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

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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 T_1- and T_2-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.

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

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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 T_1-weighted images using the three-dimensional volumetric radiofrequency spoiled gradient echo (3D SPGR) sequence and T_2-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.

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.

			PLP1 mutations	
Patient	Age	FDS	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
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	