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## Compound Heterozygous *ALDH7A1* Mutation Causes the Hemi-Allelic Expression in a Patient with Pyridoxine-Dependent Epilepsy

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Pyridoxine-dependent epilepsy (PDE) is an inherited disease with an autosomal recessive trait caused by deficiency of  $\alpha$ -amino-adipic semialdehyde (AASA) dehydrogenase encoded by the *ALDH7A1* gene. Pyridoxine administration is usually effective for the treatment of PDE. We identified compound heterozygous *ALDH7A1* mutation in a patient with undiagnosed intractable epilepsy. One of the mutations was located in the splicing region of this gene. We analyzed the RNA expression patterns and confirmed the hemi-allelic expression of this gene, which could be considered a consequence of erroneous splicing, though this was not direct evidence of a splicing error. The severe developmental delay observed in this patient could have been avoidable by prompt treatment intervention in the early neonatal period. Therefore, it is important to remind that vitamin B6 should be prescribed for neonatal patients with clustering seizures occurring soon after birth.

**Key Words:** RNA splicing, hemi-allelic expression, neonatal seizure, damaging scores, reverse transcribed-PCR

### Introduction

Pyridoxine-dependent epilepsy (PDE; MIM#266100) is an inherited disease with an autosomal recessive trait. PDE is caused by a deficiency of  $\alpha$ -amino-adipic semialdehyde (AASA) dehydrogenase that is encoded by aldehyde dehydrogenase 7 family, member A1 gene: *ALDH7A1*, also known as *antiquitin-1*.<sup>1</sup> Reduced activity of AASA dehydrogenase due to *ALDH7A1* abnormality leads to accumulation of piperidine-6-carboxylate

(P6C), which is a metabolite of lysin.<sup>2</sup> An increased level of P6C causes inactivation of pyridoxal phosphate (PLP), which is a vitamin B6-dependent activator and a coenzyme of many enzymatic reactions, including amino acids transfer, hydrolysis, and decarboxylation. Thus, seizures in PDE are most probably due to the increased metabolic activity in the brain. Although many types of *ALDH7A1* mutations have been previously reported, there is no common mutation found in this gene.<sup>3</sup> Here, we identified a rare compound heterozygous *ALDH7A1*

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mutation in a Japanese patient with PDE.

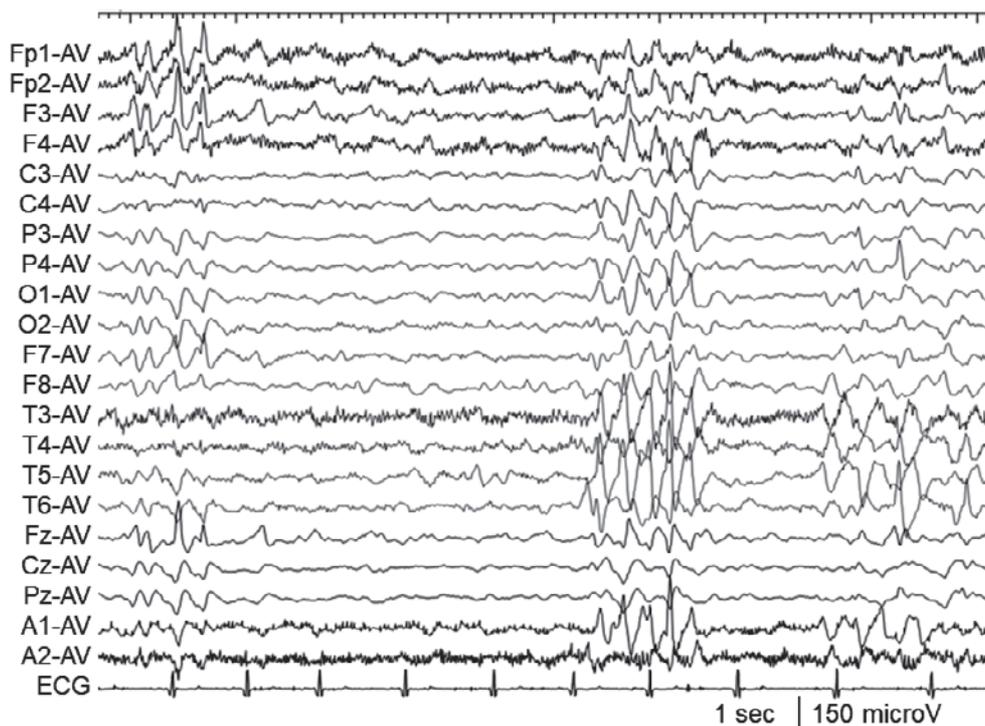
### Case Report

A 7-year-old boy was born at 37 weeks of gestation weighing 2.66 kg with 34 cm of appropriate occipitofron-



**Figure 1** T2-weighted brain magnetic resonance imaging (MRI) examined at 7 months. Diffuse brain atrophy is noted.

tal circumference through an emergency cesarean section due to a variable deceleration of fetal heart rate without asphyxia. He is the second child of unrelated healthy parents and his elder sister is also healthy. He needed to be admitted to the neonatal intensive care unit owing to signs of irritating and cramps which were observed soon after birth. Momentary motions appeared intermittently, and epileptic seizures were confirmed by electroencephalogram. The boy was then treated with continuous intravenous injections of midazolam (MDZ). Valproate and clobazam were administered and the epileptic seizures were controlled. He was discharged from hospital on day 61 after his birth. However, at 6 months of his life, epileptic seizures with hemi-lateral convulsion were observed, and intra-venous MDZ infusion was again initiated. Brain magnetic resonance imaging (MRI) examined at 7 months of age showed ventricular enlargement with diffuse atrophy of the cerebral cortex (**Figure 1**), which were retrospectively evaluated as compatible to those of the patients with PDE.<sup>4</sup> The intractable spasm-like seizures were controlled by administration of vitamin B6. An electroencephalogram, obtained at the age of 3 years showed bilateral temporal dominant sharp waves (**Figure 2**). Then, control of epileptic seizures was solely de-

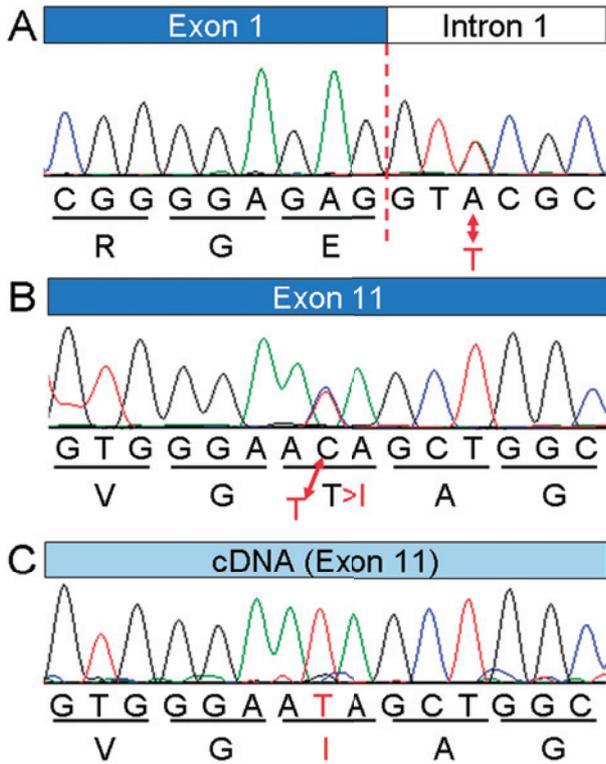


**Figure 2** Electroencephalogram (EEG) examined at 3 years shows multiple sharp wave bursts.

**Table 1** Results of metabolic examination.

Blood serum		
Pipecpli acid	6.6 $\mu\text{mol/L}$	(0.4-2.0)
$\alpha$ -AASA	8.0 $\mu\text{mol/L}$	(<0.2)
Cerebrospinal fluid		
Pipecpli acid	1.8 $\mu\text{mol/L}$	(<0.1)
$\alpha$ -AASA	5.6 $\mu\text{mol/L}$	(<0.1)

$\alpha$ -AASA,  $\alpha$ -amino-adipic semialdehyde.



**Figure 3** Results of molecular analyses. (A) The intronic mutation, c.108+3A>T, is shared with the mother. (B) The missense mutation, c.890C>T, identified in the patient is common with the father. This indicates compound heterozygous mutation in *ALDH7A1*. (C) Reverse transcribed-PCR (RT-PCR) assay followed by Sanger sequencing demonstrates only “T” in exon 11, indicating that the maternally derived allele is not expressed (hemi-allelic expression).

pendent on the dosage of administered vitamin B6. Thus, vitamin B6-dependent epilepsy was suspected when the patient was 7 years old. Metabolic analyses of the blood and cerebrospinal fluid were performed at Okayama University.<sup>5</sup> Elevated levels of pipecolic acid and  $\alpha$ -AASA were then confirmed (**Table 1**). His psychomotor development has been severely delayed; head control was obtained at 4 months, but he cannot sit or stand by himself at present. He shows no verbal communication.

From these clinical findings as well as the medical

**Table 2** Prediction scores of the identified variant.

Chromosome	chr5
Position	125894966
Reference nucleotide	G
Altered nucleotide	A
SIFT_score	0
SIFT_pred	D
Polyphen2_HDIV_score	0.967
Polyphen2_HDIV_pred	D
LRT_score	0
LRT_pred	D
MutationTaster_score	1
MutationTaster_pred	D
CADD_phred	27.5

SIFT, sorting intolerant from tolerant; pred, prediction; PolyPhen, polymorphism phenotyping; LRT, likelihood ratio test; CADD, combined annotation dependent depletion; phred, one of the base-calling programs; D, damaging

treatment courses, *ALDH7A1* was suspected as the causal candidate gene. After obtaining written informed consent from his parents, blood samples were withdrawn from the patient and his parents. This study was performed in accordance with the Declaration of Helsinki and was approved by the Gene Analysis Research Ethics Committee of Tokyo Women’s Medical University (No. 341B). Genomic DNA was extracted from peripheral blood of the patient and his parents using a QIAamp DNA extraction kit (Qiagen, Hilden, Germany). Polymerase chain reaction (PCR) and subsequent Sanger sequencing for all exons and exon-intron boundaries of *ALDH7A1* were performed.

A paternally derived missense variant, NM\_001201377.1: c.890C>T [p.Thr297Ile], was identified in exon 11 (**Figure 3B**), which has already been reported.<sup>6</sup> Previously, a similar type of a nucleotide alteration, c.890C>G [p.Thr297 Arg], was also reported as the disease-causing mutation.<sup>7</sup> “Damaging scores” of p.Thr297Ile were predicted through wANNOVAR (<http://wannovar.wglab.org/>), a web-based software (**Table 2**). Most of the scores suggested having a high probability of “damaging”. Especially, combined annotation dependent depletion (CADD)\_phred was scored as “27.5”. Thus, we considered this as the deleterious mutation.

A maternally derived intronic variant, NM\_001201377.1: c.108+3A>T, was also identified in intron 1 (**Figure 3A**), which has already been identified.<sup>8</sup>

However, the splicing abnormality has never been confirmed for this variant. We analyzed the possibility of a splicing abnormality in this variant by using an *in-silico* bioinformatics tool, Human Splicing Finder (<http://umd.be/HSF3/>), which indicates that this intronic variant “most probably affecting splicing” by breaking splicing donor site.

To confirm splicing abnormality by this intronic variant, we performed a reverse transcribed-PCR (RT-PCR), using primers located on the neighboring exonic regions. A total RNA was extracted from leukocytes for RT-PCR, as described previously.<sup>9</sup> Our mRNA analysis did not find any abnormal splicing patterns. Alternatively, only hemi-allelic expression of exon 11 was confirmed by RT-PCR and subsequent Sanger sequencing of this region (**Figure 3C**). The results indicate that only the paternally derived allele was expressed in this region and there was no expression of the maternally derived allele.

## Discussion

In this study, successful treatment with vitamin B6 for PDE was a clue to ascertain a final diagnosis of PDE in a 7-year-old patient. However, most of the PDE patients, who are treated with vitamin B6 soon after the onset of seizures in the neonatal period, show normal developmental milestones. Thus, had this patient received prompt therapeutic intervention during his neonatal period, severe developmental delay in this patient might have been avoidable. Therefore, it is important to remind that vitamin B6 should be prescribed for neonatal patients with clustering seizures occurring soon after birth.

Compound heterozygous *ALDH7A1* mutation was confirmed in the present patient. One of the mutations, p. Thr297Ile, was inherited from his father. Whereas, a suspected splicing mutation, c.108+3A>T, was inherited from his mother. Both mutations have already been reported previously.<sup>6,8</sup> However, it was unclear whether the possible splicing mutation (c.108+3A>T) would really cause a splicing abnormality. Thus, we examined to confirm if there were abnormal splicing patterns in mRNA.

As a result, we could not get direct evidence of

abnormal splicing derived from an intronic variant c.108+3A>T. Alternatively, the hemi-allelic expression in exon 11 region was confirmed, which will cause a similar effect with a homozygous mutation. Taken together, the hemi-allelic expression may be the essential reason for PDE in this patient, though we were unable to provide any evidence of abnormal splicing patterns from an intronic mutation.

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**Conflicts of Interest:** There is no conflict of interest for any of the authors.

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