Transverse Relaxation and Volumetric Neural Changes in the H67D HFE High Iron Mouse Model

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Introduction: Iron accumulation in the brain and oxidative stress are observed in a number of neurological diseases. A common mutation that leads to high iron (HFE) overload has been associated with two gene variants, C282Y and H63D. The role of the H63D HFE variant has been associated with neurodegenerative diseases such as hereditary hemochromatosis, Amyotrophic Lateral Sclerosis, Parkinson's disease, and Alzheimer's disease. H63D mutations result in iron dyshomeostasis, increased oxidative stress, glutamate release, tau phosphorylation, and alteration in inflammatory response[1]. Within Alzheimer's disease these mutations are found with increased frequency in patients (estimates are between 20-50%). A mouse model has been generated that contains the mouse homolog for the human H63D gene, the H67D knock-in[2]. The H67D mice express alterations in their iron management proteins, such as increased H- and L-Ferritin and decreased transferrin expression. The model also exhibits increased oxidative stress and astrogliosis. While the literature shows these changes upon *ex vivo* quantification, the noninvasive *in vivo* MRI evaluation of the mice has not previously been undertaken. The goal of this research is to track longitudinal neural changes in the transverse relaxation profile and relate these MR metrics to histological measures of iron concentration and iron related protein expression.

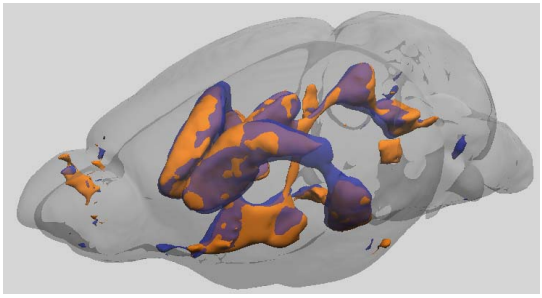

Figure 1 - Three dimensional rendering of ventricles from H67D (blue) (N=10) and WT (orange) (N=11) mice.

Methods: H67D knock-in mice were commercially generated as previously described[2]. Mice were maintained under normal housing conditions and fed food and water *ad libitum*. All procedures were conducted according to NIH and internal IACUC guidelines. Twenty-one mice, 11 HFE and 10 Wildtype C57BL/6 (WT), were anesthetized with 1.5% isoflurane and placed within a 7.0 T Bruker MedSpec MRI system and 23mm birdcage volume coil (Bruker BioSpin). Animals were imaged at baseline (10 weeks old) and twelve months later with the same imaging protocol. Eight echo T_2 weighted MSME (11-88ms) datasets were acquired at a voxel resolution of 100 x 100 x 250 µm. Parametric relaxation T_2 and R_2 maps were generated using a linear model. All datasets were coregistered and resliced to match the first echo of the MSME dataset using SPM 5 with the SPMmouse toolkit[3]. Datasets were then skullstripped, realigned to a template mouse brain, and segmented into white matter (WM), gray matter (GM) and cerebrospinal fluid (CSF) with a probability map for volumetric analysis. For parametric map analysis, the relaxation maps and first echo of the MSME datasets were normalized to the template brain and voxel based analysis was performed using a group based method in SPMmouse (cluster size ≥ 5 and pValue ≤ 0.001).

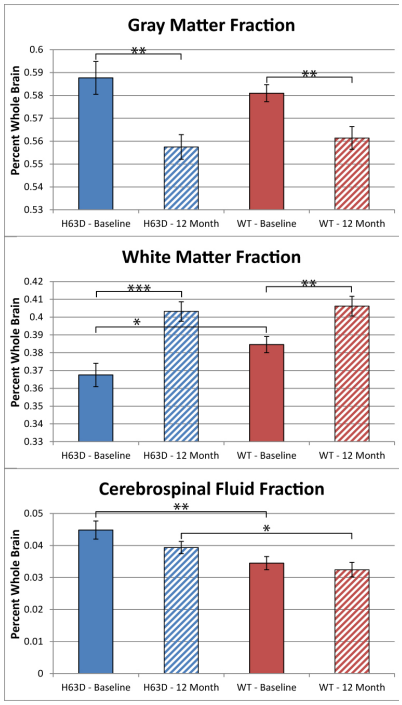

Figure 2 – Volumetric changes in gray matter , white matter, and cerebrospinal fluid. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

Results: A three dimensional rendering of the ventricles in the H67D and WT mice is seen in Fig. 1. The ventricles in the H67D mice are visually much larger than the WT mice throughout the brain. Analysis of the CSF, WM, and GM segmented ROIs to delineate longitudinal and cross sectional volumetric changes are seen in Fig.2. The change in ventricle size in Fig.1 is confirmed statistically (paired T-Test) when measuring the entire CSF fraction. The ventricles in the H67D mice are significantly larger at both baseline and 12 month time points. The gray matter fraction decreased, for both H67D and WT over the twelve month period, while the white matter fraction increased. There was a significant decrease in white matter volume in the H67D compared to the WT mice. The group based statistical parametric relaxation analysis of the transverse R_2 maps MR data is seen in Fig. 3. The analysis demonstrates a significant group based difference in transverse relaxation (R_2) between WT and H67D in both white and gray matter. Voxels highlighted have shorter transverse relaxation rate values, indicative of regions with higher iron concentration.

Discussion: The longitudinal and cross sectional H67D mouse data support the current hypothesis that increased iron loading and alteration of iron related proteins is occurring and able to be visualized and tracked non-invasively. The changes in gray matter brain volume are hypothesized to be cortical atrophy of neuronal tissue. The alterations in transverse relaxation are hypothesized to be due to increased iron loading within the highlighted regions. Changes in cortical volume and increased iron loading are congruent with previous research showing decreased cognitive ability and iron loading in these animals. The data provide evidence that the H67D HFE mutant model is a source of iron overloading and further research with this model via crossbreeding with other known mouse models presents a unique opportunity to study iron overloading in a range of neurodegenerative diseases.

References: **1** – Nandar *et al*., J Nutr., 2011 Apr 1;141(4): 1-11, **2** – Tomatsu *et al*., PNAS, 2003 Dec; 200(26): 15788-15793, **3** – Sawiak *et al* an., Neurobiol Dis. 2009 Jan;33(1):20-7.

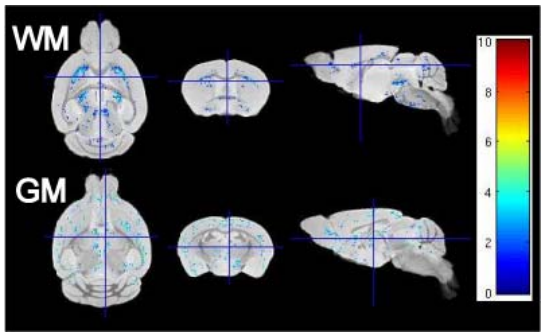

Figure 3 - Group based white and gray statistical parameter matter R_2 map differences between H67D > WT mice (N=10 and 11, respectively).