Case Study: Somatic Sprouts and Halo-Like Amorphous Materials of the Purkinje Cells in Huntington’s Disease

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Abstract

We described a 63-year-old Japanese female with genetically confirmed Huntington's disease who showed unusual pathological findings in the cerebellum. This case exhibited typical neuropathological features as Huntington's disease, including severe degeneration of the neostriatum and widespread occurrence of ubiquitin and expanded polyglutamine-positive neuronal intranuclear and intracytoplasmic inclusions. The cerebellum was macroscopically unremarkable; however, somatic sprouts and halo-like amorphous materials of Purkinje cell with a large amount of torpedoes were noteworthy. Furthermore, the Purkinje cells were found to have granular cytoplasmic inclusions. Somatic sprouting is a form of degenerated Purkinje cell exhibited in several specific conditions. Although this finding is usually appeared in developmental brains, several neurodegenerative disorders, including Menkes kinky hair disease, familial spinocerebellar ataxia, acute encephalopathy linked to familial hemiplegic migraine, and several other conditions, have been reported showing sprouting from the soma of Purkinje cell. We propose that Huntington's disease is another degenerative condition associated with these distinct neuropathological findings of Purkinje cell. Abnormally

accumulated huntingtin protein in the cytoplasm could be related to development of these structures.

(170 words)

Keywords: Huntington’s disease; somatic sprout; cactus; halo-like amorphous material; torpedo

Introduction

Huntington's disease is a neurodegenerative disorder clinically characterized by autosomal dominant inheritance, progressive dementia and choreic involuntary movement, and a CAG repeat expansion in *huntingtin* (*HTT*) gene [1]. Severe involvement of neostriatum (caudate nucleus and putamen) in addition to widespread neuronal nuclear and cytoplasmic deposits of abnormal huntingtin is noteworthy neuropathological features. Cerebellar involvement in this disorder remains controversial; however, some case studies demonstrated generalized cerebellar atrophy, loss of Purkinje cell, presence of torpedoes, and neuron loss in the cerebellar nuclei [2–5].

Here, we described an autopsy case of genetically confirmed Huntington's disease showing somatic sprouts and halo-like amorphous materials of Purkinje cell with a large amount of torpedoes.

Case report

A 63-year-old Japanese woman developed choreic involuntary movement on the upper limbs and gait disturbance at the age of 43. Although details were uncertain, her father

and elder brother demonstrated similar neurological features. She fell down frequently from the age of 50, and was admitted to our hospital at the age of 51. On examination, she could not respond properly due to severe dementia. Hasegawa's dementia rating scale was 4 out of 30. She spoke in whispers. Choreic involuntary movements were evident in the bilateral fingers and lower extremities. The extremities were hypotonic. Muscle strength was mildly impaired. Hyperreflexia was shown with extensor plantar reflex. No apparent sensory disturbance was observed. She could not continue sitting because of involuntary movement. Brain computed tomography revealed atrophy of the bilateral caudate nuclei. Under agreement of genetic analysis from her relatives, an abnormal CAG repeat expansion on *HTT* gene (46/18) was revealed. She became bed ridden at the age of 58. Severe atrophy in the cerebrum and basal ganglia was demonstrated on brain magnetic resonance imaging at the age of 62. One year later, she died of pneumonia. The whole clinical course was 20 years.

A general autopsy was performed 7 hours after death. The brain showing mild frontal atrophy weighed 900 g before fixation. On macroscopic examinations, lateral ventricles were moderately dilated. Atrophy and brownish discoloration were

conspicuous on the basal ganglia. The brain stem and cerebellum appeared to be unremarkable (Figure 1a).

The brain and spinal cord were fixed with 10% buffered formalin. For histological examination, multiple tissue blocks were embedded in paraffin, and sections of 6 μm thick were cut and stained with hematoxylin and eosin (H&E) and Klüver-Barrera (KB), and Holzer. Selected sections were also immunostained using the ABC method with a Vectastain ABC kit (Vector, Burlingame, CA, USA). Diaminobenzidine was used as the chromogen. The immunostained sections were counterstained with hematoxylin. The primary antibodies used were rabbit polyclonal antibodies against glial fibrillary acidic protein (GFAP; Dako; Glöstrup, Denmark; 1:6000), and ubiquitin (Dako; 1:15000), rabbit monoclonal antibody against S100 beta (Abcam; Cambridge, MA; 1:50000), and mouse monoclonal antibodies against phosphorylated neurofilament (SMI-31; Covance; Emeryville, CA; 1:500), calbindin D-28k (Swant; Switzerland; 1:4000), expanded polyglutamine (1C2; Millipore; Billerica, MA; 1:30000), phosphorylated tau (AT8; Innogenetics; Ghent, Belgium; 1:2000), and amyloid β protein (4G8; Covance; 1:5000). Appropriate antigen retrieval methods were

applied to each primary antibody.

Microscopically, severe neuronal loss and gliosis was observed in the neostriatum more apparent in the dorsal and caudal part (Vonsattel grade 4) [1]. The globus pallidus and amygdaloid nucleus showed moderate neuron loss with gliosis. Some degrees of neuron loss and glial proliferation were shown in the cerebral cortex, claustrum, medial part of the thalamus, and the substantia nigra. Although neither loss of Purkinje cells nor proliferation of Bergmann's glia were evident, several Purkinje cells were found to have somatic sprouts (Figure 1b). Halo-like amorphous materials were also seen around the Purkinje cell (Figure 1c). Sprouting from the Purkinje cell soma was evident in the sections stained with carbindin D-28k immunostaining (Figures 1d). Some amounts of cactus were also observed (Figure 1e). Furthermore, there was a large amount of torpedo (Figures 1f). Molecular layer, granule cells, and dentate nucleus were microscopically unremarkable. Widespread presence of 1C2 and ubiquitin-positive intracytoplasmic and intranuclear structures was shown in the central nervous system (Figure 1g, h). Most of the Purkinje cells had 1C2-positive granular cytoplasmic inclusions (Figure 1g), however, intranuclear inclusions were less amount in the

restricted areas (Figure 1h). No nuclear inclusions were demonstrated in the Purkinje cells. Furthermore, there were no S100 beta-positive cytoplasmic inclusions in the Purkinje cells. Regarding accumulation of other pathological proteins, small amount of amyloid β protein-positive leptomeningeal and cortical blood vessels were observed in the cerebral cortex. There were few phosphorylated-tau positive pretangles in the entorhinal cortex and amygdaloid nucleus.

Discussion

Ataxia is usually difficult to be recognized as clinical features in patients with Huntington's disease as a result of noticeable involuntary movement. Neither limb nor gait ataxia were manifested in our patient. Although the cerebellum was macroscopically unremarkable, there were several noteworthy microscopic findings such as the Purkinje cells with somatic sprouts and halo-like amorphous materials. Interestingly, regardless of the number of Purkinje cells was preserved, numerous torpedoes were clearly shown. No apparent proliferation of Bergmann's glia was also observed. Cerebellar atrophy with severe Purkinje cell loss was disclosed by neuropathological investigation in cases of

Huntington's disease [2, 3], although details of the pathomechanisms of Purkinje cell damages have been uncertain. Poly-glutamine expanded abnormal huntingtin could be related to neuronal damages. As shown in our case, abnormal protein accumulation was usually found to be in the nucleus and cytoplasm of Purkinje cell [5]. Mice model of Huntington's disease revealed Purkinje cell death and dysfunction of the neurons in addition to accumulation of the mutant huntingtin within the Purkinje cells [6]. Not only Purkinje cell loss, but nerve cell damages of the fastigal, globose, emboliform, and dentate nuclei were reported in autopsy cases of Huntington's disease [5]. It is important to examine cerebellar signs and pathological changes in cerebellum to confirm cerebellar involvement in patients with Huntington's disease.

The most noteworthy finding of this case is somatic sprouts and halo-like amorphous materials of the Purkinje cells. Details of pathomechanism in developing somatic sprouts are still uncertain. Newly-formed dendrites from Purkinje cell soma appear to be normally present in younger cerebellum as a course of development [7] and recovery from traumatisms [8]. Several degenerative disorders have been reported to be associated with somatic sprouts of Purkinje cell [9–13], however, pathomechanisms of

development of sprouting have never been clarified in detail. Fine structure of somatic sprouts in Menkes-kinky hair disease showed connection to presynaptic endings [9]. Although electron microscopic examination in spinocerebellar type 31 cases is inadequate, halo-like amorphous materials were immunoreactive to synaptophysin [11]. These results lead to an idea that somatic sprouts seems to represent a state of regression of the neuron in the central nervous system in addition to recover of synaptic connection. A pathological study in cases of spinocerebellar type 31 exhibited that the fragmentation of the Golgi apparatus occurred in the Purkinje cells with halo-like structure [14].

Most of the Purkinje cells in our case had normal appearance in H&E stain in spite of the cytoplasmic inclusions in the Purkinje cell. Dysfunction of Purkinje cell could precede morphological changes. Numerous torpedoes, which are related to neurofilament accumulation in the axons of Purkinje cell, were another conspicuous neuropathological finding. Normal functions of huntingtin have been unknown so far, however, disruption of axonal transport is one of molecular pathomechanisms of this fatal disease [1]. Further investigation is necessary to confirm relationship between mutant protein accumulation and morphological changes of Purkinje cell.

In conclusion, we proposed that Huntington's disease is another disease to demonstrate somatic sprouts and halo-like amorphous materials in Purkinje cell. These specific forms of degeneration seem to represent a state of regression stemmed from abnormal cytoplasmic protein accumulation.

(1,270 words)

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Compliance with ethical standards

The authors declare that they have no conflict of interest.

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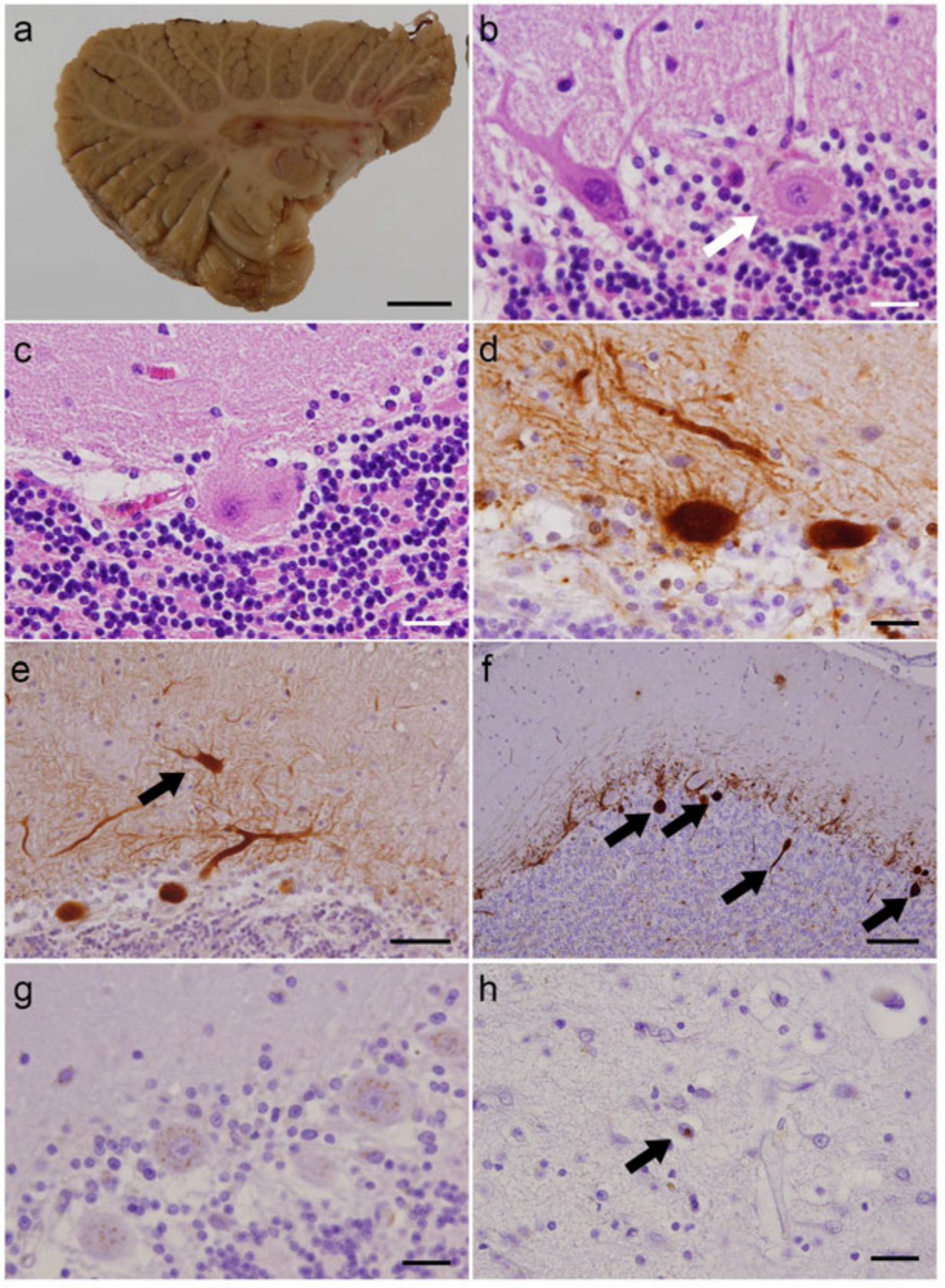
Figure legend

Figure 1. Postmortem neuropathological findings. (a) Medial view of the left cerebellar hemisphere. The cerebellar hemisphere and the dentate nucleus appear to be unremarkable. (b) A Purkinje cell showing sprouting around the soma (arrow). (c) Another Purkinje cell with halo-like amorphous materials surrounding the cytoplasm. (d) Fine processes protrudes around the Purkinje cell soma. (e) A cactus formation in the cerebellar molecular layer (arrow). (f) Numerous torpedoes are shown (arrows). (g) The 1C2 antibody reveals granular cytoplasmic inclusions in the Purkinje cells. (h) A neuronal nuclear inclusion in the putamen. H&E (b, c); immunostaining for carbindin D-28k (d, e), phosphorylated-neurofilament (SMI-31) (f), expanded polyglutamine (1C2) (g), and ubiquitin (h). Bars = 1 cm (a); 20 μm (b–d, g, h); 50 μm (e); 100 μm (F).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