within normal limits. Anthropomorphic parameters revealed accelerated height in late infancy [+2.5–3.0 standard deviation (SD)], with normal head circumference and decreased weight gain. At 8 months, her height was 76 cm (+3.0 SD), weight 6745 g (−1.7 SD), and head circumference 44 cm (−0.4 SD). Nasogastric tube feeding was started due to recurrent aspiration pneumonia. At 11 months, domiciliary oxygen was introduced because of chronic respiratory failure. At the age of 28 months, her height was 96 cm (+2.9 SD), weight 10.3 kg (−1.1 SD), and head circumference 46.5 cm (−0.7 SD). Febrile illness was consistently associated with neurological deterioration, and the patient progressed to opisthotonic posturing with generalized dystonia and segmental myoclonic jerks, which never resolved while awake.

Methods and results

Laboratory data

Routine laboratory tests were normal. Amino acid analysis showed elevated free GABA in the serum and cerebral spinal fluid (CSF) at 9 months of age (2.1 μmol/l and 1.26 μmol/l, respectively; normal range serum 0.12–0.50, CSF 0.04–0.12) (Jaeken et al 1984). Serum growth hormone was elevated (8.84 ng/ml; normal range 0.28–1.64). Insulin-like growth factor levels were relatively low (54 ng/ml; normal range 37–229). An absence of GHB in urine organic acid analysis precluded SSADH deficiency as a cause of increased GABA. β-alanine and homocarnosine were not detectable on the chromatogram, and their quantitative analyses were not performed.

Radiological findings

Bone age was 1 year 8 months at the age of 1 year 10 months (TW2 method). Initial brain computed tomography (CT) was unremarkable (Fig. 1a), and brain magnetic resonance imaging (MRI) (1.5 T) suggested mild delay in myelination (Fig. 1b, c) without structural anomalies. Diffusion weighted images (DWI) revealed high signal intensity in the internal and external capsules and much of the subcortical white matter, with restricted apparent diffusion coefficiency (Fig. 1d–f). For quantitative ^{1}H-MRS (age 8 months), locations were placed in the white matter (semioval center) and the basal ganglia (10 ml). ^{1}H-MR spectra were obtained using the stimulated-echo acquisition mode (STEAM) sequence (Frahm et al 1987) (TE/TR= 20/5,000 ms). To quantify the spectra, the LCModel (Provencher 1993) was used. The LCModel facilitates metabolite separation based upon differing linear combinations of spectra of individual metabolites and estimates the concentration of each metabolite concentration by comparing the proton concentration of water in identical voxels. The GABA concentration in the basal ganglia (Fig. 2) was

Table 1 Clinical, enzymatic, and molecular characteristics of gamma aminobutyric acid transaminase (GABA-T)-deficient patients

Sign/symptom	Patient 1	Patient 2 (sib of patient 1)	This report
Intractable seizures	+	+	+
Psychomotor retardation	+	+	+
Hypotonia	+	+	+
High-pitched cry	+	+	
Hyperreflexia	+	+	+
Lethargy	+	+	+
Acceleration of height growth	+	+	+
Age of death	25 months	12 months	Alive at 28 months
EEG/MRI/CT abnormalities	+	+	+
GABA-T (liver)[a]	70 (310–690)		
GABA-T (white cells)[b]	1.2 (20–58)		2 (23–64)
Genotype [c]	c.[659G>A (+) 1433T>C][d]		c.[275G>A]+[199_?_316+?][e]
Deduced effect	p.[Arg220Lys (+) Leu478Pro]		p.[Arg92Gln]+[?]

EEG electroencephalograph, *MRI* magnetic resonance imaging, *CT* computed tomography, + present; – absent or not determined

[a] Protein pmol/h/mg (control range in parentheses). [b] Protein pmol/min/mg (control range in parentheses). [c] Reference sequence NM_000663.3; missense mutations are considered to be pathogenic, as they were not encountered in 210 control chromosomes and involve highly conserved amino acids among GABA-T species. [d] Following the original publication (Jaeken et al 1984), we identified the second mutation (c.1433T>C) in the first described patient, confirming GABA-T deficiency at the DNA level. [e] In our patient, a presumed homozygous mutation was detected by direct sequence analysis; however, this was in contrast to the findings in DNA of the mother. The heterozygous mutation could not be detected in DNA of the father, therefore, a specific multiplex probe amplification test was developed. This showed the presence of a heterozygous exon deletion in DNA of the patient confirming compound heterozygosity

significantly elevated (2.9 mmol/l; normal 1.1 mmol/l±0.3, n=9), but in the semioval center, GABA elevation was slight (0.8 mmol/l; normal 0.5 mmol/l±0.2, n=9). Glutamine/glutamate complex (Glx) concentration was also slightly elevated in the semioval center (11.3 mmol/l in the basal ganglia, 8.3 mmol/l in the semioval center; normal 10.1 mmol/l ±1.5, 6.6 mmol/l±1.0, n=9, respectively). Follow-up ^{1}H-MRS analysis (at 9 months of age) revealed a more pronounced GABA elevation both in the basal ganglia and in the semioval center (5.9 mmol/l and 2.9 mmol/l, respectively). Based on these data and the results of quantitative ^{1}H-MRS, we suspected GABA-T deficiency, which was confirmed by enzyme and molecular studies in cultured lymphoblasts (Schor et al 2001) (Table 1).

Discussion

This report is on the third patient (second family) with GABA-T deficiency and the first patient in whom ^{1}H-MRS was performed. All three patients showed severe, nonspecific neurological manifestations, including psychomotor retardation, epilepsy, hypotonia, and hyperreflexia (Table 1), but our patient appeared less severely affected than the reported patients. All three also showed growth acceleration associated with increased serum growth hormone levels.

The underlying pathophysiology in GABA-T deficiency remains to be elucidated, and there is no animal model available. Evidence from animal studies indicates a neurotoxic role for supraphysiological GABA levels. For example, inhibition of GABA-T by the irreversible inhibitor, vigabatrin, induces intramyelinic edema in dogs via GABA elevation (Peyster et al 1995). Both GABA-T and SSADH deficiencies manifest seizures, which is paradoxical, as activation of the GABAergic system is predicted to be anticonvulsive. Nonetheless, it is important to remember that GABA is excitatory in the developing rodent brain and remains so for the first 1–2 weeks of life. Along these lines, the switch of GABA from a depolarizing to a hyperpolarizing response is critically important in the rodent substantia nigra pars reticulata (SNR), which has one of the highest concentrations of GABAergic neurons in the CNS (Iadarola and Gale 1982). In the murine model of SSADH deficiency, Jansen and coworkers (2008) demonstrated a significant increase in GABA in E10 embryos, which may predispose these animals to a hyperexcitatory state during development. This result, along with GABA(A) and GABA (B) receptor anomalies detected in developing SSADH-deficient mice, may reduce the seizure threshold in SSADH deficiency (Buzzi et al 2006; Wu et al 2006). Both disorders occupy juxtaposed positions in GABA degradation, and accordingly we speculate that the pathophysiological mechanisms observed in SSADH deficiency may be likely to be caused by high GABA levels, as observed in GABA-T deficiency. Neuropathology of the two index cases revealed spongy leukodystrophy, which may correspond to the white matter lesions seen on DWI in our patient. This observation may reflect changes in water motion in the axonal direction and/or axonal swelling associated with cortical neuronal damage.

Whereas ^{1}H-MRS estimates in vivo neurotransmitter concentrations (Provencher 1993), quantifying GABA in nonpathological states is difficult due to interference by much larger peaks of the glutamine–glutamate complex, creatine, and large peaks of N-acetylaspartic acid (Novotny et al. 2003). However, utilizing the LCModel facilitates separation of even low-concentration species (such as GABA) from other major compounds. Screening of the metabolite concentration by ^{1}H-MRS may readily reveal the pathological state, however, as in our patient. Moreover, the addition of a short exposure to ^{1}H-MRS may be acceptable, even in infants and children. Increased intracranial GABA detected by ^{1}H-MRS has been reported in SSADH deficiency (Ethofer et al 2004), but additional GABA-T-deficient patients require identification in order to determine how the concentrations of intracranial GABA compare to those in the same regions of SSADH-deficient patients.

In summary, GABA transaminase deficiency represents a human model of endogenous GABA elevation, which likely occurs during critical periods of human CNS development. This disorder may offer valuable insights into the role of the GABAergic system in human brain development. Our studies further suggest that quantitative ^{1}H-MRS may be clinically applicable to the inborn errors of GABA metabolism.

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Case report

Mild phenotype in Pelizaeus-Merzbacher disease caused by a *PLP1*-specific mutation

Hitoshi Osaka [a,e,*], Shiro Koizume [a,e], Haruhiko Aoyama [d], Hiroko Iwamoto [d], Seiji Kimura [d], Jun-ichi Nagai [b], Kenji Kurosawa [c], Sumimasa Yamashita [a]

[a] *Division of Neurology, Clinical Research Institutë, Kanagawa Children's Medical Center, Yokohama 232-855, Japan*
[b] *Division of Laboratory Medicine, Clinical Research Institute, Kanagawa Children's Medical Center, Yokohama 232-855, Japan*
[c] *Division of Genetics, Clinical Research Institute, Kanagawa Children's Medical Center, Yokohama 232-855, Japan*
[d] *Division of Pediatric Neurology, Yokohama Ryoiku-iryo Center, Yokohama 241-0014, Japan*
[e] *Molecular Pathology & Genetics Division, Kanagawa Cancer Center Research Institute, Yokohama 241-0815, Japan*

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Abstract

We present the case of a 26 year-old man who developed normally until he began having difficulty walking at age 12. He subsequently became unable to stand at 15 years old and exhibited mental regression and generalized tonic convulsions by age 20. Magnetic resonance imaging revealed incomplete myelination of cerebral white matter, which resembled that of Pelizaeus-Merzbacher disease. By sequencing the proteolipid protein 1 (*PLP1*) gene, we found a novel mutation (c.352_353delAG (p.Gly130fs)) in the latter half of exon 3 (exon 3B) that is spliced out in the DM20 isoform. Exon 3B mutations are known to cause a mild phenotype since they do not disturb DM20 production. Mutations that truncate PLP1 correlate with a mild phenotype by activating the nonsense-mediated decay mechanism that specifically detects and degrades mRNAs containing a premature termination codon. This attenuates the production of toxic mutant PLP1. The very mild presentation in the present case seems to be derived from the unique nature of the mutation, which preserves DM20 production and decreases mutant PLP1.

Keywords: Pelizaeus-Merzbacher disease; Proteolipid protein 1; *PLP1*

1. Introduction

Proteolipid protein (PLP) 1 and its splice isoform DM20 are encoded by the *PLP1* gene. PLP1/DM20 proteins are major components of myelin expressed in oligodendrocytes in central nervous system (CNS) [1]. PLP1/DM20 translated in the endoplasmic reticulum (ER) is transported to the cell surface and integrated into plasma membrane presumably via four membrane-spanning domains with both amino- and carboxy-terminal ends on cytoplasmic side. Owing to its strong hydrophobicity, PLP1/DM20 can form a stable compact myelin sheath in cooperation with other myelin proteins [1]. Expression of PLP1 and DM20 are spatially and temporally regulated. DM20 expresses preferentially in embryonic stages in a variety of cell types, whereas PLP1 expresses postnatally in oligodendrocytes. Both PLP1 and DM20 constitute the predominant protein in myelin [2].

Pelizaeus-Merzbacher disease (PMD) is a severe X-linked recessive disorder caused by mutations of the *PLP1* gene [1]. A deficiency in PLP1/DM20 at the cell

* Corresponding author. Address: Division of Neurology, Clinical Research Institute, Kanagawa Children's Medical Center, Mutsukawa 2-138-4, Minami-ku, Yokohama 232-8555, Japan. Tel.: +81 45 711 2351; fax: +81 45 721 332.
E-mail address: hosaka@kcmc.jp (H. Osaka).

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membrane by PLP1 mutation leads to the arrest of myelination. Moreover, mutant PLP1 elicits a response in the ER, which attempt to refold the misfolded mutant PLP1/DM 20 protein [3]. However, if the level of misfolded protein exceeds the controllable limit within the ER quality control system, apoptotic signals are transduced from ER [3]. This cellular process is reflected in developmental regression and atrophy of the CNS.

Exonic or intron/exon boundary mutations are found in 20–30% of PMD patients and phenotypes are severer than other mutations such as total deletion and duplication of *PLP1*. An exception has been observed in the latter half of exon 3 (exon 3B) of *PLP1* [4–8]. Mutations within this region are predicted not to disturb DM20 expression and function. Mutations that truncate PLP1 are related to a mild phenotype presumably by activating the nonsense-mediated decay (NMD) pathway, a mechanism that specifically detects and degrades mRNAs containing a premature termination codon [9]. This attenuates the production of mutant PLP1 levels and thus likely lessens the ER stress responses. In this report, we present a PMD patient with a very mild phenotype. We identified a novel *PLP1* gene mutation that is predicted to preserve DM20 production and results in a frame-shifted mutant PLP1 protein.

2. Materials and methods

2.1. Patient

This 26 year-old boy was born uneventfully at full term to Japanese parents. He was born with a body weight of 3660 g and an Apgar score of 9/9 at 1 and 5 min. No stridor or nystagmus was noted. He gained head control at 4 months, could sit without support at 8 months, and could walk without assistance at 4 years. He was pointed out spasticity of lower limbs and EEG abnormalities at 1 year. He was treated with carbamazepine for 14 years. No seizures occurred during that period. He could speak a few words at 2 years of age. He attended a special class in normal elementary and junior high school. He had no difficulties in daily conversation and writings. The patient began having difficulty walking at 12 years of age and became unable to stand at 15 years. He showed frequent urination and was diagnosed as neurogenic bladder at 15 years. MRI taken at that time revealed only mild ventricular enlargement. Myelination was not evaluated because of the motion artifact. At age 20, he showed signs of mental regression and began speaking fewer words. He exhibited generalized tonic convulsions and was treated with valproic acid at 24 years. He was subsequently referred to a hospital for evaluation. He was not small for his ages with a height of 167 cm and a weight of 54 kg. He could conversate with combining two words. He showed no nystagmus and exhibited alternating outer-nystagmus and oculomoter apraxia. He could walk with assistance. His muscle tone was hypertonic in the upper limbs. Clumsiness was observed with all extremities displaying exaggerated tendon reflexes and bilateral extensor plantar responses. Speech was slurred and dysmetry with terminal oscillation and dysdiadochokinesis were observed. Routine laboratory examinations revealed no biochemical abnormalities in the level of serum ammonia, lactate and pyruvate, very long chain fatty acids, or arylsulfatase A. Nerve conduction velocities and electromyographic studies were all normal. Measurement of auditory evoked brain responses revealed only wave I. MRI revealed a completion of myelination in the T1 signal. Myelination in the white matter was incomplete in the T2 signal (Fig. 1).

2.2. Genomic DNA sequencing

Genomic DNA from this patient was prepared from white blood cells using the Wizard Genomic DNA puri-

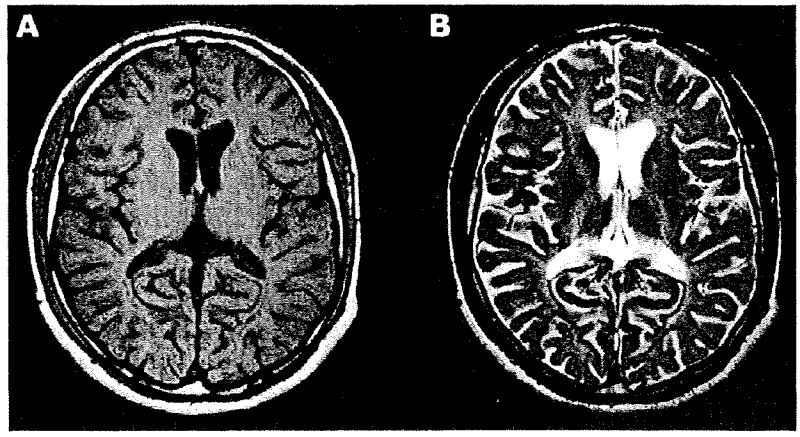

Fig. 1. Magnetic resonance imaging (MRI) at 26 year-old patient shows disappearance of contrast between cortex and white matter (A) on a T1-weighted image. T2-weighted image shows the incompletion of myelination in the white matter (B).

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fication kit (Promega, Madison, WI USA). PCR of seven exons and promoter regions of the *PLP1* gene was performed as previously described [10]. Subsequent sequencing analyses of the PCR fragments were performed by direct sequencing using the Big Dye Terminators v1.1 Cycle Sequencing kit (Applied Biosystems Foster City, CA). Duplication was screened by FISH as described [10].

3. Results

By direct sequencing of the patient's *PLP1* gene exons, exon/intron boundaries and a promoter region, we found a novel mutation in exon 3: c.352_353delAG (p.Gly130fs) (Fig. 2). No other sequence alterations were found and this mutation was not detected in more than 200 alleles. This two nucleotide deletion occurs in the latter half of exon 3 (exon 3B), which is not involved in *DM20* mRNA production (Fig. 3). FISH analysis showed normal copy numbers in this patient.

4. Discussion

Pelizaeus-Merzbacher disease belongs to leukodystrophies, one of a group of disorders that affect the white matter of the CNS. Genetic defects in PLP1/DM20, the most predominant myelin proteins, causes dual pathology: defects in CNS myelin formation (dysmyelination)

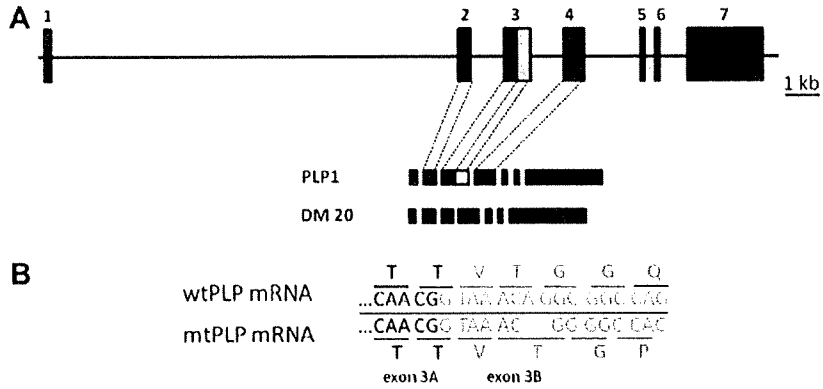

Fig. 2. Splicing the *PLP1* gene into *PLP1* mRNA and *DM20* mRNA. (A) Schematic presentation of *PLP1* gene structure. (upper panel) PLP1 gene is composed of seven exons. (lower panel) mRNA of PLP1/DM20 differs in only the latter half of exon 3 that is spliced out for the production of DM20 mRNA. (B) Two nucleotide deletion and subsequent frame shift in the Patient. Novel mutation in exon 3B, c.352_353delAG (p.Gly130fs), causes the frame shift in *PLP1* mRNA.

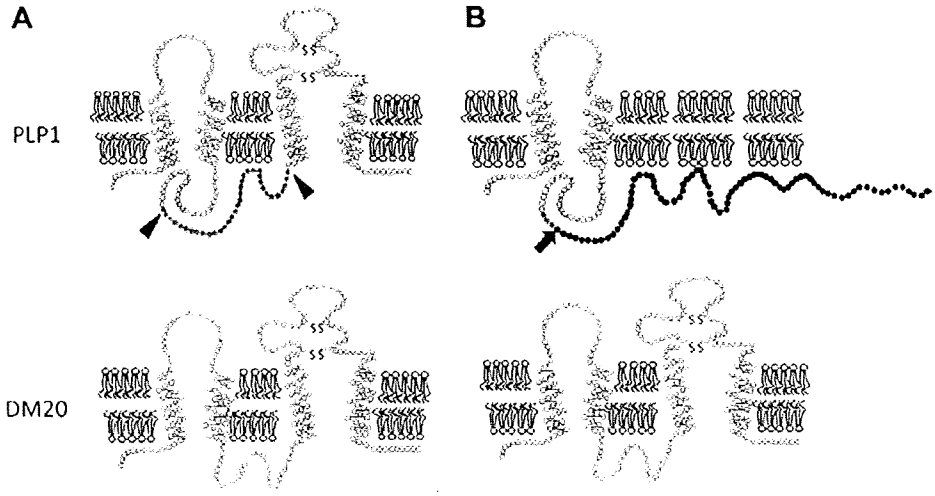

Fig. 3. Deduced PLP1 gene products; PLP1 and DM20. (A) Wild-type PLP1 (upper) and DM20 (lower) which are thought to include 4 membrane-spanning domains. Thirty-five intracellular amino acids (gray circle; between arrow head) are lacking in DM20. One circle corresponds to one amino acid. (B) PLP1 and DM20 of the patient. (upper) A two nucleotides deletion in exon 3B, c.352_353delAG (p.Gly130fs), causes a frame shift (arrow) and extension that are composed of 82 nonsense peptides. (lower) DM20 is identical to wild-type in this patient.

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and oligodendrocytes cell loss via apoptosis. PLP1 and DM20 are required for myelin compaction. Mutations in the *PLP1* gene, such as total deletion and truncation mutations, cause an inability to form normal myelin, which is easily revealed by diffuse high signals in all CNS white matter in T2-weighted MRI scans. Since PLP1/DM20 are constitute more than 50% of the protein in oligodendrocytes, mutant PLP1/DM20 cause the excessive ER stress responses and subsequent cell death that can be visualized by MRI/CT as brain atrophy.

Typically, patients with PMD show neonatal nystagmus and developmental delay that becomes apparent during infancy. Impairments of motor functions involve spastic paresis from the defect in the corticospinal tract, intention tremor from abnormalities in the cerebellar pathway, and choreoathetosis and rigidity due to basal ganglia dysfunction. Although all patients exhibit mental retardation, psycho-intellectual development is greater than motor development. Lesions are restricted in the myelinated portion in the CNS but disease severity varies considerably.

Cailloux et al. graded the clinical severity of PMD patients by their maximal motor achievements. Patients with Form 0 never gain head control ability, whereas patients with Form 1 can achieve head control. Form 2 includes the patients who are able to maintain a sitting position. Form 3 includes patients who can walk with support, while patients with Form 4 can walk autonomously. This last form overlaps the clinical phenotype of X-linked spastic paraplegia type 2, the allelic disease to PMD [8]. The patient described in the present case report belongs to Form 4, the mildest symptom group.

Amino acid substitutions, especially conserved amino acids in DM20/PLP1 within species, usually cause a severe phenotype [6]. Duplication of PLP1 causes a milder form, in which patients gain head control or sitting ability. Two types of mutations cause the mildest form of PMD. One type is total gene deletion, a truncation mutation that does not cause the mutant PLP1 that elicit the ER stress responses, and the second is the *PLP1*-specific exon 3B mutation.

Here, we described a patient with a mild form of PMD who could speak meaningful words and walk independently until 15 years of ages. He had two nucleotide deletions within exon 3B which are spliced out during *DM20* messenger RNA production. This mutation preserves the expression and function of DM20 protein. Moreover, this mutant protein is much shorter than wild-type PLP (277/241aa). It should easily be degraded via the activation of nonsense-mediated decay (NMD) pathway, a mechanism that specifically detects and degrades mRNAs containing a premature termination codon. The very mild phenotype observed is probably due to the dual effect of mutation: conservation of DM20 and the inability to elicit an ER stress response.

Thirteen different mutations have been reported in exon 3B (c.384C>G, 385C>T, 388C>T, 409C>G, 409C>T, 410delG, 418C>T, 430A>T, 434G>A, 441A>T, 442C>T, 446C>T). Twelve of them are one nucleotide changes and are predicted to preserve DM20 expressions. Clinical presentations are reported in 9 cases and 6 fit the criteria of Form 4, reinforcing the importance of DM20 function in addition to PLP1. Thus far, only one example of an exon 3B mutation that causes normal DM20 and truncated PLP1 has been reported (440delG; R137fsX8) [6]. This mutation caused two patients with Form 3 and one with Form 4. Our case is the second examples of an exon 3B mutation that produce normal DM20 and truncational PLP1. Our case, together with reports of other exon 3B mutations, supports the hypothesis that frame-shift mutations of PLP1 in exon 3B underlies the very mild phenotype in PMD.

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Case Report

A case of Brachmann-de Lange syndrome with congenital diaphragmatic hernia and *NIPBL* gene mutation

Running Title: BLS with CDH and *NIPBL* gene mutation

Shinichi Hosokawa[1)], Nobumasa Takahashi[1)], Hiroyuki Kitajima[1)], Masahiro Nakayama[2)], Kenjirou Kosaki[3)], and Nobuhiko Okamoto[4)]

[1)]Department of Neonatology, Osaka Medical Center and Research Institute for Maternal and Child Health

[2)]Department of Pathology, Osaka Medical Center and Research Institute for Maternal and Child Health

[3)]Department of Pediatrics, Keio University School of Medicine

[4)]Department of Developmental Pediatrics, Osaka Medical Center and Research Institute for Maternal and Child Health

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Corresponding author: Shinichi Hosokawa

Murodou-chou 840, Izumi City, Osaka 594-1101, Japan
Tel: +81-725-56-1220; Fax: +81-725-56-5682
E-mail: sinhoso@mac.com

Abstract

We report herein a case of Brachmann-de Lange syndrome complicated with congenital diaphragmatic hernia in which a *NIPBL* gene mutation was identified. A female infant born at 37 weeks gestation died at 134 minutes after delivery, even though endotracheal intubation and resuscitation were performed immediately after the scheduled caesarean operation. We diagnosed the infant with Brachmann-de Lange syndrome given her physical characteristics. An abnormal peak at the 29th exon in the translation area of the *NIPBL* gene was detected using denaturing high-performance liquid chromatography. In addition, a mutation of cytosine to thymine (nonsense mutation) at the 5524th base was identified using the direct sequence method. This variation was likely the cause of the syndrome.

Keywords: Brachmann-de Lange syndrome, congenital diaphragmatic hernia, denaturing high-performance liquid chromatography, direct sequence method, gene mutation

Introduction

Brachmann-de Lange syndrome (BDLS) is a multiple congenital anomaly syndrome characterized by growth and mental retardation, variable anomalies of the upper limbs and a peculiar face with hypertrichosis. A pediatrician named de Lange (1933) reported two cases of this disease while working at Amsterdam University in the Netherlands, and termed the disease Cornelia de Lange syndrome. It was subsequently revealed that Brachmann (1916) had reported on a patient exhibiting the same symptoms. As a result of these two reports, the condition is currently known as Brachmann-de Lange syndrome (Opitz 1985).

Brachmann-de Lange syndrome was originally thought to be related to 3q partial trisomic syndrome, as the clinical manifestations of the two diseases are relatively similar. More recently, Krantz et al. (2004) and Tonkin et al. (2004) reported a variation in the *NIPBL* gene in a BDLS patient, allowing the two diseases to be more easily distinguished.

We report herein a case of BDLS with congenital diaphragmatic hernia caused by a mutation in the *NIPBL* gene that was identified using denaturing high-performance liquid chromatography.

Clinical Report

A 21-year-old woman delivered a female infant at 37 weeks and 2 days gestation via a scheduled caesarean operation due to intrauterine growth retardation and congenital diaphragmatic hernia diagnosed by fetal echography at a gestational age of 30 weeks and 2 days. The infant's birth weight was 1766 g (–2.6 SD) and her Apgar score was 1 at 1 minute and 3 at 5 minutes. When she was born, her entire body was pale and she did not demonstrate spontaneous breathing patterns. Endotracheal intubation was immediately performed and artificial ventilation with high frequency oscillation (HFO) and nitric oxide inhalation therapy was initiated. Unfortunately, there was no improvement in her condition even following the administration of resuscitative medication including adrenaline and surfactant, and she died at 134 minutes after birth.

We considered that the patient had BDLS due to her characteristic facial features including synophrys, brachyrrhinia, long philtrum, thin lip, small mandible and short cervix, and the presence of hirsutism and a congenital diaphragmatic hernia. Although her limbs were small and short, and a bilateral single transverse palmar crease was recognized on each hand, the BDLS characteristics of syndactyly and limb reduction defects were not observed (Figure 1).

In regards to laboratory examination at birth, we identified slight acidosis; however, significant abnormal findings including anemia and electrolyte imbalance in

the cord blood were not observed. The infant's blood gas (venous blood) at 47 minutes after birth was also recognized as mixed acidosis of pH 6.763, PCO_2 188.0 mmHg, PO_2 3.2 mmHg and BE −16.5 mmol/l. Her hemoglobin was 6.8 g/dl, her CRP was negative and there was no elevation in liver enzyme levels. Hypernatremia was observed in her electrolytes (Table 1). Amniotic fluid chromosomes were of a normal karyotype of 46, XX. X-ray of the entire body revealed a hanging bell-shaped thoracic cage, low pneumatization in the bilateral lungs and a stomach bubble in the middle thorax (Figure 2).

Pathological autopsy was undertaken after we obtained informed consent from her parents. The placental weight was 190 g, which was small for the number of gestational weeks (our center average is 514 g), villi were immature and the umbilical cord contained a single umbilical artery. The left diaphragm was almost entirely defective and the liver, stomach, spleen, pancreas, small intestine and large intestine protruded into the intrathoracic area. Marked hypoplasia of the lungs was also recognized with a pulmonary weight ratio of 0.003 (normal is 0.012). In addition, the lungs were histologically immature. Bilateral hydroureter, annular pancreas and atrial septal defect were also observed. We did not examine the brain, as the parents did not consent to craniotomy.

After we obtained written informed consent from the parents for gene diagnosis,

we extracted genomic DNA from the patient's blood and amplified the coding region (extending from the 2nd exon to the 47th exon) of the *NIPBL* gene using PCR. An abnormal peak in exon 29 was detected when analyzed using denaturing high-performance liquid chromatography (Figure 3). Within the translation area of the *NIPBL* gene, a mutation of cytosine (C) to thymine (T) (nonsense mutation) at the 5524th base was identified using the direct sequence method. This amino acid change formed a stop codon, a result that we hypothesized would influence the complications in this patient.

Discussion

Cornelia de Lange (1933) identified 10 traits including mental retardation, low birth weight, dwarfism, microbrachycephaly, heavy eyebrows meeting at the midline, long eyelashes, low-set ears, small hands and feet, proximal placed thumb and syndactyly of the toes in two patients while working at Amsterdam University. Beck (1976) later reported the original diagnostic standards of BDLS (Table 2) and suggested that patients with BDLS could be diagnosed if they exhibited eight of these 10 traits. In regards to the current case, BDLS was not diagnosed in the fetal period, and was instead diagnosed after birth. The infant demonstrated nine of the traits described by de Lange and five of the traits described in the Beck standards. After confirming the baby's findings with both the de Lange and Beck standards, we finally made a diagnosis based on her physical characteristics.

This patient was also diagnosed based on the presence of intrauterine growth retardation and diaphragmatic hernia during the fetal period. Limb shortening was also observed. In the absence of abnormal karyotype or altered bone structures with limb shortening, BDLS is generally considered as a differential diagnosis (Beck & Fenger 1985; Jones 1988). Furthermore, the placenta of this patient weighed only 190 g, which was low for the gestational period. This finding was consistent with the hypothesis that growth of not only the fetus but also the placenta is inadequate in cases of BDLS.

There have been only a few reports of BDLS with congenital diaphragmatic hernia in Japan (Kuroiwa et al. 1990; Suzuki et al. 1999). A small number of reports (e.g., Cunniff et al. (1993), Russel et al. (1993) and Marino et al. (2002)) have been described in other countries. The reports by these groups suggested that the prognosis was worse when the patient also exhibited congenital diaphragmatic hernia. The precise causes of congenital diaphragmatic hernia remain unknown. BDLS, Fryns syndrome, Goltz syndrome and Smith-Lemli-Opitz syndrome are all associated with congenital diaphragmatic hernia (Tibboel & Gaag 1996; Bianch et al. 2000). Recently, gene analysis of these various multiple malformation syndromes has been undertaken (Holder et al. 2007). Further gene analyses in the various multiple malformation syndromes specifically associated with congenital diaphragmatic hernia are likely to shed light on which anomalies lead to diaphragmatic hernia.

In this case, a mutation of C to T (nonsense mutation) at the 5524th base in the translation area of the *NIPBL* gene was identified. As a result, we concluded that this variation was likely to be the cause of the BDLS with diaphragmatic hernia. The *NIPBL* gene is located at 5p13.1 and contains 47 exons, and its transcription is thought to be related to Notch signal transmission. There have been many confirmed gene mutations, including deletion and insertion mutations, that are associated with BDLS (Gillis et al. 2004; Bhuiyan et al. 2006; Schoumans et al. 2007). Furthermore, Musio et al. (2006)

and Deardorff et al. (2007) have presented reports relating BDLS to both *SMC1* and *SMC3* gene mutations.

DNA analysis is important for confirming BDLS diagnosis. Analysis of gene mutations in genes such as *NIPBL* also represents a useful diagnostic method. With the accumulation of cases such as ours, further description of this disease will be possible.

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