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Case Report

A Novel *PAFAH1B1* Splicing Variant Identified in a Patient with Classical Lissencephaly

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Lissencephaly is a severe brain malformation associated with abnormal formation of cerebral sulci. Until now, many genes related to lissencephaly have been identified. Among them, classical lissencephaly has been often related to the platelet activating factor acetylhydrolase 1B regulatory subunit 1 gene (*PAFAH1B1*). We identified a novel PAFAH1B1 splicing variant (NM_000430.3:c.118-2A>G) in a female patient with classical lissencephaly manifesting as developmental delay, growth failure, and epilepsy. This variant was not detected in her parents, indicating de novo occurrence of the variant in the patient. To confirm whether this variant is in fact related to splicing error, RNA expression was analyzed. As suspected, an abnormal short band excluding exon 4 was additionally detected. This evidence confirmed that the novel variant is definitely pathogenic to cause lissencephaly in the patient.

Key Words: agyria, West syndrome, Miller-Dieker syndrome

Introduction

Lissencephaly is one of the malformations of the brain, which manifests as abnormal formation of cerebral sulci. Through radiological examination using magnetic resonance imaging (MRI), lissencephaly has been classified into several types, including agyria, pachygyria, and subcortical band heterotopia.[1] These radiological classifications are related to genetic classifications. Miller-Dieker syndrome has been established as one of the congenital malformation syndromes and it has been related to the subtelomeric deletion of 17p.[2] Patients with Miller-Dieker syndrome (MIM #247200) show classical lissencephaly and facial dysmorphism. Thus, genes related to these clinical features had been suspected to be localized in 17p. In 1993, the platelet activating factor acetylhydrolase 1B regulatory subunit 1 gene (*PAFAH1B1*; former name *LIS1*) located on 17p was isolated as the gene responsible for lissencephaly.[3] Later, the tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein epsilon gene (*YWHAE*), which is located more towards the telomeric region and at a distance from *PA-*

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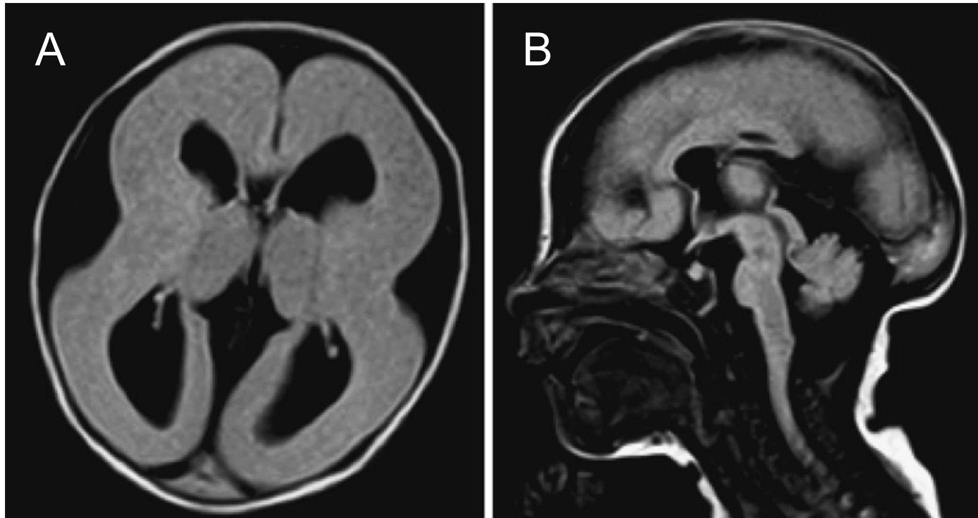

Figure 1 Brain magnetic resonance imaging of the patient. (**A**) An axial T1-weight image shows agyria and thick cortical surface. (**B**) A sagittal T1-weighted image shows reduced volume of the brain.

FAH1B1, was identified as the gene related to facial dysmorphism.[4]

Recently developed equipments for genomic analysis have promoted the understanding of the genomic basis of lissencephaly. Many genes, including the reelin (*RELN*), the tubulin alpha 1a (*TUBA1A*), and the doublecortin (*DCX*) genes, have been identified as the genes responsible for brain malformation. Intriguingly, *PAFAH1B1*-associated lissencephaly, included in Online Mendelian Inheritance in Man® (https://omim.org/) as an autosomal dominant disease of Lissencephaly 1 (MIM #607432), accounts for majority of the cases (40%) in lissencephaly cohorts.[5,6] Consequently, many disease-causing nucleotide variants of *PAFAH1B1* have been reported.[7,8]

Here, we have identified a novel disease-causing variant of *PAFAH1B1* in a patient with classical lissencephaly. As the identified variant was located on the splicing acceptor site, abnormal splicing pattern was confirmed by molecular analysis.

Case Report

A 4-month-old girl was born at 39 weeks and 5 days of gestation with birth weight of 2,370 g, height of 42.6 cm, and occipitofrontal circumference (OFC) of 31.7 cm. Apgar score was 7/9, indicating that there was no asphyxia at birth. Because dilatation of bilateral ventricles had been suggested after fetal ultrasound at 36 weeks of gestation, the girl was admitted to neonatal intensive care unit at birth. On 6th day after delivery, brain magnetic resonance imaging (MRI) revealed lissencephaly associated with no gyrus formation on the surface of the cerebrum (**Figure 1**). After discharge from the hospital, she started to show myoclonus-like movements. Although phenobarbital was prescribed for several weeks, she began to suffer from series of epileptic spasms and admitted to our hospital when she was months old.

At the time of admission, her height was 55.5 cm (−4.0 SD), weight was 4.9 kg (−2.4 SD), and OFC was 37.0 cm (−3.1 SD), indicating growth failure and microcephaly. There was no dysmorphic finding. Vital signs were within normal limit. On neurological examination, generalized hypotonia was noted because of which she had poor head control. Routine laboratory examination showed no abnormality; however, electroencephalogram revealed atypical hypsarrhythmia (**Figure 2**), indicating West syndrome. Hence, the girl was started on adrenocorticotropic hormone therapy.

For precise diagnosis, genetic diagnosis was performed. Fluorescence in-situ hybridization (FISH) showed no deletion in the 17p13.3 region, which is responsible for Miller-Dieker syndrome. Thus, comprehensive genomic analysis was performed. After obtaining written informed consent from her parents, peripheral blood samples were collected from the patient and her parents. This study was performed in accordance with the Declaration of Helsinki and was approved by the

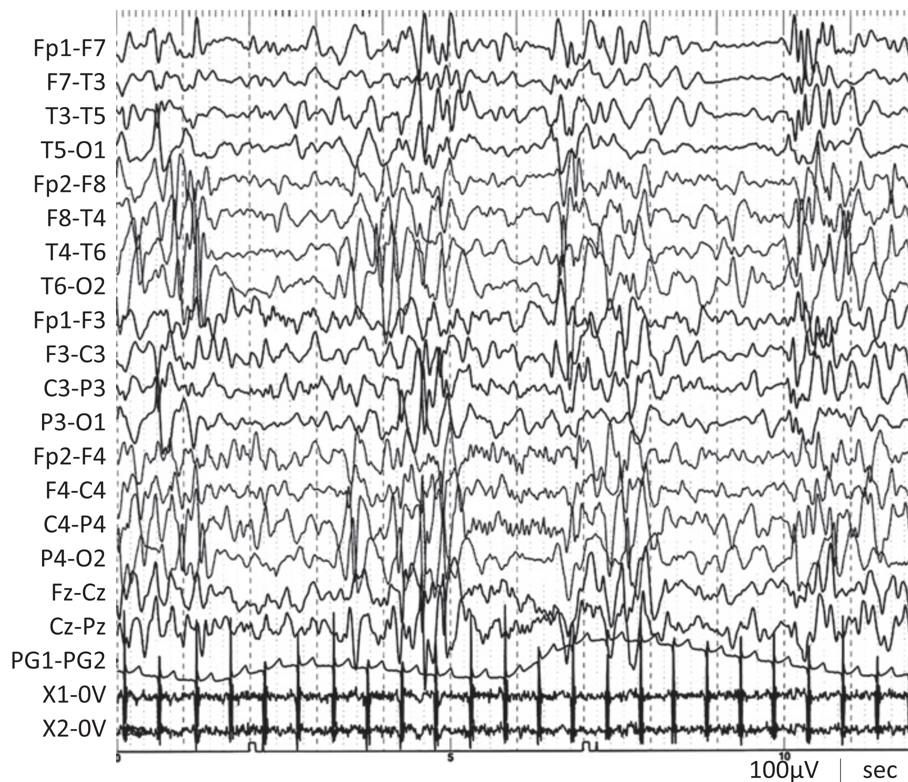

Figure 2 Electroencephalogram. Atypical hypsarrhythmia with high voltage wave bursts is shown.

Gene Analysis Research Ethics Committee of Tokyo Women's Medical University (No. 341 B). Genomic DNA was extracted from the blood samples using a QIAamp DNA extraction kit (Qiagen, Hilden, Germany). Next-generation sequencing (NGS) was performed to screen single-nucleotide variants using TruSight One ver 1.0 sequencing panel (Illumina, San Diego, CA, USA) as described previously.[9] The extracted data were mapped to a reference genome (GRCh 37/hg 19) using the BWA Enrichment ver 1.0 cloud software (Illumina), and annotated and filtered using the Variant Studio software (Illumina).

After filtering the data, a novel heterozygous variant was detected in the splicing acceptor site of exon 4 of *PAFAH1B1* (NM_000430.3:c.118-2A>G). There was no possible candidate variant in other genes related to lissencephaly. Sanger sequencing confirmed the existence of this variant in the patient but not in her parents (**Figure 3A**). This indicated de novo occurrence of the variant. To further confirm whether this variant did in fact cause the splicing abnormality, expressed RNA was examined. Total RNA extracted from Epstein-Barr virus-transformed leukocytes was reverse-transcribed (RT) into complimentary DNA (cDNA). The cDNA was amplified by RT-PCR as described previously.[10] The result showed the presence of an abnormal short band in addition to the normal band (**Figure 3B**). By use of DNA extraction kit (Qiagen), DNA amplicons were extracted through separated bands of both a normal- and an abnormal short band. Sanger sequencing performed using the extracted DNA samples confirmed exon 4 skipping (**Figure 3C**).

Discussion

In this study, we reported a patient with classical lissencephaly. The patient presented with developmental delay, hypotonia, and epileptic spasms. These clinical findings were typical of the clinical course of lissencephaly. According to the previously proposed classification of classic lissencephaly, brain MRI findings of this patient were considered as grade 1.[11] From this radiological pattern, *PAFAH1B1* located on 17 p was suspected as the most possible candidate gene.[12] However, chromosomal deletion involving 17 p was dismissed based on the results of FISH. *PAFAH1B1*

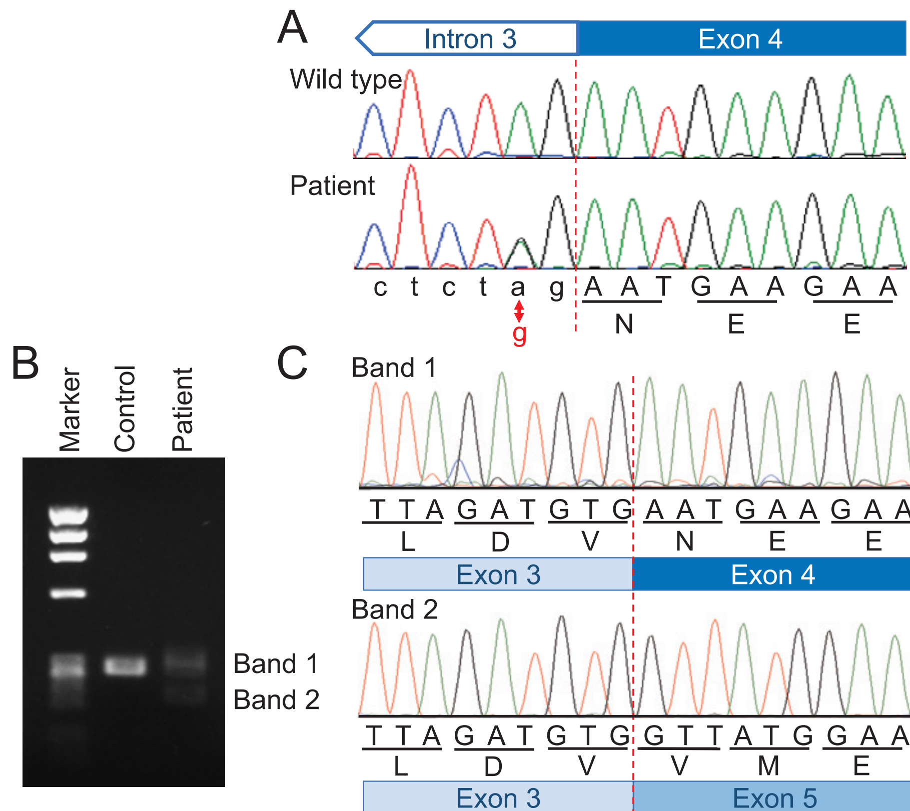

Figure 3 Results of molecular analyses. (**A**) Genomic sequence for the patient shows a nucleotide alteration (a>g) in the splicing acceptor site of exon 4, whereas control shows the wild type sequence. (**B**) Electrophoresis of the amplicons obtained after reverse transcribed (RT)-PCR revealed an additional short band (Band 2) in addition to the normal band (Band 1) in the patient. (**C**) Sanger sequence for Band 2 confirmed exon 4 skipping, whereas Band 1 (expected to be derived from the normal allele) showed normal pattern.

consists of as many as 10 exons and there are more than 10 possible candidate genes of lissencephaly other than *PAFAH1B1*. Thus, comprehensive genomic analysis using TruSight One was considered as a more efficient way for examining the status of candidate genes than targeted Sanger sequencing.

As suspected, a de novo variant was identified in *PAFAH1B1*. Splice variants are rare in *PAFAH1B1*.[13] We confirmed exon skipping by RT-PCR and subsequent Sanger sequencing. The exon 4 that was deleted in the patient consists of multiples of three nucleotides. Thus, exon 4 skipping was predicted to cause in-frame deletion and not frame-shift deletion. Generally, frameshift leads to premature termination and subsequent nonsense-mediated mRNA decay. Thus, frameshift variants are usually related to loss-of-function and most of the variants identified in *PAFAH1B1* are related to loss-of-function.[13] In comparison, in-frame deletions are generally related to milder phenotypes in genetic disorders; however, in this case, exon 4 encodes an important coiled-coil domain, which binds to microtubule-associated binding proteins.[14,15] Therefore, exon 4 skipping was considered to be related to loss-of-function mechanism.

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

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