Table 2
Clinical and MR findings of 19 patients with Pelizaeus–Merzbacher disease. (See below-mentioned references for further information.)

Sex	Age at enrollment	Clinical severity*	Age at onset	Family history	PLP1 gene abnormality	Age at first MRI assessment	MR findings								
							Evaluated by T2WI			Evaluated by T1WI	No. of MR exams	White matter signal changes	Cerebellar atrophy	Thin corpus callosum	Previous case report of point mutation
							Hypomyelination type**	High signals in brainstem	Myelination age	Myelination age					
1 M	14	F0	2 months	+	c.749C>A(p.Thr250Lys)	14	A	Diffuse	Before birth	Before birth	1		+	++	This study
2 M	4	F0	Just after birth	–	c.238_240delTTC (p.Phe80del)	0	A	Diffuse	Before birth	Before birth	2	Unchanged		+	[15]
3 M	0***	F0	1 month	–	c.725C>T(p.Ala243Val)	0	A	Diffuse	Before birth	Before birth	1			+	[16]
4 M	1****	F0	Just after birth	–	c.454-2A>C	0	A	Diffuse	Before birth	Before birth	1			++	Same mutation as [23]
5 M	6	F0	4 months	+	Duplication	0	A	Partial	Before birth–4 months	Before birth	2	Advanced		++	
6 M	14	F0	Before 12 months	+	Duplication	10	A	Partial	Before birth	0 month–before birth	1		+	++	
7 M	5	F1	Just after birth	–	c.260T>C(p.Leu87Pro)	0	A	Diffuse	Before birth–8 months	Before birth	2	Unchanged		+	Same mutation as [20]
8 M	20	F1	Before 12 months	+	Duplication	18	A	Partial	Before birth	Before birth	1			++	
9 M	9	F2	Just after birth	–	Duplication	0	A	Partial	Before birth	Before birth	2	Advanced		+	
10 M	20	F2	Before 12 months	–	c.254T>C(p.Leu85Pro)	15	B	None	0–2 months	More than 12 months (complete)	1			++	[15]
11 M	22	F2	Just after birth	–	Duplication	14	B	Partial	6–8 months	8 months	2	Unchanged	+	+	
12 M	29	F3	Just after birth	–	Duplication	28	A	None	Before birth	8–12 months	1		+	+	
13 M	16	F3	Unknown	+	Duplication	13	B	Partial	6 months	4 months	1			+	
14 M	13	F3	Before 12 months	+	Duplication	6	B	Partial	4–6 months	0–4 months	2	Unchanged		+	
15 M	9	F3	Unknown	+	Duplication	2	B	None	4–8 months	4 months	1			+	
16 M	10	F3	1 month	–	Duplication	1	B	None	0 month	0 month	1			++	
17 M	5	F3	10 months	+	c.192-2A>T	3	C	None	8 months	More than 12 months (complete)	3	Advanced		–	[22]
18 M	11	F4 SPG2	Unknown	Unknown	IVS3-9T>G	4	B	Partial	8 months	More than 12 months (complete)	1			–	Omata et al. [25]
19 M	9	F4 SPG2	3 months	–	c.589G>C(p.Gly197Arg)	8	B	None	2–8 months	8 months	1			++	[21]

"Before birth" indicates no detectable signs of myelination that is listed in the Table 1.
Shaded columns indicate gap in myelination age between T1WI and T2WI.
*Clinical severities evaluated at the time of enrollment. F0: never gained head control ability. F1: achieved head control. F2: maintains a sitting position. F3: walks with support. F4: walks autonomously.
**Type of hypomyelination on T2WI. Type A: diffuse cerebral and corticospinal high signals. Type B: diffuse cerebral high signals with corticospinal low signals in the internal capsule. Type C: patchy high signals in cerebral hemispheres.
***Died at 8 months of age.
****Dropped from follow-up at 1 year old.

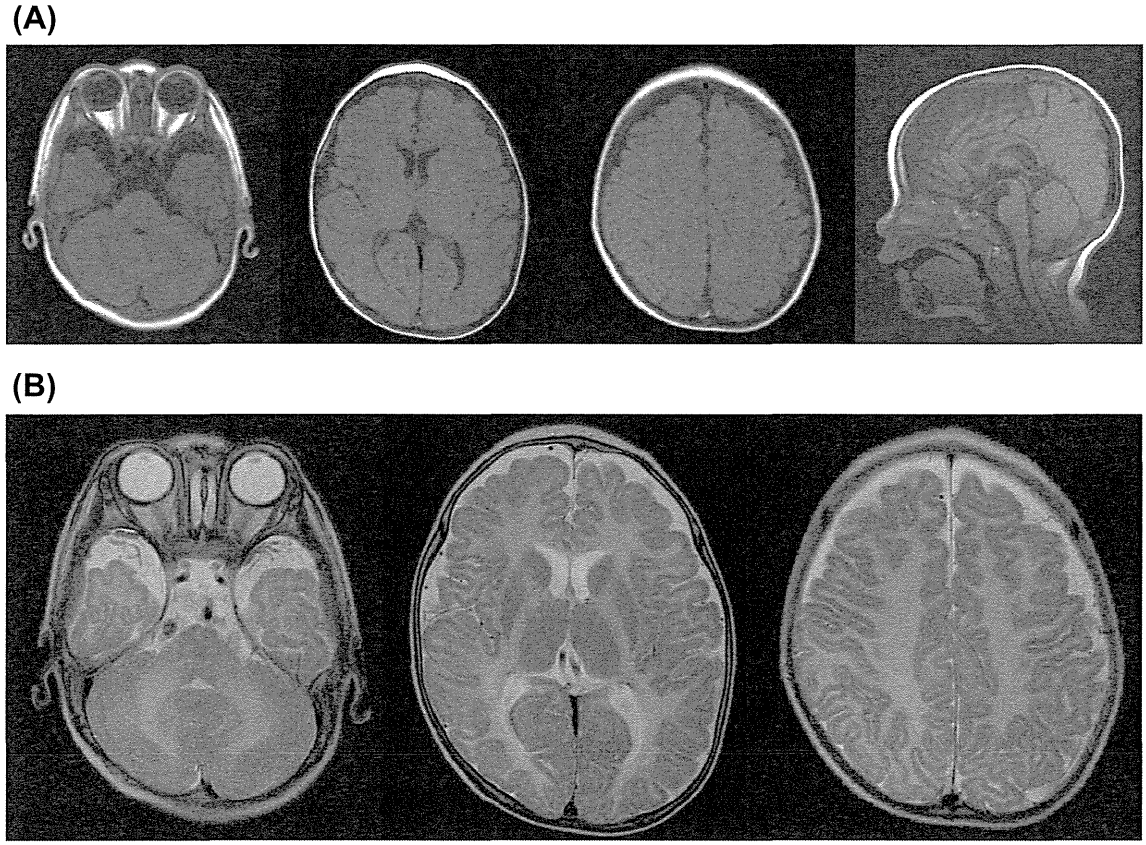

Fig. 1. (Case 3, Type A). This patient was a 7-month-old male with Ala243Val (c.725C>T) point mutation, and the clinical stage was estimated as F0. His symptoms started at 1 month old with horizontal nystagmus, developmental delay, spasticity, and persistent primitive reflex. The myelination age was "before birth" on both T1WI (A) and T2WI (B). The corpus callosum was thin, although there was no cerebellar atrophy. Low signals in the posterior limb of internal capsules were not identified on T2WI, and brainstem is diffusely hyperintense. This case manifested type A imaging.

mutation. A family history of PMD was identified in eight patients. The clinical severity, which was evaluated in all patients aged $\geqslant 4$ years except for Patients 3 and 4, showed wide variation, ranging from F0 to F4. Patient 3 died at 8 months of age and patient 4 dropped out from follow-up at 1 year old. Since these two patients showed extremely poor development with no head control throughout the course of observation and showed severe manifestations, we assumed their clinical severity as F0 [15,16].

There were 10 patients with myelination pattern type A, eight with type B, and one patient with type C. All eight patients with the severe form (F0 or F1) had type A myelination (Fig. 1). There were two patients with type A imaging manifesting F2 and F3 (Cases 9, 12), but both lacking a brainstem abnormal T2-high signal. Among the eight patients with the type B myelination pattern (Fig. 2), the clinical severity ranged from F2 to F4. The clinical form of one patient with type C (Case 17) was F3.

Brainstem abnormalities were observed in 13 patients. Five patients had diffuse T2-high signal in the brainstem, and all of them were clinically severe with *PLP1* point mutations. The remaining eight patients had partial high signals on T2WI (Table 2). Eight of the 10 patients who demonstrated a myelination age "before birth" on T1WI and T2WI showed severe phenotypes (F0 or F1). The clinical form was always milder than F2 in the eight patients whose myelination age was >4 months on T1WI. When T1WI showed an almost complete myelination pattern and there was a discrepancy in myelination age (>4 months) between T1WI and T2WI, the clinical manifestation was relatively mild (Cases 10, 12, 17, and 18) (Case 10, Fig. 3). No common type of gene mutation was found among them. The mildest cases with clinical stage F4 (Cases 18 and 19) had *PLP1* point mutations. However, we observed no other relationship between *PLP1* gene abnormalities and the clinical severity, or with the myelination age.

Follow-up MRI studies were performed in seven patients, and advancement of myelination was noted in three of them. In one point mutation case (c.192-2A>T), patchy areas of myelination appeared during follow-up, which differs from the normal course of maturation (Fig. 4). Cerebellar atrophy was found in four of

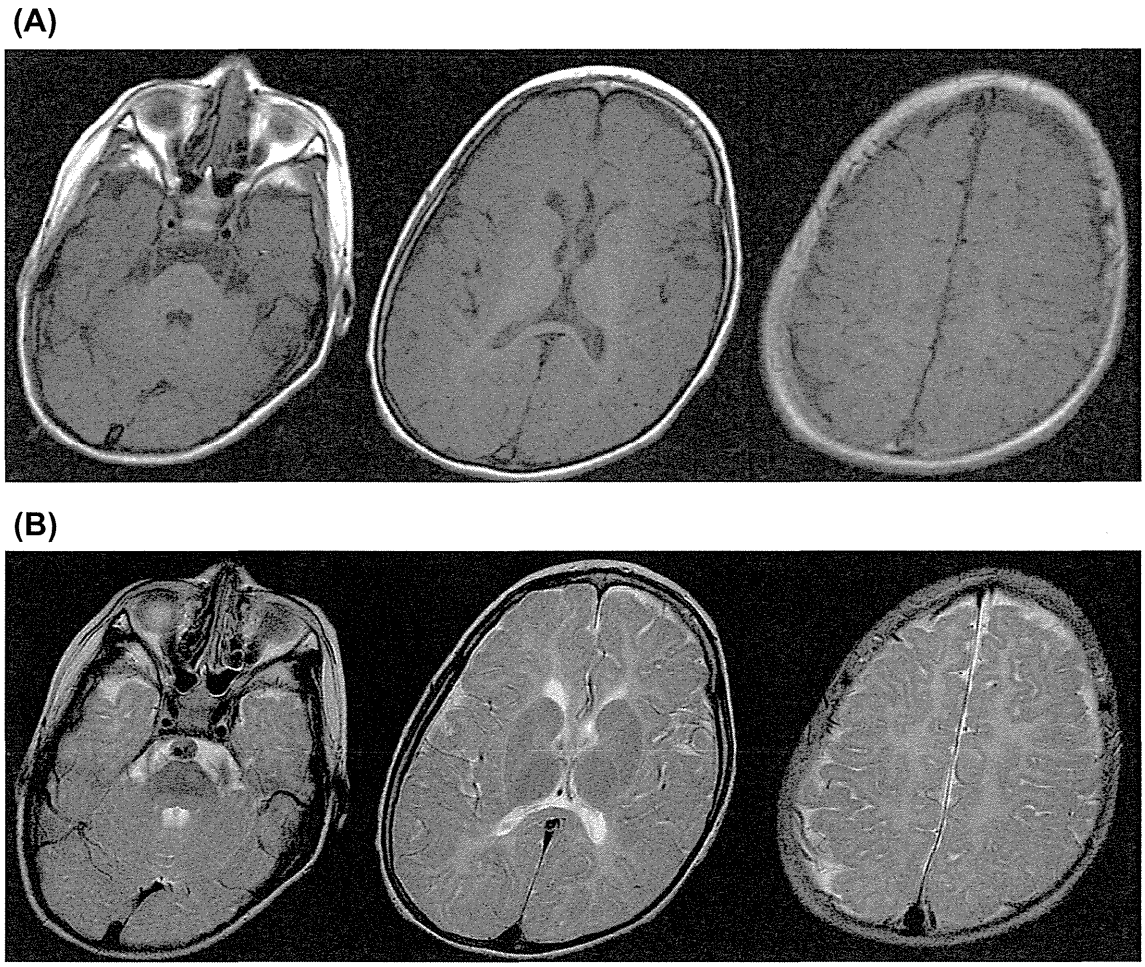

Fig. 2. (Case 14, Type B). This patient was a 13-year-old male with *PLP1* duplication, and the clinical stage was F3. His symptoms started at birth with nystagmus, hypotonia, spasticity, and pyramidal signs. The first MRI was performed at 6 years old. The myelination age was 0–4 months on T1WI (A) and 4–8 months on T2WI (B). There was atrophy of the corpus callosum without cerebellar atrophy. Low signals in the posterior limb of internal capsules were identified on T2WI. This case manifested type B imaging. Note a couple of high signals in the pons on T2WI.

the 19 patients. There was mild to moderate atrophy of corpus callosum in all but two of the patients.

4. Discussion

This retrospective study revealed various patterns of imaging findings in 19 PMD patients, with reference to the clinical course and genetic abnormalities. Our analysis showed several correlations between the imaging types and the clinical severity. The most important MR findings for predicting severe clinical form is diffuse brainstem high signals on T2WI. On the other hand, hypomyelination type B or C judged by T2WI was the most recognizable finding for predicting a mild case. Our findings also emphasized the importance of T1WI. Myelination age "before birth" on T1WI is a second manifestation correlated with the clinically severe phenotypes. In contrast, when the myelination age on T1WI was over 4 months, the patients' clinical severity was milder than F2. There were four patients whose T1WI showed an almost complete myelination pattern while T2WI revealed severely delayed myelination, showing an apparent discrepancy in myelination age between T1WI and T2WI. An unusual order of myelination or advanced myelination during the follow-up was also observed. Brainstem abnormalities were observed only in the patients with point mutation, and the mildest two cases also had point mutations. However, common genetic abnormalities associated with other findings were not obvious.

To the best of our knowledge, this is one of the largest studies of original PMD cases. Laukka et al. performed a quantitative analysis and reported a negative correlation between white matter volume and clinical severity [17]. However, a precise volumetric analysis is theoretically challenging in PMD because the ambiguous MR signal differentiation between gray and white matter may lead to inaccuracy in separating

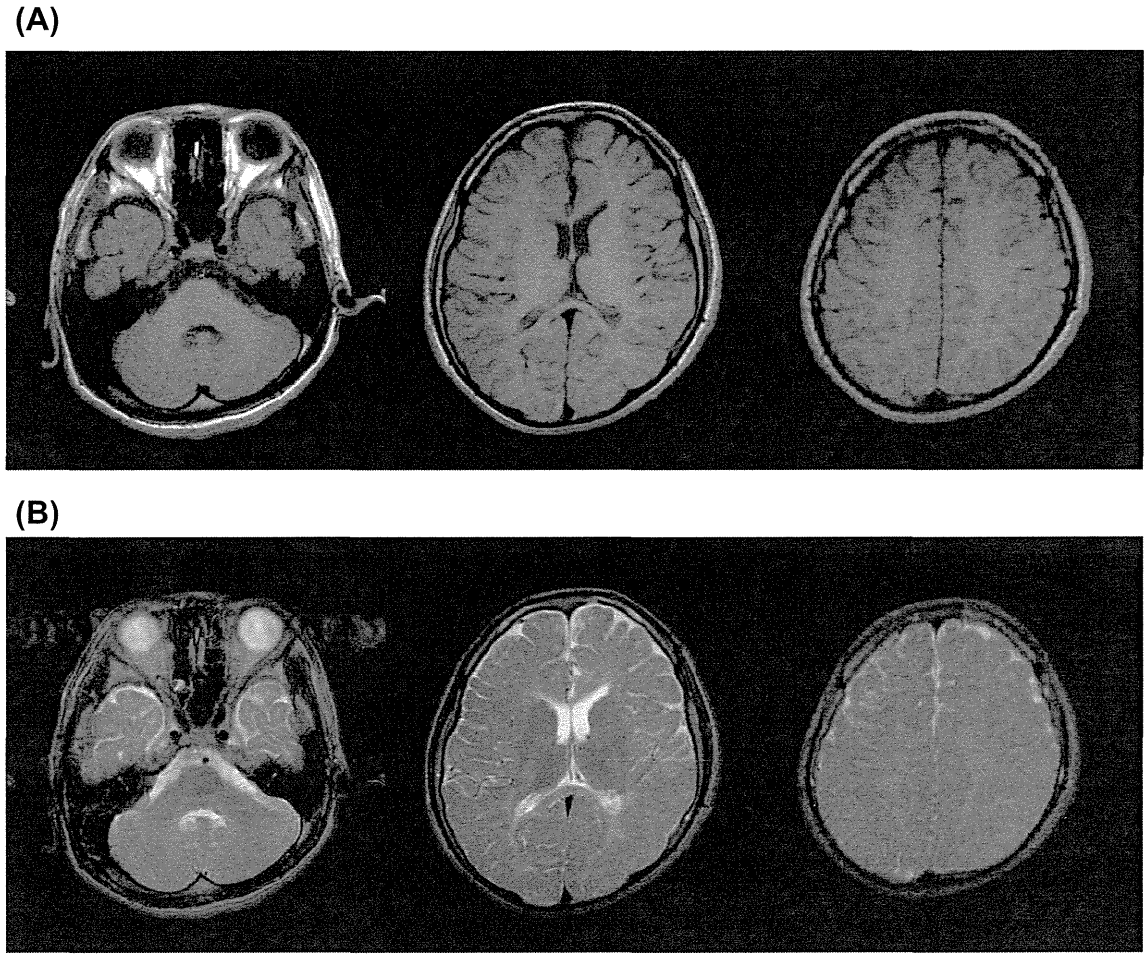

Fig. 3. (Case 10, Type B). A 20-year-old male with Leu85Pro (c.254T>C) point mutation. The clinical stage was F2. His symptoms started at birth with nystagmus, developmental delay, quadrilateral paresis, hypotonia, spasticity, and pyramidal signs. The first MRI was performed at 15 years old. The myelination was apparently completed on T1WI (A), but the myelination age was 0–2 months old on T2WI (B). There was atrophy of the corpus callosum without cerebellar atrophy. The gap between T1WI and T2WI was evident in this case. Low signals in the posterior limb of internal capsules were identified on T2WI. This case also manifested type B imaging.

these two segments based on the signal intensity. To ensure scientific accuracy, we used visual surveillance, which is more suitable for imaging analyses. Steenweg et al. analyzed the MRI patterns of patients with hypomyelinating diseases including 21 PMD patients, focusing on distinguishing different hypomyelinating leukodystrophies without presenting variable imaging patterns among the PMD patients [18].

Nezu et al. classified the MRI findings of PMD into three patterns judged by T2WI from type I to type III as described in Section 2, referring to four of their original cases and 15 cases from seven previous reports. They reported that all PMD with *PLP1* duplication cases belonged to type I [13]. Plecko et al. also suggested that *PLP1* duplication leads to type I imaging in their study including four PMD patients [19]. In contrast, type II imaging in *PLP1* duplication was reported by Takanashi et al. [20]. In the present study, we found both type I and type II imaging among the patients with *PLP1* duplication. PMD with *PLP1* duplication shows various imaging patterns, although diffuse T2-high signal of brainstem may exclude *PLP1* duplication. All patients whose brainstem showed diffuse T2-high signal were *PLP1* point mutation and their clinical findings were severe, F0 or F1. On the other hand, all patients with corticospinal T2-low signals in the posterior limb of the internal capsule (type B or C) were mild form (F2 or F3) and their gene abnormalities were various.

All patients whose T1WI showed "before birth" were F0 or F1 except for Case 9 (F2 in 9 year old). But his first MR evaluation was performed before one year old and myelination was progressive in successive examination. Therefore, the myelination age of "before birth" on T1W1 may be related to F0 and F1 without exception. These findings showed the importance of evaluating the myelination age on T1WI, which has been

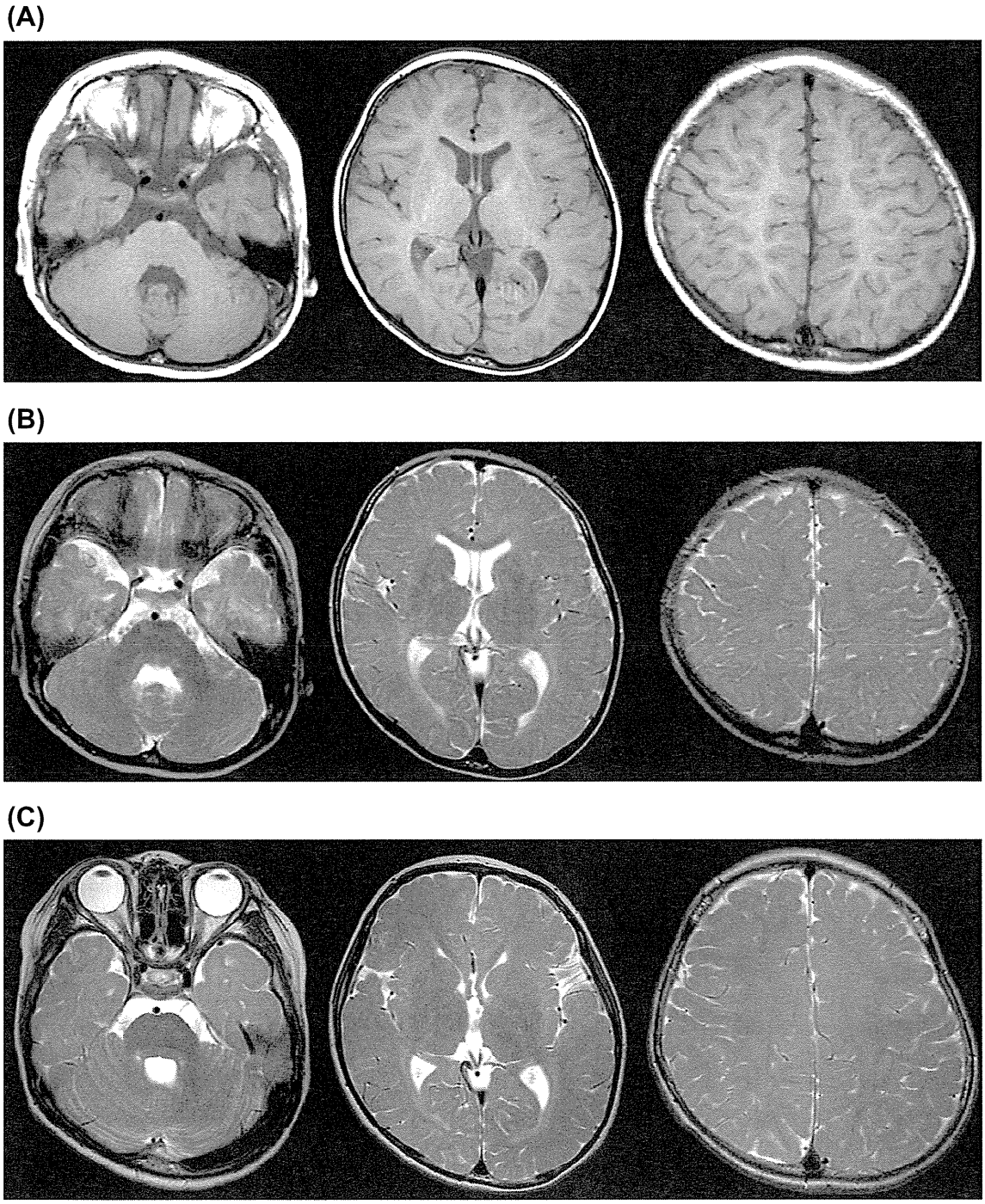

Fig. 4. (Case 17, Type C). A 5-year-old male with c.192-2A>T intronic point mutation; the clinical stage was F3. His symptoms started at 10 months old with developmental delay, paraplegia, and spasticity. The first MRI was performed at 3 years old. The myelination was apparently completed on T1WI (A), but the myelination age was 8 months old on T2WI (B). Subsequent images of the same patient acquired at 5 years old revealed advanced myelination on T2WI, but the pattern was patchy and subcortical fibers were more myelinated (C). There was no atrophy of the corpus callosum or cerebellum.

underappreciated. In the present study, the existence of the gap of myelinating age between T1WI and T2WI was virtually predictive of a mild clinical course. Well-advanced myelination on T1WI may be a cause of the T1/T2 gap, and this in turn also demonstrates that a T1WI assessment is crucial to predict the clinical stage. A case report by Osaka et al. presented a mild phenotype in PMD caused by a DM20-sparing mutation (c.352_353delAG (p.Gly130fs)); the clinical stage was F3 and T1/T2 discordance was also mentioned [21].

The patient also showed myelinated patchy areas at subcortical white matter and unmyelinated deep white matter, which corresponds to Type C. This paradoxical myelination pattern was also found in our patient (Case 17) with c.192-2A>T mutation. This is considered to be the rarest imaging pattern in PMD and may be difficult to differentiate from other demyelinating leukodystrophies that affect the deep white matter. A thin corpus callosum was apparent in most ($n = 17$) of the present cases. Three patients had cerebellar atrophy. These findings may be problematic when differentiating PMD from other hypomyelinating disorders such as Polymerase III-related leukodystrophies [22,23], and hypomyelination with atrophy of the basal ganglia and cerebellum (H-ABC) [24], where atrophy of the corpus callosum and that of the cerebellum have been noted to be key findings.

Follow up MRI studies in PMD patients were performed in seven cases and progression of myelination was observed in three of them. Surely, it is unknown when the myelination process stops. However, the hypomyelination types did not change even when progressive myelination was observed in this study (two cases; type A, and one case; type C). Clinical severity was evaluated according to motor function such as from head control to walking ability. Clinical outcome was evaluated after 4 years old in all patients except for cases 3 and 4. We think 4 years old is enough age for evaluating these motor functions. Therefore, we think the correlation between clinical and MRI stage can be reasonably discussed in this study.

In summary, the present IBISS study revealed a wide spectrum of imaging findings in each phenotype with PMD. Type B or C (corticospinal low signal in the internal capsule on T2WI) is thought to be essential for predicting a mild form. Meanwhile, diffuse high signal in brainstem on T2WI or myelination age of "before birth" on T1WI suggests a severe form. The disease course should not be predicted solely by genetic background, and clinicians' understanding of the various MRI findings in PMD will contribute to the better management of PMD patients.

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Review Article

Canavan disease: Clinical features and recent advances in research

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Abstract Canavan disease (CD) is a genetic neurodegenerative leukodystrophy that results in the spongy degeneration of white matter in the brain. CD is characterized by mutations in the gene encoding aspartoacylase (ASPA), the substrate enzyme that hydrolyzes *N*-acetylaspartic acid (NAA) to acetate and aspartate. Elevated NAA and subsequent deficiency in acetate associated with this disease cause progressive neurological symptoms, such as macrocephaly, visuocognitive dysfunction, and psychomotor delay. The prevalence of CD is higher among Ashkenazi Jewish people, and several types of mutations have been reported in the gene coding ASPA. Highly elevated NAA is more specific to CD than other leukodystrophies, and an examination of urinary NAA concentration is useful for diagnosing CD. Many researchers are now examining the mechanisms responsible for white matter degeneration or dysmyelination in CD using mouse models, and several persuasive hypotheses have been suggested for the pathophysiology of CD. One is that NAA serves as a water pump; consequently, a disorder in NAA catabolism leads to astrocytic edema. Another hypothesis is that the hydrolyzation of NAA in oligodendrocytes is essential for myelin synthesis through the supply of acetate. Although there is currently no curative therapy for CD, dietary supplements are candidates that may retard the progression of the symptoms associated with CD. Furthermore, gene therapies using viral vectors have been investigated using rat models. These therapies have been found to be tolerable with no severe long-term adverse effects, reduce the elevated NAA in the brain, and may be applied to humans in the future.

Key words aspartoacylase, Canavan disease, leukodystrophy, *N*-acetylaspartic acid, spongy degeneration.

Canavan disease (CD) is an autosomal-recessive progressive neurodegenerative disease that belongs to a group of genetic disorders recognized as leukodystrophy. CD is neuropathologically characterized by the swelling and spongy degeneration of white matter in the brain. CD was first reported by Canavan in 1931[1] and was identified as a distinct disease by Bertrand and Van Bogaert in 1949.[2] Although CD has been reported in communities throughout the world, it was shown to be more prevalent in Ashkenazi (Eastern European) Jewish people.[3] The disease has been attributed to a deficiency in aspartoacylase (ASPA) activity. ASPA is a zinc carboxypeptidase enzyme that is responsible for the breakdown of aspartic acid or *N*-acetylaspartic acid (NAA), the absence of which results in the accumulation of NAA in the brain. ASPA is normally found in oligodendrocyte progenitor cells and oligodendrocytes in the brain, with smaller amounts being reported in microglia and brainstem neurons. NAA, one of the most prevalent small molecules in the brain, is hydrolyzed to acetate and aspartate in oligodendrocytes.[4]

Many researchers initially believed that the high NAA associated with CD led to the impeded production of myelin and subsequent spongy degeneration in the brain,[5,6] but this is now being disputed. Cloning of the human ASPA gene has enabled molecular genetic studies of CD.[3]

Clinical course of CD

Three clinically distinct groups of CD have been identified: (i) the congenital form with severe symptoms in the first few weeks of life; (ii) the infantile form, the most common form in which the disease is apparent by 6 months of age; and (iii) the juvenile form, in which the disease is apparent by the age of 4 or 5.[5] Infants with CD typically appear to be normal in the first few months of life. Early signs of CD include irritability and hypotonia with poor head control. The common symptoms of CD include head lag, macrocephaly, hypotonia, ataxia, inadequate visual tracking, poor sucking ability, and intellectual disabilities.[7,8] In many cases, developmental delays and macrocephaly become noticeable after 6 months of age. In spite of profound delays, CD patients can sometimes interact with others, smile, and reach for objects. CD patients later develop optic atrophy, and hypotonia of the arms and legs converts to limb stiffness and spasticity, and axonal hypotonia persists. These patients become increasingly debilitated with age, often having seizures and being unable to move or swallow voluntarily. The long-term prognosis of a typical CD case is still poor; death typically occurs before adolescence, while some patients with milder forms survive beyond their second decade of life.

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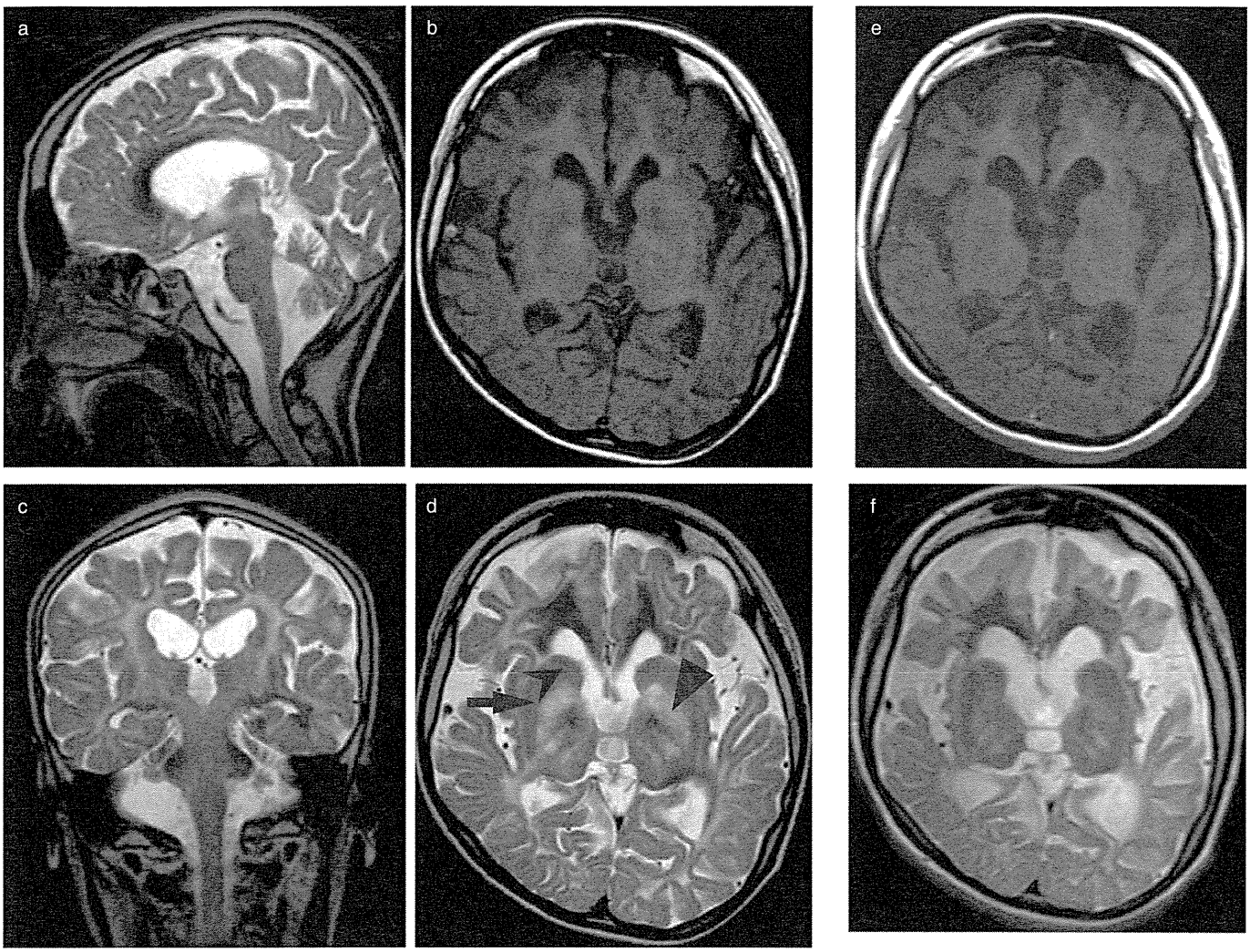

Fig. 1 Chronological features of magnetic resonance imaging (MRI) in a female Japanese Canavan disease patient at (a–d) 15 years and (e,f) 25 years of age. (b,e) T1 and (a,c,d,f) T2-weighted MRI show the involvement of the diffuse white matter, including the corpus callosum and internal capsule, as well as the (▶) globus pallidus. The (➡) putamen and (➢) caudate nucleus were spared. There are signs of diffuse cerebral and cerebellum atrophy.

Clinical examination for diagnosis

The specific method currently used to diagnose CD is urine testing.[9] CD is caused by a deficiency in ASPA, which hydrolyzes NAA to aspartate and acetate in the brain. Therefore, urinary NAA is markedly higher in CD patients, often more than 100-fold, than in normal individuals. Slightly elevated NAA (approximately 4–6-fold) have been reported in other cases of leukodystrophy.[10,11] Therefore, this diagnostic procedure is accurate for the screening and chemical diagnosis of CD, with a good cost–benefit ratio. Genetic testing for the ASPA gene mutation can also lead to a definite diagnosis of CD. Cultured skin fibroblasts were previously shown to manifest this enzyme deficiency, even in the absence of an ASPA gene mutation.[3,12] Microscopy shows spongy degeneration throughout the white matter, demonstrating vacuole formation in the myelin sheaths, astrocyte swelling, and deformed mitochondria.

In neurophysiological examinations, electroencephalograms can be diffusely slow with paroxysmal features. Evoked potentials are often delayed or absent, whereas the nerve conduction velocity is typically normal.

Neuroimaging of CD

Computed tomography of the head has shown diffuse hypodensity in the white matter of the brain, while magnetic resonance imaging (MRI) showed diffuse cerebral white matter degeneration. The most severe abnormalities are present in the subcortical white matter with a mildly swollen aspect, and central white matter structures, such as the periventricular rim of the white matter and the internal capsule, are generally preserved. The central white matter also becomes involved as the disease progresses, and white matter atrophy has been reported. The globus pallidus and thalamus are often involved, whereas the

putamen and caudate nucleus are spared, which is characteristic of CD (Fig. 1).[13] Nuclear magnetic resonance spectroscopy (MRS) has shown that NAA is higher in the brains of CD patients than in those of normal individuals (Fig. 2).[14]

Differential diagnosis

Macrocephaly has been reported in Alexander disease, Tay–Sachs disease, and other neurodegenerative diseases. Hydroxymethylglutaric aciduria also leads to macrocephaly and involvement of the white matter. Spongy degeneration of the brain can occur with viral encephalitis, mitochondrial disease, and other metabolic diseases.

Molecular basis of CD

The human ASPA gene, which is localized on the short arm of chromosome 17 (17p13-ter), was cloned by Kaul *et al.* in 1991.[15] The human ASPA gene spans 30 kb and contains five introns and six exons. Over 96% of CD patients among Ashkenazi Jewish populations have either of two mutations. One is a missense mutation in codon 285, which causes glutamic acid to be substituted with alanine (Glu285Ala). The other is a nonsense mutation on codon 231, which converts tyrosine to a stop codon (Tyr231X). The carrier frequency of these two mutations among Ashkenazi Jewish populations has ranged from 1:37 to 1:40, with a prevalence rate of 1 per 6400–13500 live births.[4,16]

Mutations are different and more diverse in non-Jewish patients. The most common mutation in non-Jewish patients, a missense mutation that substitutes alanine for glutamic acid (Ala305Glu), has been detected in codon 305.[17]

More than 50 mutations have been identified in the human ASPA gene, most of which are single base pair changes in the coding region that typically result in the loss of ASPA enzymatic activity. While all patients in whom mutations have been detected have exhibited psychomotor limitations, their onset and severity varied depending on the specific mutation.[18,19]

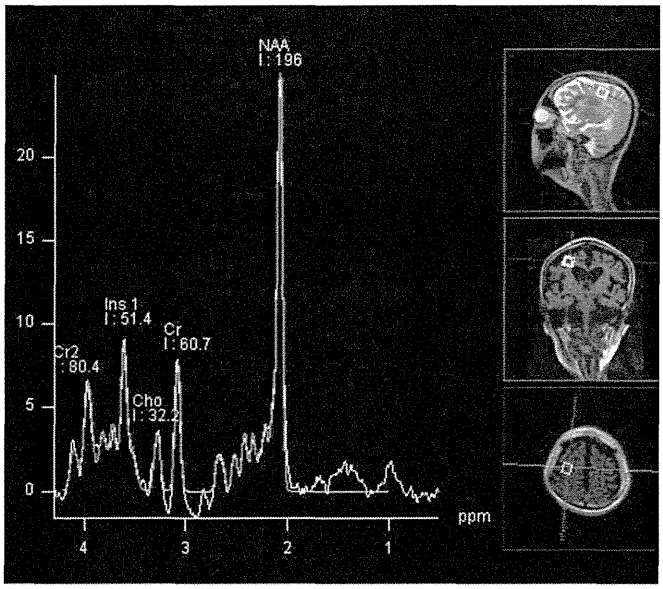

Fig. 2 Features of ^{1}H magnetic resonance spectroscopy in a female Japanese Canavan disease patient (same as in Fig. 1). The highly elevated *N*-acetylaspartic acid (NAA)/choline ratio (6.1; normal range, 1.0–2.4) was characteristic.

Japanese case of CD

Our questionnaire survey identified only one CD patient in Japan, and this is also the only case that has been reported with the missense mutation I143T.[20–22] The patient, a woman, is now 26 years old. She had macrocephaly, gross motor development retardation, and hypotonia since early infancy. She was diagnosed with CD at the age of 4. She could not sit alone due to slowly progressive spastic tetraplegia, but was relatively frequently able to attend school. She had difficulty swallowing at the age of 17 because of progressive bulbar paralysis, and was subsequently fed via a nasogastric tube. She underwent laparoscopic anti-reflux surgery for gastroesophageal reflux disease at the age of 20. Given that the bulbar paralysis is not currently considered to be severe, she does not require any respiratory devices. Although the frequency of tonic seizures increased after the age of 25, levetiracetam has effectively reduced the number of seizures experienced. Figure 1 shows MRI done at the ages of 15 and 25. Subcortical-dominant diffuse white matter was found to be involved. The anterior part of the corpus callosum was highly degenerated. The bilateral globus pallidus and thalamus were also involved, whereas the putamen and caudate nucleus appeared to be spared. Although there are signs of diffuse brain atrophy including the cerebellum, the size of the brainstem has been preserved.

Figure 2 shows MRS of a white matter region at the age of 15. Consistent with previous studies, high NAA concentration and elevated NAA/choline ratio were found to be characteristic in the Japanese patient. Figure 3 shows the ^{99m}Tc-ethyl cysteinate dimer single-photon emission computed tomography done at the age of 15. A frontal predominant decrease in cerebral blood flow was observed.

Possible pathological mechanism responsible for CD

N-Acetylaspartic acid appears to be synthesized exclusively in neurons and has been isolated from mitochondrial and microsomal fractions.[23] NAA and its related dipeptide *N*-acetylaspartyl-glutamate (NAAG) are transported from cytoplasm to the extracellular space by transporters, and NAA is taken up by oligodendrocytes through a dicarboxylic acid transporter prior to being hydrolyzed by ASPA (Fig. 4).

N-Acetylaspartic acid serves as a clinical marker of the neuronal metabolic integrity of the brain. In contrast with the decrease reported in NAA in many other neurodegenerative diseases, CD is unique because it is associated with elevated NAA in the brain. A marked rise of NAAG concentration has also been reported in patients with a Pelizaeus–Merzbacher-like syndrome, in which there is an absence of myelin.[24] Establishing why increases in NAA or NAAG lead to white matter degeneration or

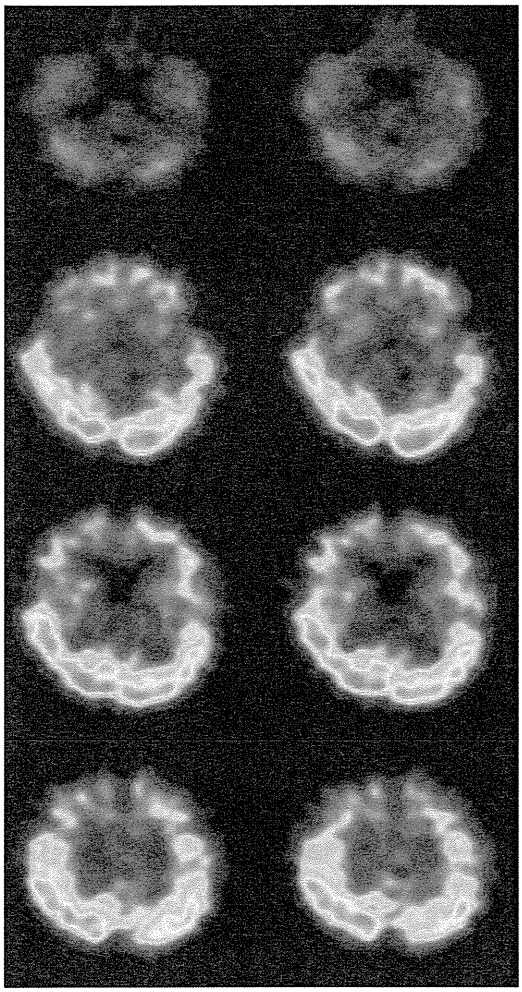

Fig. 3 ^{99m}Tc-ethyl cysteinate dimer single-photon emission computed tomography of a female Japanese Canavan disease patient. The frontal decrease in cerebral blood flow is characteristic. This was obtained from the same case as in Figures 1,2.

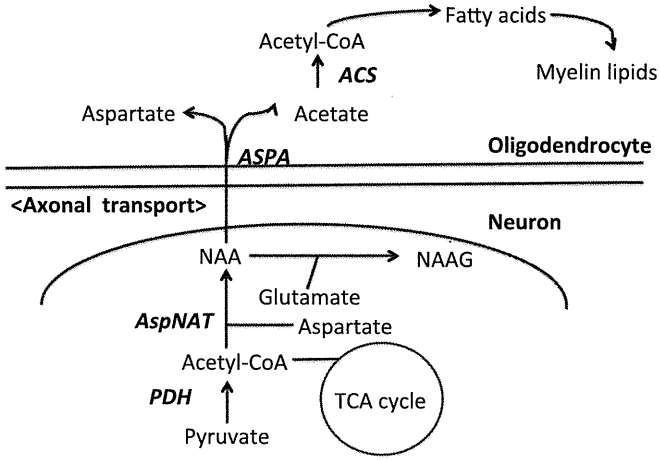

Fig. 4 Schematic representation of *N*-acetylaspartic acid (NAA) synthesis in neurons and degradation in oligodendrocytes. ACS, acetyl CoA synthetase; ASPA, aspartoacylase; AspNAT, aspartate *N*-acetyltransferase; NAAG, *N*-acetyl-aspartyl-glutamate; PDH, pyruvate dehydrogenase; TCA, tricarboxylic acid.

dysmyelination is very important, but the precise function of NAA in the development of the central nervous system (CNS) remains unknown.

Several hypotheses have been proposed to explain the pathophysiology of CD in the CNS.

First, demyelination may reflect the direct action of NAA on oligodendrocyte NMDA receptors. No current, however, was evoked by NAA in oligodendrocytes in a rat study. Therefore, the action of NAA or NAAG on oligodendrocyte NMDA receptors is unlikely to be a major contributor to white matter damage.[25]

Second, NAA may serve as a molecular water pump to remove metabolic water from mitochondria and neurons through its hydrolysis into acetate and aspartate by ASPA. This hypothesis corresponds to astrocytic edema and the formation of vacuoles in CD as a result of the accumulation of NAA.[26] A previous study, however, showed that NAA was non-toxic even at high concentration,[27] and no functional improvement was reported in CD mice even after NAA decreased due to the expression of an introduced normal ASPA gene.[28] An immunohistochemical study of the Nur7 mouse model of CD showed that aquaporin 4 (AQP4) was located throughout the cytoplasm in CD mice, but it was located exclusively in the astrocytic end-feet in control mice. This indicates that the astroglial regulation of water homeostasis may be involved in the partial prevention of spongy degeneration, and AQP4 may be a potential therapeutic target for CD.[29]

Third, NAA may be essential for lipid synthesis and myelination in the CNS during the period of postnatal myelination.[12] CD is characterized not only by an increase in NAA but also by a decrease in acetate and aspartate,[12,30,31] and a reduction in free acetate for lipid synthesis subsequent to the loss of ASPA function may contribute to the disease etiology. Spongiform degeneration in CD brains has been attributed to the failure of NAA to act as an acetate carrier from mitochondria to the cytosol, leading to impaired lipogenesis.[32,33] Therefore, one of the main causes of CD may be a decline in acetyl groups in the absence of ASPA activity. Non-polar and polar lipid levels, critical for myelin synthesis, were found to 21–38% lower in ASPA knockout mice than in wild type, whereas other lipids were not altered significantly.[32] Moreover, a reduction of cerebroside and sulfatide, component glycolipids of myelin in the white matter, was also reported both in the rat CD model and in human CD patients. The reduction observed in lipid level, however, may not have directly correlated with the clinical severity of the disease. These results suggest that the pathogenesis of CD is not restricted to a deficiency of acetate.

Fourth, NAA may play an important role in maintaining the metabolic integrity of oligodendrocytes. Elevation in oxidative stress markers was shown to precede the loss of oligodendrocytes and demyelination in the early days following birth.[34]

Last, besides the role of acetate in myelin formation and maintenance, acetylation also modulates the function of nucleosomal histones, which are components of chromatin. Therefore, a decrease in acetate may alter the expression of genes considered to be important for the maturation of

oligodendrocytes.[35] Although NAA is produced and localized primarily in neurons,[36] high NAA concentration has also been reported also in immature oligodendrocytes. NAA, however, was not detected in mature oligodendrocytes or astrocytes, which suggested the important function of ASPA in immature oligodendrocytes.[37] In rat cortical cultures, the presence of ASPA activity as well as the expression of ASPA mRNA and protein have been reported in non-myelinated oligodendrocytes.[31,38,39] Although the direct uptake of NAA by oligodendrocytes has not yet been reported, axonal transport of NAA has been demonstrated.[40] These findings suggest that the maintenance of myelin is impaired in the absence of NAA-derived acetate. ASPA plays a critical role in the maturation of oligodendrocytes and has also been shown to contribute to the pathophysiology of CD.[41] Furthermore, in an adult ASPA knockout mouse study, disruptions have been observed in cell cycle regulation, the acetylated state of nuclear histones, and continuous neurogenesis in neural cell progenitors, as well as severe reduction in certain myelin proteins.[42] ASPA may be involved in the epigenetic regulation of myelin maturation and maintenance through the supply of acetate.

Therapeutic approaches to CD

A therapy that affects the progression of CD has not yet been established. Seizures need to be controlled by anticonvulsants. Patients with CD may need nasogastric feeding or feeding by gastronomy. Acetazolamide was found to be beneficial in reducing intracranial pressure, but did not reduce white matter swelling or NAA level.

Dietary supplementation is one of the non-invasive therapies that have been positively correlated with improvement in NAA level in CD patients. Lithium citrate decreased whole-brain NAA in both rat models and human subjects.[43] More recent studies noted improved scores in gross motor functioning and visual tracking in CD patients treated with lithium citrate from an early age compared to untreated control groups of CD patients.[44] Acetate supplementation represents another potential therapy for CD that is easy and inexpensive.[45] Oral treatment with glyceryl triacetate (GTA) in CD mice led to a 17-fold increase in acetate in the brain and improved motor function, while NAA level in the brain was not significantly increased.[46] This therapy is now being used in CD patients. Although high-dose GTA (up to 4.5g/kg per dose) treatment in CD infants resulted in no improvement in their clinical status, no significant side-effects or toxicity were observed. The importance of earlier intervention has been suggested.[47] Another possible supplement is triheptanoin, an odd-carbon triglyceride, which is a dietary anaplerotic substrate that provides ketone bodies capable of traversing the blood–brain barrier and increasing the mass of tricarboxylic acid (TCA) cycle intermediates. Triheptanoin was shown to be effective in the treatment of mitochondrial oxidation and pyruvate metabolism.[48,49] A previous study reported that interventions with triheptanoin therapy reduced oxidative stress, promoted long-term survival of oligodendrocytes, and increased myelin in the brain in the nur7 mouse model.[50] The novelty of that study lies in the potentially anaplerotic substrate, with the aim of supporting TCA cycle oxidative integrity in addition to fatty acid synthesis during developmental myelination. The early provision of triheptanoin as an alternative energetic substrate to the nur7 mouse model promoted myelination by reducing the metabolic demands placed on the oxidation of glucose by fatty acid synthesis.[50] NAA was found to be higher in the brains of neonatal triheptanoin mice than in control mice, and triheptanoin had no effect on NAA. NAA is known to have a negative impact on anti-oxidant defenses; therefore, antioxidants may have therapeutic application in CD patients.[51]

Several studies recently attempted to use gene therapies for the treatment of CD. A therapy using adeno-associated virus (AAV) was used in the tremor rat, a genetic model of CD, and ASPA activity was subsequently detected in the CNS neurons of this rat. Although NAA was also reduced in the brain, motor functions remained unimproved.[28]

The application of gene therapy for CD currently faces several challenges. The varied rates of disease progression and small number of patients have confounded any interpretation of the effects of this therapy. Moreover, it is difficult to attempt a cohort study of age-matched or similar-phenotype patients due to variations in the mutations that cause CD.

Leone *et al.* reported the findings of a long-term follow up of gene therapy with an AAV vector carrying the ASPA gene (AAV2-ASPA) in 13 CD patients.[52] Each patient received 9×10^{11} vector genomes via intraparenchymal delivery at six brain infusion sites. The gene therapy was tolerable, no severe long-term adverse effects were noted, elevated NAA in the brain was reduced, the progression of brain atrophy was slowed, and improvements were observed in the frequency of seizures.[52] Moreover, neurological examination showed significant improvement in motor functions in younger cohorts of treated CD patients, indicating the possible advantage of early therapeutic intervention.[53]

Conclusion

In addition to the accumulated medical research on CD, parent and family community support has been increasing. Internet forum or family websites are also meaningful for CD patients.[19] The prognosis of childhood CD has gradually improved due to advances in comprehensive care. Future research will hopefully facilitate development of a safe and therapeutic approach for CD patients and improve their quality of life.

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神経症候群（第2版）

—その他の神経疾患を含めて—

IV

VIII　先天異常/先天奇形

先天形態形成異常

巨頭（脳）症

Canavan病

久保田雅也

先天形態形成異常
巨頭(脳)症

Canavan病

Canavan disease

久保田雅也

Key words：白質ジストロフィ，*N*-acetylaspartic acid(NAA)，アスパルトアシラーゼ(aspartoacylase: ASPA)，アセテート

1. 概念・定義

カナバン病(Canavan disease: CD)は，1931年にCanavanにより最初に記載されたアスパルトアシラーゼ(aspartoacylase: ASPA)の欠損により起こる常染色体劣性遺伝の海綿状変性を伴う白質ジストロフィである[1]．ASPAは白質に多くこの欠損により*N*-acetylaspartic acid(NAA)の蓄積が起こることが白質障害の原因とされる．生後数カ月は正常発達にみえることもあるが低緊張が目立つ発達遅滞を呈し，6カ月以降進行性の大頭，定頸の遅れが明らかとなる．大半の患者の頭囲は90％tileを超える[2]．その後，運動，言語ともに遅滞が著明となるが，限られたやりとりは可能で対象に手を伸ばすこともできることが多い．易刺激性，摂食障害，睡眠障害が目立つこともある．その後脳萎縮が進み年長になり痙性が著明になり原因不明の脳性麻痺疑いとされる例もある．約6割にてんかん発作がみられる．

2. 疫　　学

CDはすべての人種にみられるもののまれな疾患で，ほとんどはアシュケナージ・ユダヤ人であり，日本人では後述する1例が確定診断されたのみである．アシュケナージ・ユダヤ人におけるASPA遺伝子変異の保因者頻度は1：40-1：59であった[3,4]．この場合の遺伝子型は2種類(E285A;854A>C，Y231X;693C>A)の変異が98％を占める．また，非アシュケナージ・ユダヤ人でも多種の変異があるが，そのうちA305E(914C>A)変異が40％程度を占める．多くの変異は遺伝子型と表現型の関連は認めず，同一家系内でも表現型は異なるとされているが，一部の軽症型(K213E，Y288C，G212Aの複合ヘテロ接合体変異)や極度に重症なケースでは遺伝子型と表現型の関連が認められる．

3. 病因と病態

CDにおいて脳内に過剰に蓄積するNAAは大脳皮質の神経細胞内のミトコンドリアで合成され，白質のオリゴデンドロサイトへと輸送される(図1)．ASPAはNAAをアスパラギン酸とアセテートに分解する酵素で，白質のオリゴデンドロサイトに限局して発現している．ASPA欠損とCDの病態の関連はいまだ不明な部分が多いが，ASPA欠損により，オリゴデンドロサイトでのNAAの蓄積とアセテート欠乏が起こることがCDの病因として重要である．NAAは髄鞘化に必要なアセテートの供給源であり，CDの病態としてアセテートの欠乏による髄鞘化障害が一つの障害機序とされる(図1)．ASPAノックアウトマウスでは髄鞘脂質合成の欠如が認められる[5]．また，NAAは浸透圧制御活性を有し，CDでは細胞間質やオリゴデンドロサイトで浮腫性変化が起こり，髄鞘形成障害や皮質下空砲形成の原因となる[6,7]．さらにASPAは髄鞘タンパクやオリゴデンドロサイトの分化に関わ

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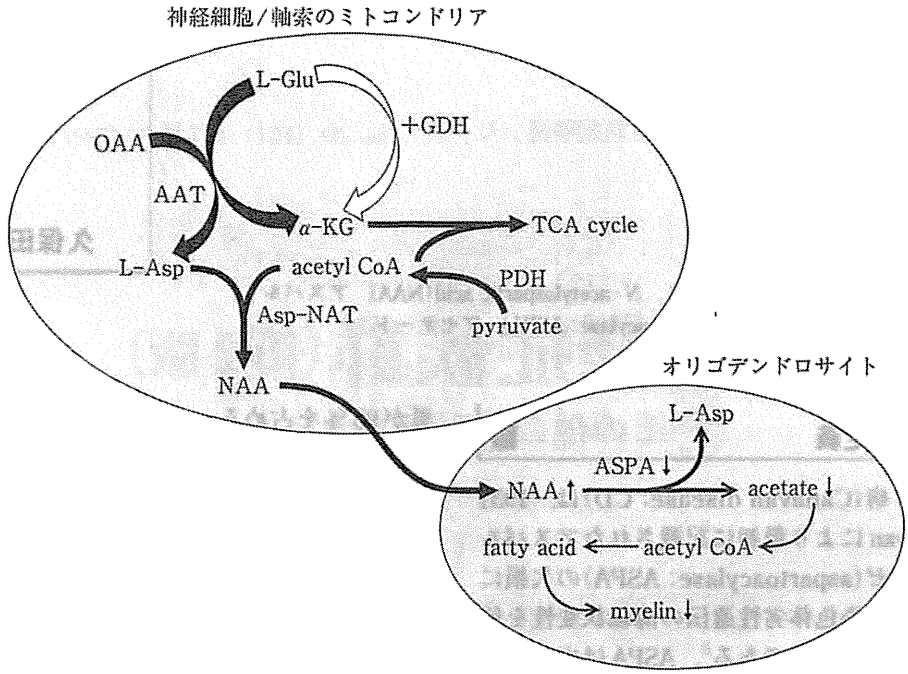

図1　NAA(*N*-acetylaspartic acid)の代謝経路

OAA: oxaloacetic acid, α-KG: α-ketoglutarate, AAT: aspartateaminotransferase, GDH: glutamate dehydrogenase, PDH: pyruvate dehydrogenase, Asp-NAT: aspartate *N*-acetyltransferase, L-Asp: L-aspartate.

る遺伝子のepigeneticな制御にも関わっている[8]．つまり，NAAの代謝系は髄鞘形成，成熟や浸透圧の調節に関係し，これらがCDの中枢障害の機序と想定される．NAA自体が神経興奮毒性を有するという仮説もあるが反論も多い．

4. 診断と鑑別診断

乳児期の低緊張型発達遅滞，著明なhead lagと大頭などの臨床徴候と画像上の白質変性所見があればまずCDが鑑別の対象に入ってくる．視神経萎縮，網膜変性，水平性眼振も認めることが多い．検査所見としてはgas chromatography-mass spectrometry(GC-MS)による尿中NAAの著明な増加が最も診断的価値がある[9,10]．**図2**に尿中NAAの正常対照の年齢変化を示した．CDではこの数10倍から100倍に相当するNAAが検出される．尿中NAAは正常では経年齢的に低下する．**図2**に示した後述する患者の尿中NAAは異常高値が持続している．培養線維芽細胞を用いた酵素活性測定も有用である(測定不能レベルとなる)が，やや煩雑であり，尿中NAA測定が簡便で感度が高いと考えられる．脳内で増加したNAAの検出にはmagnetic resonance spectroscopy(MRS)が有用である(**図3**)．通常NAAの増加，cholineの低下を認める．脱髄疾患，虚血性脳疾患，Alzheimer病など多くの神経疾患でNAAピークが低下するのと対照的である．頭部MRIはびまん性の皮質萎縮，白質変性(前頭葉深部は比較的保たれる)，淡蒼球が変性し，尾状核，被殻が保たれるパターンが特徴的である(**図4**)．脳幹，小脳は比較的保たれるが萎縮が認められる．

鑑別診断としてはAlexander病，Tay-Sachs病，3-OH glutaric acidemia，megalencephalic leukoencephalopathy with subcortical cysts，GM1 gangliosidosis，Krabbe病，異染性白質ジストロフィなどがあるがいずれも尿中NAA測定により鑑別は可能である．

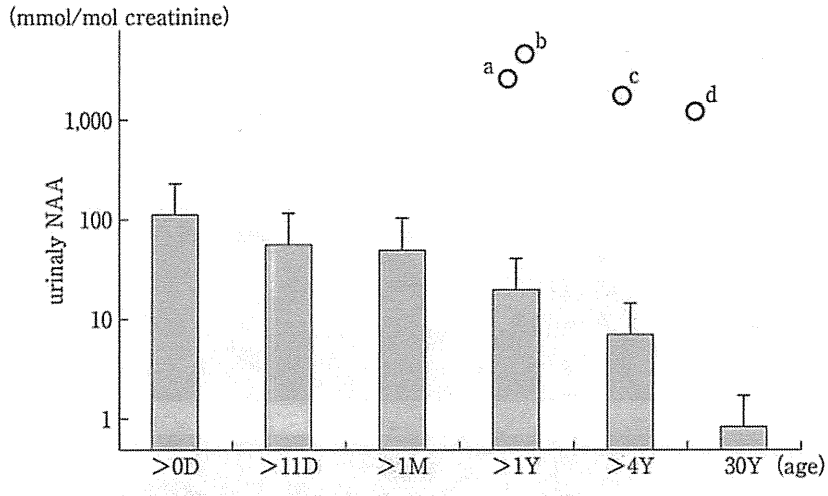

図2 尿中NAAの年齢別正常値と症例の値
a: 9カ月, b: 1歳, c: 3歳9カ月, d: 22歳.

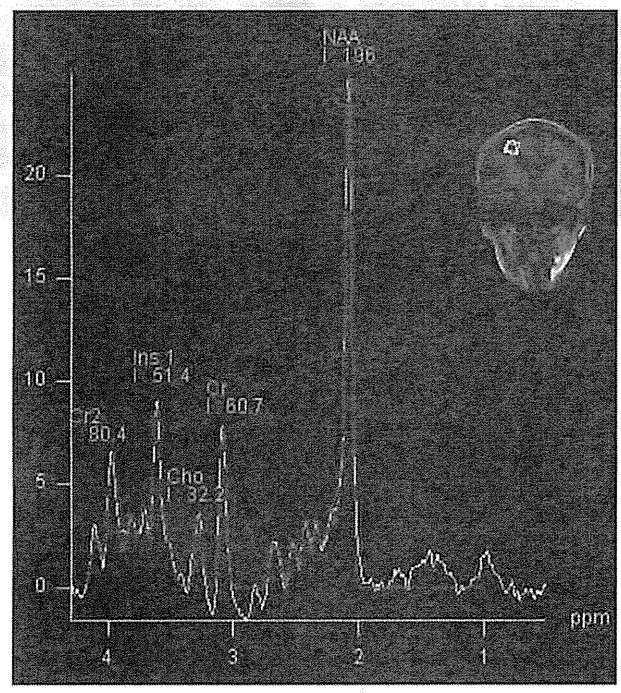

図3 症例の25歳時の頭頂葉におけるプロトンMRS (magnetic resonance spectroscopy)
NAA/Cr 3.23(正常値:1.36±0.10), Cho/Cr 0.53(正常値:0.56±0.06)とNAA高値, Cho低値を認める. 正常値は文献[20]より.

5. 治療と予後

現在CDの治療で確立されたものはないが, 幾つかの試みはなされている. アセテートの欠乏が病因と関連するというデータからアセテートの前駆体glyceryl triacetate(GTA)をサプリメントとして使う治療が行われ, マウスではアセテートの上昇が認められ, 脳内のNAAは上昇

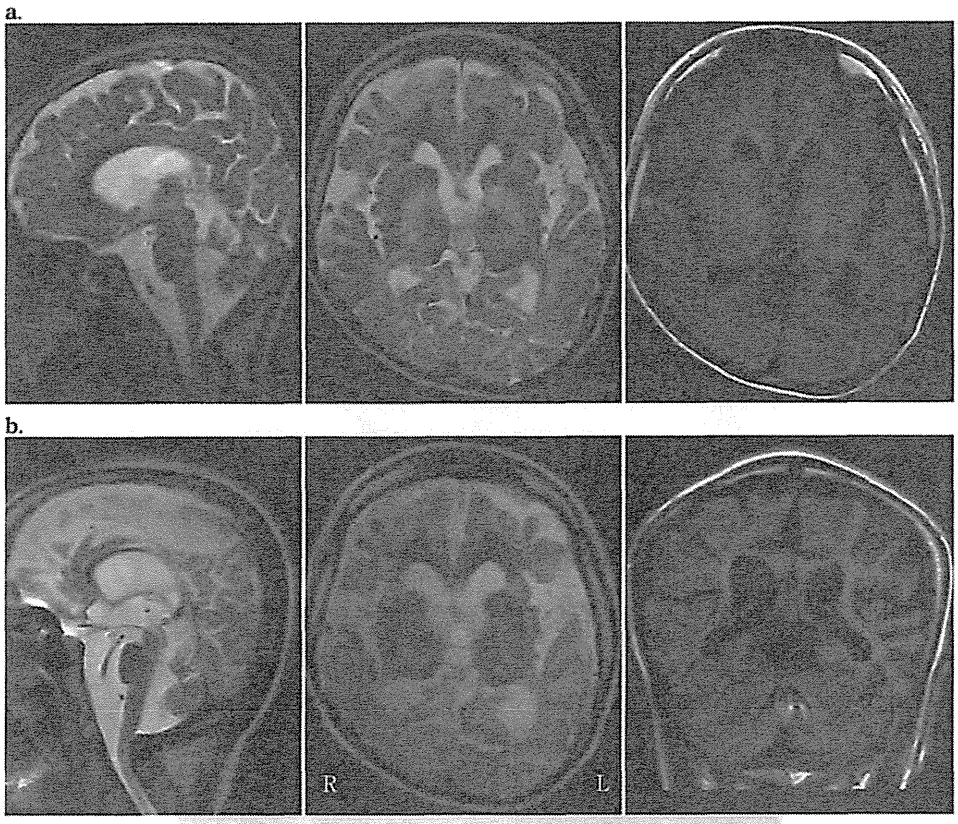

図4　症例の頭部MRI所見

全般的な皮質，小脳，脳幹の萎縮，白質病変，基底核では淡蒼球病変と尾状核，被殻が保たれるパターンを認める．a: 15歳時，b: 25歳時．

しなかった[11]．また2人の小児CD患者(8カ月と13カ月)に応用され副作用や臨床的な退行はなかったとされるが，半年以内の投与であり長期的な効果は不明である[12]．CDのモデル動物であるtremor ratへの投与では運動機能の改善と髄鞘脂質の組成の変化が観察された[13]．またtremor ratにおいて塩化リチウムが脳内NAAを減少させる効果のあることが確認され[14]，Assadiら[15]は6人の患者に応用し基底核におけるNAAの低下や髄鞘化の軽微な改善，家族の報告による日中の覚醒度や疎通性の改善を認めたが運動機能に変化はなかった．アデノ随伴ウイルスベクター(AAV)によるASPAの頭蓋内投与がノックアウトマウスやtremor ratで効果をあげ[16,17]，ヒトでの第1相試験が施行されている[18]．またLeoneら[19]の10年以上の観察を含むヒトでの同治療のコホート研究によると脳内NAAの減少，脳萎縮進行の緩徐化，痙攣頻度改善，臨床状態の安定化がみられ，特に大きな副作用は認めていないようである．より早い段階での治療群で運動機能の改善・安定化がみられている(治療最少年齢は3カ月)．今後のより非侵襲的なAAV導入法の開発による治療応用が期待される．

6. 症　　例

本症例は1993年にHamaguchiら[20]が報告した日本で唯一の症例である．

26歳女性．

家族歴：父方の祖母，母方の祖父がいとこ，姉2人は健常．

周産期歴：在胎40週6日，出生時体重3,200 g，自然分娩にて仮死なく出生した．

定頸5カ月，寝返り9カ月，座位1歳9カ月，

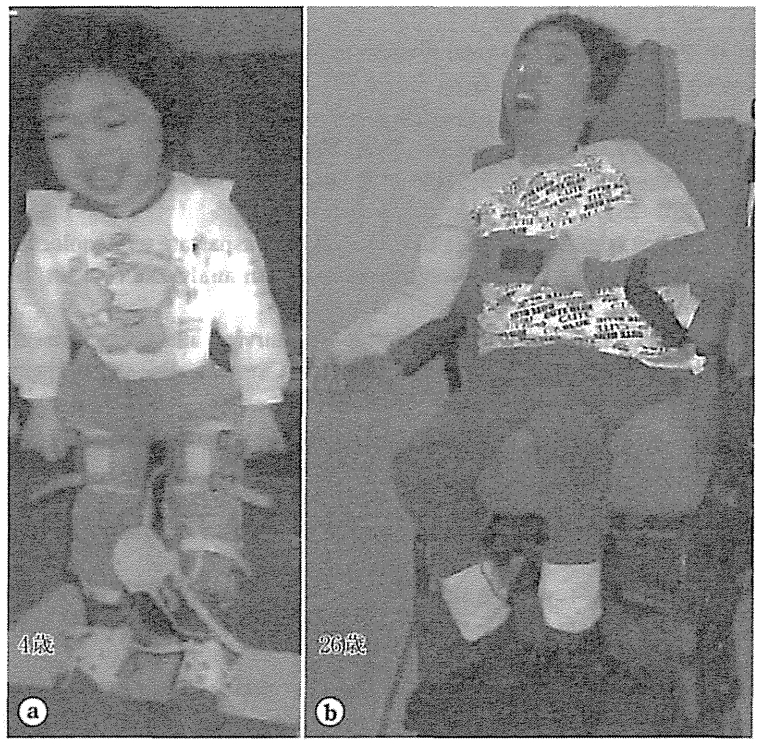

図5 症例のa: 4歳時, b: 26歳時の写真
写真掲載の同意と許可はご家族から取得済み.

3歳で膝立ちと運動発達の遅れを認め4歳から座位不安定となった. 図5-aのように装具での立位は可能であった. 身長93cm(−2SD), 頭囲51.6cm(+1.5SD)と頭囲拡大あり. 発語は数語あり, 簡単な理解は可能であった. 診察上低緊張と四肢の痙性, 小脳症状を認めた. 4歳時, 頭部CTでびまん性の大脳白質の低吸収域を認め, 尿中NAA排泄増加(図2), 皮膚線維芽細胞でのASPA活性の欠如からCDと診断された. 遺伝子検索では, I143T(428T>C)のホモ変異を認めた[21]. 5歳からミオクローヌス発作を認めたが, コントロールは容易であった. 6歳時にははいはいで移動, 友人の名前を言うことはできた. 以後, 緩徐進行性の経過をとり, 8歳で座位, ずりばいも不能となった. 痙性四肢麻痺が著明で, 生活は全介助で発語はないが, 簡単な意思表示は可能であった. 17歳で経口摂取の低下と体重減少を認め, 経管栄養を導入し, 上気道閉塞による呼吸障害, 嚥下障害を認め19歳で経鼻エアウェイを開始した. 胃食道逆流に伴う嘔吐で注入困難となり, 20歳で胃瘻-噴門形成術を行った. 重度の痙性四肢麻痺を認めるが, 意思表示は上肢大関節と頭部の運動のみによって可能で意識レベルは比較的保たれ簡単な問いかけに反応する[22]. 現在26歳となるが, 呼吸状態は安定し, 在宅にて生活を送っている(図5-b).

■文 献
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