"PrP systemic deposition disease": clinical and pathological characteristics of novel familial prion disease with 2-bp deletion in codon 178

Kosuke Matsuzono[1], MD; Hiroyuki Honda[2], MD, PhD; Kota Sato[1], MD, PhD; Ryuta Morihara[1], MD; Kentaro Deguchi[1]; MD, PhD; Nozomi Hishikawa[1], MD, PhD; Toru Yamashita[1], MD, PhD; Syoichiro Kono[1], MD, PhD; Yasuyuki Ohta[1], MD, PhD; Toru Iwaki[2], MD, PhD; and Koji Abe[1]*, MD, PhD

[1]Department of Neurology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Science, Okayama, Japan.
[2] Department of Neuropathology, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan

*Correspondence to Professor Koji Abe, Department of Neurology, Okayama University Graduate School of Medicine and Dentistry, 2-5-1 Shikata-cho, Okayama, 700-8558, Japan
Tel: +81-86-235-7365, Fax: +81-86-235-7368
E-mail: kmatsuzono51@gmail.com

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Abstract

(Background and purpose) A novel TYPE of prion disease associated mainly with autonomic-sensory polyneuropathy was reported by us previously.

(Methods) Here the autopsy pathology for patient 1 (the sister) and the clinical characteristics of her younger brother (patient 2) are newly reported. Polymerase chain reaction based restriction fragment length polymorphism analysis of the prion protein gene (*PRNP)* was performed on both patients and their father (normal control).

(Results) Polymerase chain reaction based restriction fragment length polymorphism analysis revealed a 2-bp deletion (CT) in codon 178 that causes an additional variable 25 amino acids at the C-terminal, from the mutation site to the premature stop codon at codon 203 in both patient 1 and patient 2 but not in their father. The autopsy of patient 1 showed remarkable prion protein (PrP) deposits in sympathetic ganglion and peripheral nerves, correlating to her severe autonomic sensory failure. PrP deposits were also found in the central nervous system and peripheral organs such as the heart, lung, stomach, jejunum, ileum, colon, urinary bladder, and adrenal gland. The symptoms and biopsy findings of patient 2 were nearly the same as those reported previously for patient 1. His cognitive function was well preserved, but autonomic functions were severely impaired. His biopsied samples showed PrP deposits in the sural nerve and nerve plexuses of the stomach and colon.

(Conclusion) The present unique 2-bp deletion (CT) in codon 178 induced “PrP systemic deposition disease” such as pan-autonomic failure, sensory neuropathy, and mild cognitive impairment with a specific pathology.

Introduction

In most prion diseases, cognitive functions are commonly affected and the symptoms usually worsen quickly in a short period. However, a novel type of prion disease has recently been reported by us and others that is associated mainly with autonomic-sensory polyneuropathy [1-4].

Methods

Our experience was with two patients (patient 1, sister; patient 2, brother). Their mother also had similar symptoms; the clinical findings and molecular genetics of patient 1 at age 34 were reported previously [1]. After our first report, patient 1's younger brother (patient 2) developed the same symptoms (Fig. 1a), and patient 1 died at age 37.

Clinical examinations of patient 2 and pathological analysis of the autopsied samples of patient 1 were performed, and the details were described in Appendix S1. Polymerase chain reaction (PCR)-based restriction fragment length polymorphism (RFLP) analysis of the prion protein gene (*PRNP*) was performed using the BssSI, a restriction enzyme, in Patient 1, Patient 2, and their father (normal control control).

All of the described analysis were performed with the written consent of the patients and their family. The Ethical Committee of Okayama University approved this study, and the study obtained exempted approval from the institutional review board based on our guideline because an anonymized and untraceable dataset was used (approval number 1819).

Results

Clinical characteristics

After our first report [1], the Patient 1's cognitive function declined from 27/30 on the Mini-Mental State Examination at 34 years to 22/30 at age 36 years. In addition, her heart function decreased to 39.1% of ejection fraction at 37 years of age. Because of heart failure, impaired absorption, and hypothermia (body temperature < 35.0 ˚C), she suffered severe pneumonia and died when 37 years old (11 years after the onset). No abnormal intensities were found on magnetic resonance imaging including diffusion-weighted imaging throughout her disease course of 11 years (Fig. 1c).

Table S1 shows the clinical characteristics of patient 2, her younger brother. He began to suffer from frequent diarrhea at age 20. At age 28, he developed syncope due to orthostatic hypotension. He began to suffer from urinary retention at age 30, vomiting at age 32 and thermoanesthesia at age 34.

Polymerase chain reaction RFLP analysis of *PRNP* with BssSI showed a 2-bp deletion (CT) in codon 178 that causes an additional variable 25 amino acids at the C-terminal, from the mutation site to the premature stop codon, in both Patient 1 and 2 (Fig. 1b).

Pathological analysis of the autopsied samples of Patient 1

Histological examination of the cerebral cortices showed severe spongiosis and neuropil degeneration (Fig. 1d). Immunohistochemical analysis with anti-prion protein (anti-PrP) showed coarse deposits in the cerebral cortex (Fig. 1e, upper and middle), but not in the white matter (Fig. 1e, lower). PrP was also deposited along the small vessels of the cerebral cortex (Fig. 1f, arrows), but not in white matter, the artery, or arteriole which have muscular layer.

PrP was also deposited in the spinal cord and ganglions (Fig. 1g-l). Remarkable PrP deposits were also found in her femoral and sural peripheral nerves. Double

immunofluorescence (Fig. 2a-f) also revealed PrP deposits in peripheral nerves.

Abnormal PrP deposits were also detected in almost all other organs. In the heart, PrP accumulated in the myocardium and epicardial nerve fibers (Fig. 2g, h). Unlike the normal control (Fig. 2i, arrow), immunostaining for tyrosine hydroxylase revealed a marked loss of positive nerve fibers of patient 1 (Fig. 2j, arrow). In the digestive tracts, PrP was deposited in the muscularis mucosa (Fig. 2k, arrowhead), muscularis propria, Meissner's plexus (Fig. 2l), and Auerbach's plexus (Fig. 2m), but not in the mucosa. In the urinary bladder, PrP was deposited in the muscularis propria (Fig. 2n) and nerve plexus of the bladder.

Immunohistological analysis of patient 2

Similar to patient 1, anti-3F4 staining in patient 2 revealed remarkable deposits of PrP in his biopsied sural nerve (Fig. 1m). PrP was also deposited in the muscularis mucosa, muscularis propria, Meissner's plexus and Auerbach's plexus in his biopsied stomach and colon.

Discussion

Our reported familial cases with a 2-bp deletion in codon 178 showed mild cognitive impairment with negative magnetic resonance imaging, but severe autonomic and sensory neuropathy. Of great interest is that a different type of *PRNP* mutation, a GAC→AAC point mutation at codon 178, the same position as our report, induced different phenotypes [5]. Related to the pathology of PrP systemic deposition, our cases induced with the *PRNP* mutation can be referred as “PrP systemic deposition disease”. The present familial cases provide a very important suggestion for elucidating an exact mechanism of this particular

prion disease.

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Disclosure

No disclosures.

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Figure legends

Fig. 1) (a) Family tree showing the patient 1 (=sister, arrow), patient 2 (=brother, arrow), their mother, and grandfather suffering from the same disease. (b) BssSI restriction site selectively detects a gene mutation induced with the 2-bp (CT) deletion in codon 178. PCR-RFLP analysis, shows only 567-bp PCR products in the father (normal control), but 567-bp and 280-bp (arrowhead) bands in patient 1 and patient 2 after BssSI digestion. (c) (f) The cerebrum and (g) (f) spinal cord regions of patient 1. (c) Cranial diffusion-weighted imaging at age 37, showing no abnormal signal intensity, in contrast to (d) severe spongiosis and neuropil degeneration of the autopsied brain with haematoxylin and eosin staining. (e) Immunohistochemical analysis with anti-PrP antibody 3F4 showing PrP deposits in the cerebral cortex (upper and middle) but not in the white matter (lower). (f) Coarse granular PrP deposits are present in neuropils and along the small vessels in the cerebral cortex (arrows). PrP deposits in (g) the anterior horn, (h) posterior horn, (i) ventral root, (j) dorsal root, (k) dorsal root ganglion, and (l) sympathetic ganglion. The amounts of PrP deposits were higher in posterior horn, dorsal root, and sympathetic ganglion. (m) Abnormal PrP depositions were detected in the sural nerves of patient 2. Scale bars = 100 μm.

Fig. 2) Double immunofluorescence labeling of the femoral nerve: (a) (c) with S-100 (green) plus PrP (red) antibodies and (d) (f) with myelin basic protein (MBP, green) plus PrP (red) antibodies in sagittal. (c), (f) The merge demonstrates some PrP deposits (arrow). (g) (n) Immunohistochemical analysis of patient 1's systemic organs. Anti-PrP antibody 3F4 showing abnormal PrP deposits (g) in the myocardium and (h) in the epicardial nerve fibers. In contrast to the immunostaining for tyrosine hydroxylase in epicardial nerve fiber

of the normal control (i, arrow), there is severe reduction of tyrosine-hydroxylase-positive fibers in patient 1 (j, arrow). PrP deposits in (k, arrowhead) the muscularis mucosa of the ileum, (l) Meissner's plexus of ileum, (m) Auerbach's plexus of the jejunum and (n) the muscularis propria of the urinary bladder. Scale bars = 100 μm.