[Original Article]

**A de novo *TUBB4A* mutation in a patient with hypomyelination mimicking
Pelizaeus–Merzbacher disease**

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Running head: *TUBB4A* mutation and mimicking PMD

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ABSTRACT

Objective: Hypomyelinating leukoencephalopathy is a heterogeneous disorder caused by mutations in several-different genes. Clinical entity of hypomyelination with atrophy of the basal ganglia and cerebellum (H-ABC) is one of them.

Method: A male patient showed pendular nystagmus, infantile hypotonia, an abnormal pattern of brain auditory evoked potential, and hypomyelination on brain magnetic resonance imaging, which suggested Pelizaeus–Merzbacher disease (PMD) as the candidate diagnosis; however, no abnormality was found in the proteolipid protein 1 gene (*PLP1*) that is responsible for PMD. Whole exome sequencing was performed to identify pathogenic mutations in this patient.

Results: A de novo mutation was identified in the tubulin 4a gene (*TUBB4A*), which has been recently reported to be associated with H-ABC. Although the patient did not show any neurological features suggesting H-ABC, such as extrapyramidal or cerebellar signs, radiological findings demonstrated the finding of cerebellar atrophy at the age of 36 months.

Conclusion: This study suggested us the difficulty of clinical diagnosis for H-ABC early in the life of the patient, which makes predication of prognosis and genetic counseling difficult.

Keywords

hypomyelinating leukoencephalopathy; *TUBB4A*; hypomyelination with atrophy of the basal ganglia and cerebellum (H-ABC); Pelizaeus–Merzbacher disease (PMD); genetic counseling

1. Introduction

There are heterogeneous genetic backgrounds in patients with hypomyelinating leukoencephalopathy (HLD), which are classified into eight groups by Online Mendelian Inheritance in Man (OMIM; <http://www.omim.org/>). Pelizaeus–Merzbacher disease (PMD; MIM# 312080 [HLD1]) is the most well-known disorder associated with HLD. The gene responsible for PMD, the proteolipid protein 1 gene (*PLP1*), is located on Xq22, and thus, PMD inherits as an X-linked recessive trait [1]. In the most cases, the mothers are the carriers of *PLP1* abnormalities. Therefore, there is a risk of recurrence in the families of the patients; however, only male offspring are at risk of the occurrence. Ataxia, delayed dentition and hypomyelination (ADDH; MIM #607694 [HLD7]) [2], hypomyelinating leukodystrophy with or without oligodontia and/or hypogonadotropic hypogonadism (4H syndrome; MIM #607694 [HLD7]) [3], and hypomyelination with cerebellar atrophy and hypoplasia of the corpus callosum (HCAHC; MIM#614381 [HLD8]) [4] are different clinical entities characterized by hypomyelination associated with cerebellar ataxia and others, which should be differentiated from PMD based on clinical and radiological characteristics. The polymerase (RNA) III (DNA directed) polypeptide A and B genes (*POLR3A* and *POLR3B*) have been implicated in HLD7 and 8 [5]. As HLD associated with these two genes is caused by an autosomal recessive trait, there is a risk of recurrence in the families of the patients; in this case, both male and female offspring of the parents can inherit the disease. Although it is important to distinguish between PMD and the others to provide proper genetic counseling, it is a challenge to distinguish between their symptoms in the early stages of the patient's life.

Recently, it has been reported that the tubulin 4a gene (*TUBB4A*) is also

associated with another type of HLD; hypomyelination with atrophy of the basal ganglia and cerebellum (H-ABC; MIM# 612438 [HLD6]) [6, 7]. Although a *TUBB4A* variant has been previously shown to be shared in affected family members with dystonia type 4 (DYT4), which is characterized by “whispering dysphonia” [8, 9], H-ABC is recognized as an allelic variant and all H-ABC affected patients showed de novo mutations, whereas mutations were inherited in an autosomal dominant pattern in DYT4 families. The recurrent risk of de novo mutations in the families with H-ABC caused by *TUBB4A* mutations can be ignored, excluding germ line mosaicisms.

In this study, we investigated a male patient with HLD with clinical features that mimicked those of PMD and not H-ABC early in the life of the patient.

2. Materials and methods

The subject was a male patient who showed neurological features mimicking PMD. After obtaining written informed consent upon approval of the ethics committee of Tokyo Women’s Medical University, all coding regions of the previously known genes responsible for HLD, including *PLP1*, the monocarboxylate transporter 8 gene (*MCT8*, or *SLC16A2*, MIM# 300095), the gap junction protein gamma-2 gene (*GJC2*), *POLR3A*, and *POLR3B*, were analyzed by standard Sanger sequencing as described previously [10-13], and there was no pathogenic mutation. Chromosomal microarray testing using Agilent Human Genome Microarray CGH60K (Agilent Technologies, Santa Clara, CA) showed no genomic copy number aberration [10]. Then, we performed whole exome sequencing on this patient using trio samples including patient’s parents according to the method published previously (Supplemental information) [13].

3. Results

3.1. Exome sequencing

A de novo mutation, c.785G>A: p.R262H in *TUBB4A* (MIM 602662), identified by exome sequencing was reconfirmed by Sanger sequencing (Supplemental Figure S1 and S2).

3.2. Patient report

A 3-year-old boy was born by spontaneous vaginal delivery at 40 weeks of gestation with a birth weight of 3,178 g (mean). He was the second child of healthy, nonconsanguineous parents. There was no family history of neurological disorders and his elder brother was healthy. At the age of 3 months, nystagmus was noticed, and brain magnetic resonance imaging (MRI) examined at 5 months showed marked myelination delay (Figure 1). At 7 months, he was referred to our hospital, and his height was 69.5 cm (25~50th centile), weight was 8.725 kg (50~75th centile), and occipito-frontal circumference (OFC) was 45.2 cm (75~90th centile). On examination, no dysmorphic features were recognized. Physical examination was unremarkable without stridor. Head control was incomplete, and neurological examination showed generalized hypotonia with normal deep tendon reflexes. Extrapyramidal signs such as dystonia, athetosis, and ataxia were not observed. Mild nystagmus was present, but eye pursuit was observed. Although he respond to his mother's voice, brainstem auditory evoked potentials (BAEP) showed only waves I and II, and subsequent components were not recognized. A repeated brain MRI at 9 months demonstrated a severely delayed myelination (Figure 1). From these findings, he was tentatively diagnosed with PMD. Chromosomal

G-banding showed a normal male karyotype. Peripheral blood examinations showed normal thyroid function.

At the age of 17 months, brain MRI and proton magnetic resonance spectroscopy (MRS) was performed (Figure 1, 2). A single-voxel MRS of the cerebral white matter showed elevated levels of total creatine (Cr) with normal level of N-acetylaspartate (NAA), choline (Cho), and myoinositol (Ins) (Figure 2). A brain MRI at the age of 36 months showed atrophy of the cerebellar vermis along with severely delayed myelination (Figure 1). No atrophy of the basal ganglia was observed.

His psychomotor development was severely delayed. At the last follow-up examination at the age of 39 months, his height was 86.5 cm (>3 centile), body weight was 11.2 kg (3~10 centile), and OFC was 50 cm (mean). He was aphasic and bedridden with insufficient head control. Minimal purposeful hand movement was observed. He had general hypotonia and normal deep tendon reflexes. Nystagmus was not observed from the age of 32 months. No dystonia, ataxia, or other extrapyramidal signs were observed, nor did he experience epileptic seizures.

4. Discussion

The present patient showed nystagmus since early infancy, and generalized hypotonia and psychomotor developmental delay were also noted. Brain MRI revealed a hypomyelination pattern and BAEP showed diminished evoked potentials after II waves. Because male patients with typical PMD show such characteristic findings, we established a tentative diagnosis of PMD [10]. Based on clinical assessment, we performed genomic analysis and no genomic abnormality in *PLP1* was detected. Normal expression patterns of *PLP1* in fibroblasts were found in this patient (data not

shown). Because analysis of other potential candidate genes also showed no abnormalities, we performed exome sequencing to detect the disease-causing mutation. Finally, we detected a de novo mutation in *TUBB4A*, which is now recognized as a cause of H-ABC, a rare leukodystrophy, first described by van der Knaap et al. in seven patients [7]. This syndrome is diagnosed based on MRI findings including hypomyelination and selective atrophy of the neostriatum and cerebellum.

Clinically, H-ABC is characterized by variably disturbed early development followed by extrapyramidal movement abnormalities, ataxia, and spasticity. Generally, patients with H-ABC primarily show delayed motor development in the first years of life, followed by motor deterioration and extrapyramidal signs. Although spasticity is commonly seen in these patients, a clinical course of ataxia, tremor, choreoathetosis, dystonia, rigidity and dysarthria is generally variable in patients. The degree of severity of extrapyramidal and cerebellar signs depends on the level of progression of atrophy of the basal ganglia and cerebellum. Although the present patient is 39 months old, he does not show any extrapyramidal and cerebellar signs. Therefore, we did not consider H-ABC as a tentative diagnosis. The loss of III-V waves seen on BAEP is not specific to PMD, but a common observation in H-ABC patients [14, 15].

The radiological finding of atrophy of the cerebellum is a sign of progression in H-ABC. No cerebellar atrophy was seen on the brain MRI of this patient in the first year of life. Thus, this patient did not meet the clinical criteria of H-ABC described by van der Knaap et al. on first evaluation [7]. This is another reason why H-ABC was not part of the differential diagnosis. After identification of the *TUBB4A* mutation in this patient, we retrospectively evaluated the neuroradiological findings. During infancy, no atrophy was detected in the basal ganglia and cerebellum; however, progressive cerebellar

atrophy was observed at 36 months (Figure 1). The patient reported by Simons et al. (HA107) showed atrophy of the basal ganglia and cerebellar vermis at the age of 3.5 years [6]. Because the present patient may show atrophy of the basal ganglia later, careful observation would be required in the follow-up of this patient.

On MRS, the profile of patients with PMD differs from the pattern commonly observed in demyelinating disorders, and PMD can be distinguished from other HLD; NAA, Cr, and Ins are elevated, whereas the Cho level is normal or reduced (Figure 2) [16, 17]. MRS patterns in patients with H-ABC reportedly showed normal NAA and Cho levels but elevated Ins and Cr levels; however, at the ages higher than 7 years [7]. In comparison, this patient showed elevated level only in Cr. This may be attributable to the stage of the disease, because MRS was examined at 17 months and no atrophy of the cerebellum was observed at that time. We also evaluated MRS patterns of a patient with PMD at the age of 3 months (we previously reported the splicing mutation in this patient) [10], which did not reveal typical MRS findings of PMD (data not shown). These results provide further evidence that MRS patterns do not distinguish between PMD and H-ABC in early in the patient's life.

Without a precise diagnosis, it is impossible to establish prognosis or provide genetic counseling to the parents of the patient [18]. Because progression of neurological and neuroradiological features is variable among patients with hypomyelination as shown in this study, it is difficult to diagnose them before the development of neurological findings including atrophy of the basal ganglia and cerebellum. Therefore, in young patients with hypomyelination, it is reasonable to propose a comprehensive analysis of all potential candidate genes, including *TUBB4A*, by panel sequencing using a next generation sequencer.

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Figure legends

Figure 1: Radiological examination of the present patient. The time course of the findings of the brain magnetic resonance imaging indicates progressive atrophies of the cerebellar vermis. T1WI; T1-weighted image, T2WI; T2-weighted image, M; months

Figure 2: The result of magnetic resonance spectroscopy (MRS) and the summary of the findings. (A) The patient underwent multi-voxel proton (¹H) MR spectroscopy on a 3.0-Tesla MR system (Achieva, Philips Medical Systems) with 32 channel head coil. ¹H-MRS was acquired with short echo time (TR = 5000ms; TE = 30ms; PRESS [point-resolved spectroscopy]). Volumes of interest (VOI) was selected in the left parieto-occipital white matter and which size was 4.5 mL (20mm×15mm×15mm) (left). Metabolite concentrations were calculated by using the LCModel Version 6.2 (Stephen Provencher). (B) This patient showed the elevated level only in Cr. (C) The summary of the MRS finding of this patient in comparison with typical pattern in PMD and H-ABC.

Figure 2