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Reduced White Matter MRI Transverse Relaxation Rate in Cognitively Normal H63D-HFE Human Carriers and H67D-HFE Mice

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Abstract

Mutations within the HFE protein gene sequence have been associated with increased risk of developing a number of neurodegenerative disorders. To this effect, an animal model has been created which incorporates the mouse homologue to the human H63D-HFE mutation: the H67D-HFE knock-in mouse. These mice exhibit alterations in iron management proteins, have increased neuronal oxidative stress, and a disruption in cholesterol regulation. However, it remains undetermined how these differences translate to human H63D carriers in regards to white matter (WM) integrity. To this endeavor, MRI transverse relaxation rate (R_2) parametrics were employed to test the hypothesis that WM alterations are present in H63D human carriers and are recapitulated in the H67D mice. H63D carriers exhibit widespread reductions in brain R_2 compared to non-carriers within white matter association fibers in the brain. Similar R_2 decreases within white matter tracks were observed in the H67D mouse brain. Additionally, an exacerbation of age-related R_2 decrease is found in the H67D animal model in white matter regions of interest. The decrease in R_2 within white matter tracks of both species is speculated to be multifaceted. The

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Disclosures:

All authors declare that they have no conflicts of interest.

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, and the applicable revisions at the time of the investigation. Informed consent was obtained from all patients for being included in the study.

All institutional and national guidelines for the care and use of laboratory animals were followed.

R_2 changes are hypothesized to be due to alterations in axonal biochemical tissue composition. The R_2 changes observed in both the human-H63D and mouse-H67D data suggest that modified white matter myelination is occurring in subjects with HFE mutations, potentially increasing vulnerability to neurodegenerative disorders.

Keywords

HFE; H63D; rs1799945; H67D Mouse; White Matter; MRI; Relaxometry; R_2

Introduction

Mutations within the genetic sequence coding for the HFE MHC Class 1-like protein were first identified in patients with hereditary hemochromatosis (Feder et al. 1996). The most prevalent of which has been coupled to a missense coding polymorphism (C→G) leading to a histidine (H) to aspartate (D) amino acid substitution at position 63 (HFE-H63D, rs1799945) (Feder et al. 1996; Hanson et al. 2001). The H63D-HFE variant has been associated with neurodegenerative diseases such as Amyotrophic Lateral Sclerosis, Parkinson's disease, and Alzheimer's disease (AD) for which it is believed to contribute to iron dyshomeostasis, increased oxidative stress, and amplified inflammatory response (Lin et al. 2012; Nandar and Connor 2011; Hare et al. 2013; Ward et al. 2014). The wild-type HFE (WT-HFE) gene product regulates cellular iron uptake through two known mechanisms: transferrin receptor (TfR) mediated iron intake and iron regulatory protein (IRP) interaction. The conformational change in the HFE protein due to the H63D substitution results in the dysregulation of iron transport and storage (Lebron and Bjorkman 1999; Lebron et al. 1999; Riedel et al. 1999).

The H63D-HFE polymorphism is one of the most common mutations currently found in the human population. Minor allele frequency (G) is highly prevalent within the general populace and varies dependent upon race and ethnicity (Hanson et al. 2001). The mutation has its greatest prevalence within a Caucasian population of northwestern European descent with an allelic frequency of approximately 16%; the general population has an allelic frequency of 9.8% (Flicek et al. 2014; Hanson et al. 2001). H63D-HFE genotype frequency of north-western European Caucasian descendants is approximately 29.7% when combining both homozygote and asymptomatic heterozygote carriers; the global population has a combined homozygote and heterozygote frequency of 18.3% (Flicek et al. 2014; Ryan and Vaughan 2000). The high prevalence of the mutation within descendants of Celtic origin has prompted many to believe the mutation arose in this region to facilitate iron absorption in famine conditions and promoted the "Celtic Curse" moniker for the disorder.

H63D transfected human neuronal cells and an H67D knock-in mouse model, the mouse homolog for the human H63D gene, have been generated to evaluate how the H63D-HFE mutation contributes to neurodegenerative disorders (Tomatsu et al. 2003). Cultured H63D cells exhibit prolonged endoplasmic reticulum stress, increased neuronal vulnerability, and show iron related disruption of cholesterol metabolism (Liu et al. 2011; Ali-Rahmani et al. 2014a). These findings are mirrored in the H67D mice which exhibit alterations in their iron

management protein expression, have increased neuronal oxidative stress, aberrant gliosis, and a disruption in cholesterol dynamics; all of which lead to an increase in neurodegeneration and memory deficits (Nandar et al. 2013; Ali-Rahmani et al. 2014a).

In addition to direct cellular iron regulation, HFE regulates systemic iron intake through the upstream regulation of hepcidin with ALK3 (BMPR1a) in the bone morphogenetic protein pathway (Bridle et al. 2003). HFE is involved in expression and cell surface stabilization of the ALK3/BMPR1a BMP receptor where WT-HFE inhibits the ubiquitin breakdown of ALK3, allowing increased hepcidin transcription. H63D-HFE does not inhibit the ubiquitin breakdown of ALK3, reducing the downstream transcription of hepcidin (Wu et al. 2014). ALK3/BMPR1a signaling also mediates differentiation, maturation, and myelination of oligodendrocyte cells in the brain (Feigensohn et al. 2011; Samanta et al. 2007; Mabie et al. 1997; See et al. 2007). This in addition to reduced cholesterol and iron profile in the H67D mouse model suggest that modifications in WM myelinogenesis may be altered in both H67D mice and H63D human carriers. Cholesterol and iron are both found in high quantities within oligodendrocyte cells responsible for myelination of white matter (WM) tracks in the brain. Iron is an essential factor in myelin production as it is required for energy metabolism and optimal oligodendrocyte function. This is reinforced by observations that iron deficiency results in hypomyelinated axonal tracks (Todorich et al. 2009). Iron is necessary for cholesterol synthesis as the P450 enzymes (CYP51A1) responsible for *de novo* production utilize heme-bound iron as an electron transport intermediary (Lepesheva and Waterman 2011).

The overarching focus of the current work is to evaluate MRI transverse proton relaxation in the H67D mice and contrast these to human H63D genetic carriers. Transverse relaxation rate (R_2) is sensitive to changes in white matter proton (water) compartmentalization, myelination, and lipid content in addition to iron (Koenig et al. 1990; Kucharczyk et al. 1994; Kamman et al. 1984). This method was used to specifically test the hypothesis that there are WM alterations in human H63D-HFE carriers and that the H67D mice recapitulate these MRI features. The non-invasive *in vivo* voxel-based MRI parametric evaluation of the H67D mouse model and H63D human carriers has not previously been undertaken.

Materials & Methods

Human HFE H63D and Control Subjects

Human patients were enrolled and consented to participate within an ongoing memory and aging study approved by The Pennsylvania State University – College of Medicine Institutional Review Board (IRB). A total of thirty-two healthy cognitively normal Caucasian subjects (16 male) were included in this study design. All subjects were administered a battery of cognitive tests by a neuropsychologist and were determined to be cognitively normal based on these results (see Table 1 for the complete list). All subjects reported no neurological or medical complications. Blood samples from each subject were obtained and genotyped for the H63D single nucleotide variation via conventional multiplex polymerase chain reaction amplification and H63D primer/probe binding (Stott et al. 1999). A total of 15 subjects (6 male) were heterozygous for the minor G allele (H63D/+) and 17

subjects (10 male) were homozygous for the major C allele (+/+, WT). No homozygous minor G allele patients (H63D/H63D) were identified.

Clinical MRI Protocols

All patients were scanned on a 3.0 T Siemens Tim Trio system. A 3D T₁-weighted scan was obtained with the following parameters: TR = 2300 ms, TE = 2.98 ms, FOV = 25.6 × 25.6 × 16 cm, matrix = 256 × 256 × 160, for a final isotropic resolution of 1 × 1 × 1 mm. A multi-echo T₂-weighted spin-echo protocol was used with the following parameters: average = 1, TR = 6000 ms, 9 echoes with TE = 11–99 ms with an echo spacing of 11 ms, FOV = 17.6 × 25.6 × 14 cm, matrix = 176 × 256 × 56, for a final resolution of 1 × 1 × 2.5 mm.

Clinical MRI Statistical Parametric Analysis

Parametric R₂ maps were generated from the T₂-weighted spin-echo data using a nonlinear least squares curve fitting model (Dardzinski et al. 1997; Koff et al. 2008) on a pixel-by-pixel basis with an in-house developed quantitative MRI image processing tool (qMRI, The Center for NMR Research, The Pennsylvania State University – College of Medicine, Hershey, PA) running in IDL 8.1 (Exelis, Boulder, CO, USA). The first of the nine echoes was discarded to reduce the stimulated echo artifact and obtain an accurate relaxation curve fit (Poon and Henkelman 1992). The R₂ map was spatially co-registered to the 3D-T₁ image, both then were normalized to the MNI152 atlas standard space (Mazziotta et al. 1995) at a spatial resolution of 1 × 1 × 1 mm using SPM8 (Wellcome Trust Centre for Neuroimaging, UK), followed by smoothing with a 2.5 mm isotropic Gaussian smoothing kernel.

For MRI statistical parametric analyses, the subjects were stratified based on their H63D carrier status, H63D/+ vs. +/+ (WT). A group based statistical parametric two-sample T-test was performed between the subject groups using age and gender as co-variants in the design matrix. These tests were performed within SPM8 using the normalized R₂ parametric maps with a *p* value = 0.001, an absolute threshold for voxel cluster = 100, and a regional white matter mask.

For regional analysis of R₂ data the JHU-MNI-SS “Eve” template (Oishi et al. 2009) was normalized to the MNI152 template brain. The regions of interest from this atlas were individually parcellated and normalized to the MNI152 atlas along with the template brain, consisting of 176 individual white and gray matter ROIs. The individual regions were imported into the MarsBaR toolbox for SPM (Brett et al. 2002) and relaxation rate measures were outputted for each patients’ normalized R₂ map and statistically compared with a *p* value threshold of 0.05.

Statistical Analysis of Demographic and Cognitive Tests

For statistical data analysis, the SPSS 22 program package was utilized (IBM Corporation, Armonk, New York). An analysis of co-variance (ANCOVA) was performed using the general linear model with Bonferroni correction in the design. Subject age and gender were used as covariates for all cognitive tests except the age normalized AMSS and AEMSS measures, where only gender was used. Two-tailed T-tests were used to test for significance between H63D carrier and non-carrier subjects for age. A chi-square test of independence

was used to test for significance between groups for gender bias, gene frequency, and allele frequency between the samples.

HFE H67D and WT Mice

H67D knock-in mice (homolog of human H63D) were commercially generated (Ingenious Targeting Laboratory, Inc., NY, USA) as previously described (Tomatsu et al. 2003; Nandar et al. 2013). Mice were fed food and water *ad libitum* and maintained under normal housing conditions as outlined by The Pennsylvania State University – College of Medicine Institutional Animal Care and Use Committee (IACUC). All procedures were conducted according to NIH and approved IACUC guidelines.

Preclinical MRI Protocol

Twenty mice, 10 homozygous male H67D-HFE (H67D/H67D) and 10 WT male C57BL/6 (WT, +/+), were anesthetized with 1.5% isoflurane, placed within a 35 mm birdcage volume coil using a standardized animal bed, and imaged with a 7.0 T Bruker BioSpec 70/20 MRI system (Bruker BioSpin, Ettlingen, Germany). Animals were imaged with the same protocol at 9 months old and thirteen months later at twenty-two months, to mimic midlife and aged human lifespan (respectively). One H67D mouse passed away during the study, resulting in a total of nine H67D mice included in the twenty-two month time point. A multi-echo three-dimensional RARE spin-echo T₂-weighted protocol was utilized with the following parameters: averages = 2, relaxation time (TR) = 2000 ms, rare-factor = 8, four echoes with effective echo time (TE) = 30 – 120 ms with an echo spacing of 30 ms, field of view (FOV) = 25 × 25 × 15 mm, and acquisition matrix = 256 × 256 × 32, for a final voxel resolution of 97 × 97 × 468 μm.

Preclinical MRI Statistical Parametric Analysis

Transverse relaxation rate (R₂) maps were generated using a nonlinear least squares curve fitting model on a pixel-by-pixel basis (Dardzinski et al. 1997; Koff et al. 2008) with qMRI software running in IDL 8.1. The images were then manually skull-stripped with Brainsuite v13 software (University of Southern California, USA) before spatial processing. The first echo of the spin-echo dataset was used as an anatomical image to spatially realign and co-register the parametric maps to a template mouse brain (Ma et al. 2008). Anatomical images and parameter maps were then normalized and resliced to the Magnetic Resonance Microimaging Neurological Atlas (MRM NeAt) template mouse brain (Ma et al. 2005; Ma et al. 2008) with a voxel size of 100 × 100 × 100 μm using SPM8 and the SPMmouse v1.1b toolkit (Sawiak et al. 2009), followed by smoothing with a 400 μm isotropic Gaussian smoothing kernel.

For statistical analyses, group based statistical parametric two-sample T-tests were performed between the WT and H67D groups at the nine and twenty-two month time points and between time points for both groups using SPM8. Group based comparisons were performed for the normalized R₂ parametric maps using an absolute threshold for voxel cluster 100 in size with *p* value 0.0025. The interaction effect between H67D genetics and time was determined using a modified SPM contrast matrix with a *p* value of 0.01 and 20 voxel cluster threshold.

Regional relaxation measures were obtained from the individual normalized R_2 maps using an atlas specific to the normalization template (Ma et al. 2008). Twenty parcellated ROIs were imported into the MarsBaR toolbox to generate individual R_2 measures, followed by longitudinally and between group statistical comparisons using a p value threshold of 0.05.

Results

Neurocognitive testing determined that all the human subjects were cognitively normal (Table 1). Comparison of gender, age, and cognitive tests between H63D and WT subjects demonstrated that there was not a significant difference found for all measures except a slightly lower raw DRS-2 score for H63D carriers ($p = 0.044$, Table 1). The H63D minor allele frequency in our study population was 0.234 which was determined to not be significantly different from the reported Caucasian population (Flicek et al. 2014) minor allele frequency of 0.159 ($p = 0.099$). The gene frequency of H63D carriers in the study population was 0.469 which is marginally greater than those reported for combined H63D hetero- and homozygotes within a Caucasian population of European descent (0.297, $p = 0.042$).

Voxel-wise comparison of whole brain R_2 maps between H63D and WT subjects demonstrates R_2 alterations in H63D carriers (Fig. 1). H63D subjects exhibit lower R_2 within white matter association fibers, the most prominent of which are seen in the frontal WM, extending throughout the entirety of this region. Specifically, H63D patient telencephalic white matter displays reduced R_2 in the superior longitudinal fasciculus, uncinate fasciculus of the forebrain, and superior corona radiata. Additionally, H63D patients demonstrate reduced R_2 in the arcuate, middle longitudinal, and inferior longitudinal fasciculi as well as the superior cerebellar peduncle.

Regional measures of R_2 rate demonstrate differences specific to numerous white matter tracks (Table 2). Only significant white matter regions of interest are outlined in Table 2; a complete list of all 176 regions can be found in the online supplemental documentation. Regional white matter transverse relaxation measures overlap with voxels outlined in Fig. 1, highlighting significant reductions on both a voxel-wise and regional basis.

R_2 relaxation maps of mice showed a group based decrease in R_2 in the H67D mice compared to controls at 9 months within numerous regions in the brain (Fig. 2a). These regions were WM in origin and include the middle cerebellar peduncle, lateral lemniscus, and cerebral peduncle. Analysis of 22 month relaxation data demonstrates numerous regions with lower R_2 in H67D animals compared to WT controls (Fig. 2b). Reductions in H67D R_2 at 22 months are found in the posterior commissure, superior colliculus commissure, anterior corpus callosum, and the fornix.

Longitudinal comparison of R_2 between the 9 and 22 month time points demonstrates a decrease in relaxation (R_2 rate at 9 month > 22 month) in the H67D and WT brain (Fig. 3a and 3b, respectively). Similar to the previous comparisons between H67D and WT mice, these regions were mainly WM in origin. In the H67D mice, regions include the corpus callosum, hippocampal commissure, cerebral peduncle, fimbria, internal capsule, striatum,

pallidum, hypothalamus, and the caudoputamen. When the same longitudinal comparison was made with the WT mice, a similar pattern is seen (Fig. 3b). The WT mice exhibited longitudinal R_2 decreases in the internal capsule, pallidum, and hypothalamus. There is a degree of regional overlap between the H67D and WT mice, with the H67D mice exhibiting a more pronounced longitudinal R_2 decrease. This was particularly true within the pallidum, hypothalamus, and internal capsule.

The interaction between genetics (H67D & WT) and time (9 & 22 months) demonstrates an effect in the thalamus, superior colliculus commissure, fimbria, caudoputamen, hypothalamus, and the corpus collosum (Fig. 4a). This interaction effect represents voxel groups where H67D mice had greater decreases in R_2 compared to WT mice longitudinally (Fig. 3a and b, respectively). Regional measures of transverse relaxation demonstrate a longitudinal decrease for both H67D and WT animals across all ROIs (Fig. 5). The increased negative slope of the H67D animals, compared to WT, indicates an accelerated decrease in relaxation over time. White matter (Fig. 5a – d), basal ganglia (Fig. 5e – h), and cortical (Fig. 5i – l) regions all demonstrate a similar trend with exacerbated relaxation changes in the H67D knock-ins.

Discussion

The H63D HFE polymorphism is a common mutation within the general population, especially prevalent amongst Caucasians where it is found in one-quarter of the populace. The H63D mutation and its interaction with other HFE mutations and neurodegenerative disorders remain under investigation (Nandar et al. 2014; Ali-Rahmani et al. 2014b; Xia et al. 2015; Mariani et al. 2013; Pulliam et al. 2003; Gazzina et al. 2015). The MRI data presented here demonstrate that there is an HFE genome related reduction in transverse R_2 in H63D human and H67D mouse carriers compared to WT-HFE controls.

The MRI relaxation pattern within the cognitive normal H63D-HFE carriers indicates that those regions with decreased R_2 are found in WM tracks known to myelinate later in brain development, such as the inferior temporal, temporoparietal, and prefrontal lobes through which the superior and inferior fasciculi project. Localized WM regions are characterized as early- or late-myelinating based on the temporal progression of myelinogenesis resulting in cytoarchitectural distinctions. Early myelinating regions appear largely spared from the R_2 decrease in regions such as the cortical WM in the occipital (visual) and superior frontal / parietal junction (motor and sensory cortices). The progression of myelination is highly reproducible and conserved for a given species. In the mouse, myelination is rapidly achieved in all brain regions with full myelination occurring 45 – 60 days postnatally (Baumann and Pham-Dinh 2001). In humans, myelination follows a much slower trajectory with the peak of myelin formation occurring the first year postnatally and continuing past the second decade of life in associative cortical areas (Baumann and Pham-Dinh 2001). Human regions that myelinate early, such as the sensory, visual, and motor cortices, have oligodendrocytes which exhibit robust myelination of single axon segments with over 100 myelin membrane layers (Bartzokis 2003). Conversely, regions known to incur later myelination, such as the inferior temporal, temporoparietal, and prefrontal lobe regions, have oligodendrocytes that myelinate numerous axon segments (as many as 50) with less than ten

myelin layers (Haroutunian et al. 2014; Bjartmar et al. 1994; Remahl and Hilderbrand 1990). Regions with oligodendrocytes that myelinate axons later during human brain development are more susceptible to neuronal insults compared to early myelinating regions. In addition, oligodendrocytes in late-myelinating regions re-myelinate axons to a lesser degree than those in early-myelinating regions (Baumann and Pham-Dinh 2001).

Cholesterol plays a critical function in central nervous WM development and repair. Deficient cholesterol biosynthesis within oligodendrocytes severely reduces the rate of WM myelination as cholesterol is a rate limiting factor in myelin synthesis (Saher et al. 2005). Cholesterol in the brain is produced *de novo* and endogenous cholesterol synthesis is critical for neuronal homeostasis such that nutritional supplementation of cholesterol has little therapeutic effect (Korade et al. 2009). The importance of cholesterol is highlighted in mutant mice deficient in cholesterol biosynthesis as their chronological sequence of myelination is underdeveloped (Saher et al. 2005).

The human brain is the most cholesterol rich organ in the body. While it comprises approximately 2% of total body weight, it disproportionately consists of 25% of the body's total cholesterol (Haroutunian et al. 2014). Cholesterol alters lipid membrane fluidity, function, and is critical in the formation and stability of lipid rafts (Wong et al. 2014), glycolipoprotein microdomain coordination centers for the assembly of signaling molecules, signal transduction processes, and protein trafficking. These regions are enriched in cholesterol and sphingomyelins as required elements to tightly pack, enhance rigidity, and reduce the fluidity of the lipid bilayer. Under normal circumstances the HFE protein is localized within the plasma membrane at the location of lipid rafts (Ali-Rahmani et al. 2010); as such, the functionality of HFE is intertwined with plasma membrane cholesterol.

The biochemical cholesterol composition of H63D-HFE expressing cells demonstrates that they contain 50% less cholesterol than cells expressing WT-HFE (Ali-Rahmani et al. 2010). Additionally, cholesterol levels are reduced in H67D mouse central nervous tissues (Ali-Rahmani et al. 2014a). Proteins involved in cholesterol synthesis (reduction in HMGCoAR, increase in DHCR24) and degradation (increase in CYP46A1) have altered expression in cultured H63D-HFE cells and H67D neuronal tissue, indicative of decreased cholesterol production and an increase in cholesterol efflux from the brain (Ali-Rahmani et al. 2014a; Ali-Rahmani et al. 2014b; Ali-Rahmani et al. 2010).

The widespread decrease in WM R_2 in both species is speculated to be multifaceted. The reduction of R_2 is primarily a factor of two phenomena: proton dephasing due to interactions with neighboring protons (spin-spin) and local B_0 magnetic field inhomogeneities (Chavhan et al. 2009). The transverse proton relaxation is caused by spin-spin induced proton-proton interaction in relation to biochemical alterations in tissue composition, iron content, and, more specifically, the compartmentalization of axonal WM protons. The cause for the R_2 changes in the H63D subjects and H67D mice is speculated to be due to decreased proton compartmentalization within WM. These changes would come about due to increased free / unrestricted water within and in the vicinity of axons. Less cholesterol incorporation in the myelin lipid bilayer would result in increased fluidity of the membrane and increased water

permeability. As such, the macromolecular proton environment is shifted towards a free water state as deficient myelin loses its water compartmentalization.

Similar to the human carriers, the H67D mice also exhibit reduced R_2 WM relaxation in relation to HFE mutation carrier status. WM regions with lowered R_2 appear throughout the H67D brain in decussating commissures, cerebral peduncles, lateral lemniscus, and external capsule. In the human brain these regions are outlined as early-myelinating regions, however in mice the whole of the brain's WM rapidly attains myelin maturity much earlier than the human brain. The longitudinal H67D data show that the mutant mice exhibit exacerbated longitudinal decreases in R_2 in the same regions expressed in the WT mice. The longitudinal change in R_2 matches the timing of previous data showing decreased cholesterol synthesis in the H67D brain compared to control animals as well as data showing reduced myelin basic protein in the H67D animals.

The overall degree of R_2 reduction in the H67D mouse compared to the human data could be related and proportional to the amount of myelin present in the murine brain. White matter constitutes approximately 50% of the human brain while occupying only 10% of the mouse brain (Zhang and Sejnowski 2000). Human WM volume has hyperscaled as part of the development of the our species, while GM has scaled proportionally to the increase in total brain volume (Smaers et al. 2010). This phenomenon is conserved across numerous species and is found during the evolution of the gyrencephalic (convoluted) human brain in comparison to the lissencephalic (smooth) mouse brain.

The H67D animals perform poorly on cognitive tests, especially those involving long-term memory incorporation and retrieval (Ali-Rahmani et al. 2014a). The H63D human carriers in this study did not have any apparent cognitive deficits and had a slightly lower raw DRS-2 score than WT controls. The H63D-HFE carriers place within the 60th – 71st percentile with a score of 139.6 and the controls places with the 72nd – 81st percentile with an average of 141.3 (Pedraza et al. 2010). The H63D- and WT-HFE carriers both are characterized as cognitively normal in having placed above the accepted DRS-2 threshold of 136 for mild cognitive impairment (Springate et al. 2014). The implemented cognitive tasks for the human cohort in the research design did not include a long-term memory challenge making comparison to our previous mouse cognitive data difficult. While the utilized cognitive measures are not sensitive enough to measure reductions in long-term cognition, the MRI relaxation measures are sensitive to reductions in WM R_2 associated with H63D carriers.

There is a limited amount of work that has previously studied H63D status in relation to MRI metrics (Bartzokis et al. 2010; Nielsen et al. 1995; Kohannim et al. 2012; Jahanshad et al. 2012). Our data provide evidence and demonstrate that WM R_2 alterations are related to human H63D and mouse H67D carrier status and that late-myelinating white matter brain regions are susceptible to alterations in human H63D carriers. When considered in the context of the wider literature base, these findings are congruent with prior research demonstrating that brain cholesterol content and synthesis is reduced in the H67D mice and that cholesterol levels play a role in MRI relaxometry. It is not entirely clear how HFE genetics are related to proton magnetic resonance relaxation. A plausible correlation between MRI metrics and cholesterol status in H63D subjects has been outlined; however,

this relationship needs to be further histologically verified utilizing the H67D mouse line. Additionally, the limit in patient sample size resulted in the absence of cognitively normal human H63D homozygotes. Contrasting homozygous H67D mice to heterozygous H63D humans needs to be further interpreted with heterozygous H67D mice in future study. While cholesterol is reduced in the H67D mice, it remains to be discerned if these factors are found in WM of H67D mice and H63D-HFE human carriers. The interplay between iron, proton compartmentalization, and R_2 within H63D and H67D WM remains to be elucidated. As much of the focus has been on cortical ROIs in the H67D mice, more study into the WM status of these animals is warranted. The longitudinal and cross sectional H67D mouse data support the current hypothesis that changes in human H63D WM transverse relaxation are mirrored in the animal model. The data provide further evidence that the H67D-HFE mutant model is a viable preclinical model for determining and demonstrating the impact of the highly prevalent H63D-HFE genotype variant.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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The authors would like to pay respect and dedicate this work to the late George Bartzokis, M.D. for whom the scientific community mourns. Dr. Bartzokis's research on neurodegenerative disorders will continue to inspire current and future medical scientists for years to come.

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Figure 1.

Group based parametric analysis displaying regions where H63D-HFE carriers have reduced R_2 compared to WT-HFE carriers in (a) orthogonal and (b) coronal axis orientation. White matter regions with decreased R_2 include the telencephalic white matter, superior, inferior, and frontal longitudinal fasciculi, anterior and superior coronal radiata, uncinate fasciculus, arcuate fasciculus, and the superior and inferior occipitofrontal fasciculi. All voxels of interest were thresholded at $p < 0.001$ with a 100 voxel threshold per cluster.

Figure 2.

a) Left, parametric R_2 analysis of mice at baseline (9 month) showing H67D mice with reduced R_2 relaxation rate compared to WT mice. Regions with decreased relaxation rate include the cerebral peduncle, lateral lemniscus, and middle cerebellar peduncle. b) Right, parametric R_2 analysis of mice at endpoint (22 month) showing H67D mice with reduced relaxation compared to WT mice. Regions include the posterior commissure, superior colliculus commissure, anterior corpus collosum, and the fornix. All voxels thresholded to $p < 0.0025$ with a minimum of 100 voxels per cluster.

Figure 3.

a) Left, group based statistical parametric R_2 analysis of longitudinal changes (9 month > 22 month) in H67D mice showing a decrease in R_2 related to time. The regions include the forceps major of the corpus callosum, cerebral peduncle, thalamus, hypothalamus, fimbria, hippocampal commissure, internal capsule, pallidum and striatum. b) Right, group based statistical parametric R_2 analysis of longitudinal changes (9 month > 22 month) in WT mice showing a decrease in R_2 related to time. The WT mice exhibit longitudinal R_2 decreases in the internal capsule, pallidum, and hypothalamus. Regional overlap within the pallidum, hypothalamus, and internal capsule is evident between the mice with H67D animals exhibiting a more pronounced longitudinal R_2 decrease. All voxels thresholded to $p < 0.0025$ with a minimum of 100 voxels per cluster.

Figure 4.

The time and genetic interaction effect between the H67D and WT mice and longitudinally between baseline and endpoint. An interaction where H67D mice had statistically greater decreases in R_2 compared to WT longitudinally is found in the thalamus, superior colliculus commissure, fimbria, caudoputamen, hypothalamus, and the corpus collosum. All voxels are thresholded to $p < 0.01$ with a minimum of 20 voxels per cluster.

Figure 5.

Regional measures of transverse relaxation for both H67D and WT animals across twelve ROIs in white matter (a – d), basal ganglia (e – h), and cortical (i – l) regions. The increased slope of the H67D animals, compared to WT, indicates an accelerated decrease in relaxation over time. *, $p < 0.05$; ** $p < 0.01$.

Table 1

Subject H63D Genotype, Demographics, and Cognitive Scores

Demographic and Cognitive Test	H63D Genotype		P Value
	H63D / +	+ / +	
Genetic Distribution	15 (46.9%)	17 (53.1%)	N/A
Race	Caucasian (N=15)	Caucasian (N=17)	N/A
Gender	9 Female; 6 Male	7 Female; 10 Male	p = 0.288
Age	73.9 ± 2.67	68.6 ± 1.81	p = 0.110
CDR	0 ± 0	0.03 ± 0.03	p = 0.538 [‡]
MMSE	28.3 ± 0.44	28.6 ± 0.38	p = 0.499 [‡]
DRS-2 (Raw)	139.6 ± 0.74	141.3 ± 0.41	p = 0.044 [‡]
DRS-2 (AMSS)	12.7 ± 0.61	12.8 ± 0.34	p = 0.498 [‡]
DRS-2 (AEMSS)	12.2 ± 0.61	12.0 ± 0.37	p = 0.563 [‡]
CVLT-II	58.9 ± 2.25	64.3 ± 3.01	p = 0.248 [‡]
GDS	4.1 ± 0.94	2.4 ± 0.81	p = 0.218 [‡]
BAI	3.8 ± 0.88	3.5 ± 1.28	p = 0.382 [‡]
BEHAVE-AD	0.27 ± 0.15	0.12 ± 0.08	p = 0.852 [‡]

CDR = Clinical Dementia Rating, MMSE = Mini Mental State Examination, DRS-2 = Dementia Rating Scale-2, AMSS = Age-adjusted MOANS Scaled Score, AEMSS = Age and Education adjusted MOANS Scale Score, CVLT-II = California Verbal Learning Test Second Edition, GDS = Geriatric Depression Scale, BAI = Beck Anxiety Inventory, and BEHAVE-AD = Behavioral Pathology in Alzheimer's Disease, N/A = not applicable.

[‡] Gender as covariate in statistical design.

[‡] Age and Gender as covariates in statistical design.

Table 2Regional White Matter R_2 Measures

Region	Human H63D Genotype		<i>P</i> Value
	H63D / + R_2 (1/s)	+ / + R_2 (1/s)	
Cerebral peduncle (L)	12.50 ± 0.12	12.84 ± 0.10	<i>p</i> = 0.033
Cerebral peduncle (R)	12.83 ± 0.20	13.32 ± 0.12	<i>p</i> = 0.020
Corona radiata anterior (L)	11.68 ± 0.19	12.60 ± 0.18	<i>p</i> = 0.004
Corona radiata anterior (R)	11.08 ± 0.24	12.20 ± 0.19	<i>p</i> = 0.005
Corona radiata posterior (R)	10.27 ± 0.16	10.92 ± 0.12	<i>p</i> = 0.002
Corona radiata superior (L)	10.71 ± 0.14	11.14 ± 0.19	<i>p</i> = 0.043
Corona radiata superior (R)	10.44 ± 0.20	11.18 ± 0.13	<i>p</i> = 0.005
Corpus callosum genu (R)	11.06 ± 0.32	12.24 ± 0.26	<i>p</i> = 0.014
Corticospinal tract (L)	11.82 ± 0.14	12.58 ± 0.16	<i>p</i> = 0.003
Corticospinal tract (R)	11.72 ± 0.10	12.28 ± 0.12	<i>p</i> = 0.001
External capsule (L)	11.74 ± 0.21	12.47 ± 0.12	<i>p</i> = 0.007
External capsule (R)	12.27 ± 0.17	13.09 ± 0.11	<i>p</i> = 0.001
Inferior occipital WM (L)	11.82 ± 0.12	12.24 ± 0.07	<i>p</i> = 0.011
Inferior temporal WM (R)	11.67 ± 0.09	12.09 ± 0.09	<i>p</i> = 0.006
Internal capsule posterior (R)	11.21 ± 0.12	11.66 ± 0.08	<i>p</i> = 0.004
Internal capsule retrolenticular (L)	11.52 ± 0.09	11.93 ± 0.12	<i>p</i> = 0.018
Internal capsule retrolenticular (R)	11.42 ± 0.12	12.04 ± 0.10	<i>p</i> < 0.001
Lateral fronto-orbital WM (R)	10.98 ± 0.14	11.56 ± 0.14	<i>p</i> = 0.026
Lingual WM (L)	10.76 ± 0.19	11.36 ± 0.14	<i>p</i> = 0.016
Lingual WM (R)	11.00 ± 0.24	11.74 ± 0.21	<i>p</i> = 0.049
Medial lemniscus (L)	10.48 ± 0.17	10.98 ± 0.13	<i>p</i> = 0.030
Medial lemniscus (R)	10.42 ± 0.20	11.02 ± 0.13	<i>p</i> = 0.011
Middle frontal orbital WM (L)	12.72 ± 0.12	13.16 ± 0.11	<i>p</i> = 0.014
Middle frontal orbital WM (R)	12.76 ± 0.14	13.18 ± 0.12	<i>p</i> = 0.034
Middle temporal WM (R)	10.70 ± 0.16	11.30 ± 0.12	<i>p</i> = 0.012
Superior cerebellar peduncle (R)	9.10 ± 0.22	9.91 ± 0.14	<i>p</i> = 0.009
Superior longitudinal fasciculus (L)	11.04 ± 0.09	11.39 ± 0.07	<i>p</i> = 0.006
Superior longitudinal fasciculus (R)	10.94 ± 0.10	11.28 ± 0.07	<i>p</i> = 0.008
Superior temporal WM (R)	8.95 ± 0.18	9.79 ± 0.14	<i>p</i> = 0.001
Supramarginal WM (R)	9.95 ± 0.18	10.43 ± 0.14	<i>p</i> = 0.017
Thalamic radiation (L)	10.84 ± 0.25	11.65 ± 0.13	<i>p</i> = 0.017