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ORIGINAL COMMUNICATION

Epidemiological, clinical, and genetic landscapes of hypomyelinating leukodystrophies

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Abstract To determine the epidemiological, clinical, and genetic characteristics of congenital hypomyelinating leukodystrophies, including Pelizaeus–Merzbacher disease (PMD), we conducted a nationwide epidemiological survey in Japan. A two-step survey targeting all medical institutions specializing in pediatric neurology and childhood disability (919 institutes) in Japan was performed. Detailed information was collected for 101 patients (86 males and 15 females) with congenital hypomyelinating leukodystrophies. The prevalence of congenital hypomyelinating disorders was 0.78 per 100,000 people (0–19 years old), and the incidence was 1.40 per 100,000 live births. Molecular testing was performed in 75 % of patients, and *PLP1* gene abnormalities were observed in 62 %. The incidence of PMD with *PLP1* mutations was estimated to be 1.45 per 100,000 male live births and that for congenital hypomyelinating disorders with unknown cause to be 0.41 per 100,000 live births. Patients with *PLP1* mutations showed a higher proportion of nystagmus and hypotonia, both of which tend to disappear over time. Our results constitute the first nationwide survey of congenital hypomyelinating disorders, and provide the epidemiological, clinical, and genetic landscapes of these disorders.

Keywords Hypomyelinating leukodystrophy · Epidemiology · Pelizaeus–Merzbacher disease

Introduction

Congenital hypomyelinating leukodystrophies are a heterogeneous group of inherited diseases characterized by a substantial deficit in myelin deposition in the brain. These disorders are distinguished from classic demyelinating leukodystrophies characterized by myelin degeneration. Therefore, clinical manifestations, pathogenesis, and potential treatment approaches can be distinct for these two groups of disorders. Recently, at least 11 congenital

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hypomyelinating leukodystrophies, of which Pelizaeus–Merzbacher disease (PMD) is the best characterized prototype, have been clinically and genetically recognized and differentiated [7], including Pelizaeus–Merzbacher like disease (PMLD) [19], hypomyelination with atrophy of the basal ganglia and cerebellum (H-ABC) [20], chromosome 18q-deletion syndrome (18qdel) [10], Allan–Herndon–Dudley syndrome [4], mitochondrial Hsp60 chaperonopathy (MitChap60) [11], Salla disease [22], diffuse cerebral hypomyelination with cerebellar atrophy and hypoplasia of the corpus callosum [15], hypomyelination and congenital cataract [24], ataxia, delayed dentition, and hypomyelination [23], peripheral demyelinating neuropathy, central dysmyelinating leukodystrophy, Waardenburg syndrome, and Hirschsprung disease [9]. However, epidemiological and clinical diagnostic data specific to this group of disorders are lacking.

In the diagnostic evaluation, the recognition of hypomyelination has been revolutionized by magnetic resonance imaging (MRI) technology [1, 16]. In addition, molecular detection of disease-causing mutations, especially proteolipid protein 1 (*PLP1*) gene testing for PMD, has further promoted the diagnostic clarification of hypomyelinating disorders. However, despite the availability of such neuroimaging and molecular tools, the cause of the disease remains unknown in many patients. Therefore, substantial information regarding the epidemiological and clinical picture of congenital hypomyelinating disorders remains undetermined.

Here, we conducted a nationwide epidemiological study in Japan to determine the prevalence, incidence, clinical characteristics and manifestations, and frequency of disease-causing mutations in patients with congenital hypomyelinating leukodystrophies.

Methods

Study design

This study was conducted as a part of the Rare and Intractable Disease Research Program, directed by the Ministry of Health, Labour and Welfare, Japan, and was performed in collaboration with the Japanese Society of Pediatric Neurology. The Institutional Review Board at National Center of Neurology and Psychiatry, Japan approved this study in accordance with the ethical standards. We selected all medical hospitals with one or more board-certified pediatric neurologists regardless of the size of the institution (747 institutes), all pediatric departments in medical university hospitals (48 institutes, not including the above), and institutions for children with severe physical and intellectual disabilities (124 institutes), a total of 919 institutes in Japan. All patients who visited pediatric neurologists in these institutes from 1 April 2008 to 31 March 2009 were approached. This approach enabled sufficient coverage of neuropediatric medical institutes, where most patients with hypomyelinating leukodystrophies visit for diagnosis, follow-up, treatment, and rehabilitation, and thus achieving practically a 100 % sampling rate without performing a large-scale investigation. Incidence (the rate of occurrence per birth) and prevalence (the number of patients in a population) were determined using demographic statistics obtained from the Vital Statistics of Japan yearly survey carried out by the Ministry of Health, Labour and Welfare, Japan (http://www.mhlw.go.jp/toukei/list/81-1.html). The population of Japan is calculated to be roughly 128 million.

Inclusion criteria

Diagnosis of congenital hypomyelinating leukodystrophies was defined by MRI findings and clinical features [16]. MRI diagnosis should include the observation of diffuse hyperintensity of the cerebral white matter in T2-weighted images, suggesting a persistent and substantial deficit in myelin deposition in the brain. Clinical features include developmental retardation in childhood with no or slow clinical deterioration. Demyelinating leukodystrophies were strictly excluded from this survey at the time of enrollment.

Primary and secondary surveys

In the primary survey, we sent a simple questionnaire to the 919 selected medical institutes to determine if patients with congenital hypomyelinating disorders were present in each institute by requesting the number of such patients. In the secondary survey, we sent a survey sheet to the responders of the primary survey to collect detailed clinical information. The questionnaires included questions on age, sex, family history, clinical diagnosis, clinical symptoms, changes of clinical symptoms, findings in the neuroimaging study, implementation and results of genetic testing, and availability for genetic counseling and carrier testing.

Statistical analyses

Two-tailed p values for odds ratios were calculated using Fisher's exact test.

Results

Prevalence and incidence

The primary survey (70 % recovery rate) identified 164 patients with possible congenital hypomyelinating

leukodystrophies from 95 institutes. We obtained completed and returned secondary survey questionnaire sheets from 108 patients, with a 65.5 % recovery rate. Ultimately, 101 patients (86 males and 15 females) met our diagnosis criteria for congenital hypomyelinating leukodystrophies, after exclusion of seven patients with a diagnosis of demyelinating leukodystrophies, such as Alexander disease, and duplicated registrations (Table 1). Based on these numbers, an estimation of 220 patients with congenital hypomyelinating leukodystrophies present in Japan was obtained.

We estimated the prevalence of congenital hypomyelinating leukodystrophies in Japan as 0.78/100,000 people (0–19 years old) and the incidence as 1.40/100,000 live births. More than 80 % of the patients were under 20 years old (supplementary Figure 1A). A family history of congenital hypomyelinating leukodystrophies was present in 22 patients, including 11 patients with *PLP1* gene abnormalities.

Clinical diagnosis

The most common clinical diagnosis was PMD (Table 1). Other diagnoses included PMLD, H-ABC, and 18qdel; more than 20 % of patients had no specific diagnosis and remained unclassified. These diagnoses were made primarily on the basis of MRI findings and clinical features, and the results of molecular testing were not necessarily required in the diagnostic decision, although in nearly half of the patients, conclusive diagnostic information via molecular testing was obtained (49/101 patients).

Table 1 Characteristics of the patients

Characteristics	Total $n = 101$
Range of ages (mean, median)	0–48 (12.1, 9)
Sex, *n* (%)	
Male	86 (85)
Female	15 (15)
Family history, *n* (%)	22 (22)
Clinical diagnosis, *n* (%)	Total $n = 101$
PMD	66 (65)
PMLD	6 (6)
H-ABC	4 (4)
18q deletion syndrome	1 (1)
Others	1 (1)
Unknown	23 (23)
Molecular diagnosis, *n* (%)	Total $n = 76$
PLP1 abnormalities	47 (62)
Duplication	26 (63)
Point mutation	15 (37)
Others	2 (3)
Unknown	27 (35)

PMD Pelizaeus–Merzbacher disease, *PMLD* Pelizaeus–Merzbacher like disease, *H-ABC* hypomyelination with atrophy of the basal ganglia and cerebellum

Molecular diagnosis

Almost 75 % of the patients received molecular testing for one or more genes; the majority were tested for *PLP1*, which is responsible for PMD. More than 60 % of these patients had *PLP1* abnormalities. Information about the mutation types was available in 41 patients; 26 patients had genomic duplications and 15 patients had point mutations. Besides *PLP1* mutations, chromosomal abnormality was reported in one patient with 18qdel. In 36 % of the patients, no molecular diagnosis was achieved. Based on these data, we estimated the incidence of PMD with *PLP1* mutations to be 1.45 per 100,000 male live births. *GJC2* was examined in 11 patients negative for *PLP1* testing. In all patients but one, in which a potential alteration in one allele was seen, no mutation could be found. The incidence for congenital hypomyelinating leukodystrophies with unknown cause was estimated to be 0.41/100,000 live births.

Clinical characteristics

All patients experienced their initial symptoms before 1 year of age, and 91 % showed symptoms within 6 months of birth. The most common initial symptoms were nystagmus and developmental delay (Table 2). Patients with *PLP1* mutations showed nystagmus as their initial symptom more commonly than the patients without the mutations did. In contrast, patients having no *PLP1* mutations were more likely to show developmental delay as their initial symptom. Nystagmus was a more common initial symptom in patients with earlier disease onset, whereas developmental delay was more frequent in patients with later onset (supplementary Figure 1B). Of note, five patients had respiratory problems as their initial symptom; all five patients had *PLP1* mutations. In addition, all of the patients with *PLP1* mutations showed their initial symptoms before the 5th month of life (supplementary Figure 1C). The median age for achieving clinical diagnosis was 1 year and 8 months. Almost 70 % of patients were diagnosed at <3 years of age (supplementary Figure 1D). Meanwhile, eight patients were diagnosed at age 10 years or older, including two individuals in their 40s. These individuals might have had no opportunity for *PLP1* molecular testing at their initial diagnostic evaluation before, and therefore, might have had to wait for a recent re-evaluation opportunity.

The most common symptoms over the entire clinical course were nystagmus (74 %), hypotonia (71 %), and the

Table 2 Initial symptoms

Initial symptoms	All No. (%) $n = 101$	*PLP1*+ No. (%) $n = 47$	*PLP1*− No. (%) $n = 22$	*P* value *PLP1*+ vs. *PLP1*−
Nystagmus	57 (56)	33 (70)	9 (41)	0.0332
Developmental delay	34 (34)	8 (17)	14 (64)	0.0002
Others	14 (14)	7 (15)	2 (9)	NS

Others include respiratory failure ($n = 5$)

PLP1+ patients with *PLP1* mutations, *PLP1*− patients without *PLP1* mutations

Table 3 Clinical features

Symptoms	All No. (%) $n = 101$	*PLP1*+ No. (%) $n = 47$	*PLP1*− No. (%) $n = 22$	*P* value *PLP1*+ vs. *PLP1*−
Nystagmus	75 (74)	43 (91)	12 (55)	0.0008
Hypotonia	72 (71)	37 (79)	12 (55)	0.0049
Quadriplegia	56 (55)	24 (51)	15 (68)	NS
Spasticity	59 (58)	31 (66)	11 (59)	NS
Increased DTR	62 (61)	28 (60)	13 (59)	NS
Swallowing difficulty	29 (29)	11 (23)	8 (36)	NS
Hearing loss	24 (24)	11 (23)	8 (36)	NS
Pathological reflex	36 (35)	13 (28)	9 (41)	NS
Rigidity	20 (20)	5 (11)	7 (32)	0.04
Dystonia	14 (14)	3 (6)	6 (27)	0.02
Cerebellar signs	21 (21)	9 (19)	3 (14)	NS
Athetosis	11 (11)	4 (9)	4 (18)	NS
Seizure	26 (26)	9 (19)	8 (36)	NS

DTR deep tendon reflexes, *PLP1*+ patients with *PLP1* mutations, *PLP1*− patients without *PLP1* mutations

symptoms associated with spastic diplegia/quadriplegia (increased deep tendon reflex, spasticity, quadriplegia, and pathological reflex) (Table 3). A comparison between patients with *PLP1* mutations and those without revealed that nystagmus and hypotonia were more common in patients with *PLP1* mutations (Table 3). In contrast, rigidity and dystonia were less common in those with *PLP1* mutations. No difference in the frequency of other symptoms was observed.

To determine the changes in clinical manifestations over time, we selected patients aged 7 years and older and investigated the frequency of each symptom prior to and at the time of the survey (supplementary Table 1). In patients with *PLP1* mutations, the frequencies of nystagmus and hypotonia decreased over time (Fig. 1). No such tendency was observed in patients without *PLP1* mutations, indicating that the disappearance of these symptoms is characteristic to *PLP1* mutations. Changes in the frequency of other symptoms, including quadriplegia, rigidity, dystonia, and cerebellar signs, were not observed.

Imaging findings

In accordance with the inclusion criteria, bilateral diffuse hyperintensity of cerebral white matter in T2-weighted MRI images was recognized in all patients. Other MRI findings are summarized in supplementary Table 2. Minor findings, including diffuse cerebral atrophy, cerebellar atrophy, and caudate nucleus lesions, were observed less frequently in patients with *PLP1* mutations than those without.

Clinical examination

The auditory brainstem response (ABR) was measured in more than 80 % of patients (Table 4). Compared to 83 % of patients not carrying *PLP1* mutations, all patients with *PLP1* mutations had an abnormal ABR. A nerve conduction study was performed in 37 % of patients, three of whom, including two with *PLP1* mutations, demonstrated an abnormality. Chromosomal examinations and a thyroid hormone study were performed in approximately half of the patients.

Availability for genetic counseling and carrier diagnosis

Approximately 30 % of the families of patients having conclusive molecular diagnoses proceeded to carrier testing. Genetic counseling was provided to all families. Of the institutes included in this survey, 48 % have an on-site genetic counseling service available, and 14.5 % have access to affiliated medical institutes with a genetic counseling service. However, 37.5 % have no access to genetic counseling as a part of their medical service; most of these are institutes for severely retarded children, and were responsible for the daily support and rehabilitation of children after completion of the diagnostic study.

Supportive care and treatment

Seventeen percent of patients underwent tracheotomies that included laryngotracheal separation, another 8 % required mechanical ventilation, and 27 % of patients, including those receiving a gastrotomy, required ingestion of food via a feeding tube. Rehabilitation was performed in 57 % of patients. Nearly 30 % of the patients use antiepileptic drugs and/or muscle relaxants. No difference in the frequency of care and treatment for patients with and without *PLP1* mutations was observed.

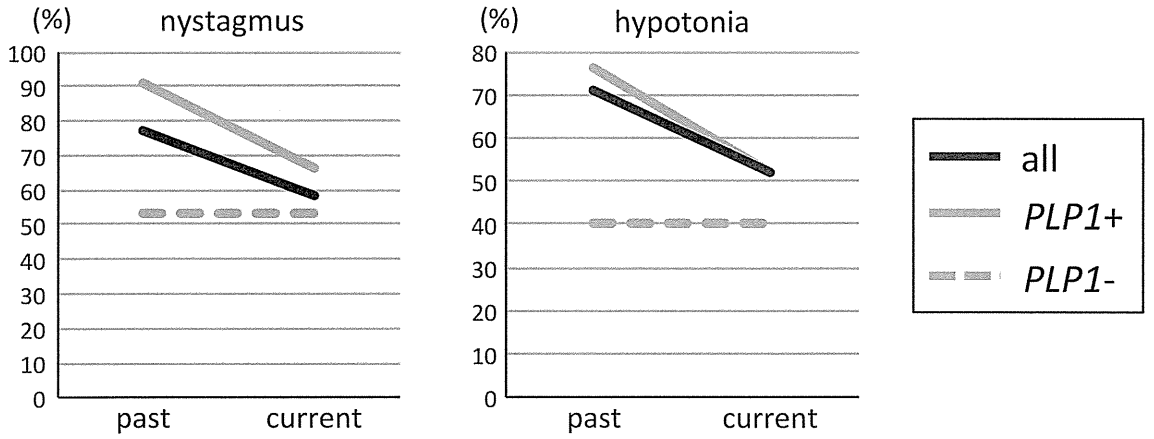

Fig. 1 Change in the clinical symptoms of patients aged 7 years and older. Patientsaged 7 years and older ($n = 66$) were selected from the cohort (*black line*). Of these, patients with genetic testing information were sub-grouped into those with *PLP1* abnormality (*grey line*; $n = 33$) and without (*dotted line*; $n = 15$). Frequencies of the symptoms present in the past and current presentation were shown for nystagmus (*left*) and hypotonia (*right*)

Table 4 Clinical examinations

Clinical examinations	All ($n = 101$)		*PLP1*+ ($n = 47$)		*PLP1*− (n = 22)		*P* value *PLP1*+ vs. *PLP1*−
	No. performed (%)	No. with abnormality (%)	No. performed (%)	No. with abnormality (%)	No. performed (%)	No. with abnormality (%)	
ABR	83 (82)	72 (87)	43 (92)	43 (100)	18 (82)	15 (83)	NS
SEP	22 (22)	10 (46)	5 (11)	2 (40)	9 (41)	7 (78)	0.008
NCV	37 (37)	3 (8)	15 (32)	2 (13)	12 (55)	1 (8)	NS
Chromosomal analysis	53 (53)	3 (6)	20 (43)	1 (5)	18 (82)	0 (0)	0.0038
Thyroid hormone	50 (50)	3 (6)	18 (38)	3 (17)	15 (68)	0 (0)	0.0374
LH, FSH	9 (9)	0 (0)	2 (4)	1 (50)	3 (14)	0 (0)	NS

ABR auditory brainstem response, *SEP* somatosensory evoked potentials, *NCV* nerve conduction velocities, *PLP1*+ patients with *PLP1* mutations, *PLP1*− patients without *PLP1* mutations, *LH* luteinizing hormone, *FSH* follicle stimulation hormone

Discussion

By using this nationwide questionnaire-based study, we sought to determine an epidemiological and molecular diagnostic view of congenital hypomyelinating leukodystrophies, and to reveal the clinical characteristics of this group of disorders in Japan. By targeting this specific group of diseases, we identified a number of findings. First, we estimated the prevalence of the congenital hypomyelinating leukodystrophies as 0.78/100,000 people (0–19 years old) and the incidence as 1.40/100,000 live births. In combination with molecular diagnostic information, we further estimated the incidence of genetically confirmed PMD as 1.45 per 100,000 male live births. Second, 62 % of patients with congenital hypomyelinating leukodystrophies have *PLP1* mutations. Third, patients with *PLP1* mutations show a higher propensity of nystagmus and hypotonia, both of which may disappear over time.

Only a few epidemiological studies have been conducted on leukodystrophies, including hypomyelinating disorders. One nationwide survey conducted in Germany determined the incidence of major forms of leukodystrophies as 2.0/100,000 live births [5]. Based on the proportion of PMD identified (via genetic testing) in this study (6.5 %), the incidence of PMD can be estimated as approximately 0.26/100,000 male live births. However, this is possibly an underestimation because molecular confirmation of *PLP1* abnormalities was required as part of the inclusion criteria; however, at the time of this study (1996), molecular testing for *PLP1* duplication was not clinically available. In another recent study conducted in Utah, USA, the incidence of leukodystrophies was 13.0/100,000 live births [3]. In this chart-based study, almost half of the patients had no known diagnosis, possibly enhancing the overall incidence. Of note, PMD was the second most frequent leukodystrophy in the Utah study, representing

7.4 % of all patients. Based on these data, an incidence of 1.9/100,000 male live births can be estimated for PMD that is consistent with the rate of 1.45/100,000 obtained in our study of a Japanese population. Neither of the aforementioned studies determined the frequency of hypomyelinating leukodystrophies other than PMD. In contrast, our study has specifically targeted congenital hypomyelinating leukodystrophies for the first time to unveil their epidemiological features. We found that 36 % of the patients with hypomyelinating leukodystrophies received no specific diagnosis, leading to an estimated incidence of 0.41/100,000 live births for congenital hypomyelinating leukodystrophies with unknown cause.

We confirmed that PMD is the most common congenital hypomyelinating leukodystrophies. Notably, *PLP1* mutations were found in 62 % of patients who underwent molecular diagnostic testing. Therefore, *PLP1* molecular testing should be recommended as a first line of examination if hypomyelinating leukodystrophies are considered a potential clinical diagnosis. Of the PMD patients, 63 % carried duplications and 37 % had point mutations. A similar proportional distribution of mutations has been estimated at the diagnostic laboratory level [8, 12, 18], but has not been determined by epidemiological studies. Meanwhile, 36 % of patients may have congenital hypomyelinating leukodystrophies caused by other genes. We found that patients possessing and lacking *PLP1* mutations showed largely overlapping clinical and MRI findings. Mutations in *SLC16A2* and *GJC2* genes were reported to be responsible for approximately 10 % of patients who did not have *PLP1* mutations [6, 21], while *SOX10* mutations were infrequent [13]. Nonetheless, comprehensive and systematic screening of the human genome is required to fully delineate the molecular diagnostics of these patients with unknown cause of the disease. In fact, exome analyses have recently identified three new genes for congenital hypomyelinating leukodystrophies [2, 14, 17].

In our comparison between patients with and without *PLP1* mutations, we found that most symptoms were present in both groups at similar frequencies (Table 3). However, nystagmus and hypotonia were both more common in patients with *PLP1* mutations than in those without. In addition, these two symptoms tend to disappear over time only in patients with *PLP1* mutations (Fig. 1). Therefore, the presence of nystagmus and hypotonia at earlier stages of the disease and the disappearance of these symptoms in later stages is the unique feature of PMD caused by *PLP1* mutations. Hypotonia is a relatively common neurological symptom in children with motor and/or intellectual disabilities, as seen in Down syndrome. However, nystagmus is not as common in other pediatric neurological disorders, and *PLP1* molecular testing is therefore recommended particularly for children exhibiting nystagmus.

Of the other diagnostic examinations, an abnormal ABR was commonly observed in patients with hypomyelinating leukodystrophies, regardless of the presence or absence of *PLP1* mutations (Table 4). Although an abnormal ABR is not linked to specific gene abnormalities, it is an easily measurable response for infants and toddlers, and has even been used for the screening of hearing in neonates. In combination with early onset nystagmus, an ABR may serve as a useful and convenient tool to detect congenital hypomyelinating leukodystrophies before an MRI reveals confirmatory evidence for hypomyelination, usually at an age of 1 year and later.

There are some limitations to this study. First, we recruited the patients mainly through pediatric neurologists; therefore, we might have excluded adult patients. The lower frequencies of enrolled adults may also be associated with the mortality of these disorders. Because our study did not determine the longitudinal outcomes of patients, we were unable to assess the mortality rate of the congenital hypomyelinating leukodystrophies. Considering these factors, we calculated the prevalence using a population aged 19 years and under; our epidemiological values should therefore be only minimally influenced by these potential biases. Second, mutational analyses of known genes other than *PLP1*, such as *GJC2*, *SLC16A2*, *HSPD1*, and *SLC17A5*, were only performed when clinically suspected as contributing to the disease. In most patients, *PLP1* was the only gene examined, because only *PLP1* could be assessed by commercial laboratory testing. Scanning of other genes requires a research-based setting, which is available for only a limited number of patients. Third, conventional *PLP1* sequencing may not detect alterations deep in introns; thus, it is possible that the proportion of PMD in our cohort may have been underestimated. Fourth, the prevalence of hypomyelinating leukodystrophies was lower than their incidence, probably because of the low number of patients older than 8 years (supplementary Figure 1A). The reason for this discrepancy is unknown, but it may be associated with the mortality of these conditions, dropout from long-term follow-up at specialized hospitals, and lower performance rate of genetic testing in older patients. It should also be noted that hypomyelinating leukodystrophies are often misdiagnosed as cerebral palsy, especially in elderly patients. This may also contribute to the underestimation of the prevalence of hypomyelinating leukodystrophies.

In conclusion, we report the results of the first nationwide epidemiological survey of congenital hypomyelinating leukodystrophies conducted in Japan. By targeting hypomyelinating leukodystrophies, we could distinguish this group of disorders from demyelinating leukodystrophies. As a result, we have provided, for the first time, data on the epidemiological, clinical, and genetic landscapes of congenital hypomyelinating leukodystrophies.

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Conflicts of interest The authors report no conflict of interest.

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Original Research

Attenuation of endoplasmic reticulum stress in Pelizaeus-Merzbacher disease by an anti-malaria drug, chloroquine

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Abstract

Pelizaeus-Merzbacher disease (PMD) is a hypomyelinating disorder caused by the duplication and missense mutations of the proteolipid protein 1 (*PLP1*) gene. PLP1 missense proteins accumulate in the endoplasmic reticulum (ER) of premature oligodendrocytes and induce severe ER stress followed by apoptosis of the cells. Here, we demonstrate that an anti-malaria drug, chloroquine, decreases the amount of an ER-resident mutant PLP1 containing an alanine-243 to valine (A243V) substitution, which induces severe PMD in human. By preventing mutant PLP1 translation through enhancing the phosphorylation of eukaryotic initiation factor 2 alpha, chloroquine ameliorated the ER stress induced by the mutant protein in HeLa cells. Chroloquine also attenuated ER stress in the primary oligodendrocytes obtained from myelin synthesis deficit (*msd*) mice, which carry the same *PLP1* mutation. In the spinal cords of *msd* mice, chloroquine inhibited ER stress and upregulated the expression of marker genes of mature oligodendrocytes. Chloroquine-mediated attenuation of ER stress was observed in HeLa cells treated with tunicamycin, an N-glycosylation inhibitor, but not with thapsigargin, a sarco/ER Ca^{2+}ATPase inhibitor, which confirms its efficacy against ER stress caused by nascent proteins. These findings indicate that chloroquine is an ER stress attenuator with potential use in treating PMD and possibly other ER stress-related diseases.

Keywords: PMD, PLP, ER stress, UPR, chloroquine, treatment

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Introduction

In the central nervous system, oligodendrocytes form the myelin sheath by wrapping axons with multiple layers of their plasma membrane; this enables rapid impulse conduction along the axons and prevents neuronal death.[1–3] Compact myelin, the lipid-rich major component of the myelin sheath, has a unique composition with more than 70% lipid by dry weight, and 80% of its protein mass consists of just two proteins, myelin basic protein (MBP), and proteolipid protein 1 (PLP1).[1] Although *shiverer*[4] (*Mbp*-deficient) mice develop a severe hypomyelinating disorder in the central and peripheral nervous systems and a corresponding shortened lifespan, the phenotypes of *Plp1*-knockout mice[5] and *PLP1*-null humans[6,7] display extremely mild myelin defects with almost normal lifespan.

While complete deficiency of *PLP1* only causes modest neurological symptoms, duplication and missense mutations of the *PLP1* gene result in a severe hypomyelinating disorder, Pelizaeus-Merzbacher disease (PMD). PMD is an X-linked recessive leukodystrophy characterized by failure of myelination in the central nervous system.[8,9] Duplication of the *PLP1* gene accounts for 60–70% of PMD cases,[10,11] but it remains unclear why an excess amount of PLP1 induces severe hypomyelination. In contrast, missense mutations of

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Short communication

A novel homozygous mutation of *GJC2* derived from maternal uniparental disomy in a female patient with Pelizaeus–Merzbacher-like disease

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ABSTRACT

Pelizaeus–Merzbacher-like disease (PMLD) is an autosomal recessive hypomyelinating disorder of the central nervous system characterized by nystagmus, motor developmental delay, ataxia, and progressive spasticity. The gap junction protein gamma-2 gene (*GJC2*), encoding the gap junction protein connexin 47, is one of the genes responsible for this condition. In this study, a novel homozygous mutation in *GJC2* (c.746C>G; p.P249R) was identified in a 21-year-old female patient with PMLD. Although her mother was a carrier of this mutation, the Mendelian inheritance pattern could not be determined because the paternal sample was unavailable. Alternatively, chromosomal microarray testing together with single nucleotide polymorphism typing (CGH + SNP) was performed to determine the gene copy number and analyze the haplotype in the 1q42.13 region in which *GJC2* is located. The result showed no deletion, but the *GJC2* region was involved in the loss-of-heterozygosity region. Furthermore, haplotype of chromosome 1, in which *GJC2* is located, revealed that both copies of chromosome 1 were derived from the patient's mother, indicating maternal uniparental disomy of chromosome 1. This study showed the advantage of the SNP genotyping microarray for detecting the origin of the mutation.

1. Introduction

Hypomyelination, which can be easily revealed by brain magnetic resonance imaging (MRI), is a consequence of the immature condition of oligodendrocytes in the brain white matter, manifesting as pyramidal signs and neurodevelopmental delay [1]. Although many genetic backgrounds are related to this condition, Pelizaeus–Merzbacher disease (PMD) is the most frequently seen in the X-linked inheritance mode of this condition as the genetic background and most of the patients are males. Patients with PMD often show nystagmus, stridor, and hypotonia in early infancy and later develop spasticity, ataxia, tremor and deterioration of psychomotor development [2]. Abnormalities of the proteolipid protein 1 gene (*PLP1*), including nucleotide alterations and gene copy number aberrations, are the cause of PMD. However, there are many related conditions associated with hypomyelination other than PMD [1].

Among them, PMD and Pelizaeus–Merzbacher-like disease (PMLD) are almost indistinguishable based on the basis of clinical and neuroradiological features at the onset of symptoms [3]. If a patient's genotype is inconsistent with an X-linked recessive inheritance pattern, genes other than *PLP1* may be related including the gap junction protein ganna-2 gene (*GJC2/GJA12* #608803) [3,4], the aminoacyl tRNA synthetase complex-interacting multifunctional protein 1gene (*AIMP1/p43*; MIM#603605) [5], and the heat shock 60 kDa protein 1 gene (*HSPD1*; MIM#118190) [6]. Because approximately 8% of all patients and 16% of female patients with PMLD showed mutations in *GJC2*, a reasonable approach for the genetic testing for female patients who have clinical symptoms of PMD/PMLD is to begin with a sequence analysis of *GJC2* [7,8]. *GJC2*, located on 1q42.13, encodes connexin 47, which is a member of a large family of homologous connexins. It has been identified in a board range of mammalian tissues. It has 4 transmembrane regions with 2 extracellular and 3 intracellular domains. It is highly expressed in oligodendrocytes and predominantly present at oligodendrocyte–astrocyte gap junctions and participates in linking these 2 types of glial cells [7,9–11].

In this study, we identified a novel homozygous mutation in *GJC2* in a female patient with PMLD. Chromosomal microarray testing revealed maternal uniparental disomy (UPD) as the cause of this homozygous

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Original article

Novel compound heterozygous mutations of *POLR3A* revealed by whole-exome sequencing in a patient with hypomyelination

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Abstract

Objective: Congenital white matter disorders are a heterogeneous group of hypomyelination disorders affecting the white matter of the brain. Recently, mutations in the genes encoding the subunits of RNA polymerase III (Pol III), *POLR3A* and *POLR3B*, have been identified as new genetic causes for hypomyelinating disorders.

Method: Whole-exome sequencing was applied to identify responsible gene mutations in a 29-year-old female patient showing hypomyelination of unknown cause. To investigate the pathological mechanism underlying the hypomyelination in this patient, the expression level of 7SL RNA, a transcriptional target of Pol III, was analyzed in cultured skin fibroblasts derived from the patient with *POLR3A* mutations.

Results: Novel compound heterozygous mutations of *POLR3A* were identified in the patient, who started to show cerebellar signs at 3 years, lost ambulation at 7 years, and became bedridden at 18 years. Brain magnetic resonance imaging showed severe volume loss in the brainstem, the cerebellum, and the white matter associated with hypomyelination. In addition to hypodontia and hypogonadism, she showed many pituitary hormone-related deficiencies. The expression level of 7SL RNA in cultured skin fibroblasts derived from this patient showed no significant abnormality.

Conclusion: The many pituitary hormone-related deficiencies identified in this patient may be an essential finding for the Pol III-related leukodystrophies spectrum. Further investigation is needed for a better understanding of the disease mechanism.

Keywords: Hypomyelination; Leukodystrophy; Hypomyelination with hypodontia and hypogonadotropic hypogonadism (4H) syndrome; *POLR3A*; Whole-exome sequencing; RNA polymerase III (Pol III)

1. Introduction

Congenital white matter disorders are a heterogeneous group of dysmyelination or hypomyelination disorders of the brain white matter and are visible by brain magnetic resonance imaging (MRI) [1,2]. Pelizaeus-Merzbacher disease (PMD; MIM#312080) is a major disease

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Original Research

Neurochemistry in Shiverer Mouse Depicted on MR Spectroscopy

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Purpose: To evaluate the neurochemical changes associated with hypomyelination, especially to clarify whether increased total *N*-acetylaspartate (tNAA) with decreased choline (Cho) observed in the thalamus of *msd* mice with the *plp1* mutation is a common finding for hypomyelinating disorders.

Materials and Methods: We performed magnetic resonance imaging (MRI) and proton MR spectroscopy (^{1}H-MRS) of the thalamus and cortex of postnatal 12-week shiverer mice devoid of myelin basic protein (mbp), heterozygous and wild-type mice with a 7.0T magnet. Luxol Fast Blue staining and immunohistochemical analysis with anti-Mbp, Gfap, Olig2, and NeuN antibodies were also performed.

Results: In the thalamus, decreased Cho and normal tNAA were observed in shiverer mice. In the cortex, tNAA, Cho, and glutamate were decreased in shiverer mice. Histological and immunohistochemical analysis of shiverer mice brains revealed hypomyelination in the thalamus, white matter, and cortex; astrogliosis and an increased number of total oligodendrocytes in the white matter; and a decreased number of neurons in the cortex.

Conclusion: The reduction of Cho on ^{1}H-MRS might be a common marker for hypomyelinating disorders. A normal tNAA level in the thalamus of shiverer mice might be explained by the presence of mature oligodendrocytes, which enable neuron-to-oligodendrocyte NAA transport or NAA catabolism.

Key Words: magnetic resonance spectroscopy; *N*-acetylaspartate; choline; hypomyelination; myelin basic protein; shiverer mouse
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THE TERM HYPOMYELINATION describes a permanent, substantial deficit of myelin deposition in the brain. The protein composition of myelin in the central nervous system (CNS) is simpler than that of other membranes; the two major components are proteolipid protein (PLP) and myelin basic protein (MBP), which account for 50% and 30% of the total myelin protein, respectively. MBP, the second major structural protein of the myelin sheath of the mammalian CNS, is associated with the major dense line (1). Shiverer (*shi/shi*) is an autosomal recessive mouse mutation of the *mbp* gene, which deletes a 20-kb region including exons 3–7, resulting in the absence of mbp (1–3). Oligodendrocytes of shiverer mice fail to assemble compacted myelin (1,2), which causes an almost total lack of myelin (hypomyelination) in the CNS.

Despite progress in understanding the molecular basis and neuroimaging characteristics of Pelizaeus-Merzbacher disease (PMD) (4,5), a representative hypomyelination disease due to derangement of the *PLP1* gene, the neurochemical changes associated with hypomyelination remains unknown. We performed proton magnetic resonance spectroscopy (^{1}H-MRS) with a 7.0T magnet on the brains of *myelin synthesis-deficient* (*msd*) mice, a model of connatal PMD, one of the most severely affected murine mutants as to the *plp1* gene. ^{1}H-MRS of *msd* mice showed increased total *N*-acetylaspartate (tNAA; NAA, 2.01 ppm, and *N*-acetylaspartylglutamate [NAAG] 2.04 ppm, which are difficult to distinguish on ^{1}H-MRS) and decreased choline (Cho) (6), as observed in

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Original Article

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Three New *PLP1* Splicing Mutations Demonstrate Pathogenic and Phenotypic Diversity of Pelizaeus-Merzbacher Disease

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Abstract

Pelizaeus-Merzbacher disease is a severe X-linked disorder of central myelination caused by mutations affecting the proteolipid protein gene. We describe 3 new *PLP1* splicing mutations, their effect on splicing and associated phenotypes. Mutation c.453_453+6del7insA affects the exon 3B donor splice site and disrupts the PLP1-transcript without affecting the DM20, was found in a patient with severe Pelizaeus-Merzbacher disease and in his female cousin with early-onset spastic paraparesis. Mutation c.191+1G>A causes exon 2 skipping with a frame shift, is expected to result in a functionally null allele, and was found in a patient with mild Pelizaeus-Merzbacher disease and in his aunt with late-onset spastic paraparesis. Mutation c.696+1G>A utilizes a cryptic splice site in exon 5, causes partial exon 5 skipping and in-frame deletion, and was found in an isolated patient with a severe classical Pelizaeus-Merzbacher. *PLP1* splice-site mutations express a variety of disease phenotypes mediated by different molecular pathogenic mechanisms.

Keywords

Pelizaeus-Merzbacher disease, *PLP1*, splice-site mutations

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Mutations in the proteolipid protein 1 gene (*PLP1*) in humans cause a spectrum of X-linked dysmyelinating disorders of central nervous system, ranging from the most severe connatal form of Pelizaeus-Merzbacher disease through classical Pelizaeus-Merzbacher disease to the mildest form of spastic paraplegia type 2.[1-2]

The *PLP1* gene is highly conserved among vertebrates and lies at Xq22.2 in humans. It has 2 major alternatively spliced transcripts, PLP1 and DM20.[3] In central nervous system myelin, proteolipid protein (PLP1) and its smaller isoform (DM20) constitute the most abundant protein compartment.[4] Expression of PLP1 and DM20 is developmentally regulated in the central nervous system and peripheral nervous system.[5] In the peripheral nervous system, DM20 is expressed in early stages of development and later predominates in the adult peripheral myelin, whereas in the central nervous system, DM20 is expressed prenatally, but after birth and during the peak of myelination, expression of PLP1 is predominant.[6-7]

The *PLP1* gene is affected by various types of mutations. Most frequent are duplications, which account for about 60-70% of Pelizaeus-Merzbacher families, followed by point mutations (missense, nonsense and splicing) and other small

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Clinical Observations

Partial *PLP1* Deletion Causing X-Linked Dominant Spastic Paraplegia Type 2

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ABSTRACT

BACKGROUND: Proteolipid protein 1 gene (*PLP1*) mutations result in a continuum of neurological findings characterized by X-linked hypomyelinating leukodystrophies of the central nervous system, from mild spastic paraplegia type 2 to severe Pelizaeus–Merzbacher disease. **PATIENTS:** We report spastic paraplegia type 2 in three individuals in one family. A 29-year-old man developed progressive spastic quadriplegia from early childhood with dysarthria, ataxia, dysphagia, and intellectual delay, but he displayed no nystagmus. His mother developed adult-onset mild spastic diplegia with dementia developing in later life, whereas his sister exhibited spastic diplegia from childhood, complicated by motor developmental delay and dysphagia. All three individuals had initially mild but progressive neurological phenotypes, no nystagmus, normal brainstem auditory-evoked potentials, and demyelinating peripheral neuropathy, but with varying clinical severity. **RESULTS:** A 33-kb deletion encompassing exon 2 to 7 of *PLP1* was identified in all three patients. Cloning of the junction fragment of the genomic recombination revealed a short palindromic sequence at the distal breakpoint, potentially facilitating a double-strand deoxyribonucleic acid break, followed by nonhomologous end joining. X-inactivation study and sequencing of the undeleted *PLP1* alleles failed to explain the differences in severity between the two female patients. **CONCLUSIONS:** *PLP1* partial deletion is a rare cause of spastic paraplegia type 2 and exhibits X-linked dominant inheritance with variable expressivity.

Keywords: proteolipid protein 1, spastic paraplegia type 2, myelin, hypomyelinating leukodystrophy, deletion, Pelizaeus-Merzbacher disease, palindrome, non-homologous end joining

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Introduction

The proteolipid protein 1 gene (*PLP1*) encodes major myelin membrane proteins (PLP1 and DM20) in the central nervous system (CNS). Phenotypically, *PLP1* mutations result in a continuum of neurological findings characterized by X-linked hypomyelinating leukodystrophies of the CNS, from Pelizaeus–Merzbacher disease (PMD) with severe CNS involvement to spastic paraplegia type 2 (SPG2), showing later onset with milder phenotype, but progressive weakness and spasticity of the lower limbs.[1] Distinct types of mutations, including point mutations and genomic duplications and deletions, result in PMD and SPG2 through different molecular mechanisms.[2]

Large genomic deletions or early truncating mutations result in null *PLP1* alleles. Patients with such null mutations show a unique clinical phenotype. Their neurological symptoms are milder than that commonly observed in other types of alterations, such as missense mutations and genomic duplications; thus, they are often diagnosed with mild PMD or a complicated form of SPG2.[3,4] Contrary to the mild disease in male patients, female carriers are more frequently symptomatic in these families, often presenting with adolescent- or adult-onset mild spastic diplegia and slowly progressive leukodystrophy, with dementia developing in later life. In addition, *PLP1* null syndrome is

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Original article

Different patterns of cerebellar abnormality and hypomyelination between *POLR3A* and *POLR3B* mutations

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Abstract

Background: Mutations of *POLR3A* and *POLR3B* have been reported to cause several allelic hypomyelinating disorders, including hypomyelination with hypogonadotropic hypogonadism and hypodontia (4H syndrome). *Patients and methods:* To clarify the difference in MRI between the two genotypes, we reviewed MRI in three patients with *POLR3B* mutations, and three with *POLR3A* mutations. *Results:* Though small cerebellar hemispheres and vermis are common MRI findings with both types of mutations, MRI in patients with *POLR3B* mutations revealed smaller cerebellar structures, especially vermis, than those in *POLR3A* mutations. MRI also showed milder hypomyelination in patients with *POLR3B* mutations than those with *POLR3A* mutations, which might explain milder clinical manifestations. *Conclusions:* MRI findings are distinct between patients with *POLR3A* and *3B* mutations, and can provide important clues for the diagnosis, as these patients sometimes have no clinical symptoms suggesting 4H syndrome.

Keywords: Hypomyelination; MRI; Hypomyelination with hypogonadotropic hypogonadism and hypodontia (4H syndrome); Diffuse cerebral hypomyelination with cerebellar atrophy and hypoplasia of the corpus callosum (HCAHC); Cerebellum; *POLR3A*; *POLR3B*; RNA polymerase III (Pol III)

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