscript{22} in a study of a FAP type IV patient of Irish descent, found no reactivity of the amyloid deposits with either amyloid A or amyloid P antiserum, whereas Darras and coworkers,\textsuperscript{6} in a study of a FAP type IV patient of French-Irish descent, found a positive immunoperoxidase reaction of corneal amyloid with antiamyloid P antiserum and a negative reaction with antiamyloid A antiserum. Based on the sensitivity of the amyloid in nerve to KMnO\textsubscript{4} treatment, Darras and associates\textsuperscript{6} suggested that the amyloid in the nerve was of amyloid A type. In a recent report, Falck and Westermark\textsuperscript{10} found positive staining with anti amyloid P component antiserum of the amyloid deposits in samples from five FAP patients, whereas commercial antiserum to transthyretin and antiserum to transthyretin-related amyloid protein of Danish familial cardiomyopathy did not stain the deposits. The authors concluded that the fibril protein of the Finnish familial amyloidosis is probably not transthyretin-related.\textsuperscript{10} The presence of amyloid P component in the amyloid of FAP type IV was confirmed in our study; however, the antiserum against FAP whole fibril protein showed positive staining of all FAP type IV amyloid deposits. In contrast, no staining was found with commercial antiserum against human plasma transthyretin suggesting different antigenic determinants in transthyretin-related amyloid fibril protein and normal plasma transthyretin. This conclusion is supported by the finding in the absorption experiments that normal transthyretin did not block the staining of the amyloid deposits.

We found decreased serum levels of transthyretin in FAP type IV patients, which is in accord with most,\textsuperscript{2,4,24} but not all,\textsuperscript{20,28} findings in FAP. The serum levels of retinol-binding protein were within the normal range, in contrast to the findings in FAP type II, which is characterized by markedly decreased levels.\textsuperscript{2} Immunohistochemistry did not reveal the presence of retinol-binding protein in the amyloid deposits in FAP type IV either, whereas in the Japanese FAP type I immunohistochemistry has suggested the presence of this protein.\textsuperscript{17}

Recent biochemical studies of the amyloid fibril pro-
teins in the various FAP syndromes suggest that they are expressions of specific alteration in the transthyretin molecules. The Swedish, Japanese, and Portuguese FAP type I syndromes are associated with a variant transthyretin molecule in which a methionine for valine substitution occurs at position 30.7,23,26 In the Jewish FAP syndrome, the transthyretin variant has an isoleucine for phenylalanine substitution at position 33.18 Finally, in FAP type II (Indiana/Swiss), a serine for isoleucine substitution at position 84 in the transthyretin monomer,8 and in the Appalachian form of FAP27 an alanine for threonine at position 60, were identified recently.

The antiserum used in this study was raised against the transthyretin-related FAP whole protein isolated from the myocardium of a FAP patient belonging to a large FAP family in the state of New York.11 The primary structure of this transthyretin-related amyloid protein has not yet been elucidated. However, preliminary examination of leukocyte DNA for RFLP revealed a pattern identical with that of family members afflicted by FAP who have the apparent substitution of alanine for threonine at position 60 (M.D. Benson and A.H. Koeppen, unpublished results).

The clinically different expression of the FAP syndrome in the Finnish FAP type IV raises the possibility that the amyloid fibril protein in this syndrome may have a unique structure. Our preliminary results indicate that FAP type IV is not associated with the FAP type I transthyretin variant characterized by a methionine for valine substitution at position 30, since radioimmunoassay for the anomalous nonapeptide 22–30 of transthyretin in serum19 was negative (M. Nakazato, C. P. J. Maury and A-M. Teppo, unpublished results).

In conclusion, our data suggest that the amyloid fibril

![Fig. 1](image1.png)


![Fig. 2](image2.png)

**Fig. 2.** The effect of absorption of antiserum to FAP amyloid whole fibril with human transthyretin. Staining of amyloid around the sebaceous gland of a patient with FAP type IV with the antiserum to FAP fibril protein before (A, left) and after (B, right) the absorption with plasma normal transthyretin.
Table 2. Serum Transthyretin, Retinol-binding, and C-Reactive Protein Levels in FAP Type IV Patients and Healthy Controls

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>TTR (mg/L)</th>
<th>RBP (mg/L)</th>
<th>CRP (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FAP type IV</td>
<td>15</td>
<td>256 ± 75*</td>
<td>52 ± 19†</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Controls</td>
<td>30</td>
<td>360 ± 56</td>
<td>48 ± 7</td>
<td>&lt;10</td>
</tr>
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* P < 0.001 (two-tailed Student's t-test).
† NS.

protein in FAP type IV, in contrast to earlier suggestions, is also related to transthyretin. The final classification of the FAP type IV amyloid fibril protein, however, awaits the results of conclusive amino acid sequence analyses.

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References