

## Pelizaeus-Merzbacher disease: Molecular diagnosis and therapy

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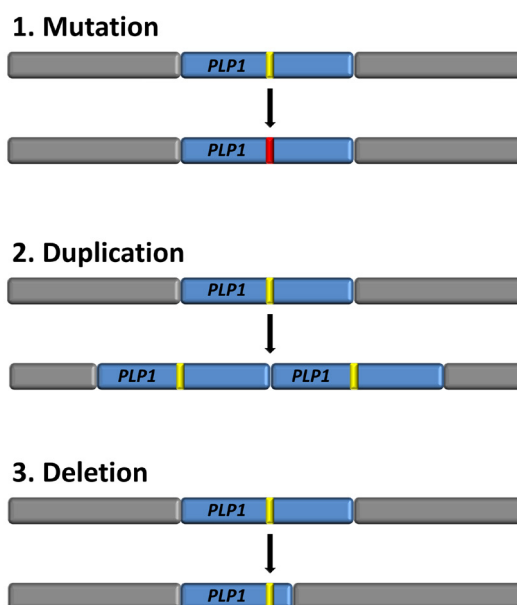
**Summary** Chromosome Xq22.2 contains the entire proteolipid protein 1 gene (*PLP1*), and a genomic duplication in that chromosome is responsible for Pelizaeus–Merzbacher disease (PMD). Duplication can be detected using several molecular diagnostic methods such as comparative multiplex PCR, fluorescent in situ hybridization (FISH), restriction site polymorphism (RSP) analysis, and multiplex ligation-dependant probe amplification (MLPA). The characteristics of these methods should be taken into account when using them. There is currently no treatment for PMD, so a cure is urgently need. Advances in research on stem cell therapies, and especially induced pluripotent stem cell therapy, offer great promise for development of a treatment for PMD.

**Keywords:** Pelizaeus-Merzbacher disease, *PLP1* gene, duplication, molecular diagnosis, iPS cell

Pelizaeus-Merzbacher disease (PMD) is an X-linked recessive disorder of the central nervous system (CNS) white matter in which coordination, motor ability, and intellectual function are delayed to a varying extent (1). In the US, PMD has an estimated prevalence of about 1/300,000 to 1/500,000, and in Germany PMD has a prevalence of 0.13 of every 10,000 live-born infants (2). In China, relevant epidemiological statistics are lacking despite the publication of numerous clinical case reports. In clinical settings, the diagnosis of PMD is often suggested when magnetic resonance imaging (MRI) scans reveal aberrant white matter (high T2 signal intensity, *i.e.* T2 lengthening) throughout the brain. This aberration is typically evident by approximately 1 year of age, but less prominent abnormalities should be evident in infancy. Unless there is a family history of sex-linked inheritance, the condition is often misdiagnosed as cerebral palsy.

PMD is caused by mutations in the proteolipid protein 1 (*PLP1*) gene on Xq22.2. *PLP1* mutations lead to a broad range of clinical syndromes from spastic

paraplegia 2 (SPG2), which is characterized primarily by leg spasticity and weakness, to the most severe, congenital, form of PMD (3). Mutations, duplication, and deletion can all affect *PLP1* gene expression (4) (Figure 1). Small mutations account for about 30% of *PLP1* mutations while *PLP1* duplications account for



**Figure 1. Pathogenetic mechanisms of PMD.** There are three major forms of genetic abnormalities in PMD.

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**Table 1. Methods for the detection of *PLP1* duplications**

Method	Advantage	Disadvantage	Ref.
Comparative/real-time PCR	Duplications can be easily detected.	Results may be ambiguous in detecting duplications.	(5)
FISH	Chromosomal translocations in metaphase and duplications in interphase can be detected.	Small duplications cannot be detected even in interphase.	(6)
RSP	Inexpensive and easy to perform.	Small duplications cannot be detected.	(1)
MLPA	Duplications can be accurately detected.	Signal intensity is not accurately detected.	(7)

about 70% of *PLP1* mutations. The duplicated region can range from about 40 Kbp to over 5 Mbp. Since the *PLP1* gene is dosage-sensitive, duplication and regulatory mutations are both likely to cause PMD. Deletion of the *PLP1* locus also leads to PMD but accounts for less than 1% of PMD cases.

Since *PLP1* duplications are noted in about 70% of PMD cases, verifying the presence of duplication is a reasonable approach when PMD is suspected in a clinical setting. Thus far, many different molecular methods have been developed to verify the presence of a *PLP1* duplication, including Southern blotting, a comparative multiplex polymerase chain reaction (PCR) assay (5), fluorescent in situ hybridization (FISH) (6), restriction site polymorphism (RSP) analysis (1), and multiplex ligation-dependant probe amplification (MLPA) (7) (Table 1). At present, these methods have been used in prenatal diagnosis to detect PMD as early as possible. The most common methods of detection are interphase fluorescent in situ hybridization (FISH) and quantitative PCR (Q-PCR). *PLP1* duplications in over 70% of interphase nuclei are diagnosed using FISH. With Q-PCR, multiplex PCR is performed with one or more pairs of primers to screen for *PLP1* duplications using a gene outside the region of duplication as a reference gene. The signal intensity of *PLP1* is compared to that of the reference gene, and a sample with duplication will have a higher signal intensity than a normal sample. These two methods have a high specificity and sensitivity but they require considerable proficiency and expensive equipment. Although RSP is a relatively inexpensive technique, it cannot be used to detect duplications in most male patients because their homozygous alleles are identical. However, these polymorphisms are very helpful when identifying females as carriers. Recently, MLPA has been increasingly used to test for duplications because of its speed and accuracy. However, there is a lack of comparative studies of these diagnostic methods indicating which method is optimal.

There is no cure for PMD nor is there a standard treatment. Treatment, which is symptomatic and supportive, may include medication for seizures and spasticity. The prognosis for individuals with Pelizaeus-Merzbacher disease varies greatly. Children with the most severe form, congenital, usually cannot survive into adolescence, but sometimes survival into the

sixties or even seventies is possible, especially with attentive care. Therefore, a cure is urgently needed. In December 2008, StemCells, Inc., a biotech company in Palo Alto, received approval from the U.S. Food and Drug Administration to conduct a Phase I clinical trial to assess the safety of transplanting human neural stem cells as a potential treatment for PMD (8). The trial began in November 2009 at the University of California, San Francisco Children's Hospital. In addition, recent studies on using induced pluripotent stem cells (iPSCs) for neural repair have found those iPSCs to show promise as a treatment for PMD. Recently, iPSCs have been used to treat many diseases related to nerve cell damage, such as Parkinson's and Huntington's diseases (9-11). In June 2013, a Japanese government panel approved the world's first clinical study using iPSCs for retinal regeneration (12). Thus, iPSCs may be able to treat other intractable diseases such as PMD.

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