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Case Report

A splicing mutation of proteolipid protein 1 in Pelizaeus-Merzbacher disease

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Abstract

A patient with an unusually mild form of Pelizaeus-Merzbacher disease was studied. Clinically, mild developmental delay with acquisition of assisted walking at 16 months and mild spastic tetraplegia were evident, but no nystagmus, cerebellar, or extrapyramidal signs were present. *PLP1* mutation analysis revealed a nucleotide substitution adjacent to the acceptor site of intron 3, NM_000533.4:c.454-9T>G. Expression analysis using the patient's leukocytes demonstrated an additional abnormal transcript including the last 118 bp of intron 3. *In silico* prediction analysis suggested the reduction of wild-type acceptor activity, which presumably evokes the cryptic splicing variant. Putative cryptic transcript results in premature termination, which may explain the mild clinical phenotype observed in this patient.
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1. Introduction

Pelizaeus-Merzbacher disease (PMD) is a rare X-linked recessive disorder caused by mutations of the *PLP1* gene, which encodes a major myelin membrane protein in the central nervous system [1]. Consequently, PMD is characterized by arrest of myelination that clinically results in psychomotor developmental delay and various neurological symptoms including nystagmus, spastic tetraplegia, dystonia, and cerebellar and extrapyramidal symptoms. One characteristic feature of PMD is the wide spectrum of clinical severity. Patients with the most severe form show essentially no achievement in developmental milestones, while those at the mildest end attain unsupported walking with well-preserved intellectuality.

Different *PLP1* mutations give rise to PMD [1]. Genomic duplication is the most common mutation, accounting for 60–70% of patients, while exonic or

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intron/exon boundary mutations are found in 20–30%. *PLP1* deletion is extremely rare. Functional studies have revealed that PMD's wide clinical spectrum is associated with distinct molecular pathogeneses of different mutations in the *PLP1* gene [1]. Meanwhile, phenotypic consequence and the molecular basis of intronic mutations are often difficult to predict [2,3]. Most intronic mutations are expected to alter splicing and result in aberrant transcripts. Here, we report one patient with a mild form of PMD who carries a mutation near the acceptor site of intron 3.

2. Materials and methods

2.1. Patient

A 7-year-old boy was born uneventfully at full term to unrelated Japanese parents. No family history was noted including his mother and an elder brother. He gained head control at 3 months, could sit without support at 10 months, and could walk with assistance at 16 months. He could speak a few words at 2 years of age. When he saw a pediatric neurologist because of his developmental delay, no stridor or nystagmus was noted, but his muscle tone was hypotonic with all extremities displaying exaggerated tendon reflexes and bilateral extensor plantar responses. No cerebellar signs or involuntary movements were observed. No biochemical abnormalities were noted in a routine laboratory examination. Nerve conduction velocities and electromyographic studies were all normal. Auditory brain response elicited only wave I. Brain MRI revealed the completion of myelination and a subependymal cyst in the right frontal region in the T1 signal. Myelination was incomplete in the insula and optic radiation in the T2 signal (Fig. 1).

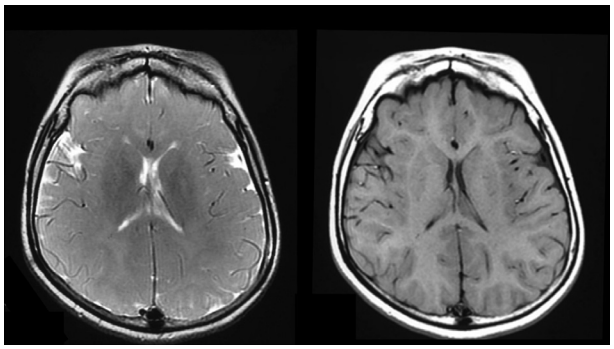

Fig. 1. Brain MRI of the patient at 5 years. Left panel; T2-weighted image shows symmetrical high intensity areas involving the insula and optic radiation, suggesting incomplete myelination. Right panel; T1-weighted image shows high intensity at whole white matter and completion of myelination.

2.2. DNA sequencing and reverse transcription-polymerase chain reaction analysis

Genomic DNA from the patient was prepared from peripheral blood. Polymerase chain reaction (PCR) amplification of seven exons and promoter regions of the *PLP1* gene was performed, followed by direct sequencing, as previously described [4]. Total RNA isolated from leukocytes was utilized for reverse transcription (RT) reactions to synthesize cDNA, which was then amplified by nested RT-PCR using a primer set spanning exons 3 and 5. Amplified PCR products were subcloned and sequenced.

2.3. In silico splice site prediction

To evaluate potential changes in the splicing efficiency, we performed computational prediction of the splice site selection using online programs: Berkeley Drosophila Genome Project, Splice Site Prediction by Neural Network [5], Human Splicing Finder including Maximum Entropy Modeling [6,7], and Alternative Splice Site Predictor [8]. The 1377-bp wild-type and mutant sequences including intron 3 and adjacent upstream and downstream exons were simultaneously analyzed.

3. Results

Direct sequencing of the patient's *PLP1* exons, exon/intron boundaries, and a promoter region revealed the novel non-coding mutation NM_000533.4:c.454-9T>G in intron 3, located 9 bp upstream of the intron 3/exon 4 boundary (Fig. 2A). No other sequence alterations were found and this mutation was not detected in more than 200 alleles from control Japanese DNA or public databases (dbSNP, 1000 Genomes, Exome Sequencing Project 6500, and Human Genetic Variation Database). The patient's mother was heterozygous for this mutation. Sequencing of the cloned RT-PCR products from the patient's cDNA revealed aberrant and normal transcripts (Fig. 2B and C). The aberrant transcripts revealed a cryptic DM20 mRNA (alternative splicing variant) in which the 5′ donor site of exon 3 is joined to intron 3 at 118 bp upstream of the normal 3′ acceptor site (r.348_349ins349-118_349-1;DM20int3δ) (Fig. 2D). This aberrant splicing is predicted to add 7 residues to the exon 3-coded amino acid sequence followed by a stop codon, leading to premature termination of translation (p.Phe117GlufsX7;DM20). Only *DM20* transcripts were found both in the patient and control; no *PLP1* transcripts were detected.

In silico prediction analyses using multiple programs commonly showed some effects of this mutation on splicing (Table 1). Likelihood scores for the native 3′ acceptor site were reduced by the mutation in 3 of 4 programs. This reduction probably results from an

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