CASE REPORT

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Neuropathological and molecular studies of spinocerebellar ataxia type 6 (SCA6)

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Abstract SCA6 is an autosomal dominant spinocerebellar ataxia (SCA) caused by a small CAG repeat expansion of the gene encoding an α-1A-voltage-dependent Ca channel gene subunit on chromosome 19p13. A Japanese woman with SCA6, with a 7-year history of progressive pure cerebellar ataxia, died of malignant lymphoma. Systematic neuropathological examination showed that neuronal degeneration was confined to the cerebellar Purkinje cells and, to a lesser degree, the granular cells, without any involvement of other central nervous system structures. Such pathological selectivity correlates with the localized expression of the responsible gene, and coincides with the neurological manifestation. These findings might contribute to establishing the phenotype of the SCA6 via comparison with other dominant ataxias.

Key words Spinocerebellar ataxia type 6 (SCA6) · Dominantly inherited ataxia · Late-onset pure cerebellar ataxia · Purkinje cell degeneration

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Introduction

Spinocerebellar ataxia (SCA) with dominant inheritance has been classified at least into seven subgroups according to the order of gene mapping and cloning: SCA1 is located on chromosome 6p22-p23 [17], SCA2 on chromosome 12q24.1 [10, 18, 20], Machado-Joseph disease (MJD/SCA3) on chromosome 14q32.1 [3, 12], SCA4 on chromosome 16q22.1 [4], SCA5 on chromosome 11 [19], SCA6 on chromosome 19p13 [26], and SCA7 on chromosome 3p12-p21.1 [1, 5]. Five of these seven dominant SCA are now known to be caused by dynamic mutation of CAG repeat expansion in the coding region of each gene.

Clinically, SCA6 manifests pure cerebellar ataxia with symptoms generally starting in the third to fifth decades of life [26]. The disorder is a genetically defined subgroup in "pure" autosomal dominant cerebellar ataxia of late onset, which has been classified as autosomal dominant cerebellar ataxia (ADCA) type III by Harding [7], and is now known to be a genetically heterogeneous disease. SCA6 is an allelic disorder of familial hemiplegic migraine and episodic ataxia (EA-2) [16] and is caused by a small expansion of CAG repeat in the coding region of the gene encoding α-1A-voltage-dependent Ca channel subunit (CACNL1A4) on chromosome 19p13 [26]: CAG repeat size was 21–27 in mutant SCA6 alleles $(n = 8)$ and 4–16 in control alleles ($n = 950$). The CACNL1A4 gene is selectively expressed in the central nervous system, predominantly in the cerebellum.

The reported clinicopathological findings on SCA6 have provided a good knowledge base for understanding the function of the gene and the pathomechanism of the disease. Few neuropathological details have been reported, however, due both to the rarity of the disease and the lack of genetic analysis of the patients' families. Here, we report an autopsy case of a patient with hereditary spinocerebellar disease, retrospectively identified as SCA6. In a study by Yagishita and Inoue [24], many of the SCAs except for SCA6 were shown to have dysfunction or pathological changes extending beyond the cerebellum to involve basal ganglia function, olivary-pontine degeneration, spinal cord changes, oculomotor disturbance, and neuropathy. Interestingly, the same study found that SCA6 was unique in its distribution of the degeneration, with only Purkinje cells of the cerebellar cortex selectively affected by a slight decrease in number of granule cells, while cerebellar white matter, dentate, olivary and pontine nuclei, and extracerebellar systems were left intact.

Case report

The female patient developed a slowly progressing ataxia at the age of 55 years. In 1987, when she was 59 years old, non-Hodgkin's lymphoma (follicular large cell type) was detected and she twice received radiation therapy to the neck, combined with a course of chemotherapy (vincristine, cyclophosphamide, and predonine). Following the chemotherapy the patient developed radiating back pain and was examined neurologically. The examination disclosed slight dysarthria, horizontal nystagmus on lateral gaze, ocular hypermetria, slight hypotonia of limb muscles, mild ataxia of the limbs and trunk, and superficial sensory disturbance of fingers and toes; limb reflexes were preserved, without any signs of pyramidal or extrapyramidal system involvement. Her cognition, memory, and sphincter control were not impaired. A brain X-ray computed tomography scan showed mild cerebellar atrophy with bilateral calcification of the globus pallidus and dentate nuclei. Because her sensory disturbance correlated with the course of chemotherapy, it was regarded a sequelae of this treatment.

The lymphoma recurred repeatedly despite the chemoradiotherapy, and bone marrow suppression with thrombocytopenia developed in February 1988. Early in January 1989, she developed herpes zoster with intractable pain, requiring epidural anesthesia. Marked thrombocytopenia and bloody stool followed, and the patient died on February 5, 1989 at the age of 61 years.

Her family history disclosed that the disease was dominantly inherited SCA as shown in the family tree (Fig. 1). Her father, who died at the age of 60, was reported to have been affected with ataxic disorders. Of her two siblings, her brother was not affected, whereas her younger sister had developed an unsteady gait and speech difficulties at the age of 54. Neurological examination of this sister at age 58 showed mild limb and gait ataxia, obvious dysarthria, and limb hypotonia. Neither gaze nystagmus nor ocular overshoot was observed at that time. Her ataxia progressed steadily. At age 66, she showed marked gaze nystagmus in addi-

Fig. 1 Family tree with number of CAG repeats. CAG repeat size of SCA6 alleles was presented in numbers of short/long repeat in each allele. The genotype of the deceased parents (presented in *parenthesis*) were deduced from the data of their three children (see also Table 1) (/ deceased, \Box man, \odot woman, *solid symbols* affected, *empty symbols* not affected)

tion to spontaneous down beat nystagmus. Her ataxia advanced to the point that she needed assistance to walk. Her mentation was normal and sphincter function was not impaired throughout the course. Magnetic resonance imaging of the brain showed cerebellar atrophy with no pathological changes in other central nervous system structures.

Materials and methods

DNA analyses

With informed consent, peripheral blood was obtained from each of the siblings and high molecular weight DNA was extracted from white blood cells or lymphoblastoid cell lines. Genotyping of the SCA6 mutation was performed according to the original report [26]. After amplification by the polymerase chain reaction method, products flanking the CAG repeat polymorphism of the α -1A-voltage-dependent Ca channel subunit gene were analyzed with an ABI PRISM 377 (PE Applied Biosystems, Foster City, Calif.), and allele size was determined by GeneScan (PE Applied Biosystems). The size of the CAG repeat was calculated by comparing the sequenced index allele.

Neuropathological examination

An autopsy was performed 3.5 h after death. Slight subarachnoid hemorrhage was found around the posterior part of the cerebellum and the spinal cord from the first lumbar to the cauda equina, but no berry aneurysm or rupture thereof was evident. The hemorrhages were considered to be caused by thrombocytopenia-related hemorrhagic diathesis. Malignant lymphoma was not detected anywhere in the organs, probably due to effective chemotherapy. To avoid postmortem autolysis the brain was fixed by perfusion fixation with 10% buffered formalin via the bilateral carotid arteries. The brain was then examined by routine histological staining using the hematoxylin-eosin, Klüver-Barrera, Bodian, and Holzer methods. The Gallyas-Braak method was also performed [2]. For the immunohistochemical analysis, antibodies against glial fibrillary acidic protein (GFAP; Dako), synaptophysin (Dako), and neurofilament triplet (Dako) were used. In addition, parvalbumin (Sigma) [23], calbindin-D (Sigma) [11], and the EAAT4 subtype of glutamate transporter [25] were used. The antibodies of the latter three proteins are known to label Purkinje cells and their dendrites. As a positive control, four age-matched cases of the cerebellum and a sporadic case of multiple system atrophy with typical olivopontocerebellar degeneration were also analyzed.

Results

SCA genotyping

The sizes of CAG repeats of the α -1A-voltage-dependent Ca channel subunit gene are shown in Table 1 for each subject. The present case had 16/22 repeats, and her affected sister had 13/22 repeats. The healthy brother had 11/16 repeats. Each of the two sisters carried one expanded allele the repeat size of which fell into the range of the SCA6 mutation.

Neuropathology

Macroscopically, the brain of the autopsied subject, which weighed 1300 g, showed no significant changes except for the slight subarachnoid hemorrhage described above. **Table 1** Family data with number of CAG repeats. Blood from the family was obtained in 1987 with informed consent (see also legend to Fig. 1) (*NE* not examined)

Fig. 2 Macroscopic view of the cerebellum. Atrophy of folia is evident in the vermis (*center*), but cerebellar white matter as well as dentate nuclei are seen well preserved

On coronal section there were no remarkable changes in the cerebral cortex, white matter, or basal ganglia, or in the midbrain, pons, medulla oblongata, or spinal cord. The cerebellum was externally symmetric. The cerebellum was sectioned tangentially through the vermis and the hemisphere through the dentate nucleus on the right side. The left side of the cerebellar hemisphere was sectioned horizontally through the dentate nucleus. Atrophy of folia was noted in the vermis (Fig. 2). The cerebellar white matter and dentate nuclei were well preserved.

Microscopically, degenerative changes were found confined to the cerebellar cortex. The number of Purkinje cells was found to have decreased to approximately onethird to one-fourth that in the age-matched controls. The loss was evident in the cerebellar vermis, and especially marked in the declive (Fig. 3a), and in the cerebellar hemisphere, less markedly in the lobuli quadragularis and simplex. Granular cells were slightly decreased in number, and torpedoes were frequently observed among their sparse nuclei (Fig. 3a, arrow). Immunostaining for neurofilament disclosed many empty basket fibers, mainly around Purkinje cells and torpedoes (Fig. 3b). Calbindin-D immunostaining demonstrated a decreased number of dendrites in the molecular layers of Purkinje cells. However, those of the remaining Purkinje cells were well preserved and among them many spiny dendrites were clearly demonstrated (Fig. 3c). Antibodies to glutamic acid transporter (GAT) and parvalbumin also disclosed a

decreased number of Purkinje cells and their dendrites in the molecular layer similar to immunostaining with calbindin-D (data not shown). A few remaining Purkinje cells and their dendritic spines were intensely labeled with GAT and parvalbumin. Torpedoes and Purkinje cell axons running in the white matter as well as around neurons of the dentate nucleus were strongly labeled by calbindin-D and parvalbumin. Synaptophysin staining disclosed numerous positive synaptic glomeruli in the granular cell layer. In addition, intensely immunolabeled synaptophysin fibers were noted around neurons of the dentate nuclei (Fig. 3d). This intense staining of synaptic protein around the neurons of dentate nuclei was also observed in a sporadic case of multiple system atrophy. There was a small concentration of GFAP-positive astrocytic fibers in the molecular layer and white matter, and around the dentate nuclei. Myelin sheaths of the cerebellar white matter were well preserved. Oligodendroglial inclusion bodies were not found in the preparation stained with Gallyas-Braak method, although the inclusions were demonstrated in the preparations of multiple system atrophy used for positive control.

There were no abnormalities found in the cerebral cortex, white matter, or basal ganglia. The putamen, pallidum, caudate nucleus, thalamus, subthalamic nucleus (Luysian body), Ammon's horn, basal nucleus of Meynert, substantia nigra, and red nucleus were well preserved. Nothing remarkable was observed in the pontine tegmentum (including the superior cerebellar peduncle), the pontine base (including ponto-cerebellar fibers), or the medulla oblongata (including the inferior olivary nuclei). The spinal cord was intact in both the pyramidal and spinocerebellar tracts. Spinal autonomic nuclei, such as nucleus intermediolateralis, and Onuf's nucleus were also intact. The only localized change of note was in the right second lumbar spinal ganglion, which was focally necrotic with lymphocytic infiltration. This was considered a sequela to herpes zoster.

Discussion

The DNA genotyping for SCA6 mutation in the present family indicates that abnormal expansion was selectively associated with two affected individuals, but not in the asymptomatic brother. The size of these two expanded alleles was consistent with the original report [26]. Our pre-

Fig. 3a–d Histological and immunohistochemical findings of the cerebellum. **a** Moderately decreased number of Purkinje cells of the vermis. The number of granule cells is also decreased. Note a few axon torpedoes among the sparsely arranged granule cells (*arrows*); H & E stain. **b** Empty baskets in the Purkinje cell layer and torpedoes in the granular layer are clearly seen; immunostaining with neurofilament.**c** Well-preserved cell body and its dendrites in the surviving Purkinje cell; immunostaining with calbindin-D. **d** Intensely visible synaptic terminals around the neurons of dentate nucleus; immunostaining with synaptophysin. $\mathbf{a}-\mathbf{d} \times 200$

vious study on a large cohort of Japanese patients indicated that such a small expansion was selectively detected in SCA6 (21–33 repeats, $n = 56$ alleles) and not in control alleles $(4-18, n = 1148$ alleles) (Yabe et al., submitted). This earlier study suggested that SCA6 is a prevalent SCA irrespective of ethnicity. The genetic data on these earlier subjects provides firm evidence for the diagnosis of the present family. Clinically, the symptoms of both the present patient and her sister began in the sixth decade of life, with cerebellar ataxia being the predominant feature throughout their clinical courses, without any neurologi-

cal signs indicating other neural system involvement. This clinical presentation would be a typical one for ADCA type III [7]. The sensory disturbances observed were thought to be related to chemotherapy, since they were relieved after withdrawal of chemotherapy and did not occur in the subject's sister. It is also unlikely that paraneoplastic cerebellar degeneration developed in association with malignant lymphoma, because cerebellar signs was slowly progressive, and the signs had not been improved after disappearance of lymphoma.

Pathologically, SCA6 is unique among SCAs in which pathological findings have been reported. SCA1 is largely characterized by degeneration of spinocerebellar (SC) and olivopontocerebellar (OPC) systems with frequent involvement of dentate, rubral and pallidal systems, and SCA2 by those of SC and OPC systems with frequent involvement of substantia nigra [24]. In SCA3 the OPC system is spared, but the involvement of the SC system and the extracerebellar systems including dentate nuclei are constant findings [24]. No pathological findings comparable to the present ones have been reported for SCA4,

SCA5, or SCA7. In contrast in SCA6, degeneration was confined to cerebellar cortex, particularly to Purkinje cells. Since the CAG repeat expansion occurred in the coding region of a gene which is important for normal Purkinje cell function and survival [13, 15], it would be reasonable to speculate that Purkinje cells were a victim of this gene abnormality.

Immunohistochemical studies using Purkinje cell markers clearly demonstrated the decreased number of Purkinje cells and subsequent reduction of their dendrites in the molecular layer. These findings were consistent with reported cerebellar disorders [11, 23]. However, these proteins were intensely expressed in the remaining Purkinje cells and their dendritic spines. This was different from the reported cases in which these protein expressions were decreased in the surviving Purkinje cells in other types of cerebellar degeneration [23]. This difference may stem not from the disorders involved, but from the different stages at which the patients died; our subject died of malignant lymphoma during the mid-stage of the ataxic disorder. However, an examination of numerous cases of different types of SCA would be needed to evaluate these protein expressions. An intense expression of synaptophysin protein around the neurons of dentate nuclei could be interpreted as reactive overexpression from the surviving Purkinje cells to compensate the lost axonal input.

Before the identification of the genetic locus of SCA6, there were reports of cerebellar ataxia which could be considered to be SCA6 based on the clinical as well as pathological findings. Key words may be (1) autosomal dominant inheritance, (2) late adult onset with relatively pure cerebellar signs, and (3) pure Purkinje cell degeneration with relatively preserved olivary nuclei. Such cases have been usually classified as Holmes type, although the case reported by Holmes (1907) had olivary degeneration, and seemed to be an autosomal recessive disorder since he described that "Neither parent of the family was afflicted with any form of nervous disease; the father died at 70 years of age, the mother at 89, with chronic rheumatism and bronchitis" [9]. Moreover, hypogonadism was also reported in Holmes' cases. Among the reports described as "Holmes type", however, we have found a few cases of autosomal dominant inheritance and no mental or gonadal abnormalities. Hall et al. [6] reported a three to four generation family of late-onset cerebellar ataxia. The reported family tree showed an autosomal dominant heredity of the disease. An autopsy of an 80-year-old man disclosed that the degeneration was confined to the cerebellar cortex with olivary involvement. Hoffman et al. [8] reported a four generation family with autosomal dominant late-onset cerebellar degeneration in northern Georgia, USA. Pathological findings of one of their cases were quite similar to our case, except for olivary involvement. Takahashi et al. [22] reported in their second case that a 54-year-old woman with dominant family history suffered from progressive ataxia and died of rectal cancer at the age of 65 years. An autopsy disclosed degenerative changes limited to the cerebellar cortex with slight loss of neurons and slight gliosis of the olivary nucleus. Muzusawa et al. [14] reported a dominantly inherited cerebellar cortical atrophy of an 84-year-old who had suffered from progressive cerebellar ataxia since he was 57. Neuropathology confirmed pure cerebellar cortical degeneration without involvement of the olivary nucleus. However, for each of these four reports, specific gene mutations are unknown. More recently, Subramony et al. [21] have described two autopsy cases of dominantly inherited cerebello-olivary atrophy. Neuropathologically, their cases were characterized by major loss of Purkinje cells, modest inferior olivary neuronal loss, and some thinning granule cell layer in the cerebellum. The family members of these two cases were later subjected in the study for SCA mutation and were subsequently demonstrated to carry SCA6 mutation [26]. Therefore, these two cases [21] represent the first description of SCA6 pathology, as far as we know, and our case is the third. Inconsistent findings in these three autopsy cases indicate that whether inferior olivary nucleus is degenerated or not is not a main characteristic of SCA6 pathology. However, it is not known whether there is further variation in SCA6 pathology, or whether there are any difference between SCA6 and non-SCA6 in ADCA type III. As SCA6 was identified in the present case by molecular analysis, there is a pressing need for neuropathologists to establish a system of DNA analysis using paraffin-embedded tissues.

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