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The morphology of amyloid fibrils and their impact on tissue damage in hereditary transthyretin amyloidosis: An ultrastructural study

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ABSTRACT

Introduction: We evaluated the morphology of amyloid fibrils in the peripheral nervous system using biopsy or autopsy specimens from hereditary transthyretin amyloidosis patients. The impact of amyloid fibril formation on neighboring tissues was also investigated.

Methods: Sural nerve biopsy specimens from 34 patients were examined using electron microscopy. Twenty-eight patients had Val30Met mutations, and the remaining 6 patients had non-Val30Met mutations (i.e., Glu54Lys, Pro24Ser, Thr49Ala, Val71Ala, Val94Gly, and Ala97Gly). The patients with the Val30Met mutation included a case from Brazil (supposedly of Portuguese origin), 6 early-onset cases from endemic foci in Japan, and 21 late-onset cases from non-endemic areas in Japan.

Results: Long amyloid fibers were abundant in the early-onset Val30Met cases from the Japanese endemic foci and Brazil, whereas the amyloid fibrils were generally short in the late-onset Val30Met and non-Val30Met cases. The amyloid fibrils seemed to mature from dotty structures among amorphous electron-dense extracellular materials and pull surrounding tissues during the maturation process. The distortion of Schwann cells close to amyloid fibril masses was conspicuous, particularly in cases with long amyloid fibrils. Atrophy was conspicuous in non-myelinating Schwann cells and bands of Büngner (i.e., Schwann cell subunits that previously contained myelinated axons), particularly those completely surrounded by amyloid fibrils. In contrast, the myelinated fibers tended to be only partially surrounded by amyloid fibrils and morphologically preserved due to their large size. Only a few demyelinated axons were found.

Conclusion: Pre-fibrillar amyloid precursors appear to play a pivotal role during the initial phase of amyloid fibril formation. The mechanical distortion and subsequent atrophy of Schwann cells resulting from the elongation of amyloid fibrils may be related to small-fiber predominant loss, which is a classical characteristic of amyloid neuropathy. Although rather rare, the destruction of myelin (i.e., demyelination) resulting from amyloid deposition may relate to nerve conduction abnormalities mimicking chronic inflammatory demyelinating polyneuropathy.

1. Introduction

Hereditary (variant) transthyretin (ATTRv) amyloidosis, also known as familial amyloid polyneuropathy (FAP), is a disease in which systemic deposition of amyloidogenic mutant TTR protein causes multi-organ failure. Although Val30Met is the most common mutation in ATTRv amyloidosis, over 130 other mutations have been reported thus far [1]. The TTR protein is mainly produced in the liver but is also produced in the choroid plexus and retinal pigment epithelium and is stable in the homotetramer form [2]. The dissociation of TTR tetramers is a crucial step in the formation of amyloid fibrils [2]. The process of amyloid formation in ATTRv amyloidosis has been well investigated in vitro, and a therapeutic strategy to stabilize TTR tetramers in the plasma is now available in clinical practice [3].

However, the in vivo mechanisms of tissue damage resulting from mutant TTR have not been fully elucidated. Although some studies highlight the toxicity of pre-fibrillar TTR (i.e., the precursor of amyloid fibrils) [4–7], the amyloid deposits are more widely believed to exert harmful effects on neighboring tissues [7–11]. Previous studies have demonstrated differences in the morphology of amyloid fibrils in

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ATTRv amyloidosis patients with the Val30Met mutation depending on the age at onset [7,12,13]. In Japanese ATTRv amyloidosis patients with the Val30Met mutation, long and thick amyloid fibrils are common in early-onset cases from endemic foci, whereas the fibrils are usually short and thin in late-onset cases from non-endemic areas [7,13]. As the neuropathic features and modality of the nerve fiber loss in these two forms of ATTRv amyloidosis are distinct [6,14], the difference in the morphology of the amyloid fibrils may be strongly related to the mechanisms of its neuropathy.

In the present study, we evaluated the electron microscopic morphology of amyloid fibrils in the peripheral nervous system using biopsy or autopsy specimens from various types of ATTRv amyloidosis patients. We also evaluated the impact of the amyloid fibrils on neighboring tissues in these patients.

2. Patients and methods

2.1. Patients

Biopsy or autopsy specimens from 34 patients with ATTRv amyloidosis who were referred to Nagoya University Graduate School of Medicine and exhibited endoneurial amyloid deposits on electron microscopic examinations were investigated. The patients included 28 patients with the Val30Met mutation and 6 patients with non-Val30Met mutations (i.e., Glu54Lys, Pro24Ser, Thr49Ala, Val71Ala, Val94Gly, and Ala97Gly) (Table 1). The patients with the Val30Met mutation included a 42-year-old man from Brazil, 6 early-onset cases from endemic foci in Japan, and 21 late-onset cases from non-endemic areas in Japan. Since the case from Brazil was a first-generation immigrant to Japan without any apparent Japanese blood relatives, his disease was considered to have originated in Portugal [15]. Except for the patients with the Val94Gly and Ala97Gly mutations, who were autopsied, the specimens were obtained at diagnostic sural nerve biopsy. Autopsy findings in Val94Gly and Ala97Gly patients were previously published [16,17]. In these patients, cardiac amyloid deposition was conspicuous as in late-onset Val30Met patients from non-endemic areas of Japan [6]. Amyloid deposition and myelinated fibrer loss in the ventral and dorsal spinal roots were minimal or not apparent. Mild to moderate degrees of amyloid deposition and neuronal loss in the parenchyma of the dorsal root ganglia and thoracic sympathetic ganglia were seen. In the median and sciatic/tibial nerves, amyloid deposition in the endoneurium was mild to moderate, and myelinated fiber loss was severe in both patients. Six early-onset Val30Met cases from endemic foci in Japan and 19 late-onset Val30Met cases from non-endemic areas in Japan were included in a previous study [7]. Informed consent was obtained, and this study was approved by the Ethics Committees of Nagoya University Graduate School of Medicine and conformed to the Ethical Guidelines for Medical and Health Research Involving Human Subjects endorsed by the Japanese government.

2.2. Pathological assessment

Biopsy of the sural nerve was performed as previously described [6,7,18]. The specimens were divided into two portions. The first portion was fixed in 2.5% glutaraldehyde in 0.125 M cacodylate buffer (pH 7.4) and embedded in epoxy resin for ultrastructural assessments. The epoxy resin-embedded specimens were cut into ultra-thin transverse sections and stained with uranyl acetate and lead citrate for electron microscopic observation. In this study, the morphology of the amyloid fibrils in the endoneurium was assessed, and at least 6 fascicles were assessed in each case. In this study, amyloid deposits were defined as aggregations of non-branching fibrils in the extracellular space [7]. In addition to the amyloid fibrils, the morphology of the Schwann cells was examined, focusing on their relationship to neighboring amyloid deposits. Schwann cells associated with unmyelinated fibers were designated non-myelinating Schwann cells in this study. The bands of Büngner, which were defined as Schwann cell subunits that previously contained myelinated axons, were distinguished by the following morphologic criteria [19]: (1) the subunits had a larger diameter (3 to 8 μm) than the subunits that formerly contained unmyelinated axons; (2) their profiles were larger than those of the subunits of the non-myelinating Schwann cells; (3) they contained remnants of myelin and lamellated inclusions, suggesting the presence of degeneration; and (4) their shape was more irregular, and their basement membrane was folded. The second portion of the specimen was fixed in a 10% formalin solution and embedded in paraffin. The sections were cut by routine methods and stained with hematoxylin and eosin and Congo red.

Among the patients with the Val94Gly and Ala97Gly mutations who were autopsied, the median and sciatic/tibial nerves were obtained and processed as previously described [20].

3. Results

3.1. Morphology of amyloid fibrils

In general, amyloid deposits were observed both with and without a relationship to the endoneurial microvessels, although they tended to be present around them. As described in previous studies [7,13], the morphology of the amyloid fibrils of the Japanese early-onset Val30Met cases from endemic foci were distinctly different from the late-onset Val30Met cases from non-endemic areas. The amyloid deposits were burred in the low-power view and mainly consisted of long and thick

Table 1
Summary of patients.

Mutation	Number of patients	Region of origin	Sex	Age at onset (years)	Age at biopsy/autopsy (years)	Morphology of* amyloid fibrils	Atrophy of Schwann cells apposed to amyloid fibrils*
Val30Met	6	Endemic foci in Japan	4 M and 2 F	32.8 ± 4.5**	35.7 ± 4.3**	Long	Conspicuous
Val30Met	21	Non-endemic areas in Japan	19 M and 2 F	64.6 ± 7.5**	68.6 ± 7.6**	Short***	Inconspicuous
Val30Met	1	Brazil	M	39	42	Long	Conspicuous
Glu54Lys	1	Japan	F	42	44	Short	Inconspicuous
Pro24Ser	1	Japan	M	70	75	Short	Inconspicuous
Thr49Ala	1	Japan	F	49	54	Short	Inconspicuous
Val71Ala	1	Brazil	F	31	35	Short	Inconspicuous
Val94Gly	1	Japan	F	58	65	Short	Inconspicuous
Ala97Gly	1	Japan	M	56	74	Short	Inconspicuous

* Sural nerve biopsy specimens were assessed in all patients, except for the patients with the Val94Gly and Ala97Gly mutations. Autopsy specimens of the median and sciatic/tibial nerves were assessed in patients with the Val94Gly and Ala97Gly mutations.

** Values are expressed as the mean ± SD.

*** Long fibrils were also found in three patients.