Pelizaeus-Merzbacher Disease, Pelizaeus-Merzbacher-Like Disease 1, and Related Hypomyelinating Disorders

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Abstract

The purpose of this article is to present contemporary information on the clinical and molecular diagnosis and the treatment of Pelizaeus-Merzbacher’s disease (PMD) and related leukodystrophies. Various types of mutations of the X-linked proteolipid protein 1 gene (PLP1) that include copy number changes, point mutations, and insertions or deletions of a few bases lead to a clinical spectrum from the most severe connatal PMD, to the least severe spastic paraplegia 2 (SPG2). Signs of PMD include nystagmus, hypotonia, tremors, titubation, ataxia, spasticity, athetotic movements and cognitive impairment; the major findings in SPG2 are leg weakness and spasticity. A diffuse pattern of hypomyelination is seen on magnetic resonance imaging (MRI) of PMD/SPG2 patients. A similar constellation of signs and pattern of hypomyelination lead to the autosomal recessive disease called Pelizaeus-Merzbacher-like disease 1 (PMLD1) and the less-severe spastic paraplegia 44 (SPG44), caused by mutations of the gap junction protein, gamma-2 gene (GJC2), formerly known as the gap junction protein, α12 gene (GJA12). Magnetic resonance spectroscopy (MRS) and brainstem auditory evoked potentials (BAEP) may assist with differential clinical diagnosis of PMD and PMLD1. Supportive therapy for patients with PMD/SPG2 and PMLD1/SPG44 includes medications for seizures and spasticity; physical therapy, exercise, and orthotics for spasticity management; surgery for contractures and scoliosis; gastrostomy for severe dysphagia; proper wheelchair seating, physical therapy, and orthotics to prevent or ameliorate the effects of scoliosis; special education; and assistive communication devices.

Keywords
- Pelizaeus-Merzbacher disease
- spastic paraplegia 2
- Pelizaeus-Merzbacher-like disease
- spastic paraplegia 44

Background

Pelizaeus-Merzbacher Disease and Spastic Paraplegia 2

Pelizaeus-Merzbacher disease (PMD; MIM #312080), also called leukodystrophy hypomyelinating 1 (HDL1), and the less severe spastic paraplegia 2, X-linked (SPG2; MIM #312920) are inherited allelic leukodystrophies caused by mutations of the proteolipid protein 1 gene (PLP1; MIM #300401). PLP1 encodes the major protein of the myelin sheath, proteolipid protein (PLP), a 4-pass transmembrane protein with two extracellular and three intracellular domains. The PLP1 gene also encodes the DM20 isoform by alternative splicing at exon 3. PMD/SPG2 is inherited as an X-linked recessive trait. Males with PLP1 mutations have the disease; females are usually unaffected carriers. PMD/SPG2 is caused by various types of mutations of the PLP1 gene. When strict clinical criteria for PMD are applied, more than 50% of cases are caused by duplication of a genomic region, including the entire PLP1 gene.1 Duplications can range in size from less than 100 kilobases to 9 or more megabases.2–5 They are usually tandem direct repeats at Xq22.2 where the PLP1 gene is located, but duplicated segments have been reported at Xq26, Xp, 19pter, and Y.2,6,7 The duplications include other
genes in addition to PLP1, but it is not known whether these other genes affect the disease phenotype. Size of the duplication does not correlate with clinical severity of the disease.6, 5 Point mutations and indels (insertion or deletion of one to a few bases) of PLP1 account for ~25% of PMD/SPG2 cases.1

Point mutations that result in amino acid substitutions (missense mutations) have been reported throughout coding regions. Frameshift mutations that result in truncated protein or are null, and mutations that result in aberrant splicing are less common than missense mutations but have also been reported.8–11 Genomic triplication and higher copy numbers of PLP1, partial triplication, and complete and partial deletion are rare, but also result in PMD.7, 12, 13 Reported mutations of the PLP1 gene are cataloged at http://www.ncbi.nlm.nih.gov/books/NBK1182/.14 PMD is a rare disease that is not limited to nationality or race. Incidence was reported to be 0.13 in 100,000 live births in Germany and 1 in 90,000 in the Czech Republic.15, 16 The reason for the discrepant incidence numbers is unknown.

**Pelizaeus-Merzbacher-Like Disease 1 and Spastic Paraplegia 44**

When patients have symptoms of PMD but do not have mutations of PLP1, their disease is called Pelizaeus-Merzbacher-like disease (PMLD).17 Only 8% of PMLD cases are caused by mutations of the gap junction protein, gamma-2 gene (GJC2, MIM #608803), formerly known as the gap junction protein, α-12 gene (GJA12). GJC2 encodes a member of a large family of connexin proteins. Like PLP, the gap junction protein gamma-2, also called connexin 47 (Cx47) or connexin 46.6 (Cx46.6), encoded by the GJC2 gene is a 4-pass transmembrane protein with two extracellular and three intracellular domains. PMLD caused by GJC2 mutations is called PMLD1 (MIM #608804), or leukodystrophy hypomyelinating 2 (HDL2). Molecular etiology of the remaining cases of PMLD is unknown. PMLD1 is inherited as an autosomal recessive trait, affecting males and females with equal frequency. Patients with PMLD1 have homozygous or compound heterozygous mutations.18–23 Missense, nonsense, frameshift, and indel mutations have been reported. A mutation in the promoter of GJC2 was also reported to cause PMLD1.24 Five patients with PMLD were reported to have only one mutation of the GJC2 gene, leaving their disease not fully explained at the molecular level.22 A milder disease, spastic paraplegia 44 (SPG44), was reported in one family with individuals who have a homozygous mutation of GJC2.25 Incidence of PMLD1 and SPG44 has not been reported. It should be noted that mutations of the GJC2 gene have also been reported to cause autosomal dominant lymphedema.26

**Clinical and magnetic resonance imaging (MRI) Features**

**Clinical Features of Pelizaeus-Merzbacher Disease and Spastic Paraplegia 2**

Early signs suggestive of PMD that are present at birth or in the first few months of life include nystagmus and hypotonia, and in the more severe cases there may be stridor, sometimes with vocal cord paralysis, feeding difficulty, and seizures. Subsequently, developmental milestones are delayed. By around 6 months or later, ataxia of the limbs becomes apparent and over several years, the limb hypotonia is replaced by spasticity of the limbs. Dystonic posturing and athetotic movements may be present, and cognitive impairment is present in most children. The cognitive delay often leads to difficulty in clinical educational assessment. When sufficient time is given for children to respond to questions or instructions, they are often found to be more capable than they initially appear. Clinical phenotypes reported range from the severe so-called connatal form of PMD to the milder complicated and pure SPG2.27–29

**Connatal PMD** is observed at birth or in the first 2 weeks of life. Stridor and seizures are common in this form. Hypotonia in infancy evolves into severe spasticity during childhood. Patients do not develop the ability to walk, gain use of their upper extremities, or talk, although there may be comprehension of verbal language. Patients may have short stature and have poor weight gain. They often require gastrostomy feeding tubes for nutritional support. Dystonia may occur. Most children with connatal disease die in the first one to two decades of life.

**Classic PMD** is the phenotype originally described by Pelizaeus and Merzbacher. It is the most common phenotype because patients with duplication most frequently have classic PMD. Patients develop nystagmus, usually noticed within the first 2 months of life, but occasionally, patients do not develop nystagmus. It typically resolves within 2 to 5 years of age. Patients develop head titubation that may resolve. Hypotonia in infancy begins to evolve to spastic quadriplegia in the first 5 years. Patients usually develop purposeful use of the upper extremities and dysarthric language. At the milder end of the classic PMD range, patients may begin to walk in childhood, usually with an assistive device, but as spasticity becomes greater, they lose the ability to walk. Dystonia and athetosis may occur at the severe end of the classic PMD range. Seizures are rare. Lifespan may be shortened, but patients with classic PMD have lived to the seventh decade.

The PLP null phenotype occurs when no PLP protein is present and is typically a complicated SPG phenotype that develops in the first 1 to 5 years of life. It is characterized by mild to moderate developmental delay, spastic gait, and mild demyelinating peripheral neuropathy.30 Over time, progression of signs after puberty is typical and ultimately leads to severe spastic quadriplegia, with loss of communication abilities.

**Complicated SPG2** begins in the first 5 years of life with nystagmus, ataxia, and autonomic dysfunction, such as spastic urinary bladder. Patients are able to walk with a spastic gait. Patients are able to talk, but speech may be slow. Lifespan may be normal.

Pure SPG2 is usually apparent in the first 5 years, but onset may be delayed until the third or fourth decade. Patients are able to walk with a spastic gait. Patients are able to talk, but speech may be slow. Lifespan is normal.

The wide clinical spectrum in PMD/SPG2 is a reflection of the diverse molecular etiology. Disease severity increases...
with increasing copy number of PLP1 and is not affected by the size of the duplicated region.\(^4\),\(^5\),\(^12\) Duplications of the PLP1 gene usually result in classic PMD, while triplications are reported to cause a more severe phenotype. Complete deletion of the PLP1 gene and point mutations that disrupt the methionine codon or that cause truncation of the protein near the amino-terminus result in the milder null phenotype.\(^30\) Other point mutations (missense, frameshift, nonsense) and indels are reported to result in severity across the entire spectrum of PMD/SPG2 phenotypes.\(^11\),\(^29\) Mutations that affect the PLP-specific region and not the DM20 isoform result in disease toward the mild end of the spectrum, including spastic paraplegia phenotypes.\(^29\) There can be some intrafamilial phenotypic heterogeneity.\(^28\),\(^31\),\(^32\) Females can exhibit mild or moderate disease signs, especially in families in which the males are mildly affected.\(^33\),\(^34\)

### MRI and MRS Features of Pelizaeus-Merzbacher Disease and Spastic Paraplegia 2

Patients with PMD have a diffuse pattern of hypomyelination on MRI.\(^35\)–\(^37\) This is seen best as increased signal intensity on T2-weighted or fluid-attenuated inversion recovery (FLAIR) scans. Affected white matter regions include the cerebral hemispheres, cerebellum, and brainstem. Thinning of the corpus callosum is sometimes noted.\(^36\) Atrophy of the cerebral hemispheres may be seen in severe PMD cases. Sparing of the corticospinal tracts can occur in classical PMD. Patients with the PLP null phenotype have milder diffuse abnormalities of the white matter on MRI scans.\(^30\) Patients with the milder SPG2 phenotype may have patchy areas of hypomyelination on MRI. Children with PMD may not have definitively abnormal MRI scans until the age of 1 or 2 when rapid myelination is completed. However, absence of normal myelin-related changes in the pons and cerebellum of a newborn or absence of normal myelin-related changes in the posterior limb of the internal capsule, the splenium of the corpus callosum, and the optic radiations of a 3-month-old child could suggest PMD or other leukodystrophies.\(^38\) It should be noted that T1-weighted images may be more sensitive to these early changes than T2-weighted images.\(^38\)

Metabolite levels on magnetic resonance spectroscopy (MRS) are not necessarily consistent across the forms of PMD. Patients with duplications have increased levels of N-acetyl aspartate (NAA),\(^39\) whereas patients with other mutations, especially those that lack PLP protein, have reduced NAA levels.\(^36\),\(^40\),\(^41\) Most patients with point mutations or duplications have low levels of choline consistent with hypomyelination of PLP1, but increased levels of choline are seen in some cases, suggesting ongoing demyelination.\(^41\) In PMD duplication patients, increased levels of glutamine, inositol, and creatine are seen on MRS in addition to increased NAA.\(^42\) This pattern is distinctive and may assist with distinguishing PMD from other leukodystrophies.\(^42\)

### Clinical Features of Pelizaeus-Merzbacher-Like Disease 1 and Spastic Paraplegia 44

PMLD1 has only recently been reported, so the full phenotypic spectrum may not yet be known. The clinical phenotype of most PMLD1 patients reported to date is not appreciably different from that of the classic phenotype of PMD. The phenotypic spectrum of GJC2 mutations was recently extended to include a milder complicated spastic paraplegia phenotype, SPG44 (MIM #613206), with the description of a homozygous mutation in three members of one mildly affected family.\(^25\)

### MRI/MRS Features of Pelizaeus-Merzbacher-Like Disease 1 and Spastic Paraplegia 44

The MRI of PMLD1 patients shows a diffuse pattern of hypomyelination, quite similar or indistinguishable from those of true PMD patients (see above).\(^17\)–\(^21\),\(^37\) Sparing of the corticospinal tracts, as can occur in classical PMD, has been reported. However, unlike PMD patients, two PMLD1 patients had normal or almost normal levels of choline, NAA and creatine on MRS, suggesting that MRS might be useful in distinguishing PMD and PMLD1.\(^21\)

### Diagnosis and Testing of PMD/SPG2 and PMLD1/SPG44

Diagnosis of PMD/SPG2 is made based on clinical presentation, MRI indicative of diffusely abnormal myelin, X-linked inheritance pattern, and molecular testing indicating duplication or other mutation of the PLP1 gene.\(^27\) Diagnosis of PMLD1/SPG44 is made based on clinical presentation, MRI indicative of diffusely abnormal myelin, autosomal recessive inheritance pattern, and molecular testing indicating mutations of the GJC2 gene.\(^22\)

Brainstem auditory evoked potentials (BAEP) may be a helpful tool to distinguish between classic PMD and PMLD1.\(^43\) In one study, waves III, IV, and V generated in the pons and midbrain, were absent in 10 patients with PMD, but were present in 11 of 13 investigations in 8 patients with PMLD1.

### Molecular Testing of Male Patients

When PMD is suspected clinically in male patients, PLP1 gene duplication testing is a reasonable first step because duplications are the most common PLP1 gene defect occurring in over 50% of the patients who fit strict clinical criteria for PMD.\(^1\) The tests for duplication will also detect less common deletions. Several methods are currently in use by clinical diagnostic laboratories for duplication/deletion testing: quantitative multiplex polymerase chain reaction (PCR), multiple ligation probe analysis (MLPA), real time PCR, array comparative genomic hybridization (aCGH), and fluorescent in situ hybridization (FISH). Considerations and limitations for these methods are as follows:

- Quantitative multiplex PCR, MLPA, real time PCR, aCGH, and interphase FISH can detect copy number changes. These methods cannot be used to determine whether the duplication is tandem or inserted elsewhere in the genome. Tandem duplications that are smaller than 100 kilobases may not be resolved by interphase FISH.
- Most PMD duplications are too small to resolve by metaphase FISH. However, metaphase FISH can be used to
determine location of duplicated segments that are inserted in a region of the genome that is distant from the *PLP1* gene.

- Quantitative multiplex PCR, MLPA, and real-time PCR are generally designed to detect only copy number changes in the *PLP1* gene itself. They do not give information about the extent of the region that is duplicated unless they are designed to do so.

- aCGH can give information about the extent of the region that is duplicated. Size and spacing of the probes on the array should be taken into consideration. When aCGH is performed with closely spaced oligonucleotide probes, it is more sensitive than when performed with larger probes such as BACs or cosmids. If probes are spaced too far apart on aCGH, false-negatives may result.

If duplication or deletion of the *PLP1* gene is not detected in male patients, sequence analysis of the *PLP1* gene is the next step. Mutations of *PLP1* that can be detected by sequence analysis account for ~25% of cases that fit strict clinical criteria for PMD. Male patients who are negative for *PLP1* mutations should next be tested for *GJC2* mutations. Approximately 8% of PMLD patients (those who test negative for *PLP1* duplications or other mutations) have mutations in the *GJC2* gene. However, the percentage of *GJC2* mutations in consanguineous PMLD cases was 30%, suggesting that sequence analysis for *GJC2* mutations prior to sequence analysis for *PLP1* mutations might be justified in these cases.

When a mild spastic paraplegia phenotype is determined clinically in male patients, it is reasonable to undertake DNA sequence analysis of the *PLP1* gene prior to duplication analysis.

**Molecular Testing of Female Patients**

A reasonable approach for testing females who have clinical symptoms of PMD/PMLD in families with no affected male family members is to begin with sequence analysis for mutations of the *GJC2* gene. Approximately 16% of females with PMLD had mutations of *GJC2*. If an affected female tests negative for *GJC2* mutations, then duplication analysis and sequence analysis of the *PLP1* gene might be justified, especially if the phenotype is mild because carrier females in families with *PLP1* duplications and mutations can be mildly affected.

**Carrier Testing, Prenatal Testing, and Preimplantation Genetic Diagnosis for Family Members**

Carrier testing for at-risk females and prenatal testing for at-risk pregnancies in PMD families require prior identification of the disease-causing mutation in a male family member. Preimplantation genetic diagnosis has been reported for point mutation of *PLP1*.

Carrier testing for at-risk individuals and prenatal testing in PMLD1 families require prior identification of the disease-causing alleles. Carrier testing for parents of patients with PMLD1 may help determine whether heterozygous individuals are compound heterozygotes (two mutations are on different alleles) and whether individuals with one mutation are homozygous or have a deletion of one allele. Preimplantation genetic diagnosis should be possible for PMLD1 families.

**Other Considerations**

When only one heterozygous *GJC2* mutation is identified in a PMLD patient, the following may be considered: (1) the mutation may be unrelated to the disease in the patient; (2) the patient may have a second mutation in a region of the other *GJC2* allele that was not sequenced, such as a transcriptional or splicing regulatory region; (3) the mutation in the patient may be causing autosomal dominant disease; and (4) the mutation detected may combine with mutation of a different gene to produce the disease phenotype (digenic inheritance). Both options 3 and 4 have been reported for gap junction protein genes that cause congenital deafness.

Other diseases that may be considered when PMD/SPG2 and PMLD1/SPG44 mutations are not identified:

- Hypomyelinating leukodystrophy 3 (HDL3; MIM #260600)—autosomal recessive, caused by mutations of the aminoacyl-tRNA synthetase complex-interacting multifunctional protein 1 gene (*AIMP1*; MIM #603605)

- Hypomyelinating leukodystrophy 4 (HDL4; MIM#612233), also called mitochondrial HSP60 chaperonopathy and mitchop 60 disease—autosomal recessive, caused by mutations of the heat-shock 60-kd protein 1 gene (*HSPD1*; MIM#118190)

- Hypomyelinating leukodystrophy 5 (HLD5; MIM#610532), also called hypomyelination and congenital cataract (HCC)—autosomal recessive, caused by mutations of the family with sequence similarity 126, member A gene (*FAM126A; MIM# 610531)

- Allan-Herndon-Dudley’s syndrome (AHDS; MIM#300523), also called Allan-Herndon’s syndrome, monocarboxylate transporter 8 deficiency, triiodothyronine resistance, T3 resistance, X-linked mental retardation with hypotonia, and mental retardation and muscular atrophy—X-linked, caused by mutations of the solute carrier family 16 (monocarboxylic acid transporter) member 2 gene (*SLC16A2; MIM#300095), also known as MCT8

- Leukodystrophy, demyelinating, adult-onset, autosomal dominant (ADLD; MIM# 169500), also known as Pelizaeus–Merzbacher disease, autosomal dominant or late-onset type, multiple sclerosis-like disorder—autosomal dominant, caused by duplications of the lamin B1 gene (*LMNB1; MIM# 150340)

- Sialuria, Finnish type (MIM# 604369), also known as Salla’s disease (SD) and the allelic infantile sialic acid storage disorder (ISSD; MIM# 269920), also known as sialuria, infantile form, N-acetylmuramidase acid storage disease, and nana storage disease (NSD)—autosomal recessive, caused by mutations of the solute carrier family 17 (sodium phosphate cotransporter), member 5 gene (*SLC17A5; MIM# 604322)

- Peripheral demyelinating neuropathy, central dysmyelination, Waardenburg’s syndrome, and Hirschsprung’s