

Identification of Proteolipid Protein 1 Gene Duplication by Multiplex Ligation-Dependent Probe Amplification: First Report of Genetically Confirmed Family of Pelizaeus-Merzbacher Disease in Korea

Pelizaeus-Merzbacher disease (PMD) is a rare X-linked recessive disorder with a prototype of a dysmyelinating leukodystrophy that is caused by a mutation in the proteolipid protein 1 (*PLP1*) gene on the long arm of the X chromosome in band Xq22. This mutation results in abnormal expression or production of PLP. We here present a Korean boy with spastic quadriplegia, horizontal nystagmus, saccadic gaze, intentional tremor, head titubation, ataxia, and developmental delay. The brain magnetic resonance imaging (MRI) showed abnormally high signal intensities in the white matter tract, including a subcortical U fiber on the T2-weighted and fluid attenuated inversion recovery (FLAIR) image. The chromosomal analysis was normal; however, duplication of the *PLP1* gene in chromosome Xq22 was detected when the multiplex ligation-dependent probe amplification (MLPA) method was used. We also investigated the pedigree for a genetic study related to PMD. This case suggests that the duplication mutation of the *PLP1* gene in patients with PMD results in a mild clinical form of the disorder that mimics the spastic quadriplegia of cerebral palsy.

Key Words : Gene Amplification; Pelizaeus-Merzbacher Disease; Quadriplegia; Proteolipid Protein Duplication

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INTRODUCTION

Pelizaeus-Merzbacher disease (PMD) is a rare, X-linked recessive dysmyelinating leukodystrophy of the central nervous system (CNS). It is caused by mutations in the proteolipid protein 1 (*PLP1*) gene on the long arm of the X chromosome in band Xq22 that encodes a major component of CNS myelin protein (1, 2). There are several related tests available for the diagnosis of PMD; however, no definitive test exists. In spite of this, molecular testing of *PLP1* is now essential to confirm diagnosis. Here, we present a Korean boy with PMD who had clinical symptoms mimicking cerebral palsy and was confirmed to have a *PLP1* duplication mutation using a specific molecular technique.

CASE REPORT

A 52-month-old boy with developmental delay was admitted to the department of rehabilitation medicine for evaluation and treatment. He was born post-maturely at the gestational age of 41 weeks with an initial body weight of 3,400 g. The mother underwent a cesarean section due to failure of

progression during labor; however, the pregnancy and prenatal period of the patient had been uneventful. The boy showed significantly delayed development. He could turn over and pull-to-stand, but he could not stand without support. Also, genu recurvatum was observed while standing. He could not walk nor keep balance in a sitting position. His kneeling and crawling were inadequate. He could not speak any meaningful word. Physical examination showed horizontal nystagmus, saccadic gaze, intentional tremor, head titubation, and ataxia. The deep tendon reflexes were hyperactive in all extremities and positive ankle clonus was observed, bilaterally. Electromyography was normal but somatosensory evoked potential showed no response on median nerve stimulation. Creatine phosphokinase was normal and lactic dehydrogenase was slightly increased (525 IU/L). Thyroid function tests, liver function tests, and infant hereditary metabolic screening tests (very-long-chain fatty acid, lactic acid, pyruvic acid, taurine acid, urine organic acid, mucopolysaccharide, urine and serum amino acids) were also normal. The brain magnetic resonance image (MRI) studies revealed diffuse abnormal high signal intensities in the white matter tract including observation of subcortical U fiber on the T2-weighted and fluid attenuated inversion recovery (FLAIR) image (Fig. 1). The pattern

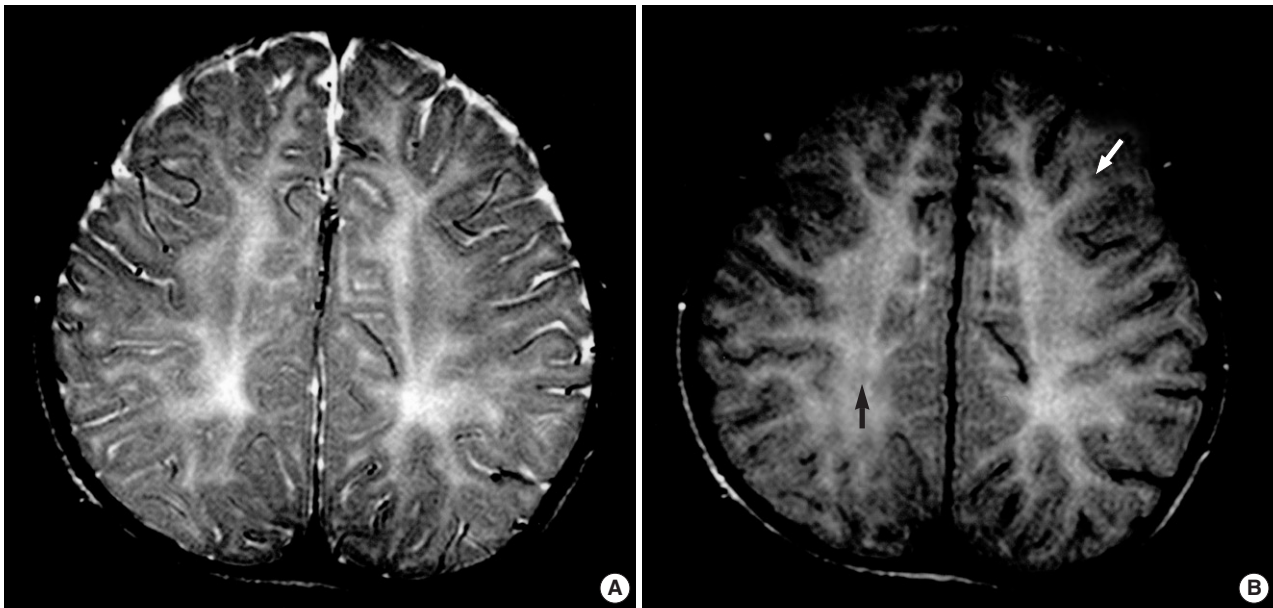


Fig. 1. Axial T2-weighted (A) and FLAIR (B) brain MRI scans at the level of the centrum semiovale in a 52-month-old boy with PMD. Extensive high signal intensity in white matter including subcortical U fiber is seen. Note the residual myelinated fibers (arrows), showing iso-signal intensity and giving the “tigroid” appearance sometimes presented in PMD, within the diffuse demyelinating white matter.

of the demyelination, which manifested as high SI on T2-weighted or FLAIR images, was patchy, with characteristic sparing of perivascular white matter creating a “tigroid” or “leopard-skin” pattern. There was no abnormal enhancement found during a gadolinium-enhanced study. The overlying cortex was normal.

His younger brother, a 27-month-old boy at present with developmental delay, visited our outpatient clinic. The boy revealed also significant developmental delay. He could creep, but could not sit without support and, could not pull to sit. He showed poor sitting balance while in the sitting position. On physical examination, he also showed horizontal nystagmus, saccadic gaze, rhythmic tremor, and head titubation. The deep tendon reflexes were brisk in all extremities and positive ankle clonus was observed, bilaterally. He was also unable to say any meaningful words.

After obtaining informed consent, blood samples were collected from the patient, his parents and younger brother for a genetic study. The genomic DNA was isolated from peripheral blood leukocytes using a Wizard genomic DNA purification kit according to the manufacturer’s instructions (Promega, Madison, WI, U.S.A.). We used multiple ligation-dependent probe amplification (MLPA) to screen all exons of the *PLP1* gene using a MLPA kit (SALSA P022; MRC Holland, Amsterdam, Netherlands). The kit contains a total of 32 probes, including 1 probe for each of the 7 *PLP1* exons, 8 control probes of other parts of the X chromosome, 1 probe that detects a Y-chromosome, and 16 probes that detect sequences on autosomes. All thermal reactions were carried out on a thermal cycler (Model 9700, Applied Biosys-

tems, Foster City, CA, U.S.A.). One hundred nanograms of sample DNA were diluted to 5 μ L with deionized water and denatured at 98°C for 5 min before the addition of 3 μ L MLPA probe mix and buffer. The reaction mixture was denatured for 1 min, then incubated for 16 hr at 60°C to ensure specific hybridization of the oligonucleotide probes with their target sequences. After hybridization, a buffer/ligase mixture (32 μ L) was added according to the manufacturer’s instructions and incubated at 54°C for another 15 min to stabilize the ligation reaction. Ligation was terminated by heating to 98°C for 5 min. A fluorescent multiplex polymerase chain reaction (PCR) amplification using a single universal primer pair suitable for all 40 probes in each kit was carried out under standard conditions (annealing temperature 60°C, 36 cycles). PCR products were analyzed on a fluorescent capillary sequencer (ABI 3100, Applied Biosystems, Foster City, CA, U.S.A.) using Genescan software. Five healthy female control samples were included in each MLPA test to calibrate unknown samples. Data analysis was performed with GeneMarker® software (SoftGenetics LLC, State College, PA 16803, U.S.A.). The *PLP1* gene dosage was determined using a population normalization method. Expected *PLP1* values for non-PMD males, non-PMD females, males with a *PLP1* duplication, and female carriers of a *PLP1* duplication are 0.5, 1.0, 1.0 and 1.5, respectively.

We determined the relative *PLP1* copy numbers from peak areas by normalizing each *PLP1* probe to each autosome, using normal female DNA as a calibrator. The patient and younger brother had all 7 *PLP1* exons duplicated. His father showed normal relative copy numbers of the *PLP1* gene but his moth-

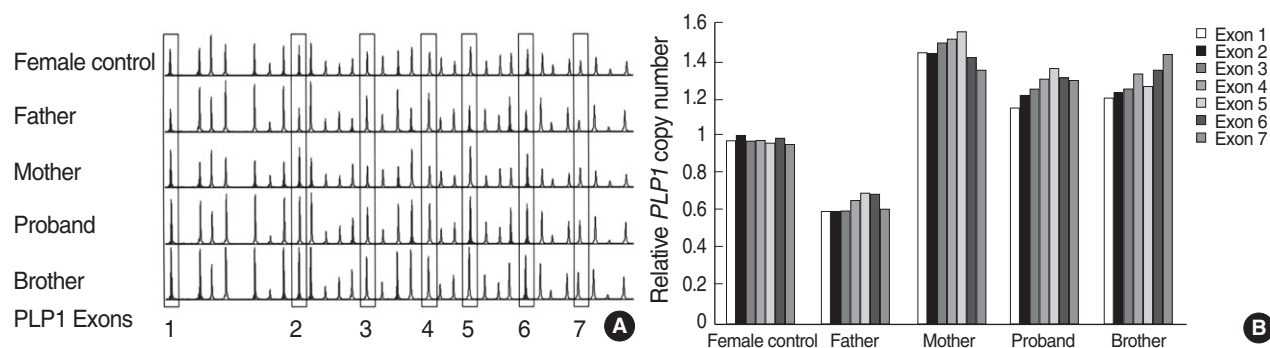


Fig. 2. Multiplex ligation-dependent probe amplification (MLPA) results. (A) Electropherograms after MLPA analysis of the family. All peaks corresponding to the 7 exons of the *PLP1* gene in the proband and his brother are higher than peaks in the proband's father but similar to peaks in female control due to *PLP1* gene duplication. The proband's mother has even higher peaks than female control. (B) The relative copy numbers for each *PLP1* exon were shown.

er showed findings consistent with carriers of a duplication. The relative dosage of *PLP1* exons in the patient and his family members are summarized in (Fig. 2). Taken together, these findings indicate the patient and his younger brother could be diagnosed with PMD caused by duplicated *PLP1* gene, inherited from their carrier mother.

DISCUSSION

Clinical findings in cases of PMD are very different and few studies have been reported. Golomb *et al.* conducted a study of 10 boys and reported their clinical findings (3). They found that prenatal complication occurred in eight patients and that their patients demonstrated defect of social interaction, dysphasia, nystagmus, hypotonia, and spasticity, that affected the legs more than the arm, as well as ataxia. The patient in our study demonstrated horizontal nystagmus, saccadic gaze, intentional tremor, head titubation, and ataxia. The deep tendon reflexes were hyperactive in all extremities. Unlike general cases, the leg and arm were equally involved and frequently showed spastic quadriplegia as seen in cases of cerebral palsy, and initially, our clinics suspected that the boy had spastic quadriplegia due to cerebral palsy. We focused on clinical diagnosis such as spasticity, inability to walk, and MRI findings around the periventricular white matter. The patient in our study showed only mild spasticity and genu recurvatum. Due to mild spasticity, the patient did not show equines of foot or contracture of hip adductors, which have been frequently shown in spastic quadriplegic type cerebral palsy.

PMD has been reported several times in Korea since 1995, but they were diagnosed by the clinical features and MRI findings (4-6). In 2004, the first Korean case of PMD with a confirmed point mutation in *PLP1* was reported (7); however, aside from this case, there have been no reports of confirmation based on a genetic study.

Barkovich (8) suggested that in PMD, MR imaging showed

reduction of normal myelin and normal oligodendroglial cells in the brain, resulting in delayed myelination. Due to the marked reduction of myelin, both diffusion anisotropy and magnetization transfer were decreased compared to age-matched control subjects. Our case showed abnormally high signal intensities in the white matter tract, including subcortical new fiber on the T2-weighted and FLAIR image, which indicates abnormal myelination of white matter in the brain.

Complete duplication of the *PLP1* gene on Xq22 is the cause of 60-70% of PMD cases, whereas deletions of this gene as well as point mutations in coding or splice site regions are involved in most of the remaining cases. Duplications of *PLP1*, indicating an increased *PLP1* dosage, prevent them from making normal myelination. Patients with an increased *PLP1* dosage have variable phenotype ranging from severe congenital to mild PMD, but most have a classic form of disease. Carrier females with a *PLP1* duplication are usually asymptomatic (9).

Various methods have been used to identify *PLP1* gene duplications, including fluorescent in situ hybridization (FISH) (10), array comparative genomic hybridization (CGH) (11), quantitative PCR (12), MLPA (13), real-time PCR (14), and multiplex amplification and probe hybridization (MAPH) (15). MLPA is based on the ligation of 2 adjacent annealing oligonucleotides followed by quantitative PCR amplification of the ligated products. Previous studies have confirmed the efficiency of MLPA for detecting *PLP1* gene duplications and deletions with good intra- and interassay precision (16). The precision of MLPA analysis was excellent and comparable to or better than quantitative real-time PCR, with CVs of 4.3-9.8% and 3.0-14%, respectively. Furthermore, MLPA analysis using customized probe sets has proven to have significant advantages over other methods such as FISH or array-CGH or MAPH as a rapid, reliable, economical, and easy molecular test (17-19).

In summary, this is the first reported Korean case of PMD in two affected male siblings with a confirmed duplication of the *PLP1* gene, inherited from their mother, an asymp-

tomatic carrier. Family study of the *PLP1* gene in Korean patients with suspected PMD, therefore, should be considered to better understand the mechanism of genetic disorders as well as for counseling and family planning.

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