article

Quantifying the carrier female phenotype in Pelizaeus-Merzbacher disease

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Purpose: Pelizaeus-Merzbacher disease and spastic paraplegia type 2 are allelic *X*-linked disorders that principally affect males and are caused by mutations in the proteolipid protein 1 gene. Neurologic symptoms are occasionally observed in carrier females, and anecdotal evidence suggests that these clinical signs are more likely in families with affected males. We analyze 40 pedigrees to determine whether such a link exists. **Methods:** From a chart review of patients from Wayne State University, we categorize patients according to disease severity and type of genetic lesion within the proteolipid protein 1 gene. We then analyze the clinical data using nonparametric *t* tests and analyses of variance. **Results:** Our analyses formally demonstrate the link between mild disease in males and symptoms in carrier female relatives. Conversely, mutations causing severe disease in males rarely cause clinical signs in carrier females. The greatest risk of disease in females is found for nonsense/indel or null mutations. Missense mutations carry moderate risk. The lowest risk, which represents the bulk of families with Pelizaeus-Merzbacher disease, is associated with proteolipid protein 1 gene duplications. **Conclusions:** Effective genetic counseling of Pelizaeus-Merzbacher disease and spastic paraplegia carrier females must include an assessment of disease severity in affected male relatives. ***Genet Med* 2006:8(6):371–378.**

Key Words: *X-inactivation, leukodystrophy, oligodendrocytes, myelin, lyonization*

Pelizaeus-Merzbacher disease is a rare *X*-linked genetic disorder initially described by Pelizaeus in 1885[1] and subsequently by Merzbacher in 1910.[2] Pelizaeus-Merzbacher disease stems from hypomyelination in the central nervous system (CNS), and, until recently, diagnosis has been based on clinical findings. Linkage of Pelizaeus-Merzbacher disease to the *proteolipid protein 1* (*PLP1*) gene was established by Willard and Riordan[3] and subsequently demonstrated in patients by Gencic et al. and Hudson et al.[4,5] The spectrum of phenotypes in males is broad and ranges from severe disease (connatal Pelizaeus-Merzbacher disease) associated with quadriparesis, severe cognitive impairment, and death before the third decade of life to the classic form associated with paraparesis, cognitive impairment, and death in the third to seventh decades, which overlaps clinically with spastic paraplegia type 2 (SPG2), to mild disease associated with lower limb spasticity, normal intelligence, and life span, which is designated as pure spastic paraparesis.[6–8]

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Two gene products arise from the *PLP1* gene, PLP1 and its smaller splice isoform DM-20, which lacks 35 amino acids in the central cytoplasmic domain.[9] Both proteins share the same topology in the membrane and have four transmembrane domains with the amino- and carboxyl-termini exposed to the cytoplasm.[10] Together, these structural proteins comprise approximately 50% of the total protein in CNS myelin where they appear to serve an adhesive or membrane-stabilizing function. In addition, PLP1 is required to maintain the integrity of the myelin-axon unit to ensure the long-term stability of white matter tracts.[11]

More than 100 missense mutations, small insertions or deletions (indels), and nonsense mutations have been identified in the coding region of the *PLP1* gene (http://www.geneclinics.org/profiles/pmd), which account for 15% to 20% of Pelizaeus-Merzbacher disease pedigrees. Gene duplications predominate, accounting for 50% to 75% of pedigrees, and molecular studies indicate that chromosome breakpoints are heterogeneous between pedigrees but may correlate with disease severity.[12] Gene deletions and other presumed mutations in regulatory regions of the *PLP1* gene constitute the remaining percentage of Pelizaeus-Merzbacher disease cases.[13,14] Studies in vitro and in animal models suggest that each of these genetic lesions causes disease by distinct mechanisms.[15] Thus, missense and indel mutations destabilize the three-dimensional structure of *PLP1* gene products and cause intracellular protein accumulation.[16–21] Duplications appear to disrupt cholesterol trafficking,[22] and null mutations disrupt axonal transport mechanisms.[23,24]

To date, most studies seeking to define correlations between disease severity and *PLP1* mutations have been limited to male patients, whereas characterization and analysis of clinical signs in carrier females has received scant attention beyond documentation in case studies. Undoubtedly, disproportionate attention to cause in carriers stems in part from incomplete penetrance in this patient group, particularly in pedigrees harboring gene duplications or deletions.[25] Such variability necessitates an examination of many pedigrees to achieve statistically meaningful data. Furthermore, symptomatic carriers often exhibit only transient signs in childhood or late-onset Pelizaeus-Merzbacher disease,[13,25] and relevance to the pathophysiology in males is unclear.

In the current study, we undertake a quantitative analysis of Pelizaeus-Merzbacher disease-related symptomatology in carrier females from 40 pedigrees evaluated at Wayne State University (WSU) to determine whether clinical symptoms in female carriers at some point in their life are correlated with disease severity or particular types of genetic lesions in the *PLP1* gene. We find that mutant *PLP1* alleles conferring mild disease in males are more likely to cause disease in carrier females. Conversely, severely affected males harbor mutations that rarely cause clinical signs in female relatives.

METHODS

Chart review of Wayne State University study participants

Participants in this study are derived from two institutional review board-approved Pelizaeus-Merzbacher disease research protocols at WSU. All participants harbor known *PLP1* gene mutations and have undergone a standardized evaluation to determine the degree to which disease affects several neurologic metrics, including education level and employment history, speech, writing ability, personal hygiene and self-care (feeding, dressing, toileting), breathing, sitting, and walking. From this examination, disease severity for each patient has been assigned a numeric score based on a phenotypic assessment scheme developed from earlier studies.[8,13,26–29] This scheme, designated the Phenotypic Classification Score (PCS), is a 6-point scale from 0 (neurologically normal) to 5 (connatal Pelizaeus-Merzbacher disease) that correlates with generally accepted clinical subtypes of Pelizaeus-Merzbacher disease and spastic paraparesis as described in the literature. Table 1 shows the details of the PCS and lists typical clinical signs observed in affected males. Autonomic dysfunction refers to urinary incontinence resulting from bladder spasticity. In females, these symptoms largely overlap but, rarely, may include cognitive decline, psychosis, and mild peripheral neuropathy. Symptoms in females may resolve over time unless unfavorable skewed *X*-inactivation is overlaid on the Pelizaeus-Merzbacher disease phenotype.

The PCS assigned to each carrier female has been paired with the PCS from a first-degree male relative (i.e., sibling or son) to enable an assessment of the relative extent to which females are affected by *PLP1* mutations. In pedigrees with more than one female or male examined, PCSs have been averaged.

Table 1
Equating male and female clinical phenotypes with the Phenotypic Classification Score

Phenotype	PCS	Major clinical features
Normal	0	Neurologically normal
Pure spastic paraparesis (SPG)	1	Spastic gait, autonomic dysfunction, normal intelligence and life span
Complicated spastic paraparesis (SPG2)	2	±Nystagmus, ataxia, spastic gait, mild to moderate cognitive impairment, autonomic dysfunction, survival into sixth decade
Classic PMD	3	Nystagmus, initial hypotonia followed by spastic quadriparesis, usually never walks, titubation, ataxia, mild to moderate cognitive impairment, survival into sixth decade
Transitional/severe PMD	4	Nystagmus, spastic quadriparesis, moderate to severe cognitive impairment, survival to third or fourth decade
Connatal PMD	5	Neonatal nystagmus, seizures, stridor and severe hypotonia followed by spastic quadriparesis, severe cognitive impairment, nonverbal, usually fatal by second decade

PMD, Pelizaeus-Merzbacher disease; PCS, Phenotypic Classification Score; SPG, spastic paraplegia; SPG2, spastic paraplegia type 2.

Analyses of the Wayne State University study cohort

In this study, we examine the WSU study cohort data using three analyses. The first analysis examines correlations between female scores and paired male scores. The second analysis examines correlations between female scores and male scores after dividing pedigrees into five subgroups that are based on the consequence of mutations identified in the *PLP1* gene (Mutation-Consequence). The subgroups are as follows: Overexpression, which includes duplications and triplications of the entire *PLP1* gene; Null, comprising deletion of the entire gene; Missense, including single-base changes in the open reading frame (ORF) that cause the substitution of one amino acid for another; Nonsense/Indel, including single-base changes or small insertions or deletions (indels) in the ORF or RNA splice junctions that truncate the ORF by generating a translation stop codon or shifting the reading frame, and; Other, which includes disease in two families caused by either the deletion of an evolutionarily conserved motif located at the 5′ end of intron 3[30] or a point mutation in exon 3B that may interfere with DM-20 splicing by antagonizing splicing factor binding.[31]

The third analysis examines correlations between female scores and male scores after dividing pedigrees into three subgroups that are based on categories of mutations within the *PLP1* gene (Mutation-Category). The subgroups are as follows: Entire Gene, which refers to duplications or deletions of the entire *PLP1* gene; Exon, which refers to missense and nonsense mutations, as well as small insertions or deletions (indels) located within the coding region of the *PLP1*; and Intron, which refers to mutations and indels located within introns and usually found in proximity to RNA splicing signals.

Statistical analyses

In the current study, we organized the patient data into 40 pedigrees and calculated the arithmetic mean of PCS for pedigrees in which multiple family members were clinically evaluated. Frequency distributions were determined, and statistical analyses were performed on these data using GraphPad Prism version 4.0c for MacIntosh OSX (GraphPad Software, San Diego, CA).

We used nonparametric *t* tests and one-way or two-way analysis of variance (ANOVA) to analyze the frequency data summarized in the current study. We chose these nonparametric tests because of the non-Gaussian distributions of some of the data and the relatively small sample sizes in some subgroups. The tests used include the following: the two-tailed Wilcoxon signed rank-sum test (paired *t* test) to determine whether disease severity in all females is of similar or differing severity compared with all males; the Spearman rank-order test (correlation test) to examine the correlation between female PCSs and those of their paired male relatives; the Kruskal-Wallis one-way ANOVA to evaluate relationships between PCS and Mutation-Consequence (see details of these categorizations above) with Dunn's multiple comparison tests to perform post-test pair-wise comparisons of the Mutation-Consequence subgroups; the Friedman two-way repeated-measures ANOVA to evaluate relationships between gender and PCSs categorized according to Mutation-Consequence; the two-tailed Mann-Whitney test (unpaired *t* test) to evaluate relationships between PCS and Mutation-Category (see details of these categorizations above), and; contingency tables using the Fisher exact test to examine the relationship between female carrier PCSs in the Entire Gene Mutation-Category.

RESULTS

The clinical data analyzed in this study was compiled from a chart review at WSU (the WSU study cohort), for which details of female and paired male phenotypes are available. These details enabled us to assign a PCS for all patients according to the criteria defined in Table 1. A total of 56 carrier females and 55 males from 40 pedigrees (i.e., n = 40) are included in the WSU study cohort.

Disease severity is lower in carrier females than paired males

Initially, we analyzed PCS frequency distributions from the WSU study cohort using a two-tailed Wilcoxon rank-sum test to determine whether disease severity in carrier females differs statistically from paired males (Fig. 1). In this cohort, the highest PCS we have assigned is 2, and approximately 80% of female carriers have no clinical symptoms or history of disease. Male patients in 23% of families are mildly affected ($1 \leq PCS \leq 2$), and 23% are severely affected (PCS = 5). The remaining 54% have intermediate forms of Pelizaeus-Merzbacher disease.

The PCS distribution for females is to the left of the male distribution, and the Wilcoxon test confirms this difference

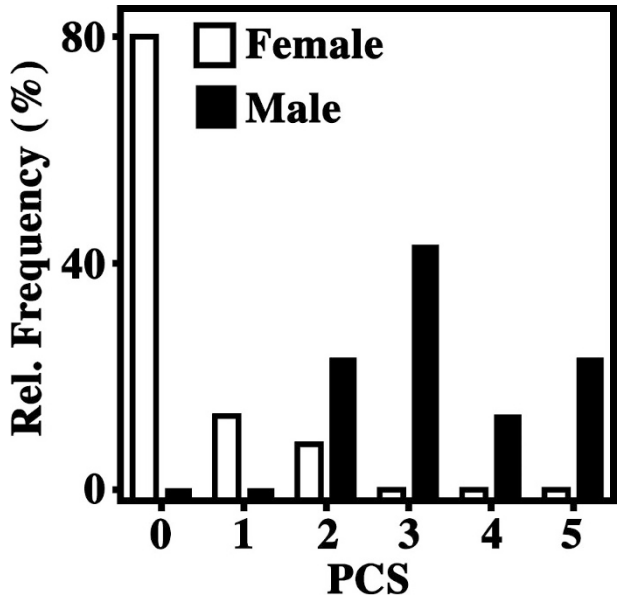

Fig. 1. Histogram showing frequency distribution of disease severity for females and males within the Wayne State University (WSU) study cohort. The distribution for disease severity in carrier females is to the left of that for the males. Most females are asymptomatic.

($P < .0001$). Thus, we formally demonstrate that disease conferred by *PLP1* mutations in female carriers will almost certainly be less severe than for males in the same pedigree.

Strong correlation between female and paired male PCSs

To determine whether disease severity in carrier females is correlated with that in first-degree male relatives, we performed a Spearman rank-order analysis on PCSs within pedigrees (Table 2). Indeed, we find a strong negative correlation between carrier female and paired male PCSs in the WSU study cohort, where $r = -0.70$ (95% confidence interval, -0.50 to -0.84; $P = .0001$). Thus, we formally demonstrate that mutations associated with mild clinical disease in males are likely to

Table 2
Carrier female and paired male Phenotypic Classification Score frequencies in the Wayne State University study cohort

PCS	Female carriers [a]	Paired males [a]
0	32	0
1	5	0
2	3	9
3	0	17
4	0	5
5	0	9
Total pedigrees	40	40
Spearman, *r*	−0.70	
(95% CI)	(−0.50 to –0.84)	

[a]Data represent single PCSs or averaged pedigree PCSs if more than one family member (female or male) was available for evaluation.
CI, confidence interval.

cause symptoms in carrier female relatives. Conversely, mutations that cause intermediate or severe forms of disease in males are likely to be asymptomatic in carrier females. These correlations have important implications for genetic counseling of Pelizaeus-Merzbacher disease carrier females.

Strong correlations between Mutation-Consequence and clinical signs

Mutations in Pelizaeus-Merzbacher disease families can be broadly categorized using a variety of clinical, molecular genetic, or functional criteria. To date, most classification schemes have relied on clinical criteria that provides an assessment of disease severity but does not take into account the underlying disease mechanisms.[26,27] To develop a more comprehensive scheme using a molecular genetic approach, we classified mutations into five groups according to the consequence for the gene (Mutation-Consequence). Table 3 summarizes the PCS frequency distributions for females when classified according to Mutation-Consequence. A single pedigree comprises the Null group, and these data were excluded from our analysis because they provide insufficient statistical power.

A Kruskal-Wallis one-way ANOVA of the WSU study cohort indicates an overall strong effect for the consequence of a mutation on disease severity ($P = .0002$). Dunn's multiple comparison post hoc test reveals that the majority of this effect is associated with the Overexpression versus Nonsense/Indel groups ($P < .001$) and the Missense versus Nonsense/Indel groups ($P < .05$). All other pair-wise comparisons are not significantly different ($P > .05$). Thus, these data indicate that disease severity is likely to be more severe in carrier females harboring nonsense/indel mutations than for those harboring overexpression or missense mutations. On the basis of an examination of paired male PCSs in the WSU study, the Kruskal-Wallis analysis also reveals a strong effect for Mutation-Consequence on disease severity ($P < .006$), and Dunn's post-test indicates that nonsense/indel mutations are generally less severe than mutations associated with missense mutations (Fig. 2; $P < .01$).

In addition to one-way ANOVA, we performed a two-way repeated-measures ANOVA for the WSU study cohort to examine interactions between gender and Mutation-Consequence. The purpose of this analysis is to determine whether PCSs from the five different Mutation-Consequence subgroups have any relationship to the gender of the patients. Indeed, this Friedman test reveals a strong overall interaction between Mutation-Consequence and gender of the patients ($P < .0003$), which is consistent with the negative Spearman correlation shown in Table 2.

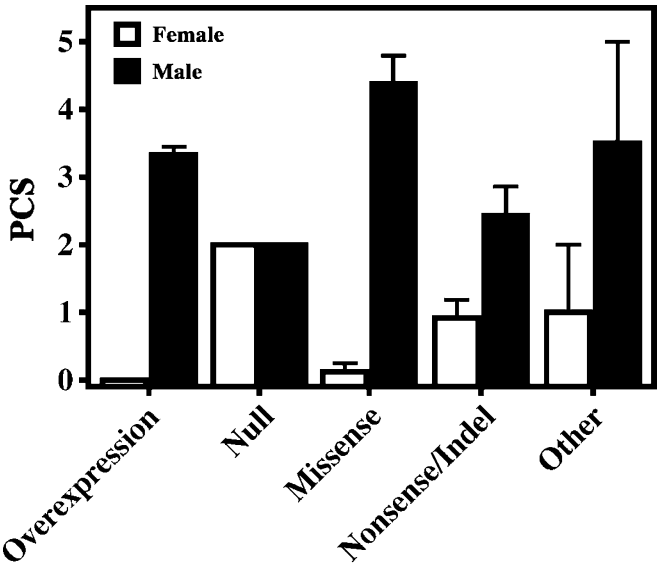

Fig. 2. Comparisons of average PCSs for WSU study females and males when *PLP1* mutations are classified in terms of the consequence for the gene or its products. Error bars reflect standard deviations of the means.

Mixed correlations between Mutation-Category and clinical signs

In view of the correlations between severe forms of disease and particular Mutation-Consequence groups in the WSU study cohort, we sought a simplified classification scheme with fewer groups that would, nonetheless, preserve the strong correlations revealed in the Mutation-Consequence classification. Such a scheme would be advantageous for genetic counseling of patients and other family members because it reduces the complexity of information that must be conveyed by the counselor, while still serving as a useful prognostic tool. Toward this end, we used a molecular genetics approach to classify mutations according to three categories (Mutation-Category) based on the general region of the *PLP1* gene that is affected.

Table 4 summarizes carrier female PCS frequencies for the

Table 3
Frequencies of carrier female and male scores for the Wayne State University study cohort correlated with the types of mutations in the *PLP1* gene

Mutation-Consequence	Female PCSs				Male PCSs						
	0	1	2	Total	0	1	2	3	4	5	Total
Overexpression	22	0	0	22	0	0	0	16	5	1	22
Null	0	0	1	1	0	0	1	0	0	0	1
Missense	7	1	0	8	0	0	1	1	0	6	8
Nonsense/Indel	2	4	1	7	0	0	6	0	0	1	7
Other	1	0	1	2	0	0	1	0	0	1	2

PCS, Phenotypic Classification Score.

Table 4
Frequencies of carrier female and male scores for the Wayne State University study cohort correlated with the locations of mutations in the *PLP1* gene

Mutation-Category	Female PCSs				Male PCSs						
	0	1	2	Total	0	1	2	3	4	5	Total
Entire gene	22	0	1	23	0	0	1	16	5	1	23
Exon	10	5	1	16	0	0	7	1	0	8	16
Intron	0	0	1	1	0	0	1	0	0	0	1

PCS, Phenotypic Classification Score.

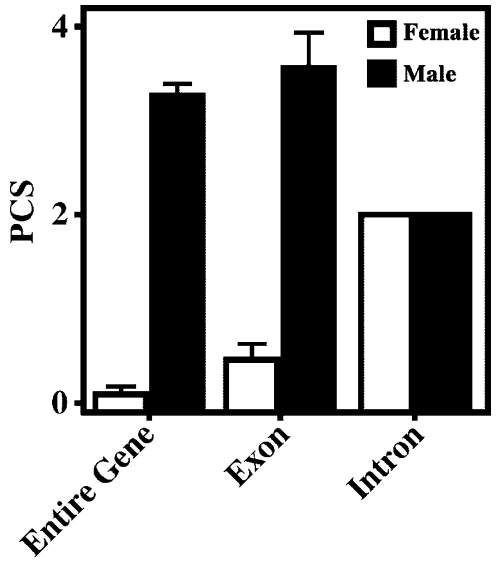

Fig. 3. Comparisons of average Phenotypic Classification Scores (PCSs) for Wayne State University (WSU) study females and males when *PLP1* mutations are classified in terms of the region of the gene that is affected. Error bars reflect standard deviations of the means.

WSU study cohort. We have an insufficient number of pedigrees for analysis of the Intron category, which has been excluded from our analysis. By using a two-tailed Mann-Whitney test, we find that disease severity in carrier females does not differ substantially between the Entire Gene and Exon categories ($P = .09$). Furthermore, we find no correlation between Mutation-Category and disease severity for paired males (Fig. 3; $P = .84$). Thus, categorizing the WSU study cohort using the Mutation-Category scheme has little prognostic value.

DISCUSSION

Despite intense interest in the molecular characterization of *PLP1* gene mutations for 15 years, few studies have attempted to determine whether disease severity in patients with Pelizaeus-Merzbacher disease can be defined using a broad classification scheme based on molecularly defined lesions in the *PLP1* gene. As part of our ongoing studies, we have examined possible neurologic effects of Pelizaeus-Merzbacher disease and spastic paraplegia (SPG) in heterozygous females. We and other investigators have observed a paradoxic increase in the likelihood of finding symptomatic females in families in whom affected males have a less severe phenotype.[32–34] Previous studies have not attempted to carefully quantify disease symptoms in carrier females and correlate them with those in first-degree male relatives. In the current study, we correlate several features of disease between males and females. First, symptomatic carrier females are more mildly affected than their first-degree male relatives, as is often observed for *X*-linked disorders. Second, strong negative correlations for disease severity between males and females indicate that carriers are more likely to exhibit clinical signs when first-degree male relatives are mildly affected. Third, mutations causing severe disease in males rarely cause symptoms in carrier females.

Multiple mechanisms account for phenotypes associated with different genetic lesions

In early studies, we formulated a hypothesis to accommodate mild and severe forms of disease arising in male patients with Pelizaeus-Merzbacher disease from coding region mutations in the *PLP1* gene.[16,17,35–38] Such mutations account for approximately 25% of families with Pelizaeus-Merzbacher disease. The hypothesis states that coding region mutations perturb the three-dimensional conformation of *PLP1* gene products and disrupt their trafficking through the secretory pathway of oligodendrocytes leading to toxic accumulation in the endoplasmic reticulum. In support of this notion, we demonstrated in transfected cells, animal models, and patients with Pelizaeus-Merzbacher disease that mutant forms of PLP1 and DM-20 accumulate in the cell bodies of oligodendrocytes and activate a stress signaling pathway called the unfolded protein response.[18,19] This pathway comprises a serine/threonine signaling cascade originating in the endoplasmic reticulum that signals to the nucleus and cytoplasm to attenuate protein synthesis and induce expression of molecular chaperones.[15] In the best case scenario, activation of this pathway restores homeostasis of the cell so it can return to normal function. However, if the pathway remains activated then the cell undergoes apoptosis. Thus, the greater the accumulation of mutant proteins, the more oligodendrocyte apoptosis and the greater the disease severity.

Although studied in much less detail, disease in males that stems from supernumerary copies of the *PLP1* gene is caused by a distinct mechanism from coding region mutations. The unfolded protein response is not activated,[15] and studies in vitro suggest that cholesterol trafficking is perturbed in oligodendrocytes.[22] Presumably, the greater the level of overexpression of *PLP1* (e.g., gene duplication vs. triplication) the greater the disease severity, although there is currently insufficient data to explore this correlation in detail. Likewise, null mutations cause disease by a distinct mechanism which seems to be related to oligodendrocyte-neuron interactions that maintain axonal transport in myelinated axons.[23,24]

A correlation between disease in males and the appearance of clinical signs in carrier female relatives

To explain the occurrence of disease symptoms in females harboring disease-causing mutations in the *PLP1* gene, we favor a mechanism that accounts for the cellular toxicity effects of the mutant protein on cell viability and incorporates the developmental features of *X*-inactivation in heterozygotes. A hypothesis that describes the mechanism by which carrier females exhibit the clinical signs of Pelizaeus-Merzbacher disease has been proposed,[13,39] but this hypothesis has not been rigorously tested in humans. This hypothesis states that oligodendrocytes expressing severe alleles of mutant *PLP1* are selected against and the remaining oligodendrocytes expressing wild-type *PLP1* populate the CNS. Such a selection process results in favorable cell-type specific skewed *X*-inactivation for

oligodendrocytes, which is formally defined as 85% or more of a cell population inactivating the same copy of the *X*-chromosome.[40] We emphasize that in carrier females harboring severe disease-causing alleles, favorable skewing patterns do not arise early in development during the stochastic *X*-chromosome inactivation process, but rather stem from cell-autonomous apoptosis of oligodendrocytes expressing mutant *PLP1* gene products. These cells are eventually replaced through gradual expansion of the subpopulation of oligodendrocyte progenitor cells that differentiate and express the wild-type *PLP1* gene. Indeed, the dynamic nature of these cellular selection and recovery processes are clinically observable in heterozygotes who have early neurologic signs and clinical test abnormalities that resolve over time.[25,39]

The hypothesis also stipulates that oligodendrocytes expressing mild alleles of the mutant *PLP1* gene are rarely negatively selected during development and that skewed *X*-inactivation is not observed in the oligodendrocyte population. Consequently, the CNS is myelinated both by wild-type and mutant *PLP1*-expressing oligodendrocytes. Several studies indicate that PLP1 is critical to local interactions between the myelin sheath and underlying axon,[23,41] and, for reasons that are currently poorly understood, myelin synthesized by oligodendrocytes expressing mutant *PLP1* alleles is unstable and presumably lost over time. Thus, carrier females may exhibit symptoms at some point in their life. Indeed, evidence from several animal models of Pelizaeus-Merzbacher disease is consistent with this hypothesis.[42–44]

Toxic gain-of-function effects of mutant *PLP1* gene expression notwithstanding, we do expect that clinical signs in carrier females will occasionally arise from unfavorable skewing of *X*-inactivation during early development. In cases in which carriers harbor severe mutations, unfavorable skewing will result in almost all oligodendrocytes in a brain region or possibly throughout the CNS expressing the mutant *PLP1* gene and subsequently undergoing apoptosis. With a minute population of proliferating cells to give rise to wild-type oligodendrocytes, very little myelin will be synthesized, and these patients may exhibit relatively severe clinical signs from an early age that persist into adulthood. Conversely, mild mutations may cause less severe disease in females that, nevertheless, begins early and persists into adulthood. Females with such distinctive clinical signs are rare, and we believe that most affected females have relatively normal *X*-inactivation patterns.

Strong correlations for disease severity between males and females

Our data reveal significant relationships between female carrier PCSs and Mutation-Consequence in the WSU study cohort. These data demonstrate that *PLP1* duplications, which are found in more than half of all Pelizaeus-Merzbacher disease pedigrees,[45] are as likely to confer symptoms in carrier females as are missense mutations, but less likely than null alleles or nonsense/indel mutations.

In light of the strong negative Spearman correlations for PCSs between females and males (Table 2), the ANOVA for the WSU study female cohort (Table 3) implies that nonsense/indel mutations should confer less severe clinical disease in males than either duplications or missense mutations. Indeed, this inference is largely derived from direct Kruskal-Wallis analyses of male PCSs (Fig. 2). Dunn's post-tests reveal that nonsense/indel mutations are generally less severe than gene duplications and missense mutations ($P < .05$).

Limitations of the current study

Several limitations of the current study will require evaluation of additional patients and longitudinal studies to remedy. First, Pelizaeus-Merzbacher disease is a progressive condition. Females in the WSU study cohort were examined only once. Of those who were asymptomatic up to the time of examination, at least a portion may have subsequently developed clinical signs that have not been evaluated. In addition, some carriers may have exhibited symptoms in childhood but, at the time of examination for the current study, were neurologically normal. The net effect of such deficiencies is likely to be underreporting of carrier female phenotypes. To minimize this risk, we carefully documented the clinical history of WSU study patients.

A second limitation arises because of the relative lack of detailed characterization of clinical signs in females. Symptoms that are typically exhibited by males have been assumed to represent the entirety of the clinical spectrum. However, there is currently insufficient information to determine whether symptoms unique to carriers, such as dementia or other psychiatric illnesses, might be manifest as part of a broader clinical phenotype. Male patients with severe disease may not survive long enough to develop dementia, but even if this is so, the archetypal symptoms may be difficult to recognize because of the other substantial disabilities that these patients exhibit.

A third limitation of the current study rests with the relative proportions of genetic lesions observed in the patient pool. By far the lesion of greatest prevalence is *PLP1* gene duplication, where pedigrees belonging to this Mutation-Consequence account for 55% of the WSU study cohort. We believe that this imbalance has limited effect on the statistical analyses using the Mutation-Consequence scheme; however, duplications overwhelm contributions to the sample population from null alleles in the Mutation-Category analyses.

Comparison of this study with published case reports

A major advantage of the current study over a review of published case studies is the absence of interrater variability; Dr. Garbern has evaluated all patients in the WSU study cohort. Nevertheless, some level of comparison between our study and the literature is warranted. Toward this end, we performed a meta-analysis and compiled the phenotypes and genotypes of 82 carrier females and 81 affected male relatives from 54 pedigrees (the Literature cohort) from a keyword search of the PubMed database (data not shown).

Two issues beset the Literature cohort; the possibility of interrater variability and the relative lack of clinical descriptions in a portion of the case studies, particularly for carrier females. To

accommodate these issues, we simplified our classification to a dichotomized PCS by which carrier females were classified as asymptomatic or symptomatic. In addition, we classified most males according to the criteria in Table 1 with the remainder assessed as having classic Pelizaeus-Merzbacher disease when a *PLP1* duplication is the cause of disease or SPG2 unless the reports specifically indicate a mild form of SPG, which we interpret as pure SPG.

These problems with the Literature cohort notwithstanding, we find a high degree of conformity to the WSU study cohort in terms of the conclusions we can draw from the analysis. Thus, there is a strong negative Spearman correlation for disease severity between carrier females and male relatives where $r = -0.48$ (95% confidence interval, -0.24 to -0.67; $P = .0002$). Moreover, we find further evidence that the classification of *PLP1* mutations by Mutation-Category is not a useful scheme for genetic counselors. Under this scheme the Entire Gene category includes 38 pedigrees harboring *PLP1* duplications, all of which are asymptomatic, and six null allele pedigrees, all of which are symptomatic. Clearly, this category is composed of two differing subpopulations (a Contingency table analysis using the Fisher exact test is highly significant ($P < .0001$). We also note that the pathophysiology of duplications primarily affects the CNS whereas that for null mutations is distinct and appears more closely associated with PNS dysfunction.[15,18,24]

Implications of this study for genetic counseling of Pelizaeus-Merzbacher disease carrier females

Skewed *X*-inactivation patterns arising as a consequence of *PLP1* gene expression, rather than through the *X*-inactivation process itself, have a dramatic effect on the apparent Mendelian mode of transmission. Severe mutations are almost always transmitted with an *X*-linked recessive mode, whereas mild mutations are consistent with *X*-linked dominant inheritance, but with reduced penetrance. Accordingly, unequivocal categorization of all Pelizaeus-Merzbacher disease and SPG2 alleles as exhibiting recessive or dominant transmission may not be possible or practical. However, Dobyns and colleagues[46] suggest that these terms be avoided for *X*-chromosome disorders. Under such new guidelines for *X*-linked inheritance in mammals, severe Pelizaeus-Merzbacher disease alleles are consistent with Category 1 diseases for which the mutant gene products act cell autonomously and cause cell death. On the other hand, mild alleles are consistent with Category 2 diseases for which mutant gene products act cell autonomously but do not (or infrequently) cause early cell death. Regardless of nomenclature, understanding both the genetic and cellular aspects of pathogenesis are critical to the interpretation of the clinical observations, and their underlying mechanisms, and ultimately benefits the counseling of families.

In summary, genetic counseling of carrier females harboring *PLP1* mutations is rarely straightforward because of the complexities associated with the *X*-linked mode of genetic inheritance, the variable effect of different mutations on oligodendrocyte function and survival, and the stochastic effects arising from patterns of *X*-inactivation. The results of this study demonstrate the importance of developing a molecular genetics-based approach such as the Mutation-Consequence scheme as a tool for counseling carrier females who may be caring for an affected male and who may be faced with the possibility of exhibiting clinical signs at some point in the future. In general, the risk of clinical disease for carrier females is relatively low but is greatest for nonsense/indel or null mutations. Carriers harboring missense mutations are at moderate risk of disease and those with the lowest risk, who represent the bulk of families with Pelizaeus-Merzbacher disease, typically harbor *PLP1* gene duplications.

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