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Neuroradiologic correlates of clinical disability and progression in the X-Linked leukodystrophy Pelizaeus–Merzbacher disease

Jeremy J. Laukka^{a,e}, Jeffrey A. Stanley^f, James Y. Garbern^{a,b}, Angela Trepanier^a, Grace Hobson^c, Tori Lafleur^g, Alexander Gow^{a,b,d}, and John Kamholz^{a,b,*}

^aCenter for Molecular Medicine and Genetics, Wayne State University School of Medicine, Detroit, MI 48201, United States

^bDepartment of Neurology, Wayne State University, School of Medicine Detroit, MI 48201, United States

^cNeurogenetics Research Laboratory, A.I. duPont Hospital for Children, Nemours Biomedical Research A/R, Wilmington, DE 19803, United States

^dCarman and Ann Adams Department of Pediatrics, Wayne State University, School of Medicine, Detroit, MI 48201, United States

^eDepartment of Neurosciences & Neurology, University of Toledo College of Medicine, Toledo, OH 43614, United States

^fDepartment of Psychiatry & Behavioral Neurosciences, Wayne State University School of Medicine, Detroit, MI 48201, United States

^gWayne State University School of Medicine

Abstract

Objective—To determine whether quantitative measure of magnetic resonance imaging data from patients with the inherited leukodystrophy, Pelizaeus–Merzbacher disease (PMD) correlates with clinical severity or progression.

Methods—In our current work we have analyzed the clinical phenotypes and MRI scans of 51 male patients with PMD and 10 female carriers for whom the *PLP1* genotype had been determined. In addition, we developed a 32-point functional disability scoring (FDS) system for PMD, and validated it for inter-rater reliability. Using conventional T₁- and T₂-weighted MRI images of the whole brain, we measured white matter and total brain volume (WMV and TBV), inter-caudate ratio (ICR), and corpus callosum area.

Results—There was a significant positive correlation of FDS with white matter fraction (WMV/TBV) and corpus callosum area. Also, when applying a median split based on FDS, patients with lower FDS showed reduced white matter fraction and corpus callosum area, and increased ICR compared to patients with relatively higher FDS, regardless of age.

Conclusion—Although this patient population is heterogeneous, with multiple genetic and molecular mechanisms causing PMD, these data imply that white matter atrophy is a major pathological determinant of the clinical disability in most patients. Development of reliable non-invasive quantitative biomarkers of disease activity would be useful not only for following the natural history of the disease, but also raising the potential for evaluating future therapies.

Keywords

Proteolipid protein; Pelizaeus–Merzbacher disease; Magnetic resonance imaging; White matter atrophy; Clinical disability; Genetics

1. Introduction

Pelizaeus–Merzbacher disease (PMD), an X-linked dysmyelinating disorder, is caused by mutations in the gene encoding proteolipid protein (*PLP1*), the major structural protein in the central nervous system (CNS) myelin [1-5]. Patients with PMD display a variety of neurological signs and symptoms, including spastic paraparesis, nystagmus, cognitive and visual impairment. The majority of patients with PMD have a variable sized duplication of a region of the X-chromosome containing the *PLP1* gene [6], suggesting that overexpression of *PLP1* is the cause of the disease [7]. More than 100 point mutations in the *PLP1* coding region have also been identified, accounting for approximately 15–25% of the PMD patients and have been shown to have a variety of deleterious effects.

The predominant pathological abnormality in PMD consists of thinning and/or absence of myelin in the CNS. Gow and colleagues have proposed that mutations alter the structure of PLP, which can cause protein misfolding, activation of the unfolded protein response and oligodendrocyte apoptosis, thereby accounting for the disease's severity [8-10]. In contrast, complete absence of *PLP1* is associated with well-formed, compact myelin, and a late onset of a length-dependent pattern of axonal degeneration [11]. The pathogenesis of myelin and axonal injury in rodents and humans with a *PLP1* duplication, however, is less well understood [12].

Brain MRI studies in patients with PMD have demonstrated patterns consistent with hypomyelination, both in patients with duplications and in patients with point mutations [13-17]. A study by Garbern and coworkers has also shown that some patients with PMD caused by a *PLP1* null mutation, and have decreased levels of N-acetylaspartate (NAA) due to a length-dependent axonal degeneration [11]. In contrast, a study of patients with *PLP1* duplications has found increased brain levels of NAA [18,19], as has an MRI study of the *msd* mouse mutant that has a point mutation in *PLP* [20]. In general, however, very little is known about the natural history of patients with PMD, either those with point mutations or gene duplications. In addition, there are no biomarkers identified to follow the disease progression, or to aid in understanding the nature of the disease pathogenesis.

In this current study we have analyzed a large number of PMD patients by brain MRI in conjunction with a functional disability scoring system to identify aspects of PMD pathogenesis that are responsible for the clinical severity of the disease. These patients were entered into this study over a 10 year period by both their availability to travel to our medical center and their ability and willingness to undergo MRI scanning, and thus do not represent a random sample of patients with PMD. The cohort of patients is heterogeneous, comprising individuals with different *PLP1* mutations and a wide range of ages. In spite of this heterogeneity, however, there is a statistically significant correlation in this group between clinical disability, as measured by our functional disability scoring system, and white matter fraction, as measured by MRI scanning and volumetric analysis. Taken together, these data suggest that white matter fraction is a predictor of disease severity and patient disability in individuals with a variety of *PLP1* mutations.

2. Materials and methods

2.1. Study population

The study population included 61 individuals with known *PLP1* mutations, including 51 males and 10 heterozygous females (Table 1). The patients were entered into this study from a population of patients with known *PLP1* mutations and selected based on their willingness to travel to our medical center and to undergo MRI scanning, and for this reason, do not represent a random sample of PMD patients. Ten patients in this group had *PLP1* duplications, two had *PLP1* triplications, while the other 49 individuals had one of 27 different *PLP1* point mutations, including missense, nonsense and splice site mutations. The mean age of this patient group was 21.7 ± 16.2 , with a range of ages from 2 to 57.6 years. Sixteen patients were less than 6 years of age. The study was approved by the Wayne State School of Medicine Institutional Review Board, and all patients and/or their parents gave informed consent to participate.

Because all the individuals analyzed in this study did not obtain their MRI scans at the same institution using a uniform protocol, we were not able to evaluate each of the 61 patients for brain volume, inter-caudate ratio and corpus callosal area. As can be seen in the data sections, brain volumes were obtained on 45 individuals, all of whom had their MRI at the Children's Hospital of Michigan using a uniform protocol. In contrast, inter-caudate ratios were obtained on 52 individuals, and corpus callosal area was obtained on 35 individuals. Thirty-five patients from the study population had all three of these measures. Although each of these three subgroups has a slightly different composition with respect to age, mutation and disease severity, the statistical analysis used for our analysis takes these differences into consideration.

2.2. MR imaging acquisition and tissue volume estimation procedure

Forty-five individuals, including 8 patients who were less than 6 years of age, were evaluated with routine clinical brain MRI examinations on a 1.5 Tesla GE Signa scanner (General Electric, Milwaukee, WI) at the Children's Hospital of Michigan. The MRI protocol T₁-weighted images using the three-dimensional volumetric radiofrequency spoiled gradient echo (3D SPGR) sequence and T₂-weighted images. The total brain volume (TBV) and, white and gray matter volumes (WMV and GMV) were manually measured from these scans using the program NIH 1.62, see (<http://rsbweb.nih.gov/nih-image/>). Seven patients were excluded from this analysis because the contrast between gray and white matter was insufficient for volume segmentation. An automated segmentation software FreeSurfer was also used for all of the scans in this study. Although this program works quite well for scans with normal white matter–gray matter contrast, it did not provide useful information for this group of scans from patients with PMD. Manual segmentation was thus necessary to measure total volume, white matter volume and gray matter volume. The manual segmentation was performed twice for each case by an experienced rater (JL), and the results were averaged for the final volumes. The standard deviation of these separate measurements was, however, quite small. Four examples of coronal sections from SPGR series with varying gray matter-to-white matter contrast are shown in Fig. 1. Volume segmentation could not be performed from image (D) of this figure because of lack of white-to-gray matter contrast.

The raw images data were imported into the program NIH 1.62 for visualization, manual tracing, and quantitative analysis. To prepare the stacks for volumetric measurements, non-brain structures, including the skull, dura, subarachnoid spaces, ventricles, cerebellum, and brainstem were excluded from the region of interest (ROI) by manually tracing around them using the NIH program 1.62 as shown in Fig. 2. The color threshold was manually adjusted

for each slice to ensure accurate segmentation of white and gray matter boundaries. This methodology was used to measure the ROI for each of the 62 slices available for each MRI scan. The brain volumes are expressed in units of cubic centimeter and white matter fraction was calculated by dividing the WMV by the TBV.

2.3. Inter-caudate distance and transverse skull diameter ratio measurements

To establish a simple MRI measure to follow the disease progression, we measured the inter-caudate ratio for 52 individuals with PLP1 mutations, 16 of whom were less than 6 years of age. We chose to measure the inter-caudate ratio (ICR), since it has been validated as a surrogate marker of brain atrophy in multiple sclerosis, traumatic brain injury and evaluating cognitive decline in Alzheimers disease [21-25]. A major advantage of measuring the ICR is that it can be performed quickly without the requirement of special training to operate computer-based software programs, and for MRI scans from heterogeneous protocols. It could thus be used to follow real patients in the real world. An increased ICR is probably the result of surrounding white matter loss, and is thus well suited for analyzing diseases of white matter.

The inter-caudate distance (ICD) and transverse skull diameter (TSD) were measured from the most caudal axial T₁-weighted MRI image for 52 individuals. The ICD was defined as the linear distance on this image between the medial borders of the head of the caudate nucleus; the TSD was defined as the distance separating the inner table of the skull on this same image. The inter-caudate ratio was then calculated by dividing the ICD by the TSD at the same axial section level, as described previously by Caon and co-workers [21], and does not depend on precise alignment of the axial sections used to make the measurement. NIH image was used to perform all measurements. A set of typical measurements for the inter-caudate ratio is shown in Fig. 3.

2.4. Corpus callosum area measurements

The corpus callosum, the largest white matter track in the brain, is easily identified and measured on both axial and sagittal MRI images. A decrease in the thickness of the corpus callosum as in patients with multiple sclerosis, would thus be an additional indicator of white matter disease. For this study, the mid-corpus callosum area was analyzed in 35 individuals in which the genu, body, and splenium of the corpus callosum could be visualized, as shown in Fig. 4. Eight of these patients were less than 6 years of age. The corpus callosum area was measured using the program NIH Image 1.62 as described above by manually tracing around the corpus callosum and the area expressed in squared centimeters.

2.5. Statistical approach

Pearson correlations were first computed to determine associations between FDS and the structural MRI measures (i.e., WMV, white matter fraction, inter-caudate ratio and the corpus callosum area). To further assess FDS associations, a median split of patients based on FDS was performed to generate two subgroups (lower and higher FDS subject groups). FDS subgroup differences were tested using a generalized linear model (GLM) analysis based on the GEE (generalized estimating equation) methodology with FDS subgroup as the main effect term and age as a covariate (JMP; SAS Institute Inc.). The TBV was used as an additional covariate when comparing white matter volume and the corpus callosum area. The statistical results of the GLM analyses were based on the “Chi-Square” statistics and associated p-values. P-values of 0.05 or less were considered significant.

3. Results

3.1. FDS in patients with PMD

In order to analyze clinical disability in patients with PMD caused by a variety of PLP1 mutations, we developed a simple and reproducible clinical scale adapted from a similar scale used for patients with ALS [26]. This functional scale (0 to 32) shown in Table 2, measures the ability of patients to perform routine tasks of daily living, such as feeding, bathing, dressing, and walking. The scoring system does not depend on any specific disease process or neurological sign, and can be assessed from a short interview with the patient's caregiver. Because the scoring system has been adapted from an ALS scale for adult patients, however, it is most useful for PMD patients greater than 6 years of age.

Using this scale, four neurologists at the Wayne State University School of Medicine have estimated the functional disability of a group of 20 patients with genetically confirmed PMD. The inter-rater reliability of this scoring system, shown in Table 3A, is greater than 95%. The scores, however, shown in Table 3B, suggest that functional disability is not equally distributed throughout the possible variable range, but instead occurs in 3 clusters: 0–10 (severe); 10–20 (moderate); and 20 and above (mild). Interestingly, these clusters probably represent patients with congenital PMD, classic/transitional PMD and progressive spastic paraparesis, similar to the categories originally proposed by Seitelberger [27–29].

The FDS assessment was then administered by JYG and JK to 61 individuals with a confirmed PLP1 mutation. The scores were determined either by direct examination of the patient, interview with the patient's parents, or both, and are displayed in Table 1 along with the patient's age at the time of examination and the PLP1 mutation. The MRI scans were obtained at the same visit as that of the patient's neurological examination. All patients were rated using this scale regardless of age.

3.2. FDS and white matter

Forty-five individuals with PLP1 mutations had MRI scans that could be used to measure tissue brain volumes, including total brain volume and white and gray matter volumes, as described in detail in the materials and methods section.

As can be seen from the data in Table 4, white matter volume tended to be smaller but the white matter fraction (WMV/TBV) is significantly lower in the group with lower FDS compared to higher FDS patients. In addition, as shown in Fig. 5, the white matter fraction is significantly correlated with functional disability, but not with patient age across the entire patient population or the measured gray matter. A similar correlation between white matter fraction and functional disability was also found after removing the 8 patients from the group who were less than 6 years of age (data not shown). These data suggest that a relative decrease in white matter volume in individuals carrying PLP1 mutations is an important cause of clinical disability, regardless of age or mutation type.

3.3. FDS and inter-caudate ratio

The ICR is significantly higher in the group with lower FDS compared to higher FDS patients (see Table 4). In addition, the ICR also is inversely correlated with functional disability across the entire patient population but failed to reach significance (Fig. 6).

When the 16 individuals less than 6 years of age were removed from this calculation, however, the inverse correlation of ICR with functional disability was statistically significant (data not shown). These data, as those for the white matter fraction, further

suggest that a relative decrease in white matter volume in individuals with PLP1 mutations is an important cause of their clinical disability.

3.4. FDS and the corpus callosum area

As an additional check on our data relating white matter atrophy to functional disability, we measured the area of the corpus callosum at its midpoint for 35 individuals for which this MRI section was available. Since the corpus callosum is the largest white matter track in the brain, the functional disability of these individuals, as with white matter fraction and ICR, should be correlated with the cross sectional area of this structure. The corpus callosum area is significantly smaller in the group with lower FDS compared to higher FDS patients (see Table 4). In addition, the corpus callosum area is significantly correlated with functional disability across the entire patient population, but is also significantly correlated with patient age to a lesser degree (Fig. 7). A similar result was found after removing the patients who were less than 6 years of age from this data set (data now shown). These data, as those for the white matter fraction and ICR, further suggest that a relative decrease in white matter volume in individuals with PLP1 mutations of all ages and mutation types is an important cause of their clinical disability.

4. Discussion

PMD is a clinically and genetically heterogeneous disease caused by mutations in the gene encoding the major CNS myelin protein, proteolipid protein (PLP1). Myelin is a major target of disease pathogenesis in most cases of PMD, but how the various mutations cause clinical disability is not fully understood. As a step in further understanding the molecular pathogenesis of PMD, we have analyzed the MRI scans of a group of 61 individuals with *PLP1* mutations, including point mutations, deletions and duplications, and have correlated these findings with their clinical disability score, determined by a system we have established for this purpose. Our data demonstrates that a relative decrease in brain white matter volume, analyzed by volumetric fractionation, inter-caudate ratio, and/or corpus callosal area, is significantly correlated with the patient's functional disability. These data suggest that white matter atrophy is the main cause of clinical disability in individuals with PLP1 mutations, regardless of their age or mutation type.

Gray matter volumes were measured in this study, and did not correlate with the patient's functional disability in this cohort of patients. White matter changes are not specific in PMD, but do appear to predict disease severity. We do not think that gray matter is spared from pathological changes in PMD, since these have been previously demonstrated [30]. In fact, loss of cortical neurons may be involved in the disease progression in PMD, which is important for understanding the natural history of this disease. Although white matter atrophy may be the cause of clinical disability in PMD, this does not imply that the molecular pathogenesis is the same for all types of PLP1 mutations.

White matter is composed of axons of differing caliber, oligodendrocytes producing myelin sheaths of variable thickness, oligodendrocyte progenitors, astrocytes, and the occasional macrophage or microglia [31,32]. *PLP1* mutations can cause dysmyelination, with reduced myelin thickness and axon caliber, gliosis, demyelination with associated myelin debris and inflammation, and/or axonal damage or destruction. All of these processes, either singly or together, could cause white matter atrophy, and all have been previously described in pathological specimens or animal models of PMD [28,33-38]. Our findings are thus consistent both with the underlying genetic cause of PMD, and with its classification as a leukodystrophy [27,39,40]. Future imaging studies on this group of patients using magnetic resonance spectroscopy (MRS), diffusion tensor imaging (DTI), and magnetization transfer imaging (MTI), however, will allow us to probe more deeply into the cellular and molecular

pathogenesis of the white matter atrophy for different *PLP1* mutations, and to identify the mechanisms both common and unique to each.

The functional disability scoring system we have developed is easy to use and clearly identifies patients with differing disease severities. This system will also be useful for following patients over time, since the functional disability score can be determined by talking with a patient's caregivers by phone. A more complete scoring system appropriate for both adults and children that formally incorporates elements of the neurological exam will be required for use in clinical trials of PMD patients, since a patient's response to treatment may change the exam, but not the functional disability. In addition, a scoring system that takes into account the normal development of the nervous system will also be necessary for clinical trials and treatment of younger patients less than 6 years of age. These clinical instruments are currently under development.

MRI analysis of patients with PMD will not only further our understanding of this inherited leukodystrophy, but will also provide important insights into the molecular pathogenesis of other more common, acquired white matter diseases, such as multiple sclerosis (MS). For example, the role of demyelination in causing the clinical signs and symptoms in MS is complex, and long-term disability in this disease may be caused by axonal degeneration, not by demyelination and/or abnormalities of nerve conduction.

5. Conclusion

Although we do already know that PMD is a white matter disease, this study demonstrates that white matter atrophy is a major determinant of the clinical progression in PMD. This is a novel finding with implications for both the natural history of the disease and for predicting the clinical severity of individual patients. These findings may also help elucidate the role of axonal degeneration and disease progression. In addition, this may be relevant for future clinical trials in patients with PMD.

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Fig. 1.

Comparison of gray matter–white matter contrast in patients with PMD. Three patients with PMD and *PLP1* duplications (B–D) and a normal control (A) were analyzed using a three dimensional volumetric radiofrequency spoiled gradient echo (SPGR) series. (A) Control showing normal myelination of the subcortical white matter and internal capsule. (B) PMD patient with mild disease (FDS = 20). The subcortical white matter is iso-intense with a gradual decrease in hyperintensity of the internal capsule. (C) PMD patient with moderate disease (FDS = 12). The subcortical white matter is thin and the signal is hypointense in the temporal lobe and internal capsule. (D) PMD patient with severe disease (FDS = 5). There is diffusely reduced signal in the subcortical white matter, internal capsule and temporal lobes with enlargement of the lateral ventricles.

Fig. 2.

Example of mask placement for determination of white matter volume. (A) Coronal section from a three dimensional volumetric radiofrequency spoiled gradient echo series. (B) The same section as in (A) with the mask for volumetric measurement shown in red.

Fig. 3. Measurement of the inter-caudate ratio (ICR). The ICR is determined by dividing the inter-caudate distance by the transverse width of the inner skull table.

Fig. 4.

Comparison of corpus callosum in patients with PMD. Mid-sagittal T1-weighted MRI sections from (A) a normal subject and (B–C) two PMD patients. (B) PMD patient with a complete deletion of the *PLP1* gene (FDS = 18). (C) PMD patient with a *PLP1* duplication (FDS = 9).

Fig. 5.

White matter fraction is correlated to FDS but not age in individuals with PLP1 mutations. The white matter fraction from forty-five patients with PLP1 mutations for whom this metric was measured is plotted against the functional disability score (a) and age (b).

Fig. 6.

The inter-caudate ratio (ICR) is correlated to FDS but not age in individuals with PLP1 mutations. The ICR from 52 patients with PLP1 mutations for whom this metric was measured is plotted against the functional disability score (a) and age (b).

Fig. 7.

Corpus callosal area is correlated to FDS but not age in individuals with PLP1 mutations. The corpus callosum area from 35 individuals with PLP1 mutations for whom this metric was measured is plotted against the functional disability score (a) and age (b).

Table 1

List of PMD patients' age and FDS at the time of evaluation, as well as their mutation at the DNA and Protein Level.

Patient	Age	FDS	<u><i>PLP1</i> mutations</u>	
			DNA	Protein
1	0.95	4	Triplication	
2	1.8	4	c.106_108del	p.Gly36del
3	2.0	2	c.103T>C	p.Cys35Arg
4	2.1	30	c.619T>C	p.Tyr207His
5	2.2	14	c.409C>G	p.Arg137Gly
6	2.9	5	c.151T>G	p.Phe51Val
7	3.4	12	Duplication	
8	3.7	10	c.254T>G	p.Leu85Arg
9	3.7	4	c.242T>G	p.Leu81Arg
10	3.8	11	c.763-1G>T	
11	3.8	9	Duplication	
12	3.9	13	Unknown	p.Tyr157His
13	4.0	6	c.517C>T	p.Pro173Ser
14	4.6	6	c.736G>T	p.Gly246Trp
15	5	5	c.260T>C	p.Leu87Pro
16	5.2	4	c.242T>G	p.Leu81Arg
17	6.5	15	Duplication	
18	8.9	27	c.418C>T	p.His140Tyr
19	10	32	c.409C>T	p.Arg137Trp
20	10.0	25	c.834A>G	p.*278fs*15
21	10.0	6	Triplication	
22	10.1	12	Duplication	
23	10.8	20	Duplication	
24	11.8	21	c.282delC	p.Gly 95fs*19
25	11.8	9	Duplication	
26	12.3	20	c.453+28_+46del	
27	14.0	18	Deletion	Deletion
28	15.6	32	c.G4del/hetero	p.Gly2fs*3
29	16.2	25	c.430A>T	p.Lys144*
30	16.2	5	Duplication	
31	18.4	20	c.G4del	p.Gly2fs*3
32	18.8	18	c.406_422del	p.Glu136fs*62
33	19.0	27	c.434G>A	p.Trp145*
34	23.0	20	c.282delC	p.Gly 95fs*19
35	23.0	25	c.434G>A	p.Trp145*
36	23.0	3	c.762+3G>T	
37	23.1	4	c.44C>T	p.Pro15Leu

Patient	Age	FDS	<i>PLP1</i> mutations	
			DNA	Protein
38	25.0	3	c.762+3G>T	
39	25.4	15	c.655G>T	p.Val219Phe
40	26.0	32	c.834A>G/hetero	p.*278fs*15
41	28.1	29	c.676T>C	p.Ser226Pro
42	28.2	25	c.619T>C	p.Tyr207His
43	31.0	32	c.676T>C/hetero	p.Ser226Pro
44	34.2	32	c.619T>C/hetero	p.Tyr207His
45	35.0	31	c.834A>T	p.*278fs*15
46	35.5	9	c.406_422del	p.Glu136fs*62
47	38.0	11	c.406_422del	p.Glu136fs*62
48	38.0	31	c.834A>T	p.*278fs*15
49	40.0	32	c.44C>T/hetero	p.Pro15Leu
50	40.0	32	c.G4del/hetero	p.Gly2fs*3
51	40.5	16	Duplication	
52	43.9	16	Duplication	
53	45.6	30	c.560T>C	p.Ile187Thr
54	45.8	30	c.676T>C	p.Ser226Pro
55	45.9	8	Duplication	
56	46.1	29	c.434G>A/hetero	p.Trp145*
57	47.0	30	c.G4del/hetero	p.Gly2fs*3
58	49.0	30	c.762+3G>T/hetero	
59	56.0	32	c.676T>C/hetero	p.Ser226Pro
60	57.6	32	c.619T>C/hetero	p.Tyr207His
61	n/a	26	c.436C>T	

Table 2

The PMD functional disability scoring system.

<i>Education</i>
0 — no formal schooling; 1 — special school or special education classes; 2 — regular classes, but not at grade level; 3 — regular school, grade appropriate for age (within 2 years)
<i>Employment: If beyond school age</i>
0 — unable to work/homebound; 1 — sheltered workshop (i.e. works at institution dedicated to disable employees); 2 — special job (i.e. works at conventional workplace, but requires special supervision); 3 — regular job
<i>Speech</i>
0 — no verbal communication; 1 — rare understandable words with nonverbal communication; 2 — speech understandable with difficulty; 3 — detectable speech disturbance but readily understood; 4 — normal speech
<i>Feeding</i>
0 — tube feedings only; 1 — some oral feeding, with supplemental tube feedings; 2 — oral feedings with consistency changes to diet; 3 — normal diet with occasional choking; 4 — normal swallowing
<i>Dressing</i>
0 — total dependence; 1 — Can assist with dressing but dependent on others 2 — independent, but with decreased efficiency; 3 — normal
<i>Toileting</i>
0 — total dependence; 1 — needs assistance; 2 — independent, but with decreased efficiency; 3 — normal
<i>Writing</i>
0 — total dependence; 1 — needs assistance; 2 — independent, but with decreased efficiency; 3 — normal
<i>Sitting</i>
0 — cannot sit without support; 2 — can sit without support
<i>Walking</i>
0 — wheelchair or bedbound; 1 — can crawl/bunny hop; 2 — can walk a few steps, but needs adaptive aids or other support; 3 — needs adaptive aids to walk 20 ft; 4 — impaired gait, but uses no assistive devices; 5 — normal gait
<i>Breathing</i>
0 — ventilator or constant respiratory support; 1 — intermittent use of non-invasive respiratory support; 2 — has respiratory symptoms but does not use ventilatory support; 3 — normal breathing

Table 3**A** Inter-rater reliability estimates from the scores shown in Table 3B.

	Pairwise correlation	Average Spearman correlation
R1–R2	0.99467	
R1–R3	0.99086	
R1–R4	0.95802	
R2–R3	0.98935	
R2–R4	0.99164	
R3–R4	0.99696	
	5.9215	0.986916667

B Verification of the PMD functional disability scoring system.

	Phys. #1	Phys. #2	Phys. #3	Phys. #4	PLP1 mutation
1	13	12	12	10	Duplication
2	27	27	27	27	DelG4 heterozygote
3	7	7	6	6	Del G4 male
4	9	10	9	9	Del G4 male
5	20	20	20	20	Del G4 male
6	14	14	13	12	Unknown
7	4	4	4	4	Pro14Leu
8	5	5	5	5	Lys150Asn
9	27	27	27	27	Ile186Thr (rsh)
10	27	27	27	27	Ile186Thr (rsh)
11	14	14	16	16	Duplication
12	16	15	15	15	Duplication
13	20	17	17	20	Del 19 bp intron 3
14	16	14	16	15	Duplication
15	2	2	2	2	+3 IVS 6 (skips exon 6)
16	4	4	4	3	Duplication
17	6	7	6	6	Duplication
18	16	16	16	16	Duplication
19	27	27	27	27	Unknown
20	9	8	8	7	Duplication

Table 4

MRI metrics analyzed with respect to functional disability score for individuals with PLP1 mutations. A generalized linear model (GLM) analyses based on the GEE (generalized estimating equation) methodology was performed with FDS subgroups as the main effect term and age as a covariate to test FDS subgroup differences (JMP; SAS Institute Inc.). The total brain volume (TBV) was used as an additional covariate when comparing white matter volume and the corpus callosum area. The statistical results of the GLM analyses were based on the “Chi-Square” statistics and associated p-values. P-values of 0.05 or less were considered significant. Since all three measurements were not performed on all subjects, a median split for each measure was conducted.

	Lower FDS group [†]	Higher FDS group [†]	Chi Square (p-value)
	FDS < 21	FDS ≥ 21	
Sample size	21	24	
Age (years)	16.5 ± 13.3	31.4 ± 15.6	
White matter volume (cm ³)	69.5 ± 42.3	112.8 ± 40.1	
Total brain volume (cm ³)	188.4 ± 76.7	253.1 ± 80.3	
WM fraction (%)	34.8 ± 9.5	43.6 ± 7.1	9.83 (p = 0.0017) **
	FDS < 18	FDS ≥ 18	
Sample size	25	27	
Age (years)	12.7 ± 14.1	27.2 ± 15.5	
Inter-caudate distance (cm)	1.47 ± 0.63	1.32 ± 0.46	
Transverse width of inner skull table (cm)	13.2 ± 4.7	13.5 ± 3.3	
ICD/TWS	0.111 ± 0.027	0.097 ± 0.016	6.28 (p = 0.012) **
	FDS < 20	FDS ≥ 20	
Sample size	17	18	
Age (years)	16.0 ± 15.1	25.5 ± 14.6	
Corpus callosum area (cm ²)	2.73 ± 0.75	4.30 ± 1.68	7.52 (p = 0.0061) *

[†] Mean values ± 1SD.

* With TBV and age as covariates.

** With age as a covariate.