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Familial amyloidotic polyneuropathy (ATTR Ser50Ile): the first autopsy case report

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Abstract We report an autopsy case of a pedigree of familial amyloidotic polyneuropathy (FAP) with a mutation of isoleucine-50 transthyretin (ATTR Ser50Ile). A 47-year-old man started developing severe diarrhea and weight loss at age 41 years, followed by urinary incontinence, autonomic-nervous-system abnormalities and serious heart failure; the diagnosis of FAP (ATTR Ser50Ile) was made on the basis of genetic, histochemical and immunohistochemical analysis. Six years after the initial symptoms, he died of septic shock. Autopsy revealed suppurative peritonitis, perforation of the sigmoid colon and marked systemic amyloid deposition. The total amount of amyloid deposited in the heart was greatly increased and was much lower in the thyroid gland and kidneys compared with amyloid deposits in ordinary FAP (ATTR Val30Met). Amyloid deposition in peripheral vessel walls was prominent, particularly in lymphatics and veins. His elder sister, 54 years old, started to develop orthostatic hypotension at age 49 years, followed by dysesthesia, diarrhea and severe congestive heart failure. Endomyocardial biopsy revealed severe TTR–amyloid deposition; ultrastructural examination demonstrated that amyloid fibrils were deposited disproportionately and extended radially around microvessels. These characteristic patterns of systemic amyloid deposition and distinct clinical manifestations, especially in the cardiovascular system, are considered to be a characteristic feature of ATTR Ser50Ile amyloidosis.

Key words Amyloidosis · ATTR Ser50Ile · Autopsy · Familial amyloidotic polyneuropathy · Transthyretin

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Introduction

Familial amyloidotic polyneuropathy (FAP) is a generic term for a heterogeneous group of familial diseases characterized by progressive peripheral polyneuropathy. The precursor proteins of FAP include mutant transthyretin (TTR), apolipoprotein AI and gelsolin [2, 3, 5]. The most common TTR mutation in this disorder is the single amino acid substitution of the 30th valine with methionine (ATTR Val30Met). In addition to this common type, more than 70 other mutations in TTR have been reported, including cases without pathological abnormalities [5, 8]. Although most of these mutant TTRs can form amyloid fibrils and result in serious clinical symptoms, their manifestations are extremely different [5, 8]. The major symptoms of the common FAP (ATTR Val30Met) are peripheral neuropathy, including disturbances of the autonomic nervous system, and insufficiency of visceral organs, such as heart and renal failure. However, the severity of these manifestations varies, and some patients lack heart or renal failure [1]. In contrast, some types of FAP, such as FAP (ATTR Ala45Thr), FAP (ATTR Ala45Asp), FAP (ATTR Ser50Ile), FAP (ATTR Thr59Lys), FAP (ATTR Thr60Ala), FAP (ATTR Ile68Leu), FAP (ATTR Glu89Gln), FAP (ATTR Ile111Met) and FAP (ATTR Val122Ile), are characterized by severe heart failure rather than peripheral neuropathy [8]. FAP (ATTR Ser50Ile) patients commonly present with serious heart failure, fatal arrhythmia and cardiac-conduction block; however, peripheral neuropathy is less frequent, as was well documented in previous case studies [4, 6, 7]. On the basis of clinico-pathologi-

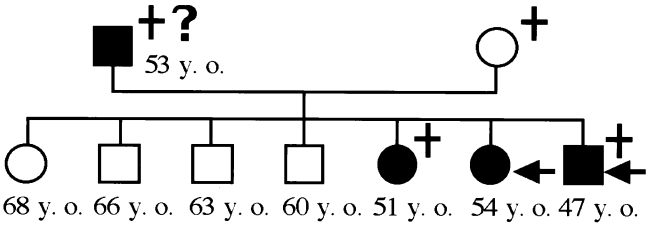

Fig. 1 Family pedigree of two cases with familial amyloidotic polyneuropathy (FAP) (ATTR Ser50Ile). □ Male; ○ female; ●, ■ symptomatic FAP; ? presumed affected FAP; † dead. *Arrows* indicate cases described in this report. The age of death of the patient's mother is unknown

cal features in autopsy cases of the common FAP (ATTR Val30Met), it was established that the severity of clinical symptoms is closely related to the quantity of amyloid deposited [9, 10]. In addition, although there are only a few case reports describing autopsy findings in other types of TTR-related FAP [11, 12], the documented findings were limited. Little is known about the pathological and ultrastructural changes of FAP (ATTR Ser50Ile).

The present case study describes clinico-pathological features based on autopsy findings of FAP (ATTR Ser50Ile). Autopsy findings and amyloid deposition are recorded and compared with those of the common type of FAP (ATTR Val30Met), especially for the cardiovascular system. Furthermore, ultrastructural findings of the endomyocardial biopsy from the subject's sister are shown.

Clinical history

A 47-year-old man started to develop uncontrolled diarrhea and weight loss at age 41 years, followed by urinary incontinence, severe heart failure, autonomic nervous symptoms and sensorimotor disturbance. His father complained of similar symptoms, especially heart failure, and died. However, the final diagnosis of FAP was not settled. Two elder sisters closer in age to the subject also suffered from FAP, and one of them died of FAP. However, we could not determine any details of her clinical history (Fig. 1). His mother and other older siblings did not have this disease. FAP (ATTR Ser50Ile) was diagnosed on the basis of histochemical and immunohistochemical analysis of rectal-biopsy specimens and genetic examination of peripheral leukocytes of the patient using the polymerase-chain-reaction-restriction-fragment length-polymorphism (PCR-RFLP) method. PCR-RFLP analysis revealed a mutation in the transthyretin gene: a T-to-A substitution resulting in a single amino acid substitution of serine by isoleucine at the 50th residue of the amino acid sequence of normal TTR. After 6 years, his symptoms gradually deteriorated and he became bedridden from age 47 years on. Because his severe diarrhea caused uncontrolled weight loss, he received daily nutrition via an intravenous hyperalimentation technique. Six years after the initial symptoms, he had sudden high fever and acute abdominal pain and died of septic shock.

His elder sister, 54 years old, noticed orthostatic hypotension and constipation at age 49 years. Severe congestive heart failure, glove-and-stocking dysesthesia and diarrhea occurred at age 51 years. Chest X-rays and echocardiographic examinations revealed marked cardiomegaly (a chest:thorax ratio of 64%), and cardiac catheterization studies revealed poor myocardial kinetics. Pathological stenosis was not demonstrated in coronary arteries. Electrocardiographic study showed a first-degree atrioventricular-conduction block and myocardial damage. Endomyocardial biopsy was performed, and specimens were submitted for electron microscopy. The patient underwent pacemaker implantation, which partially relieved her cardiac symptoms.

Materials and methods

All tissues were removed from the autopsy case, fixed in 10% formalin and embedded in paraffin. In addition to processing for routine histological examination, paraffin sections were stained via the Congo-red method (with or without potassium permanganate treatment). Congo-red-stained sections were observed with a polarization microscope to detect an apple-green birefringence emitted from amyloid deposits.

The indirect immunoperoxidase method using mouse monoclonal antibodies for human TTR, amyloid A (AA), λ-chain and κ-chain as primary antibodies was used to determine amyloid precursor proteins; all antisera were purchased from DAKO Japan (Kyoto, Japan). To block endogenous peroxidase activity, 3-μm-thick de-paraffinized sections were immersed for 10 min in methanol containing 0.3% hydrogen peroxide. Each primary antibody was applied to the specimens for 1 h at room temperature; specimens were washed in phosphate-buffered saline and reacted with appropriate peroxidase-labeled secondary antibodies for 45 min. After the specimens were rinsed with phosphate-buffered saline, the immunoreaction was visualized by using 3,3-diaminobenzidine solution containing hydrogen peroxide. To ascertain specific immunoreaction, all immunostaining was carried out by comparing appropriate positive and negative control studies; no positive immunoreaction was seen in the background when primary antibodies were omitted.

For electron microscopy, the endomyocardial-biopsy specimen was fixed in 2.5% glutaraldehyde for 2 h and was washed in cacodylate buffer. The specimen was cut into small blocks, postfixed in 1% osmium tetroxide for 1 h and dehydrated with a graded series of ethanol and propylene oxide solutions. These blocks were embedded in Epok 812. Ultra-thin sections were cut by using an ultra-microtome (MT7000 ULTRA; Tucson, AZ, USA) and observed with an H-7500 electron microscope (Hitachi, Tokyo, Japan) after staining with uranyl acetate and lead citrate. For comparison, tissue specimens obtained from two cases of ATTR Val30Met amyloidosis underwent the similar processing for electron microscopy.

Pathologic findings

Autopsy revealed suppurative peritonitis due to perforation of the sigmoid colon, acute splenitis, myeloid hyper-

Fig. 2 Histochemical demonstration of amyloid deposits in familial amyloidotic polyneuropathy (FAP) (ATTR Ser50Ile) and FAP (ATTR Val30Met). All micrographs demonstrate results of Congo-red staining. A great amount of amyloid, which emits green birefringence under polarized light (B), is seen in the endocardial and interstitial regions of the heart in an FAP (ATTR Ser50Ile) patient (A). **C** Less amyloid is deposited in the heart in patients with FAP (ATTR Val30Met). In the kidney, amyloid deposition is prominent in the glomerulus and around vessels in patients with FAP (ATTR Val30Met) (E), but no amyloid is shown in the glomerulus in FAP (ATTR Ser50Ile) (D). *Asterisks* in **D** and **E** indicate vessels, and *arrowheads* point at glomeruli. **F** Amyloid in the thyroid gland is mainly observed in the vessel walls (*asterisks*) in FAP (ATTR Ser50Ile), and there are additional deposits in the interfollicular space in FAP (ATTR Val30Met) (G). **I** In the peripheral vessels in digestive tracts, clear amyloid deposits are seen in the vascular walls (*arrowheads*) but not lymphatics (*asterisks*) in FAP (ATTR Val30Met) patients, but amyloids are mainly observed around lymphatics in patients with FAP (ATTR Ser50Ile) (H). Magnification: **A–C** ×45; **D**, **E**, **H, I** ×70; **F, G** ×90

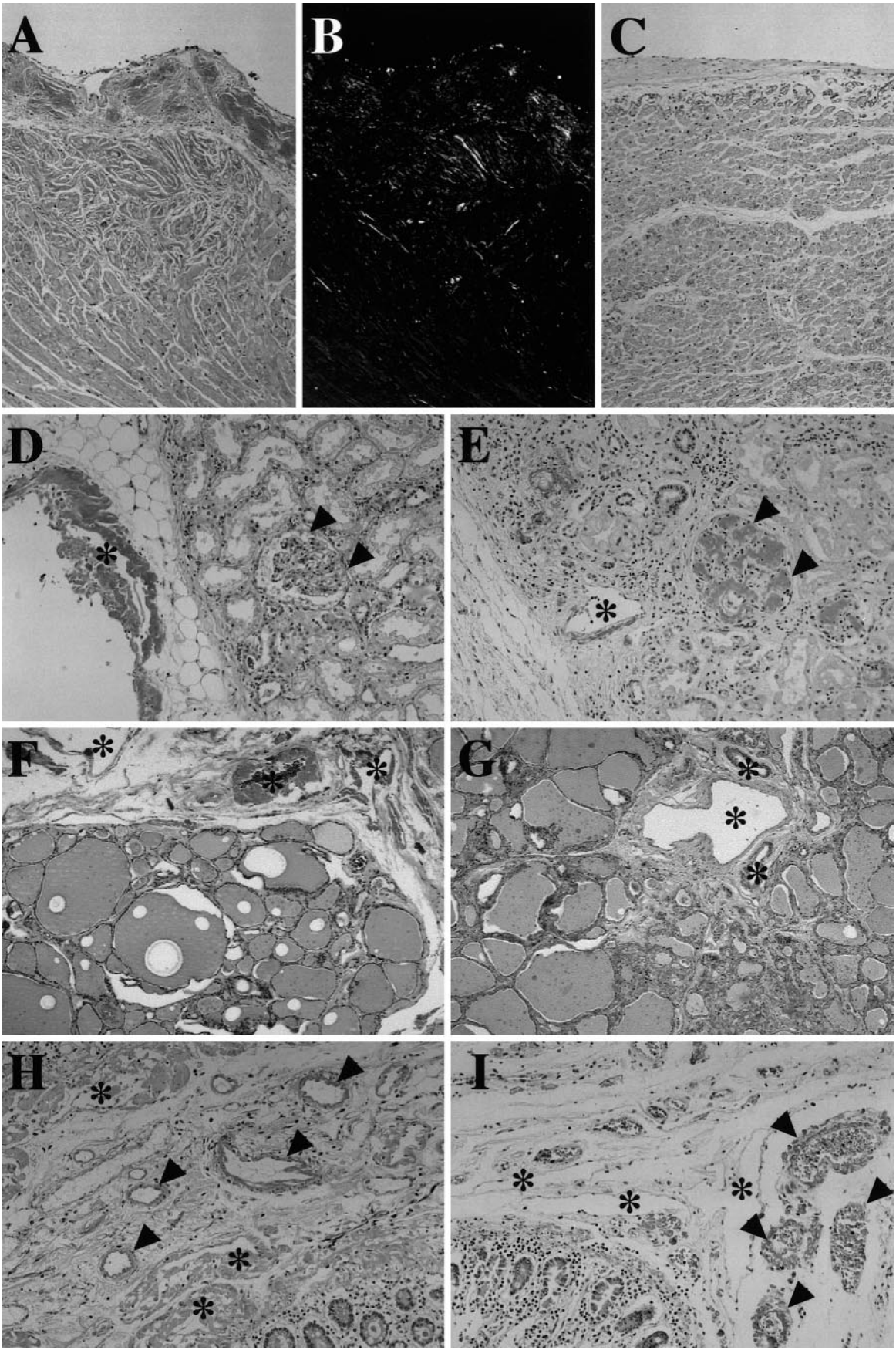
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Table 1 Comparison of amyloid deposition in various organs and tissues between familial amyloidotic polyneuropathy (FAP) (ATTR Ser50Ile) and FAP (ATTR Val30Met), – Absent; *0* vascular wall only; *1–3* both vascular wall and *extracellular interstitium* (*1* slight; *2* moderate; *3* marked; *4* extremely marked)

Tissue (site)	FAP (ATTR Ser50Ile)	FAP (ATTR Val30Met)[a]
Heart	4	2
Lungs		
Alveoli	1	0
Interstitium	1	0
Liver		
Lobule	–	–
Periportal	0	0
Spleen	1	1
Pancreas		
Interstitium	2	2
Islet	1	–
Kidneys		
Cortex	0	2
Medulla	0	1
Adrenals	0	1
Thyroid gland	1	3
Testis	1	1
Cerebrum	–	–
Cerebellum	–	–
Arachnoid membrane	–	1
Choroid plexus	0	3
Spinal cord	–	–
Sciatic nerve	2	3

[a] These data are based on 31 autopsy cases, including 17 previously reported cases [9] and 14 unreported cases

plasia in the bone marrow and marked systemic amyloid deposition. Major pathological findings leading to the direct cause of death were a perforation (3 cm×4 cm in diameter) of the sigmoid colon and perforative diffuse peritonitis; the ascites was suppurative and contained stool. Thick purulence covered the pelvic wall. Histologically, the perforated site of the sigmoid colon was necrotic, accompanied by prominent atrophy of the proper muscle layer in the periphery of the perforation. Amyloid deposition in various organs and tissues is summarized in Table 1 and compared with that in typical FAP (ATTR Val30Met). Massive amyloid deposits in the heart stained positively for Congo-red and emitted a green birefringence under polarized light (Fig. 2A–C). Considerable amyloid deposition was found in peripheral nerves (including the autonomic nervous system) and in the digestive tract, lungs, bile-duct system and testis. However, amyloid deposition was slight in the thyroid gland, kidneys (Fig. 2D–G), adrenal glands, liver and spleen (data not shown). Amyloid deposition in peripheral nerves was not different in the two types of FAP (data not shown). In the peripheral vascular system, massive amyloid deposits were around veins and lymphatics; however, deposition was negligible around arteries and in arterial walls (Fig. 2H, I). This peculiar amyloid deposition around small vessels was also shown around the perforation site of the sigmoid colon (data not shown). Immunohistochemical examination demonstrated that the massive amyloid deposits were composed of TTR but not AA, λ- or κ-chain (data not shown).

Electron microscopy showed that amyloid fibrils accumulated just outside the basement membrane of capillaries in the myocardial interstitium (Fig. 3A). In FAP (ATTR Val30Met), amyloid fibrils were uniformly deposited around microvessels (Fig. 3B); this observation is identical to that of our previous investigation of 17 autopsy cases with FAP (ATTR Val30Met) [9]. However, in FAP (ATTR Ser50Ile), amyloid fibrils were not uniformly distributed; most of fibrils near the vessels were short, and there were many long fibrils in regions distant from the vessels (Fig. 3C, D).

Discussion

This report has described clinico-pathological findings for two cases of FAP with the single amino acid mutation ATTR Ser50Ile and has demonstrated a systemic amyloid deposition in various organs and tissues of an autopsy case. Initial signs and symptoms in FAP (ATTR Ser50Ile) do not differ from those of the typical FAP (ATTR Val30Met). However, cardiac symptoms, especially congestive heart failure, became prominent in the early clinical course. Consistent with the present cases, previous reports noted (1) that the most critical problems in this type mutation were severe cardiac failure and fatal arrhythmia and (2) that pacemaker implantation could improve prognosis [6]. Our present examination revealed significant amyloid deposition in the cardiovascular system, similar to that described in previous autopsy reports of Ser50Arg and Tyr114Cys types of TTR-related FAP [11, 12]. The total amount of amyloid in the heart of the present autopsy case was extremely large, compared with that in 20 cases of FAP (ATTR Val30Met) previously reported in our laboratory [9, 10]. In the FAP (ATTR Val30Met), amyloid accumulation in the vascular system is predominant around arteries, especially in the adventitia of arterioles, and shows a fine granular pattern. In contrast, amyloid deposition in the present cases was massive in both veins and lymphatics. In contrast, it was absent or negligible around arteries and arterioles. Amyloid in the venous wall is prominent in the region of atrophic proper muscle layer around the necrotic perforation focus. Furthermore, such amyloid distribution in the vascular system was not observed in our previous autopsy cases of FAP (ATTR Val30Met), and colonic perforation is extremely uncommon in the ordinary type [9, 10]. This suggests that the unique amyloid deposition in the vascular system of the present case might be related to the atrophic colonic wall and the perforation of the sigmoid colon. Ultrastructural observation in the present case revealed an uneven distribution of amyloid fibrils. All these observations suggest that the mechanisms of amyloid formation in the ATTR Ser50Ile type of FAP are different from those in typical ATTR Val30Met FAP.

Generally speaking, the systemic deposition of amyloid is common for TTR-related FAP but differs in its

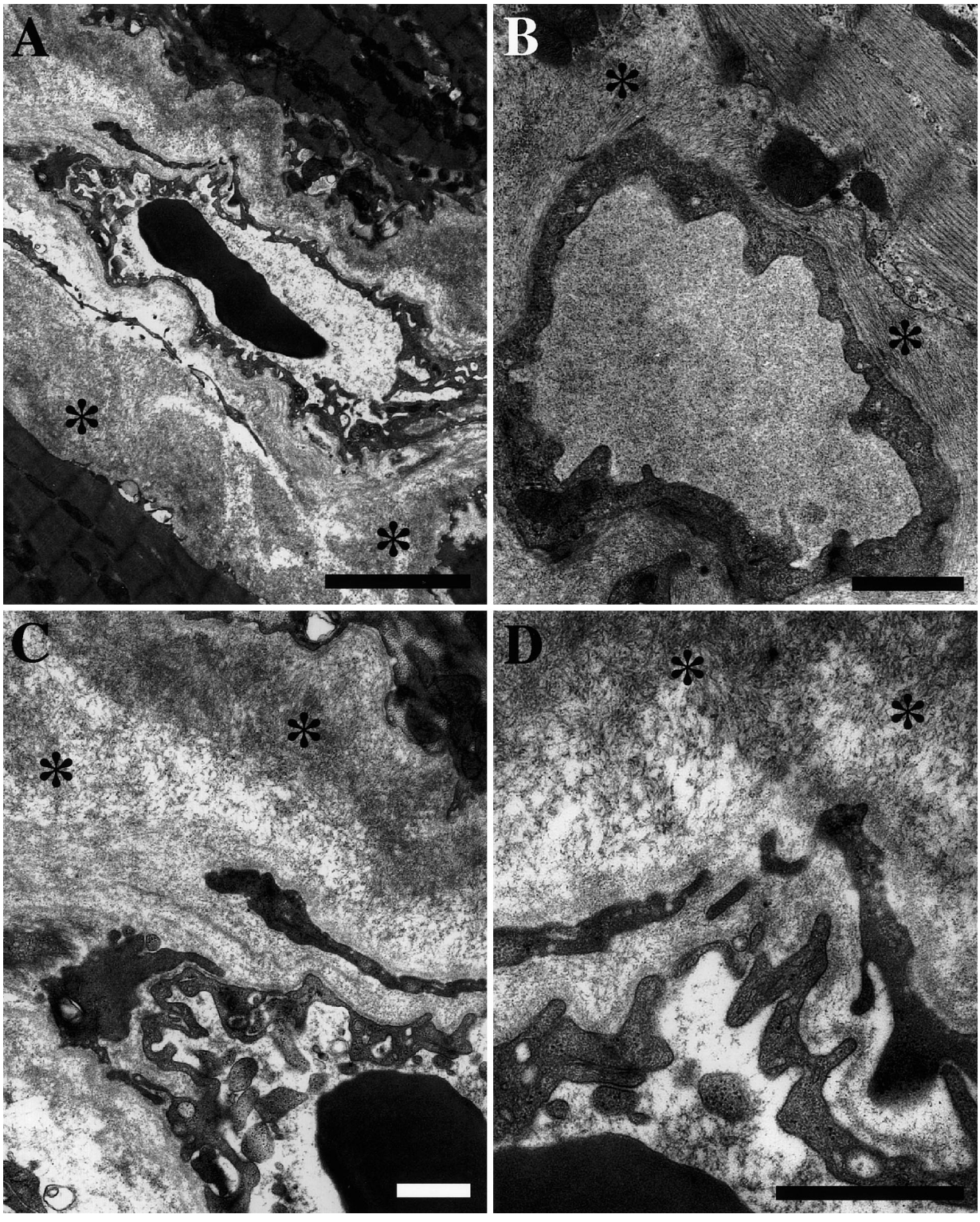

Fig. 3 Ultrastructure of amyloid fibrils in the endomyocardium of cases with familial amyloidotic polyneuropathy (FAP) (ATTR Ser50Ile). All micrographs demonstrate intermyocardial capillaries and amyloid fibrils; **A**, **C**, and **D** are from the FAP (ATTR Ser50Ile) patient; **B** is from an FAP (ATTR Val30Met) patient. *Asterisks* in each micrograph indicate amyloid fibrils. Amyloid fibrils in both types of FAP are found just around capillaries. Uniform fibrils are seen in FAP (ATTR Val30Met) (B); however, fibrils in FAP (ATTR Ser50Ile) are not uniform, and extending fibrils are seen in regions distant from capillaries (A, C and D). *Scale bar:* **A** 5 μm; *other bars* 1 μm

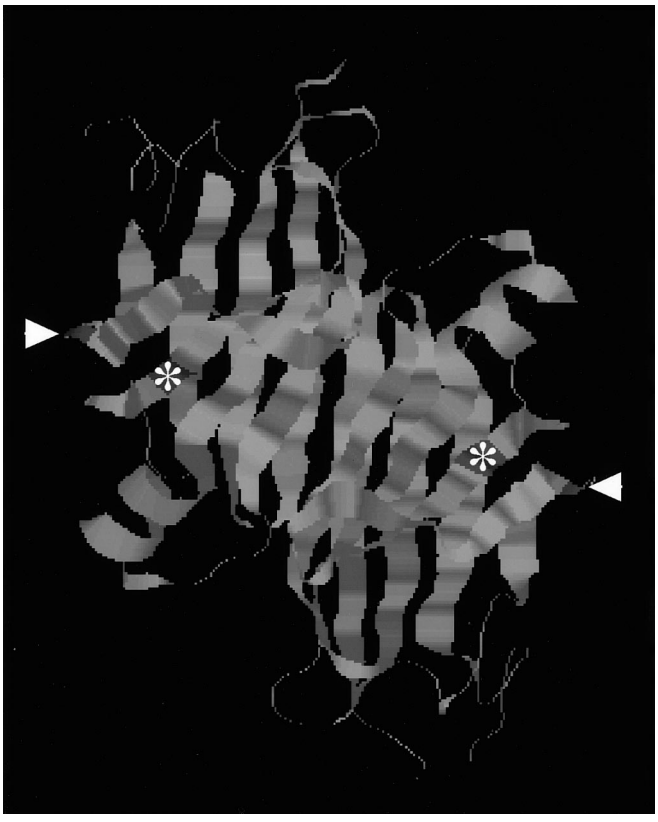

Fig. 4 Mutation points of mutant transthyretin (ATTR) Val30Met and ATTR Ser50Ile in the dimer form of the normal TTR molecule. The mutation in ATTR Val30Met is at the mid-portion of the b-strand of the molecule (*asterisks*), and ATTR Ser50Ile is located at the start of the c-strand (*arrowheads*)

distribution pattern among the different mutations. For example, although amyloid deposits in alveolar walls and pancreatic islets were quite rare in FAP (ATTR Val30Met), a small but significant amount of amyloid was found in these areas in the present case. A large amount of amyloid in the thyroid gland and kidneys (especially glomeruli) is known to be common in the FAP (ATTR Val30Met). However, in FAP (ATTR Ser50Ile), amyloid deposition in these organs is extremely small. Previous reports of FAP (ATTR Ser50Ile) showed that clinical manifestations were compatible with these characteristic distributions of amyloid deposition and described no renal failure [4, 6]. Because the pathological features in both FAPs described in this paper were based on autopsy samples and because the patients seemed to be in the end stage of the disease, there is little possibility that these clinico-pathological differences between FAP (ATTR Ser50Ile) and FAP (ATTR Val30Met) may be involved in the clinical stage. On the contrary, as shown in Fig. 4, a mutation point in ATTR Val30Met is located in the mid-portion of the b-strand of the molecule, whereas a mutation in ATTR Ser50Ile is found at the start of c-strand of the molecule. This discrepancy might be related to the differences in the patterns of amyloid deposition between FAP (ATTR Val30Met) and FAP (ATTR Ser50Ile).

In summary, we have reported pathological features of an autopsy case of the FAP (ATTR Ser50Ile). This case was characterized clinically by peripheral neuropathy, severe cardiac failure and autonomic nervous-system symptoms and revealed marked cardiac amyloid deposition with a unique pattern in the vascular system. Ultrastructural observation of an endomyocardial biopsy specimen from his elder sister demonstrated a characteristic distribution of amyloid fibrils around intermyocardial capillaries. However, amyloid deposition in the thyroid and kidneys of the present autopsy case was not prominent and is sharply contrasted with the massive amyloid involvement in both organs found in the common FAP (ATTR Val30Met). These features suggest that the mechanisms of amyloid formation are different between the ATTR Ser50Ile and ATTR Val30Met amyloidosis types. To confirm this suggestion, subsequent studies of the autopsy materials of further accumulated cases of FAP (ATTR Ser50Ile) are required.

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