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## **Novel approaches to quantify CNS involvement in children with Pompe disease**

Aditi Korlimarla<sup>1</sup> MBBS, Gail A. Spiridigliozzi<sup>2</sup> PhD, Kelly Crisp<sup>3</sup> MA, CCC-SLP, Mrudu Herbert<sup>4</sup> MD, MPH, Steven Chen<sup>5</sup> BS, Michael Malinzak<sup>5</sup> MD, PhD, Mihaela Stefanescu<sup>1</sup> BS, Stephanie L. Austin<sup>1</sup> MA, MS, Heidi Cope<sup>1</sup> MS, CGC, Kanecia Zimmerman<sup>6</sup> MD MPH, Harrison Jones<sup>3</sup> PhD, James M. Provenzale<sup>5</sup> MD, FACR, Priya S. Kishnani<sup>1</sup> MD

<sup>1</sup> Division of Medical Genetics, Department of Pediatrics, Duke University Medical Center, Durham, NC, USA

<sup>2</sup> Department of Psychiatry and Behavioral Sciences, Duke University Medical Center, Durham, NC

<sup>3</sup> Department of Surgery, Duke University Medical Center, Durham, NC

<sup>4</sup> Department of Pediatric Neurology, University of Kentucky Medical Center, Lexington, KY

<sup>5</sup> Department of Neuroradiology, Duke University Medical Center, Durham, NC

<sup>6</sup> Duke Clinical Research Institute, Durham, NC, USA

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**Corresponding Author:** Priya S. Kishnani MD ([priya.kishnani@duke.edu](mailto:priya.kishnani@duke.edu))

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**ABSTRACT**

*Objective:* To characterize the extent of central nervous system involvement in children with Pompe disease using brain magnetic resonance imaging (MRI) and developmental assessments.

*Methods:* The study included fourteen children (ages 6-18 years) with infantile Pompe disease (IPD) (n=12) or late onset Pompe disease (LOPD) (n=2) receiving enzyme replacement therapy. White matter (WM) hyperintense foci seen in the brain MRIs were systematically quantified using the Fazekas scale (FS) grading system with a novel approach; the individual FS scores from ten anatomical areas were summed to yield a total FS score (range: absent-0 to severe-30) for each child. The FS scores were compared to developmental assessments of cognition and language obtained during the same time period.

*Results:* Mild to severe WM hyperintense foci were seen in 10/12 children with IPD (median age-10.6 years) with total FS scores ranging from 2 to 23. Periventricular, subcortical and deep WM were involved. WM hyperintense foci were seen throughout the path of the corticospinal tracts in the brain in children with IPD. Two children with IPD had no WM hyperintense foci. Children with IPD had relative weaknesses in Processing Speed, Fluid Reasoning, Visual Perception, and receptive vocabulary. The two children with LOPD had no WM hyperintense foci, and high scores on most developmental assessments.

*Conclusion:* This study systematically characterized WM hyperintense foci in children with IPD; which could serve as a benchmark for longitudinal follow up of WM abnormalities in

patients with Pompe disease and other known neurodegenerative disorders or leukodystrophies in children.

## **GLOSSARY**

*PD = Pompe disease, IPD = infantile Pompe disease, LOPD = late-onset Pompe disease, CRIM = cross reactive immune material, ERT = enzyme replacement therapy, GAA = acid alpha-glucosidase, CNS = central nervous system, MRI = magnetic resonance imaging, WM= white matter, FS = Fazekas scale, Leiter-3=Leiter International Performance Scale-Third Edition, CELF-5=Clinical Evaluation of Language Fundamentals – Fifth Edition, CCC-2=Children’s Communication Checklist – Second Edition, m=month, y=year*

## **MAIN TEXT OF THE MANUSCRIPT**

### **INTRODUCTION**

Pompe disease (PD) encompasses a continuum of patients broadly classified into two groups; infantile PD (IPD; with cardiomyopathy in the first year of life) and late-onset PD (LOPD) to describe all others<sup>1</sup>. IPD includes classic (most severe) and non-classic (less severe) disease forms<sup>2</sup>. IPD was once a fatal disease; brain autopsies showed several histopathological changes, including glycogen accumulation, in the brain and spinal cord<sup>3-9</sup>. However, patients with IPD are now living longer, since the advent of intravenous enzyme replacement therapy (ERT) with alglucosidase alfa in 2006<sup>1</sup>. There is increasing evidence of nervous system involvement among these survivors. Neurological symptoms include sensorineural hearing loss, foot-slapping gait, bulbar weakness with dysarthria and oropharyngeal dysphagia, and small

fiber neuropathy<sup>10-13</sup>. However, there are limited data delineating the extent of central nervous system (CNS) involvement in children with PD and its impact on developmental functioning. Using brain magnetic resonance imaging (MRI), white matter (WM) hyperintense foci have been described in young children with IPD, the clinical significance of which is unclear<sup>14-20</sup>. Prior studies have not utilized standardized grading scales in MRIs to determine severity of the affected areas.

This cross-sectional study aims to better characterize the extent of CNS involvement in children with IPD and LOPD by 1) quantifying WM hyperintense foci in the brain using MRI and a comprehensive grading system-the Fazekas scale, 2) simultaneously assessing cognitive functioning and language with standardized measures, and 3) exploring potential relationships between the degree of WM hyperintense foci and performance on these developmental measures.

## **METHODS**

### ***Participants***

The study included fourteen children (age 6-18 years) with a confirmed diagnosis of IPD (n=12) and LOPD (n=2) receiving long term ERT (20 mg/kg/biweekly up to 40 mg/kg/weekly). For the IPD group, records were reviewed to confirm cardiomyopathy in the first year of life. The LOPD group includes children presenting with clinical features of PD, but no cardiac findings in the first year of life.

### ***Standard Protocol Approvals, Registrations, and Patient Consents***

The Duke University Institutional Review Board (Protocol #Pro00072329) approved the study protocol (2016-2018). The developmental outcomes component of the study is in [clinicaltrials.gov](https://clinicaltrials.gov) (NCT02950298). Written informed consent was obtained from each patient (at age 18 years) or patient's parent/guardian prior to all assessments. Verbal assent was obtained from children 6-11 years of age and additional written assent was obtained from children 12 years of age and older. All children traveled to Duke University for their assessments, with the exception of Patient 12. Medical records and MRIs of Patient 12 were obtained through a collaborative effort with a geneticist at another institution, abiding with the Duke Institutional Review Board and the study protocol.

#### ***Data Availability***

Any additional data would be shared by requests from any qualified investigator; contingent on the study protocol.

#### ***Medical records review***

Medical records from each patient's most recent clinic visit with an experienced medical geneticist (PSK) were reviewed. Speech, vision, hearing evaluations, examination of deep tendon reflexes, ventilation status, ambulation status, genetic variants, and CRIM status at the time of this study were reviewed. Baseline and most recent echocardiographic data, and alglucosidase-alfa (ERT) IgG antibody titers at most recent follow up, peak antibody titers, and titers over time were also reviewed.

### *Neuroimaging*

Brain MRI scans were acquired on a Siemens 3.0 T MR Scanner (MAGNETOM Trio) using an 8-channel head coil at Duke University; with the exception of Patient 12 who had an MRI at another institution. All scans at Duke were performed on the same scanner without contrast administration or sedation. T1-weighted, T2-weighted, and fluid-attenuated inversion recovery (FLAIR) images were obtained. An expert neuroradiologist (JP) examined all MRI scans for abnormalities of the brain and adjacent structures. Particular attention was paid to the presence or absence of WM hyperintense foci in the brain and the severity of involvement. WM hyperintense foci on T2-weighted and FLAIR images on brain MRI were independently quantified by two experienced raters (SC, MM). These raters were blinded to the patients' clinical information. Discrepancies were adjudicated by JP, whose decision was considered final.

Neuroimaging data from previous studies in IPD have described one or more areas of WM involvement in the brain<sup>14,15,17-19,21</sup>. Based on the data available from these studies, and from patients followed at Duke, ten anatomical areas of the brain were found to be involved and were selected for the assessment of WM hyperintense foci in a systemic manner in this study. These ten areas reside within the superficial and deep WM areas of the brain. The superficial WM included 1) juxtacortical U-fibers, 2) subcortical WM, 3) periventricular WM in the centrum semiovale and 4) periventricular WM in the frontal and parietal areas at the level of corona radiata; and deep WM included 5) corpus callosum, 6) anterior limb of the internal capsule, 7) posterior limb of the internal capsule 8) external capsule, 9) corticospinal tracts at cerebral peduncles, midbrain, pons (brainstem) and 10) medullary decussation of corticospinal tracts. To quantify WM hyperintense foci in the ten areas, the Fazekas scale was used; a well-

known MRI visual grading scale in adults<sup>22</sup>. The superficial areas were graded as 0=absent, 1=capping or pencil-thin lining (mild), 2=smooth halo (moderate), or 3=irregular WM hyperintense foci extending into the deep white matter (severe)<sup>22</sup>. The deep areas were graded as 0=absent, 1=punctate foci (mild), 2=beginning confluence of foci (moderate), or 3=large confluent areas (severe)<sup>22</sup>. A novel approach was developed in which the individual FS scores from these ten areas were summed to obtain a total FS score for each child, ranging from zero (absent) to 30 (extensive, severe WM hyperintense foci). This was done to characterize the extent of CNS involvement in children with PD. A mid-point value (15; i.e. 50%) between 0 and 30 was considered to be a cut-off to denote severe WM involvement. This was based on the expertise of the neuroradiologist (JP).

### *Developmental Assessments*

One clinical child psychologist (GAS) and one speech and language pathologist (KC), each with expertise in PD, assessed the children. All measures were completed within the same week as the brain MRI scan, with the exception of Patients 4, 5, and 13 who had a time difference of 1-4 months between their MRI and developmental assessments.

### *Cognition:*

Depending on the patient's age, cognitive abilities were assessed using either the Wechsler Intelligence Scale for Children-Fifth Edition (ages 6-16 years) or the Wechsler Adult Intelligence Scale-Fourth Edition (older than 16 years)<sup>23,24</sup>. As an alternative to the Wechsler scales, children with significant hearing and/or speech and language difficulties or those who were not native English speakers completed the Leiter International Performance Scale-Third Edition (Leiter-3),

a nonverbal intelligence test with minimal motor demands<sup>25</sup>. Overall composite scores were computed for the Wechsler scales (Full Scale IQ) and/or the Leiter-3 (Nonverbal IQ) as an estimate of each patient's global intellectual ability. Children also completed all subtests of the Beery-Buktenica Developmental Test of Visual-Motor Integration, 6<sup>th</sup> Edition as a measure of their visuomotor ability and the Peabody Picture Vocabulary Test-4 as a measure of their one-word receptive vocabulary<sup>26,27</sup>. All tests yielded age standard scores with a mean of 100 and a standard deviation of 15. Thus, scores between 85 and 115 were considered to be in the average range in comparison to same-aged, typically developing peers. The tests were generally administered in two morning sessions to minimize the potential impact of fatigue on each patient's performance.

### ***Language:***

Language abilities were assessed using the Clinical Evaluation of Language Fundamentals – Fifth Edition (CELF-5), which yields three index scores: Core Language Score (a measure of general language ability), the Receptive Language Index Score (listening and auditory comprehension skills), and the Expressive Language Index Score (expressive language skills)<sup>28</sup>. Caregivers of children who were under the age of 17 years completed the Children's Communication Checklist – Second Edition (CCC-2) to describe their child's articulation/phonology, language structure, vocabulary, discourse, and pragmatic communication skills<sup>29</sup>. This measure yielded a General Communication Composite score. All CELF-5 and CCC-2 scores are age standard scores with a mean of 100 and a standard deviation of 15.

### ***Statistical analyses***

Descriptive statistics were used to summarize the distribution of continuous and categorical variables using medians (5th and 95th percentiles) and counts (percentages), respectively. The distributions of cognitive and language assessments were compared by age of disease onset, age of ERT initiation, and between the IPD and LOPD groups using scatter and box plots.

Relationships between clinical characteristics and MRI findings were explored. Possible relationships between genetic variants in the two affected GAA alleles and the extent of WM hyperintense foci were explored. Where appropriate, Wilcoxon rank-sum tests or Kruskal-Wallis tests with Bonferroni correction for multiple comparisons were used to evaluate statistical differences between the IPD and LOPD groups on various scales and other clinical findings. The relationships between neuroimaging findings and developmental assessments were explored for children with IPD using the Kruskal-Wallis tests with Bonferroni correction. Statistical significance was defined as the calculated probability (p-value) of less than 0.005 for comparisons of disease duration or duration of treatment and brain MRI findings; and a calculated probability of less than 0.003 was required for comparisons of MRI findings with cognitive and language assessments.

## RESULTS

### *Patient demographics*

**Table 1** summarizes the clinical characteristics of twelve children with IPD (Patients 1-12) and two children with LOPD (Patients 13 and 14). Patients 1-10 were CRIM-positive and 11-12 were CRIM-negative. Cardiac data was available in all; data on left ventricular mass index and

ejection fraction were available in seven patients with IPD. *Table 2* summarizes the patients' genetic variants.

### *Neuroimaging*

#### **IPD group:**

Of the twelve children with IPD, MRIs from two (Patients 1 and 11) had a total FS score of zero, indicating no WM hyperintense foci in the selected anatomical areas. MRIs from ten children (Patients 2-10, 12) had total FS scores ranging from 2 to 23, indicating WM hyperintense foci in the brain. Superficial WM areas (n=10) were more affected (with individual area FS scores of 2 and 3) than those of the deep WM (n=7) (*Table 3 and Figure 1*). *Table 3* summarizes the total number of children affected in the ten WM areas, and the individual FS scores.

Corticospinal tracts were affected throughout its pathway in the brain: the fronto-parietal areas (n=10; Patients 2-10, 12), the posterior limb of internal capsule (n=5; Patients 2-3, 9-10, 12), corticospinal tracts at the level of cerebral peduncles, midbrain, and pons (n=6; Patients 2, 3, 7, 9, 10, 12), and at the level of medullary decussation (n=2; Patients 2 and 3) (*Table 3*).

Assessment of the other structural areas in the brain was overall normal, except for two children who had mild volume loss (Patients 5 and 8) (*Table 3*).

There were no statistically significant relationships between total FS scores and each of the following variables: age of children with IPD, anatomic areas of the brain, duration of treatment, and duration of disease. There were no statistically significant relationships between individual area FS scores and the duration of the disease or duration of treatment. However, there

was an association between duration of disease and individual FS scores for fronto-parietal areas (p-value=0.08), and the posterior limb of internal capsule (p-value=0.09).

Genetic variant combinations were evaluated, of which the combination of missense-missense was the most frequent (**Table 2**). There were no significant relationships between genetic variants in the two affected GAA alleles and the extent of WM hyperintense foci.

#### **LOPD group:**

Both children with LOPD had a total FS score of zero. Overall, their structural anatomy of the brain was normal (**Table 3**). Variant combinations included splice site-frameshift deletion and missense-missense (**Table 2**).

#### ***Cognitive assessments***

Ten children with IPD (Patients 2-11) and two children with LOPD (Patients 13, 14) completed the cognitive assessments (**Table 4**). Patient 1 was not evaluated due to scheduling issues. Patient 12 did not travel to Duke University or complete the developmental assessments locally. The psychologist did not administer the Wechsler Intelligence Scale for Children-V verbal subtests to Patients 2, 5 and 9 due to reduced speech intelligibility. Patient 8 completed the Wechsler Adult Intelligence Scale-IV, which does not yield Visual Spatial and Fluid Reasoning composite scores.

#### **IPD group:**

Available Full Scale IQ scores from the Wechsler scales (n=7) ranged from significantly below average (n=3) to significantly above average (n=1) in comparison to same-aged peers. The

median score for the Processing Speed domain (76) was the lowest among the Wechsler domains. Nonverbal IQ scores from the Leiter-3 (n=7) ranged from below average (n=2) to average (n=5) in comparison to same-aged peers. Standard scores on the Peabody Picture Vocabulary Test-4 (n=9) ranged from significantly below average (n=5) to significantly above average (n=2). Standard scores on the Visual-Motor Integration (n=8) and the Motor coordination subtests (n=8) clustered at the lower end of the average range (*Table 4*).

#### **LOPD group:**

All standard scores for the two children with LOPD were within the average range or above average in comparison to same-aged peers, with the exception of Patient 13's below average score on the Wechsler Intelligence Scale for Children-V Processing Speed composite.

#### ***Language assessments***

Eight children with IPD (Patients 1-3, 5, 7-9, 11) and both children with LOPD (Patients 13, 14) completed the language assessments (*Table 4*). Core Language and Expressive Language Index scores could not be calculated due to limited speech intelligibility in Patients 2 and 9, and due to researcher error in test administration in Patient 14. Patient 8 exceeded the upper age limit for CCC-2. As per test guidelines, CCC-2 could not be administered in Patients 2, 3, 5, and 7 due to permanent hearing loss.

#### **IPD group:**

On the CELF-5, scores on Core Language (n=6), Receptive Language Index (n=8), and Expressive Language Index (n=6) ranged from below average to above average in comparison to

same-aged peers. All median standard scores were within the average range, including the General Communication Composite score from the CCC-2 (n=3).

**LOPD group:**

All language standard scores for Patient 13 were significantly above average in comparison to same-aged peers. Available scores for Patient 14 were average and below average.

***Statistical relationships between neuroimaging, cognition, and language assessments***

There were no significant relationships between total or individual area FS scores and standard scores on each cognitive or language domain. However, there was an association between the degree of WM hyperintense foci in the corpus callosum and scores on the Beery-Buktenica Visual-Motor Integration subtest (p-value=0.02) in children with IPD; though not statistically significant.

**DISCUSSION**

Our study utilized the Fazekas scale (FS), a 4-point grading system, to quantify WM hyperintense foci seen on brain MRIs of children and adolescents with IPD and LOPD. The rationale for choosing the FS over other visual grading scales was that it is well-validated, reliable, reproducible, and modifiable (for use in multiple anatomical regions in the superficial and deep areas). The MRIs used for the scoring did not require the children to be sedated or be given any contrast. Novel approaches used in a well-established grading system enables a better characterization of the extent and location of CNS involvement, and could serve as a model for

longitudinal follow up with serial brain MRIs in patients with Pompe disease and other known neurodegenerative disorders or leukodystrophies.

Data from this study showed considerable WM hyperintense foci in the superficial areas of the brain in the majority of children with IPD (10/12) and additional WM hyperintense foci in the deep areas (7/12). The individual area FS scores in the superficial regions of the brain were higher than those in the deeper regions (**Table 3**). These MRI findings suggest that WM hyperintense foci may develop first in superficial WM and progress to deep WM. A similar pattern of progression was proposed in a prior report of repeated MRIs for six children with IPD (ages 2y, 8m to 17y, 1m), where WM hyperintense foci appeared to progress from periventricular WM to subcortical as well as the deeper areas<sup>15</sup>.

To better understand the pattern of involvement in children with IPD, the current findings were compared with other inborn errors of metabolism with leukodystrophy, such as Krabbe disease, adrenoleukodystrophy (ALD), metachromatic leukodystrophy (MLD), and Alexander disease<sup>30-33</sup> (**Table 5**).

Anatomically, fibers of the corticospinal tracts originate from the motor compartments (fronto-parietal areas) in the brain, pass along the posterior limb of internal capsule, the brainstem, decussate at the medulla, and then traverse in the spinal column. In our study, WM hyperintense foci were seen at each of these levels in the brain (n=10 children with IPD) (**Results: Neuroimaging section**). These abnormalities typically produce motor deficits, which are measured by the Motor Coordination subtest of the Beery-Buktenica. In our study, for the IPD group, Motor Coordination subtest scores were low in 6/7 children with WM involvement along the path of corticospinal tracts, and one child with no WM involvement had a high score (**Table 4**). Moreover, there was an association between duration of disease and FS scores in the

fronto-parietal areas and the posterior limb of internal capsule. Although not statistically significant, perhaps due to the small sample size in our study, trends in the route of the corticospinal tracts need to be closely observed with follow up studies.

Additionally, abnormalities in the corticospinal tracts are often associated with hyperreflexia or spasticity. In our study, children were either areflexic, hyporeflexic, or normal; spasticity was not observed. However, spasticity was described in a CRIM-negative 4 year old girl who also had severely progressive WM hyperintense foci<sup>17</sup>. The mechanisms involved in spasticity are complex and as a result, the lack of spasticity could still mean that either upper or lower motor neurons or both are involved<sup>34</sup>. There is early and severe myopathy below the spinal level in children with IPD. This may be one reason why clinicians are unable to elicit any signs of upper motor neuron disease.

In our study, five of the younger children with IPD (6y 7m-12y 6m; Patients 2, 3, 9, 10, 12) had extensive involvement of the u-fibers, along with extensive brain WM hyperintense foci (total FS scores range: 16-23). The older children with IPD (ages 12y 5m-18y) (Patients 4-8) had no u-fiber involvement and had lower total FS scores (range: 2-7). Previously, however, sparing of u-fibers was described in children with IPD (age range: 32m-7y, 1m)<sup>15,17,19</sup> as seen in Krabbe disease, ALD, and MLD<sup>30-33</sup> (**Table 5**). More research is needed to better understand the impact of early versus late u-fiber involvement in children with IPD.

Our study explored the relationship between genetic variants and WM hyperintense foci, but did not find any significant relationships. Children with higher total FS scores (>15) had missense-missense, in-frame deletion-missense, frameshift deletion-missense, splice site-splice site, and nonsense/missense-missense combinations (**Table 2**). One should recognize that children with more severe variant combinations might have either succumbed to the disease

earlier than survivors of IPD or have not been able to obtain MRIs due to their functional limitations, and therefore, the extent of the most severe CNS involvement cannot be determined. Four children in our study did not show WM hyperintense foci: two younger children with IPD (one CRIM-negative and one CRIM-positive) and the two children with LOPD. The effects of the pathogenic variants were known in three of the four children, and were found to be less severe (**Table 2**). Two children had complex genotypes with multiple variant types in one allele (**Table 2**). Such complex genotypes make it difficult to analyze the typical genotype-phenotype correlations and may not be completely informative about the disease severity. Patients 7 and 8 have low FS scores (6 and 2, respectively) and are pursuing post-secondary education. These children may have survived to this age due to their less severe genetic variants or a better response to ERT. Therefore, our study showed that there is a spectrum of CNS involvement in IPD, where some children are more affected than others. While there were no WM hyperintense foci in the two children with LOPD in our study, there is an isolated case report in the literature of a 6 year old male with LOPD showing WM hyperintense foci in the right middle frontal gyrus/frontal cortex<sup>35</sup>. With such limited data, one cannot state whether or not children with LOPD are at a risk of developing any WM hyperintense foci.

In an attempt to determine the clinical significance of WM hyperintense foci, cognitive functioning and language were simultaneously assessed in this study using standardized measures. Overall, due to the small sample size, there was limited evidence of a statistical relationship between the severity of WM hyperintense foci and cognitive measures, using the Kruskal-Wallis tests with Bonferroni correction. The association of Beery-Buktenica Visual-Motor Integration and the degree of WM hyperintense foci in the corpus callosum was evident, though not statistically significant. Since the corpus callosum helps to integrate the motor,

sensory, and cognition, further studies with a larger patient cohort are required to understand how this relationship would be affected as PD progresses in these children.

Analyses of the cognitive and language measures were largely in concordance with previous reports in the IPD population; with scores ranging from below normal to above normal range<sup>36</sup>. The median Full Scale IQ for the IPD group (85) in this study was very consistent with our previous reports, falling at the lower end of the average range<sup>36</sup>. The median Nonverbal IQ score on the Leiter-3 (99) was higher, as reported previously<sup>36</sup>. The Leiter-3 measures a child's IQ when the language demands are minimized, which may account for the higher scores in comparison to the Wechsler scales. Median scores on the Processing Speed (76) and Fluid Reasoning (78.5) domains of the Wechsler scales, the Visual Perception (79) subtest of the Beery-Buktenica, and the Peabody Picture Vocabulary Test-4 (80) were more than one standard deviation below the mean, suggesting that these may be relative areas of weaknesses in IPD. In contrast, short-term Working Memory (103) appears to be a relative area of strength for the IPD group.

In our LOPD group, the two children earned average or above average scores on most cognitive domains. Adults with LOPD were reported to have significant impairments in executive functions and short-term memory<sup>37,38</sup>. Therefore, to understand the developmental trajectory of children with LOPD, further studies are warranted.

The language assessments among children with IPD showed that the scores on the core language, receptive, and expressive language domains ranged from below average to above average on the Clinical Evaluation of Language Fundamentals (CELF-5) tests. However, the median scores on the language domains for the IPD group suggest that language may be a relative area of strength for those children who were able to complete these measures.

Our study is limited by the sample size of both groups (IPD and LOPD) and the current neuropsychological battery, which may not be sensitive to the impact of PD on the developing brain. Moreover, myelination (WM growth) is a dynamic process in a child. The cross-sectional design of this study and lack of an age-matched control group without PD limit our ability to understand the significance of the WM hyperintense foci at one time-point in children with PD. With only two children with LOPD, our study could not compare WM involvement between the IPD and LOPD groups. Despite these limitations, ours is the first study to use the FS to quantify WM hyperintense foci in multiple anatomic areas of the brain in children with PD, to assess the extent of involvement, and have the ability to monitor disease progression in each area. Our study showed that in children with IPD, WM hyperintense foci were seen throughout the path of the corticospinal tract within the brain.

Variable degrees of WM hyperintense foci were present in children with IPD. There could be several factors contributing to this; including genotype and allelic variations, classic versus non-classic forms of the disease, modifier genes, complex genotypic combinations, and CRIM status. However, with the small number of patients in the subgroups and presence of unique/complex genotypes, it was difficult to make genotype-phenotype correlations.

Our findings of variable WM abnormalities support the importance of obtaining baseline and serial MRIs, and the use of visual grading scales (such as the FS) to understand possible advancement of WM lesions. A better understanding of whether these WM abnormalities represent an advancement of cerebral damage in CNS is needed. Additionally, repeated neuroimaging studies over time are critical to our understanding of the development of brain abnormalities, their potential impact on developmental outcomes, the probable mechanisms, and to explore the possibility of a time lag between the emergence of WM hyperintense foci in the

brain and problems in cognition. A time lag was previously described in Krabbe disease and ALD, where the WM hyperintense foci in the brain precede symptom onset<sup>39,40</sup>.

Moreover, the Fazekas scale grading system is a semi-quantitative attempt to depict the severity of WM abnormalities (screening tool). It does not allow the determination of the cause-demyelination, gliosis, vascular problems such as ischemia, cerebrospinal fluid leaks, edema, or inflammatory processes. Based on the findings in the current study, likely mechanisms for WM abnormalities in young patients include dysmyelination (i.e., failure of development of normal myelin) or demyelination. In the absence of early childhood MRI scans, it is difficult to distinguish between the two entities. To the best of the authors' knowledge, no articles have been published to determine the cause of these WM hyperintense foci in children with IPD. Further research with advanced neuroimaging techniques would help in understanding the pathogenesis and in the development of targeted therapies for CNS involvement. Even though the FS is a widely used visual rating scale with good reliability, the validity of using the FS in Pompe disease could not be evaluated in our study. Statistical validation of the FS in the PD population would be a valuable future step.

Our study highlights the importance of a quantitative approach to systematically describe WM hyperintense foci in ten anatomical areas of the brain in patients with PD. With our novel approach of using the Fazekas scale in a sample of children with PD, the extent of WM hyperintense foci can be quantified at any given point of time, and the severity can be compared between any two patients. If done by the same neuroradiologist, subjective errors can be reduced. Our scoring system is flexible and modifiable; with a growing understanding of the natural history and the anatomical areas of the brain involved in Pompe disease, one can add or remove certain areas from the total scoring. Since it is a standard grading system, a multi-center

collaboration would allow a deeper insight about the CNS involvement in a larger cohort of patients. Our study also outlines the importance of simultaneously assessing developmental measures such as cognition and language to explore potential relationships with neuroimaging findings. Standardized assessments of WM hyperintense foci in the brain and developmental outcomes, as outlined in this study, will contribute greatly to our efforts to assess the effectiveness of new therapies targeting the CNS in patients with PD.

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## APPENDIX 1: AUTHORS

Author Name	Location	Contribution
Aditi Korlimarla, MBBS	Duke University Medical Center, Durham, NC	Designed and conceptualized study; major role in data acquisition; analyzed and interpreted the data; drafted and revised the manuscript for intellectual content; coordinated with all the study collaborators
Gail A. Spiridigliozzi, PhD	Duke University Medical Center, Durham, NC	Major role in the acquisition of data; analyzed the data; revised the manuscript for intellectual content; designed and conceptualized study
Kelly Crisp, MA, CCC-SLP,	Duke University Medical Center, Durham, NC	Major role in the acquisition of data; analyzed the data; revised the manuscript for intellectual content; designed and conceptualized study
Mrudu Herbert, MD, MPH	University of Kentucky Medical Center, Lexington, KY	Drafted part of the manuscript for intellectual content; role in data acquisition

Steven Chen, BS,	Duke University Medical Center, Durham, NC	Major role in the acquisition of data; analyzed and interpreted the data; conceptualized part of the study
Michael Malinzak, MD, PhD,	Duke University Medical Center, Durham, NC	Analyzed and interpreted the data; conceptualized part of the study
Mihaela Stefanescu, BS	Duke University Medical Center, Durham, NC, USA	Major role in the acquisition of data
Stephanie L. Austin, MA, MS,	Duke University Medical Center, Durham, NC, USA	Major role in the acquisition of data; designed and conceptualized study; reviewed the manuscript for intellectual content
Heidi Cope, MS, CGC,	Duke University Medical Center, Durham, NC	Revised the manuscript for intellectual content; played a role in data acquisition
Kanecia Zimmerman, MD MPH,	Duke Clinical Research Institute, Durham, NC	Analyzed and interpreted all the statistical data; reviewed the manuscript for intellectual content
Harrison Jones, PhD,	Duke University Medical Center, Durham, NC	Analyzed and interpreted the data; reviewed the manuscript for intellectual content; designed and conceptualized study
James M. Provenzale, MD, FACR	Duke University Medical Center, Durham, NC	Major role in the acquisition of data; analyzed and interpreted the data; reviewed the manuscript for intellectual content; designed and conceptualized study
Priya S. Kishnani, MD	Duke University Medical Center, Durham, NC	Study PI; designed and conceptualized study; analyzed and interpreted the data; revised the manuscript for intellectual content; played a role in data acquisition

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## TABLES

Table 1. Clinical features and characteristics of children with IPD (Patients 1-12) and LOPD (Patients 13 &amp; 14)

Pt ID	Age at MRI assessment t/sex	Age at diagnosis (months)	Age at ERT start (months)	ERT dose (mg/kg)	History of delay/d milestones (<4 years of age)	DT R (on both sides)	Baseline LVMI in g/m <sup>2</sup> (EF in %)	Echocardiography (first year of life) Other details available	most recent ERT IgG Antibody titers	ACE poly morphism	Wheelch air/scooter used for ambulation	SNH L	Corrected with hearing aids	Dysarthria (hyper-nasality, lingual weakness)	Impaired vision requiring glasses (myopia/astigmatism/amblyopia)	Inv asi ve ve ntil ati on
1 <sup>i</sup>	6y, 1m/F	3	3	20 <sup>w</sup>	+	NL		severe LVH and HCM	<sup>u</sup>		+	-		+	+	-
2 <sup>i</sup>	6y, 7m/M	6	6	20 <sup>bw</sup>	+	↓	225.6	biventricular hypertrophy	3200 <sup>a</sup>	D/D	+	+	No <sup>h</sup>	+	+	-
3 <sup>i</sup>	8y, 9m/F	2.25	2.25	40 <sup>w</sup>	+	↓		cardiomegaly on chest x-ray	6400 <sup>a</sup>	I/D	-	+	+	+	+	-
4 <sup>i</sup>	12y, 5m/M	2	3	40 <sup>w</sup>	+	<sup>u</sup>	239 (45)	severe concentric LVH	800 <sup>a</sup>		+	+	+	+	+	-
5 <sup>i</sup>	14y, 9m/F	3	5.5	40 <sup>w</sup>	+	↓/-	114 (67)	HCM	100 <sup>a</sup>	I/I	+	+	No <sup>h</sup>	+	+	-
6 <sup>i</sup>	13y, 9m/M	6	7	40 <sup>w</sup>	+	↓/-	179 (58)	severe LVH	<100 <sup>a</sup>		+	+	+	+	+	-
7 <sup>i</sup>	16y, 3m/M	1	3	30 <sup>w</sup>	+	↓	221 (76)	biventricular hypertrophy	None <sup>a</sup>	I/D	-	+	+	+	+	-
8 <sup>i</sup>	18y/M	prenatal	2	40 <sup>w</sup>	+	↓/-		cardiomegaly on chest x-ray, PFO	800 <sup>a</sup>	I/I	+	-		+	+	-
9 <sup>i</sup>	8y/F	1	2	20 <sup>bw</sup>	+	↓		HCM	1600 <sup>a</sup>	I/I	+	+	No <sup>h</sup>	+	+	-
10 <sup>i</sup>	12y, 6m/M	prenatal	0.5	40 <sup>w</sup>	<sup>u</sup>	↓	69.9 (51)	biventricular hypertrophy, PFO	None <sup>a</sup>	I/D	-	+	+	+	+	-
11 <sup>i,n</sup>	7y, 3m/F	prenatal	0.25	20 <sup>bw</sup>	-	↓		severe HCM	<sup>u</sup>		-	-		-	NL	-
12 <sup>i,n</sup>	7y, 2m/M	3.5	4.5	20 <sup>bw</sup>	+	<sup>u</sup>	445.8	HCM	None <sup>b</sup>		+	+	No <sup>h</sup>	<sup>u</sup>	+	+ <sup>t</sup>
<b>MEDIAN</b>	<b>10y, 6m</b>	<b>1.7</b>	<b>3</b>													
13 <sup>l</sup>	9y, 3m/M	24	27	20 <sup>bw</sup>	+	↓	NL (NL)	NL	200 <sup>a</sup>	I/I	-	-		+	NL	-

14<sup>l</sup> 14y, 6m/F 66 72 40<sup>w</sup> + ↓/- NL (NL) NL; except aortic root dilatation<sup>r</sup> 1600<sup>a</sup> I/I + - + + -

*Patient data was de-identified and IDs were assigned at random. y years, m months, F female, M male, SNHL - sensorineural hearing loss, DTR- deep tendon reflexes in lower limbs, LVMI- left ventricular mass index, EF- ejection fraction, PFO- patent foramen ovale, HCM- hypertrophic cardiomyopathy, LVH- left ventricular hypertrophy; <sup>i</sup> children with IPD, <sup>l</sup> children with LOPD, <sup>n</sup> CRIM-negative, <sup>w</sup> weekly, <sup>bw</sup> biweekly, <sup>h</sup> hearing aids recommended, <sup>u</sup> unavailable details, + present, - absent, ↓ hyporeflexia, NL- normal, <sup>t</sup> tracheostomy in Patient 12 (invasive ventilation for 24 hours/day), <sup>e</sup> The most recent Echocardiography (2D or M mode) reports showed normal values for LVMI and EF in all the patients. <sup>r</sup> aortic root was 2.2cm/m<sup>2</sup> - borderline increase (normal: <2.1 cm/m<sup>2</sup>). <sup>a</sup> The antibody titers remained below 12,800 IU throughout (low titers) and therefore, these patients did not receive immune modulation during the course of ERT. <sup>b</sup> Patient 12(CRIM-negative) received immune modulation (with Rituximab, Methotrexate, and IVIG) at ERT start. No other patients in the study received immune modulation. ACE polymorphism (a modifier gene): I- insertion and D- deletion type.*

Table 2. Types and clinical effects of genetic variants in children with Pompe disease (PD)

ID	Fa zek as sca le	Complementary DNA and amino acid changes				GAA allele type <sup>a</sup>		Clinical effects <sup>a</sup>	
		GAA allele 1		GAA allele 2		GAA allele 1	GAA allele 2	GAA allele 1	GAA allele 2
		Complementary DNA changes	Amino acid changes	Complementar y DNA changes	Amino acid changes				
1 <sup>i</sup>	0	c.1856G>A	p.Ser613Asn	c.1841C>A	p.Thr614Lys	Missense	Missense	less severe	potentially less
		c.-32-13T>G	r.=, r.-32_546del, r.- 32_486del			Splice site		potentially mild	severe
2 <sup>i</sup>	23		p.Tyr407Stop			Nonsense			potentially less
		c.1281G>T	p.Met427Ile	c.1564 C>T	p.Pro522Ser	Missense	Missense	VOUS	severe
		c.2296T>A	p.Tyr766Asn			Missense			
3 <sup>i</sup>	21	c.1293_1312del2 0	p.Gln433AspfsX66	c.1716C>G	p.His572Gln	In-frame deletion	Missense	very severe	potentially less severe
4 <sup>i</sup>	6	c.1933G>A	p.Asp645Asn	c.1933G>A	p.Asp645Asn	Missense	Missense	potentially less severe	potentially less severe
5 <sup>i</sup>	7	c.1802C>T	p.Ser601Leu	c.1099T>C	p.Trp367Arg	Missense	Missense	potentially less severe	potentially less severe
6 <sup>i</sup>	5	c.2297A>C	p.Tyr766Ser	c.2297A>C	p.Tyr766Ser	Missense	Missense	VOUS	VOUS

7 <sup>i</sup>	6	c.1933G>A	p.Asp645Asn	c.1933G>A	p.Asp645Asn	Missense	Missense	potentially less severe	potentially less severe
8 <sup>i</sup>	2	c.1438-1G>T	r.0?	c.1655T>C	p.Lue552Pro	Splice site	Missense	very severe	potentially less severe
9 <sup>i</sup>	18	c.925G>A	p.Gly309Arg	c.1841C>A	p.Thr614Lys	Missense	Missense	potentially less severe	potentially less severe
10 <sup>i</sup>	16	c.525delT	p.Glu176ArgfsX45	c.1642G>T	p.Val548Phe	Frameshift deletion	Missense	very severe	VOUS
11 <sup>i</sup> <sub>n</sub>	0	c.546+2_546+5delTGGG	r.spl?	c.1650_1651dupG	p.Thr551Aspfs*85	Intronic deletion	Frameshift duplication	very severe	very severe
12 <sup>i</sup> <sub>n</sub>	23	c.546+2T>C	r.spl?	c.546+2T>C	r.spl?	Splice site	Splice site	very severe	very severe
13 <sup>1</sup>	0	c.2501_2502delCA	p.Thr834Argfs*49	c.-32-13T>G	r.=, r.-32_546del, r.-32_486del	Frame shift deletion	Splice site	very severe	potentially mild
14 <sup>1</sup>	0	c.1477C>T	p.Pro493Ser	c.1978C>T	p.Arg660Cys	Missense	Missense	VOUS	potentially less
		c.2221G>A	p.Asp741Asn			Missense		VOUS	severe

<sup>i</sup>children with IPD, <sup>1</sup>children with LOPD, <sup>n</sup>CRIM-negative. <sup>a</sup>Based on Erasmus database for human acid alpha-glucosidase (GAA) variants (Last accessed in October 2019) and ClinVar data from National Center for Biotechnology Information (NCBI) (Last accessed in April 2019), VOUS- Variants of uncertain significance

**Table 3. Findings on brain MRI of children with IPD (Patients 1-12) and LOPD (Patients 13 & 14) with total Fazekas Scale scores to measure the degree of white matter hyperintense foci**

ID	Age at MRI	Superficial White Matter					Deep White Matter					Total Fazekas Scale Score	Other Anatomical Features							
		Periventricular WM: centrum semiovale	Periventricular WM: frontal/parietal- corona radiata	Subcortical WM	Juxtacortical U-fibers	External Capsule	Posterior limb of internal capsule	Corticospinal tracts at cerebral peduncles, midbrain, and pons	Corpus callosum	Anterior limb of internal capsule	Medullary decussations of corticospinal tracts		Brain volume	Gray matter	Developmental brain abnormalities	Intracranial vessels	Hemorrhage/mass	Ventricular size	Orbits, cranium, paranasal, and mastoid sinuses	
1 <sup>i</sup>	6y, 1m	0	0	0	0	0	0	0	0	0	0	0	0	NL	NL	No	NL	NL	NL	NL
2 <sup>i</sup>	6y, 7m	3	3	3	3	3	3	2	1	0	2	23	NL	NL	No	NL	No	NL	NL	
3 <sup>i</sup>	8y, 9m	3	3	3	3	3	3	2	0	0	1	21	NL	NL	No	NL	No	NL	NL	
4 <sup>i</sup>	12y, 5m	2	2	2	0	0	0	0	0	0	0	6	a	a	a	a	a	a	a	
5 <sup>i</sup>	14y, 9m	2	2	2	0	0	0	0	1	0	0	7	v		No	NL			NL	
6 <sup>i</sup>	13y, 9m	2	2	1	0	0	0	0	0	0	0	5		NL	No	NL			NL	
7 <sup>i</sup>	16y, 3m	2	1	1	0	0	0	2	0	0	a	6	NL	NL	No		NL	NL	NL	
8 <sup>i</sup>	18y	1	1	0	0	0	0	0	0	0	0	2	v	NL	No	NL			NL	
9 <sup>i</sup>	8y	3	3	3	3	2	1	1	1	1	a	18	NL	NL	No	NL	No	NL	NL	
10 <sup>i</sup>	12y, 6m	3	3	3	2	2	1	1	1	0	0	16	NL	m	No	NL	No	NL	NL	
11 <sup>i,m</sup>	7y, 3m	0	0	0	0	0	0	0	0	0	0	0	NL	NL	No	NL	No		NL	
12 <sup>i,m</sup>	7y, 2m	3	3	3	2	2	3	2	3	2	0	23	NL	NL	No	NL	No	NL	NL	
13 <sup>l</sup>	9y, 3m	0	0	0	0	0	0	0	0	0	0	0	NL	NL	No	NL	No	NL	NL	

14 <sup>l</sup>	14y, 6m	0	0	0	0	0	0	0	0	0	0	0	0	NL	NL	No	NL	No	NL	NL
<b>Total patients</b>																				
<b>with WM</b>																				
<b>hyperintense</b>		<b>10</b>	<b>10</b>	<b>9</b>	<b>5</b>	<b>5</b>	<b>5</b>	<b>6</b>	<b>5</b>	<b>2</b>	<b>2</b>									
<b>foci in each area</b>																				

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*y - years, m- months, a- Indeterminate due to pulsation artifact, <sup>l</sup> children with IPD, <sup>n</sup> CRIM-negative, <sup>l</sup> children with LOPD, v- mild volume loss, m-mild involvement of the lentiform nucleus, NL- normal*

ACCEPTED

Table 4. Standard scores from cognitive and language assessments for children with IPD (Patients 1-12) and LOPD (Patients 13 &amp; 14)

Patient ID	Age at developmental assessment	Total Fazekas Score	Cognitive Assessments										Language Assessments				
			Wechsler Composites					Leiter-3 Nonverbal IQ	Beery-Buktenica		PPVT-4	CELF-5		CCC-2			
			Verbal Comprehension	Visual-Spatial	Fluid Reasoning	Working Memory	Processing Speed	Wechsler Full Scale IQ		Visual-Motor Integration	Visual Perception	Motor Coordination	Core Language Score	Receptive Language Index	Expressive Language Index	General Communication Composite Score	
1 <sup>i</sup>	6y, 1m	0											101	76	90	106	
2 <sup>i</sup>	6y, 7m	23							108	87	107	87	131	111			
3 <sup>i</sup>	8y, 9m	21	98	75	91	103	83	85	100	93	75	87	77	95	102	92	
4 <sup>i</sup>	12y, 1m	6	59	67	69	69	69	60	99				79				
5 <sup>i</sup>	14y, 8m	7		61	67				77	87	51	85	74	61	61		
6 <sup>i</sup>	13y, 9m	5	62	84	67	67	69	60	93				53				
7 <sup>i</sup>	16y, 3m	6	103	94	112	112	103	108		92	89	82	104	111	104	120	
8 <sup>i</sup>	18y	2	143			119	102	124		101	82	93	126	107	117	106	
9 <sup>i</sup>	8y	18		86	85		69		101	77	76	77	80	65		91	
10 <sup>i</sup>	12y 6m	16	84	86	72	76	63	71	84	66	72	73					
11 <sup>i,n</sup>	7y, 3m	0	89	89	109	120	95	98		90	113	109	95	105	115	100	115
12 <sup>i,n</sup>	7y, 2m	23															
<b>For the IPD group</b>		<b>N</b>	7	8	8	7	8	7	7	8	8	8	9	6	8	6	3
		<b>Mean</b>	91.1	80.2	84	95.1	81.6	86.5	94.5	86.6	82.3	86.6	91	96.7	94.9	94.8	104

		<b>Median</b>	89	85	78.5	103	76	85	99	88.5	79	86	80	103	103	96	106
		<b>SD</b>	28.3	11.4	18.5	23.7	16.4	24.7	10.7	10.7	28	11	32	18.3	21	19.8	12.1
<b>13<sup>1</sup></b>	9y, 4m	0	133	117	118	110	75	118		93	106	93	127	131	120	126	125
<b>14<sup>1</sup></b>	14y, 6m	0	95	111	100	94	114	104	113	95	97	102	113		79		91

*y, m- years, months, <sup>1</sup> children with IPD, <sup>n</sup> CRIM-negative, <sup>1</sup> children with LOPD, SD- standard deviation, n- total number of patients, PPVT-4- Peabody Picture*

*Vocabulary Test 4<sup>th</sup> edition; CELF-5- Clinical Evaluation of Language Fundamentals 5<sup>th</sup> edition, CCC-2- Caregiver Communication Checklist 2<sup>nd</sup> edition.*

*Patient 12 did not complete any developmental assessments at Duke*

Table 5. Comparing the pattern of involvement of WM hyperintense foci in other inborn errors of metabolism with leukodystrophy

Other Inborn errors of metabolism with leukodystrophy	Similarities with IPD	Differences when compared with IPD
<b>Krabbe disease</b> <sup>30</sup>	<ul style="list-style-type: none"> <li>Periventricular WM predominance: centrum semiovale</li> <li>Pyramidal tract involvement: early in the disease course</li> </ul>	<ul style="list-style-type: none"> <li>Involvement of occipital areas and deep grey matter</li> <li>Subcortical U-fiber may be spared until late in the course of the disease</li> </ul>
<b>Adrenoleukodystrophy (ALD)</b> <sup>31-33</sup>	<ul style="list-style-type: none"> <li>Early stages of the disease usually involves the splenium of corpus callosum (deep WM)</li> <li>Corticospinal tracts involved</li> </ul>	<ul style="list-style-type: none"> <li>Involvement of occipital WM, with a prominent distinction between the parietal and occipital zones</li> <li>Spreads outwards to the cerebral WM in a cephalad manner (deep WM&gt;periventricular WM)</li> <li>Delayed subcortical WM involvement</li> </ul>
<b>Metachromatic leukodystrophy (MLD)</b> <sup>15,30,33</sup>	<ul style="list-style-type: none"> <li>Involvement of the periventricular WM (especially the centrum semiovale) and deep WM (corpus callosum, internal and external capsules and the corticospinal tracts)</li> <li>Tigroid/ leopard skin pattern of alternating demyelination and normal myelination areas (periventricular areas in MLD; occipital areas in PD)</li> </ul>	<ul style="list-style-type: none"> <li>Sparing of subcortical u-fibers</li> </ul>
<b>Alexander disease</b> <sup>30,33</sup>	<ul style="list-style-type: none"> <li>Subcortical WM is affected early in the course of the disease</li> </ul>	<ul style="list-style-type: none"> <li>Predominantly frontal WM abnormalities in early phases; progress posteriorly to parietal WM and internal/external</li> </ul>

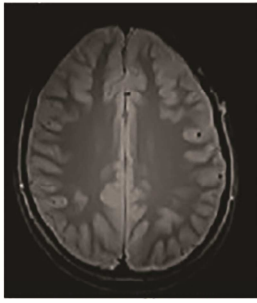
capsules

- Additional slight signal abnormalities in the basal ganglia

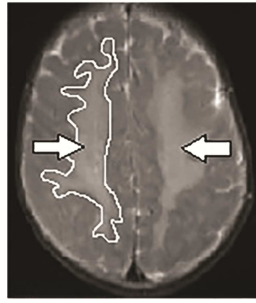
## FIGURE LEGEND

### Figure 1. Brain MRI in children with Pompe disease

*FS = Fazekas scale; White arrows show the abnormal, bilateral white matter hyperintense foci in the brain MRI of five children with IPD (Patients 2, 3, 9, 10, and 12). The abnormal areas (hyperintense foci on T2-FLAIR MRI) are depicted as lighter gray areas (outlined on one side of each patient) compared to the surrounding normal darker gray areas. A normal brain MRI of a child with LOPD (Patient 14) has been included here for comparison.*



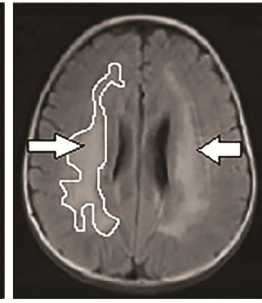
Patient 14: Normal study  
in a child with LOPD  
(centrum semiovale)  
Individual area FS score: 0



Patient 2  
(centrum semiovale)  
Individual area FS score: 3



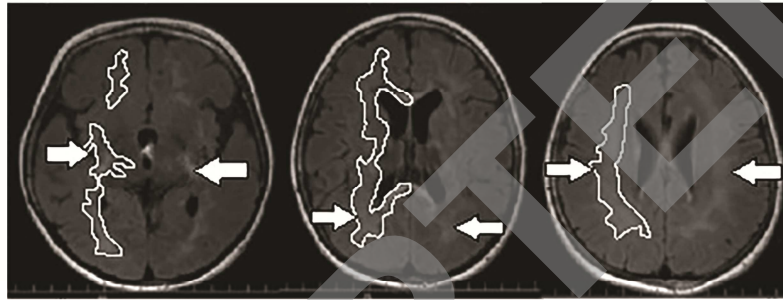
Patient 3  
(at the level of basal  
ganglia-PLIC,  
external capsule,  
fronto-temporo-occipital)  
Individual area FS score: 3



Patient 9  
(at the level of corona radiata)  
Individual area FS score: 3



Patient 10:  
(centrum semiovale)  
Individual area FS score: 3



Patient 12  
(at the level of basal ganglia, corona radiata/lateral ventricle)  
Individual area FS score: 3

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Aditi Korlimarla, Gail A. Spiridigliozzi, Kelly Crisp, et al.

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