

Table 2 Neuropathological features

Location	Degree of neuronal loss	Other remarkable changes	Gliosis	White matter changes
Cerebrum				
Cerebral cortex				
Superior frontal gyrus	0	-	0	/
Precentral gyrus	0	-	0	/
Parietal lobule	0	-	0	/
Visual cortex	0	-	0	/
Parahippocampal gyrus	0	-	0	/
Middle temporal gyrus	0	-	0	/
Superior temporal gyrus	0	-	0	/
Inferior temporal gyrus	0	-	0	/
Cingulate gyrus	0	-	0	/
Cerebral gray matter	0	-	0	/
Caudate nucleus	0	-	0	/
Putamen	0	-	0	/
Globus pallidus (external and internal)	0	-	0	/
Thalamus	0	-	0	/
Clastrum	0	-	0	/
Hippocampus (CA1-CA4)	0	Moderate number of NFTs	0	/
Hippocampus, dentate gyrus	0	-	0	/
Cerebral white matter	/	-	0	0
Brainstem				
Substantia nigra, pars reticulata and compacta	0	-	0	/
Red nucleus	0	-	0	/
Periaqueductal gray	0	-	Mild	/
Oculomotor nucleus	0	-	0	/
Basal pontine nuclei	0	Lacunar infarct	Mild	/
Paramedian pontine reticular formation	0	-	0	/
Cranial nuclei: V, VI, VII	0	-	0	/
Inferior olivary nucleus	0	-	Mild	/
Vestibular and cochlear nuclei, trapezoid bodies	0	-	0	/
Cranial nuclei: IX, X, XI, XII	0	-	0	0
Pyramidal tract	/	-	0	0
Cerebellar peduncle (superior)	/	-	0	0
Cerebellar peduncle (middle)	/	-	0	0
Cerebellar peduncle (inferior)	/	-	0	0
Nucleus gracilis	0	Moderate number of spheroids	Mild	Mild myelin pallor
Nucleus cuneatus	0	-	0	0
Dentate nucleus	0	-	Moderate	
Fastigial nucleus	0	-	0	
Spinal cord (thoracic and lumbar spine)				
Anterior horn	0	-	0	/
Posterior horn	0	-	Mild	/
Lateral horn and Clarke's column	0	-	0	/
Funiculus, (anterior)	/	-	0	0
Funiculus (posterior: fasciculus gracilis)	/	-	Mild	Mild myelin pallor
Pyramidal tract	/	-	0	0
Anterior and posterior spinocerebellar tract	/	-	Mild	Mild myelin pallor
Anterior root of spinal nerve (4th lumbar)	/	-	/	0
Posterior root of spinal nerve (4th lumbar)	/	-	/	0
Dorsal root ganglia (7th thoracic spine)	Not apparent	A few Nageotte's nodules	0	

The degree of neuronal loss was rated in four steps: 0, mild, moderate and severe. /, sites that do not contain white matter.

stage of amorphous material formation. The presence of ubiquitin-immunoreactive granules, mimicking calbindin-immunoreactive granules, may suggest that a certain protein degradation system is taking place in the amorphous material. The second component is the presynaptic termi-

nals innervated by certain neurons, which is evidenced by synaptophysin immunoreactivity. The third component is the astroglial processes. Further study using a larger number of patients will be needed to clarify the present hypothesis. Such effort will lead to the establishment of the

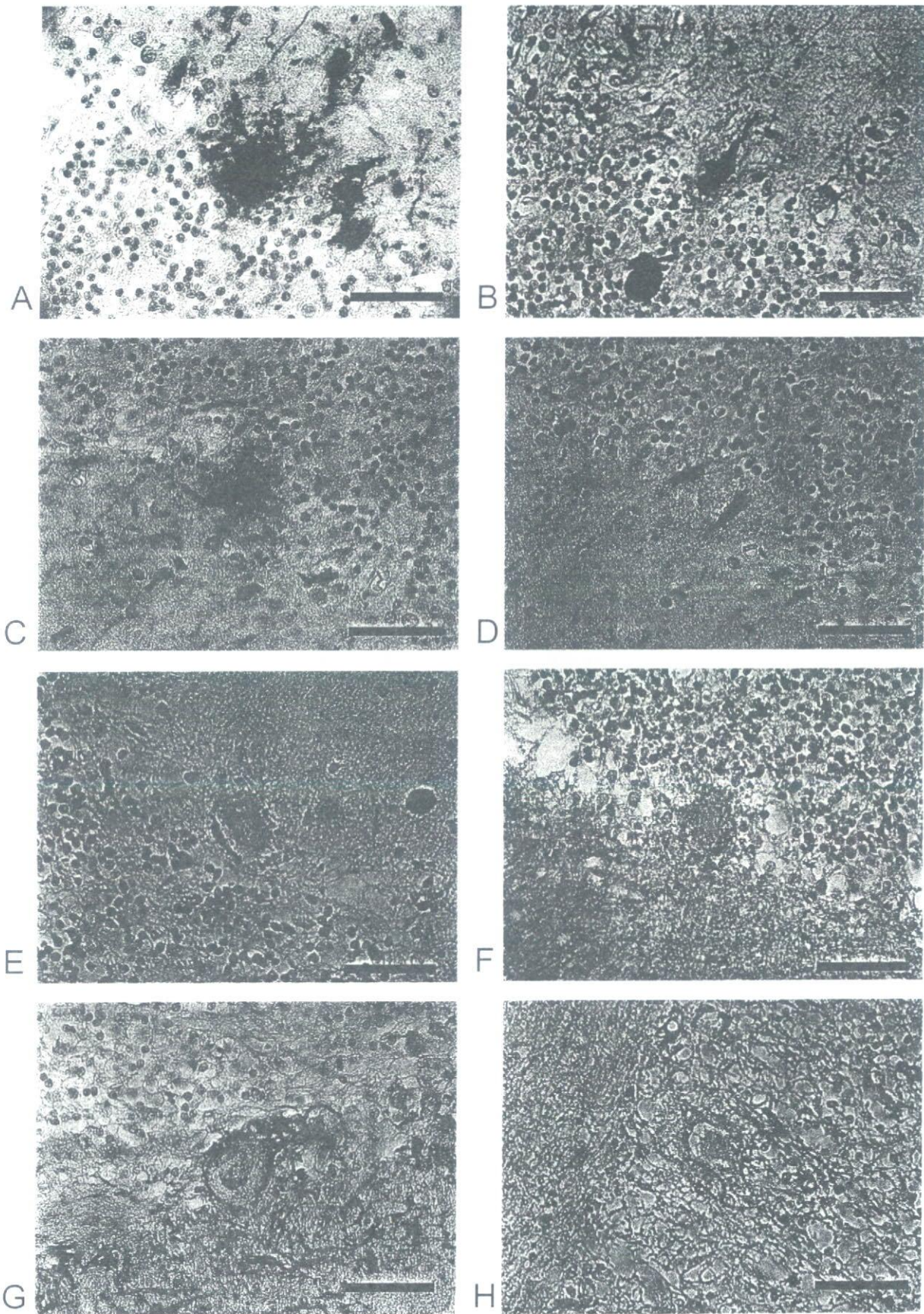


Fig. 3 Immunohistochemistry of the Purkinje cells of a patient with 16q-linked autosomal dominant cerebellar ataxia type III. (A) A Purkinje cell is a bizarre shape, with an abnormal dendritic structure stemming from its plump cell body (calbindin-D28k immunohistochemistry, bar indicates 50 μ m). (B) Granular immunoreactive structures are seen nearby a shrunken Purkinje cell body (calbindin-D28k immunohistochemistry, bar indicates 50 μ m). (C) A diffuse calbindin-D28k immunoreactive area is seen surrounding the Purkinje cell body (calbindin-D28k immunohistochemistry, bar indicates 50 μ m). (D) Two Purkinje cells are surrounded by amorphous material without obvious calbindin-D28k immunoreactivity (calbindin-D28k immunohistochemistry, bar indicates 50 μ m). (E) Ubiquitin immunoreactive granules are seen within amorphous material (ubiquitin immunohistochemistry, bar indicates 50 μ m). (F) Synaptophysin immunohistochemistry shows increased immunoreactivity at the amorphous material (bar indicates 50 μ m). (G) Immunohistochemistry against phosphorylated neurofilament shows immunoreactivity at the outer rim of the amorphous material (bar indicates 50 μ m). (H) Astroglial processes are occasionally seen within the amorphous material (GFAP immunohistochemistry, bar indicates 50 μ m).

Table 3 Neuropathological features specifically in relation to the Purkinje cells and granule cells

Location	Degree of neuronal loss		Other remarkable findings	Gliosis	White matter changes
	Purkinje cells	Granule cells			
Cerebellum					
Cerebellar cortex					
Culmen and declive	Severe	Moderate	Reduced thickness of the molecular layer	Prominent	Mild myelin pallor
Nodulus	Moderate	Mild	–	Mild	None
Uvula	Moderate	Mild	–	Mild	None
Lobules (quadangularis and simplex)	Moderate	Mild	–	Mild	None
Lobules (semilunaris caudalis and gracilis)	Mild	0 to mild	–	Mild	None
Flocculus	Mild	0 to mild	–	Mild	None
Tonsils	Mild	0 to mild	–	Mild	None
Cerebellar white matter	None	None	–	Moderate	Mild myelin pallor

The degree of neuronal loss was rated in four steps: 0, mild, moderate and severe.

clinical and neuropathological features of a newly identified ADCA that is common in Japan.

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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 heterogeneous 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.

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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 pallidolusian 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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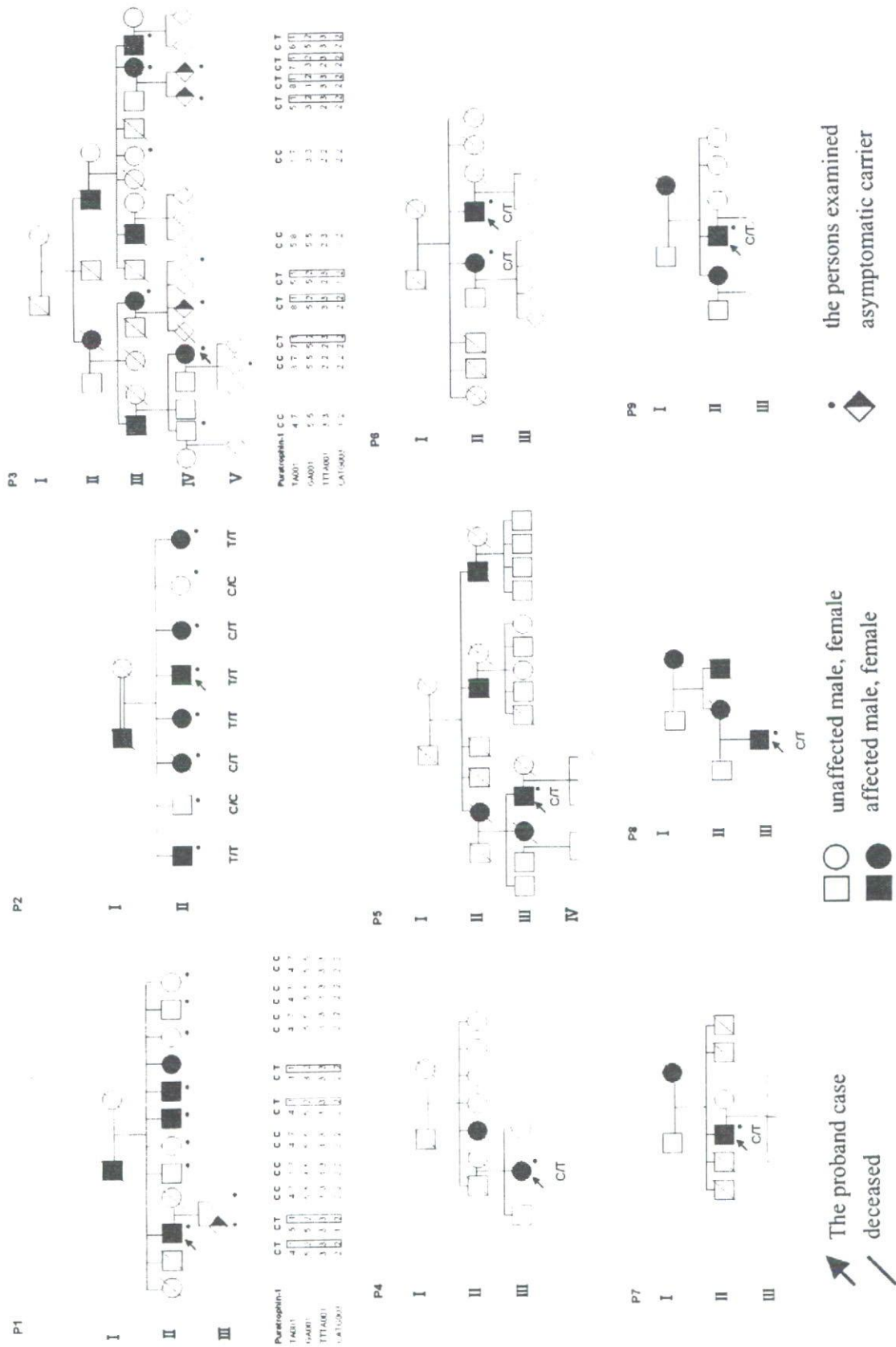


Fig. 1. The pedigrees of nine Japanese families with 16q-linked ADCA. In pedigrees 1 and 3, the gender is concealed in those individuals, including asymptomatic ones, denoted by diamonds to maintain the anonymity of the families.

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.

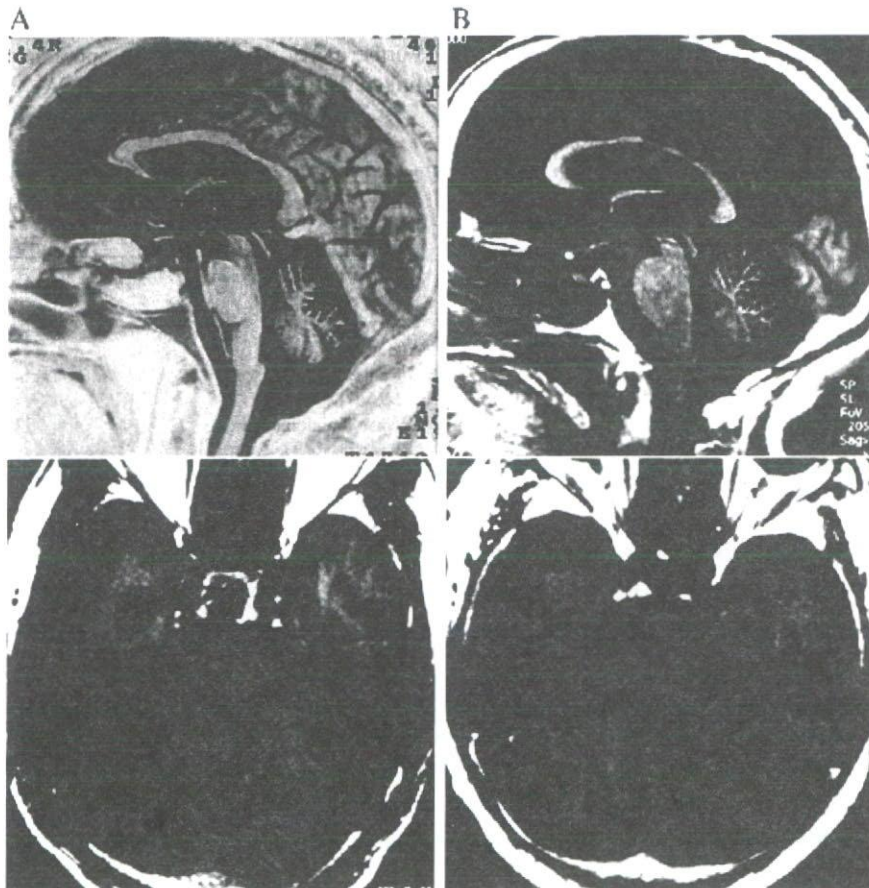


Fig. 2. (A) Brain MRI in a homozygous patient (disease duration, 20 years). Top: Reversed T2-weighted, sagittal slice. Bottom: T1-weighted, axial slice. (B) Brain MRI in a heterozygous patient (disease duration, 22 years). Top: T1-weighted, sagittal slice. Bottom: T1-weighted, axial slice. Both patients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

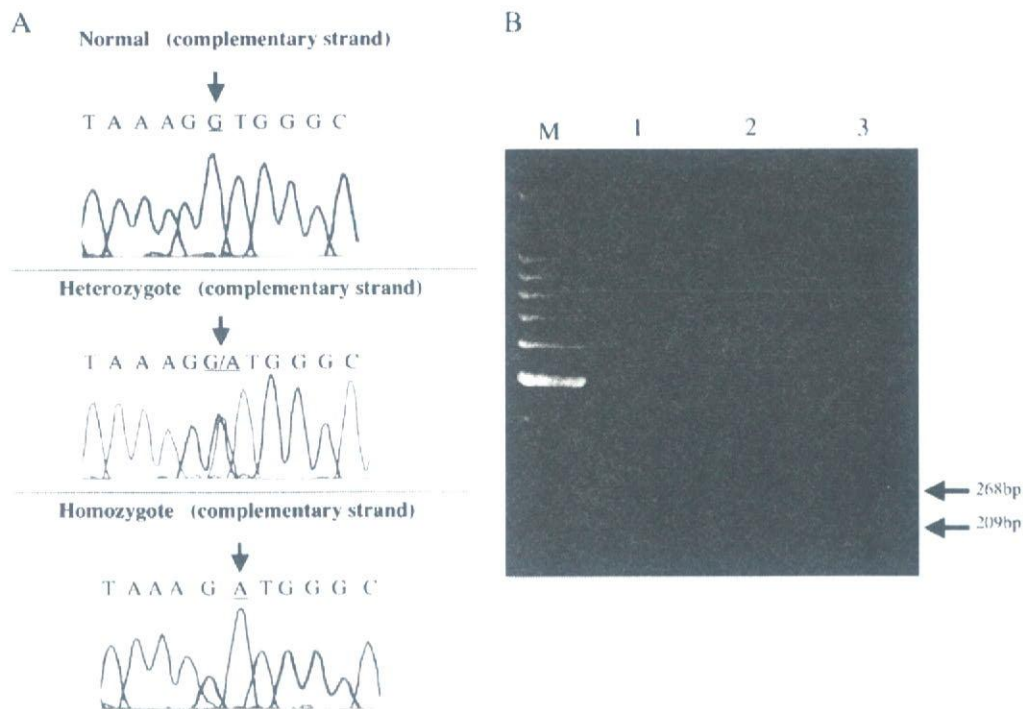


Fig. 3. (A) Nucleotide sequences of exon 1 in the *Puratrophin-1* gene. A G-to-A change (complementary strand) in a patient with a heterozygous or a homozygous state, and the normal sequence in a normal individual are shown. (B) The PCR products after *Eco*NI digestion. Lane M, 100 bp size markers; lane 1, a normal individual; lane 2, a patient with a heterozygous C-to-T change; lane 3, a patient with a homozygous C-to-T change. The wild-type *Eco*NI-digested PCR products gave three bands (209, 92, and 59 bp). Meanwhile, the *Eco*NI-digested PCR products with a heterozygous C-to-T change gave four bands (268, 209, 92, and 59 bp), and those with a homozygous C-to-T change gave two bands (268 and 92 bp). Two *Eco*NI-digested bands (92 and 59 bp) cannot be seen.

3A shows the results of GeneScan analysis of the nucleotide sequence flanking the C-to-T change (complementary strand) in the *puratrophin-1* gene in a heterozygous or homozygous patient and a normal individual in the same family. Fig. 3B shows the results as to the *Eco*NI-digested PCR products in the heterozygous or homozygous patient and the normal individual.

We constructed haplotypes for pedigrees 1 and 3, the two families large enough for haplotype analysis, and the results

revealed that all patients in the two families were segregated with the haplotype 1-2-3-2 for the chromosome 16q markers TA001, GA001, TTTA001 and CATG003 (Fig. 1). The genotypes of the remaining patients and carriers were also identical with the haplotype 1-2-3-2 (data not shown). Furthermore, the specific allele, 2, of GA001 was only seen in all 22 patients and the four carriers with 16q-linked ADCA. The results indicate that the GA001 marker is very specific for the diagnosis of patients with 16q-linked ADCA.

3.3. Frequency of 16q-linked ADCA

The frequencies of various subtypes of ADCA in Japan are shown in Table 2. The results showed that the frequency of 16q-linked ADCA families is 8.2%, this being lower than those of MJD/SCA3 (32.7%) and SCA6 (24.5%) ones, and thus it is the third most frequent ADCA together with DRPLA in Japan. Similarly, concerning the number of patients, 16q-linked ADCA was the third-most frequent next to MJD/SCA3 and SCA6.

4. Discussion

In the present study, we found 22 Japanese patients with 16q-linked ADCA, and revealed some characteristic clinical features of this disease in comparison with those found in

Table 2
Frequencies of various subtypes of ADCA in 110 Japanese families

	Number of families	%	Number of patients	%
MJD/SCA3	36	32.7	79	41.6
SCA6	27	24.5	44	23.1
16q-linked	9	8.2	20	10.5
DRPLA	9	8.2	11	5.8
SCA2	7	6.4	12	6.3
SCA1	6	5.5	6	3.2
SCA8	1	0.9	1	0.5
Unknown	15	13.6	17	9.0
Total	110	100	190	100

Approximately 80% of the 110 families were from the Kanto region, in a central region of the mainland of Japan. Five of the nine families with 16q-linked ADCA were from the Kanto region, whereas the remaining families were from the other regions of Japan.

earlier studies on 16q-linked ADCA in Japan, and SCA4 in Utah and Germany, most of which involved linkage analyses [4–10,20,21]. First, the mean age at onset in our patients was 61.8 years, this being later than those in two earlier reports on Japanese families with 16q-linked ADCA (mean, 55.9 and 56.7 years old) [6,20]. Moreover, the mean age at onset in our patients was much later than that in the SCA4 patients in Utah and Germany (mean, 39.3 and 38.3 years old) [4,5]. The age at onset in our patients with 16q-linked ADCA is much later than that in the patients with SCA6 (mean, 45.0 years old) [6], which indicates late-onset pure cerebellar ataxia. Therefore, 16q-linked ADCA appears to exhibit the oldest age at onset among the ADCA subtypes with assigned loci [9]. Second, we found that although cerebellar ataxia was the most common and predominant feature in 16q-linked ADCA, 54.5% of our patients showed exaggerated deep tendon reflexes. Furthermore, moderate spasticity in the lower limbs was noted in three of the four patients examined in pedigree 3. Thus, although we observed no Babinski signs in our patients, possible pyramidal tract signs can accompany cerebellar ataxia in 16q-linked ADCA, as described for SCA4 [4,5]. Since spasticity in the lower limbs was noted only in one pedigree, the presence of some modifying genetic factors for this phenotype is suggested. Meanwhile, the sensory axonal neuropathy described in SCA4 [4,5] was absent in our patients, as in the earlier reports of 16q-linked ADCA [6–10,20,21]. Sensorineural hearing impairment was considered to be another important clinical feature of the disease [9,10], and 6 (42.8%) of 14 families were reported to have this condition in addition to age-related hearing loss [9]. In our study, audiograms revealed that 37.5% of the patients examined had hearing impairment. However, since we examined only eight patients by means of audiograms, further examinations including audiograms and brainstem auditory evoked potential measurement will be necessary to clarify whether or not hearing impairment is associated with 16q-linked ADCA. Third, we found two asymptomatic carriers with transient nystagmus and mild hyperreflexia, suggesting they are early clinical signs of this disease.

It is noteworthy that we found two sporadic patients with 16q-linked ADCA who had been diagnosed as having LCCA. The parents of the two sporadic patients were all normal until death in their 40s and at 73, 74, and 94 years old, and there were no individuals with cerebellar ataxia in their families. Since the age at onset in our patients with 16q-linked ADCA is very late, the parents who could have harbored a C-to-T mutation in the *puratrophin-1* gene appeared to be neurologically free until their death. Otherwise, incomplete penetrance can be suspected in 16q-linked ADCA. Thus, there is a possibility that a patient with this disease can be misdiagnosed as having sporadic LCCA, and we should analyze the *puratrophin-1* gene even in an apparently sporadic case with cerebellar ataxia.

In pedigree 2, four of the six patients were homozygous for the C-to-T substitution in the *puratrophin-1* gene. Comparing the mean age at onset in homozygotes with that in

heterozygotes in this pedigree, the former was earlier than the latter. Unfortunately, we could not accurately compare the phenotypic severity during the disease course in them. Since the number of observation is low, we should be prudent in interpretation for a gene dosage effect in 16q-linked ADCA. In SCA6, although a gene dosage effect is considered [22–24], the increase in the severity of symptoms with homozygosity is not as great as that observed in MJD/SCA3 [13]. Similarly, a gene dosage effect in 16q-linked ADCA, if one exists, might be mild and similar to that in SCA6. Further studies are required to clarify whether a gene dosage effect indeed exists in 16q-linked ADCA or not, because the brain MRI findings revealed similar atrophy of the cerebellum in a homozygous patient and a heterozygous one.

Our study revealed that 16q-linked ADCA was the third-most frequent subtype of ADCA next to MJD/SCA3 and SCA6 in 110 Japanese families with ADCA. Although SCA6, MJD/SCA3, and DRPLA are considered to be the most prevalent subtypes of ADCA in Japan despite considerable variation in the frequency of each subtype among districts [25], our study showed that 16q-linked ADCA is also frequently seen among Japanese patients with ADCA, and thus this disease may be widespread in Japan. Meanwhile, 13.6% of our ADCA families still remained to be caused by an unknown molecular basis. The clinical features of these families showed adult-onset cerebellar ataxia with or without extracerebellar neurological dysfunction. Although a linkage analysis could not be performed on these families because of a small number of the family members, we should elucidate the molecular etiology of these ADCA families in the near future.

We confirmed that a C-to-T single nucleotide substitution in the 5' UTR of exon 1 in the *puratrophin-1* gene is strongly associated with a distinct form of ataxia. This substitution appears to be the mutation that causes 16q-linked ADCA for the following reasons. First, this change was completely segregated with the disease in 52 Japanese ADCA families, whereas such a change was not seen in 1000 control chromosomes [9]. Second, the C-to-T change resulted in reduced expression in the *in vitro* luciferase assay, which was consistent with the tendency for reduction in mRNA expression in the cerebellum in 16q-linked ADCA [9]. Third, *puratrophin-1* was aggregated in the major target neurons, i.e., Purkinje cells, in 16q-linked ADCA [9]. In the present study, we also confirmed that allele 2 of GA001 was only seen in all affected and asymptomatic carriers with the C-to-T substitution. Since allele 2 ("allele 4" in the previous report) has been seen in all affected individuals in all 52 families with 16q-linked ADCA, but in only 1 in 1000 control chromosomes, GA001 shows strong linkage disequilibrium [9]. Although we could perform haplotype analysis in only two families, the haplotype of "1-2-3-2" (TA001-GA001-TTTA001-CATG003) was common in the two families, suggesting a founder effect in 16q-linked ADCA. Similarly, a strong founder effect has been observed for 16q-linked ADCA in Japan [7, 9].

Finally, it is interesting as to whether 16q-linked ADCA and SCA4 are allelic or not [26]. Since the possible pyramidal tract signs with cerebellar ataxia seen in our patients are common in 16q-linked ADCA and SCA4 despite the absence of sensory axonal neuropathy in the former, the two disorders might be allelic. There is a possibility that patients with 16q-linked ADCA will hereafter be found throughout the world. Further investigations are necessary to clarify the molecular mechanisms underlying 16q-linked ADCA and SCA4.

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Unexpected clinicopathological phenotype linked to small elongation of CAG repeat in SCA1 gene

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Sirs: We report a patient with spinocerebellar ataxia type 1 (SCA1), whose clinical and pathological pictures are not expected from the genetic abnormality [4, 5]. The patient was a 74-year-old man, who developed progressive gait disturbance, dysphagia and dysarthria for several years, as noted in his mother and a sister. He was fully conscious and well oriented. Extraocular movements were restricted in vertical directions and to a lesser extent in horizontal directions. Generalized muscle wasting and weakness involving bulbar muscles were prominent especially in the distal portion of the lower extremities. Patellar tendon reflexes were normal and Achilles tendon reflexes were decreased with positive right Babinski sign. Sensory disturbance, ataxia and extrapyramidal signs were not evident. Needle electromyogram demonstrated neurogenic changes. Laboratory examination was normal except for elevated blood glucose (320 mg/dl) and creatine kinase (1760 U/l). His general

condition deteriorated so rapidly that severe respiratory distress led to a fatal outcome. The clinical diagnosis was motor neuron disease.

The brain weighed 1260 g. The pons and spinal cord was atrophic while inferior olives (Fig. 1A) and cerebellum were relatively preserved. The brain was otherwise normal except for nigral discoloration.

Marked degeneration of the pontocerebellar fibers and of pontine neurons, occasionally containing intranuclear inclusions (NIs) immunopositive for ubiquitin and expanded polyglutamine (1C2, Fig. 1B) was noted [1]. By contrast, degeneration was relatively mild but consistently accompanied by a few NIs, in the inferior olives, dentate nucleus, substantia nigra and lower motor neurons including those in the oculomotor nucleus. Depletion of Purkinje cells and glial reactions were mild to moderate. NIs were absent in Pj cells [3]. Dilatation of perineuronal space and mild spongiosis was noted in the cerebral cortex and striatum, where NIs were identified (Fig. 1C). Neurons were mildly degenerated

in the subthalamic nucleus, where gliosis was slight (Fig. 1D). Neuronal degeneration and gliosis were evident in the globus pallidus, where difference between its external and internal segments was not apparent. Neither Bunina bodies nor skein-like inclusions were detected and anterior and lateral corticospinal tracts were preserved relative to the spinocerebellar tracts. Skeletal muscles exhibited neurogenic changes. With the consent of the family, genomic DNA was extracted and a small elongation (n = 41, normal < 39) of CAG repeat was noted in SCA1 gene [4], while CAG repeat size was normal in SCA2, SCA3, SCA6, SCA17, DR-PLA genes.

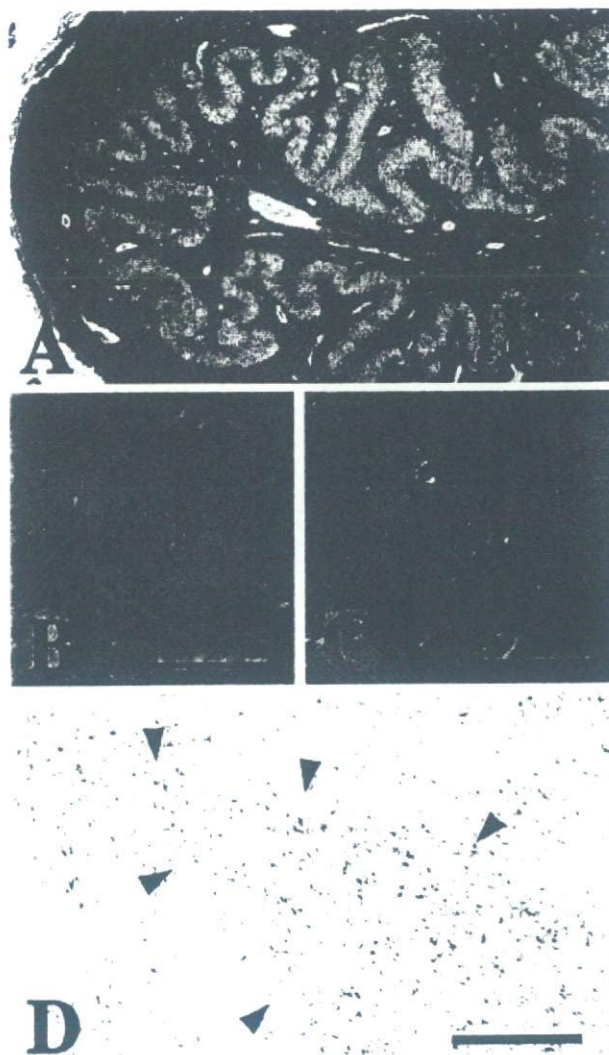
Preferential involvement of pontine nucleus, spinocerebellar system, substantia nigra and lower motor neurons in the presence of NIs, noted in this patient are compatible with reported autopsy findings of SCA1 [1]. In typical SCA1 cases, apparent involvement of inferior olivary nucleus and cerebellar cortex and dentate nucleus, is the rule [2], all of which were not evident in this case (Table). Al-

Table Regional distribution of lesions and their severity

Area	representative SCA1 cases [2]	this case
Cerebellar system including its afferents and efferents		
Purkinje cells	± - ++	+
Dentatofugal system	+ - ++	+
Pontocerebellar system	± - ++	++
Inferior olivary nucleus	+ - ++	+
Spinocerebellar tracts	+++	++
Clarke's column	++	+++
Extrapyramidal system		
Substantia nigra	± - ++	++
Pallidum interna	-	++
Pallidum externa	± - ++	++
Subthalamic nucleus	± - +	+Oculomotor system + - + + + +
Anterior horns	+ - ++	++
Dorsal column	- ++	+

ext external; int internal; nucl. nucleus; - absent; ± very mild; + mild, ++ moderate; +++ severe

Fig. 1 **A:** Inferior olivary nucleus. The width of ribbon is slightly reduced, but the entire structure is relatively preserved. (Klüver-Barrera stain). **B:** Nuclear inclusion in a pontine neuron (1C2 immunostaining after pretreatment with formic acid, bar = 25µm). **C:** Nuclear inclusion in cerebral cortex (ubiquitin immunostaining, bar = 25µm). **D:** Subthalamic nucleus (arrowheads, GFAP immunostaining, bar = 50µm). Atrophy is not evident and proliferation of GFAP-positive cells is slight



though the lesion in the globus pallidus is one of the pathological features of SCA1, typical SCA1 cases are characterized by preferential involvement of its external segment [2], again not detectable in this case. Mild spongiosis with minimal glial proliferation may represent an influence not directly linked to degeneration but possibly related to circulatory disturbance in the agonal state. However, identification of NIs in these areas (Fig. 1C), as well as in the thalamus indicates that degenerative process with NI is extended to these areas, not described so far in SCA1

brains. Small expansion of the CAG repeat in this patient may be correlated not only with late disease onset [4] but also with relative preservation of these regions, which may explain predominant manifestation of lower motor involvement without apparent abnormality on motor control. Variability of pathological lesion as seen in this case may provide an opportunity to gain further insight into how lesions are engendered in human brains.

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Dynamic imbalance in gait ataxia. Characteristics of plantar pressure measurements

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Abstract

The present study was designed to evaluate the interaction between disequilibrium and irregular stepping components of ataxic gait. For this purpose, we compared the walking patterns of patients with cerebellar dominant multiple system atrophy (MSAc, $n=8$), spinocerebellar ataxia type 6 (SCA6, $n=4$) and 16q-linked autosomal dominant cortical cerebellar atrophy (16q-linked ADCA, $n=6$), and 6 normal subjects, by measuring toe and heel plantar pressures. In healthy subjects, the heel contacted the floor at step-in followed by an immediate shift of the center of pressure (COP) to the contacted leg. In ataxic gait, however, both the heel and toes simultaneously contacted the floor and the disappearance of the immediate shift of the COP was noted. These changes appeared to be nonspecific compensations for the instability. Examination of two parameters of ataxia-specific changes showed that prolongation of the double support period was associated with proportionate increase in the coefficients of variance of the plantar pressures and the step lengths on walking of patients with SCA6, but not those with MSAc and 16q-linked ADCA. Our results suggest that disequilibrium and irregularity are two separate and independent components of cerebellar ataxic gait.

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Keywords: Ataxia; Gait; Disequilibrium; Double support period; Walking; Center of pressure; Instability; Cerebellar ataxia; Compensatory changes

1. Introduction

Ataxic gait is a cardinal and noticeable clinical feature of cerebellar disorders. The clinical features of ataxic gait comprise compensatory reactions against the imbalance and specific changes proper for ataxia [1,2]. Due to the profound instability, compensatory reactions occur prominently in ataxic gait compared with those in other gait disorders, which result in hiding ataxia-induced changes. Thus, to determine the exact characteristics of ataxic gait, it is necessary to define the extent of the nonspecific compensations that are superimposed on the ataxia-specific changes.

Previous studies reported that ataxic patients walk with widened stance, prolonged double support period and

augmented activities of the proximal muscles. The angular displacements of the hip, knee and ankle joints become small and walking speed becomes slow [1–7]. Ataxic patients give priority to the maintenance of balance over propulsive locomotion. An intriguing question about this strategy is the extent of disturbance of the propulsion of the center of body mass in such a protective walking pattern. On the other hand, characteristic changes in ataxic gait have been documented, which consist of large and abrupt body sways and irregular stepping with interjoint incoordination [1–7]. These changes have been considered as a single symptom based on the assumption that their severity is correlatively advanced. However, recent physiological studies suggest heterogeneity in the ataxic gait.

The neural structures that control walking can be divided into two functionally discrete systems: (1) the equilibrium center, which assumes the upright posture, and (2) the

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Table 1
Clinical profile of participating patients

Age (years), sex	Duration from onset	Ataxia	Eye movement
Patients with MSAC			
1. 61, F	6 years	+	Saccadic, nystagmus (-)
2. 64, M	10 months	+	Saccadic, nystagmus (-)
3. 51, M	1 year	+	Saccadic, nystagmus (+)
4. 73, M	2 years	++	Saccadic, nystagmus (+)
5. 69, M	2 years	+	Saccadic, nystagmus (+)
6. 54, F	8 months	+	Saccadic, nystagmus (+)
7. 64, F	5 years	+	Saccadic, nystagmus (-)
8. 54, M	1 year	+	Nystagmus (-)
Patients with SCA6			
1. 56, M	13 years	++	Saccadic, nystagmus (+)
2. 66, M	3 years	+	Saccadic, nystagmus (-)
3. 62, F	4 years	+	Saccadic, nystagmus (+)
4. 60, M	12 years	+	Saccadic, nystagmus(+)
Patients with 16qADCA			
1. 61, F	4 years	+	Nystagmus (-)
2. 56, F	6 years	+	Saccadic, nystagmus (+)
3. 63, F	2 months	+	Saccadic, nystagmus (-)
4. 59, M	3 months	+	Saccadic, nystagmus (+)
5. 75, M	6 years	++	Saccadic, nystagmus (-)
6. 61, M	2 years	+	Nystagmus(-)

locomotion center, which initiates and maintains rhythmic stepping [8]. These centers exist in the brainstem and spinal cord and are functionally integrated for proper execution of gait. Thus, adaptive control by the cerebellum on walking could be classified into two aspects: the dynamic regulation of postural stability and the fine tuning of rhythmic stepping. For example, the former includes integrated control of proprioceptive reflexes, whereas the latter includes adjustment of the timing of locomotor movements of the limb and the interjoint coordination during locomotion [9]. If so, disequilibrium and irregular stepping would be also independent components of clinical appearances in ataxic gait.

The present study was designed to evaluate the interaction between disequilibrium and irregular stepping components of ataxic gait. For this purpose, we analyzed the walking pattern of patients with spinocerebellar degeneration. We first measured toe and heel plantar pressures to define how efficient propelling is sacrificed in unstable gait.

Second, we examined the relationship between disequilibrium and irregular stepping in ataxic gait. Thus, we measured the double support period as index for disequilibrium, and then calculated the coefficients of variance (CV) of toe and heel plantar pressures and that of strides as index for irregularity. Based on deviations in these two parameters, we estimated the disequilibrium and irregular stepping in each patient. The results suggested that disequilibrium and irregularity seem to be two separate and independent components of cerebellar ataxic gait.

2. Methods

2.1. Subjects

The study subjects were 24; 18 patients with cerebellar ataxia (mean age, 61.6 years, 11 men and 7 women) and 6 age-matched normal controls (mean age, 58.3 years, 3 men and 3 women) free of gait disturbances. The background conditions of ataxic patients were ataxia dominant multiple system atrophy (MSAC, $n=8$, mean age 61.3 years, 5 men and 3 women), 16q-linked autosomal dominant cortical cerebellar atrophy (ADCA, $n=6$, mean age 62.5, 3 men and 3 women), and spinocerebellar ataxia type 6 (SCA6, $n=4$, mean age, 61.0 years, 3 men and 1 woman). Table 1 shows the clinical profile of participating patients. The diagnosis of MSAC was made according to the clinical criteria [10]. The diagnoses of 16q-linked ADCA and SCA6 were made based on clinical findings and genetic analyses [11,12]. In the standing and gait portions of the International Ataxia Cooperative Rating Scale (ICARS) [13], patients participating in this study were included in 'walk without cane or any assistance'.

The study was explained in detail to each subject and a signed consent was obtained before the start of the study.

2.2. Study protocol

The Peak Motus 3D Motion Analysis System (PEAK Performance Technologies Inc., Englewood, CO) was used to examine the gait kinematics. Anima Plantar Pressure Measuring System MP-1200 (ANIMA Inc., Tokyo, Japan)

Table 2
Kinematic data

	Patients with ataxia			Normal control
	MSAC	SCA6	16qADCA	
<i>N</i>	8	4	6	6
Gait velocity (meter/s)	0.848±0.22	0.641±0.29	0.710±0.19	1.330±0.12
Cadence (steps/min)	98.8±12.7	89.6±26.2	89.2±8.7	113.6±4.9
Stride/height ratio	0.632±0.13	0.527±0.17	0.613±0.11	0.873±0.09
Step width/height ratio	0.150±0.02	0.148±0.05	0.179±0.04	0.095±0.03

Data are mean±S.D.

Compared with normal subjects, each ataxic group showed a significantly slow gait velocity ($p<0.05$), shortened stride/height ratio ($p<0.05$). There were no significant differences in the parameters among the three ataxic groups ($p<0.05$).

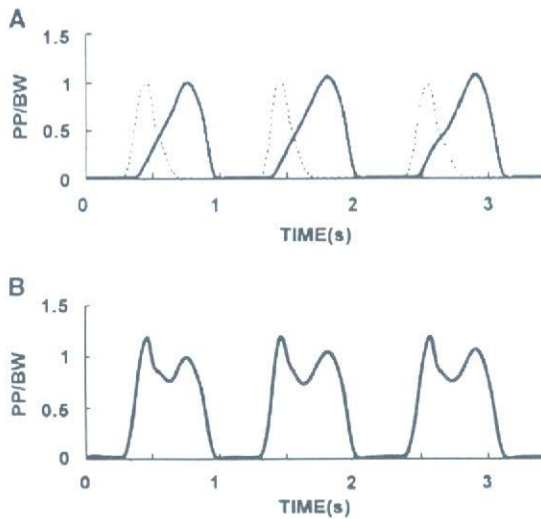


Fig. 1. An example of plantar pressures of a normal control gait. (A) Discriminative recordings of toe (solid line) and heel (dotted line) pressures. (B) Summation of toe and heel pressures. PP: plantar pressure, BW: body weight.

was used for recording plantar pressure. The latter system consists of a pair of shoe-type devices (in which two insole pressure sensors are embedded: one under the toes and the other in the heel), two wireless telemeters (connected to each shoe-type device), a receiver unit and a personal computer with a data-analysis software (MMMP1200). The signals were A–D converted at a sampling rate of 1000 times per second, digitized and stored on a personal computer.

For the test, the subject wore the ANIMA shoe-type devices and was asked to walk at his/her free walking speed without assistance. The three-dimensional gait parameters measured included stride, gait cycle, step length, and step width. Gait velocity and cadence were calculated from these parameters (Table 2). For each subject, values of gait velocity and step width were expressed relative to body height for standardization. Plantar pressures at the toes and the heel were also measured on both sides. The peak pressure values were expressed relative to body weight. For these parameters, the mean and standard deviation were calculated from six consecutive steps in rhythmic phase. The coefficients of variance (CV) were also calculated for step lengths and plantar pressures. Kinematic and plantar pressure data were processed and gait parameters were calculated using Microsoft Excel. Statistical analysis was conducted using SPSS for Windows (Windows ver. 13, SPSS Inc., Chicago, IL).

3. Results

3.1. Nonspecific compensation in plantar pressures

Compared with plantar pressure patterns recorded during walking of a healthy (*herein abbreviated as normal*

walking) (Fig. 1), those recorded during walking of ataxic patients (*abbreviated as ataxic walking*) (Fig. 2A and B) showed the following three characteristic features.

First, ataxic patients showed simultaneous floor contact of the heel and toes. In normal walking, the heel pressure (dotted lines) was followed by the toe pressure (solid lines), resulting in two peaks in the sum tracing (Fig. 1B). Thus, the heel contacted the floor for step-in in normal subjects. In ataxic gait, however, the toe plantar pressure increased from an early stage (Fig. 2A). Furthermore, severely ataxic gait showed simultaneous increases of pressures in both the heel and toes (Fig. 2B). These changes indicate that at the foot contact, ataxic patients supported their body weights using the whole foot sole.

Second, differences were observed in the shift of center of pressure (COP) in the contacted foot. In healthy walking, the plantar pressure curve of the contacted foot increased steeply and the ratio of plantar pressure to body weight (PP/BW) immediately reached 1.0 (Fig. 1). Thus, when the foot contacted the floor, it immediately bore the body weight. In contrast, at step-in by ataxic patients, the maximum plantar pressure of the contacted foot was always below the body weight. During walking, the PP/BW ratio of the heel ranged from 0.6 to 0.8 (Fig. 2A) in patients with moderate ataxia,

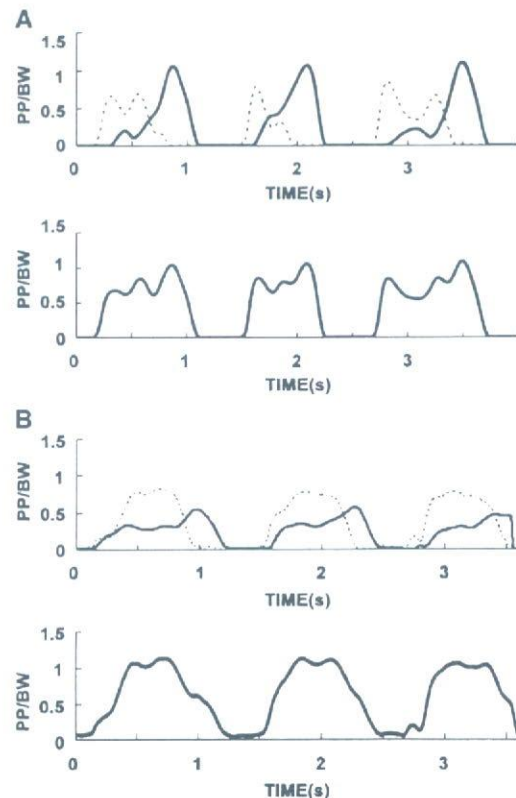


Fig. 2. Examples of plantar pressures of (A) moderate ataxic gait (patient with MSAc) and (B) severely ataxic gait (patient with 16q-linked ADCA). Top recordings: discriminative recordings of toe (solid line) and heel (dotted line) pressures. Bottom recordings: summation of toe and heel pressures. PP: plantar pressure, BW: body weight.

while even the sum of toe and heel PP/BW was only 0.5 to 0.6 in patients with severe ataxia (Fig. 2B). Thus, the PP/BW ratio of the contacted foot gradually increased to 1.0. These findings indicate that the body weight of the ataxic patient was supported by both feet, the contacted foot and the contralateral foot, coupled with a lack of immediate shift of COP to the contacted foot.

Thirdly, the toe plantar pressures decreased in proportion with the stage of ataxia. In normal walking, the toe pressure for kick-off was similar to the heel pressure for kick-in and their peak values of the PP/BW were 1.0 (Fig. 1). In

severely ataxic gait, however, the peak value of the PP/BW ratio diminished to 0.5. The kick-off component appeared as a hump (Fig. 2A and B).

3.2. Relationship between double support period and coefficients of variance

Fig. 3 shows the relationships between double support period and CVs for the toe and heel plantar pressures and the stride length. In ataxic patients with SCA6, the double support period correlated with CV in toe pressure; i.e., the CV values increased proportionately and significantly ($p < 0.05$) with prolongation of the double support period (Fig. 3A). In contrast, no such relationship was noted in patients with MSAc and 16q-linked ADCA (Fig. 3A). Similar characteristics were noted between the double support period and heel pressure (Fig. 3B) and between the double support period and stride length (Fig. 3C). Analysis of walking of patients with SCA6 showed that prolongation of the double support period was associated with a proportionate increase in CV values. In contrast, analysis of the gait of patients with MSAc and 16q-linked ADCA showed that the CV value was less affected compared with the double support period. Slightly increased CV was also noted even in patients with prolonged double support period. Thus, the CV values were smaller in patients with MSAc and 16q-linked ADCA compared with those with SCA6. These results indicate that the two parameters, the double support period and CV, were not necessarily equally impaired.

4. Discussion

4.1. Compensation for decrease and absorption of body sways in ataxic gait

Cerebellar ataxic gait is characterized by marked instability [1–7]. The ataxic patients walk with large sways of COP, mainly in an anteroposterior direction [1]. Previous studies indicated that cerebellar dysfunction causes exaggeration of the stretch reflex and, consequently, small forward or backward deviations of the COP from a neutral position produces large sways [1,9]. Large displacements of the COP occur especially in association with movements of contact of the foot on the floor and the subsequent forward swing of the foot, since these movements produce reaction forces toward the leg. In ataxic gait, therefore, elaborate compensatory mechanisms are necessary during the period of foot swing and foot contact.

The most well-known compensatory changes in ataxia are slow walking speed, widened stance, increased rate of double support period and augmented activities of the thigh and leg muscles [1–7]. Conrad and colleagues named this gait pattern protective walking [14]. In patients with various neurological disorders, we also demonstrated previously the presence of augmented muscle activities during walking,

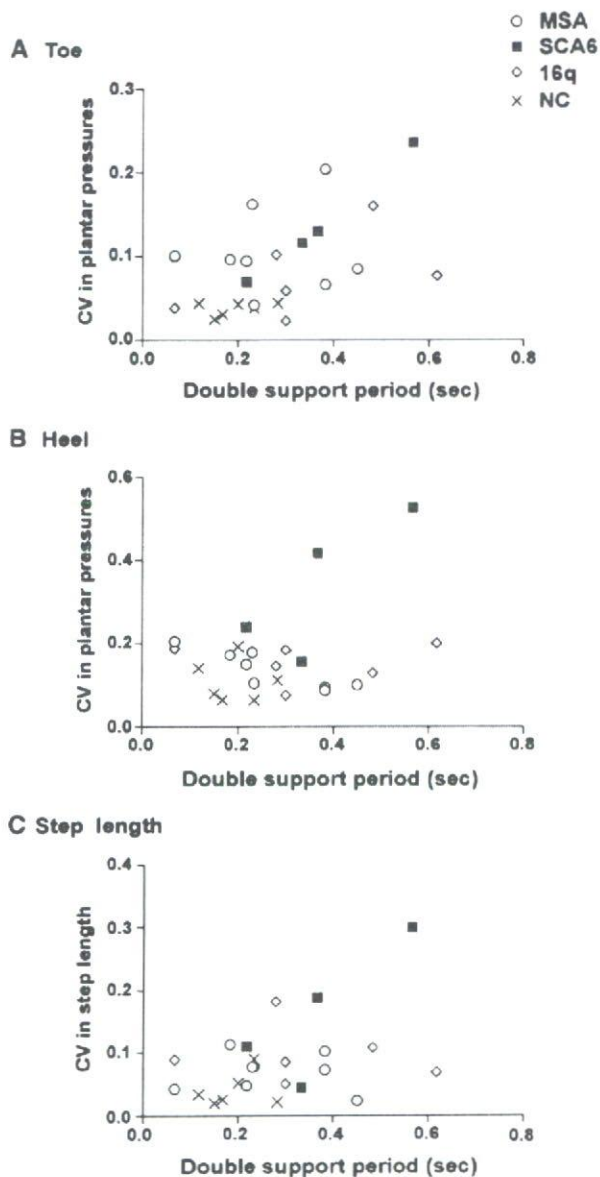


Fig. 3. Correlation between double support period and coefficients of variance (CV) of toe (A) and heel (B) plantar pressures, and correlation between double support period and CV of step lengths (C). These parameters were recorded during walking of patients with MSAc, SCA6 and 16q-linked ADCA, and normal subjects.

which resulted in co-contraction between the gastrocnemius and tibialis anterior muscles [1,2]. The co-contraction increases the stiffness of the ankle joint to produce optimal absorption of the momentum of perturbations. The present study also revealed two types of compensatory reactions in the heel and toe plantar pressures. The first compensatory changes serve to absorb body sways at the foot contact. At step-in of healthy subjects, the heel contacts the floor and then the COP immediately shifts to the contacted leg. In ataxic gait, however, both the heel and toe simultaneously contact the floor and the disappearance of the immediate shift of COP. Thus, to endure the foot contact-induced body sways, ataxic patients appear to increase the plantar areas for touching the floor and, at the step-in, put their body weights evenly on the two legs. After cessation of the body sway, ataxic patients slowly bear their body weight on the contacted leg for preparation of the swing of the opposite leg. The second compensatory change is reduction in toe plantar pressure. A decrease in toe plantar pressure serves to minimize perturbations at kick off. Through these sequential compensations in plantar pressures, ataxic patients give priority to compensatory reactions against any imbalance over efficient forward movement. Consequently, the standing posture is markedly pronounced in ataxic walking.

4.2. Pathophysiological indices of instability and irregularity of ataxic gait

The present study was designed to examine whether the two factors, instability and irregular stepping, correlatively deviate in ataxic patients. Our results indicate that in patients with SCA6, the coefficients of variance of plantar pressures and those of the strides increased proportionately with prolongation of the double support period. In contrast, in patients with MSAC and 16q-linked ADCA, prolongation of the double support period did not correlate with CV values of plantar pressures or strides. Such discrepancy might be due to the heterogeneous degeneration of the cerebellar cortex; the cerebellar area responsible for the coordinated control of stepping might be preserved. Alternatively, the cerebellar learning mechanisms in MSAC and 16q-linked ADCA might operate so as to improve the irregular stepping. In this regard, one experimental study indicated that stepping is adjusted by modifications of cerebellar synaptic transmissions [15]. There might be a difference in the efficacy of compensatory learning between instability and stepping irregularity. Our results show that the double support period and the CV of plantar pressures were independently modified, suggesting that the disequilibrium and irregularity are two separate components of the cerebellar ataxic gait. The clinical characteristic of the ataxic gait is a combination of these two components, and its

pathophysiological profile might vary from a predominantly unstable walking to a walking pattern characterized by both instability and irregular stepping. Thus, further studies are necessary to confirm our hypothesis by measuring various types of patients with cerebellar degenerations and vascular diseases.

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Redefining the disease locus of 16q22.1-linked autosomal dominant cerebellar ataxia

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Abstract The 16q22.1-linked autosomal dominant cerebellar ataxia (16q-ADCA; Online Mendelian Inheritance in Man [OMIM] #117210) is one of the most common ADCAs in Japan. Previously, we had reported that the patients share a common haplotype by founder effect and that a C-to-T substitution (–16C>T) in the *puratrophin-1* gene was strongly associated with the disease. However, recently, an exceptional patient without the substitution was reported, indicating that a true pathogenic mutation might be present elsewhere. In this study, we clarified the disease locus more definitely by the haplotype analysis of families showing pure cerebellar ataxia. In addition to microsatellite markers, the

single nucleotide polymorphisms (SNPs) that we identified on the disease chromosome were examined to confirm the borders of the disease locus. The analysis of 64 families with the –16C>T substitution in the *puratrophin-1* gene revealed one family showing an ancestral recombination event between SNP04 and SNP05 on the disease chromosome. The analysis of 22 families without identifiable genetic mutations revealed another family carrying the common haplotype centromeric to the *puratrophin-1* gene, but lacking the –16C>T substitution in this gene. We concluded that the disease locus of 16q-ADCA was definitely confined to a 900-kb genomic region between the SNP04 and the –16C>T substitution in the *puratrophin-1* gene in 16q22.1.

Keywords 16q-ADCA · Pure cerebellar ataxia · Haplotype · SNP · Founder effect · SCA4

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Introduction

Autosomal dominant cerebellar ataxia (ADCA) is a clinical entity of heterogeneous neurodegenerative diseases that show dominantly inherited, progressive cerebellar ataxia that can be variably associated with other neurological and systemic features (Harding 1982). ADCA is now classified by the responsible mutations or gene loci. Subtypes of ADCA of which causative genes or gene loci have been identified are known as spinocerebellar ataxia type (SCA) 1, 2, 3 (or Machado-Joseph disease), 4–8, 10–19, 21–23, 25, 26, 28, dentatorubral and pallidolusian atrophy (DRPLA), and ADCA with mutation in the fibroblast growth factor (FGF) 14 gene (Schöls et al. 2004; Yu et al. 2005; Cagnoli et al. 2006).

Among these, mutations in SCA1, SCA2, SCA3/MJD, SCA6, SCA7, SCA17, and DRPLA have been identified as