Pre-symptomatic Degeneration and Dysmyelination of Axons in a Huntington's Mouse Model Revealed by Diffusion Tensor MRI

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Target audience – Investigators in the MR community interested in developing new tools for evaluating axonal degeneration in Huntington's Disease (HD).

Purpose – HD results from accumulation in the brain of a malformed Huntingtin protein that expresses an abnormal repetition of an amino acid, glutamine. Extensive pathological and electrophysiological evidence[1] suggest that the motor and cognitive symptoms characteristic of HD involve axonal degeneration in specific neuronal populations within the cortex and the striatum. Nevertheless, the molecular mechanisms underlying these deficits remain unknown, making the development of new pre-symptomatic, non-invasive imaging tools for evaluating HD animal models imperative. The double transgenic YFP-R6/2 mouse, which allows the direct visualization of HD through intrinsic fluorescent properties of Huntingtin has been proposed as a way to monitor disease progression (via laser confocal microscopy). In this study, we supplemented the optical imaging with MR longitudinal studies to quantify the development of HD as it develops using diffusion tensor imaging. In addition, we compared the differences between animals with low (YFP-R6/2[120q]) and high (YFP-R6/2[160Q]) glutamine repeats, specifically in the corpus callosum. To further validate the MR findings, we also compared DTI parameters with results obtained from microscope-based quantitative fluorescence experiments.

Methods – Brains of control animals expressing YFP only and YFP-R6/2 mice were excised at 30, 60, and 90 days and fixed with a 4% formalin perfusion using an approved animal protocol. All MR images were acquired on a horizontal Agilent 9.4 T animal imaging system with samples immersed in Fluorinert to reduce magnetic susceptibility. Spin echo images were acquired with TR = 2000 ms and TE = 35 ms at b = 1000 s/mm^2 in 12 and 30 directions with an in-plane resolution of 117 µm and a slice thickness of 1 mm. Post-image processing used MRIStudio[2] and TrackVis[3]. ROIs were segmented, according to imaging atlases, across sagittal (nine ROIs) and coronal sections (four ROIs) of the corpus callosum in order to measure mean fractional anisotropy (mFA), axial diffusivity (AD), and radial diffusivity (RD). To evaluate axonal integrity and organization, brain tissues were further evaluated by quantitative fluorescent microscopy using ImageJ[4] and a pixel-based coherence algorithm[5].

Results – The mean fractional anisotropy decreased in the pre-frontal and parietal regions of the corpus callosum in R6/2 brains compared with controls in a time-dependent manner. Consistent with the pathology of HD, the mFA was lower in YFP-R6/2 [160Q] than in YFP-R6/2 [120Q] (**Figure 1**). In addition, both the RD and AD increased with age in affected sagittal regions and coronal ROIs (data not shown in figures). Color maps of both control and R6/2 brains at 30, 60 and 90 days are displayed in **Figure 2**.

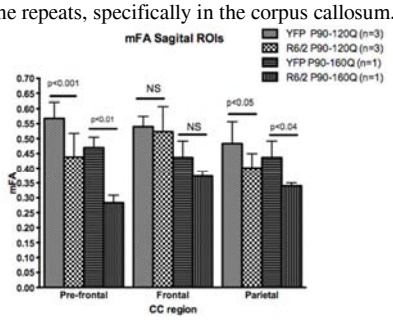

Figure 1: mFa values for 90 day-old YFP and YFP-R6/2 mutant mice expressing mutant Huntingtin with low (120Q) and high (160Q) glutamine repeat.

Discussion – The mean fractional anisotropy describes the integrity of white matter fiber tracts. Our results suggest that mFA decreases with the onset and progression of HD in axonal tracts such as the corpus callosum. In the pre-frontal and parietal areas of the corpus callosum there was a decrease in mFA with a largely spared segment in the frontal region. These observations match the functional progression observed in human HD. Furthermore, quantitative increases in the axial and radial diffusivity have been associated with axonal degeneration and demyelination[6] respectively (increases in AD and RD). Finally, the measured mFA results were consistent with fluorescent microscopy (**Figure 3**), showing axonal degeneration in the pre-frontal and parietal regions of the corpus callosum with frontal region preservation.

Conclusion – These results indicate a high degree of spatial correlation between DTI and fluorescent microscopy techniques and suggest a basis for further investigation into the staging and treatment of HD. DTI can visualize axonal degeneration in YFP-R6/2 mice and quantifies parameters that can be utilized successfully as biomarkers for degeneration in this HD mouse model. Further fluorescent studies will be done to profile and quantify changes in specific populations of neurons. We are also implementing fractional diffusion measurements to interrogate the changing relative changes in cell populations during the evolution of the disease.

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References – [1] Han, I., *et. al, J. Neurochem* (2010). doi:10.1111/j.1471-4159.2010.06672.x [2] Mori, S., *et. al. Ann. Neurol*. **45,** 265–269 (1999). [3] Wang, R., *et. al. ISMRM Abstr.* (2007). [4] Schneider, C. A., *et. al. Nat Methods* **9,** 671–675 (2012). [5] Rezakhaniha, R. *et al. Biomech Model Mech* **11,** 461–473 (2011). [6] Phillips, O. *et al. PLoS ONE* **8,** e73280 (2013).

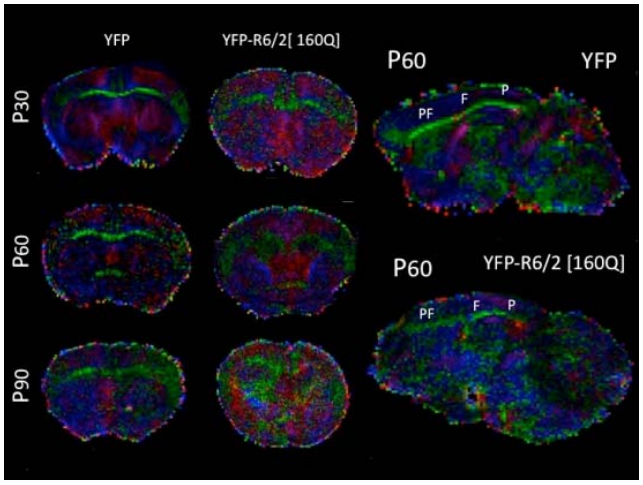

Figure 2: Graphic showing mFA color maps of axial views of 30/60/90 day-old YFP and YFP R6/2 mouse brains with sagittal views at 60 days.

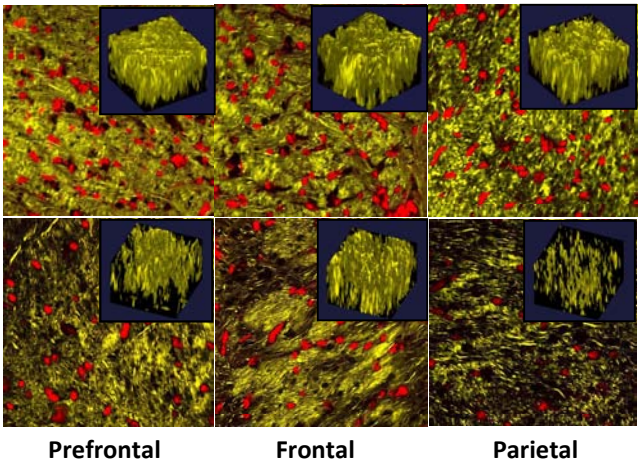

Figure 3: Optical imaging of P90 YFP control (top) and P90 YFP-R6/2 160Q mutant (bottom). Note the relative lack of degeneration in the frontal area of the corpus callosum (blue insert).