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Late-onset familial amyloid polyneuropathy type I (transthyretin Met30-associated familial amyloid polyneuropathy) unrelated to endemic focus in Japan

Clinicopathological and genetic features

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Summary

Clinicopathological and genetic features were assessed on 35 Japanese families affected by late-onset familial amyloid polyneuropathy type I (transthyretin Met30-associated familial amyloid polyneuropathy, FAP TTR Met30) whose siblings were unrelated to endemic Japanese foci. In these patients (50 years or older), the most common initial symptom was paraesthesias in the legs. Autonomic symptoms were generally mild and did not seriously affect daily activities. The male-to-female ratio was extremely high (10.7 : 1). A family history was evident in only 11 out of 35 families, and other patients were apparently sporadic. The rate of penetrance was very low. Symptomatic siblings of familial cases showed a late age of onset, male preponderance and clinical features similar to those of the probands. Asymptomatic carriers, predominantly female, were detected relatively late in life. The geographical distribution of these late-onset, FAP TTR Met30 cases was scattered throughout Japan. In three autopsy cases and 20 sural nerve biopsy specimens, neurons in sympathetic and sensory ganglia were relatively preserved. Amyloid deposition was seen in the peripheral nervous system, particularly in the sympathetic ganglia, dorsal root ganglia and proximal nerve trunks such as sciatic nerve. These abnormalities were milder than those seen in typical early-onset FAP TTR Met30, as observed in two Japanese endemic foci of this disease. While axonal degeneration was prominent in myelinated fibres, resulting in severe fibre loss, unmyelinated fibres were relatively preserved. Our cases of late-onset FAP TTR Met30 showed features distinct from those of typical early-onset FAP TTR Met30 that occurred in the two Japanese endemic foci. Factors responsible for clinicopathological differences between these two forms of FAP TTR Met30 need to be identified.

Keywords: transthyretin Met30-associated familial amyloid polyneuropathy; transthyretin; clinicopathological study; genetic study; late onset

Abbreviation: FAP TTR Met30 = transthyretin Met30-associated familial amyloid polyneuropathy

Introduction

Familial amyloid polyneuropathy type I (transthyretin Met30-associated familial amyloid polyneuropathy, FAP TTR Met30), associated with a mutant transthyretin substituting methionine for valine at position 30 (Met30), is the most common type of genetic familial amyloid polyneuropathy in the Western countries, as well as in Japan (Andrade, 1952; Araki *et al*., 1968; Andersson, 1976; Benson and Cohen, 1977; Coutinho *et al*., 1980; Kito *et al*., 1980; Ikeda *et al*., 1987; Nakazato *et al*., 1992; Drugge *et al*., 1993; Sousa *et al*., 1993, 1995; Holmgren *et al*., 1994; Reilly *et al*., 1995; Plantê-Bordeneuve *et al*., 1998). Typical clinical features include a relatively young age of onset (20–40 years), prominent autonomic symptoms that disrupt the activities of the patient's daily life and sensory impairment that is predominantly superficial in type, with relatively preserved deep sensation (Andrade, 1952; Araki *et al*., 1968; Ando *et al*., 1992; Alves *et al*., 1997). In Japan, typical FAP TTR Met30 is endemic in a restricted area of Arao City in Kumamoto Prefecture and Ogawa Village in Nagano Prefecture (Araki *et al*., 1968; Kito *et al*., 1980; Ikeda *et al*., 1987; Nakazato *et al*., 1992). These endemic FAP TTR Met30 foci in Japan have been compared with the large Portuguese and Swedish foci of FAP TTR Met30 (Andrade, 1952; Andersson, 1976; Benson and Cohen 1977; Coutinho *et al*., 1980). In contrast, patients with Met30 transthyretin showing a late age of clinical onset (50 years or older), no apparent family history, no kinship with families in the endemic focus and features that mimic chronic sensorimotor neuropathy without pronounced autonomic symptoms, have been reported anecdotally among the Japanese and Portuguese populations (Ikeda *et al*., 1981, 1992; Libbey *et al*., 1984; Nitta *et al*., 1986; Saraiva *et al*., 1986; Yamada *et al*., 1987; Kincaid *et al*., 1989; Fujitake *et al*., 1991; Matsushima *et al*., 1992; Aoki *et al*., 1993; Tashima *et al*., 1995). However, details of the clinical and pathological features of these patients have not been fully described. Although liver transplantation is currently the most effective curative treatment for symptomatic patients with FAP TTR Met30 (Holmgren *et al*., 1991, 1993; Ando *et al*., 1995; Bergethon *et al*., 1996; Ikeda *et al*., 1997*a*, *b*; Parrilla *et al*., 1997; Suhr *et al*., 1997; Pomfret *et al*., 1998), factors affecting the age of onset and clinical features of polyneuropathy among FAP TTR Met30 patients with the same transthyretin mutation need to be determined to refine therapeutic approaches to FAP TTR Met30.

In the present study, we analysed the clinicopathological and genetic features of late-onset FAP TTR Met30 patients in 35 families in Japan, particularly those unrelated to the endemic areas of Japan, and compared them with those of early-onset FAP TTR Met30 patients in endemic areas.

Patients and methods

Patients in this study were from 35 Japanese families with late-onset FAP TTR Met30, sometimes limited to one individual per family. The patients in this study were seen at the Department of Neurology, Nagoya University School of Medicine and its affiliates from 1989 to 1997, and at three familial amyloid polyneuropathy research centres in Japan [Department of Medicine (Neurology), Shinshu University School of Medicine, First Department of Internal Medicine, Kumamoto University School of Medicine and Third Department of Internal Medicine, Miyazaki Medical College], and in addition those collected from information given in the annual report of regional meeting of the Japanese Society of Neurology for 1981–1997. In the latter case, the clinical and genetic information and biopsy information were collected from either the patients' doctors or the hospitals. Inclusion criteria were polyneuropathy, Met30 transthyretin mutation and onset at age 50 years or older. Patients whose two most recent prior generations of family members were related to families in the two Japanese endemic FAP TTR Met30 foci were excluded. Late-onset was designated as age of onset at age 50 years or older based on previous reports (Ikeda *et al*., 1987; Sequeiros *et al*., 1991; Nakazato *et al*., 1992).

Routine examinations

All patients underwent neurological examination, nerve conduction studies, CSF examination, routine blood and urine examinations, ECG, cranial MRI and CT, and other radiological studies.

Nerve conduction

The nerve conduction study was performed using standard methods (Kimura, 1989*a*, *b*).

Nerve biopsy

The sural nerve biopsy was performed as described previously (Sobue *et al*., 1990; Hattori *et al*., 1999): specimens were fixed with 2% glutaraldehyde solution in 0.025% cacodylate buffer (pH 7.4) and processed for semi-thin, ultra-thin or teased-fibre studies. The density of myelinated fibres was assessed directly from toluidine blue-stained transverse semi-thin sections of sural nerve using a computer-assisted image analyser (Luzex FS, Nireco, Tokyo) as described previously (Sobue *et al*., 1989, 1990; Hattori *et al*., 1999). Unmyelinated fibre density was assessed using the same system based on electron microscope photographs (magnification $\times$5000) taken randomly of uranyl acetate-stained transverse ultra-thin sections (Sobue *et al*., 1989; Hattori *et al*., 1999). Isolated single nerve fibres were also prepared from the specimens by teasing, and the condition of each fibre was evaluated pathologically according to criteria described previously (Sobue *et al*., 1989, 1997; Dyck *et al*., 1993) A portion of the biopsy specimen was fixed in 10% buffered formalin solution, embedded in paraffin and observed after

haematoxylin and eosin, Klüver–Barrera and Congo red staining. Congo red staining was assessed using a polarizing microscope.

Autonomic involvement

Autonomic involvement was recorded with the items of orthostatic hypotension, syncope, diarrhoea/constipation, urination, sweating and impotence, which were evaluated in most cases by neurological examinations and patient interviews. Orthostatic hypotension was defined as a fall of 30 mmHg in the systolic blood pressure in the standing position from the recumbent position. Recurrent episodes of diarrhoea and constipation, urinary retention or incontinence, apparent hypohidrosis or episodes of excessive sweating and complaints of impotence were defined as a positive for these autonomic symptoms. $CV_{R–R}$ (coefficient of variation ECG R–R interval) examination of heart rate variation, thermographic examination of skin temperature, tilt-table testing for orthostatic hypotension and urodynamic studies also were performed for assessment in some patients.

Autopsy patients

For the three patients examined at autopsy, the spinal cord, thoracic sympathetic ganglia, lumbar sensory ganglia, sciatic nerves, tibial nerves and sural nerves were removed within 6 h of death, in addition to muscle specimens and the brain. All specimens were fixed in 10% buffered formalin solution, embedded in paraffin, and sections were observed after haematoxylin and eosin, Klüver–Barrera and Congo red staining. To assess neuronal loss in sympathetic and dorsal root ganglia, residual neurons with obvious nucleoli were counted on five 10-μm thick transverse sections. Sections represented every tenth section in a consecutive series and were stained by the Klüver–Barrera method. Neuronal counts were correlated with the control values as described previously (Sobue *et al*., 1990). Portions of the sciatic, tibial and sural nerves were fixed with 2% glutaraldehyde in 0.025% cacodylate buffer and processed for assessment of myelinated fibre density as in the sural nerve biopsy specimens (Sobue *et al*., 1989, 1990). Myelinated fibre loss was determined relative to control values as described previously (Sobue *et al*., 1989, 1990).

Some specimens fixed with 10% buffered formalin and embedded in paraffin were deparaffinized with xylene and processed for immunohistochemical detection of human transthyretin as described previously (Sobue *et al*., 1990). Anti-human transthyretin rabbit polyclonal antibody (Dako, Denmark) was used as the first antibody.

Genetic analysis

Genomic DNA was extracted from the patient's leucocytes, and exon 2 of the transthyretin gene was amplified by PCR (polymerase chain reaction) as described previously (Saraiva *et al*., 1986; Sakaki *et al*., 1989; Nakazato *et al*., 1992). After the PCR product was digested with the restriction enzyme *Nsi*I, we determined whether digestion at the new site was shown on agarose gels stained with ethidium bromide. In some families, asymptomatic carriers with a mutant transthyretin gene were diagnosed. Genetic diagnostic testing was performed after informed consent was obtained.

The study was approved by The Research Ethical Committee of the Department of Neurology, Nagoya University School of Medicine.

Results

Clinical features

The clinical features of the probands in the 35 families are summarized in Table 1. The age of onset was 52–80 years (mean ± SD, 62.7 ± 6.6); subjects included 32 men and three women (male-to-female ratio, 10.7 : 1). Initial symptoms were paraesthesias, usually bilateral in the distal portion of the leg (30 patients), and weakness in distal leg muscles (five patients). Heart failure due to cardiomyopathy occurred in one patient. Autonomic dysfunction was not observed as an initial symptom. The duration of illness from onset to study ranged from 0.5 to 10 years, and neurological assessment was performed during this period. When studied, all patients were ambulatory. Cranial nerve defects including difficulty in swallowing, dysarthria, atrophy and fasciculation of the tongue were only seen in five patients. Muscular weakness and atrophy were noted in the upper and lower limbs from a mild to moderate degree, in a distally accentuated manner. In most patients, muscular wasting was more severe in the lower limbs than in the upper limbs. Somatic sensory impairment was distributed in the pattern of a symmetric polyneuropathy for pain and touch sensation as well as vibration and joint position sense. In most patients, sensory involvement was similar for superficial and deep sensory modalities. Dissociation of sensory impairment between modalities was not prominent, nor did painless burns occur in the areas of impaired sensation. Deep tendon reflexes were diffusely absent in all patients.

Most patients did not complain of autonomic symptoms in the initial phase. Orthostatic hypotension was present in 18 of the 35 patients, but syncopal attacks occurred in three patients. Mild to moderate alternation of diarrhoea and constipation was noted in 13 patients, and mild to moderate disturbances of urination (mainly hesitancy) were present in 15 patients. Decreased sweating, particularly in the legs, was observed in 19 patients. Sexual impotence was encountered in 17 out of 23 male patients assessed. These autonomic symptoms generally were mild to moderate in severity, and did not substantially affect the quality or level of activity of the patient's daily life, except in three patients (patients 22, 26, 35), who had syncopal attacks.

Motor nerve conduction velocity in the median nerves was slowed (range 14–54 m/s; mean ± SD, 40.5 ± 9.7), and the

Table 1 *Clinical features of late-onset FAP TTR Met 30 in Japan (probands in 35 families)*

Patients	Onset age (years)/sex	Initial symptom	Duration from onset (years)	Muscle weakness		Sensory involvement		Autonomic involvement						Cardiac involvement
				UE	LE	Pain/touch	Vibration/ joint sense	OH	Syncope	Diarrohea/ constipation	Urination	Sweating	Impotence	
1	67/M	P	3	1+	2+	3+	3+	0	0	0	+	+	+	0
2	77/M	P,HF	0.5	1+	2+	3+	3+	0	0	0	0	0	+	CH
3	56/M	W	1	2+	3+	1+	3+	+	0	+	0	+	0	CV,CH
4	64/M	P	2	2+	2+	3+	2+	+	0	+	0	+	+	A
5	62/M	P	3	2+	2+	3+	2+	+	0	+	+	+	+	Q,CH
6	61/M	P	4	1+	2+	3+	3+	+	0	0	+	+	+	A,CH
7	52/M	P	4	1+	2+	3+	3+	0	0	0	0	0	0	CH
8	59/M	P	3	0	2+	3+	3+	0	0	+	0	+	+	CH
9	63/M	P,W	1	2+	2+	2+	3+	0	0	0	+	+	+	ND
10	58/M	P	0.6	1+	2+	2+	2+	+	0	0	+	+	+	CV,CH
11	64/M	P,W	3	2+	2+	3+	2+	+	0	+	+	+	+	CV,A,CH
12	56/M	W	1	0	2+	2+	1+	0	0	0	0	0	0	CH
13	61/M	P	10	1+	2+	2+	2+	0	0	0	0	0	ND	CH
14	63/F	P	4	1+	1+	2+	1+	+	0	0	+	ND	−	Q
15	61/M	P	2	1+	1+	3+	2+	+	0	+	+	+	+	CV,CH
16	56/F	P	2	1+	1+	1+	2+	+	0	0	0	+	−	0
17	57/M	P	4	2+	2+	3+	3+	0	0	0	0	ND	0	CV
18	59/M	H	2	2+	2+	3+	3+	+	0	0	0	+	ND	CV,A
19	54/M	P	5	0	1+	1+	1+	0	0	+	0	0	+	0
20	63/M	P	7	1+	1+	3+	3+	+	0	+	+	+	+	CV,CH
21	70/M	P	6	ND	ND	ND	ND	0	0	0	0	+	ND	CV
22	73/M	P	3	1+	1+	3+	3+	+	+	+	+	+	+	CV
23	71/M	P	2	1+	1+	1+	1+	+	0	0	0	0	ND	CV,CH
24	52/M	P	3	1+	1+	2+	1+	0	0	0	0	+	0	CV
25	63/F	P	6	0	1+	2+	2+	0	0	0	0	+	−	CH
26	62/M	P	5	1+	1+	2+	2+	+	+	+	+	0	+	CH,A
27	62/M	P	3	1+	2+	2+	2+	+	0	0	0	+	0	A
28	65/M	P	4	1+	1+	3+	3+	+	0	+	+	+	+	CH,A
29	67/M	P	5	2+	3+	3+	3+	+	0	+	+	0	ND	CV
30	80/M	W	3	1+	3+	3+	3+	0	0	0	+	ND	ND	CV,A
31	69/M	P	2	1+	1+	3+	3+	0	0	0	+	ND	ND	0
32	64/M	P	2.5	1+	2+	2+	2+	0	0	0	0	ND	ND	ND
33	67/M	P	2	2+	2+	3+	3+	+	0	+	0	ND	ND	0
34	62/M	P	1.5	1+	1+	1+	1+	0	0	0	0	0	+	0
35	53/M	P*	7	1+	1+	2+	0	0	+	0	0	ND	+	CV

P = paraesthesias in lower extremities; P* = paraesthesias in upper extremities; H = hypaesthesia in lower extremities; W = weakness in lower extremities; UE = upper extremities; LE = lower extremities; OH = orthostatic hypotension (>30 mmHg fall in systolic pressure); HF = heart failure; ND = not determined; A = arrhythmia; Q = presence of Q wave; CV = conduction abnormalities including bundle branch block, atrioventricular conduction block; CH = cardiac hypertrophy. 0 = absent; + = present. 1+, 2+ and 3+ represent minimal, moderate and severe involvement for muscle weakness and sensory deficits. Sensory involvement was assessed in the distal portion of the legs. Only spontaneous complaints of impotence were noted.

Table 2 *Genetic background of late-onset FAP TTR Met30*

Proband patient	Family history	Symptomatic siblings			Asymptomatic carriers Age (years)/sex	Kinship with Arao or Ogawa village families
		Age of onset (years)/sex	Initial symptom	Autonomic dysfunction		
1	+	68/M,70/M	P	OH	58/M	No
2	0				ND	No
3	0				ND	No
4	0				60/F	No
5	0				28/M,77/M	No
6	0				ND	No
7	0				52/M	No
8	0				56/M,60/F	No
9	+	40/M,52/M,52/M,57/M	P,W	Heart failure	73/F	No
10	0				54/F,56/F	No
11	+	59/M	P	OH, diarrhoea, impotence	41/M	No
12	+	52/M	P	No autonomic symptoms	ND	No
13	0				35/F,65/F	No
14	0				ND	No
15	0				32/F	No
16	0				30/F,33/M,56/F	No
17	+	30/M,51/F,58/M, 62/M,62/F,66/M	P	Diarrhoea	36/F,49/M	No
18	0				ND	No
19	0	72/F,82/F	P	No autonomic symptoms	ND	No
20	0				ND	No
21	0				ND	No
22	0				ND	No
23	+	57/M	P	PH, diarrhoea, impotence	37/M,45/F,61/F,63/F,70/F	No
24	+	59/M	P	No autonomic symptoms	29/F	No
25	0				59/F	No
26					38/F,49/M,69/M,94/F	No
27	0				ND	No
28	0				41/F	No
29	0				ND	No
30	+	71/M	P	No autonomic symptoms	44/F,53/M	No
31	0				ND	No
32	0				ND	No
33	0				40/F	No
34	+	60/M,60/M,62/F,70/M	P	No autonomic symptoms	37/M	No
35	+	60/M,85/F	P	A–V block diarrhoea	47/F	No

+ = symptomatic patients present in family; ND = not determined; P = paraesthesias in lower extremities; W = weakness in lower extremities; OH = orthostatic hypotension. Kinship was assessed for two generations preceding the probands.

distal latency was prolonged (range 3.1–10 ms; mean ± SD, 5.3 ± 2.2). The amplitude of compound muscle action potentials was significantly but variably diminished (range 0.3–6.5 mV; mean ± SD, 2.2 ± 1.8). In the tibial nerves, compound muscle action potentials were diminished (range 0.04–1.9 mV; mean ± SD, 0.4 ± 0.6) when successfully recorded, and no compound muscle action potentials could be elicited in 11 patients. Slowing of the motor nerve conduction velocity (range 22–43 m/s; mean ± SD, 35.2 ± 5.5) and prolonged distal latency (range 5.4–13.6 ms; mean ± SD, 7.3 ± 2.9) were observed when potentials were recorded. Sensory nerve action potentials were not elicited in the median nerves in 13 patients. Sensory conduction velocity was slowed (range 34–56 m/s; mean ± SD, 44.6 ± 8.4) in the remainder. Sensory nerve action potentials were not elicited in the sural nerves except in three patients, who showed slowing (range 33–48 m/s) and low sensory nerve action potential (range 0.6–2 μV). Denervation potentials were detected widely by needle EMG.

The protein content of the CSF was moderately elevated in 10 patients (47–148 mg/dl). CT, MRI and routine blood and urine test results were essentially normal in most patients. Subsequently, one patient developed metastasis of lung cancer to the cerebellum, which was diagnosed by MRI.

Genetic background and family history

The probands proved to have symptomatic siblings in 11 out of 35 families (Table 2). The other 24 probands did not have siblings with manifestations and appeared to have a sporadic

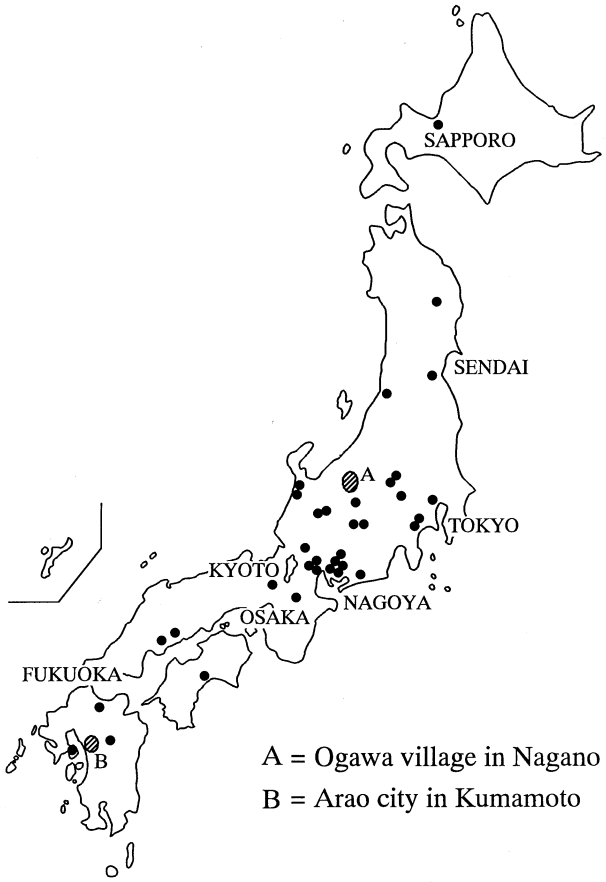

Fig. 1 Geographical distribution of late-onset FAP TTR Met30 in Japan. The 35 families occurred widely throughout Japan.

occurrence of the mutation. The ages of onset in symptomatic siblings were late in life, between 51 and 85 years, except in two siblings, who were 30 and 40 years old. An extreme male preponderance (19 : 6) was noted, as among the probands. The initial symptoms in affected siblings were sensory, such as paraesthesias in the lower legs. No dissociation of impairments by sensory modality was apparent in their sensory examinations. Autonomic nervous dysfunction symptoms did not represent a major complaint in symptomatic siblings which was similar to the situation with probands. Thirty-six asymptomatic carriers were detected in 21 families by DNA analysis. Most of the cases of asymptomatic carriers with TTR Met30 were only assessed by bedside neurological examinations and interview. In a few cases, a nerve conduction study was carried out, and the results were normal. These carriers showed a female preponderance (13 : 23), in contrast to the symptomatic siblings and probands; 18 of the 36 carriers were at least 50 years or older. These features of symptomatic siblings, asymptomatic carriers, and probands indicate that late onset, extreme male preponderance, and mild autonomic symptoms are common among patients in a late-onset FAP TTR Met30 family, and female preponderance is the rule among asymptomatic carriers. The 35 families presented in this study were distributed widely across Japan (Fig. 1). Kinship with families in Arao City in Kumamoto Prefecture or in Ogawa Village in Nagano Prefecture, two large FAP TTR Met30 foci in Japan, was assessed for at least the two generations preceding the probands, but no such relationship was evident in any of the 35 families.

Pathology of the peripheral nervous system

Sural nerve biopsy findings

In the 20 sural nerve biopsy specimens, myelinated fibres were severely diminished (range 79–3466/mm; mean ± SD, 929.9 ± 937.3). Unmyelinated fibre density also was diminished (range 701–16 757/mm; mean ± SD, 7391.8 ± 5165.0), but showed better preservation than myelinated fibres. In teased-fibre preparations, axonal degeneration was predominant (range 11–37%; mean ± SD, 21.9 ± 8.4) and only mild segmental demyelination and remyelination (range 0–19%; mean ± SD, 7.6 ± 6.3) was seen in some patients. The Schwann cell population was increased in number. Amyloid deposits were assessed using the Congo red stain and immunohistochemistry for human transthyretin, and was only detected in 18 patients to a minimal or mild degree in the endoneurium of the sural nerves, either associated or unassociated with the endoneurial capillaries (Fig. 2). These amyloid deposits were extremely limited, particularly when compared with those at autopsy in proximal peripheral nerve trunks, such as the sciatic nerves (Fig. 2). Amorphous material present in the endoneurium that as not stained by Congo red, was stained by the anti-human transthyretin antibody.

Autopsy findings

Spinal motor neurons were well preserved in number, but most showed central chromatolysis. Myelinated fibre loss in the dorsal columns of the spinal cord was seen particularly in the fasciculus gracilis, but was minimal in all three patients. Multiple nodular proliferations of Schwann cells resembling Schwannoma were observed in the ventral sulcus or in the central grey matter of the thoracic spinal cord in two patients. Neuronal loss was evident in the dorsal root ganglia and in the sympathetic ganglia, but was only mild to moderate (Table 3; Fig. 2). Residual nodules of Nageotte were seen frequently, particularly in dorsal root ganglia. Mild to moderate amyloid deposits were noted in dorsal root ganglia and sympathetic ganglia (Table 3; Fig. 2). Myelinated fibre loss was mild in the segmental nerves and was only minimal in the ventral and dorsal spinal roots, but was prominent in the sciatic, tibial and sural nerves (Table 3). Myelinated fibre loss with distal accentuation was seen in peripheral nerve trunks and clusters of myelinated fibres of nerve sprouts were present throughout these trunks. The Schwann cell population was increased, and cytoplasmic process formation was prominent. Amyloid deposits were prominent in the endoneurium of proximal nerve trunks such as the sciatic

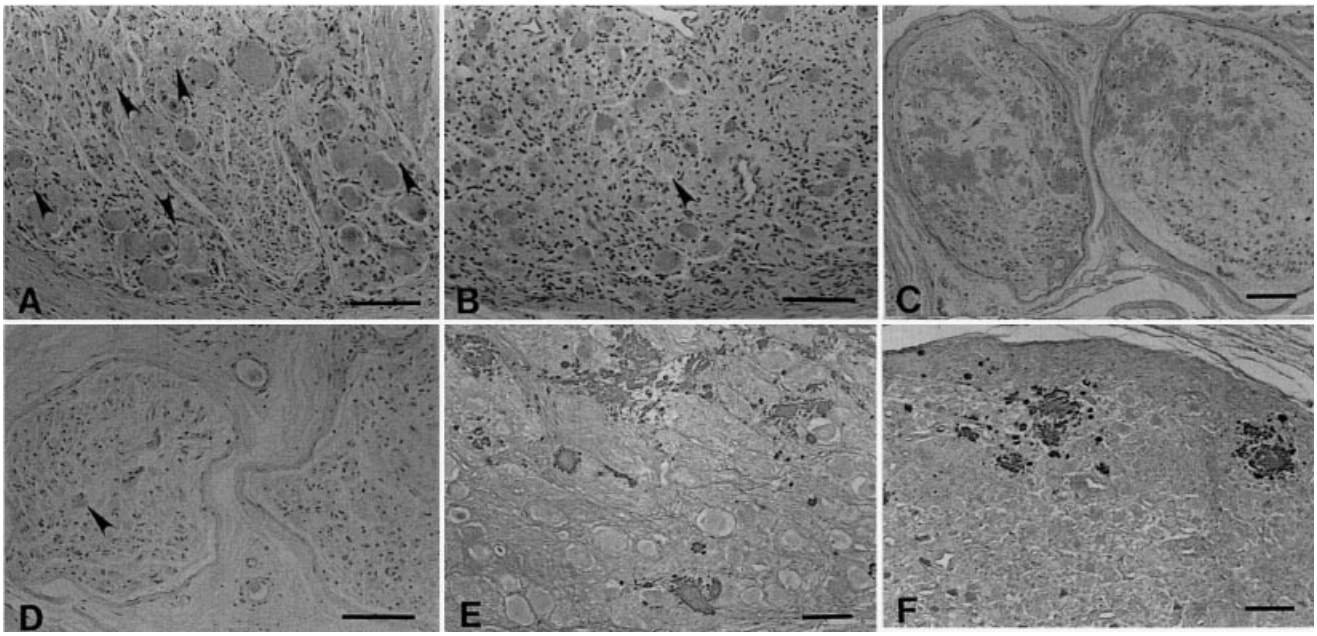

Fig. 2 Amyloid deposits and pathological findings in the peripheral nervous system in late-onset FAP TTR Met30. Dorsal root ganglion neurons (**A**) and sympathetic ganglion neurons (**B**) are relatively well preserved. Arrowheads indicate amyloid deposition. Amyloid deposition is abundant in the endoneurial space of the sciatic nerve (**C**) but minimal in the sural nerve (**D**); arrowhead shows where it is seen in relation to a capillary. Oedematous-amorphous deposits are also present in the sciatic and the sural nerves. Amyloid deposits stained by anti-human transthyretin antibody are present to a small extent in dorsal root ganglia (**E**) and sympathetic ganglia (**F**). All specimens are from patient 5; **D** is from the biopsy specimen. **A**, **B**, **C** and **D**, Congo red stain. **E** and **F**, stained by anti-human transthyretin immunostaining. The bars indicate 100 μm in **A**–**F**.

Table 3 *Autopsy findings in late-onset FAP TTR Met30*

Region	Patient 4		Patient 5		Patient 7	
	Neuron/fibre loss	Amyloid deposits	Neuron/fibre loss	Amyloid deposits	Neuron/fibre loss	Amyloid deposits
Spinal cord						
Motor neurons	0	0	0	0	0	0
Clarke's column neurons	0	0	0	0	0	0
IML neurons	0	0	0	0	0	0
Posterior columns	+1	0	+1	0	+1	0
Sympathetic ganglia	+1	+2	+1	+1/+2	ND	ND
Dorsal root ganglia	+1	+2/+3	+1/+2	+2	+1/+2	+2
Ventral root	0	0	0	0	0	0
Dorsal root	+1	0	+1	0	+1	0
Segmental nerve	ND	ND	+1	+1/+2	ND	ND
Sciatic nerve	+2	+2	+3	+3	+3	+3
Tibial nerve	ND	ND	+3	+3	ND	ND
Sural nerve	+3	+1	+2	+1	+3	+1
Remarks	Central chromatolysis in motor neurons. Schwann cell proliferation in spinal cord. Metastatic tumour in cerebellum. Severe neurogenic change in muscles.		Central chromatolysis in motor neurons. CNS well preserved. Severe neurogenic change in muscles.		Central chromatolysis in motor neurons. CNS well preserved. Schwann cell prpoliferation in spinal cord. Severe neurogenic change in muscles.	

Neuronal loss was assessed as: 0 = no detectable neuronal loss; +1 = mild neuronal loss; +2 = moderate to severe neuronal loss. Myelinated fibre loss was graded as: 0 = no detectable fibre loss; +1 = mild fibre loss up to 30%; +2 = moderate fibre loss up to 70%; +3 = severe fibre loss exceeding 70% of control values. The myelinated fibre population was estimated as described in the Patients and methods section and compared with control values (Sobue *et al*., 1990). Amyloid deposits were assessed by Congo red staining as: 0 = not detectable; +1 = detectable but minimal deposition; 2+ = moderate deposition; +3 = massive deposition. ND = not determined; IML = intermediolateral columns. Two values, when given, describe different segmental levels.

and tibial nerves, but they were minimal or few in the spinal nerve roots and sural nerves (Table 3; Fig. 2). They were frequently associated with endoneurial capillaries. Amorphous material that was not stained by Congo red but was immunoreactive for human transthyretin were present in the endoneurium throughout the nerve trunks. Cellular infiltrates were not observed.

The central nervous system was essentially intact except that a small cell carcinoma of the lung had metastasized to the cerebellum in one patient.

Discussion

The clinical features of late-onset FAP TTR Met30, which had clinical onset at age 50 or later and was unrelated to endemic foci in Japan, included a marked male preponderance, sensorimotor complaints in the legs as an initial symptom, essentially parallel involvement of superficial and deep sensation without sensory dissociation, mild autonomic dysfunction not interfering with activities of daily living of most patients, and little family history of symptomatic siblings. These features resemble those of chronic, sporadic somatic sensorimotor neuropathy of late onset. Before availability of molecular diagnosis, these patients frequently were misdiagnosed with chronic inflammatory demyelinating polyradiculoneuropathy or polyneuropathy of undetermined cause (Ando *et al*., 1993). Corticosteroid therapy had been administered to some patients in the present series under a diagnosis of chronic inflammatory demyelinating polyradiculoneuropathy. The late-onset patients resided in many parts of Japan, as opposed to the two major foci of early-onset FAP TTR Met30 in Arao City and Ogawa Village. Moreover, the ancestors of the siblings were not related to FAP TTR Met30 families in these two Japanese endemic foci. The clinical features of late-onset FAP TTR Met30 differ strikingly from those of early-onset FAP TTR Met30, which occurs in one of the endemic foci (Table 4). Early-onset FAP TTR Met30 generally manifests by a superficial loss of pain sensation leading to painless burns or skin ulcers, early autonomic dysfunction such as alternating diarrhoea and constipation or syncopal attacks due to orthostatic hypotension or A–V (atrioventricular) conduction block (Andrade, 1952; Araki *et al*., 1968; Ikeda *et al*., 1987; Sobue *et al*., 1990; Ando *et al*., 1992; Alves *et al*., 1997). All of these symptoms critically disturb the quality and activities of patients' daily lives (Andrade, 1952; Araki *et al*., 1968; Coutinho *et al*., 1980; Ikeda *et al*., 1987; Sobue *et al*., 1990).

The penetrance rate of early-onset FAP TTR Met30 is very high, representing an essentially completely penetrant autosomal dominant trait. Male preponderance in early-onset FAP TTR Met30 in endemic foci (up to 1.7 : 1) is much less striking than in late-onset FAP TTR Met30 (10.7 : 1 in proband cases). In contrast, the sex ratio in asymptomatic carriers detected late in life in late-onset FAP TTR Met30 families showed an extreme female preponderance. Some non-genetic factors specific to females, such as hormonal milieu, might suppress clinical onset of FAP TTR Met30 more effectively in the late-onset form.

The peripheral nerve pathology of the two onset-defined forms of FAP TTR Met30 includes both similarities and differences. In late-onset FAP TTR Met30, neuronal loss in sensory and sympathetic ganglia occurred, as well as a length-dependent, distally accentuated axonal loss. Amyloid deposition was prominent in proximal nerve trunks and mild in the sural nerves and spinal nerve roots. The spatial distribution pattern of neuronal and fibre loss as well as amyloid deposition in the peripheral nervous system in late-onset FAP TTR Met30 closely resembled that in early-onset FAP TTR Met30 (Said *et al*., 1984; Hanyu *et al*., 1989; Sobue *et al*., 1990; Takahashi *et al*., 1991, 1997), but the pathology differed in severity between these two conditions. Sensory and sympathetic neuronal loss was mild in dorsal root ganglia and sympathetic ganglia, and the amount of amyloid deposition in these ganglia was also rather small in late-onset compared with early-onset FAP TTR Met30, as documented in our previous study and in other reports (Hofer *et al*., 1975; Yamada *et al*., 1984; Ikeda *et al*., 1987; Hanyu *et al*., 1989; Sobue *et al*., 1990; Takahashi *et al*., 1991). Myelinated fibre loss involving distal portions of long axons such as those of sural nerves was as severe in late- as in early-onset FAP TTR Met30 (Ikeda *et al*., 1987; Hanyu *et al*., 1989; Sobue *et al*., 1990; Takahashi *et al*., 1991), but in the late-onset form, unmyelinated fibre loss was milder than myelinated fibre loss, even in the sural nerves. Thus, although the process of amyloid deposition and subsequent axonal and neuronal loss is indistiguishable between the early-onset and late-onset FAP TTR Met30 groups, the pathological extent of severe involvement is less in late- than early-onset FAP TTR Met30. This difference correlates well with the differences in the severity of clinical symptoms particularly of autonomic symptoms, which are milder in late-onset FAP TTR Met30. Takahashi and colleagues have reported that in autopsy cases with late-onset FAP TTR Met30, amyloid deposition was marked in the heart, kidney and thyroid, but was slight to moderate in the peripheral nervous system, including autonomic pathways (Takahashi *et al*., 1997), in agreement with our present pathological findings.

The question arises as to whether differences in pathological involvement between early- and late-onset FAP TTR Met30 result from a difference in the mechanism underlying amyloid deposition or simply from a difference in the duration of the disease process; all three of our patients with late-onset FAP TTR Met30 studied at autopsy had a relatively short disease duration from onset (4–10 years). This issue should be addressed further by assessing the findings according to the disease duration in further selected autopsy cases. However, relatively mild pathological and clinical involvement in late-onset FAP TTR Met30, even at the autopsy stage, may support the view that late-onset FAP TTR Met30 is clinicopathologically distinguishable from

Table 4 *Clinical and pathological features of early- and late-onset FAP TTR Met 30 in Japan*

Clinical and pathological features	Early-onset	Late-onset
Clinical features		
Age of onset (years)	33–37 (average)	52–80
Geographic distribution	Endemic	Sporadic
Family history	Common	Rare
Penetrance rate	High	Low
Sex ratio (M : F)	1–1.7 : 1	10.7 : 1
Initial symptoms	Sympathetic dysfunction Sensory deficit	Sensory deficit
Sensory dissociation	Common	Rare
Autonomic dysfunction in early stages	Severe with ADL disturbance	Mild without ADL disturbance
Bulbar signs	Rare	5/35
Pathological findings in the peripheral nerves		
Loss of sensory neurons	Severe	Mild to moderate
Loss of sympathetic neurons	Severe	Mild to moderate
Loss of myelinated fibres		
Sciatic nerve	Severe	Severe
Sural nerve	Severe	Severe
Loss of unmyelinated fibres		
Sural nerve	Severe	Moderate
Amyloid deposition		
DRG	Severe	Moderate
Sympathetic ganglia	Severe	Moderate
Sciatic nerve	Severe	Severe
Sural nerve	Mild to moderate	Mild to moderate

Clinical and pathological features of early-onset disease were summarized based on previous reports of patients from two large Japanese endemic foci in Arao City in Kumamoto Prefecture and Ogawa Village in Negano Prefecture, for patients with onset at 40 years or less (Araki *et al*., 1968; Kito *et al*., 1980; Ikeda *et al*., 1987; Sobue *et al*., 1990; Takahashi *et al*., 1991; Ando *et al*., 1992). Clinical and pathological features of late-onset disease were summarized based on our probands. ADL = activities of daily living; DRG = dorsal root ganglia.

early-onset FAP TTR Met30 and should be considered as a distinct clinicopathological condition.

Another important issue is the identification of factors determining the contrasting clinicopathological manifestations among the FAP TTR Met30 cases with the same Met30 transthyretin mutation. Symptomatic siblings in our late-onset FAP TTR Met30 families generally had late onset of symptoms resembling those in the probands. Furthermore, most asymptomatic carriers in their families were identified late in life. Such familial clustering of relatively old symptomatic siblings and asymptomatic carriers in a late-onset family suggests the presence of a haplotype background differing between the late-onset and early-onset FAP TTR Met30 families and capable of influencing gene expression or tissue deposition of mutant transthyretin. FAP TTR Met30 patients with Met30 in Swedish endemic foci have been reported to have a relatively late onset and relatively mild clinical manifestations, as well as a low rate of penetrance (Andersson, 1976; Benson and Cohen, 1977; Drugge, 1993; Sousa *et al*., 1993); their clinical features are also dissimilar to those of Japanese early-onset FAP TTR Met30 patients in endemic foci (Araki *et al*., 1968; Ikeda *et al*., 1987). On the other hand, age of onset and phenotype of patients in an endemic area in Portugal with Met30 are similar to those of early-onset FAP TTR Met30 patients in Japan (Andrade, 1952; Coutinho *et al*., 1980; Sousa *et al*., 1995). These phenotypic similarities and dissimilarities of FAP TTR Met30 cases involving the same transthyretin mutation in different ethnic populations also appear to reflect different genetic backgrounds. Interestingly, Portuguese FAP TTR Met30 patients whose parents are both unaffected show a tendency toward a later onset and a somewhat atypical geographical distribution, suggesting that the *FAP* gene may have a lower expression in certain families (Coelho *et al*., 1994). For elucidation of the genetic background of these phenotypic differences, the late- and early-onset FAP TTR Met30 families with similar ethnic backgrounds in the Japanese population would be an effective model for haplotype analysis.

Another possible explanation for these phenotypic differences involves environmental factors. Discordant phenotypic expression of FAP TTR Met30 in monozygotic twins supports this view (Holmgren *et al*., 1997). Transgenic mice carrying the human mutant transthyretin gene express the phenotype of amyloid deposition in non-neural organs (Yi *et al*., 1991; Araki *et al*., 1994; Kohno *et al*., 1997) but not in peripheral nerves (Yi *et al*., 1991; Araki *et al*., 1994). In these transgenic mice, amyloid deposition was observed in different organs at different times in the course of the experimental disorder, despite a consistently high plasma level of mutant transthyretin (Yi *et al*., 1991; Takaoka

et al., 1997). These results suggest that environmental factors unrelated to the mutant gene are involved in the distribution and timing of amyloid deposition in each organ. When these transgenic mice are maintained under germ-free conditions, amyloid deposition is significantly inhibited, suggesting that external environmental conditions can influence phenotypic expression. A feminized environment in terms of gonadal factors may also influence phenotypic expression, particularly in late-onset FAP TTR Met30, as mentioned above. Proteins binding to transthyretin such as T4 (thyroxine) (Kohno *et al*., 1997) or retinol-binding protein (Kohno *et al*., 1997), as well as oxidative stress in tissues (Ando *et al*., 1997), may modify amyloid deposition. Recently, post-translational modification of the transthyretin protein has been suggested as a factor in determining tissue amyloid deposition (Suhr *et al*., 1998), specifically the ratio of the free form to the cysteineylated form of transthyretin in plasma (Ando *et al*., 1998). Many environmental and genetic factors could also influence these binding protein expression levels. The factors differentiating phenotypic expression among ethnic Portuguese, Swedish and Japanese populations could be at least partly environmental. Elucidation of factors influencing age of onset and phenotype is important in devising an optimally effective therapeutic approach for FAP TTR Met30 capable of ameliorating symptoms or delaying onset.

As we found in the present study, the wide geographical distribution of cases and the difficulty of correct diagnosis without genetic testing suggest that late-onset FAP TTR Met30 is more prevalent in Japan and elsewhere than is generally believed. The early-onset form is considered to be the prototype of FAP TTR Met30. However, although further studies should be performed with respect to haplotype and environmental factors, one might speculate that late-onset FAP TTR Met30 with low penetrance may have been the original form of this Met30 transthyretin mutation. Unknown genetic or environmental factors might have induced an early-onset form with a high penetrance rate in two large endemic foci in Japan. Anticipation of the age of onset observed in the FAP TTR Met30 families in two endemic foci in Japan (Tashima *et al*., 1995; Yamamoto *et al*., 1998) would relate to this speculation.

In summary, a distinctive type of FAP TTR Met30 with a late onset exists in Japan. This form is a clinicopathologically mild, somatic sensorimotor neuropathy. Often occurring sporadically, it has a wide distribution throughout Japan that is unrelated to the two endemic foci. A genetic difference is likely to separate the late-onset group from early-onset families, but environmental factors should also be considered.

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