

DATA REPORT

A novel *PLP1* mutation F240L identified in a patient with congenital type Pelizaeus-Merzbacher diseaseYongping Lu¹, Keiko Shimojima¹, Tomoko Sakuma², Sachiko Nakaoka² and Toshiyuki Yamamoto¹

Pelizaeus-Merzbacher disease (PMD) is an X-linked recessive hypomyelination disorder caused by mutations in the proteolipid protein 1 gene (*PLP1*) located on chromosome Xq22. A male patient showed severe developmental delay, pendular nystagmus and laryngeal wheezing. The auditory brain stem response showed only the first wave and brain magnetic resonance imaging showed white matter hypomyelination, suggesting typical PMD. A novel *PLP1* mutation, F240L, which was inherited from his mother, was identified.

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Pelizaeus-Merzbacher disease (PMD; MIM #312080) is a rare X-linked recessive hypomyelination disorder caused by mutations in the proteolipid protein 1 gene (*PLP1*) located on chromosome Xq22.¹ *PLP1* is highly conserved among vertebrates and encodes two major compact myelin proteins, PLP1 and its splicing variant DM20.² More than 100 mutations have been reported in PMD, including duplications, point mutations, insertions and deletions. Chromosomal Xq22 microduplications involving *PLP1* are the most frequent mutations, which have been detected in ~60–70% of PMD patients. Point mutations, including missense, nonsense and splicing, have been detected in 10–25% of patients and deleterious mutations are rare.³

Most patients with typical PMD manifest pendular nystagmus in early infancy as well as generalized hypotonia that later changes into spasticity, tremor and motor developmental delay.⁴ According to the severity of clinical manifestations, PMD is classified into subgroups, including congenital, classical, X-linked spastic paraplegia and a *PLP1* null syndrome. These subgroups correspond to the different types of *PLP1* mutations, namely missense mutations, chromosomal Xq22 microduplications, missense mutations in the specific region in exon 4 and whole-gene deletions, respectively.^{2,5} Here we report the case of a patient with PMD with a novel *PLP1* mutation.

A 3-year-old boy was born at 38 weeks of gestation with a birth weight of 2774 g. This boy is the only child of healthy and non-consanguineous parents. Family history is unremarkable. Soon after birth, automated auditory brainstem response hearing screening detected abnormalities with no detection of waves after the first wave. At that time, pendular nystagmus was also noted. Because he had feeding difficulties due to laryngeal wheezing, tube feeding was initiated. Brain magnetic resonance imaging at 6 months of age revealed a hypomyelination pattern (Figure 1). The patient exhibited severe developmental delay with no head control or meaningful language development. From these findings, congenital type PMD was suggested as a candidate diagnosis.

This study was performed in accordance with the Declaration of Helsinki and was approved by the ethics committee of Tokyo

Women's Medical University. After receiving written informed consent, we obtained blood samples from the patient and his mother for genomic DNA extraction. Because *PLP1* duplications are predominantly observed in PMD patients, chromosomal microarray testing was first performed as described previously,¹ but *PLP1* duplication was not observed. Sanger sequencing for all *PLP1* exons was then performed as described in a previous report.⁶

Sanger sequencing of the patient's sample revealed a nucleotide alteration (c.718T>C) in exon 6 of *PLP1*, resulting in a protein change, p.Phe240Leu (F240L). The mother was heterozygous for this mutation, indicating that this mutation was maternally inherited (Figure 2). The prediction scores of this mutation were evaluated using wANNOVAR (<http://wannovar.usc.edu/>); the results were: SIFT_score=0 (Damaging), Polyphen2_HDIV_score=0.979 (Damaging), Polyphen2_HVAR_score=0.982 (Damaging), MutationTaster_score=1 (Damaging) and CADD_phred=34. From these findings, c.718T>C (p.Phe240Leu) was considered to be the cause of disease.

Previously reported nucleotide alterations in coding regions are summarized in Supplementary Table S1. In total, 112 mutations had been reported. Since the c.718T>C (p.Phe240Leu) mutation identified in this study has not been reported previously, we determined it to be a novel mutation.

Previous studies have shown that PLP1 is important for the central nervous system because it maintains and stabilizes the lamellar structure of oligodendrocytes.^{7,8} PLP1 is a membrane protein with four alpha-helix transmembrane (TM) domains that can mediate its conformational structure through sequence-specific interactions between the TM alpha-helices of the protein monomers.⁹

As mentioned above, 112 nucleotide alterations have been reported. Among them, 48 mutations are located in the TM domains. As previously suggested, most of the missense mutations in the TM domains were related to the very severe congenital type PMD.^{9,10} The mutation identified in this study altered the amino acid phenylalanine (Phe) to leucine (Leu) in TM4. A similar Phe-to-Leu mutation in TM1 (p.Phe32Leu) was previously

¹Institute of Medical Genetics, Tokyo Women's Medical University, Tokyo, Japan and ²Department of Pediatrics, Kouseiren Takaoka Hospital, Takaoka, Japan.

Correspondence: T Yamamoto (yamamoto.toshiyuki@twmu.ac.jp)

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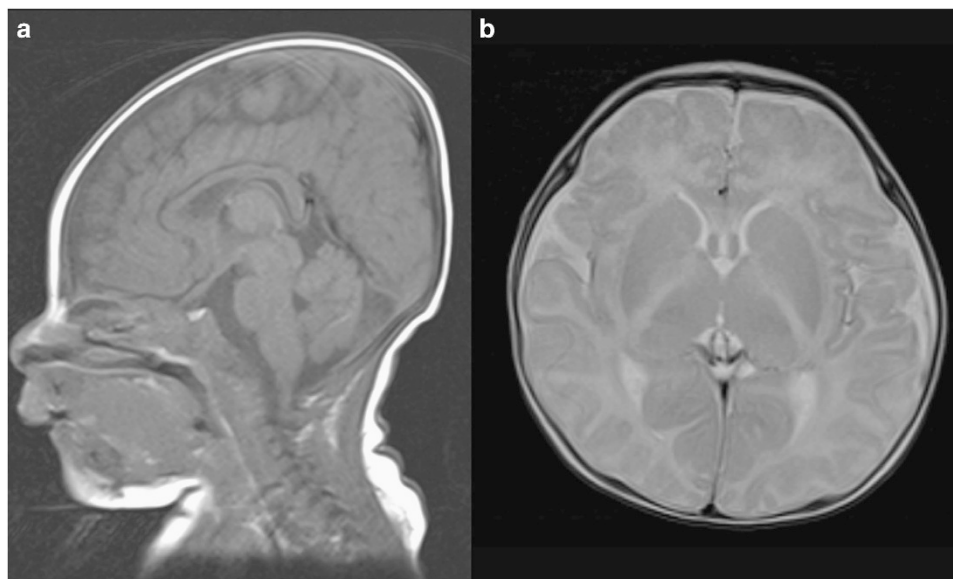


Figure 1. Brain magnetic resonance imaging at 6 months of age. **(a)** T1-weighted sagittal image. There are no abnormalities in the corpus callosum or cerebellum. **(b)** T2-weighted axial image shows high intensity in the white matter, indicating hypomyelination.

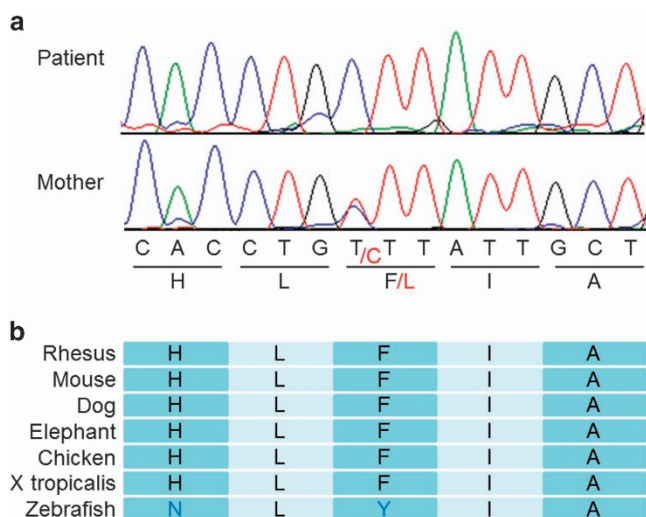


Figure 2. Sanger sequencing results. **(a)** Hemizygous and heterozygous mutations of c.718T > C (p.Phe240Leu [F240L]) are shown in the patient and his mother, respectively. **(b)** The amino acid sequences of the different species are shown, indicating conservation among species.

reported,⁵ and the patient exhibited a very severe connatal type similar to the patient reported in this study.

Amino acid changes in the TM domain may lead to changes in protein conformational structure or result in an unfolded protein, affecting TM transport of the protein and resulting in accumulation of PLP1 in the endoplasmic reticulum.² Previously, we identified the following four missense substitutions located within the TM domain: p.Gly83Arg, p.Leu85Pro, p.Ala243Val and p.Ala247Thr.^{6,11,12} All four mutations were related to connatal type PMD.

In conclusion, we identified a novel *PLP1* mutation, c.718T > C (p.Phe240Leu), in a patient with connatal type PMD. This novel mutation on the fourth alpha-helix in the TM domain

supports the genotype–phenotype correlation in PMD that relates missense mutations in TM domains to severe clinical manifestations.

HGV DATABASE

The relevant data from this Data Report are hosted at the Human Genome Variation Database at <http://dx.doi.org/10.6084/m9.figshare.hgv.920>.

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COMPETING INTERESTS

The authors declare no conflict of interest.

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