The Molecular Pathogenesis of Pelizaeus-Merzbacher Disease

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In 1885, Pelizaeus\(^1\) described 5 boys in a single family with nystagmus, spastic quadripare-sis, ataxia, and delay in cognitive development. In 1910, Merzbacher\(^2\) reexamined this family, which then included 14 affected individuals, including 2 girls, and found that all affected family members shared a common female ancestor. Also, he noted that the disease was passed exclusively through the female line without male-to-male transmission. Pathological analysis of brain tissue from one affected individual showed that most of the central white matter lacked histochemical staining for myelin, although there were occasional small regions of preserved myelin, giving the sections a “tigroid” appearance. The description of this family provides the clinical, genetic, and pathological basis for Pelizaeus-Merzbacher disease (PMD): an X-linked disorder of myelination classically characterized by nystagmus, spastic quadriparesis, ataxia, and cognitive delay in early childhood.

In the succeeding years, studies of PMD focused mainly on its interesting neuropathological features rather than on its genetics, thus creating considerable nosological confusion in the field. Seitelberger’s\(^3\) influential article, for example, which reanalyzed the clinical neurological and neuropathological features of PMD, contains a genetically diverse set of sporadic and dominantly inherited disorders that share the pathological finding of tigroid or patchy preservation of central nervous system (CNS) myelin, but that have little clinical similarity to the original cases of Pelizaeus and Merzbacher. Reconsideration by Zeman et al\(^4\) and Bouloche and Aicardi\(^5\) of the original descriptions of PMD by Pelizaeus and Merzbacher, however, reincorporated the genetic and clinical, as well as the neuropathological, criteria for its diagnosis. This comprehensive approach to the diagnosis of PMD has been critical for the discovery of the mutations responsible for causing the disease.

With the use of both clinical and genetic descriptions for the identification of families with PMD, mutations in the proteolipid protein (PLP) gene (PLP), which encodes one of the major CNS myelin proteins, have been found in the majority of PMD cases. More than 60 point mutations in the PLP coding region have been identified, and these account for approximately 15% to 20% of PMD cases.\(^6\) (A database of these mutations is maintained at [http://www.med.wayne.edu/Neurology/plp.html](http://www.med.wayne.edu/Neurology/plp.html).) Recent data have demonstrated that PMD can also be caused by duplication of a portion of the X chromosome that contains the PLP gene, and this mutation probably accounts for 50% to 75% of the cases of PMD.\(^7\) Individuals with clinical PMD but without a PLP coding mutation have also been identified, however, suggesting that mutations in the PLP regulatory regions or mutations at other gene loci can also cause PMD.

As with other genetic disorders, the range of the clinical phenotype in PMD has expanded since the identification of its cause. Mutations in PLP have been subsequently found to produce a wide spectrum of neurological disease, from mild X-linked spastic paraparesis type 2 (SPG2) without other significant neurological findings to severe quadriplegia associated with congenital nystagmus, seizures, and cognitive impairment (“congenital PMD”). The molecular basis for this phenotypic vari-
ability is not completely understood, but is probably attributable to differences in the cellular effects of individual PLP mutations. In addition, similar clinical variability can also occur within families with the same PLP mutation, suggesting that modifier genes and/or genetic background plays a role in producing clinical variability. Since considerable progress has been made in the last several years in understanding the molecular pathogenesis of PLP mutations, both in patients with PMD and in animal models, we will focus the remainder of this review on the molecular pathogenesis of PLP mutations. The information gleaned from these studies not only is relevant for PMD, but also provides important clues into the molecular pathogenesis and possible treatment of other myelin diseases, such as multiple sclerosis.

**MYELIN AND PLP**

Myelin is a multilamellar membrane structure that surrounds axons in both the CNS and the peripheral nervous system (PNS). The myelin membrane is synthesized by glial cells, and consists of multiple wraps of extended glial membrane processes that compact to form the mature myelin sheath. Myelination is an important developmental process, since it is necessary for rapid conduction of axonal impulses, and accounts for a significant proportion of brain growth after birth.

In the CNS, myelin is synthesized by oligodendrocytes. Each oligodendrocyte myelinates up to 50 axonal segments, synthesizing approximately 1000-fold more membrane than its perikaryal surface area in the process. Lipids make up approximately 70% of the total mass of myelin, while a set of myelin-specific structural proteins make up the rest. Proteolipid protein is the major structural protein of CNS myelin, and makes up about 25% of the protein mass of myelin. Although PLP was initially identified as a major myelin structural protein in the CNS, it is also expressed by Schwann cells, which are the myelinating cells of the PNS. In the PNS, however, PLP is only a minor constituent and composes less than 1% of the mass of PNS myelin proteins.

Proteolipid protein, which is an extremely hydrophobic membrane protein of 276 amino acids, has 4 putative membrane-spanning domains (Figure) and is covalently coupled to fatty acids at cysteine residues, making the protein even more hydrophobic. The PLP gene undergoes alternative splicing to produce 2 proteins, PLP and DM20, that are identical except for a portion of the second intracellular membrane loop (amino acid residues 116-150), which is absent in DM20 (Figure). Because of the absence of these amino acid residues, the alternatively spliced DM20 is even more hydrophobic than PLP itself.

Although PLP and DM20 have been studied extensively for many years, their biological functions are still not known in detail. The amino acid sequence of PLP is identical in mouse, rat, and human and is highly conserved among other vertebrate species, implying that PLP plays an important structural and functional role in myelin. Mice in which PLP expression has been inactivated, however, demonstrate only subtle defects in the ultrastructure of CNS myelin,6 demonstrating that neither PLP nor DM20 is necessary for normal myelin assembly to occur. Eventually, these mice do develop clinical signs of neurological dysfunction, which is, surprisingly, caused by widespread Wallerian degeneration of CNS axons, rather than by demyelination.9 Since Wallerian degeneration is not found in shiverer mice, which do not express the other major CNS myelin protein, myelin basic protein, these data demonstrate that PLP expression in oligodendrocytes is necessary for the maintenance of normal axonal integrity, probably through direct oligodendrocyte-axonal interactions. This important point will be discussed further below.

As stated above, PLP is also expressed in Schwann cells in the PNS, but in considerably smaller amounts than in oligodendrocytes in the CNS. Unexpectedly, the absence of PLP and DM20 expression in individuals carrying a PLP null mutation causes a demyelinating peripheral neuropathy,10 demonstrating that PLP or DM20 is also necessary for normal peripheral myelin function. The effect of other types of PLP mutations on peripheral nerve function, however, has not been studied in detail.

**GENETIC MECHANISMS CAUSING PMD**

Clinical observations and studies of PLP mutations in animals and cell cultures suggest that there at least 3 distinct genetic mechanisms that cause PMD. The first of these mechanisms produces loss of PLP function, in which PLP does not accumulate in the cell. To date, 4 so-called null mutations that cause PMD have been identified, including a deletion of the entire PLP gene, all of which produce a similar, relatively mild clinical phenotype, characterized by moderate spastic quadriplegia, mild cognitive delay, ataxia, and demyelinating peripheral neuropathy (see above).10

The second of these genetic mechanisms produces a gain-of-toxic function. Experimental evidence supports this mechanism, which was originally suggested by Gow et al.11 Gain-of-function mutations, typically amino acid substitutions, prevent PLP from reaching the cell surface by disrupting normal PLP folding. The mutant protein then accumulates in the endoplasmic reticulum, somehow triggering increased oligodendrocyte cell death by apoptosis, with resultant dysmyelination.12,13 The clinical phenotype caused by a gain-of-function mutation depends on the location of the altered amino acid, as well as on the particular amino acid substituted. For these reasons, these mutations produce a range of PMD phenotypes, including the most severe, characterized by congenital nystagmus, hypotonia, severe spastic quadriplegia, and seizures, as well as a relatively mild phenotype, characterized by only mild spastic paraparesis.14

The third and most common genetic mechanism that causes PMD is duplication of the region of the X chromosome that contains the PLP gene.2 Since overexpression of PLP and/or DM20 is sufficient to cause both CNS dysmyelination and subsequent demyelination in transgenic mice,15,16 the human duplication probably produces PMD for similar reasons. The molecular mechanisms underlying the PLP duplication have not yet been elucidated, but appear to be more complex than those observed in the duplications responsible for Charcot-
Marie-Tooth disease 1A, in which a large repeat sequence facilitates nonreciprocal homologous recombination.\(^\text{18}\) The breakpoints of the PLP duplication often vary between patients, and inclusion of flanking genes in addition to PLP and/or disruption of a flanking gene may explain differences in phenotypic severities among patients with PLP duplication.\(^\text{19,20}\) Quite remarkably, 2 patients have been identified in whom the duplicated region was inserted at loci on the X chromosome that are quite distant from the normal location at Xq22.\(^\text{21}\) The most common clinical phenotype of PMD caused by PLP duplications corresponds to the classic form of PMD.\(^\text{7,22}\) Some infants with the duplication, however, develop the severe connatal syndrome.\(^\text{23}\)

The nomenclature of clinical syndromes caused by PLP mutations has caused confusion and some controversy, and stems from the variability of syndromes caused by different mutations and from the variable expressivity of an individual mutation among family members. It is also likely that environmental as well as genetic background factors modify the neurobiological consequences of an individual mutation. The most consistent features of PMD include spasticity, a lack of evidence of male-to-male transmission in the family, and generalized leukodystrophy on magnetic resonance imaging scans. However, even these relaxed criteria, applied too strictly, might exclude some patients who have only ataxia and tremor, or those infants with severe mutations who have hypotonia at onset. Since duplications now appear to be the most prevalent mutation mechanism resulting in the PMD phenotype, duplication analysis should be performed before a search for point mutations is begun. There is potential for mutations lying in regulatory regions of the PLP gene that at this time are not being examined by current clinical testing methods. In addition, there may be other gene loci that can also give rise to a PMD phenotype.

Proteolipid protein sequence depicted in its likely conformation in the lipid bilayer. The clinical severities were scored as follows: severe, apparent in the first few months of life and never ambulated or acquired significant verbal communication (includes the “connatal” and “intermediate” forms); moderate, spastic quadriplegia or paraplegia with some cognitive impairment, dystonia, and/or ataxia and typically learned to walk and speak; and mild, predominantly X-linked spastic paraparesis type 2 (SPG2).
MOLECULAR PATHOGENESIS OF PMD: THE PROTEIN-MISFOLDING HYPOTHESIS

As mentioned above, gain-of-function mutations inhibit the ability of PLP and/or DM20 to be transported through the endoplasmic reticulum to the cell surface,\textsuperscript{12,13} probably because of protein misfolding. The differential effect of these mutations on the folding and transport of either PLP and/or DM20 can explain, at least in part, the observed differences in clinical severity of PMD among families with point mutations. Mutations that affect the folding and transport to the cell surface of both PLP and DM20 are associated with the most severe PMD phenotypes, and also cause increased oligodendrocyte cell death. Mutations that affect the transport of PLP but not of DM20, however, produce a less severe PMD phenotype, and do not cause increased oligodendrocyte cell death.\textsuperscript{12} In contrast, null mutations, in which no functional PLP or DM20 is made, are usually associated with a relatively mild clinical phenotype, and do not cause increased oligodendrocyte cell death or arrest of myelin gene expression. The molecular mechanism(s) by which accumulation of misfolded PLP and DM20 in the endoplasmic reticulum causes apoptosis of oligodendrocytes is not known, but is clearly important for further understanding and treatment of this disease.

Not only does the protein-misfolding hypothesis explain the differential clinical effect of PLP mutations, it also explains the effect of these mutations on female carriers. Female dogs that are heterozygous for a severe mutation in the canine PLP, for example, have neurological abnormalities early in life, but by adulthood are clinically normal, have normal numbers of oligodendrocytes, and express very little mutant PLP messenger RNA.\textsuperscript{23} Female PMD carriers are also usually clinically unaffected, although some may have transient neurological abnormalities as children.\textsuperscript{25} In some PMD families, however, female heterozygotes are clinically affected, as in the family described by Pelizaeus\textsuperscript{1} and Merzbacher.\textsuperscript{2} Because of random inactivation of the X chromosome on which the PLP gene is located, females who are heterozygous for PLP mutations should express the abnormal protein in approximately 50\% of their oligodendrocytes. Oligodendrocytes expressing a more severe PLP mutation, however, in which both PLP and DM20 are affected, undergo increased cell death and are eliminated during myelination and replaced by normal oligodendrocytes. In contrast, oligodendrocytes expressing a less severe PLP mutation, which does not cause cell death, are not eliminated, thus producing abnormal myelin and neurological dysfunction. Paradoxically then, females who are heterozygous for the less severe PLP mutations are more likely to experience neurological difficulties as adults than are females who are heterozygous for the more severe PLP alleles.\textsuperscript{10,20} Similar observations have been observed with experimental and naturally occurring murine PLP mutations (Ian Griffiths, DVM, PhD, oral communication, February 1998).

PLP MUTATIONS CAUSE AXONAL DAMAGE IN THE CNS

Evidence of axonal damage has been recently found in both PMD and its animal models, a finding that is important for future understanding of the pathogenesis of demyelinating disease and its treatment. In his original description of the neuropathological features of PMD, Merzbacher\textsuperscript{2} noted: \textit{Es stellt sich n"amlich heraus: daß dort, wo die Markscheiden fehlen, auch keine Achsencylinder nachweisbar sind} (It is evident that there are no axons demonstrated where the myelin sheaths are absent). Merzbacher, however, was not convinced that there was axonal damage, and he subsequently concluded that axons were much thinner and did not stain well with axonal stains. Conclusive evidence for axonal damage in PMD, however, has been found in several rodent models, including those caused by PLP point mutations, increased PLP gene dosage, or PLP null mutation.\textsuperscript{9,13,15,17} Consistent with this interpretation, we have found a significant decrease in the N-acetyl aspartate/creatine ratio using magnetic resonance spectroscopy both in mice overexpressing PLP due to increased gene dosage and in patients with PMD (J.G., J.K., and Gregory Moore, PhD, unpublished data, 1998). In animals carrying a PLP null mutation, this axonal injury is not caused by demyelination, since myelin in these animals is intact, or by oligodendrocyte cell death, since these cells appear healthy and ensheathe axons. Also, the extent of axonal injury increases with the age of the animal, and probably accounts for the onset of its neurological signs and symptoms.\textsuperscript{9} These data thus demonstrate that progressive axonal damage is a common feature of the pathogenesis of PMD and that it is clinically relevant. Furthermore, they suggest that axonal damage is caused by abnormalities of oligodendrocyte-axon interactions that are, at least in part, mediated by PLP or DM20.

Interestingly, the axonal abnormalities in PMD are very similar to those recently described by Trapp et al\textsuperscript{27} in multiple sclerosis (MS), suggesting that the axonal abnormalities in MS, like those in PMD, may also be caused by disruption of oligodendrocyte-axonal interactions rather than by direct immune attack on neurons. Axonal degeneration is also clinically relevant in MS, since the N-acetyl aspartate/creatine ratio is decreased in the brains of patients with MS, even in regions outside of MS lesions, and correlates well with clinical disability.\textsuperscript{28} Also, axonal damage in MS may underlie the secondary progressive phase of the disease, which does not respond significantly to immune modulation. Further understanding of the mechanisms of axonal degeneration in PMD will thus also be important in MS, and may lead to the development of new treatment strategies for both diseases.

MANAGEMENT AND FUTURE PROSPECTS

Currently, there is no specific therapy for patients with PMD. The observation that most of those affected by PMD either overexpress PLP or have a gain-of-function mutation precludes simple replacement gene therapy, even if appropriate delivery vehicles were to become available. Indeed, for most patients, the more appropriate goal might be the reduction of PLP expression, such as through antisense gene therapy, since absence of PLP results in a less severe syndrome. The finding that axoglial interactions are disrupted in PMD also raises the possibility that therapy directed at maintaining the integrity of axons may be ef-
fective in this syndrome. Cellular therapy, such as transplantation of oligodendrocyte precursors into the CNS, has shown potential in animal models of PMD and may therefore be effective in patients with PMD. However, for maximum effectiveness, therapy may need to be started in utero or shortly after birth.²⁹

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