Transverse Relaxation and Volumetric Neural Changes in the H67D HFE Mouse Model and Cognitively Normal Healthy H63D-HFE Human Genotype Carriers

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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 systemic iron overload has been associated with a missense coding polymorphism leading to an amino acid substitution at position 63 within the hereditary hemochromatosis (HHC) protein (H63D-HFE). The H63D-HFE variant is a known factor in hereditary hemochromatosis and has been associated with neurodegenerative diseases such as Amyotrophic Lateral Sclerosis, Parkinson's disease, and Alzheimer's disease. H63D mutations result in iron dyshomeostasis, increased oxidative stress, glutamate release, tau phosphorylation, and alterations in inflammatory response[1]. The H63D-HFE mutation is prevalent within the general population with the greatest frequency in Caucasians; approximately 29.7% when combining both homo- and heterozygote carriers. To evaluate how the H63D-HFE mutation contributes to neurodegenerative disorders, a mouse model has been generated that contains the mouse homolog for the human H63D gene, the H67D knock-in. H67D mice exhibit alterations in their iron management protein expression, have increased neuronal oxidative stress, aberrant gliosis, and a disruption in cholesterol dynamics; all which leads to an increase in neurodegeneration and memory deficits. The overarching goal of this research was to evaluate the transverse relaxation profiles and volumetric measures in the mice and contrast these to human H63D carrier metrics with the hypothesis that the H67D mice recapitulate MRI features of human H63D carriers. The voxel-based MRI parametric evaluation of H63D patients and the H67D mouse model has not previously been undertaken.

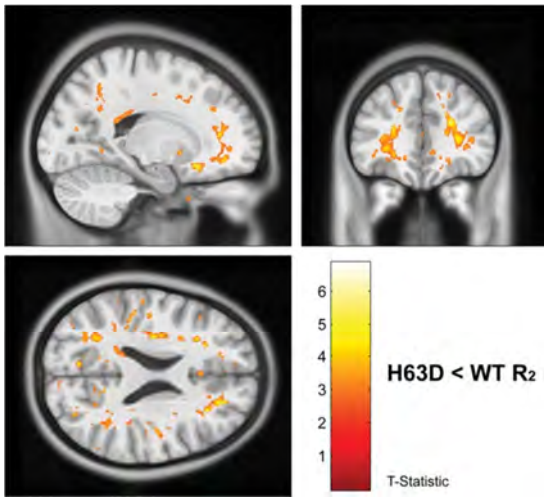

Figure 1 - Group based parametric analysis showing H63D-HFE carriers have reduced R_2 rates compared to WT-HFE carriers extensively throughout white matter ROIs.

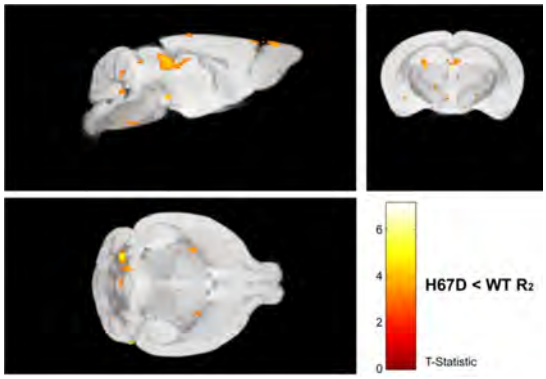

Figure 2 – Group based parametric analysis showing that H67D mice have reduced R_2 rates compared to WT-HFE carriers throughout white matter ROIs.

Methods: Human Work: Thirty-two cognitively normal healthy Caucasian subjects (16F, 16M) were selected for inclusion in this study design. All subjects were administered a battery of cognitive tests by a neuropsychologist, were determined to be cognitively normal, and did not have hemochromatosis. Blood samples were obtained and genotyped for the H63D single nucleotide variation. A total of 15 subjects (9F, 6M) were heterozygous for the minor G allele (H63D/+). A 3DT1-weighted scan and a multi-echo T_2-weighted spin-echo (9 echoes, 11 – 99 ms) protocol were obtained. Parametric relaxation R_2 rate maps were generated. All datasets were coregistered, resliced, realigned to a template brain, and segmented using SPM 8. For parametric map analysis, the relaxation maps were normalized to the template brain and voxel based analysis was performed using a group based method (cluster size ≥ 10 and $p \leq 0.005$). Mouse work: Twenty mice, 10 HFE-H67D knock-in mice and 10 Wildtype C57BL/6 (WT), were anesthetized with 1.5% isoflurane and placed within a 7.0 T MRI system and 35 mm birdcage volume coil (Bruker BioSpin). Animals were imaged at baseline (9 months) and twelve months later with the same imaging protocol. A four-echo 3D-T_2-weighted RARE dataset was acquired at a voxel resolution of 98 x 98 x 468 µm. Parametric relaxation R_2 maps were generated using a linear model. All datasets were coregistered, resliced, realigned to a template mouse brain, and segmented using SPM 8 with the SPMmouse toolkit[2]. For parametric map analysis, the relaxation maps were normalized to the template brain and voxel based analysis was performed using a group based method in SPMmouse (cluster size ≥ 10 and $p \leq 0.005$).

Results: Voxel-wise comparison of H63D and wild-type cognitively normal whole human brain R_2 relaxation rates demonstrate alterations in the neuronal R_2 relaxation rate within H63D carriers compared to the wild-type patients (Fig. 1). H63D patients exhibit a decrease in R_2 relaxation rate within white matter throughout the brain. White matter regions with a decreased bilateral R_2 rate include the superior, inferior, and frontal longitudinal fasciculi, cingulum bundle, anterior and superior coronal radiata, superior and inferior occipitofrontal fasciculi, internal capsule, arcuate fasciculus, thalamus, red nucleus, and the orbital frontal cortex. The most prominent decrease in R_2 rate is seen in the frontal white matter, extending throughout the entirety of this white matter region. Parametric relaxation maps of mice show a group based decrease in R_2 relaxation rate within the H67D mice compared to controls. These regions are exclusively white matter in origin and include the cerebral peduncle, supramammillary decussation, external capsule, medial lemniscus, lateral lemniscus, and the alveus of the hippocampus.

Discussion: The MRI data presented here demonstrate that cognitively normal H63D HFE carriers have relaxometry alterations in their brains despite not reporting difficulties with HHC and remaining asymptomatic. The data demonstrate that there is an HFE genome related alteration in the transverse R_2 relaxation rate in cognitively normal H63D human and H67D mouse carriers compared to WT-HFE controls. The widespread decrease in R_2 relaxation rate in both species is limited to white matter tracks for which the cause is speculated to be multifaceted. The transverse proton decay rate changes are hypothesized to be due to enhanced proton spin-spin induced relaxation in relation to alterations in biochemical tissue composition and, more specifically, the compartmentalization of protons in the biological system. As cholesterol levels are reduced in both H63D-HFE expressing neuronal cells and H67D mouse central neural tissue, the R_2 changes observed in both the human-H63D and mouse-H67D data suggest that the sequential process of myelinogenesis is refashioned, resulting in modified myelin membrane proton compartmentalization in patients with HFE mutations.

References: **1** – Nandar *et al*., J Nutr., 2011 Apr 1;141(4): 1-11, **2** – Sawiak *et* al., Neurobiol Dis. 2009 Jan;33(1):20-7.