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Case Report

Severe cortical involvement in MV2 Creutzfeldt–Jakob disease: An autopsy case report

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MV2 type sporadic Creutzfeldt–Jakob disease (sCJD) is reported to have a long duration and marked involvement of the cerebral deep gray matter. We describe an autopsied long-surviving sCJD case of MV2. In the early stages, the patient exhibited memory impairment, attention deficit and semantic memory disorder. Diffusion-weighted MRI showed abnormal hyperintensity signals along the cerebral cortex, sparing the thalami and basal ganglia. Pathological observations included: severe spongiosis throughout the cerebral cortex, several kuru plaques and plaque-like PrP deposits in the cerebellum, with only minimal degeneration in the thalami and basal ganglia. Our case suggests that MV2 has a wide clinicopathological spectrum, which ranges from "VV2" to "MM2" type.

Key words: Creutzfeldt–Jakob disease, MRI, MV2, pathology.

INTRODUCTION

Cases of sporadic Creutzfeldt–Jakob disease (sCJD) present with a variety of symptoms and courses. In 1999, Parchi *et al.* proposed a classification of sCJD based on molecular and phenotypic features of 300 cases, using a combination of two factors: polymorphic codon 129 of the prion protein gene, that is, methionine homozygote (MM), heterozygote (MV) or valine homozygote (VV); and the physicochemical properties of protease-resistant prion protein (PrP) accumulated in the brain, i.e. type 1 or type 2.[1] Most cases of classical sCJD are MM1 or MV1. In this classification, MV2 cases present with a long clinical illness, ataxia and cognitive impairment in the initial stage, and characteristic kuru-plaques in the cerebellum.

Here, we report a case of MV2 type sCJD presenting with atypical pathological findings.

CLINICAL SUMMARY

Case report

A 73-year-old woman was brought to our hospital by her daughters because of 6-month history of forgetfulness and abnormal behavior. She could not recall the names of her acquaintances or daily objects and used pronouns frequently because she was unable to remember the names of persons or things to which she was referring. She could feed and dress herself. Her family had no history of neurological disease. She had had a brainstem hemorrhage (right pontine base) at age 66. She had never undergone neurosurgery involving a dura mater graft, deep brain electrodes or corneal transplantation.

On admission, she was oriented to date and place, was polite and showed no antisocial or disinhibitory behavior. She showed attention deficit and semantic memory disorder. On the revised edition of the Wechsler adult intelligence scale she scored verbal IQ score of 57, performance IQ score of 61, and full-scale IQ score of 56. Her cranial nerves were unremarkable, aside from palatal myoclonus and left-sided facial sensory loss. Her visual field showed no deficiency or visual extinction. The muscle tone was slightly spastic in her left upper and lower extremities. As a result of involuntary movement, such as pseudoathethosis and hyperkinesie volitionelle, her coordination was disturbed in left upper and lower extremities. She could walk using a cane in the right hand. She had sensory disturbance in all modalities on the left side of the body. These motor

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and sensory symptoms were sequelae of the right pontine hemorrhage.

Her laboratory findings were within the normal range, including tests for syphilis, thyroid function and vitamins. The examination of cerebrospinal fluid was normal aside from a slightly elevated protein concentration (61 mg/dL). An assay of 14-3-3 protein was equivocal. An analysis of the prion protein gene showed no mutation. She had the methionine and valine heterozygote alleles for codon 129.

Brain MRI revealed hyperintense signals along the parieto-occipital lobe cortex, predominant on the left side, in the diffusion-weighted and fluid attenuated inversion recovery (FLAIR) images (Fig. 1). No abnormal finding was detected in the basal ganglia and thalami. In the T_2-weighted images, there was a high-signal lesion located at the right inferior olivary nucleus and right central tegmental tract, suggesting an old vascular disorder. The EEG showed almost symmetrical diffuse slow alpha activity and did not show periodic sharp wave complexes (PSWC).

Her condition gradually worsened, leaving her bedridden and requiring total parental nutrition within 6 months of admission. At this time, she developed myoclonus in the right upper extremity. A second assay for 14-3-3 protein in the cerebrospinal fluid was positive. Her speech output gradually decreased, but she could respond to an examiner's speech by nodding, shaking head, or speaking simple words, such as "yes" or "good morning". The EEG did not reveal typical PSWC.

About 6 months before her death, she developed complete akinetic mutism. She died of acute cardiac failure after about 4 years after her family first noticed her memory impairment. The clinical diagnosis was atypical sCJD of long duration.

Pathological findings

Histological examination of only the brain was performed. Sections from the brain were stained with HE, Klüver-Barrera, Bodian's, and PAS stains. Immunohistochemistry was performed with a monoclonal antibody 3F4 (Senetek, Maryland Heights, MO; 1:500), which recognizes the human prion protein residues 109–112, as previously described.[2]

The fixed brain weighed 960 g. Macroscopically, the brain was markedly atrophic, especially in the frontal lobe. Serial coronal sections of the cerebrum showed dilatations of the lateral and third ventricles, volume loss in the white matter, and brown pigmentation in the medial portion of the hippocampi. Serial horizontal sections of the brainstem showed linear pigmentation along the right medial leminis-

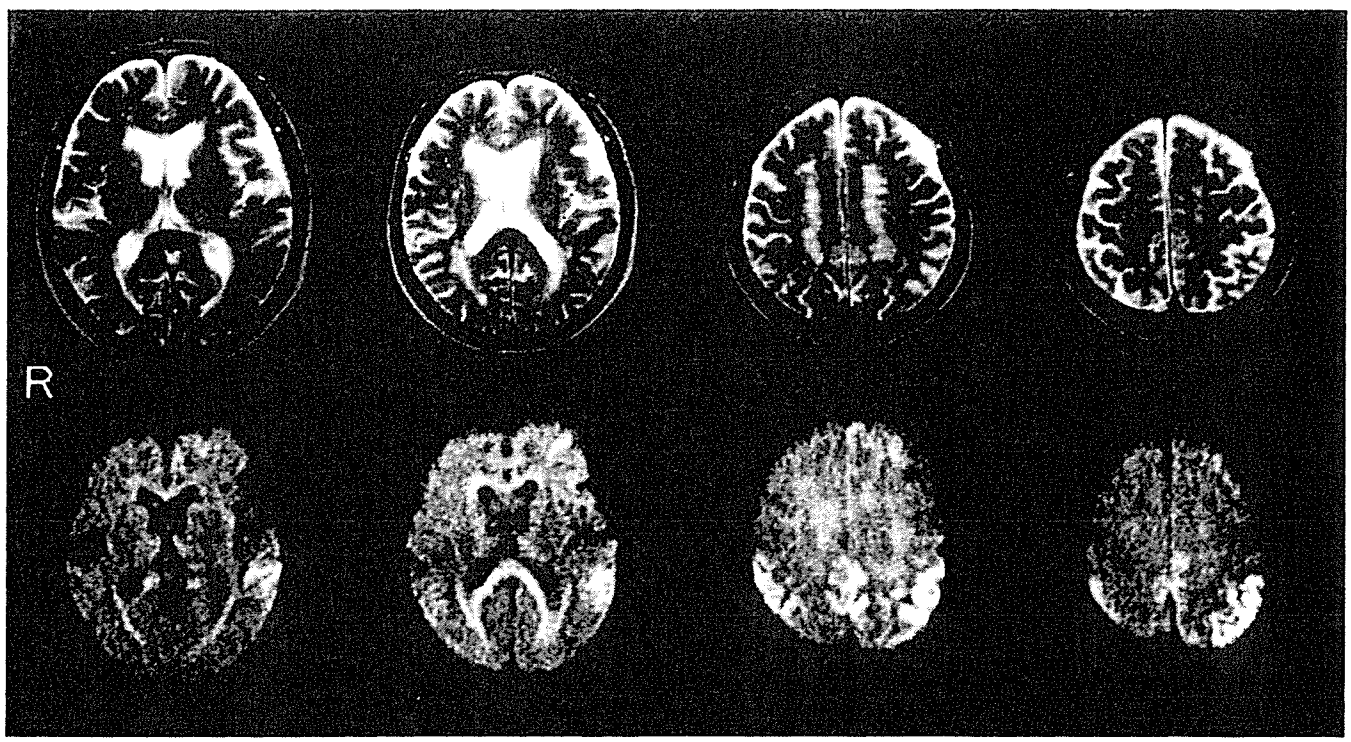

Fig. 1 MRI performed 6 months after the emergence of symptoms. An abnormally high signal is seen along the parieto-occipital lobe cortex, predominant on the left side. In the basal ganglia and thalami, no abnormal findings are seen. T_2 (upper row) and diffusion (lower row) weighted images.

cus at the pons and discoloration in the right inferior olivary nucleus. These changes were suggestive of the old pontine hemorrhage.

Diffuse spongiform change was observed throughout the cerebral cortex, including the cingulate gyrus, except for the hippocampus and subiculum (Fig. 2A). All layers showed moderate to severe status spongiosis with a tendency to spare the middle layers, giving the appearance of laminar involvement, especially around the calcarine fissure. There was moderate neuronal loss in the affected areas, but mild reactive astrocytosis only. Immunohistological staining using anti-PrP showed cortical coarse perivacuolar (Fig. 2B) and dot-like PrP deposits.

In the basal ganglia and thalami, only mild neuronal loss and mild spongiform change were observed (Fig. 2C,D). The subcortical white matter was unremarkable. Dot-like synaptic deposits of PrP were observed in the basal ganglia and thalami.

In the cerebellum, a severe loss of Purkinje cells and proliferation of Bergmann's glia were observed in the hemisphere and upper vermis (Fig. 2E). Several kuru-plaques were observed by HE and PAS staining (Fig. 2F). Many plaque-like PrP deposits were observed, especially in the upper vermis (Fig. 2G).

In the right pontine tegmentum, an irregular, old necrotic lesion, with a few macrophages containing free melanin, was observed. In the right inferior olivary nucleus, severe neuronal loss and glial proliferation, with a few vacuolated neurons, were observed, suggesting pseudohypertrophy.

Western blot analysis

Brain tissue from the right frontal lobe was homogenized, and Western blot analysis of proteinase K-resistant prion protein was performed using prion protein monoclonal antibody 3F4, as previously described.[3] In this case, type 2 prion protein was detected (Fig. 3).

DISCUSSION

Clinically, MV2 sCJD has a long course, producing cognitive impairment and ataxia initially.[1] Our case had a long clinical illness (more than 3 years from the onset of the initial symptoms to akinetic mutism) and cognitive impairment in the early stage, which is clinically compatible with MV2, with the exception of a lack of ataxia in the initial stage.

In MV2 sCJD, high signals in the basal ganglia and thalami are frequently seen on MRI T2-weighted images.[4,5] Our case exhibited abnormal hyperintensity along the cerebral cortex, but not in the basal ganglia and thalami, on MRI diffusion-weighted and FLAIR images. These image features are unusual for MV2 sCJD.

The combination of cortical hyperintensity signals on diffusion-weighted MRI, late-onset and slowly progressive dementia, and elevated levels of CSF 14-3-3 protein are characteristic findings in clinical MM2 cortical type sCJD.[6] Together with the lack of ataxia in the initial stage, our case mimicked cortical type MM2 clinically.

Neuropathologically, our case had unique findings. So far, all reported cases of MV2 sCJD show severe involvement of the cerebral subcortical nuclei and kuru-plaques and plaque-like PrP deposits in the cerebellum.[1] The degree of cerebral cortical lesions varies with the disease duration, with a tendency toward marked sponginess limited to the entorhinal cortices and deep layer of the neocortex. Cerebellar pathology is less severe, compared with the VV2 cases with similar disease duration. By contrast, our case had only mild involvement of the basal ganglia and thalami, while the entire neocortex showed marked status spongiosis, and severe loss of the Purkinje cells were observed in the cerebellum. Although kuru-plaques and plaque-like PrP deposits, observed in our case, are consistent with the pathology of MV2 sCJD, these other pathological findings are quite atypical for this type of sCJD (Table 1).

Our intensive literature search failed to find a report of MV2 sCJD with clinicopathological features similar to ours. Only one clinicopathological study of MV2 sCJD with cortical involvement in diffusion-weighted MRI has been reported.[7] However, this case showed abnormal hyperintensity signals in the basal ganglia and thalami other than the cerebral cortex, and pathological findings were restricted to the right frontal lobe cortex.

Pathologically atypical features for MV2, observed in our case, are severe extensive cortical sponginess, only mild changes in the basal ganglia and thalami and severe cerebellar pathology. Although kuru plaques, the pathological hallmarks of MV2, were also observed in our case, the cortical coarse perivacuolar PrP deposits, observed in our case, are always observed in MM2 cortical type. Plaque-like PrP deposits are always observed not only in MV2 but also in VV2.[1] Therefore, our case might have the combination of pathological features; i.e. both MM2 cortical type and VV2, besides typical MV2 features (Table 1).

In cases of MV2, valine is a candidate as the determinant factor of type 2 PrP, because most cases of MV2 show many plaque-like PrP deposits, as in VV2 cases.[1] Theoretically, methionine may also be the determinant factor of type 2 PrP. Our case suggests that MV2 has a wide clinicopathological spectrum, which ranges from "VV2 (or cerebellar)" to "MM2 (or cortical)" type. Further detailed pathological study of MV2 cases is required to justify this hypothesis.

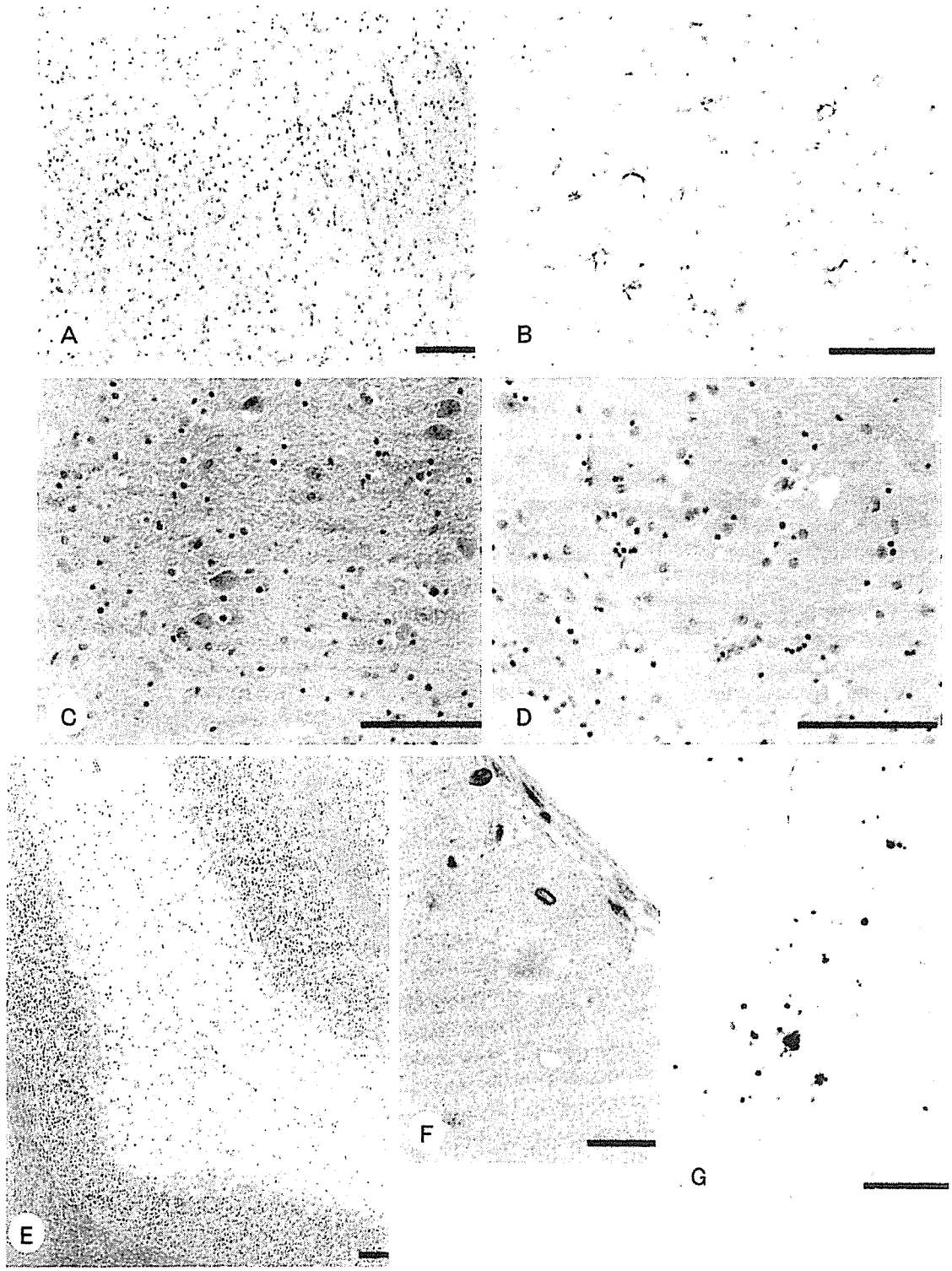

Fig. 2 Pathological findings. (A) Severe status spongiosis is observed in the left temporal lobe cortex (HE stained; scale bar = 50 μm). (B) Perivacuolar prion protein deposits are seen in the right temporal lobe cortex (immunostaining with anti-PrP antibody; scale bar = 50 μm). (C) Mild glial proliferation is seen in the left thalamus (HE stained; scale bar = 50 μm). (D) Sparse vacuolation is seen in the left putamen (HE stained; scale bar = 50 μm). (E) Severe loss of Purkinje cells and proliferation of Bergmann's glia are seen in the cerebellum (Klüver-Barrera stained; scale bar = 50 μm). (F) A kuru-plaque is observed in the molecular layer of the upper vermis of the cerebellum (HE stained; scale bar = 10 μm). (G) Many plaque-type prion protein deposits are seen in the molecular layer of the upper vermis (immunostaining with anti-PrP antibody; scale bar = 50 μm).

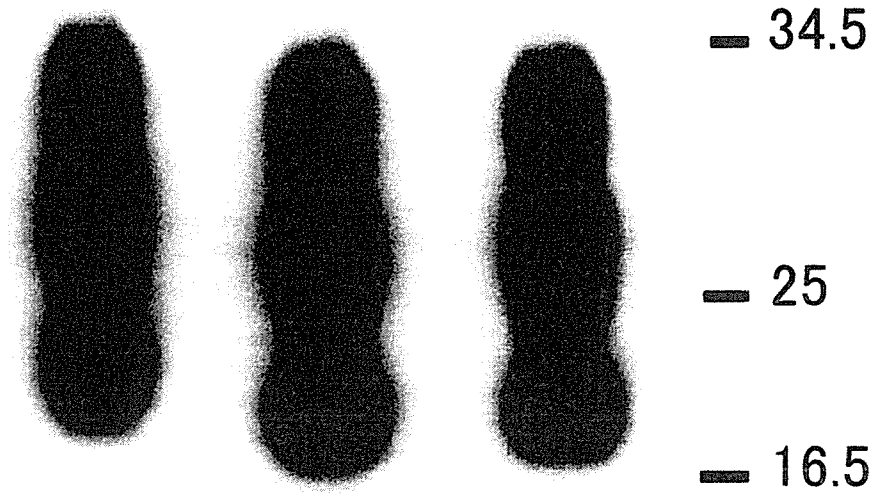

Fig. 3 Western blot analysis of the brain. Left lane: type 1 PrP, Center lane: type 2 PrP (our case), Right lane: another type 2 PrP case.

Table 1 Comparison of pathological features

Pathology	MM2 cortical	MV2	VV2	Our case
Cerebral cortex	○			○
Basal ganglia/thalamus		○		
Cerebellum			○	○
Kuru plaque		○		○
Plaque-like PrP deposit		○	○	○
Coarse perivacuolar PrP deposit	○			○

○ indicates constantly observed pathological findings.

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Case Report

An autopsy case of frontotemporal dementia with severe dysarthria and motor neuron disease showing numerous basophilic inclusions

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We report a clinicopathological study of a patient suffering from frontotemporal dementia (FTD) with severe dysarthria and concomitant motor neuron disease (MND). The patient was a 52-year-old woman with almost simultaneous emergence of severe dysarthria and FTD. The severe dysarthria subsequently evolved into anterior opercular syndrome. Motor neuron signs then emerged, and the patient developed akinetic mutism approximately 2 years after the onset of the disease. The patient died of pneumonia after a 7-year clinical illness. Pathologically, severe and widespread degeneration in the frontal and temporal lobes, including the anterior opercular area, limbic system, basal ganglia, spinal cord and cerebellum, and frequent ubiquitin- and tau-negative basophilic inclusions were observed. The pyramidal tracts and anterior horns of the cervical cord also showed marked degeneration. Cases showing basophilic inclusions reported so far have been divided into two groups: early onset FTD and MND with basophilic inclusions. Our case presented clinicopathological features of both FTD and MND, which suggests that cases showing basophilic inclusions may constitute a clinicopathological entity of FTD/MND.

Key words: anterior opercular syndrome, basophilic inclusion, frontotemporal dementia, motor neuron disease, Pick's disease.

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INTRODUCTION

Although mutism is a late symptom of frontotemporal dementia (FTD), disordered speech is usually not observed in the early stages.[1] On the other hand, in FTD with motor neuron disease (FTD/MND), which is a subgroup of FTD, severe dysarthria attributable to bulbar palsy is observed within approximately 6 months to 1 year after the onset of the disease.[1,2] This subgroup shows a rapid progression to akinetic mutism, and most patients die within 3 years of the disease onset. The pathological hallmarks of FTD/MND are ubiquitin-positive intraneuronal inclusions in the cortical layer II of the frontal and temporal cortices and granule cells in the hippocampal dentate gyrus,[2–4] and focal degeneration of the rostral CA1-subiculum border.[5]

We report an autopsied case of FTD with MND that showed severe dysarthria in the early stage and a rapid progression to akinetic mutism but was pathologically different from FTD/MND, because this case lacked ubiquitin-positive inclusions but showed numerous ubiquitin-negative basophilic inclusions in the widespread lesions in the central nervous system.

CASE REPORT

Clinical summary

A 52-year-old right-handed Japanese woman visited our hospital because of speech difficulties. Around this time, the patient's activity level decreased, and she became disinterested in performing activities of daily living, such as cooking and washing clothes. Her medical history was unremarkable except for chronic thyroiditis, and she had

no family history of neurological diseases. At the clinic, her voice was weak, hoarse and coarse owing to prominent breath sounds, and her speech duration time was short. The movements of her speech organs (tongue, jaw, soft palate and cheek) were not limited in range, but were slow. Spontaneous speech was not substantially distorted, but was monotonous with impaired prosody. Singing was also disturbed. Although she was able to understand oral and written language, she had mild attention deficit and cognitive impairment. She scored a verbal IQ 68, performance IQ 62 and full-scale IQ 61 on the revised edition of the Wechsler adult intelligence scale. She was aware of her difficulty in speech.

The patient was admitted to our hospital approximately 1 year later because her speech had deteriorated. On admission, her general condition was unremarkable. She was apathetic with poor facial expression and paid no attention to her surroundings. She was almost mute and unconcerned with her clothes or appearance. Only occasionally her speech was so nasal and monotonous that the vowels and consonants were too distorted to be comprehended. In addition, her voice was severely hoarse and coarse, with prominent breath sounds. The patient could not sing, and she had no forced crying or laughter. There was no wasting or fasciculation of the tongue, but movements of the speech organs were disturbed. She could not intentionally move the facial muscles, tongue or soft palate, but she could open her mouth naturally while yawning, and her tongue moved sufficiently at meals (automatico-voluntary dissociation). The results of a nasolaryngoscopy showed normal symmetrical movements of the vocal cords. The limb muscle tone was normal. The tendon reflexes were within normal range, and pathological reflexes were not observed. Based on these clinical findings, we made a diagnosis of progressive anterior opercular syndrome (AOS).

Routine laboratory examinations did not show any abnormalities, such as thyroid dysfunction, syphilis or vitamin deficiencies. A brain MRI showed bilateral enlargement of the anterior horn of the lateral ventricles (Fig. 1).

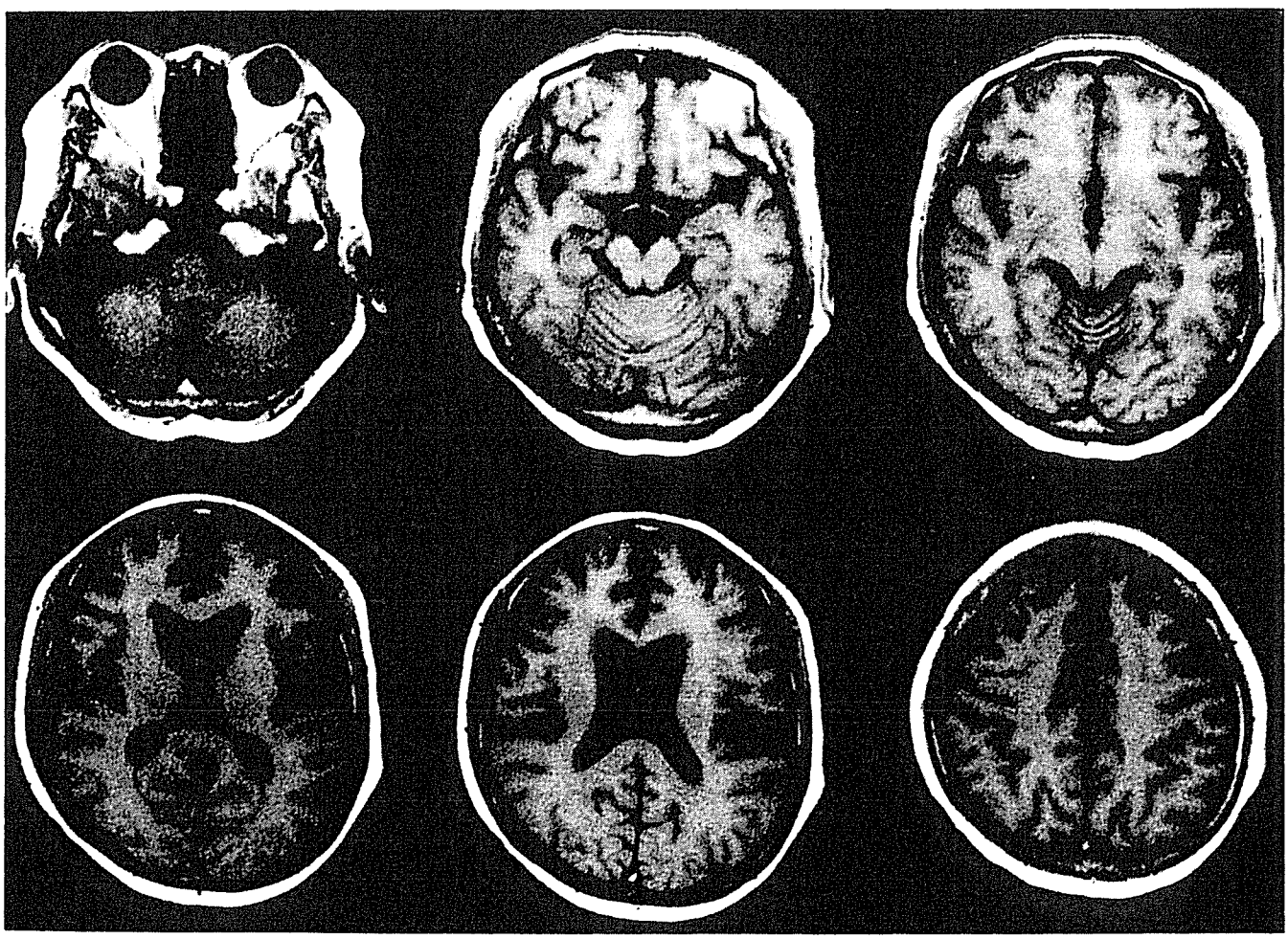

Fig. 1 MRI performed on the first admission. The anterior horns of the bilateral lateral ventricles are enlarged, suggesting atrophy of the caudate head.

The SPECT image showed hypoperfusion in the fronto-temporal cortex and subcortical area bilaterally. The EEG did not show any abnormality.

After she was discharged, the patient repeatedly went to the toilet but did not urinate or defecate. She aimlessly wandered in her house and frequently fell. At meals, she continuously brought food to her mouth without swallowing, and her behavior had to be monitored by her family all day.

Six months later, she was readmitted because of exacerbated neurological conditions. She had bilateral pyramidal tract signs (brisk tendon reflexes, spasticity in the lower limbs and positive Babinski's sign), extrapyramidal symptoms (akinesia and postural instability), and frontal lobe signs (palmomental reflexes and snout reflexes). She showed stereotypical behaviors. For example, she repeatedly brought an empty cup to her mouth and mimicked drinking water; she also aimlessly repeated standing up and sitting down on the bed. A brain MRI showed a rapid progression of atrophy in the bilateral frontal-temporal lobes (Fig. 2). Electromyography performed in the right hand and leg showed neurogenic changes, suggesting anterior horn cell degeneration. A feeding tube was eventually inserted due to her difficulty with swallowing.

Approximately 2 years after the onset of the disease, the patient developed akinetic mutism and quadriparesis. She died of pneumonia about 7.5 years after the initial manifestation of the speech disorder. The clinical diagnosis was FTD/MND presenting with progressive AOS in the early stage.

Pathological findings

Pathological examination was performed only on the brain. Paraffin-embedded sections obtained from the appropriate regions of the cerebrum, brainstem, cerebellum and spinal cord were stained with HE, Klüver-Barrera's, Bodian's and Nissl stains. Immunostaining using

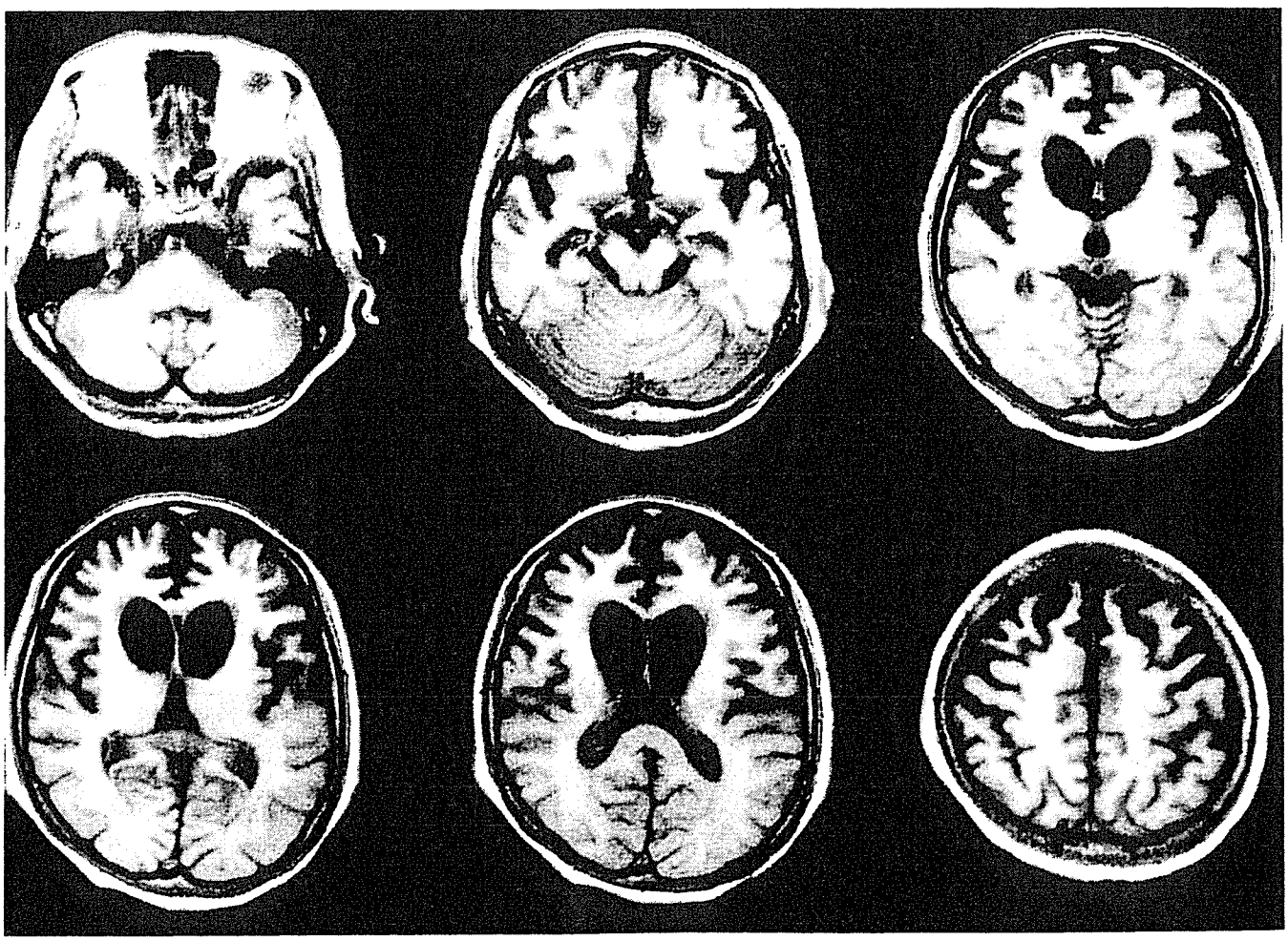

Fig. 2 MRI performed about 1 year after the first admission. Prominent atrophy of the bilateral frontal and anterior temporal lobes can be seen.

antitau (AT8; Innogenetics, Ghent, Belgium; 1:1000), antiubiquitin (Dako; Glostrup, Denmark; 1:100) and antialpha-synuclein (monoclonal; LB509; 1:100) antibodies was also performed in some sliced sections.

The fixed brain weighed 710 g. Macroscopically, severe atrophy of the bilateral frontal and anterior temporal lobes involving the precentral gyrus was observed (Fig. 3), while the parietal and occipital lobes were preserved in volume. The brainstem was atrophic. The medial two-thirds of the cerebral peduncle showed prominent brownish discoloration. The coronal sections of the cerebrum showed prominent enlargement of the lateral ventricles and severe atrophy of the bilateral frontal and anterior temporal lobes and the limbic system (Fig. 3). The cortex in these lobes was thin, and the volume of the white matter was reduced. The deeply located structures were also degenerated to the point that the caudate nucleus, putamen, globus pallidus, subthalamic nucleus and thalamus were difficult to recognize separately. The horizontal sections of the brainstem and cerebellum showed severe atrophy of the brainstem. The bilateral pyramids were also atrophic.

Histopathological examination showed severe and diffuse neuronal loss, gliosis and rarefaction of the neuropil in the frontal and temporal lobes, the hippocampus, amygdala, basal ganglia, thalamus and substantia nigra (Fig. 4). In contrast, the cerebral cortices in the parietal and occipital lobes were preserved. There were numerous basophilic inclusions in the frontal and temporal lobes, limbic systems, basal ganglia, subthalamic nucleus, nucleus basalis of Meynert, red nucleus, substantia nigra, locus ceruleus, pontine nucleus, inferior olivary nucleus and anterior horn of the higher cervical cord (Fig. 5). The basophilic inclusions were weakly argyrophilic and stained with Nissl stain, but were not stained with antitau (AT-8), antiubiquitin or antialpha-synuclein antibodies. Neurofibrillary tangles or senile plaques were scarcely observed even in their preferential areas. In the cerebellum, several basophilic inclusions in the neurons of the dentate nucleus, loss of Purkinje cells and many torpedoes were observed. Pallor of the myelin sheath and severe reduction of the myelinated nerve fibers in the pyramidal tracts, at the level of cerebral peduncle, medullary pyramids and higher cervical cord were observed (Fig. 6). Motor neurons in the facial and hypoglossal nucleus and high cervical anterior horns were obviously reduced in number. The intramedullary roots of the hypoglossal nerve showed degeneration. How-

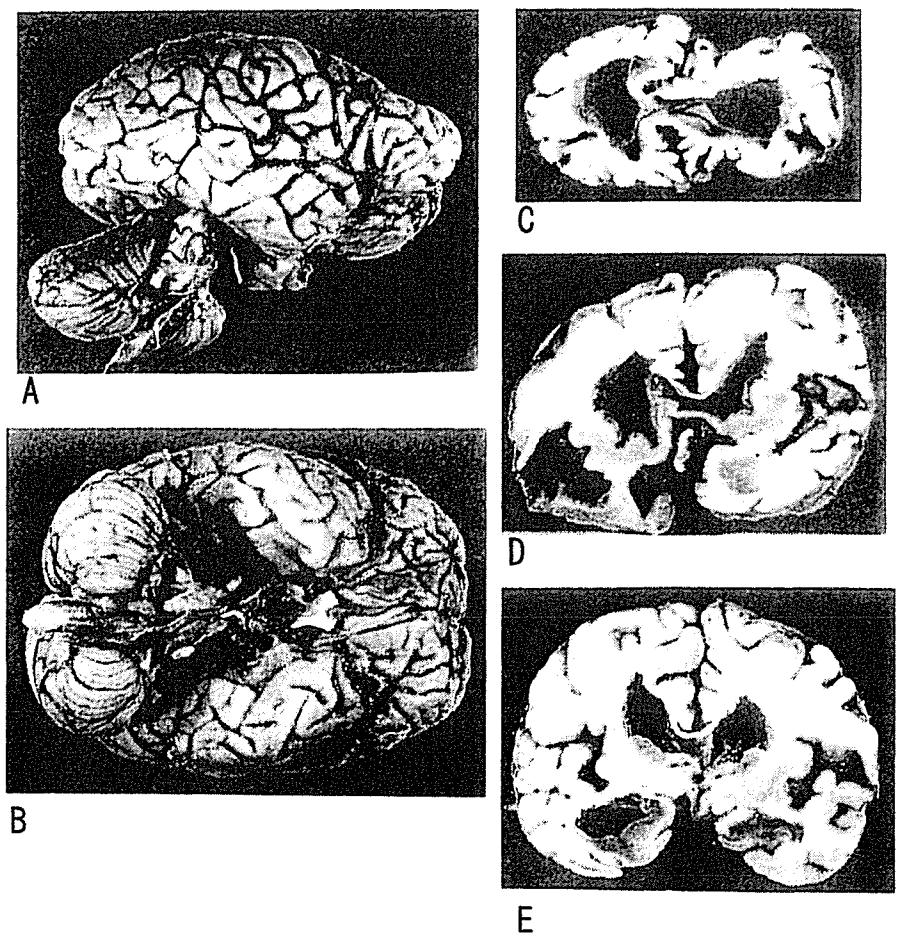

Fig. 3 Macroscopic appearance of the fixed brain. (A,B) Severe atrophy of the frontal lobes, including the precentral gyrus, anterior temporal lobes, and the brain stem, can be seen. (C,D,E) In the coronal sections, prominent enlargement of the lateral ventricles and severe atrophy of the bilateral frontal and anterior temporal lobes and the limbic system can be seen. (A) Lateral view from the right side. (B) Basal surface of the brain and brain stem. Coronal sections through the (C) genu of the corpus callosum, (D) amygdala and (E) hippocampus.

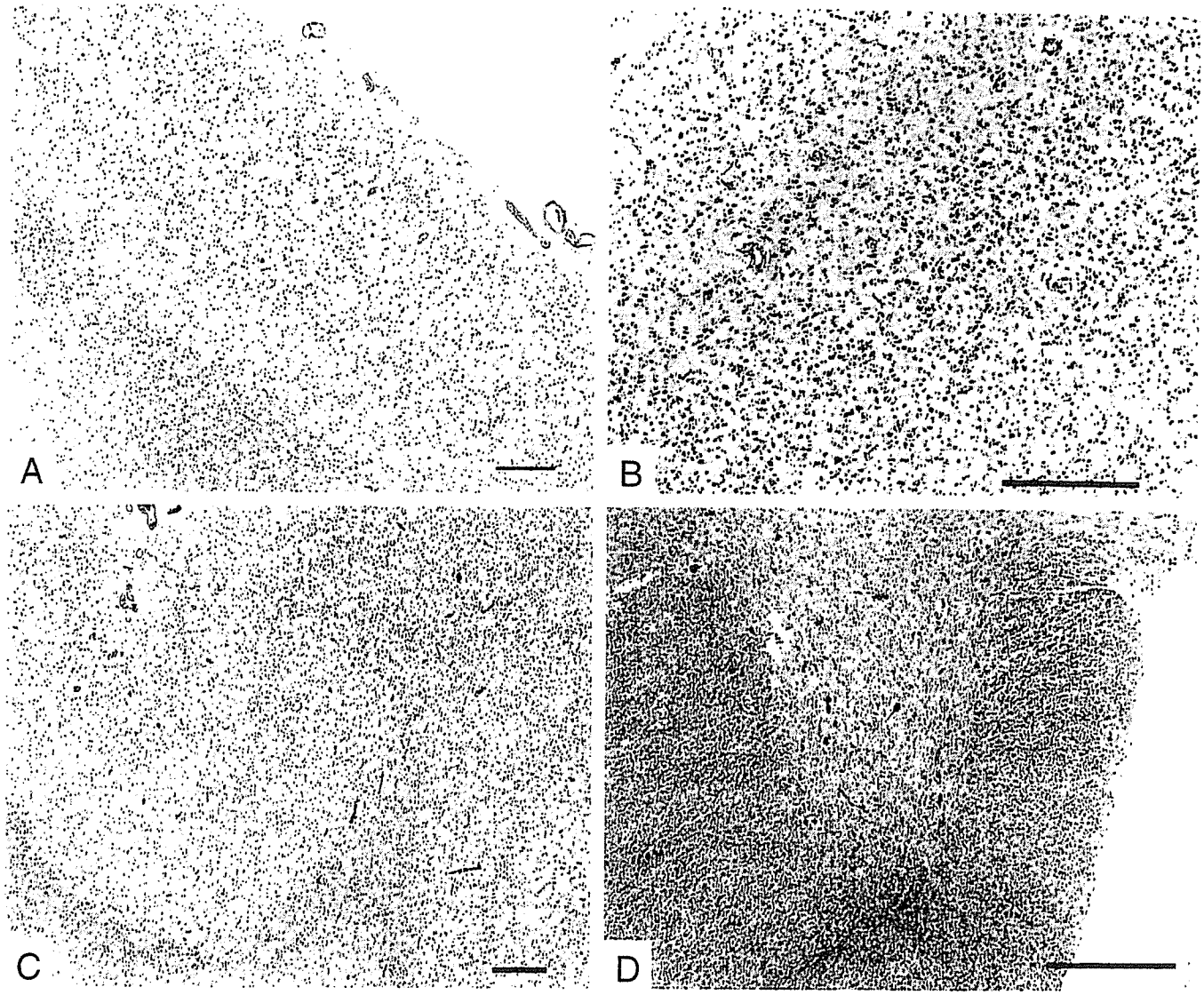

Fig. 4 Microscopic findings. (A–C) Severe neuronal loss, gliosis, and rarefaction of the neuropil can be seen. (A) Frontal lobe (HE; bar = 200 μm). (B) Precentral gyrus (HE; bar = 200 μm). (C) Temporal lobe (HE; bar = 400 μm). (D) Motor neurons in the anterior horn of the cervical cord are markedly reduced in number (HE; bar = 200 μm).

ever, there were no Bunina bodies or ubiquitin-positive intraneuronal inclusions.

DISCUSSION

The early decline in social interpersonal conduct, decline in personal hygiene and grooming, perseverative and stereotypical behaviors, and late mutism observed in the presented case are clinical features of FTD.[1,6] The bilateral pyramidal tract signs and neurogenic findings in EMG observed in the middle stage support the clinical diagnosis of MND. Therefore, with the rapid progression into akinetic mutism, this case is clinically compatible with FTD/MND.

The dysarthria observed in the initial stage was characterized by hoarseness, followed by the emergence of a nasal voice and volitional movement disorders of the speech organs, which ultimately evolved into AOS. MND signs, such as tongue atrophy and fasciculation, were not observed even in this stage; therefore, the severe dysarthria was thought to be attributable to AOS, not bulbar palsy. This is a unique clinical feature of our case because severe dysarthria is caused by bulbar palsy in FTD/MND.[1] Progressive AOS is a rare condition,[7] and to the best of our knowledge, no other case of progressive AOS with FTD has been reported in the literature. We propose that the combination of FTD and progressive AOS in our case was derived from severe and widespread lesions in the bilateral

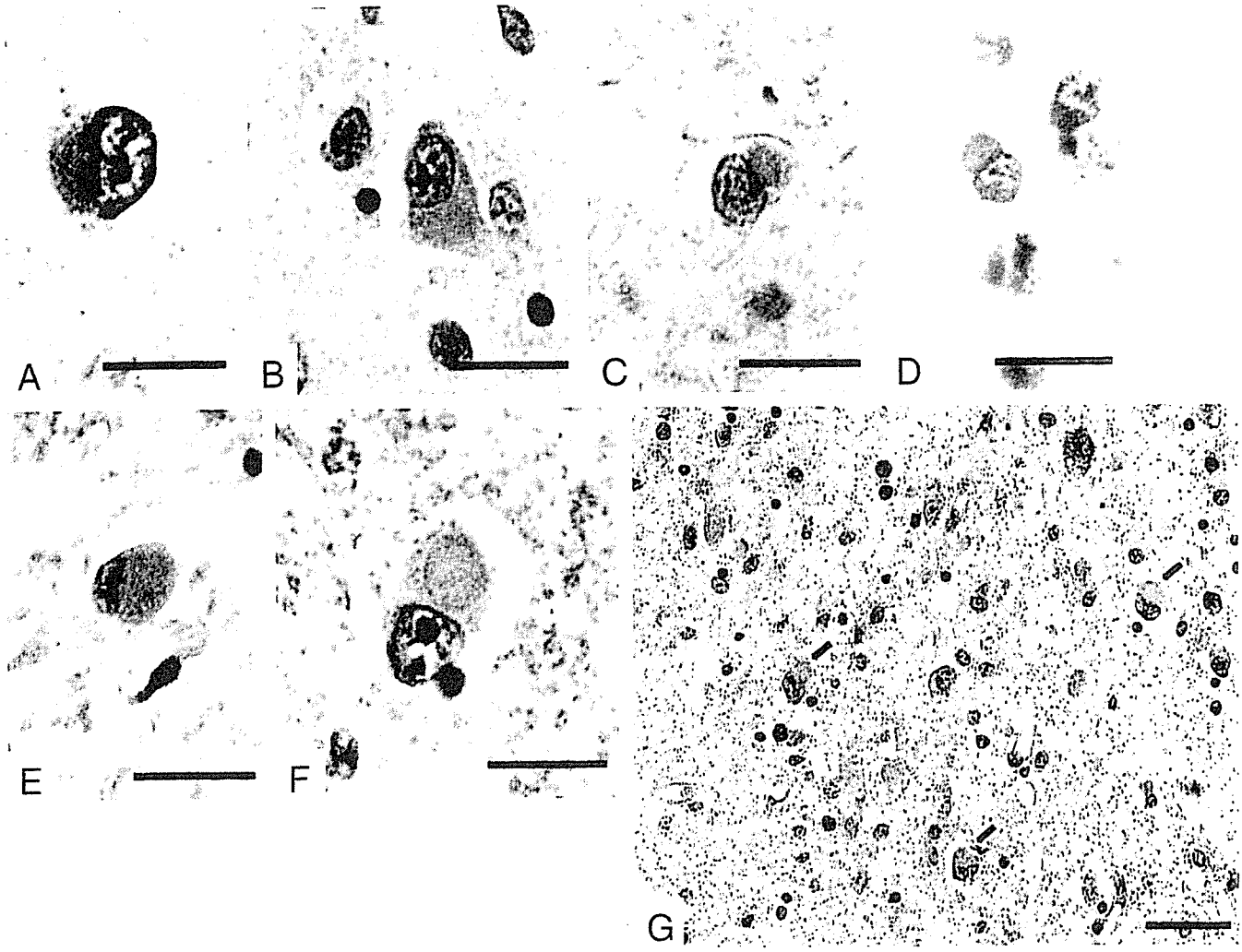

Fig. 5 Basophilic inclusion bodies observed in the brain. (A) Frontal lobe (HE; bar = 10 μm). (B) Temporal cortex (HE; bar = 20 μm). (C) Precentral gyrus (HE; bar = 20 μm). (D) Insular cortex (KB; bar = 20 μm. (E) Subthalamic nucleus (HE; bar = 20 μm). (F) Red nucleus (HE; bar = 10 μm). (G) Hippocampus, basophilic inclusions are indicated by arrows (HE; bar = 20 μm).

frontal lobes, including the anterior opercular area, which is supported by the SPECT and pathological findings.

Intraneuronal inclusions observed in the present case were basophilic with HE and KB stain, well stained with Nissl stain, weakly argyrophilic with Bodian's stain, and negative with AT8 and antiubiquitin immunostaining. Although electromicroscopic examination was not performed, these findings coincide well with a previous report of basophilic inclusions.[8] Basophilic inclusions have been described in cases of juvenile onset amyotrophic lateral sclerosis.[9-13] So far, adult-onset cases showing basophilic inclusions consist of two major subgroups:[14] one is a generalized variant of Pick's disease or relatively early onset FTD,[15] and the other is motor neuron disease with basophilic inclusions. Although only a few cases in each subgroup have been reported,[8,15-18] some of these cases show a widespread distribution of lesions as was observed in our case.

The present case showed clinicopathological features of both FTD and MND, which suggests that cases showing basophilic inclusions may constitute a clinicopathological spectrum of FTD/MND between a generalized variant of Pick's disease (FTD) and motor neuron disease with basophilic inclusions (MND). This condition clinically mimics FTD/MND with ubiquitin-positive inclusions, but differs pathologically.

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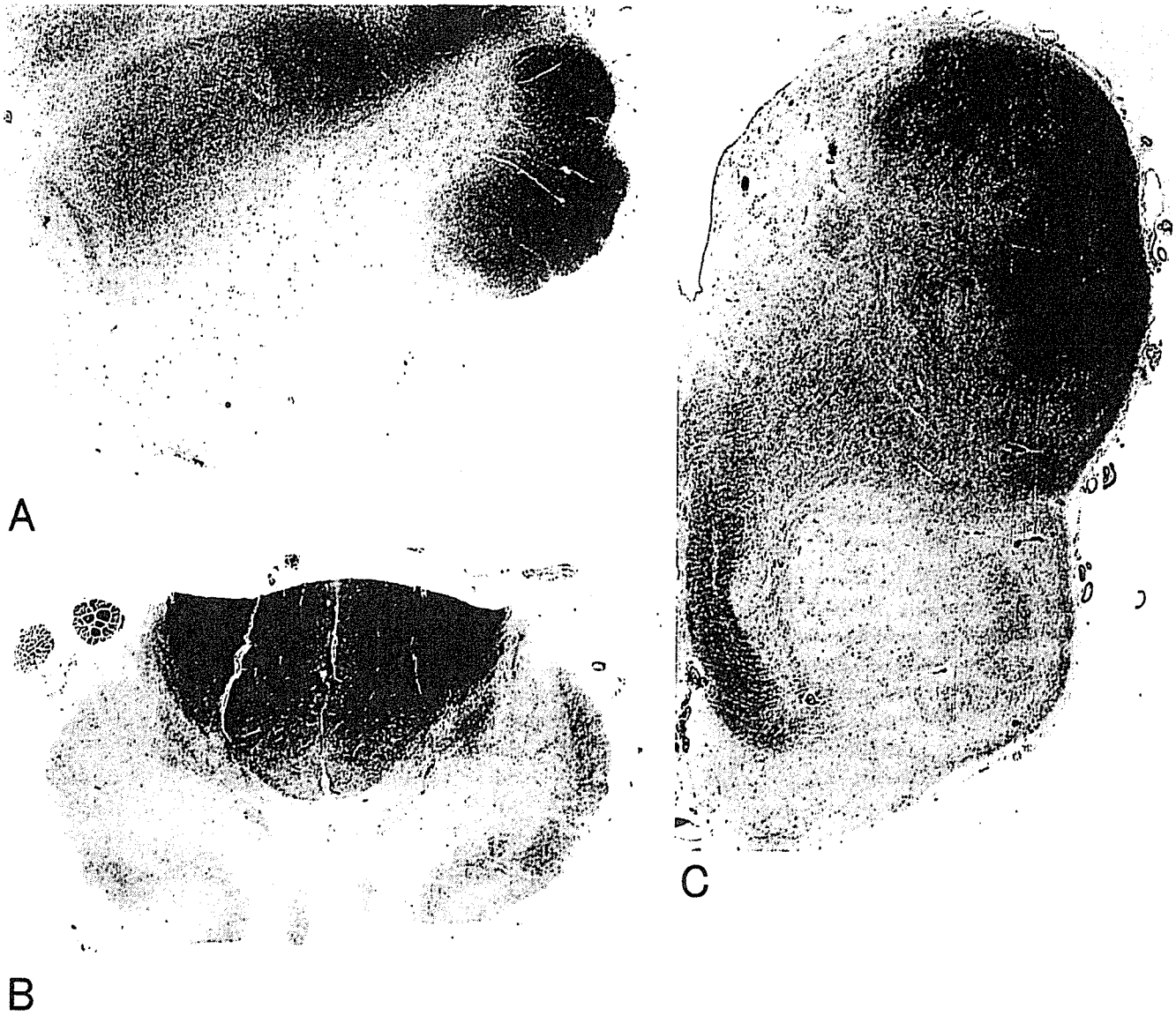

Fig. 6 Semimacroscopic findings of the brainstem and cervical cord. (A) Cerebral peduncle (KB). (B) Medulla oblongata (KB). (C) Upper cervical cord (KB). Prominent pallor of the myelin sheath of the corticospinal tract can be seen.

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Sacsin-related ataxia (ARSACS): Expanding the genotype upstream from the gigantic exon

Abstract—The authors describe a Japanese autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS) patient with a compound heterozygous mutation (32627-32636delACACTGTTAC and 31760delT) in a new exon of the *SACS* gene. The new exons upstream of the gigantic one should be analyzed when a case is clinically compatible with ARSACS, even without any mutation in the gigantic exon.

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Autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS; MIM 270550) was first reported in the late 1970s.[1] ARSACS is characterized by early-onset spastic ataxia, dysarthria, nystagmus, distal muscle wasting, finger or foot deformities, and retinal hypermyelination.[1]

In 2000, the gene responsible for ARSACS (*SACS*) was identified in Quebec patients.[2] The *SACS* gene consists of a single gigantic exon spanning 12,794 bp with an 11,487-bp open reading frame and encodes the protein sacsin.[2] To date, over 20 mutations in the gigantic exon have been found in Quebec[2] and non-Quebec patients including ones in Japan,[3-5] Italy,[6] Tunisia,[7] Turkey,[8] and Spain,[9] and ARSACS thus shows a worldwide occurrence. We sometimes encounter patients with clinical features identical to those of ARSACS who have no mutation in the gigantic exon. Eight new exons located upstream of the gigantic one were recently found (GenBank, AL157766). We report a Japanese patient with ARSACS with a compound heterozygous mutation in a new exon of the *SACS* gene.

Methods. *Clinical study.* We encountered a 25-year-old woman with early-onset spastic ataxia. The patient was an only child born to nonconsanguineous parents. Detailed neurologic examinations were performed on the family members including the patient and her unaffected parents. In addition, we performed brain MRI and a nerve conduction study of the patient.

Molecular analysis. Blood samples were obtained with informed consent from the patient and her parents. Genomic DNA was extracted from peripheral blood leukocytes. Including the gigantic exon described previously,[2] nine exons were initially retrieved from the National Center for Biotechnology Information (NCBI). The accession number is AL 157766, and the protein product number is CAI 13923 (4579 aa) in NCBI. Exons 1 through 9 are shown according to their location in figure 1. Primer pairs were designed to amplify each exon including the gigantic one (exon 9) (the primer sequences are available on request). Each exon was amplified by PCR from 200 ng of genomic DNA and sequenced directly with an ABI PRISM 310 genetic analyzer. To confirm the mutations, the amplified fragments were subcloned into a TA-cloning plasmid vector (TOPO TA Cloning Kit; Invitrogen). Each mutation was screened in the chromosomes from 100 Japanese controls in order to exclude polymorphisms. This study was approved by the Medical Ethical Committee of Jichi Medical School.

Case report. This 25-year-old woman first walked at 18 months of age, but the speed of her gait and running was low in her first decade. In her school days, she could not run as fast as her classmates. Her gait disturbance progressed slowly, and from age 23, she needed some assistance when walking. Her speech also became slow and dysarthric at age 23.

Neurologic examination at age 25 revealed marked spasticity and moderate distal weakness in the lower extremities. Tendon reflexes were markedly increased with Babinski signs, but absent in the ankles. She showed limb and truncal ataxia, slurred speech, and a defect in conjugate pursuit ocular movements. Vibration sensation in the toes was reduced. She showed pes caves and pes varus. Her gait was markedly ataxic and spastic. Myelinated retinal nerve fibers were not observed. Brain MRI revealed cerebellar atrophy, especially in the upper vermis (data not shown). Motor nerve conduction velocity was mildly reduced in the median and ulnar nerves. A compound muscle action potential was not evoked in the posterior tibial nerve. A sensory nerve action potential was not evoked in the sural nerve (data not shown).

Results. *Molecular analysis.* No mutation in gigantic exon 9 was found in the patient, and a compound heterozygous deletion mutation (32627-32636delACACTGTTAC and 31760 delT) was identified in a new exon, 7, of the *SACS* gene, which results in a frameshift and a subsequent stop codon at amino acid residues 407 (W395-fsX407) and 713 (V687-fsX713) (figure 2A). This mutation leads to truncation of the predicted sacsin protein. This mutation was found in a heterozygous state in the unaffected father (32627-32636delACACTGTTAC) and mother (31760delT) (figure 2B and C). None of these mutations were found in the chromosomes from 100 Japanese controls.

Discussion. We consider the present compound heterozygous mutation (32627-32636delACACTGTTAC and 31760delT) responsible for our patient's condition. First, this mutation in the new coding exon results in premature termination of the predicted protein (W395-fsX407 and V687-fsX713). Second, 32627-32636delACACTGTTAC was found in the father and 31760delT in the mother, supporting autosomal recessive inheritance as in ARSACS.

All causative mutations previously reported were in the gigantic exon.[3-9] Our results, however, show

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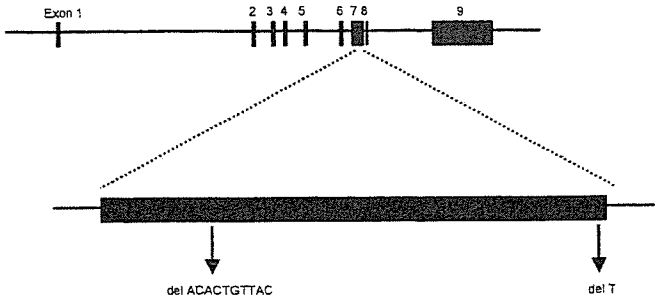

Figure 1. Schematic representation of the SACS gene. Nine exons of the SACS gene, including gigantic exon 9 originally described,[2] and their locations are shown. The arrows indicate the compound heterozygous mutation of 32627-32636delACACTGTTAC and 31760delT.

the need to analyze the new exons when a patient without any mutation in the gigantic one is clinically suspected to have ARSACS. A Turkish family with a mutation linked to the ARSACS region on chromosome 13q, the clinical symptoms being identical to those in the other ARSACS patients studied, showed

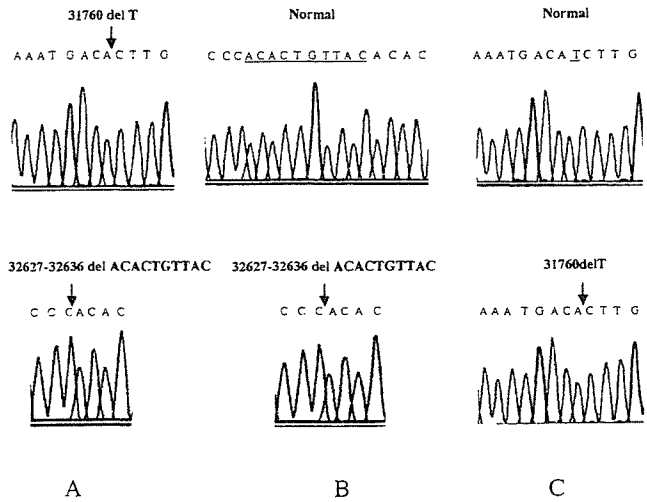

Figure 2. Identification of mutations of the SACS gene. The sequences in the patient (A), father (B), and mother (C) are shown. A compound heterozygous mutation (32627-32636delACACTGTTAC and 31760delT) was identified in exon 7 of the SACS gene in the patient, which results in a frameshift and a subsequent stop codon at amino acid residues 407 (W395-fsX407) and 713 (V687-fsX713). This mutation leads to truncation of the predicted sacsin protein. This mutation was found in a heterozygous state in the unaffected father (32627-32636delACACTGTTAC) and mother (31760delT).

no mutation in the gigantic exon of the *SACS* gene.[8] It is possible that this family also had a mutation in the new exons in the *SACS* gene.

In Quebec patients, two ancestral haplotypes (6594delT/6594delT and C5254T/6594delT in exon 9) have been identified,[2] and patients with these two haplotypes are known to show clinical homogeneity of ARSACS with the core clinical features of early-onset spastic ataxia and prominent myelinated retinal fibers.[1,2] Meanwhile, although early-onset spastic ataxia is the core clinical features in non-Quebec patients,[3-9] retinal hypermyelination and mental retardation are variable. Furthermore, we previously reported a phenotype without spasticity in a Japanese family with ARSACS,[4] reinforcing the notion that the clinical features are heterogeneous in non-Quebec patients. However, the genotype-phenotype correlation in ARSACS has remained unclear so far. In the present study, our patient showed the core clinical features of early-onset spastic ataxia without retinal hypermyelination, and we did not observe any difference in the clinical features between our patient with a truncated protein encoded by the new exon and patients with a truncated protein encoded by or a missense mutation in the original gigantic exon. However, as more *SACS* mutations are identified in the new exons, the clinical spectrum of sacsinopathies will expand, and a finer genotype-phenotype correlation study will be possible.

Acknowledgment

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16q-linked autosomal dominant cerebellar ataxia: A clinical and genetic study

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Abstract

The autosomal dominant cerebellar ataxias (ADCAs) comprise a genetically and clinically heterogenous group of neurodegenerative disorders. Very recently, a C-to-T single nucleotide substitution in the *puratrophin-1* gene was found to be strongly associated with a form of ADCA linked to chromosome 16q22.1 (16q-linked ADCA; OMIM 600223). We found the C-to-T substitution in the *puratrophin-1* gene in 20 patients with ataxia (16 heterozygotes and four homozygotes) and four asymptomatic carriers in 9 of 24 families with an unknown type of ADCA. We also found two cases with 16q-linked ADCA among 43 sporadic patients with late-onset cortical cerebellar atrophy (LCCA). The mean age at onset in the 22 patients was 61.8 years, and that of homozygous patients was lower than that of heterozygous ones in one family. Neurological examination revealed that the majority of our patients showed exaggerated deep tendon reflexes in addition to the cardinal symptom of cerebellar ataxia (100%), and 37.5% of them had sensorineural hearing impairment, whereas sensory axonal neuropathy was absent. The frequency of 16q-linked ADCA was about 1/10 of our series of 110 ADCA families, making it the third most frequent ADCA in Japan.

Keywords: 16q-linked ADCA; *Puratrophin-1* gene; Heterozygote; Homozygote; Haplotype analysis

1. Introduction

Autosomal dominant cerebellar ataxias (ADCAs) comprise a genetically and clinically heterogeneous group of neurodegenerative disorders characterized by progressive cerebellar ataxia that can be variably associated with other neurological features [1]. ADCAs are now classified on the basis of the causative genes or gene loci. To date, at least 26 subtypes of ADCA have been identified including spinocerebellar ataxia (SCA) type 1, 2, Machado-Joseph disease (MJD/SCA3), 4–8, 10–19/22, 21, 23, 25–28, and dentatorubral and pallidoluysian atrophy (DRPLA) [2,3].

Among these subtypes, SCA4 was mapped to chromosome 16q22.1 in a Scandinavian family residing in Utah and Wyoming in 1996 [4]. This family showed prominent sensory axonal neuropathy and pyramidal tract signs in addition to cerebellar ataxia. In 2003, a German family characterized by cerebellar ataxia and sensory axonal neuropathy was assigned to the same locus as SCA4 [5].

Meanwhile, the gene locus responsible for six Japanese families with ADCA was mapped to the same region as SCA4 in 2000 [6]. Although SCA4 and this form of ADCA might be allelic, the clinical features of the Japanese families

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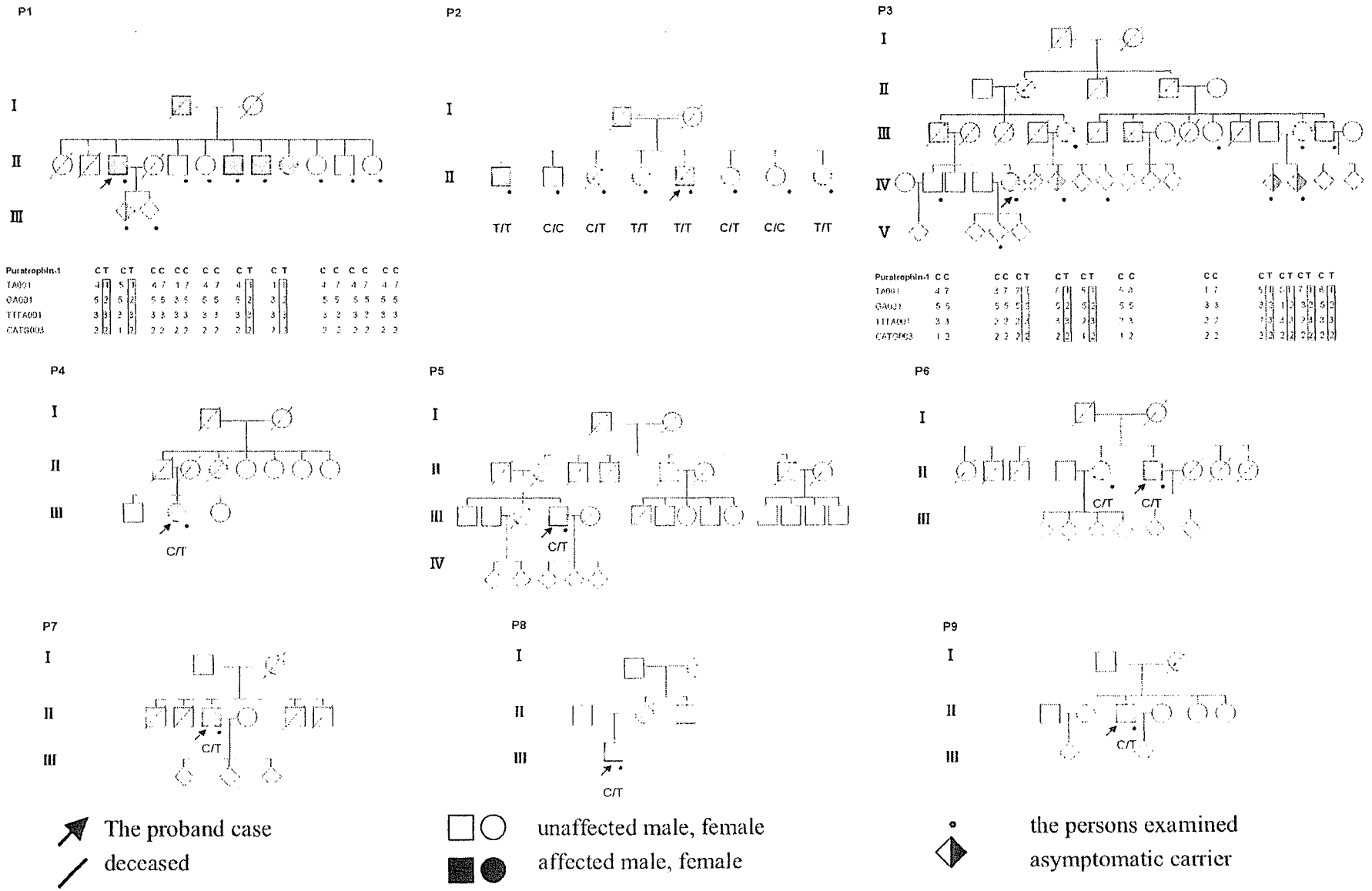

Fig. 1. The pedigrees of nine Japanese families with 16q-linked ADCA. In pedigrees 1 and 3, the gender is concealed in those individuals, including asymptomatic ones, denoted by diamonds to maintain the anonymity of the families.

were somewhat different from those in the case of SCA4, i.e., pure cerebellar ataxia without obvious evidence of extracerebellar neurological dysfunction. Therefore, the term "16q-linked ADCA" instead of "SCA4" was used to describe these Japanese families [7]. It is considered that 16q-linked ADCA shows prominent cerebellar ataxia with a later age at onset (>55 years) than that in SCA4 [8]. Very recently, a heterozygous C-to-T single nucleotide substitution in the 5′ untranslated region (UTR) of the *puratrophin-1* gene was found to be strongly associated with 16q-linked ADCA [9]. Thereafter, a substantial number of patients with this mutation showed progressive sensorineural hearing impairment in addition to cerebellar ataxia [10]. The clinical spectrum and the prevalence of 16q-ADCA, however, remain unclear.

We report here the clinical and molecular features of 20 patients including four homozygotes and four asymptomatic carriers in nine families, and two apparently sporadic patients with 16q-linked ADCA. Furthermore, we describe the frequency of 16q-linked ADCA in our series of 110 Japanese families with ADCA.

2. Subjects and methods

2.1. Clinical study

Clinical data were collected for 20 patients, four asymptomatic carriers in nine Japanese ADCA families, and two sporadic patients with a C-to-T substitution in the *puratrophin-1* gene (16q-linked ADCA). Fig. 1 shows the pedigrees of the nine families. Pedigrees 1 and 2 were partially described in the previous reports [6,8,9]. In pedigree 2, the parents (generation I) were first cousins, and thus consanguinity was present. In addition to neurological examination, brain MRI ($n=15$), peripheral nerve conduction studies ($n=8$), and audiograms ($n=8$) were performed in the patients as much as possible.

2.2. Molecular analysis

Blood samples were obtained with informed consent from 190 patients in 110 Japanese families with ADCA seen in the past 14 years (from 1992 to 2005). Genomic DNA was extracted from peripheral blood leukocytes. Screening for CAG repeat expansion for SCA1, SCA2, MJD/SCA3, SCA6, SCA7, SCA8, SCA12, SCA17, and DRPLA was performed by PCR as described elsewhere [11–19]. In this study, the SCA10, SCA14, and FGF mutations were not analyzed.

The C-to-T substitution in the *puratrophin-1* gene were analyzed in 33 patients, 16 at risk individuals, and 5 normal spouses in 24 of 110 families after exclusion of SCA1, SCA2, MJD/SCA3, SCA6, SCA7, SCA8, SCA12, SCA17, and DRPLA gene mutations (unknown ADCA families), and 43 sporadic patients with LCCA (late-onset cortical cerebellar ataxia without apparent extracerebellar signs or genetic inheritance). Using the primer pair of UK1-E1F1 (5′-CAGCGCGTTCACACTGAGA-3′) and UK1-E1R1 (5′-GGCCCTTTCTGACAGGACTGA-3′), exon 1 flanking the C-to-T change in the 5′ UTR of the *puratrophin-1* gene was amplified by PCR from 200 ng of genomic DNA [9], and then sequenced directly with an ABI PRISM 310 genetic analyzer; analysis was performed with Sequencing Analysis software, ver. 3.4.1 (Applied Biosystems). The PCR products of exon 1 with the primers were digested with *Eco*NI at 37 °C, subjected to electrophoresis on 2% agarose gels, and then stained with ethidium bromide. In addition, we performed haplotype analysis for the family members in pedigrees 1 and 3 using chromosome 16q markers TA001, GA001, TTTA001 and CATG003 [9].

This study was approved by the Medical Ethical Committee of Jichi Medical School.

3. Results

3.1. Clinical study

We found 20 patients (16 heterozygotes and four homozygotes) with 16q-linked ADCA and four asymptomatic carriers (two with and two without clinical signs) in 9 of 24 families with an unknown type of ADCA (Fig. 1). Furthermore, we found two sporadic patients with 16q-linked ADCA among 43 with LCCA.

Table 1
Clinical features in the patients with 16q-linked ADCA

Number of patients	22 (Male 13, Female 9)
Age at examination (years)	
Range	61–88
Mean	74.5
Age at onset (years)	
Range	50–83
Mean	61.8
Disease duration (years)	
Range	1–13
Mean	12.5
Initial symptoms (%)	
Unsteadiness of gait	77.3
Dysarthria	13.6
Tremor	9.1
Clinical features (%)	
Cerebellar	
Ataxic gait	100
Dysarthria	100
Nystagmus	77.3
Pyramidal	
Spasticity	13.6
Brisk DTRs	54.5
Babinski signs	0
Peripheral	
Depressed DTRs	13.6
Decreased vibration sense	13.6
Hearing impairment	37.5[a]
Tremor	13.6

[a] Audiograms revealed hearing impairment in three of the eight patients examined.

Table 1 summarizes the clinical features in the 22 patients with 16q-linked ADCA. The age at onset in the patients ranged from 50 to 83 years, the mean age at onset being 61.8 years. In pedigree 2, the mean age at onset in homozygous patients ($n=4$) was 55.6 years and that in heterozygous ones ($n=2$) was 68.5 years, showing an earlier age at onset in the former than in the latter. In pedigrees 1 and 3, anticipation was not noted.

The cardinal clinical feature was cerebellar ataxia including ataxic gait (100%), dysarthria (100%), and nystagmus (77.3%). Fifteen patients showed lateral gaze nystagmus, and two showed down-beat nystagmus. Oscillopsia was noted in one patient with down-beat nystagmus. Although external ophthalmoparesis was not evident, 13.6% of the patients complained of diplopia. Brisk deep tendon reflexes were found in the majority of the patients (54.5%), but Babinski signs were absent. In pedigree 3, three of the four patients examined showed moderate spasticity of the lower extremities in addition to brisk deep tendon reflexes. Meanwhile, 13.6% of the patients showed depressed deep tendon reflexes and depressed vibration sense in the toes. Audiograms revealed hearing impairment in three (37.5%) of the eight patients examined. Tremor was noted in 13.6% of the patients. Unfortunately, since we examined each homozygous or heterozygous patient in pedigree 2 only one time, we could not compare the disease course progression in them. However, there seemed to be no apparent differences in clinical phenotype between them. Among the four asymptomatic carriers, two individuals (mean, 46.0 years old) showed transient nystagmus and mild hyperreflexia.

Brain MRI ($n=15$) revealed cerebellar atrophy whereas the brainstem was of normal size and shape. Brain MRI of a homozygous (disease duration, 20 years) and a heterozygous patient (disease duration, 22 years) showed cerebellar atrophy of the same degree (Fig. 2). The results of a motor and sensory nerve conduction study ($n=8$) including two patients with depressed deep tendon reflexes or depressed vibration sense were normal, there being no sensory axonal neuropathy.

3.2. Molecular study

Fig. 1 shows the results of a heterozygous or homozygous C-to-T substitution of exon 1 in the *puratrophin-1* gene. Fig.

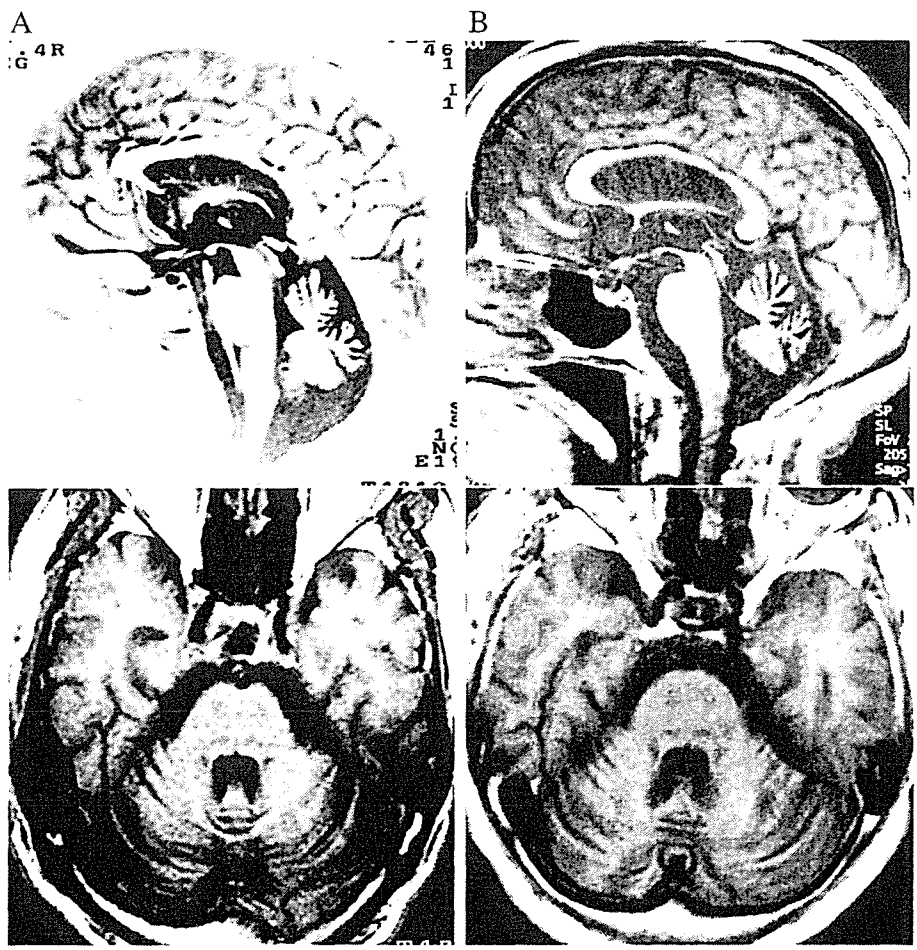

Fig. 2. (A) Brain MRI in a homozygous patient (disease duration, 20 years). Top: Reversed T2-weighted, sagittal slice. Bottom: T1-weighted, axial slice. (B) Brain MRI in a heterozygous patient (disease duration, 22 years). Top: T1-weighted, sagittal slice. Bottom: T1-weighted, axial slice. Both patients showed cerebellar atrophy of the same degree.