Heterogenous PLP1 Mutations Express Differing Pathology Of The Corpus Callosum in Pelizaeus-Merzbacher Disease.

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Introduction: Oilgogdendrocyte health and viability is a central component in the pathogenesis of Pelizaeus-Merzbacher disease (PMD). The proteolipid protein (PLP) is an integral membrane protein found predominately in the CNS myelin [1]. Mutations affected PLP1 gene that include *1)* deletion of the entire gene *2)* point mutations affecting splicing or regulation of gene expression and *3)* increased dosage of the *PLP1* gene [2] all have deleterious effects on oligodendrocyte viability. The corpus callosum is the largest white matter fiber bundle providing connectivity between homologous cortical areas and begins the process of myelination during the 7th month [3]. Congenital anomalies, demyelinating diseases, and transient signal changes are among many disorders affecting this structure [4]. In this study we are investigating white matter integrity and fiber intactness of 3 *PLP1* gene mutation categories: null (gene deletion), moderate and severe all affecting *PLP1* in a cohort of PMD patients and to determine the sensitivity of DTI to probe the potential mutational differences.

Methods: DTI was performed on 12 PMD patients (age: min=2 y, max=45 y, mean=17 ± 16). The images were acquired on 1.5T scanner using 8-channel head coils, parallel imaging (factor of 2) and double refocusing pulses. Six diffusion gradient directions were applied with diffusion sensitivity = 1000 s/mm^2. The acquisition matrix was 128x128 and reconstructed with homodyne in 256x256, the slice thickness was 3 mm, and we performed six repetitions. All PMD subjects underwent genetic testing, neurological examination and an overall assessment of functional disability to assign the severity of PLP mutation. Three patients were diagnosed with a null mutation, 4 with severe mutation and 5 with moderate mutation. The genu and splenium were manually delineated in the directional encoded color maps (Figure 1) then copied to the axial (E_1), radial (E_{23}), mean diffusion (ADC) and FA maps. Between group differences (null, moderate, severe mutations) was tested with multivariate analysis of covariance and age was entered as covariate.

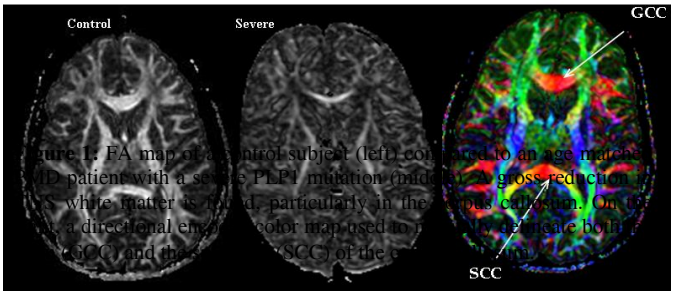
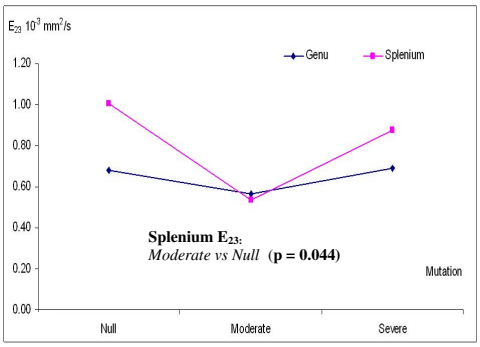
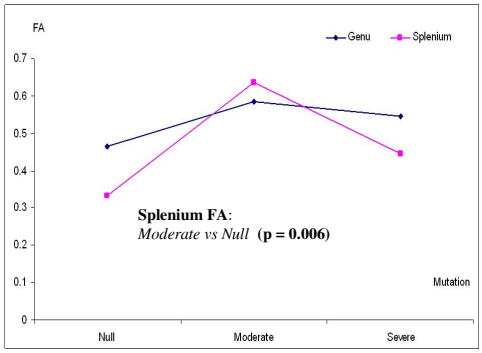


Figure 1: FA map of a control subject (left) compared to an age matched PMD patient with a severe PLP1 mutation (middle). A gross reduction in CNS white matter is found, particularly in the corpus callosum. On the right, a directional encoded color map used to primarily delineate both the genu (GCC) and the splenium (SCC) of the corpus callosum.

Results: In the genu we did not find any significant difference in any DTI index when comparing moderate to null, severe to null and severe to moderate mutations. Radial diffusion and ADC were smaller in the moderate mutation group than that of the null and severe mutations. The FA was larger in the moderate mutation than the other two groups (Figure 2). With regards of the splenium, E_{23} was significantly lower *(p=0.044)* in patients with moderate severity compared to null mutations (-46%). The anisotropy FA on the splenium was significantly higher *(p=0.006)* in patients with moderate severity compared null mutations (+100%). No other difference in any DTI index was measured significantly between severe and null mutation groups or between severe and moderate mutations groups.

Figure 2: Estimated marginal means corrected for age in the genu and splenium of the corpus callosum measured among 12 PMD patients with null, moderate and severe *PLP1* mutation. radial diffusion E_{23}, (left) and fractional anisotropy FA (right). The splenium E_{23} of the moderate mutation group was significantly lower (p= 0.044) than that of the null group (b). The anisotropy in the splenium was significantly higher (p=0.006) in patients with moderate severity compared to that of null mutation.





Conclusion: Our study of the genu and splenium of the corpus callosum revealed pathologic insights describing *PLP1* mutations that characterizes the clinical spectrum of PMD. The increased radial diffusivity found in the splenium of patients with a deletion (null syndrome) of the PLP1 gene compared to severe mutations likely suggest that more than one PLP mutation mechanism is responsible for the observed imaging pathology. Absence of PLP causes structural instability of CNS myelin resulting in the tendency for extracellular fluid to infitrate into the interlammellar space, revealing the existence of a "radial component" [5], whereas the decrease in FA describes the lack of orientational coherence of white matter fiber bundles characterized by the cytoarchitecture of the CNS tissue, resulting from axonopathy that may occur as a late-onset phenonmenon. The increase in axial and radial indices observed in severe *PLP1* mutations describes both hypomyelination and axonopathy, suggesting water has a greater amplitude of motion and freedom. The respective contribution of the extracellular and intracellular compartments to the measured ADC remains unclear. Amplification of axial diffusion may reflect extensive cytoplasmic extension of astrocytes in response to the severe hypomyelnation and reduction in fiber density. The remarkable distinguishing difference between the genu and splenium is not fully understood, however investigating the correlation between fiber tract integrity in the corpus callosum and cortical gray matter volume would support the notion that a decline in FA reflects a loss of intracortical projecting neurons.

References: [1] Griffiths I. et. al. *Microsc Res Tech* (1998) [2] Woodward K. and Malccolm S. *Trends Genet* (1998). [3] Kinney HC et al. *J Neuropathol Exp Neurol* (1988) [4] Deoni SC et al., *J Neurosciences* (2011) [5] Rosenbluth J et al., *Glia* (2006);