Special Article

Pelizaeus-Merzbacher Disease and Spastic Paraplegia Type 2: Two Faces of Myelin Loss From Mutations in the Same Gene

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ABSTRACT

Pelizaeus-Merzbacher disease and X-linked spastic paraplegia type 2 are two sides of the same coin. Both arise from mutations in the gene encoding myelin proteolipid protein. The disease spectrum for Pelizaeus-Merzbacher disease and spastic paraplegia type 2 is extraordinarily broad, ranging from a spastic gait in the pure form of spastic paraplegia type 2 to a severely disabling form of Pelizaeus-Merzbacher disease featuring hypotonia, respiratory distress, stridor, nystagmus, and profound myelin loss. The diverse disease spectrum is mirrored by the underlying pathogenesis, in which a blockade at any stage of myelin proteolipid protein synthesis and assembly into myelin spawns a unique phenotype. The continuing definition of pathogenetic mechanisms operative in Pelizaeus-Merzbacher disease and spastic paraplegia type 2, together with advances in neural cell transplant therapy, augurs well for future treatment of the severe forms of Pelizaeus-Merzbacher disease. (*J Child Neurol* 2003;18:616–624).

PELIZAEUS-MERZBACHER DISEASE AND THE ALLELIC DISORDER X-LINKED SPASTIC PARAPLEGIA TYPE 2 SPAN A BROAD CLINICAL SPECTRUM

Friedrich Pelizaeus provided the first clinical picture of the disease and managed to correctly identify the X-linked mode of inheritance of this disorder by including a quotation from the affected family "that the disease is passed on by the mother but does not hurt her."[1] This observation on the sparing of the carrier mother, like many others made on families with Pelizaeus-Merzbacher disease over the subsequent century, was subject to revision. That a fraction of female carriers express features of the disease and, counterintuitively, that families with the least severe type of Pelizaeus-Merzbacher disease are most likely to have these afflicted carrier mothers illustrates the opportunities afforded by families with Pelizaeus-Merzbacher disease for examining oligodendrocyte behavior and function. Twenty-five years later, Ludwig Merzbacher followed up on the original family examined by Pelizaeus and discovered a widespread loss of myelin in the cortical white matter.[2] The 12 affected male subjects in this family displayed a high degree of similarity in symptoms, another feature challenged as additional families with Pelizaeus-Merzbacher disease were studied that presented within-family variability suggestive of segregating modifier genes. Merzbacher's exhaustive description of what was subsequently categorized as the classic form of Pelizaeus-Merzbacher disease (OMIM 312080) begins postnatally. Initially, the disease manifests itself by aimless, wandering eye movements, followed by nystagmus. Infants fail to develop normal head control and display tremors or shaking movements of the head. The disease is slowly progressive, with additional signs including bradylalia, scanning speech, ataxia, and intention tremor of the upper limbs, spastic contractions of the lower limbs, athetotic movements, and mild dementia.[1,2] Death ensues in the second decade.

A more severe disorder (the connatal form of Pelizaeus-Merzbacher disease), in which the nearly complete absence

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Table 1. Clinical Spectrum of PLP Mutations

	Most Severe Phenotype →			*Least Severe Phenotype*
			Spastic Paraplegia Type 2	
Phenotype	*Connatal PMD*	*Classic PMD*	*Complicated*	*Pure*
Age of onset	Neonatal	Year 1	Year 1–5	Year 1–5
Age of death	Childhood to 3rd decade	3rd–7th decade	Normal	Normal
Nystagmus	Present	Present	Often present	Absent
Hypotonia	Present	Initially present	Absent	Absent
Ataxia	Present	Titubation	Present	Absent
Spasticity	Severe	Spastic quadriparesis	Spastic gait	Spastic gait
Other neurologic signs	Stridor Pharyngeal weakness Seizures	Dystonia Athetosis	Spastic urinary bladder	Spastic urinary bladder
Cognition	Impaired	Impaired	Normal	Normal
Ambulation	Absent	Partial	Present	Present
Speech	Absent	Present	Present	Normal

PMD = Pelizaeus-Merzbacher disease.

of myelin sheaths is accompanied by a profound loss of myelin-forming oligodendrocytes, was reported by Seitelberger.[3] Seitelberger correctly identified the connatal and classic forms of Pelizaeus-Merzbacher disease as leukodystrophies, that is, conditions in which the absence of myelin is primary. Whether this lack of myelin arises from a failure of synthesis or maintenance is not always clear in the different forms of Pelizaeus-Merzbacher disease. Although originally described as a "demyelinating" (destruction of myelin) disorder, the pathology of the connatal form of Pelizaeus-Merzbacher disease resembles that of a "dysmyelinating" (inability to form normal myelin) disease.[4] Zeman and coworkers hypothesized that the defect in Pelizaeus-Merzbacher disease resided in the proteolipid protein (also known as lipophilin or Folch-Lees protein) component of myelin.[4] A quarter century later, this hypothesis was verified by the sequencing of mutations in the *PLP* gene of several patients with the connatal or classic form of the disease.[5–7]

Long before mutation analysis pinpointed the cause of Pelizaeus-Merzbacher disease, the wide-ranging phenotypic variation in this disorder frustrated classification efforts.[4,8,9] A discovery by Boespflug-Tanguy and colleagues magnified the disease spectrum of Pelizaeus-Merzbacher disease.[10] Clinically distinct from Pelizaeus-Merzbacher disease, X-linked spastic paraplegia type 2 was also found to arise from mutations in the *PLP* gene.[11] The observations leading to this surprising finding included the following: (1) linkage of spastic paraplegia type 2 to the chromosome Xq22 region where Pelizaeus-Merzbacher disease also mapped; (2) commonalities in the clinical presentation of Pelizaeus-Merzbacher disease and the complicated form of spastic paraplegia type 2, namely spasticity, nystagmus, cerebellar ataxia, and a pyramidal syndrome; (3) detection of hypomyelination in a patient with spastic paraplegia type 2; and (4) collection of several families with Pelizaeus-Merzbacher disease in which a family member was diagnosed with the rare spastic paraplegia disorder.[12] The X-linked form of spastic paraplegia type 2 exists as either a "complicated" form or a milder "pure" one in which the clinical phenotype is confined to lower limb spasticity. The detection of families in which Pelizaeus-Merzbacher disease and spastic paraplegia type 2 coexist emphasizes the broad clinical continuum of these disorders, all of which share a phenotype of spasticity and hypomyelination.[13–16]

The severity and clinical course of Pelizaeus-Merzbacher disease and spastic paraplegia type 2 vary widely, as illustrated in Table 1. Despite the variability arising from different mutations at the *PLP* locus and variable expressivity within a family, three features are consistently encountered: (1) spasticity, (2) a diffuse myelin loss on magnetic resonance images (MRIs), and (3) absence of male-to-male transmission. Although not observed in the pure form of spastic paraplegia type 2 or in *PLP* null mutations, nystagmus is a prominent feature of Pelizaeus-Merzbacher disease and spastic paraplegia type 2, one that is particularly helpful in the differential diagnosis of this disorder. None of the other leukodystrophies (eg, metachromatic leukodystrophy, adrenoleukodystrophy, Krabbe's disease, Cockayne's syndrome, and Canavan's disease) present with nystagmus. These other leukodystrophies can also be discounted by analyses of lysosomal/peroxisomal proteins and MRIs displaying a regional loss of myelin, as opposed to the generally diffuse character of myelin loss in Pelizaeus-Merzbacher disease. Patients with Pelizaeus-Merzbacher disease with a null mutation have the additional feature of mild demyelinating peripheral neuropathy, in which conduction velocities are detectably slower.[17,18] Axonal damage was also evident in these patients,[19] consistent with alterations in axonal morphology originally reported by Merzbacher.[2] Decreased *N*-acetylaspartate in some patients with *PLP* mutations is also in accordance with a diagnosis of neuroaxonal injury.[19,20]

The diagnosis of Pelizaeus-Merzbacher disease/spastic paraplegia type 2 can be made by applying a combination of criteria (Table 2). The characteristic set of neurologic signs includes nystagmus, spastic paraparesis, and limb ataxia. Neurologic symptoms vary widely among the different forms of Pelizaeus-Merzbacher disease and spastic paraplegia type 2, as illustrated in Table 2. MRI analysis reveals diffusely increased signal intensity within the central white

Table 2. Diagnostic Criteria and Screening Centers for Pelizaeus-Merzbacher Disease and Spastic Paraplegia Type 2

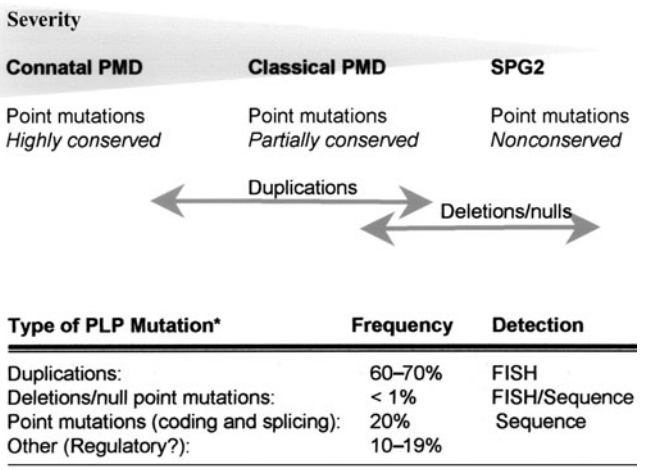
Neurologic signs:	*Nystagmus, spastic paraparesis, limb ataxia*
Family history:	*X-linked recessive pattern of inheritance*
MRI:	*Diffuse central nervous system abnormalities of myelin*
Genetic screening:	*PLP1 gene duplication, deletion, or point mutation*

US Clinical Centers for Mutation Screening at the PLP Locus

Alfred I. duPont Hospital for Children, Wilmington, DE
Main contact: Grace Hobson, PhD (ghobson@nemours.org)
Tel: 302-651-6829
Fax: 302-651-6881
Methodology: DNA sequencing

Baylor College of Medicine, Houston, TX
Kleberg Cytogenetics Laboratory
Tel: 713-798-6555 or 800-411-4363
Fax: 713-798-3157
Methodology: FISH

FISH = fluorescent in situ hybridization to detect *PLP* duplications, a test that is recommended as the first screen for *PLP* mutations.



Type of PLP Mutation*	Frequency	Detection
Duplications:	60–70%	FISH
Deletions/null point mutations:	< 1%	FISH/Sequence
Point mutations (coding and splicing):	20%	Sequence
Other (Regulatory?):	10–19%	

Figure 1. Genotype-phenotype correlations and frequencies of different mutations in Pelizaeus-Merzbacher disease (PMD) and spastic paraplegia type (SPG2). Point mutations exist in each category of Pelizaeus-Merzbacher disease and spastic paraplegia type 2, but the severity of the mutation is directly correlated with the level of conservation of the amino acid.[55] The relative severity of the disease is indicated by the *arrowhead*. Although duplications typically present as the classic form of Pelizaeus-Merzbacher disease and deletions or nulls as the minimally affected spastic paraplegia type 2, individual mutations in each of these categories can be associated with more or less severe phenotypes, as indicated by the *arrows*. An updated list of mutations in the *PLP* gene is provided by Dr James Garbern at <http://www.med.wayne.edu/Neurology/ClinicalPrograms/Pelizaeus-Merzbacher/plp.html>. FISH = fluorescent in situ hybridization.

matter of the cerebral hemispheres, cerebellum, and brain stem.[21,22] Owing to the ongoing synthesis of myelination during postnatal development, hypomyelinated white matter can be difficult to detect until a child reaches 2 years of age. Brain structure is largely normal, although the volume of white matter, including that of the corpus callosum, can be reduced. Less pronounced MRI changes are evident in patients with the milder spastic paraplegia type 2 phenotype, and areas of normal myelin intermingle with hypomyelinated regions.[23,24] The family history of the disease is consistent with an X-linked recessive pattern of inheritance. In the great majority of cases with positive neurologic symptoms, imaging results, and family history, the diagnosis can be unequivocally established with molecular genetic testing to identify a *PLP* gene duplication or a *PLP* gene mutation.

MUTATIONS OF THE MYELIN *PLP* GENE CAUSE PELIZAEUS-MERZBACHER DISEASE AND SPASTIC PARAPLEGIA TYPE 2

Proteolipid protein is an exceptionally well-conserved protein as mammals encode nearly identical proteolipid proteins and no amino-acid polymorphisms exist in the human *PLP* gene. *PLP* gene structure is similarly highly preserved among vertebrates, with an ancestral *PLP* gene found in invertebrates.[25] Proteolipid protein is encoded by a single gene composed of seven exons located on the X chromosome (chromosome Xq22.2).[26–29] The third exon contains an internal donor splice site that is used to generate transcripts encoding the smaller (20 kDa) DM20 isoform. DM20 lacks a portion of the intracellular loop that contains two acylation sites, an absence that may account for the altered conformation and physical properties observed for DM20[30–32] and the inability of DM20 to compensate for a loss of proteolipid protein in myelin.[25,33] The intriguing hypothesis that proteolipid protein acts as a sensor in transmitting information across the lipid bilayer[34] was validated by the discovery that proteolipid protein, but not DM20, interacts with α_v-integrin as part of a signaling complex.[35] Apart from oligodendrocytes, the *PLP* gene is transcriptionally active in the nervous system in olfactory ensheathing cells,[36] satellite cells,[37] and Schwann cells,[18,38,39] in which the predominant isoform expressed is DM20.[40] Schwann cell expression of proteolipid protein and DM20 is an order of magnitude lower than that observed in oligodendrocytes, and most of the proteins produced are not normally incorporated into the myelin sheath.[18,41]

Duplications Predominate in Patients With Pelizaeus-Merzbacher Disease and Spastic Paraplegia Type 2

As predicted by the conserved nature of the *PLP* gene, all types of mutations at the *PLP* locus have a discernible impact in humans. Duplications are most frequently encountered, representing 60 to 70% of cases,[42–45] as depicted in Figure 1. *PLP* duplications occur typically in tandem, involving a large genomic segment that includes neighboring genes.[42,46–48] Striking variation in the size of the duplicated segment exists between different families with Pelizaeus-Merzbacher disease.[46,47] Moreover, occasionally the duplicated copy translocates to another region of the X chromosome.[46,49] Together, the large range in both size and position of the duplicated proteolipid protein segment contributes to the nonuniform phenotypes between families with *PLP* duplications. Different sets of genes will be duplicated and therefore overexpressed in different patients with Pelizaeus-Merzbacher

disease and spastic paraplegia type 2, and another set of genes, those that are interrupted at the breakpoints, will be selectively inactivated in different patients with Pelizaeus-Merzbacher disease and spastic paraplegia type 2. Although gene disruption is likely to yield a discernible phenotype, very few genes are sensitive to dosage effects. In the segments of the X chromosome duplicated in patients with Pelizaeus-Merzbacher disease, *PLP* is apparently the sole gene for which changes in copy number spawn phenotypic aberrations. The majority of patients with duplications present with the classic form of Pelizaeus-Merzbacher disease, although heterogeneity in the clinical course is evident among patients with duplications.[44,46,47] Patients with a milder spastic paraparesis have been described (see Figure 1). The more severe connatal form of Pelizaeus-Merzbacher disease has also been documented in patients who appear to harbor three copies of the *PLP* gene.[46,50]

Mutations That Eliminate Proteolipid Protein Are Rare in Patients With Pelizaeus-Merzbacher Disease and Spastic Paraplegia Type 2

Loss-of-function mutations (absence of proteolipid protein) arise from either deletions of the *PLP* locus or point mutations that eliminate proteolipid protein synthesis. Unlike duplications, deletions of *PLP* occur rarely and involve much smaller segments.[10,51–53] Probably the deletion of larger sections of the X chromosome, which would comprise the majority of reciprocal recombination events arising from duplications of proteolipid protein, would cause lethality or infertility. In addition to the loss-of-function mutations arising from deletion events, two point mutations in the *PLP* coding region at the initiation codon[54] or the second codon[18] are null for proteolipid protein expression. Unlike the *PLP* deletions characterized to date, these null point mutations allow for a direct examination of *PLP* loss without complicating considerations from deletion of those genes neighboring *PLP*, namely, the *ras* superfamily member *RAB9L* and the thymosin β family member *TMSNB*.[52] Although only a handful of patients with the null phenotype have been identified, emerging features of the clinical course point to a Pelizaeus-Merzbacher disease and spastic paraplegia type 2 form of intermediate severity. Onset is within the first 5 years and features ataxia and mild spastic quadriparesis, with peripheral neuropathy as a distinguishing trait of this form.[19] Patients are ambulatory and speech is present but diminishes after adolescence. Life span extends to the fifth to seventh decade.

Point mutations in the most highly conserved domains of proteolipid protein create the most severe phenotype. One fifth of patients with Pelizaeus-Merzbacher disease have point mutations (single base changes or small deletions or insertions) at the *PLP* locus that alter the amino-acid sequence of the proteolipid and DM20 proteins. Approximately 100 distinct missense, nonsense, frameshift, and splicing mutations, all of which produce abnormal proteolipid DM20 proteins, have been identified (for an up-to-date accounting, refer to <http://www.med.wayne.edu/Neurology/ClinicalPrograms/Pelizaeus-Merzbacher/plp.html>). From comparing the genotype and phenotype of 33 families with *PLP* point mutations, Boespflug-Tanguy and colleagues found that changes within the most evolutionarily conserved sites are generally associated with the most severe disease.[10,54] Substitutions of less conserved amino acids, together with protein truncations, null mutations, and mutations within the *PLP1*-specific region (amino acids 116–150), produce a milder phenotype. Although mutations are distributed throughout the proteolipid protein/DM20 coding sequence, mutational "hot spots" exist at the *PLP* locus. The entire external loop has an excessive number of mutations as half of the missense mutations occur within this loop. The susceptibility of this region hints at conformational cues that could be important in proteolipid protein/DM20's maintenance of the intraperiod line in compact myelin.

Additional Screening Efforts Are Required for Splice Site and Regulatory Mutations

Of the handful of splice site mutations uncovered in patients with Pelizaeus-Merzbacher disease, of most interest are the splicing mutations that are not located at the strictly conserved positions in the donor and acceptor splice sites, including a deletion of 19 bp within intron 3 and 26 bp in intron 5.[55–57] This atypical splicing mutation predicts that even more splicing mutations might be found in patients with Pelizaeus-Merzbacher disease and spastic paraplegia type 2, mutations that have eluded detection because sequencing efforts usually concentrate on coding regions and intron/exon junctions. Only one potential regulatory mutation, at position −34 of the *PLP* gene, has been reported in a family with Pelizaeus-Merzbacher disease.[58] Because 10 to 20% of patients with Pelizaeus-Merzbacher disease and spastic paraplegia type 2 lack an identifiable mutation at the *PLP* locus (duplication, deletion, or point mutation in the coding region or splice sites), a significant proportion of Pelizaeus-Merzbacher disease and spastic paraplegia type 2 could be attributable to mutations in the promoter region or *cis* regulatory elements of the *PLP* gene (see Figure 1). Another possibility is the existence of a nearby gene that, when mutated, can also give rise to a proteolipid protein-like Pelizaeus-Merzbacher disease phenotype, as suggested by Cambi and coworkers for families with an X-linked pure spastic paraplegia that map to the *PLP* region yet do not have identifiable mutations in the *PLP* gene.[15,23,57]

Because duplication of the *PLP* region is the most common cause of Pelizaeus-Merzbacher disease, screening for *PLP* gene duplications is the most efficient initial genetic screening test for diagnosing Pelizaeus-Merzbacher disease. Both interphase fluorescent in situ hybridization and quantitative polymerase chain reaction (PCR) have been used to detect *PLP* duplications,[42,47,56,59] and a combination of these methods is optimal for both identifying small duplications and disclosing the cytogenetic location of the duplicated segment. If neither interphase fluorescent in situ hybridization nor quantitative PCR reveals a *PLP* duplication, direct sequence analysis of the *PLP* gene should be performed. Contact infor-

mation for the Pelizaeus-Merzbacher disease and spastic paraplegia type 2 screening centers is provided in Table 2.

DIFFERENT PATHOGENETIC MECHANISMS ACCOUNT FOR THE VARIABLE EXPRESSIVITY OF PROTEOLIPID PROTEIN MUTATIONS IN PELIZAEUS-MERZBACHER DISEASE AND SPASTIC PARAPLEGIA TYPE 2

PLP and proteolipid protein function to maintain the structure of central nervous system myelin and are best described in superlatives: *PLP* is one of the most highly conserved genes, and proteolipid protein is the most abundant protein in central nervous system myelin and one of nature's most hydrophobic proteins. Each of these traits figures in the pathogenesis of mutations in the *PLP* gene. Proteolipid protein is synthesized in the rough endoplasmic reticulum as a tetraspan intrinsic membrane protein oriented with both termini on the cytoplasmic face[32,60] and is subsequently transported through the Golgi complex, where other myelin lipid constituents, such as cholesterol and galactocerebroside, associate with proteolipid protein in "rafts."[61] Raft formation is one of the initial stages of myelin assembly and is followed by the vesicular transport of proteolipid protein to the myelin membrane. Six fatty acid chains are posttranslationally attached to each proteolipid protein molecule,[62] and those fatty acids covalently linked to the intracellular loop of proteolipid protein have been proposed to mediate the association of proteolipid protein with the adjacent lipid leaflet in compact myelin.[33]

A block at any stage of synthesis and assembly of proteolipid into myelin sheaths can have detrimental consequences to oligodendrocytes. An examination of the extensive collection of mutations in the *PLP* gene reveals that individual mutations can exert their influence at different checkpoints. These stopping points at various stages of proteolipid protein biosynthesis are illustrated in Figure 2, and the pathogenetic mechanisms associated with each block are described below. The three mechanisms of pathogenesis are not mutually exclusive. For example, a missense mutation resulting in a malfolded proteolipid protein or DM20 could waken the cell to the need to monitor abnormally folded proteins (pathogenetic mechanism I). If the mutation retards but does not prevent trafficking, the mutant protein can subsequently pose additional problems to oligodendrocytes on assembly into myelin as the abnormal protein could be unable to function properly in myelin maintenance. Apart from the pathogenetic mechanisms described below, additional diversity of phenotypic expression in Pelizaeus-Merzbacher disease and spastic paraplegia type 2 could be attributable to modifier genes that mitigate or aggravate the phenotype within a family.

Pathogenetic Mechanism I: Activation of the Unfolded Protein Response by Abnormal Proteolipid and DM20 Proteins

Gow and Lazzarini originally proposed that the spectrum of clinical severity in patients with different *PLP* coding region mutations can be accounted for by differential effects of individual mutations on the folding and intracellular trafficking of the proteolipid and DM20 proteins.[34] Coding region mutations that curtail the folding and transport to the cell surface of both proteolipid protein and DM20 are associated with the most severe Pelizaeus-Merzbacher disease phenotypes, including oligodendrocyte cell death, whereas mutations that minimally impede proteolipid protein or DM20 transport or selectively impair transport of proteolipid protein but not DM20 produce a less severe Pelizaeus-Merzbacher disease phenotype that is not associated with oligodendrocyte cell death.[34,63] Accumulation of misfolded DM20 or proteolipid protein 1 in the rough endoplasmic reticulum of oligodendrocytes triggers the unfolded protein response, a network of genes that are induced in response to unfolded proteins and serve to regulate expression of molecular chaperones, transcription factors, caspases, and other genes relevant to rough endoplasmic reticulum trafficking.[64] Two transcription factors of the unfolded protein response network, CCAAT/enhacer-binding protein (CEBP) homologous transcription factor (CHOP) (CEBP-homologous protein) and activated transcription factor 3 (ATF3), as well as several rough endoplasmic reticulum–resident molecular chaperones, are overexpressed in oligodendrocytes synthesizing mutant proteolipid and DM20 proteins. Not all components of the unfolded protein response network are induced to the same extent by different mutations, and therein lies the basis for a graded response tailored to individual mutations.[64] The absence of CHOP aggravates the condition of *rumpshaker (rsh)* mice, which harbor an I186T mutation that results in a mild disease phenotype in humans.[13] Thus, CHOP figures prominently in the pathogenesis of Pelizaeus-Merzbacher disease. CHOP, together with the other genes induced during the unfolded protein response in oligodendrocytes, might partially protect oligodendrocytes from the toxic effects of misfolded DM20 and proteolipid protein. Why numerous proteolipid protein missense mutations activate CHOP and other unfolded protein response genes still elicit cell death is best understood in quantitative terms. Small amounts of incorrectly folded proteins fail to activate the unfolded protein response pathway. But as the most abundant myelin protein, proteolipid protein is produced on a monumental scale, and large amounts of any misfolded proteins invariably swing the unfolded protein response to an apoptotic one.

Pathogenetic Mechanism II: Disruption of the Secretory Pathway by Excessive Amounts of Normal Proteolipid and DM20 Proteins

Overexpression of proteolipid and DM20 proteins occurs in patients with duplications of the *PLP* gene. Excessive amounts of normal proteolipid proteins accumulate in the late endosome and lysosomal compartments of rodent cells overexpressing proteolipid protein.[65] Because proteolipid protein typically associates with cholesterol and other lipids to form myelin "rafts" during trafficking through the Golgi complex,[61] the shunting of excess proteolipid protein into the endosomal or lysosomal compartment effectively drains

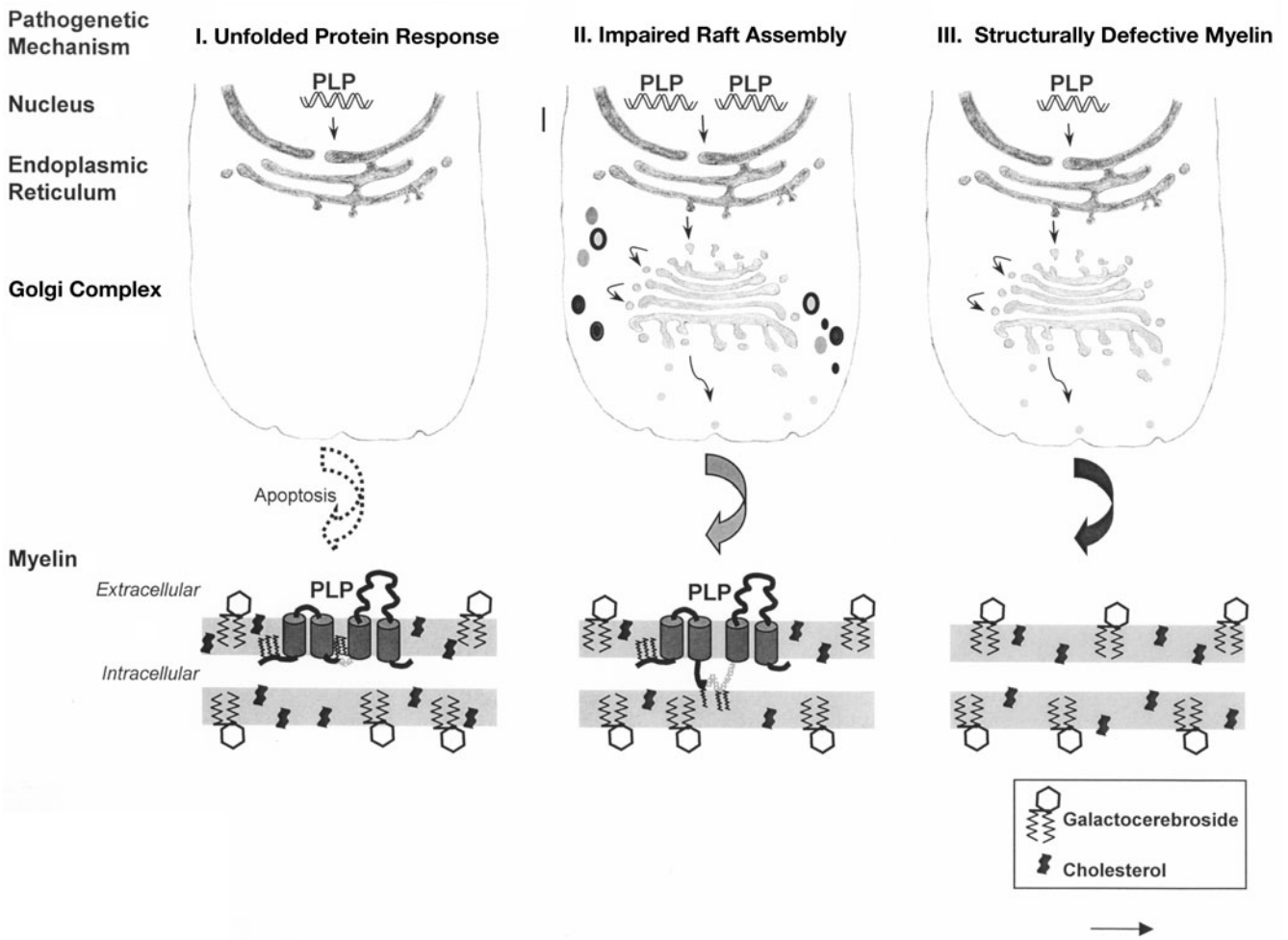


Figure 2. Pathogenesis of Pelizaeus-Merzbacher disease and spastic paraplegia type 2. The diversity of phenotypic expression in Pelizaeus-Merzbacher disease and spastic paraplegia type 2 is attributable in part to three distinct pathogenetic mechanisms. In the unfolded protein response, many point mutations create abnormally folded proteolipid proteins (PLP) in the rough endoplasmic reticulum, which triggers the unfolded protein response pathway.[64] In severe cases, the cellular responses culminate in cell death and the consequent absence of myelin. In less severe cases, oligodendrocytes survive, but their myelin contains abnormal proteolipid proteins, which might not be able to link adjacent myelin bilayers (as proposed by Stoffel and coworkers[33] and illustrated for the normal proteolipid proteins in mechanism II). In impaired raft assembly, overexpression of normal proteins from a duplication of the *PLP* gene, which occurs in the great majority of patients with Pelizaeus-Merzbacher disease and spastic paraplegia type 2, disrupts a subsequent stage of myelin biosynthesis in the Golgi complex. Excess proteolipid proteins, together with lipids such as cholesterol and galactocerebroside that normally associate with proteolipid protein rafts in the Golgi complex, can be found in lysosomal and endosomal vesicles.[65] The sequestration of particular lipids can alter the composition of rafts destined for myelin assembly. Consequently, despite the presence of normal proteolipid protein, the assembled sheaths could be unbalanced in other myelin constituents. In the third mechanism, structurally defective myelin, deletions of the *PLP* gene, or null point mutations give rise to myelin sheaths lacking proteolipid protein. These loss-of-function mutations are unable to maintain compact myelin and to promote interactions with axons.[19] The six fatty acids attached to proteolipid protein are depicted by the zigzag lines, and the segment of the proteolipid protein that is missing in DM20, which contains two acylation sites, is a lighter shade.

myelin lipids from the Golgi complex.[65] Consequently, the transport and assembly of myelin constituents are altered in cells overexpressing proteolipid protein and DM20. Thus, whereas abnormal proteolipid proteins trigger a protein misfolding response in the rough endoplasmic reticulum, excessive proteolipid proteins create an imbalance in myelin constituents that adversely affects the subsequent stage of nascent myelin assembly in the Golgi network. The myelin produced in such cells contains normal proteolipid and DM20 proteins but can be structurally flawed from deranged ratios of myelin lipid and protein constituents.

Pathogenetic Mechanism III:
Breakdown in Myelin Maintenance When
Proteolipid and DM20 Proteins Are Absent

Loss of function occurs in patients with a deletion of the *PLP* gene[10,51,52] or with point mutations at the beginning of the coding region that preclude translation.[18,54] These patients have less severe forms of the disease, with the *PLP* deletions giving rise to either a complicated form of spastic paraplegia type 2 or a mild form of Pelizaeus-Merzbacher disease.[52] In mice lacking proteolipid protein, oligodendrocytes develop normally and manage to assemble a myelin sheath, yet

defects in the intraperiod line of these sheaths translate into reduced conduction velocities and impaired motor coordination.[66–70] In addition, null mutations are associated with axonal pathology.[19,20] In toto, these pathologic changes document an absolute requirement for proteolipid protein both to maintain the structure of compact myelin and to maintain axonal integrity and function. Thus, the absence of proteolipid protein would neither trigger the unfolded protein response nor derail myelin assembly but would instead negatively affect maintenance of the myelin sheath.

PARADOXICAL EXPRESSION OF DISEASE IN HETEROZYGOUS FEMALE SUBJECTS

Females heterozygous for a *PLP* gene mutation typically do not display neurologic symptoms. Surprisingly, families with very mildly affected male subjects tend to have female "carriers" with late-onset clinical manifestations of Pelizaeus-Merzbacher disease and spastic paraplegia type 2.[16,71,72] One other class of female subjects with signs of Pelizaeus-Merzbacher disease and spastic paraplegia type 2 has been described. Female subjects with a duplication of the *PLP* gene have infrequently presented as index cases, but, in these cases, the relatively mild Pelizaeus-Merzbacher disease and spastic paraplegia occur with an early onset, and sustained clinical improvement has been noted over time.[73] Transient neurologic symptoms during childhood have likewise been observed in rare cases of female heterozygotes with missense *PLP* mutations that result in a severe form of the disease in male members of the family.[74]

What pathogenesis underlies the expression of a mutant X-linked gene in female subjects? The X chromosome is subject to random inactivation, so female subjects heterozygous for a *PLP* mutation theoretically express the mutant gene in half of the oligodendrocyte population. Skewed X inactivation can arise stochastically or can appear secondarily as a result of selective survival of one cell population. The latter scenario appears to apply to those *PLP* mutations that cause moderate to severe Pelizaeus-Merzbacher disease in affected male subjects.[75] The most severe *PLP* mutations trigger not only the unfolded protein response but also one of the apoptotic pathways. Presumably, in female carriers, the oligodendrocyte population expressing the *PLP* mutation undergoes cell death during the major build-up of myelin and is gradually replaced by normal oligodendrocytes. Such a phenomenon has been documented in the canine model of Pelizaeus-Merzbacher disease, in which female heterozygotes have neurologic abnormalities early in life but by adulthood are neurologically asymptomatic, have a normal complement of oligodendrocytes, and express very little mutant *PLP* messenger ribonucleic acid.[76] The transient neurologic abnormalities detected in some female children who are carriers of severe *PLP* mutation[74] therefore represent the "catch-up" phase during which the normal oligodendrocyte population fills the void left by apoptotic cells.

Skewed X-inactivation or selective survival does not figure in the pathogenesis of the mild *PLP* mutations. Although oligodendrocytes expressing mild *PLP* mutations can elicit an unfolded protein response, that response does not lead to cell death. Consequently, two populations of oligodendrocytes will coexist in the central nervous system of these female subjects, one that churns out normal myelin and the other manufacturing a flawed product. Over time, the structurally unstable myelin, which cannot be readily displaced by the normal population of oligodendrocytes, becomes functionally inadequate. Paradoxically then, female carriers who are heterozygous for the less severe *PLP* mutations are more likely to experience neurologic difficulties as adults than are female carriers who are heterozygous for the more severe *PLP* alleles.[72–74]

PROSPECTUS

Noteworthy advances on the Pelizaeus-Merzbacher disease and spastic paraplegia type 2 front over the past decade include (1) the enhanced screening capabilities for *PLP* mutations, (2) the formation of a Pelizaeus-Merzbacher disease foundation, and (3) progress in the development of cellular therapies for treating neurologic disorders. Screening for mutations in the *PLP* gene is available from three centers in the United States (see Table 2) and is definitive in approximately 80% of cases. Prenatal diagnosis of duplications by fluorescent in situ hybridization or point mutations by DNA sequencing is also possible.[48,59,77] The Pelizaeus-Merzbacher Disease Foundation promotes awareness of the disorder, links families with clinicians and researchers, and helps to fund research initiatives. Driven by a core group of families with Pelizaeus-Merzbacher disease, the foundation maintains an active Web site (<http://www.pmdfoundation.org/>). Strategies for treatment of Pelizaeus-Merzbacher disease and spastic paraplegia type 2 must be guided by our understanding of the pathogenesis of the disease. The fact that defective oligodendrocytes are replaced by normal oligodendrocytes in heterozygous female subjects[76] highlights the potential for transplanted oligodendrocyte precursors to correct Pelizaeus-Merzbacher disease. Indeed, most of the progress in glial cell transplantation has occurred in animal models of Pelizaeus-Merzbacher disease,[78–80] and the results are therefore directly relevant to future clinical trials with patients with Pelizaeus-Merzbacher disease. Although promising in terms of the extent to which transplanted cells produce myelin, cellular therapy still needs optimization to reverse clinical deficits. Also, transplantation could yield the maximal benefit when initiated shortly after birth. The future convergence of several events, namely the rapid diagnosis of the disease (made possible by an awareness of the disease within the pediatric and neurologic communities and the availability of screening centers), the active participation of families with Pelizaeus-Merzbacher disease (through the Pelizaeus-Merzbacher Disease Foundation) and further breakthroughs in transplantation research should propel treatment strategies for Pelizaeus-Merzbacher disease.

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