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ORIGINAL COMMUNICATION

Nationwide survey of Alexander disease in Japan and proposed new guidelines for diagnosis

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Abstract Alexander disease (AxD) is a rare neurodegenerative disorder characterized by white matter degeneration and formation of cytoplasmic inclusions. *Glial fibrillary acidic protein* (*GFAP*) mutations have been reported in various forms of AxD since 2001. However, a definitive diagnosis remains difficult because of uncertain prevalence, and different clinical features seen in infantile AxD and adult AxD may lead to confusion and misdiagnosis. Here we report an epidemiological study conducted in Japan. Two nationwide questionnaire-based surveys were conducted using tentative diagnostic criteria. We gathered information regarding prevalence, neurological findings, magnetic resonance imaging (MRI) findings, electrophysiological findings, genetic information, and the results of therapeutic interventions and home care. Prevalence of various forms of AxD was determined as 27.3% (infantile), 24.2% (juvenile), and 48.5% (adult). Prevalence of AxD in Japan was estimated to be approximately 1 case per 2.7 million individuals. The main characteristics of infantile and juvenile AxD include delayed psychomotor development or mental retardation, convulsions, macrocephaly, and predominant cerebral white matter abnormalities in the frontal lobe on brain MRI. The main characteristics of adult AxD include bulbar signs, muscle weakness with hyperreflexia, and signal abnormalities and/or atrophy of medulla oblongata and cervical spinal cord on MRI. To ensure correct diagnosis of AxD, the physician should understand the importance of the process of *GFAP* genetic testing, which provides definitive diagnosis. Therefore, we propose new clinical guidelines for diagnosing AxD based on simplified classifications: cerebral AxD (type 1), bulbospinal AxD (type 2), and intermediate form (type 3).

Keywords Glial fibrillary acidic protein · Alexander disease · Genetics · Magnetic resonance imaging · Prevalence

Abbreviations

AxD	Alexander disease
GFAP	Glial fibrillary acidic protein
EEG	Electroencephalogram
ABR	Auditory brainstem response
TRH	Thyroid-releasing hormone

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Introduction

Alexander disease (AxD) is a rare neurodegenerative disorder characterized by white matter degeneration and formation of cytoplasmic inclusions known as Rosenthal fibers, which accumulate primarily in the astrocyte end-feet of subpial and perivascular zones and consist of glial fibrillary acidic protein (GFAP), heat shock protein 27, and αB-crystallin [1–3]. *Glial fibrillary acidic protein* mutations have been reported in various forms of AxD since 2001 [4]. AxD in adults was identified following research on various clinical forms of AxD and their associated findings detected by magnetic resonance imaging (MRI) [5]. Although adult AxD demonstrates a *GFAP* mutation, the clinical symptoms and MRI findings are different from those found in infantile AxD. Clinical features and MRI findings characteristic of AxD were recently identified by performing case studies and a systematic review [6–10]. However, a definitive diagnosis remains difficult because of uncertain prevalence; furthermore, different clinical features seen in infantile AxD and adult AxD may lead to confusion and misdiagnosis.

This study reports the results of a nationwide survey conducted in Japan to gather information on the prevalence, neurological findings, MRI findings, electrophysiological findings, genetic information, and the results of therapeutic interventions and home care.

Materials and methods

Inclusion criteria specified for the diagnosis of AxD are summarized in Table 1. These criteria were decided on the basis of a literature review on AxD, and were decided for the three common age-dependent clinical subtypes, namely infantile, juvenile, and adult. MRI findings were based on previously reported proposed criteria [11].

First survey

To determine the proportion of AxD patients in Japan, a survey questionnaire was sent to the members of educational facilities listed by the Japanese Societies of Neurology, Pediatrics, and Child Neurology, requesting information on patients who fulfilled the clinical inclusion criteria. Cases reported between 2004 and 2009 were examined in this survey.

Second survey

A second set of survey questionnaires was mailed in November 2009 to facilities that were reported as having AxD patients in the first survey. Information was requested on patients' neurological, electrophysiological, and pathological findings, results of genetic analyses, treatment provided, and their clinical outcomes. We confirmed that these cases did not overlap with other reported cases by documenting age and place of birth.

Receipt of responses to the second survey was closed in February 2010, and the collected data were then analyzed.

The survey aimed at collecting information without many details, to obtain the maximum possible answers. Therefore, results were superficial, e.g., normal/abnormal, presence/absence of muscle weakness.

Table 1 Requirements for the proposed diagnosis of Alexander disease for the first survey

Definite Alexander disease: existence of numerous Rosenthal fibers in addition to gliosis and loss of myelin in pathological study or *GFAP* gene mutation, and satisfaction of the following neurological and MRI findings 1, 2, or 3

1. Infantile Alexander disease: onset age is under 2 years with one or more items of (a) and one or more items of (b)

   (a) Neurological findings: psychomotor developmental delay/mental retardation, convulsion, macrocephaly spastic paralysis, bulbar or pseudobulbar signs, cerebellar ataxia

   (b) MRI findings: cerebral white matter abnormalities with frontal lobe predominance, signal abnormalities with swelling or atrophy of basal ganglia and thalami, periventricular rim, brainstem lesions, contrast enhancement

2. Juvenile Alexander disease: onset age is between 2 and 12 years with one or more items of (a) and one or more items of (b) (i) or (b) (ii)

   (a) Neurological findings: mental retardation or dementia, convulsion, macrocephaly spastic paralysis, bulbar or pseudobulbar signs, cerebellar ataxia, autonomic dysfunction, nystagmus, palatal myoclonus

   (b) MRI findings

   (i) Cerebral white matter abnormalities with frontal lobe predominance, signal abnormalities with swelling or atrophy of basal ganglia and thalami, periventricular rim, brainstem lesions, contrast enhancement

   (ii) Signal abnormalities or atrophy of medulla, oblongata, and/or cervical cord

3. Adult Alexander disease: onset age is over 12 years with one or more items of (a) and one or more items of (b)

   (a) Neurological findings: paralysis, bulbar or pseudobulbar signs, cerebellar ataxia, autonomic dysfunction, nystagmus, palatal myoclonus, dementia

   (b) MRI findings: signal abnormalities or atrophy of medulla oblongata and/or cervical cord

Results

The results of the first and second surveys are summarized in Fig. 1.

Thirty-five patients with definite AxD were identified at the end of the surveys and were further analyzed. Prevalence of various forms of AxD was determined as infantile, 9/33 (27.3%); juvenile, 8/33 (24.2%); and adult, 16/33 (48.5%). Cases of twins with infantile AxD, and those with familial adult AxD were considered as one case; this was reflected in the calculations of the proportion of patients in each category.

Patient profiles are summarized in Table 2. Infantile AxD predominantly affected males (8 boys, 2 girls), while no gender predominance was observed in juvenile or adult AxD. Sixty-five percent of adult AxD patients had family members with AxD-like symptoms. Only one pair of identical twins presenting with similar clinical findings were diagnosed with infantile AxD, because brain biopsy of one of the twins showed characteristic Rosenthal fibers. In both cases, a *GFAP* mutation (R79C) was identified later.

Neurological findings are summarized in Table 3. Incidence of delayed psychomotor development or mental retardation, convulsions, macrocephaly, hyperreflexia, dysarthria, and dysphagia was high, whereas incidence of palatal tremor was zero in infantile AxD patients. Incidence of muscle weakness, hyperreflexia, dysarthria, dysphonia, dysphagia, and sphincter abnormalities was high in adult AxD patients. Three cases described as having "unilateral or asymmetric muscle weakness" were included. In addition, three cases that showed asymmetry in the initial stage of the disease, but not at the time of reporting, were also included. Asymmetry was identified in at least 35.0% of the adult AxD cases. Dementia and muscle rigidity were observed in 25.0% and 29.4% of adult AxD

Fig. 1 Flow of the survey

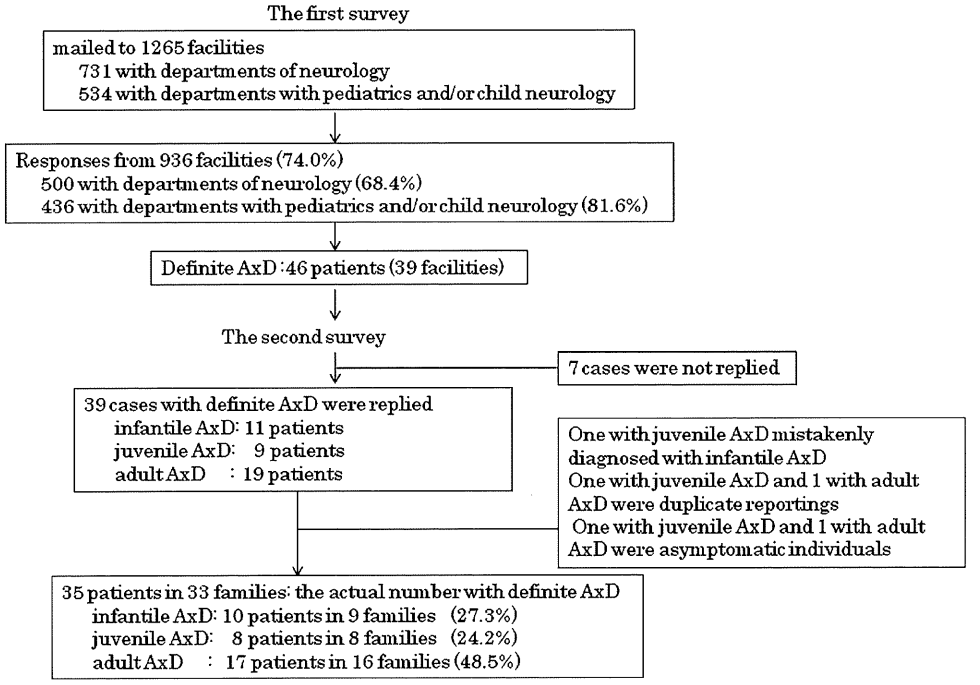

Table 2 Patient profiles in the second survey

	Infantile form	Juvenile form	Adult form
Cases	10	8	17
M:F	8:2	4:4	9:8
Age at onset (m: months, y: years)	10.7 ± 6.7 m (3 to 24 m)	4 0 ± 2.3 y (2 y 10 m to 9 y)	44.1 ± 12.9 y (26 to 61 y)
Family history	2 (twins)	0	10 (9 families)
Period from onset to diagnosis	60 ± 4 4 m (0 to 14 m)	6.3 ± 3.1 y (<1 y 2 m to 10 y)	6.9 ± 6.3 y (0 to 22 y)
GFAP gene analysis[a]	10	8	17
Pathological examination	1 (brain biopsy)	0	2 (autopsy)

[a] *Glial fibrillary acidic protein* gene analysis was also preformed in two asymptomatic individuals

Table 3 Summary of neurological signs of Alexander disease in the second survey

	Infantile form	Juvenile form	Adult form
Muscle weakness		42.9% (3/7)	82.4% (15/17)
Tender reflex abnormality	85.7% (6/7)	71.4% (5/7)	94.1% (16/17)
Hyperreflexia	85.7% (6/7)	71.4% (5/7)	94.1% (16/17)
Hyporeflexia or areflexia			12.5% (2/16)
Babinski sign		57.1% (4/7)	82.4% (14/17)
Parkinsonism		0.0% (0/7)	29.4% (5/17)
Sensory disturbance		0.0% (0/7)	17.6% (3/17)
Dysarthria	100.0% (6/6)	100.0% (8/8)	88.2% (15/17)
Dysphonia	66.7% (6/9)	37.5% (3/8)	70.6% (12/17)
Dysphagia	77.8% (7/9)	25.0% (2/8)	88.2% (15/17)
Nystagmus	0.0% (0/6)	0.0% (0/8)	64.7% (11/17)
Limb ataxia	20.0% (1/5)	37.5% (3/8)	30.8% (4/13)
Truncal ataxia	20.0% (1/5)	50.0% (4/8)	50.0% (6/12)
Palatal myoclonus	0.0% (0/6)	0.0% (0/7)	37.5% (6/16)
Orthostatic hypotension		20.0% (1/5)	7.7% (1/13)
Sphincter abnormalities	33.3% (3/9)	12.5% (1/8)	57.1% (8/14)
Sleep disorder		25.0% (1/4)	30.8% (4/13)
Convulsions	100.0% (9/9)	87.5% (7/8)	6.3% (1/16)
Mental retardation/psychomotor developmental delay	100.0% (8/8)	87.5% (7/8)	6.3% (1/16)
Dementia			25.0% (4/16)
Macrocephaly	75.0% (6/8)	50.0% (4/5)	
Scoliosis	44.4% (4/9)	50.0% (4/8)	13.3% (2/15)

Table 4 Summary of MRI findings of Alexander disease in the second survey

	Infantile form	Juvenile form	Adult form
White matter lesion	100% (10/10)	100% (8/8)	37.5% (6/16)
Abnormalities of basal ganglia, thalamus	100% (9/9)	50.0% (4/8)	50.0% (8/16)
Abnormalities of brainstem			
Medulla oblongata	14.3% (1/7)	62.5% (5/8)	100% (16/16)
Pons	14.3% (1/7)	62.5% (5/8)	75.0% (12/16)
Midbrain	28.6% (2/7)	57.1% (4/7)	75.0% (12/16)
Abnormalities of cervical cord	25.0% (1/4)	20.0% (1/5)	100% (16/16)
Abnormalities of cerebellum	37.5% (3/8)	50.0% (4/8)	62.5% (10/16)
Periventricular rim	100% (7/7)	62.5% (5/8)	31.3% (5/16)
Enhancement	75.0% (3/4)	50.0% (1/2)	8.3% (1/12)

patients, respectively. Three cases with clinical findings similar to frontotemporal dementia were mentioned in the free comments section of the questionnaire. Sleep disorders, such as sleep apnea syndrome or rapid eye movement (REM) sleep behavior disorder, and palatal tremor were observed in 30.8% and 37.5% of the adult AxD patients, respectively. Incidence of dysarthria, convulsions, mental retardation, and hyperreflexia was high in juvenile AxD patients.

MRI findings are summarized in Table 4. Cerebral white matter abnormalities in the frontal lobe, signal abnormalities indicating swelling or atrophy of the basal ganglia and thalami, and periventricular rim of low signal intensity on T2-weighted images and high signal intensity on T1-weighted images were predominantly observed in all infantile AxD patients, whereas brainstem lesions were present in only a small number of these patients. Medulla oblongata and cervical spinal cord abnormalities were observed in all adult AxD patients, while abnormalities of other structures related to the brainstem and cerebellum were observed in 60–70% of adult AxD patients. Furthermore, abnormal signals of nucleus dentatus and middle cerebellar peduncle were observed in a few cases reported in the free comments section of the second survey form.

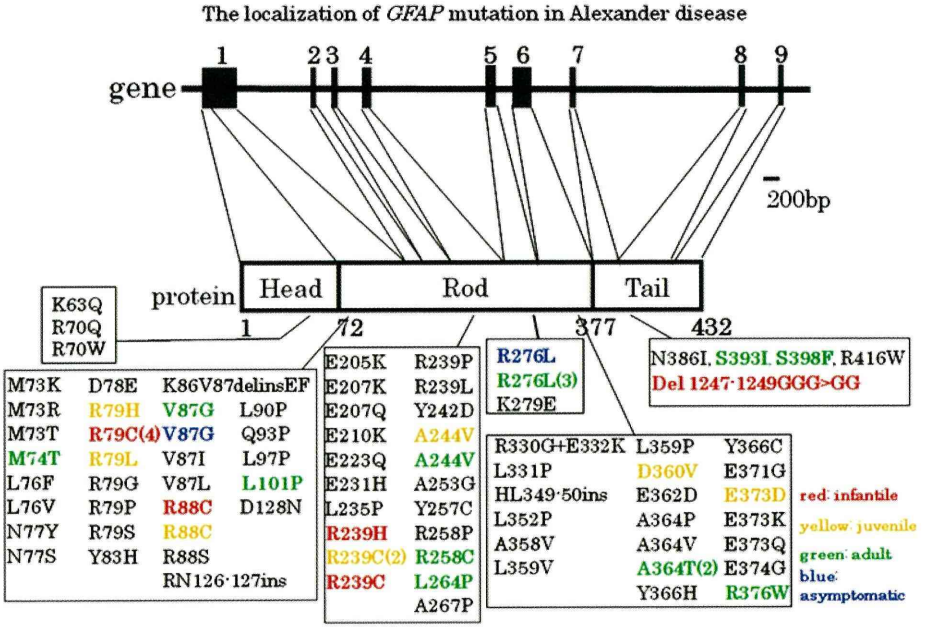

Fig. 2 Schematic demonstrating the *GFAP*, corresponding proteins, and localization of *GFAP* mutations in AxD. Colors indicate mutations reported in this survey: *red* infantile AxD, *yellow* juvenile AxD, *green* adult AxD, *blue* asymptomatic individuals. Mutations shown in *black* indicate published *GFAP* mutations that were excluded from this survey

Although white matter abnormalities were observed in all juvenile AxD patients, abnormalities of the basal ganglia and thalami, periventricular rim, and abnormalities of the brainstem were only observed in approximately 50% of these patients.

Gene analysis is summarized in Fig. 2. *Glial fibrillary acidic protein* analysis was performed for all 35 AxD patients. The mutation sites were unknown for five cases despite the existence of genetic abnormalities, because each patient underwent gene analysis at different facilities; hence a total of 30 patients were available for further analysis. All mutations were identified as *GFAP* point mutations except for one infantile AxD patient, who showed one base deletion in exon 7. The residues affected by point mutation in 70.0% of infantile AxD patients included R79, R88, and R239. Amino acid mutations in juvenile AxD included mutations at R79, R88, R239, A244, D360, and E373. Mutations detected in adult AxD were spread throughout *GFAP* and included M74T, V87G, L101P, A244V, L264P, R258C, R276L, A364T, R376W, S393I, and S398F. The A244V mutation was detected in juvenile and adult AxD.

Electrophysiological findings, treatments, and prognosis are summarized in Table 5. Electroencephalogram (EEG) recordings revealed abnormalities in 8 of 9 (88.9%) infantile AxD patients, 6 of 7 (85.7%) juvenile AxD patients, and 6 of 13 (46.2%) adult AxD patients. Auditory brainstem response (ABR) findings revealed abnormalities in four of five (80.0%) infantile AxD patients, five of seven (71.4%) juvenile AxD patients, and four of five (80.0%) adult AxD patients. Nerve conduction studies were conducted in 13 adult AxD patients, which revealed abnormalities in motor and sensory nerve conduction in 4 (30.8%) and 2 (15.4%) of these patients, respectively. However, data for these studies contained information only about whether it was normal or abnormal. There was no information about the sites of examination or details of the abnormalities.

The survey results did not specify any therapy for AxD. However, some symptomatic treatments were described and evaluated. Antiepileptic drugs seemed to be effective for treating convulsions in infantile or juvenile AxD patients. The efficacy of thyroid-releasing hormone (TRH) therapy was evaluated in three patients, being effective for treating ataxia and some brainstem abnormalities in one juvenile AxD patient. In this patient, envelope-area improvement was observed by balance testing using a stabilometer. Two adult AxD patients were treated with L-dopa, an antiparkinsonism drug, and one adult AxD patient was treated with tizanidine, an antispasticity drug.

The disease duration of the surviving cases ranged from 7 months to 18 years for infantile AxD, from 4 years 6 months to 21 years for juvenile AxD, and from 1 to 16 years for adult AxD patients. Nine of the ten infantile AxD patients were still alive and receiving home care when the first survey was conducted. All juvenile AxD patients were also alive; seven were receiving home care, and one was undergoing treatment in a hospital. Three adult AxD patients failed to survive, and 13 were alive. There was no information on one patient. One nonsurvival case was a patient with V87G who died at the age of 67 years with disease duration of 13 years. In the second case, a patient with R276L died at the age of 51 years with disease duration of 18 years. In the third case, the patient with R258C died at the age of 77 years with disease duration of 9 years.

Table 5 Summary of abnormalities of electrophysiological examinations, therapy, and prognosis in the second survey

	Infantile form	Juvenile form	Adult form
Abnormalities of electrophysiological examinations			
Motor nerve conduction study	0% (0/5)	0% (0/4)	30.8% (4/13)
Sensory nerve conduction study	0% (0/3)	0% (0/2)	15.4% (2/13)
Electroencephalogram	88.9% (8/9)	85.7% (6/7)	46.2% (6/13)
Sensory evoked potential	100% (2/2)	50.0% (2/4)	42.9% (3/7)
Motor evoked potential	100% (1/1)		100% (3/3)
Auditory brainstem response	80.0% (4/5	71.4% (6/7)	80.0% (4/5)
Visual evoked potential	100% (2/2)	50.0% (12)	50.0% (12)
Therapy			
Antiepileptic drugs	8 cases (7 were effective)	3 cases (all were effective)	2 cases (1 was effective)
TRH	1 case	1 case (effective)	1 case
Other			L-Dopa antispasticity dugs
Prognosis	9 cases: alive	8 cases: alive	13 cases: alive
	1 case unknown		3 cases: dead
			1 case: unknown

Discussion

A definitive diagnosis of Alexander disease requires genetic analysis or pathological diagnosis, and diagnoses are almost exclusively given by neurologists or neuropediatricians. Therefore, the estimated number of patients was reported as (patients in the second survey)/(sampling rate × response rate in the first survey) = (reported number of patients)/(response rate in first survey) = 35/0.74 = 47 patients, which is a minimal prevalence assuming that it is unlikely that other units have diagnosed AxD. Considering that the population in Japan is approximately 128 million, we estimate that the disease prevalence is approximately 1 in 2.7 million people, which indicates the frequency of AxD for a 5-year period. The number of actual patients indicates only the number reported in this study, and does not include affected family members who were not reported. Also, the time parameter for prevalence was 5 years, which was also the duration of investigation for this study. Although genetic testing for AxD is mainly conducted in the facility where the present authors work, we cannot rule out the existence of uncertain cases because of the small number of Japanese facilities that conduct genetic testing for AxD. Therefore, the actual prevalence may possibly be higher than that indicated by this study.

Regarding the frequency of the various forms of the disease, adult AxD accounted for 48.5% of the total cases evaluated in this study, and was the most frequent form reported in the survey. However, it was previously reported that infantile AxD accounts for 51% of the total number of AxD cases and is the most frequent form, while adult AxD is the least frequent form, accounting for only 27.2% of total cases [12]. Nevertheless, we consider the results of the present study to be reliable, based on the high response rate to both the first and the second survey. Adult AxD is more difficult to diagnose than infantile AxD, because the MRI criteria and typical clinical symptoms in infantile AxD patients show more regions of brain involvement [11]. Therefore, the frequency of occurrence of adult AxD is not related to that of diagnosis of infantile AxD patients.

We based this study on the presently conducted surveys. However, one case of infantile AxD (1247-1249GGG>GG [13]), two cases of juvenile AxD (D360V [14], R79H [15]), and nine cases of adult AxD (S398F [16], L264P [17], V87G [18], R276L [19, 20], R376W [21], L101P [22], R258C [23], and M74T [24]) have been previously reported. Two cases included in this study, A364T (juvenile AxD) and E373D (adult AxD), are new variations that have not been previously reported.

Cerebral symptoms, such as delayed psychomotor development or mental retardation, convulsions, and macrocephaly, are the most significant neurological findings considered for the diagnosis of infantile AxD. The general characteristics of AxD on brain MRI include predominant cerebral white matter abnormalities in the frontal lobe, signal abnormalities indicating swelling or atrophy of the basal ganglia and thalami, and periventricular rim. Furthermore, progressive bulbospinal symptoms, such as bulbar signs, motor signs, and autonomic dysfunction, may aid

diagnosis of adult AxD. Characteristic findings on MRI in adult AxD patients included signal abnormalities and/or atrophy of medulla oblongata and cervical spinal cord, and abnormalities of other structures related to the brainstem and cerebellum. These results support the results of previous MRI studies [7–9, 23].

Glial fibrillary acidic protein mutations at R79, R88, and R239 accounted for 75.0% of mutations identified in infantile and juvenile AxD patients; however, these mutations were not detected in any of the adult AxD cases. The abnormalities at R79, R88, and R239, which are reported worldwide, have a tendency to be associated with infantile or juvenile AxD. R79 mutations are reported in adult AxD patients, although this is rare [23]. Approximately 60% of the *GFAP* mutations identified in adult AxD cases in this survey were only detected in the Japanese population. Sixty-five percent of adult AxD patients had family members with AxD. In contrast, infantile or juvenile AxD patients had no family histories of AxD, except for one pair of twins. The R276L mutation was observed in three independent families from the same region of Japan. This mutation was not a mutational site with many known cases seen across different races, such as R79 and R239, but was observed in cases found in the same area. Although we cannot rule out that it was a de novo mutation, this mutation is suggestive of the founder effect. Two unrelated patients from the same prefecture in the northern part of Japan were identified with the A244V mutation. However, this mutation was also observed in a patient of other race [6]. Therefore, the A244V mutation may not be the result of a founder effect in Japan.

All infantile or juvenile AxD patients suffered seizures and hence showed EEG abnormalities. However, approximately 50% of adult AxD patients also showed EEG abnormalities, suggesting subclinical cerebral dysfunction resulting from pathological abnormalities in the cerebral matter. ABR abnormalities were independent of AxD type, indicating that radiological and pathological abnormalities of medulla oblongata were observed in all AxD types.

Unfortunately, this survey did not specify any therapies for AxD. However, antiepileptic drugs were reported to be effective in controlling seizures, although seizures that present in infantile or juvenile AxD are considered intractable. The efficacy of TRH therapy was evaluated in three patients. Thyroid-releasing hormone is a neuromodulator of the cerebellum and brainstem and is expected to improve cerebellar ataxia and symptoms of brainstem dysfunction [14]. However, the effectiveness of TRH needs to be confirmed by further large-scale studies. Ceftriaxone, which was not reported in this survey, was recently reported as a potential therapy for halting the progression of some neurological symptoms of AxD [25, 26].

The prognosis of AxD was reported to be relatively good in this survey, which may be due to various reasons. First neonatal AxD, which results in severe disability or death within 2 years [12], was not reported. Second, improvements in general care, nutritional requirements, and respiratory care have contributed to extending the lifespan of AxD patients. However, most patients with AxD receive care in their own homes; therefore, better care is required for such patients.

We inferred the prevalence of AxD in Japan from the high response rate achieved in both our studies. However, we cannot rule out that there may be more than a few undiagnosed cases, one reason being the lack of enough facilities that perform genetic testing for definitive diagnosis of the disease. Another reason is that diagnosis of juvenile AxD may be difficult, particularly in Japan. This is supported by the fact that, in our first survey, no cases of onset during the teens (there are no reports of this originating in Japan) were found, which may be due to several reasons. First, extremely varied cases were found such as cases that presented clinical features of infantile AxD, of adult AxD, of both, and even cases that presented changes in medulla oblongata and cervical spinal cord but that were seen as tuberous in imaging findings [27–29]. These variations may lead to confusion among physicians and misdiagnosis. Second, in Japan, patients in their teens are examined by pediatricians, and MRI diagnostic standards established by van der Knaap et al. [11] are very well known, hence symptoms that are similar to adult AxD, which falls outside those guidelines, may be overlooked. We also inferred this because no such cases of juvenile AxD were reported. Furthermore, although neurologists usually diagnose cases in older teens, we speculate that some cases of AxD are confused for other diseases (such as multiple sclerosis or cancer). This confusion could be similar to cases of nodular lesions that do not present with atrophy, but rather are believed to be observed in juvenile AxD, when patients present atrophy of medulla oblongata or cervical spinal cord, or in cases where signal abnormality that accompanies atrophy indicates possible adult AxD [8, 9, 23]. Therefore, to ensure correct diagnosis of AxD, the physician should understand the importance of the process of *GFAP* genetic testing, which provides definitive diagnosis, but before that the neuropediatrician and neurologist should understand the importance of suspecting AxD from the patient's clinical condition and MRI findings, and place high importance on identifying juvenile AxD, which presents the most complex clinical features. For this reason, we believe that a new classification that helps physicians to suspect AxD based only on neurological and neuroradiological findings, instead of the age at which symptoms present, would be beneficial.

Table 6 Guideline for diagnosing Alexander disease

1. Cerebral Alexander disease (type 1)
I. Neurological findings
(a) Core features
Psychomotor developmental delay/mental retardation, convulsions, macrocephaly
(b) Supportive features
Dysarthria, dysphagia, dysphonia, hyperreflexia, cerebellar ataxia, sphincter abnormalities, scoliosis
II. MRI findings
(a) Core feature
Cerebral white matter abnormalities with frontal lobe predominance
(b) Supportive features
Signal abnormalities with swelling or atrophy of basal ganglia and thalami, periventricular rim, brainstem lesions, contrast enhancement
2. Bulbospinal Alexander disease (type 2)
I. Neurological findings
(a) Core features
Muscle weakness, hyperreflexia (sometimes hypo- or areflexia), positive Babinski sign, dysarthria, dysphagia, dysphonia
(b) Not frequent but specific features
Palatal myoclonus
(c) Supportive features
Cerebellar ataxia nystagmus, scoliosis, sleep disorder (i.e., sleep apnea syndrome, REM behavior disorder), parkinsonism, dementia, psychosis, sphincter abnormalities
II. MRI findings
(a) Core feature
Signal abnormalities or atrophy of medulla oblongata and/or cervical cord
(b) Supportive features
Signal abnormalities and/or atrophy of cerebellum, white matter lesion, signal abnormalities of basal ganglia and thalami, contrast enhancement
3. Intermediate form (type 3)
I. Neurological findings
At least one of the core features in type 1 and at least one of the core features in type 2
II. MRI findings
Core feature of type 1 and core feature of type 2
For a case satisfying any of the above-mentioned types, the following definite diagnosis is recommended
Definite diagnosis
I. Pathological findings
Existence of numerous Rosenthal fibers in addition to gliosis and loss of myelin
II. Gene analysis
GFAP mutation

Approximately 10% of Alexander disease cases seem to show negative *GFAP* mutation in spite of showing typical clinical features of Alexander disease and pathological findings. Therefore, cases satisfying the above clinical features but with negative *GFAP* mutation may be Alexander disease ("possible" Alexander disease)

Based on the above findings, we propose a new guideline where the clinical forms of AxD are classified into the following three types based on neurological and MRI findings: (1) cerebral (type 1), (2) bulbospinal AxD (type 2), and (3) intermediate form (type 3) (Table 6). The primary objective of our guidelines is to increase diagnostic yield by suspecting AxD based on neurological symptoms and MRI findings, which will lead to genetic or pathological testing. Hence, we decided to increase the sensitivity instead of increasing the positive predictive value. On the basis of these new guidelines, we have corrected and updated the neurological and MRI findings for the cases included in our study (Table 7, 8) to 12 cases of type 1 (previously, 9 cases were classified as infantile AxD and 3 as juvenile AxD), 16 cases of type 2 (all cases were previously classified as adult AxD), and 7 cases of type 3 (previously, 1 case was classified as infantile AxD, 5 cases as juvenile AxD, and 1 case as adult AxD).

The phenotypes may seem to vary greatly between type 1 and type 2, but from changes in the images of

Table 7 Summary of neurological signs of Alexander disease classified by the proposed guideline

	Type 1 ($n = 12$)	Type 2 ($n = 16$)	Type 3 ($n = 7$)
Age at onset	3 m to 5 y	26 to 61 y	9 m to 30 y
Muscle weakness	33.0% (1/3)	87.5% (14/16)	60.0% (3/5)
Tendon reflex abnormality	77.8% (7/9)	93.8% (15/16)	83.3% (5/6)
Hyperreflexia	77.8% (6/9)	93.8% (15/1)	83.3% (5/6)
Hyporeflexia or areflexia		12.5% (2/16)	
Babinski sign	33.0% (1/3)	81.3% (13/16)	80.0% (4/5)
Parkinsonism	0.0% (0/3)	25.0% (4 16)	20.0% (1/5)
Sensory disturbance	0.0% (0/3)	18.8% (3/16)	0.0% (0/5)
Dysarthria	100.0% (8/8)	87.5% (14/16)	100.0% (7/7)
Dysphonia	63.8% (7/11)	68.8% (11/16)	42.9% (3/7)
Dysphagia	54.5% (6/11)	87.5% (14/16)	57.1% (4/7)
Nystagmus	0.0% (0/8)	68.8% (11/16)	0.0% (0/7)
Limb ataxia	14.3% (1/7)	33.3% (4/12)	42.9% (3/7)
Truncal ataxia	0.0% (0/7)	50.0% (6/12)	83.3% (5/6)
Palatal myoclonus	0.0% (0/8)	40.0% (6/15)	0.0% (0/6)
Orthostatic hypotension	0.0% (0/3)	7.7% (1/13)	50.0% (1/2)
Sphincter abnormalities	27.3% (3/11)	53.8% (7/13)	28.6% (2/7)
Sleep disorder	0.0% (0/2)	30.8% (4/13)	50.0% (l/2)
Convulsions	90.9% (10/11)	0.0% (0/15)	100.0% (7/7)
Mental retardation/psychomotor developmental delay	90.0% (9/10)	0.0% (0/15)	100.0% (7/7)
Dementia		26.7% (4/15)	0.0% (0/1)
Macrocephaly	80.0% (8/10)		50.0% (3/6)
Scoliosis	45.5% (5/11)	13.3% (2/15)	50.0% (3/6)

Table 8 Summary of MRI findings of Alexander disease classified by the proposed guideline

	Type 1 ($n = 12$)	Type 2 ($n = 16$)	Type 3 ($n = 7$)
White matter lesion	100.0% (12/12)	33.3% (5/15)	100.0% (7/7)
Abnormalities of basal ganglia, thalamus	81.8% (9/11)	46.7% (7/15)	71.4% (5/7)
Abnormalities of brainstem			
Medulla oblongata	0.0% (0/9)	100.0% (15/15)	100.0% (7/7)
Pons	0.0% (0/9)	73.3% (11/15)	100.0% (7/7)
Midbrain	11.1% (1/9)	73.3% (11/15)	100.0% (6/6)
Abnormalities of cervical cord	0.0% (0/6)	100.0% (15/15)	75.0% (3/4)
Abnormalities of cerebellum	20.0% (2/10)	60.0% (9/15)	94.3% (6/7)
Periventricular rim	77.8% (7/9)	26.7% (4/15)	94.3% (6/7)
Enhancement	66.7% (4/6)	8.3% (1/12)	

recently reported long-term survival infantile cases [30], and from the progression of medulla oblongata and cervical spinal cord atrophy seen in an adult cases [31], we believe that certain factors related to the developmental stage determine the severity of cerebral white matter pathological change.

In conclusion, we report a large number of AxD patients in Japan, and provide an estimate of the overall prevalence of the disease with relative frequencies of the three forms. In addition, we propose new clinical guidelines for diagnosing AxD based on simplified classifications. We hope that this report and the guidelines we propose will lead to higher diagnostic yield in the future.

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Conflicts of interest None.

Appendix

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Clinical aspects and pathology of Alexander disease, and morphological and functional alteration of astrocytes induced by *GFAP* mutation

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Alexander disease (AxD) is pathologically characterized by the presence of Rosenthal fibers (RF), which are made up of GFAP, αB-crystallin and heat shock protein 27, in the cytoplasm of perivascular and subpial astrocyte endfeet. Since GFAP mutation has been confirmed in reported cases of AxD, clinical or experimental research is being conducted on the relationship between GFAP mutation and the onset pathology as well as the clinical form. We conducted a nationwide survey and a clinical study, and classified AxD into three types: cerebral AxD (type 1), which primarily has an infantile onset with presence of seizures, psychomotor developmental retardation, macrocephaly, and abnormalities in the superior frontal cerebral white matter observed in a brain MRI; bulbospinal AxD (type 2), which primarily has an adult onset with presence of muscle weakness, hyperreflexia, bulbar or pseudobulbar symptoms, signal abnormalities, and atrophy observed in an MRI of the medulla oblongata and upper cervical spinal cord; and an intermediate form (type 3) which has the characteristics of both. A research on GFAP mutations and aggregate formation concluded that GFAP mutations decreased the solubility of GFAP. According to our cell model experiment, the formation of mutant GFAP aggravates depending on the site of the GFAP mutation. Furthermore, there is a possibility that polymorphism in the GFAP promoter gene regulates the degree to which GFAP is expressed; it may have an effect on clinical heterogeneity. Recent research using cell and animal models suggests that the pathology of AxD involves not only mere functional abnormalities in intermediate filaments but also functional abnormalities in astrocytes as well as in neurons. Clarification of the glia–neuron interactions will prove the disease to be very interesting.

Key words: Alexander disease, astrocyte, glial fibrillary acidic protein, guideline, Rosenthal fiber.

INTRODUCTION

In 1949, Dr W. Stewart Alexander reported a case of an infant with mental retardation accompanied by convulsions and progressive hydrocephalus.[1] Histopathological examination revealed numerous fuchsinophilic bodies in the white matter and beneath the ependyma and pia. He observed that these fuchsinophilic bodies were a result of fibrinoid degeneration in the fibers and cell bodies of the fibrillary neuroglia. These bodies appear identical to the Rosenthal fibers first described by Rosenthal[2] and became the hallmark of Alexander disease (AxD). Subsequently, studies revealed that these Rosenthal fibers were mainly comprised of GFAP, αB-crystallin and heat shock protein 27, in addition to other components.[1–6] Although MRI findings, such as extensive white matter changes with frontal predominance and periventricular rim, can assist in the diagnosis of AxD, this disease can only be definitively diagnosed by pathological examination. Until 2001, AxD was considered a rare leukoencephalopathy affecting infants and older children, and it was characterized by motor and developmental delay, seizures and macrocephaly. In 2001, Brenner *et al.* discovered missense mutations in 10 of the 11 neuropathologically established cases of sporadic infantile AxD in which the GFAP-coding region had been sequenced.[7] Since then, it has become possible to diagnose

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AxD by gene analysis. Following the identification of these GFAP mutations, milder forms of AxD with juvenile or adult onset and characterized by muscle weakness, hyperreflexia, bulbar or pseudobulbar signs, ataxia and palatal myoclonus, have been described with increasing frequency. The characteristic MRI findings of juvenile or adult-onset AxD, which are completely different from those of the infantile form, include signal abnormalities and/or atrophy of the medulla oblongata and cervical spinal cord.[8–11] To date, approximately 100 GFAP mutations have been reported in patients with AxD. Most mutations are identified as point mutations in the coding region of the GFAP gene.

CLINICAL DIAGNOSIS OF ALEXANDER DISEASE

AxD has been classified into three subtypes according to the age of disease onset; infantile AxD (under 2 years old), juvenile AxD (2–12 years old), and adult AxD (over 12 years old). However, several cases of teenage onset of AxD were difficult to diagnose; this may be attributed to several factors. First, the varied symptoms and MRI findings in the medulla oblongata and cervical spinal cord, which are similar to those of nodular lesions, may lead to confusion and subsequent misdiagnosis by physicians.[12–14] Second, in Japan, patients in their teens are usually examined by pediatricians who are quite familiar with the MRI diagnostic standards established by van der Knaap *et al.*[15] Hence, some symptoms of adult AxD that fall outside the present classification may be overlooked. Therefore, to ensure the correct diagnosis of the type of AxD, physicians should be made aware of the importance of *GFAP* testing, which provides a definitive diagnosis. Moreover, in order to accurately diagnose the disease, pediatricians and neurologists should be familiar with the clinical features and MRI findings.

We reported an epidemiological study conducted in Japan, and proposed new guidelines for classifying AxD into three distinct types, namely: cerebral (type 1), bulbospinal (type 2) and intermediate (type 3), on the basis of neurological and MRI findings (Fig. 1).[16] This classification will assist in further genetic or pathological testing. The main characteristics of type 1 AxD include delayed psychomotor development or mental retardation, convulsions, macrocephaly and predominant cerebral white matter abnormalities in the frontal lobe on brain MRI. The main characteristics of type 2 AxD include bulbar signs, muscle weakness with hyperreflexia, and signal abnormalities and/or atrophy of the medulla oblongata and cervical spinal cord on MRI. Intermediate phenotypes that fall between types 1 and 2 and long-term survivors with type1 are considered as having type 3 AxD.[16] Based on these new guidelines, the overall prevalence of the disease with relative frequencies of the three forms were 34.3% for type 1, 45.7% for type 2 and 20.0% for type 3.[16]

Although the phenotypes of types 1 and 2 AxD may seem to vary greatly, based on changes in the MRIs of recently reported long-term survival infantile cases[17] and on the progression of medulla oblongata and cervical spinal cord atrophy in adult cases,[18] we hypothesized that

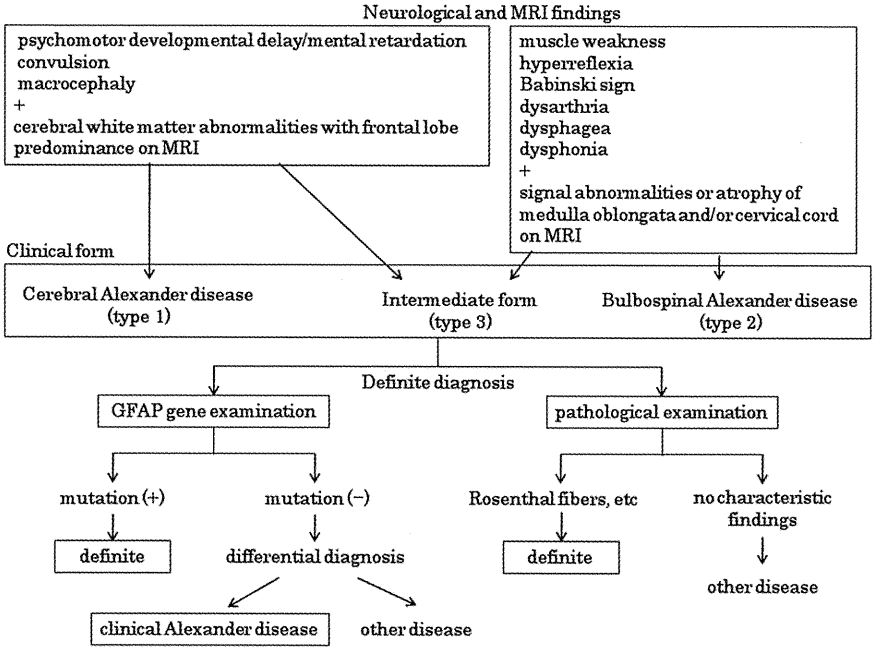

Fig. 1 Algorithm of the diagnosis of Alexander disease.

certain factors related to each glial developmental stage determine the severity of cerebral white matter pathological changes.

PHENOTYPE GENOTYPE CORRELATION IN AXD

The relationship between genotypes and phenotypes in AxD remains unknown. However, *GFAP* mutations in R79, R88 and R239 account for 75.0% of mutations identified in type 1 and type 3 AxD patients; furthermore, these mutations have not been detected in any of the type 2 cases.[12] In contrast to types 1 and 3, the hot spots of *GFAP* mutations in type 2 AxD have not been identified. Sixty-five percent of the type 2 AxD patients had a family history of AxD, whereas the types 1 and 3 AxD patients, except for a few pairs of twins, had no family histories of AxD.[16,19] R416W, which is located in the tail domain of GFAP and is present in multiple forms of AxD, may be another hot spot mutation.[7,20,21]

THE PROPERTIES OF MUTANT GFAP

GFAP is expressed in neuroglial cells and is a member of the intermediate filament superfamily, which consistis of α-helical polypeptides that intertwine in a coiled-coil fashion to form dimer subunit structures of 10 nm filaments. The most prominent feature of the GFAP molecule is a central α-helical domain that is called the rod. These rods are flanked by an amino terminal nonhelical head domain and a carboxy tail end domain. The head domain is thought to be involved in lateral associations, end-to-end subunit stabilization, or both. The main role of the rod domain is in network formation and filament assembly, while the tail domain is passively involved in the stabilization of protofibrillar interactions and filament diameters.[22]

In immunohistochemical studies using astrocytoma-derived cells, most mutant GFAPs demonstrated a filamentous pattern that could not be distinguished from normal GFAPs; however, some mutant GFAP cells are known to demonstrate an irregular aggregation with few filamentous structures or appear as cells where the filament networks are destroyed.[23] Hsiao *et al.* demonstrated that a R239C mutant GFAP, which was extracted from transfected cells using a strong extraction buffer, exhibited relatively little solubility. However, this mutant GFAP assembled into 10 nm filaments with a similar morphology *in vitro*.[24] Der Perng *et al.* demonstrated that R416W mutant GFAP demonstrated decreased solubility; moreover, when wild-type GFAP was mixed with R416W mutant GFAP in different proportions, the effects of the latter on *in vitro* filament assembly were greater than those of the former.[25]

To clarify the aggregation process, we performed a time-lapse recording of R239C and R416W mutant GFAP cells (Fig. 2).[26] Real-time images of wild-type GFAP and mutant GFAP, which were labeled with green fluorescent protein (GFP), demonstrated an apparently normal filamentous network or aggregate in the two initial phenotype patterns. Approximately 85% GFP-labeled wild-type GFAP cells initially appeared to have normal filamentous networks. Among these, almost all cells maintained the filamentous network and were capable of cell division. The remaining 15% of cells initially appeared as aggregates, of which almost all remained unchanged and incapable of cell division. In GFP-labeled R239C cells, one-third of cells initially appeared as a cluster of aggregates in the cytoplasm. These clusters tended to move inward until they finally formed amorphous aggregates that were incapable of cell division. On the other hand, at high magnification, R416W mutant GFAP exhibited filaments in a bubble-like or ring-like pattern. In approximately 20% of these cells, clusters of small aggregates emerged from the cytoplasm and formed large aggregates. Similar to the R239C cells, cells with this type of aggregate were incapable of cell division. However, the remaining 80% of these cells were capable of cell division despite their apparently abnormal GFAP structure. Our real-time imaging study indicates that the mechanism of GFAP aggregation depends on the domain in which the point mutation is located.[26]

Mutant *GFAP* sites located in the rod domain may be unable to maintain the fundamental filamentous architecture of GFAP; therefore, they induce the formation of aggregates. This finding suggests that the degree of severity of mutant GFAP in the rod domain depends on the degree of disruption of the fundamental structure and may have a dominant effect on wild-type GFAP. In contrast, mutant GFAP in the tail domain could result in abnormal GFAP structure. However, most of the astrocytes with R416W gene mutations were capable of cell division. This result suggests that mutant GFAP in the tail domain could maintain the fundamental structure of GFAP, and that the alteration of R416W GFAP function in astrocytes may depend on other elements interacting with GFAP. This may support the belief that the phenotypes of mutant GFAP in the tail domain include a variety of clinical features of AxD with varying severity.[26]

FUNCTIONAL IMPAIRMENT OF ASTROCYTES AND NEURONS DUE TO *GFAP* MUTATIONS

Tang *et al.* proposed a chain of events that led to astrocytic dysfunction following R239C mutations.[27–29] They demonstrated that accumulation of mutant GFAP impairs proteasome function in astrocytes. This impairment activates

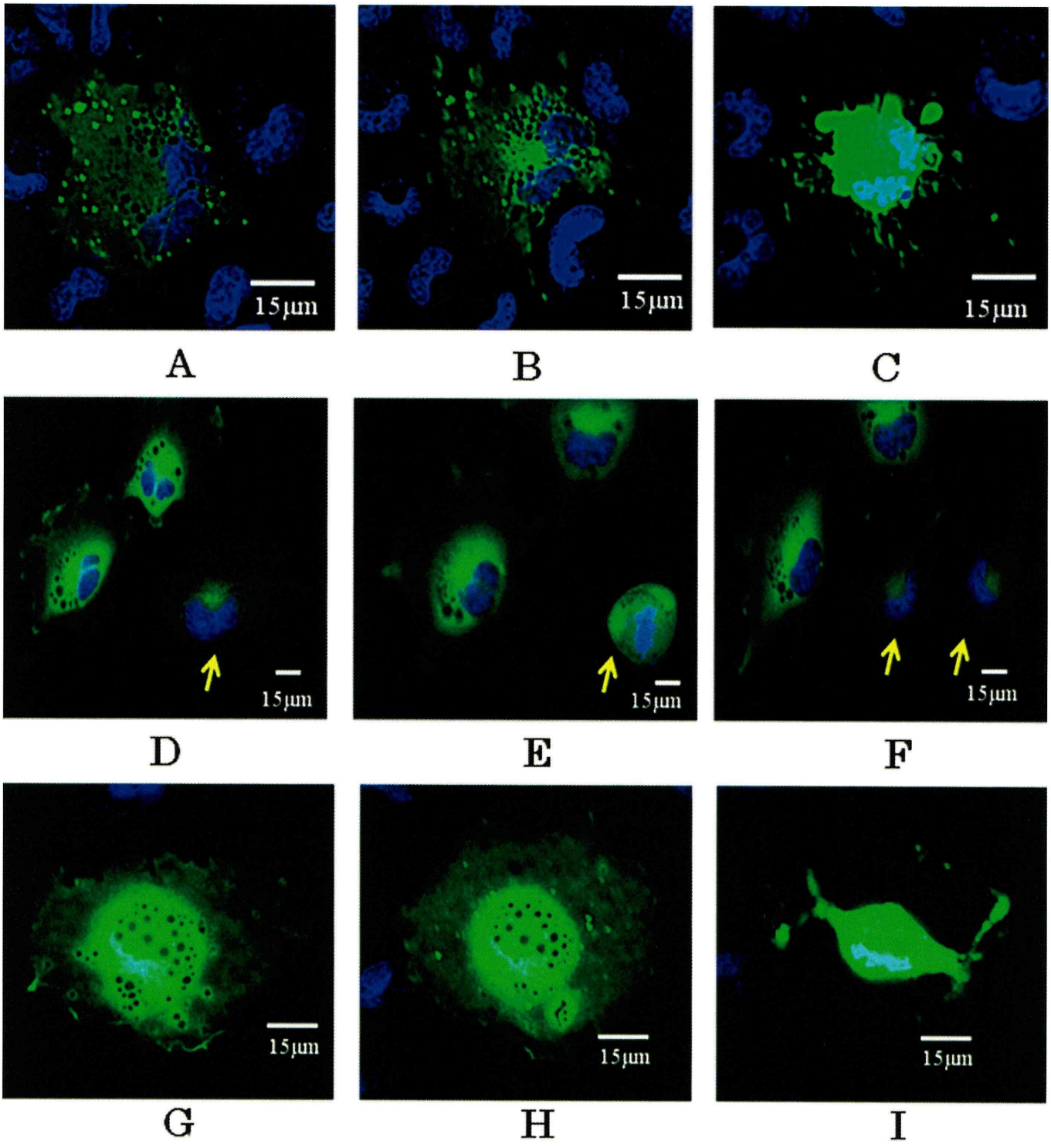

Fig. 2 Time-lapse recording of cells transfected with mutant GFAP, GFP-R239C cells (A–C) and GFP-R416W cells (D–I). Approximately 30% of GFP-R239C cells first showed a cluster of aggregates in the cytoplasm (A) and tended to move inward (B), and finally formed amorphous aggregates (C). This type of aggregate had impaired cell division. On the other hand, GFP-R416W cells showed filaments constructed of a bubble- or ring-like structure when viewed at a high magnification (D,G). Approximately 80% of these cells maintained this structure and were capable of cell division (E, F) (arrows). However, in 20% of the cells, clusters of small aggregates emerged from the cytoplasm (H) and formed large aggregates (I).

mixed lineage kinase (MLK)/JNK/p38 stress kinase pathways that upregulate αB-crystallin transcription and activate autophagy. The small heat-shock protein αB-crystallin accumulates in large numbers in AxD astrocytes, reverses the inhibitory effects of R239C GFAP on proteasomal activity, and promotes degradation of the mutant GFAP, which may be due to shifting of the size of the mutant protein from larger oligomers to small oligomers, which comprise 4–6 molecules of GFAP, or monomers. Oligomeric forms of GFAP are particularly effective in inhibiting proteasomal activity.[29]

In order to analyze glutamate metabolism in neuron and astrocyte in AxD, Meisingset *et al.* simultaneously injected labeled glucose into a transgenic mouse line that over-expresses human GFAP and analyzed the brain extracts by magnetic resonance spectroscopy.[30] Most of the glutamate in the brain is stored in glutamatergic neurons. Synaptically released glutamate is transported into astrocytes. Thereafter, some glutamate enters the astrocytic tricarboxylic acid (TCA) cycle, where some are directly converted to glutamine. Glutamine is then released back to the neurons and used to resynthesize glutamate. Therefore, astrocytes and neurons interact in glutamate metabolism through this glutamate-glutamine cycle. They found reduced utilization of labeled acetate, which is selectively taken up by astrocytes, for the synthesis of glutamine and glutamate.[30] Moreover, the concentration of N-acetylaspartate, a marker of neuronal mitochondrial metabolism, was decreased.[30] This result suggests impaired astrocytic and neuronal metabolism and decreased transfer of glutamine from astrocytes to neurons.

GFAP PROMOTER POLYMORPHISM ALTERS ITS GFAP EXPRESSION?

Approximately 10% of AxD cases seem to show a negative *GFAP* mutation despite showing typical clinical features and pathological findings of AxD. Indeed, recent studies revealed that mutant GFAP induced decreased solubility;[24,25] in addition, the oligomeric forms of mutant GFAP inhibited the proteasomal system and demonstrated a toxic effect in astrocytes.[29] However, in brain tissue of transgenic mice that overexpress wild-type GFAP, aggregates similar to Rosenthal fibers were detected.[31] Furthermore, the overexpression of GFAP was much higher in the lines with the shortest lifespan.[31] Therefore, an excess of wild-type GFAP may modify the increase of GFAP aggregates, even in the absence of mutant GFAP, and promote formation of aggregates. On the basis of these data, we presume that the GFAP promoter may play a role in modifying the expression level of GFAP protein.

In knock-in mice expressing the R236H AxD mutant, GFAP promoter activity is only transiently elevated and may not entirely account for the accumulation of GFAP protein that occurs in these mice.[32]

Bachetti *et al.* demonstrated that a single-nucleotide polymorphism (SNP) of the GFAP promoter mediates the effects of GFAP mutations.[33] They identified a new activator protein 1 (AP-1) binding site that is −250 bp upstream from the GFAP transcriptional start site. In the −250 C/A locus, they expected that the A allele would primarily modify the sequence of the site by inducing interactions with the AP-1 complex. However, they observed that differences in the extent of binding between the C and A alleles demonstrated a greater association with the transcriptional activity of the C allele than that of the A allele. Therefore, their results indicated that higher GFAP expression occurs in the presence of the C allele than that in the presence of the A allele.[33] As such, polymorphisms of the GFAP promoter may mediate GFAP mutations that affect the heterogeneity of AxD phenotypes.

GFAP gene duplication for AxD has not been reported. We examined gene multiplication of GFAP using real-time PCR in four AxD cases with GFAP gene mutation and eight clinically suspected AxD cases without GFAP gene mutation, resulting in no multiplication being found (data was not shown).

ANIMAL MODELS

Transgenic mice overexpressing the wild-type human GFAP exhibit an AxD-like phenotype by demonstrating the formation of aggregates, which are similar to Rosenthal fibers, and an enlarged stress response, which induces small heat-shock proteins and results in activation of microglia and compromised neuronal function. Furthermore, mice in the highest GFAP-expressing lines had the shortest life-spans.[31,34]

Knock-in mice models with missense mutations homologous to those found in humans (R79H, R236H) have been created. These mice exhibit GFAP aggregates that are similar to Rosenthal fibers and are more susceptible to kainate-induced seizures than are wild-type mice. However, these mice demonstrated normal life-spans and no overt behavioral defects.[35,36] Cho *et al.* revealed cytoplasmic inclusions and functional alterations in primary cultures obtained from knock-in mice. These alterations included decreased cell proliferation, increased cell death, reduced proteasomal function, and compromised astrocyte resistance.[37] Hagemann *et al.* revealed an important role of αB-crystallin, which suppress GFAP toxicity, by demonstrating that overexpression of αB-crystallin results in a markedly reduced stress response by the CNS, restores expression of EAAT2, and protects from death AxD mouse models that lose αB-crystallin.[38]

More recently, a drosophila model of AxD, in which a mutant GFAP gene is expressed in the glia, has been created. In this model, protein aggregation and oxidative stress were induced. This drosophila model is a simple genetic model that may greatly aid in our understanding of the genetics of AxD, as well as in drug screening.[39]

CONCLUSION

Over 60 years has passed since Alexander reported the first case of AxD. Although the characteristics of its pathology, patterns on MRI, and most recently gene mutations are widely known, there may still be undiagnosed cases because AxD is a very rare disease with a variety of clinical phenotypes. The new guidelines we have proposed to classify AxD into three types on the basis of neurological and MRI findings are expected to assist physicians in making an accurate diagnosis. The researches using animal or cell models suggest that the pathology of AxD involves not only mere functional abnormalities in intermediate filaments but also functional abnormalities in astrocytes as well as in neurons. To date, the mechanisms mediating AxD remain unclear. However, the elucidation of these mechanisms using cellular, murine and most recently, drosophila models, continues apace.

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Glial fibrillary acidic protein mutations in adult-onset Alexander disease: clinical features observed in 12 Japanese patients

Yoshida T, Sasayama H, Mizuta I, Okamoto Y, Yoshida M, Riku Y, Hayashi Y, Yonezu T, Takata Y, Ohnari K, Okuda S, Aiba I, Nakagawa M. Glial fibrillary acidic protein mutations in adult-onset Alexander disease: clinical features observed in 12 Japanese patients. Acta Neurol Scand: 2011: 124: 104–108.

Objective – To clarify the clinical manifestations of adult-onset Alexander disease (AOAD) in Japanese patients with glial fibrillary acidic protein (GFAP) gene mutations. *Methods and materials* – Twelve patients of AOAD with *GFAP* mutations detected in our centre were examined for neurological and magnetic resonance imaging (MRI) findings. *Results* – Major symptoms were pyramidal and bulbar signs. In addition, three patients presented abnormal behaviour and/or memory disturbance. Two of the three patients also had Parkinsonism and had been diagnosed with fronto-temporal dementia or progressive supranuclear palsy until *GFAP* mutations were detected. Abnormalities of the medulla oblongata and cervical spinal cord were observed on MRI in all patients. *Conclusions* – Patients presenting with pyramidal and/or bulbar signs with abnormalities of the medulla oblongata and cervical spinal cord on MRI should be considered for *GFAP* analysis as this is the typical presentation of AOAD. Abnormal behaviour and cognitive disorders including deterioration of memory were rare symptoms but could be an obstacle to diagnosing Alexander disease.

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Key words: Alexander disease; glial fibrillary acidic protein; gene analysis; MRI

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Introduction

Alexander disease is a rare neuro-degenerative disorder characterized by white matter degeneration and formation of cytoplasmic inclusions called Rosenthal fibres, which have been observed in astrocytes in previous pathological studies (1). Glial fibrillary acidic protein (GFAP) gene mutations have been recently reported in patients with various clinical forms of Alexander disease (2–6). Adult-onset Alexander disease (AOAD) was first recognized after identification of *GFAP* mutations, and it demonstrates different clinical symptoms and magnetic resonance imaging (MRI) findings from infantile forms (6). The correlation between the genotype and various phenotypes of AOAD, however, remains unresolved in Japan, because there were no reports based on a series of cases.

Since 2001, *GFAP* mutations have been analysed in patients suspected of Alexander disease in our centres throughout Japan. The aim of this study was to investigate the neurological manifestations of AOAD in Japanese patients with *GFAP* mutations and to characterize the clinical features observed in these patients.

Materials and methods

In this study, AOAD was defined as the onset of Alexander disease in individuals older than 12 years of age (6). Twenty-eight patients suspected of AOAD were referred from hospitals all over Japan to Kagoshima University (2001–2002) and Kyoto Prefectural University of Medicine (2002) for analysis of possible *GFAP* mutations. The patients were suspected of AOAD because of leuko-encephalopathy without brainstem abnormalities (nine patients); motor disturbance and/or bulbar signs with atrophy of the medulla oblongata and upper cervical spinal cord present on MRI (16 patients); pathological findings demonstrating Rosenthal fibres accumulation, particularly in the astrocyte end-feet in the subpial and perivascular zones (one patient) and motor disturbance and/or bulbar signs without atrophy of the brainstem (two patients).

After obtaining written informed consent from all patients, genomic DNA was extracted from their peripheral blood. Sequence analysis of the genomic DNA was performed to detect the presence of any *GFAP* mutations. Briefly, the coding region and adjacent splice sites were amplified by direct sequence analysis using an ABI PRISM 3100 auto-sequencer (PE Applied Biosystems, Foster City, CA, USA) and Big Dye terminators according to the manufacturer's instructions. The mutations identified were described according to recent nomenclature recommendations. The presence of mutations was confirmed either by other strands or restriction enzyme digestion. Each nucleotide variant detected was tested in 100 unrelated healthy individuals, a comparison that made it possible to distinguish between disease-causing mutations and neutral common variants.

Information on genetic analysis as well as that on the neurological and MRI findings of patients with Alexander disease was obtained from neurologists and neuro-radiologists of each hospital.

Results

Twelve of the 28 referred patients showed heterozygous missense mutations in *GFAP*, of which 11 patients were referred because of atrophy of the medulla oblongata and upper cervical spinal cord and one patient was referred because of pathological findings consistent with Alexander disease. *GFAP* mutations were not detected in any of the nine patients who presented with leuko-dystrophy without atrophy of the medulla oblongata and upper cervical spinal cord.

The clinical features of AOAD patients with neurological manifestations are summarized in Table 1. Patient age at the time of gene analysis ranged from 24 to 73 years and that at AOAD, onset ranged from 18 to 64 years. Patients 1–5 were from the same family, wherein patients 1 and 4 were siblings; patients 2 and 3 were the daughter and son of patient 1, respectively, and patient 5 was a son of patient 4. Each family member presented with different clinical forms of Alexander disease of which three patients presented with adult form (patients 1, 2 and 4), two patients presented with only pyramidal signs and atrophy of the medulla oblongata and spinal cord on MRI (patients 3 and 5) and one patient, who was the other son of patient 4, presented with the juvenile form. The details of clinical symptoms in patient 1–3 have been described (7). Patient 6 has been described as the first case of AOAD with *R416W* mutation (8). Patient 7, who carried *M74T* mutation and has also been described, who clinically presented with bulbar and pyramidal signs, showed abnormal high intensities in the ventral medulla oblongata and marked atrophy of the medulla oblongata and spinal cord (9). Patient 11 also carried the *M74T* mutation and showed bulbar and pyramidal signs with atrophy of the medulla oblongata and spinal cord. However, patients 7 and 11 did not have a blood relationship. *R258C* mutation, which was detected in patient 8, is a novel mutation. Patient 8 seemed to have a family history of AOAD. The son of patient 8 had gait disturbance and dysphagia for several years, but gene analysis could not be performed for him because he had committed suicide at the age of 45. A brother of the patient exhibited abnormal behaviour at the age of 58 and was later diagnosed with progressive supranuclear palsy (PSP). He passed away at the age of 68 because of brain infarction. The patient 8 presented with abnormal behaviour at the age of 59. Visual and auditory hallucinations occurred at the age of 62. One year later, gait disturbance occurred. At the age of 64, she presented with vertical supra-nuclear palsy, balance difficulty, falling and retrocollis, and she was, therefore, diagnosed with PSP. She also showed euphoria, confabulation and positive snout reflex. The score of Hasegawa dementia scale revised, the maximum