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MILD PELIZAEUS-MERZBACHER DISEASE CAUSED BY A POINT MUTATION AFFECTING CORRECT SPLICING OF *PLP1* mRNA

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Abstract—We describe a 28-year-old male patient with a mild course of Pelizaeus-Merzbacher disease (PMD) who presented with developmental delay in his second year of life and was able to walk until 12 years of age. Several computed tomography scans in infancy and youth were normal, the diagnosis of PMD was eventually suggested by magnetic resonance imaging at the age of 24 years. Analysis of the proteolipid protein gene (*PLP1*) revealed a nucleotide exchange (c.762G>T) at the 3′ border of exon 6, which did not entail an amino acid exchange but adversely affected splicing. PCR analysis of fibroblast cDNA showed that c.762G>T resulted in partial skipping of exon 6 in the PLP1 mRNA. Exclusion of exon 6 does not alter the reading frame but leads to absence of amino acids 232–253 that constitute a main part of the fourth transmembrane helix of the PLP protein. Remarkably, residual wild-type splicing was also detected in the patient's cultured fibroblasts. This might explain the mild phenotype in this case, as exon 6 skipping mutations resulted in a severe course of disease in other patients.
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Key words: Pelizaeus-Merzbacher disease, PLP1 gene, splice mutation.

Mutations in the proteolipid protein gene (*PLP1*) may cause a spectrum of dysmyelinating disorders ranging from Pelizaeus-Merzbacher disease (PMD) to X-linked spastic paraplegia (SPG2). *PLP1,* which maps to Xq22, encodes two proteolipid proteins, PLP and DM20, as a consequence of differential splicing of exon 3. Both polypeptides are abundant in oligodendrocytes and account for more than half of the total protein mass of myelin in the CNS. Larger intrachromosomal duplications, which include the *PLP1,* are found in the majority of PMD cases whereas point mutations, mainly missense mutations, account for 15–20% of PMD disease alleles (Garbern et al., 1999; Sistermans et al., 1998; Inoue et al., 1999; Mimault et al., 1999; Hübner et al., 2005).

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Abbreviations: CT, computed tomography; ER, endoplasmatic reticulum; MRI, magnetic resonance imaging; PLP1, proteolipid protein gene; PMD, Pelizaeus-Merzbacher disease; SPG, spastic paraplegia; SSCP, single strand conformation polymorphism.

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Clinical diagnosis of PMD has been defined by strict criteria that can be applied at any time in life of an affected male (Boespflug-Tanguy et al., 1994) and include specific clinical features, electrophysiological studies and brain magnetic resonance imaging (MRI). The clinical picture of PMD can be categorized in classic and connatal forms. The severe connatal form shows an absence of psychomotor development and results in early death. Classic PMD presents with hypotonia in the first 3 months, nystagmus within the first year, dystonia and choreoathetotic movements at 6–18 months, spastic pareses, scanning speech, ataxia and mental deficiency (Boulloche and Aicardi, 1986; Hudson, 2003). Abnormal central conduction is present and by MRI, dysmyelination can be seen in all cases. The disease is slowly progressive and death usually occurs in adolescence or adulthood. Disease severity in PMD has been correlated with the presence of aberrant *PLP1* product. Missense mutations resulting in misfolding and retention of both PLP and DM20 in endoplasmic reticulum (ER) are often associated with early oligodendrocyte death and a more severe phenotype than mutations which result in misfolding or retention of PLP alone (Gow and Lazzarini, 1996). In contrast, absence of the proteins, e.g. due to functional null alleles by severely truncating mutations, is usually associated with mild forms of PMD or SPG2 (Cailloux et al., 2000). Clinical phenotypes associated with point mutations at splice sites are largely variable (Hobson et al., 2000).

Here we report a 28-year-old man with PMD caused by a nucleotide substitution c.762G>T of the *PLP1*. Functional studies of the splice site mutation identified in *PLP1* provide a possible explanation for the unusually mild phenotype.

Case report

The patient was born at term after a normal pregnancy and uneventful delivery. Developmental delay was noted for the first time at the age of around 1 year. Neurological examination at 13 months did not show relevant abnormalities; muscle tone and tendon reflexes were normal. He sat alone at the age of 18 months and was able to walk with assistance at 2.5 years. At that time there was still no evidence of an abnormal muscle tone or pyramidal tract signs, merely a delay in motor development was noted. Two months later, spasticity and nystagmus developed and a cerebral palsy was suspected. Funduscopy at 6 years showed pale optic discs, visual evoked responses were abnormal. The boy was operated for divergent strabism at the age of 7 years. Lower limb spasticity without progression and normal brain imaging stipulated the diag-

nosis of infantile cerebral palsy. At 12 years, the patient was confined to a wheelchair. Since spasticity increased and the patient developed atactic limb movements, the diagnosis of spastic tetraplegia with ataxia of unknown origin was considered at the age of 15 years. At 16 years, a scoliosis developed and the patient underwent spinal surgery 7 years later. Mental development was normal with a slowly progressive dysarthria from childhood on. At 22 years, visually evoked potentials were absent, while sensory evoked potentials of the tibial nerve and peripheral nerve conduction were normal. Cranial computed tomography (CT) scans at the age of 3 (1979), 6 (1982), and 15 years (1991) did not reveal any abnormality. When the patient was 24, the younger sister wanted to know the genetic risk of her future children, and a new attempt was made to reach a diagnosis. At this time, cranial MRI showed dysmyelination (Fig. 1) suggestive of PMD.

DNA and RNA analysis

Genomic DNA was isolated from peripheral blood lymphocytes. All seven exons of the *PLP1* were PCR amplified using intronic primers. Single strand conformation polymorphism (SSCP) analysis was performed for all amplified exons essentially as reported previously (Gu et al., 1996). PCR products with an aberrant SSCP pattern were sequenced. The G>T change identified resulted in loss of an ScrFI site and was confirmed by restriction analysis. Restriction fragments were separated by non-denaturing polyacrylamide gel electrophoresis (8%) and visualized by silver-staining.

Total RNA was extracted from cultured fibroblasts using the High Pure RNA purification kit (Qiagen). Fibroblast cDNA was synthesized by reverse transcription with Superscript II (Invitrogen) using random hexamers according to the manufacturer's instructions. *PLP1* cDNA fragments were amplified with primers binding in exon 3 (forward: 5′-GGC CAG AAG GGG AGG GGT TCC AG-3′) and exon 7 (reverse: 5′-GGC AAA GAG A AGA TGG GAG ACG C-3′). One microliter of the reaction product was subsequently used as template for nested polymerase chain reactions (PCRs), which were performed with a primer binding in exon 4 (forward: 5′-ACC TAT GCC CTG ACC GTT GTG TG-3′) and a reverse primer binding either in exon 7 (reverse: 5′-GCT GTG TGG TTA GAG CCT CGC-3′) or in exon 6 (reverse: 5′-CAG GGA AAC CAG TGT AGC TGC-3′). For both PCR-rounds a cycle with 30 s of denaturation at 94 °C, 60 s for annealing at 55 °C, and 60 s for elongation at 65 °C was repeated 30 times. The expected wild-type cDNA fragment size of the exon 4/exon 7 product was 420 bp, that for the exon 4/exon 6 product was 300 bp. PCR products were visualized after agarose gel electrophoresis (2%) with ethidium bromide. The *PLP1* reference cDNA sequence (GenBank ID number NM_000533.3) was used to describe the sequence variant identified. The historical numbering is given in brackets.

RESULTS

Mutation screening by SSCP of genomic DNA of the patient detected a mobility shift for amplicon 6. Direct sequencing revealed a nucleotide substitution c.762G>T (historical numbering: c.759G>T; Hübner et al., 2005) that destroys the ScrFI site present in the wild-type sequence of *PLP1*, a feature that can be used to confirm the presence of the mutation. Both the mother and sister of the patient carried the c.762G>T change in heterozygous form. The patient's sister requested prenatal diagnosis in her first pregnancy. Molecular genetic analysis excluded the c.762G>T change in the fetus and the pregnancy resulted in a healthy boy.

The G>T transversion was not expected to result in an amino acid change as both CTG and CTT encode for leucine. However, it was hypothesized that the nucleotide change may affect correct splicing as it alters the sequence at the 3′ boundary of exon 6.

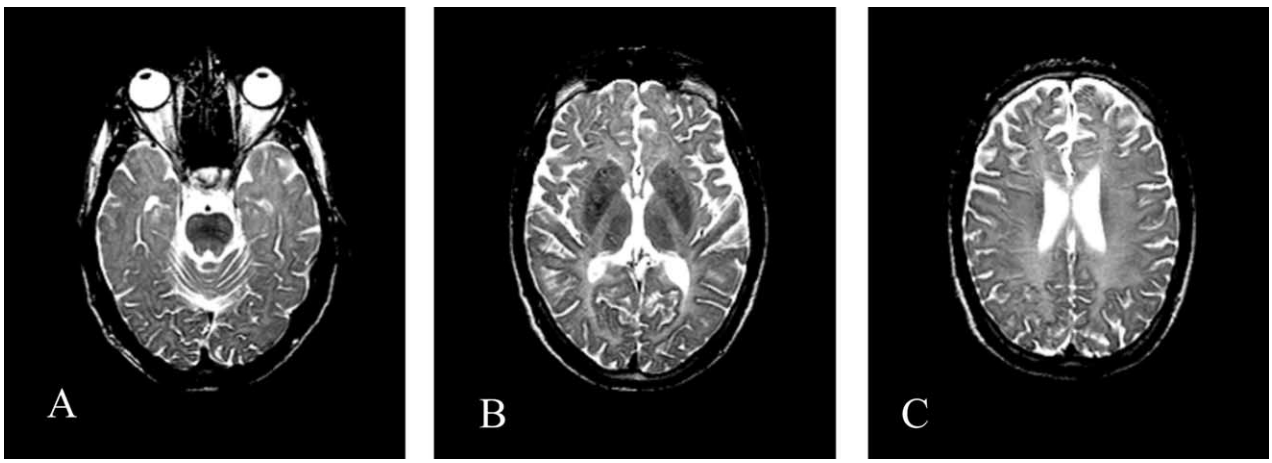

Fig. 1. MRI scans of the patient at the age of 24 years. Axial, 5 mm thick, T2-weighted fast spin echo images (A) at the level of the mesencephalon, (B) basal ganglia, and (C) at the roofs of the lateral ventricles. Although disturbed by motion artifacts, increased signal intensity of the white matter suggestive of lack of myelination is clearly visible. Note that both mesencephalon and globus pallidus have low signal intensity indicating that some myelin is preserved within these structures.

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