Two Dutch Families with FAP Type I

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TWO DUTCH FAMILIES WITH FAP TYPE I
Clinical Data and Biochemical Characterization of the TTR Variant

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Summary: Two Dutch families with familial amyloidotic polyneuropathy (FAP) type I are described. Histologic confirmation of amyloid deposition could be obtained in four members of family A and in one member of family B. Their clinical data are presented. A late onset of the disease was a prominent feature in both families. The serum precursor of this type of amyloid, TTR Met 30, could be demonstrated by RIA in the serum of both an affected and an unaffected member of each family.

TTR was isolated from the plasma of one patient of family A by affinity chromatography and HPLC, and characterized by amino acid sequence analysis up to position 34. As expected a valine residue at position 30 appeared to be substituted by methionine. CNBr cleavage of this variant TTR was followed by trypsin digestion. In the resulting tryptic peptide map immunoreactivity for the FAP Met 30 nonapeptide could be detected.

INTRODUCTION

The first description of familial amyloidotic polyneuropathy (FAP) type I with onset in the lower limbs has been made by Andrade in 1952 (1). In 1968 Araki reported the first kinship with FAP type I in Japan (2). Many reports concerning other affected families in different countries have been published already (3).

In 1967 two patients with FAP type I from a Dutch kinship (family A) were published by our collegue Jan Scholten (4); unfortunately this early report was written in Dutch and consequently remained unnoticed by other investigators. Recently two members of a younger generation appeared to be affected too. In addition we saw a patient with FAP type I from another Dutch kinship (family B), not related to family A.

Clinical data of these five patients and some relatives are presented below. Serum from an affected member of family A was used for the isolation and characterization of transthyretin (TTR).

MATERIAL AND METHODS

Isolation of serum TTR

TTR was isolated from serum following the procedure as outlined by one of us (5). In short this comprises the following steps:

1. **Affinity chromatography.**

Serum (5 ml) was applied on a column packed with Blue-Sepharose CL.6B (Pharmacia, Sweden) equilibrated with 30 mM phosphate buffer pH 7.0. After collection of the void-volume fractions, the bound proteins were eluted after application of an 1 M NaCl solution followed by 0.5 M sodium thiocyanate.

2. **HPLC.**

The TTR-positive peak obtained after the previous described Affi-Gel Blue chromatography, was subsequently analysed by anion exchange HPLC using a Mono Q column (Pharmacia, Sweden). After injection of the sample, a

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linear gradient of NaCl (0-0.5 M) was started. The TTR-positive peak was rechromatographed on the HPLC column applying the same sodium-gradient.

Identification of immunoreactivity for the FAP Met 30 nonapeptide

TTR purified as described above was cleaved by CNBr and digested with trypsin. The resulting digests were separated by reverse-phase HPLC as outlined in detail previously (5). The obtained tryptic peptide map was compared with that of normal human TTR and screened for immunoreactivity with the FAP Met 30 nonapeptide.

Amino acid sequence analysis

To determine the amino acid sequence unambiguously the primary structure of the isolated TTR protein was determined, using an Applied Biosystems model 477A protein sequencer (pulse-liquid sequenator), on-line connected with a 120A PTH-analyzer (6).

Radioimmunoassay for TTR Met 30

The level of TTR Met 30 in the sera of the patients tested as well as in the fractions obtained in the tryptic peptide map analysis was measured by means of a radioimmunoassay specific for the FAP-nonapeptide of the variant TTR molecule as described by Nakazato et al (5).

ELISA for TTR

The serum levels of normal TTR (prealbumin) were established by means of an enzyme-linked immunosorbent assay (ELISA) performed according to our own design. Microtiter plates (Inotech) were coated with the gamma fraction of anti-prealbumin antiserum (DAKO A002) (Dakopatts A/S, Denmark), diluted 1:750 in coating buffer (0.1 M sodium carbonate buffer pH 9.6), 100 µl per well, for 45 minutes at 37 ºC. After washing the plates with washing solution (0.15 M NaCl, 0.05% Tween-20), standards and test sera were added and incubated for 45 minutes at 37 ºC. Standard human serum (Behring ORDT 07) (Behringwerke AG, Marburg, FRG) was used for the calibration curve providing a range from 1.5

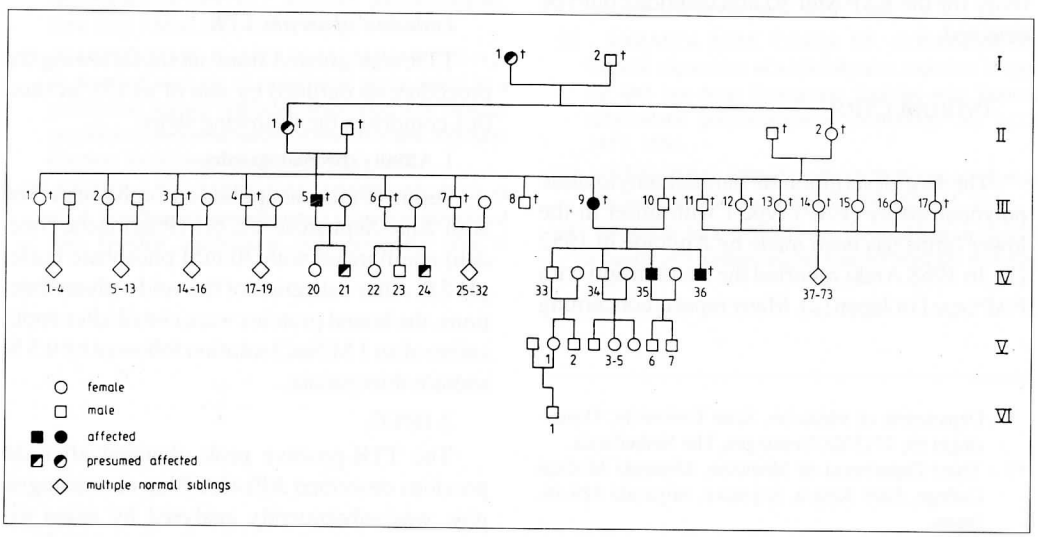

Fig. 1 - Pedigree of a Dutch kinship (family A) with FAP type I.

Table I — Dutch patients with FAP type I, histologically confirmed.

	Family A				*Family B*
	III.5	*III.9*	*IV.35*	*IV.36*	*Case 1*
Sex	M	F	M	M	M
Presenting symptoms	impotence	fatigue	diarrhoea	LL-neur.	LL-neur.
Age pres. symptoms	61	55	44	35	58
Histol. confirm.	rectum nerve kidney	rectum	gingiva rectum jejunum colon subcutis	rectum nerve	gingiva nerve
Age hist. confirm.	67	58	48	37	63
TTR Met 30 (mg/l)	ND	ND	124.9	ND	96.6
Organ dysfunction:					
Lower limb neurop.	++	++	++	++	++
Upper limb neurop.	–	–	+	+	++
Autonomic neurop.	+	ND	++	++	++
Kidney	+	ND	++	+	ND
Heart	–	ND	–	–	–
Eyes	–	ND	–	–	–
Survival (years):					
After pres. symtom.	10	10	>9	12	10.5
After hist. confirm.	4.5	7	>5	10	6

ND denotes not determined; (–) = absent; (+) = present; (++) = severe.

to 200 ng/ml. Test samples were prediluted 1:1250 in incubation buffer (0.05 M tris-HCl pH 8.0, 0.3 M NaCl, 0.05% Tween-20, 5% BSA). The amount of bound antigen (TTR) was established using biotinylated anti-prealbumin as second antibody and streptavidinperoxidase (Amersham UK) as label. After incubation with OPD (o-phenylenediamine di HCl) (Eastman Kodak, Rochester, N. Y., USA), the reaction was stopped by adding 100 µl H_2SO_4 (0.5 M) to each well. The absorbance was measured at 492 nm using a Titertek Multiscan (Flow Laboratories, Irvine, UK). Results were obtained after log-logit transformation according to Rodbard et al (7). The intra-assay and inter-assay coefficients of variation were 4.0% and 8.7% respectively.

RESULTS

1. Clinical evaluation

Case histories (suspected and proven) of both families are presented below. Both families are not known to have any Portuguese, Japanese or other foreing ancestry. In Fig. 1 the pedigree of kinship A is presented, showing 102 members of 6 generations. The pattern of heredity appears to be autosomal dominant. Although many of them were very cooperative, some were not and seemed to be frightened or refused any contact. So we have only tested two serum samples for TTR Met 30 by RIA at the moment as a pilot study. Both were positive: 124.9 mg/l in patient IV.35, and 109.7 mg/l in his 53 year old, asymptomatic niece IV.2 with hypothyroidism and vague complaints (restless legs).

Case I.1: Leg ulcers, died at age 58 of cachexia.

Case II.1: Leg ulcers, died also at age 58 of cachexia like her mother.

Case III.5: Impotence at age of 61 (4). See table I.

Case III.9: Fatigue at the age of 55 (4). See table 1.

Case IV.21: Neuropathy, unstable gait, sensory loss. 55 years old. No histologic examination performed.

Case IV.24: Neuropathy, unstable gait, sensory loss. 60 years old. No histologic examination performed.

Case IV.35: Diarrhoea and fecal incontinence at age 44. See table I. He developed many other problems like progressive renal function loss, proteinuria, orthostatic hypotension, impotence,

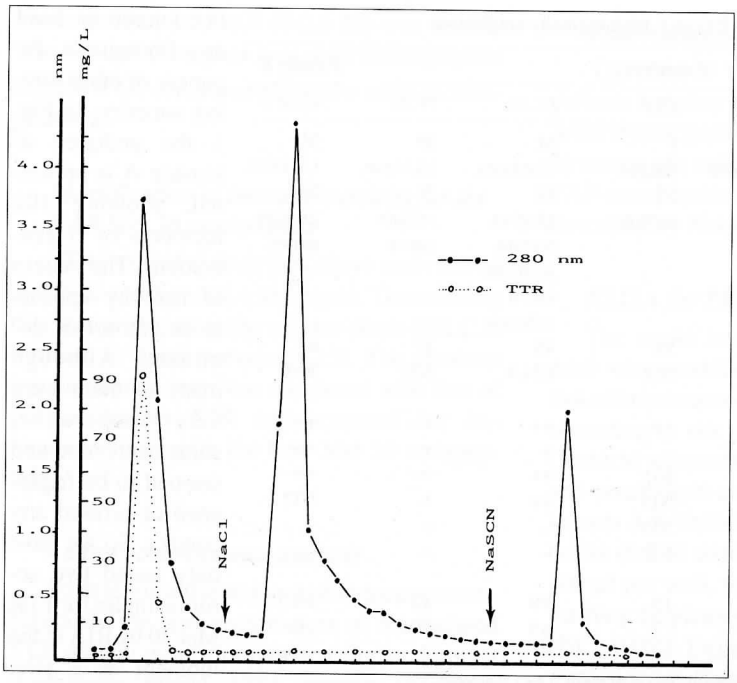

Fig. 2 - Affinity chromatography. Serum (5 ml) of FAP patient (A) IV.35 was applied on a 1 x 40 cm column packed with Blue-Sepharose CL.6B, equilibrated with 30 mM phosphate buffer pH 7.0. Fractions of approx. 7.5 ml were collected. Arrows indicate the change of elution buffer, 1 M NaCl and 0.5 M sodiumthiocyanate respectively. The absorbance at 280 nm (solid line) as well as the TTR-activity of the fractions (ELISA) were measured.

self catheterization with recurrent urinary infections, renal calculi, non-painful pretibial ulcers, lower limb neuropathy, gastrointestinal motility disturbances. No signs of an immunocyte dyscrasia in blood, urine and bone marrow were seen. Congo red stain of the rectum was positive for amyloid, immunohistologic examination was positive with anti-TTR and negative with anti-AA, -Kappa, -Lambda and $ß_2$-microglobulin. TTR Met 30 was detected in the serum.

Case IV.36: Neuropathy diagnosed at age 35. See table 1.

Case IV.2: Asymptomatic, TTR Met 30 detectable.

Cases III.1 and III.6 might have been affected too, but have died (before the appearance of symptoms?) at an unknown age and at age 51 (car accident), respectively.

Family B: Recently detected. Thorough investigation of this family has not been done yet.

Case 1: Lower limb neuropathy at the age of 58. See table I. Very progressive neuropathy, later also in the upper limbs. His grandmother, mother and two nephews also had a progressive neuropathy. All were about 60 years old at the onset of the disease and they died about 10 years after the first manifestation of the disease. Two sisters have had surgery for a carpal tunnel syndrome: no amyloid could be detected. His other brothers and sisters do not have signs of neuropathy at the moment.

Case 2: A close related and asymptomatic young member of the family of case 1 had a TTR Met 30 serum level of 109.8 mg/l.

Table I summarizes some clinical data from the 5 patients both with a clinical picture of FAP type I and with a histological confirmation of amyloidosis, made by a positive Congo red stain.

2. *TTR characterization*

TTR was isolated from the serum of patient IV.35 of family A. Results of the affinity chromatography are shown in Fig. 2. TTR-positivity was only observed with respect to the void-volume fraction.

HPLC: Analysis of the collected fractions revealed a single protein peak immunoreactive for TTR (Fig. 3), which after rechromatography resulted in a further separation, revealing a peak

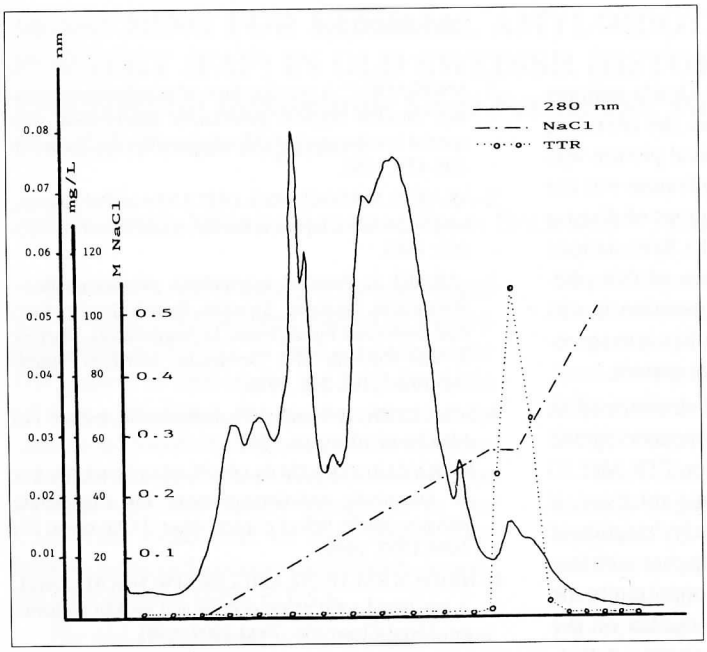

Fig. 3 - HPLC-analysis. The void-volume peak obtained after affinity chromatography (Fig. 2) was injected on a HPLC Mono Q column. Elution was achieved by application of a linear salt-gradient (0-0.5 M NaCl). The absorbance at 280 nm as well as the TTR-activity were established as indicated.

suitable for biochemical analysis.

Immunoreactivity for FAP Met 30 nonapeptide: The patient's tryptic peptide map was compared with that of normal human TTR. The FAP Met 30 nonapeptide was detected in the patient's map by radioimmunoassay.

Amino acid sequence analysis: The sequence data – performed up to position 34 – were highly suggestive for a valine residue at position 30 substituted by methionine in this isolated TTR protein. The complete amino acid sequence of this variant TTR molecule will be published elsewhere.

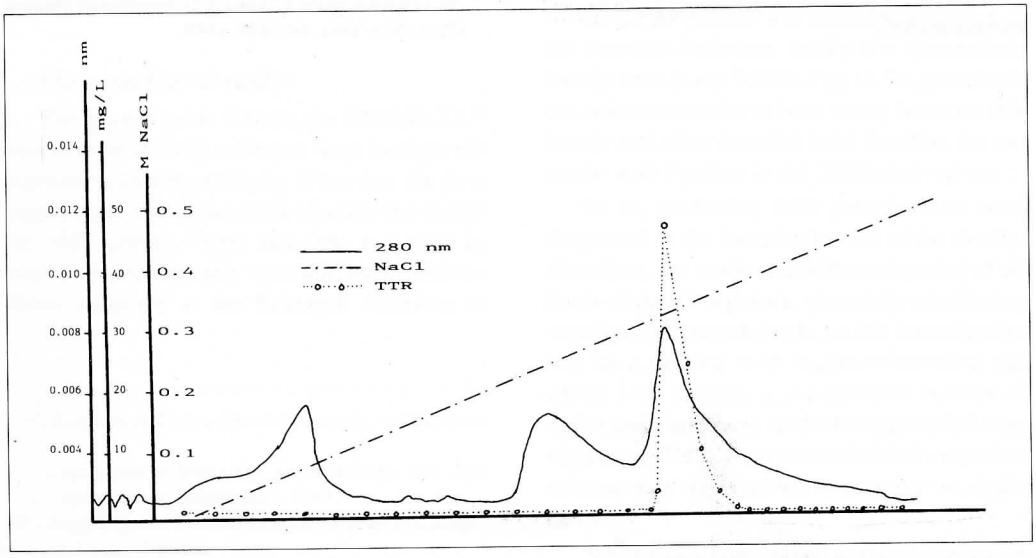

Fig. 4 - HPLC-rechromatography. The TTR-immunoreactive peak as obtained after the first HPLC-run (Fig. 3) was rechromatographed on the same column applying a somewhat less steep salt-gradient.

DISCUSSION

Clinical data from our five Dutch patients were in agreement to those from the literature concerning FAP type I. The clinical picture was diverse in family A and a striking feature was the late onset in both families. Late-onset of disease tends to cluster in some families (8). Speculations have been made about the nature of this phenomenon, but a satisfactory explanation is still lacking. The heredity in both families is in agreement with an autosomal dominant pattern.

The variant TTR could be characterized as TTR Met 30 both by amino acid sequencing and by immunoreactivity. The RIA for TTR Met 30 was positive in two asymptomatic relatives. It appears to be a good tool for the early detection of affected family members in this disease with late onset. However, by offering this opportunity the clinician lays a new and heavy burden on the shoulders of (young) persons in whom a detectable TTR Met 30 serum level has been found.

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