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Gender and Iron Genes May Modify Associations Between Brain Iron and Memory in Healthy Aging

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Brain iron increases with age and is abnormally elevated early in the disease process in several neurodegenerative disorders that impact memory including Alzheimer's disease (AD). Higher brain iron levels are associated with male gender and presence of highly prevalent allelic variants in genes encoding for iron metabolism proteins (hemochromatosis H63D (HFE H63D) and transferrin C2 (TfC2)). In this study, we examined whether in healthy older individuals memory performance is associated with increased brain iron, and whether gender and gene variant carrier (IRON+) *vs* noncarrier (IRON−) status (for HFE H63D/TfC2) modify the associations. Tissue iron deposited in ferritin molecules can be measured *in vivo* with magnetic resonance imaging utilizing the field-dependent relaxation rate increase (FDRI) method. FDRI was assessed in hippocampus, basal ganglia, and white matter, and IRON+ *vs* IRON− status was determined in a cohort of 63 healthy older individuals. Three cognitive domains were assessed: verbal memory (delayed recall), working memory/attention, and processing speed. Independent of gene status, worse verbal-memory performance was associated with higher hippocampal iron in men ($r=-0.50$, $p=0.003$) but not in women. Independent of gender, worse verbal working memory performance was associated with higher basal ganglia iron in IRON− group ($r=-0.49$, $p=0.005$) but not in the IRON+ group. Between-group interactions ($p=0.006$) were noted for both of these associations. No significant associations with white matter or processing speed were observed. The results suggest that in specific subgroups of healthy older individuals, higher accumulations of iron in vulnerable gray matter regions may adversely impact memory functions and could represent a risk factor for accelerated cognitive decline. Combining genetic and MRI biomarkers may provide opportunities to design primary prevention clinical trials that target high-risk groups.
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INTRODUCTION

Iron is essential for cell function, however, elevated tissue iron can promote tissue-oxidative damage to which the brain is especially vulnerable (Halliwell and Gutteridge, 1985; Kell, 2009; Zecca *et al*, 2004). Abnormally high brain iron levels are observed in age-related degenerative diseases such as Alzheimer's disease (AD), dementia with Lewy bodies (DLB), and Parkinson's disease (PD) (Bartzokis *et al*, 2007a; Kell, 2009). Brain iron levels increase with age (Bartzokis *et al*, 2007c; Hallgren and Sourander, 1958) and recent studies reveal elevated levels even in preclinical stages of AD (Lavados *et al*, 2008; Smith *et al*, 2010), suggesting an accelerated trajectory of brain iron accumulation may be occurring during the transition from healthy aging into preclinical stages and eventually dementia (Bartzokis, 2009).

Age-related dementing disorders such as AD are characterized by progressive memory deficits that begin developing years before the diagnosis can be made (Amieva *et al*, 2008; den Heijer *et al*, 2006). The hippocampus (Hip) is a key region in memory function that is severely affected in aging and dementing disorders such as AD (Braak and

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Braak, 1991; Squire and Zola-Morgan, 1991). Hippocampal iron levels increase with age in healthy individuals (Bartzokis *et al*, 2007a) and postmortem studies have shown that hippocampal iron is increased in AD beyond levels of non-demented controls (Bouras *et al*, 1997; Deibel *et al*, 1996; Pankhurst *et al*, 2008; Smith *et al*, 1997).

Magnetic resonance imaging (MRI) can be used to indirectly assess relationships between cognition and brain iron in living individuals. Several methods of varying sensitivity and specificity have been published (reviewed in Haacke *et al*, 2005; Pfefferbaum *et al*, 2009). MRI can measure brain iron levels through the effect of iron on transverse relaxation rates (R_2) (Bartzokis *et al*, 1993; Bartzokis *et al*, 1994; Vymazal *et al*., 1996a; Yao *et al*, 2009). The bulk of brain iron is stored in ferritin molecules (Floyd and Carney, 1993; Morris *et al*, 1992) and an *in vivo* MRI method called field-dependent relaxation rate (R_2) increase (FDRI) can measure their iron content (Bartzokis *et al*, 1993; Bartzokis *et al*, 1994). Briefly, FDRI is the difference in measures of brain R_2 obtained with two different field-strength MRI instruments. The FDRI is specifically associated with the total iron contained in ferritin molecules (Bartzokis *et al*, 1993; Vymazal *et al*, 1996a) and has been shown to be independent of the amount of iron loading (number of iron atoms per molecule of ferritin) (Vymazal *et al*, 1996b) and to increase linearly with field-strength (Bartzokis *et al*, 1993; Gossuin *et al*, 2004; Vymazal *et al*, 1996a; Vymazal *et al*, 1995a; Vymazal *et al*, 1996b; Yao *et al*, 2009). *In vivo* FDRI data has been validated by demonstrating very high correlations with published postmortem data on adult human brain iron distribution as well as replicating the striking age-related increases in iron levels in basal ganglia regions documented in postmortem studies (Bartzokis *et al*, 2007c; Hallgren and Sourander, 1958; Klintworth, 1973). Thus, FDRI measures the iron contained in ferric oxyhydroxide particles that form the mineral core of ferritin molecules. In human tissue, ferritin and its breakdown product (hemosiderin) are the only known physiologic sources of such particles (Bartzokis *et al*, 1993; Bulte *et al*, 1997; Vymazal *et al*, 1996a; Vymazal *et al*, 1995b). The FDRI measure will therefore be referred herein as ferritin iron (Bartzokis *et al*, 1999; Bartzokis *et al*, 2000).

Recently, Ding *et al* (2009) used a phase-shift imaging MRI technique that, amongst other things, is affected by tissue iron, and reported that increased iron in the Hip of subjects with AD may be related to worse cognitive performance and duration of illness. Whether age-related increases in hippocampal iron levels in healthy individuals may represent a trajectory of increasing risk of cognitive decline into AD (Bartzokis, 2009) and are associated with decreased memory performance have yet to be assessed. Herein we present the first study that examines associations between ferritin iron levels in the Hip as well as basal ganglia and white matter regions on memory and processing speed of healthy older individuals.

We, recently, observed higher ferritin iron in men than women (Bartzokis *et al*, 2007c) and suggested that the increased levels may contribute to the risk of developing neurodegenerative diseases at earlier ages in men (Bartzokis *et al*, 2007a; Bartzokis *et al*, 2004; Raber *et al*, 2004). We also observed that gene variants involved in iron metabolism (hemochromatosis H63D (HFE H63D) and transferrin C2 (TfC2) variants) are associated with higher brain iron levels in healthy older men (Bartzokis *et al*, 2010). These two gene variants are highly prevalent (affecting approximately 50% of the population), and some studies have shown an association of these variants with higher risk of developing AD (Connor and Lee, 2006; Lehmann *et al*, 2010; Sampietro *et al*, 2001; Moalem *et al*, 2000). We therefore examined memory function in the context of gender and the presence (IRON+) or absence (IRON−) of these iron gene variants.

We hypothesized that even in healthy individuals, age-related increases in ferritin iron levels in the Hip, which is damaged early and severely in dementia-causing diseases such as AD and DLB (Braak *et al*, 1996; Kotzbauer *et al*, 2001), will negatively impact memory function (Bartzokis *et al*, 2007a; Bartzokis *et al*, 2007c; Bartzokis *et al*, 2004). Based on data that men may develop neurodegenerative diseases at younger ages (Barker *et al*, 2002; Friedman, 1994; Miech *et al*, 2002; Pantelatos and Fornadi, 1993; Raber *et al*, 2004) (reviewed in Bartzokis *et al*, 2004), we also hypothesized that healthy older men may be at increased risk for such iron-associated declines in memory function compared to women (Bartzokis *et al*, 2007c; Bartzokis *et al*, 2004). In exploratory analyses we also examined the effects of gender and iron gene variants (HFE H63D and TfC2) in basal ganglia and white matter regions on working memory/attention and processing speed functions.

SUBJECTS AND METHODS

The subjects, imaging, and genetic methods were described in detail in prior publications (Bartzokis *et al*, 2010; Bartzokis *et al*, 2007c) and will only be summarized here.

Subjects

Normal older adult volunteers were recruited from the community and hospital staff for a study of healthy aging (Bartzokis *et al*, 2007c). Potential subjects were excluded if they had a history of neurological disorder or a family history of AD or other neurodegenerative disorder, psychiatric illness (including drug or alcohol abuse), or head injury resulting in loss of consciousness for more than 10 min. The subjects were physically very healthy and were excluded if they were obese, or if they had a current or prior serious illness, or a medical history of diabetes, cardiovascular disease, or difficult-to-control hypertension. They were independently functioning and had no evidence of neurocognitive impairment or gross neurological abnormalities on clinical interview and brief neurological examination with the study PI. The final population of 63 individuals contained 33 men and 30 postmenopausal women ranging in age from 55 to 76 years (mean = 67.0 years, SD = 6.1). The sample averaged 15.8 years of education (SD = 2.5; range = 10–23 years) and their reported racial composition was 45 (71%) Caucasian, 13 (21%) Asian, and 5 (8%) African-American. All subjects received written and oral information about the study and signed written informed consents approved by the local Institutional Review Board prior to study participation.

The participants were first assessed and evaluated with MRI, clinical assessment, and neurocognitive measures. The

subjects were later genotyped for the presence of TfC2 or HFE H63D and C282Y genes. There were no carriers of the rare HFE C282Y variant in this sample so the only genes present were TfC2 and HFE H63D (Bartzokis *et al*, 2010). Of the total sample, 32 subjects had one or both genes (IRON + group; 18 males, 14 females) while 31 had neither gene (IRON− group; 15 males, 16 females).

Neurocognitive Measures

The neurocognitive measures were collected within one month of the MRI scan.

Memory related. The California Verbal Learning Test (CVLT; Delis *et al*, 1987) is a measure of rote verbal learning and memory in which a list of 16 words is presented over 5 trials and recalled after an interference list and again after a 20-min delay. The total number of words recalled after 20 min serves as a measure of delayed memory for unstructured verbal material.

Verbal working memory/attention related. Auditory Consonant Trigrams (ACT; Peterson and Peterson, 1959) is a sensitive measure of working memory, requiring the subject to sustain information in short-term memory while performing other cognitive operations. Basic and complex attention span was assessed using the Digit Span subtest from the WAIS III (Wechsler, 1997).

Processing speed related. Trailmaking Test—part A and B (Reitan and Wolfson, 1985) assesses information processing speed, visuomotor tracking, and mental flexibility. Part A requires subjects to rapidly connect consecutively numbered circles, and part B requires subjects to consecutively connect circles containing numbers and letters by alternating between the two sequences (e.g., 1-A-2-B). Time to complete the task serves as the variable of interest. Digit Symbol subtest from the WAIS-R (Wechsler, 1981) involves rapid copying of symbols and integrates several cognitive processes including psychomotor speed, visual scanning, and simple constructional ability. The score reflects number of symbols copied within 90 seconds.

MRI Protocol

The methods have been previously described in detail (Bartzokis *et al*, 1993; Bartzokis *et al*, 1994; Bartzokis *et al*, 2007c). The participants were scanned using two MRI instruments (1.5 and 0.5 T) within 1 h of each other using the same imaging protocol. Coronal and sagittal pilot scans were first obtained to specify the location and spatial orientation of the head and the position of the axial image-acquisition grid. The axial image-acquisition sequence acquired interleaved contiguous slices using a Carr–Purcell–Meiboom–Gill dual spin–echo sequence (time to repetition (TR) = 2500 ms; time to echo (TE) = 20 and 90 ms; 2 excitations; 3 mm slice thickness; 192 gradient steps; and 25 cm field-of-view). The coronal and sagittal pilot scans were used to determine the alignment and accuracy of head repositioning in the second MRI instrument (Bartzokis *et al*, 1993; Bartzokis *et al*, 1994).

Data for each region of interest (ROI; depicted in Figure 1) were obtained from contiguous pairs of slices. The slice containing the anterior commissure and the slice immediately superior to it were used to obtain the putamen (P) and globus pallidus (G) transverse relaxation time (T_2) data. The third and fourth slices above the anterior commissure were used to obtain the T_2 data for caudate nucleus (C) and the second and third slices superior to the orbitofrontal gray matter were used to obtain the frontal lobe white matter (Fwm) data. For the genu of the corpus callosum (Gwm), the two slices on which the angle formed by the left and the right sides of the genu appeared the most linear were chosen in order to obtain a sample that would be consistently in the middle of the structure, which contains primarily fibers connecting the prefrontal cortices. For the splenium of the corpus callosum (Swm), the second and third lowest slices on which the fibers of the splenium connected in the midline were chosen in order to sample primarily the lower half of the splenium that contains predominantly primary sensory (visual) fibers. For thalamus (T), the second and third highest slices on which thalamus appears are chosen. The lateral border of the ROI is drawn along the white matter of the internal capsule using the TE = 20 image. The medial and inferior portions bordering CSF are defined using the TE = 90 image. For Hip, the two contiguous slices

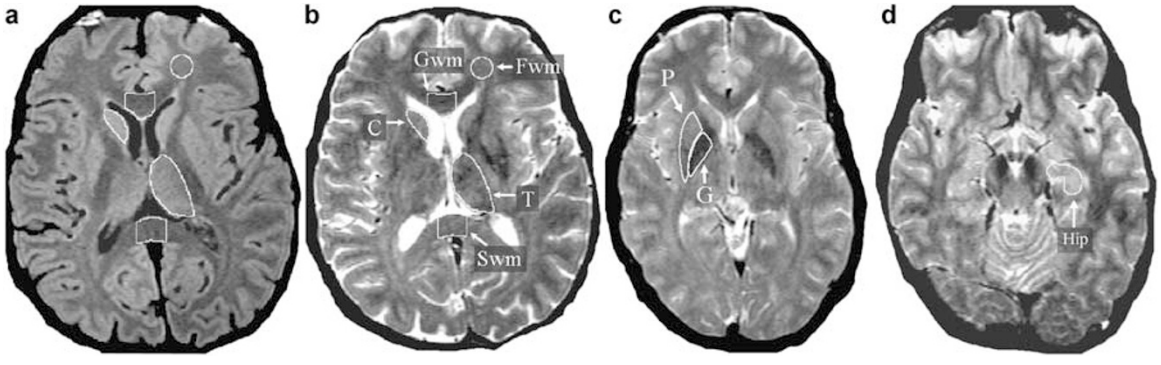

Figure 1 Regions of interest (ROIs). ROI definition is depicted on axial MRI TE20 (a) and TE90 (b–d). The TE20 has optimal contrast between gray (appears light gray) and white matter (appears dark gray). The TE90 has optimal contrast between brain (appears gray) and CSF (appears white). Both TE20 and TE90 images are used to draw each ROI as this combination of images maximizes contrast needed for accurate ROI definition. As an example, the use of both contrasts is depicted in the thalamus ROI that borders CSF medially and white matter laterally and posteriorly (a and b). Data for each ROI are obtained from contiguous pairs of slices. Only one hemisphere ROI is depicted on the figures although ROIs are measured bilaterally for all regions except for midline corpus callosum regions (Gwm and Swm). Please see text ('MRI Protocol') for further details.

that contained the largest areas of these structures were used in the data analysis. The Hip measure was obtained from the anterior third of the structure and was limited by drawing a horizontal line at the level of the cerebral peduncle to exclude any tissue posterior to that line (Bartzokis *et al*, 2010; Bartzokis *et al*, 2007c).

Image Analysis

Transverse relaxation times (T_2) were calculated for each voxel by an automated algorithm from the two signal intensities (TE = 20 and 90) of the dual spin–echo sequence to produce gray-scale encoded T_2 maps of the brain (Bartzokis *et al*, 1994). The T_2 measures were extracted using an Apple Macintosh-configured image analysis workstation. T_2 data for each of the ROIs were obtained from contiguous pairs of slices. The R_2 was calculated as the reciprocal of $T_2 \times 1000$ ms/s. The average R_2 of the two slices from both hemispheres were the final measures used in the subsequent analyses. The FDRI measure was calculated as the difference in R_2 (high-field R_2−low-field R_2). Test–retest reliability for FDRI measures was very high with intraclass correlation coefficients ranging from 0.88 to 0.99 ($p < 0.0023$) (Bartzokis *et al*, 1993; Bartzokis *et al*, 1994).

Data Analyses

Parametric approaches were used to assess the impact of the iron gene variants and gender on the association between brain iron and cognition. We first performed multiple regression analysis that crosses cognitive scores with measures of brain iron. To supplement the regression analyses, Pearson's correlations were conducted to examine the relationship between brain iron measures and cognitive performance in relevant subgroups (eg, stratified by gender and gene grouping). Differences between correlations were then tested by the non-parametric approach of normal curve test on Fisher's *z*-transformed values. As in this restricted age range the FDRI measure was not significantly associated with age in any of the regions, age was not included in the analyses.

Hippocampal Iron and Episodic Memory

A specific hypothesis regarding hippocampal iron and episodic memory performance was tested by performing a multiple regression analysis that crosses the CVLT delayed recall score with the FDRI measure of iron in the Hip. The memory measure was the dependent variable, and the independent variables were gender, iron genes (presence or absence of genes), and the Hip FDRI measure. The independent variable factors were fully crossed and our interest focused on the two-factor interactions of gene × brain iron measure and the three-factor gene × brain iron × gender interactions.

Basal Ganglia and White Matter Iron and Non-Memory Cognitive Performance

We then assessed whether the iron genes impact the association between iron levels in other brain regions and non-memory aspects of cognitive functioning. For the purpose of data reduction, principal components analysis was performed separately for the cognitive tasks and the measures of brain iron. In each case, the analysis suggested retention of two components for rotation, and the interpretation was based on eigenvalues >1 and a clear break in the scree curve. Substantive interpretations of the components were based on highest loadings for each variable. In the cognitive variables, the components were interpreted as being related to processing speed (Trailmaking Test—parts A and B, Digit Symbol) and auditory working memory/attention (ACT, Digit Span). In the FDRI measures of brain iron, the components represented measures of the basal ganglia (caudate, globus pallidus, and putamen) and white matter (frontal, splenium of the corpus callosum, genu of the corpus callosum, and thalamus). Component scores were computer generated by regression-based scoring, producing standardized scores with mean = 0 and standard deviation = 1.

Using the same statistical methodologies detailed above, four multiple regression analyses were generated by crossing the two cognitive factors (working memory/attention and processing speed) with the two measures of brain iron (basal ganglia and white matter). The two cognitive measures were the dependent variables and the independent variables were gender, iron genes (presence (IRON+) or absence (IRON−) of genes), and either the basal ganglia or white matter component score. The independent variable factors were fully crossed, and our interest focused on the two-factor interactions of gene × brain iron measure and the three-factor interactions of gene × brain iron × gender.

All *p*-values were assessed as significant at an α level of 0.05. The *p*-values for the multiple regression and Pearson's correlation analyses testing the associations between basal ganglia and white matter iron with nonmemory cognitive performance were assessed as significant at an α level of 0.0125 to reflect Bonferroni correction for the four comparisons.

RESULTS

The iron absorption genes did not have a significant effect on the relationship between Hip iron and CVLT. The analysis of Hip iron and CVLT performance yielded a significant interaction of gender by iron level ($F = 10.56$, $df = 1, 55$, $p = 0.002$). Pearson's correlation analyses indicated a significant negative association between the Hip iron and CVLT in men ($N = 33$, $r = -0.50$, $df = 31$, $p = 0.003$), and a nonsignificant positive association between these two measures in women ($N = 30$, $r = 0.19$, $df = 28$, $p = 0.31$). The difference between these correlation coefficients was significant and similar to the result from the more complex regression model above (Fisher's *z*-test: $t = 2.74$, $p = 0.006$). The results are depicted in Figure 2 and were not substantially altered after controlling for age in the analyses.

The regression models based on iron and cognitive components yielded a significant effect of the iron genes on the association between basal ganglia iron level and the working memory/attention score (interaction $F = 8.15$, $df = 1, 55$, $p = 0.006$). As gender was not a significant factor

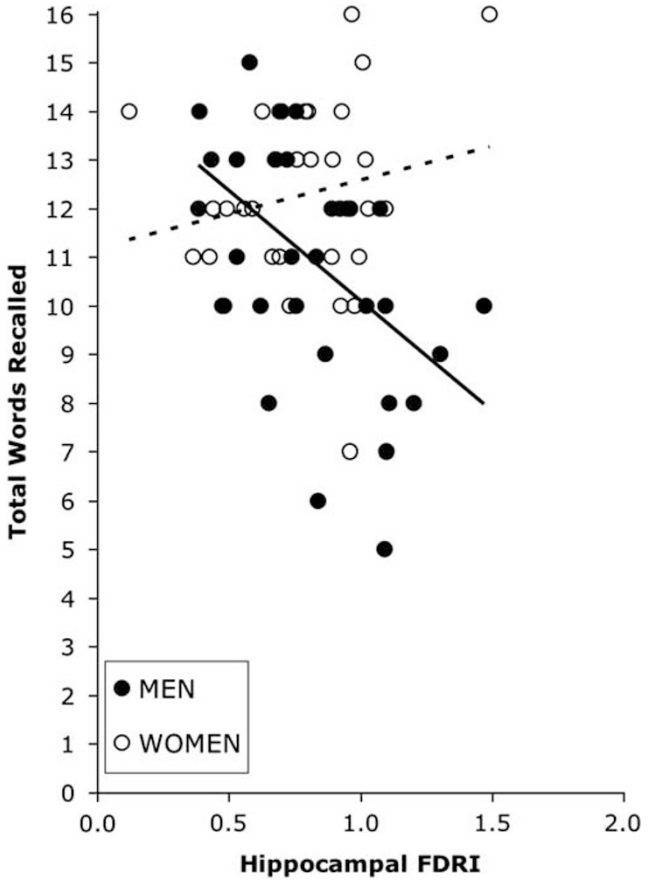

Figure 2 Memory function *vs* hippocampal ferritin iron in healthy older men and women. Men: $r=-0.50$, $p=0.003$. Women: $r=0.19$, $p=0.31$. Memory function: assessed using The California Verbal Learning Test (CVLT) (Delis *et al*, 1987), which is a measure of rote verbal learning and memory (number of words recalled after a 20-min delay). FDRI, field-dependent transverse relaxation rate (R_2) increase, an MRI measure of ferritin iron (the iron content of ferritin molecules).

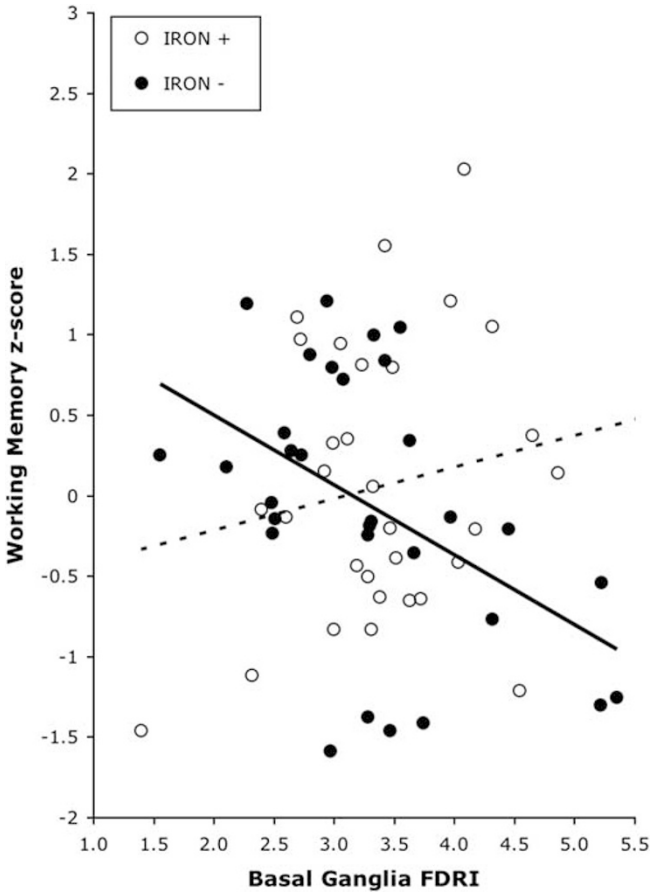

Figure 3 Working memory function *vs* basal ganglia ferritin iron in healthy older men and women with and without iron genes. IRON−: wild type gene carriers ($r=-0.49$, $p=0.005$). IRON+: carriers of either transferrin C2 (TfC2) and/or hemochromatosis H63D (HFE H63D) gene variants ($r=0.19$, $p=0.30$). Working memory function: assessed using a composite score of Auditory Consonant Trigrams (ACT; Peterson and Peterson, 1959) and Digit Span, which require subjects to either manipulate information or simultaneously perform another cognitive operation while sustaining the original information in short-term memory. Basal ganglia: composite score of caudate, putamen, and globus pallidus. FDRI, field-dependent transverse relaxation rate (R_2) increase, an MRI measure of ferritin iron (the iron content of ferritin molecules).

in this model, it was not examined separately. We computed the Pearson's correlation between basal ganglia iron and working memory/attention and found a significant negative relationship in the IRON− group ($N=31$, $r=-0.49$, $df=29$, $p=0.005$); thus for those without either of the iron genes (H63D and TfC2), increased basal ganglia iron was significantly associated with worse performance in working memory/attention. A positive but nonsignificant association was observed in the IRON+ group ($N=32$, $r=0.19$, $df=30$, $p=0.30$). The difference in correlation coefficients between the IRON+ and IRON− groups were statistically significant and mirrored the regression result (Fisher's z-test: $t=2.75$, $p=0.006$). The results are depicted in Figure 3 and were not substantially altered by controlling for age in the analyses. The results from the multiple regression and Pearson's correlation analyses remain significant after Bonferroni correction ($\times 4$ tests) for multiple comparisons (corrected: $p=0.024$ and $p=0.020$, respectively). None of the analyses involving white matter iron approached significance.

When the above regression analyses were confined to only Caucasian subjects ($N=45$), all the findings remained essentially unchanged.

DISCUSSION

This is the first demonstration that in healthy older men, declarative memory function may be adversely affected by increased Hip ferritin iron. A male-specific association is in line with our previously observed higher brain iron levels in men compared to women across the lifespan (Bartzokis *et al*, 2007c) and increased iron levels in male carriers of highly prevalent allelic variants of genes (HFE H63D and/or TfC2) that encode proteins involved in iron metabolism (Bartzokis *et al*, 2010). These observations are consistent with the proposition that a portion of the suggested earlier age at onset in men of neurodegenerative diseases such as AD, PD, and DLB (Barker *et al*, 2002; Raber *et al*, 2004) may be accounted for, at least in part, by their higher brain ferritin iron (Bartzokis *et al*, 2004) (reviewed in Bartzokis *et al*, 2007c). In women, the iron–memory association was significantly different from the one observed in men. The

association could be substantially modified in women because of the gender-specific pattern of iron requirements such as those produced by menstruation and/or metabolism (see below) (Coppus *et al*, 2009; Whitfield *et al*, 2003) (reviewed in Bartzokis *et al*, 2007c).

Animal data suggest that during early postnatal development, increases in Hip ferritin iron are necessary for normal cognitive function and memory (Carlson *et al*, 2009; Georgieff, 2008; Schmidt *et al*, 2007; Shoham and Youdim, 2002; Siddappa *et al*, 2004) and that early deficits can have long-lasting detrimental effects on cognition even when corrected through supplementation (Schmidt *et al*, 2007). Differentiating oligodendrocytes have very high iron requirements and may have a critical role in brain iron uptake (reviewed in Bartzokis *et al*, 2007a; Bartzokis *et al*, 2007b). This may be especially important in the hippocampus where 50% of oligodendrocytes are juxtaposed directly on blood vessels and may thus be in a position to acquire iron directly from the vasculature (Vinet *et al*, 2010). On the other hand, iron overload may also cause cognitive deficits (Maaroufi *et al*, 2009) and increased neonatal iron intake as well as overexpression of ferritin may be associated with increased risk of neurodegenerative disease in late life (Carlson *et al*, 2008; Kaur *et al*, 2006a; Kaur *et al*, 2006b). It is thus becoming evident that regulating the processes underlying brain iron accumulation trajectories has important consequences throughout the lifespan. Furthermore, many neurodegenerative diseases are associated with dysregulated iron metabolism (Bartzokis, 2009; Kell, 2009; Roth *et al*, 2010; Zecca *et al*, 2004). Such dysregulations are often manifested as increases in tissue iron that go beyond those observed in healthy individuals (reviewed in Bartzokis *et al*, 2007c; Kell, 2009; Zecca *et al*, 2004). One mechanism leading to such iron increases could result from abnormal intracellular and extracellular protein deposits (proteinopathies) triggering inappropriate iron accumulation in multiple disorders (Singh *et al*, 2009; Smith *et al*, 1997) (reviewed in Bartzokis, 2009; Bartzokis *et al*, 2007a).

Iron regulation in neurons seems to be strongly dependent on the ferroxidase activity of amyloid precursor protein (APP) that is responsible for removing excess iron (Duce *et al*, 2010) and may thus mitigate intracellular iron-catalyzed oxidative damage (Halliwell and Gutteridge, 1988). The iron-management function of APP is also supported by the observation that APP transcription is controlled, at least in part, by the same iron-sensing mechanism that controls transcription of two canonical iron metabolism proteins: ferritin and transferrin (Cahill *et al*, 2008). Neurons may be especially susceptible to excess iron because of the disproportionately long processes (axons) connecting neuron body to synapses, and APP is well suited for its iron management function because of its presence throughout the neuron. The other well-known function of APP is to adhere the 'cargo' (eg, vesicles) to the 'motors' powering the fast axonal transport (FAT) that moves supplies from the neuron body to axons and synapses, and back. The two-way FAT traffic thus automatically provides the ferroxidase function of APP throughout, and especially near mitochondria that amass all along the axon at the nodes of Ranvier as well as at distant synapses. Removal of iron liberated from any source, such as damaged iron-rich mitochondria being transported back to the neuronal body for degradation, would be crucial throughout the FAT transport system (reviewed in Bartzokis, 2009).

The Hip region has been shown to have elevated iron levels in AD (Deibel *et al*, 1996; Good *et al*, 1992; Smith *et al*, 1997; Thompson *et al*, 1988) (Bartzokis *et al*, unpublished data), and is affected early and severely in age-related proteinopathies such as AD and DLB (Braak *et al*, 1996; Kotzbauer *et al*, 2001) that cause the vast majority (over 70%) of dementia (Barker *et al*, 2002; Fratiglioni *et al*, 2000; Lobo *et al*, 2000). The observation that increased hippocampal iron is associated with poorer memory function even in healthy older individuals supports the suggestion that the 'normal' trajectory of age-related increases in brain ferritin iron may represent an underlying risk factor for age-related degenerative brain diseases (Chen *et al*, 2009) (reviewed in Bartzokis, 2009). Age-related accumulations of brain iron in structures such as basal ganglia and Hip of healthy individuals (Bartzokis *et al*, 2007c; Hallgren and Sourander, 1958) may be conceptualized as 'normal' trajectories toward brain iron overload of vulnerable regions (Bartzokis, 2009) that may be modified by gender and genetic differences (Bartzokis *et al*, 2010; Bartzokis *et al*, 2007c). These differences may modify trajectories of iron accumulation from normal age-related memory declines (Figures 2 and 3) into preclinical stages of degeneration and eventually dementias (Bartzokis, 2009; Lavados *et al*, 2008; Smith *et al*, 1997; Smith *et al*, 2010).

As reviewed above, however, both iron deficiency and brain iron excess can have deleterious consequences on cognition. In old age, adequate iron levels are essential for the continual process of myelin repair/replacement, a key process in maintaining cognitive functions (reviewed in Bartzokis, 2009). Thus the opposite association we observed in men compared to that in women in the Hip–memory correlations are not entirely unexpected given known gender differences in iron status. Women begin their postmenopausal years in a state of relative peripheral iron deficiency compared with men and their iron levels increase for the first 15–20 years after menopause without fully 'catching up' to those in men (Whitfield *et al*, 2003). As the peripheral iron levels can influence brain iron accumulation (Bartzokis *et al*, 2007c; House *et al*, 2010; Li *et al*, 2010), these peripheral effects could manifest in the brain. Thus, as the two genders approach older ages, it is possible that the already higher levels of iron observed in men push them into a toxic range earlier as they enter older ages, whereas women, who start with considerably lower iron levels, may initially experience a cognitive benefit from increasing postmenopausal iron levels.

The basal ganglia accumulate markedly more iron with age than most other brain structures (Bartzokis *et al*, 2007c; Hallgren and Sourander, 1958) and may thus require additional protection from iron-associated toxicity. Further analyses revealed an unexpected modifying effect of prevalent iron metabolism gene variants on the association between basal ganglia ferritin iron and working memory function (Figure 3). This observation is consistent with the direct involvement of the basal ganglia in working memory networks (Battig *et al*, 1960; Chorover and Gross, 1963) (reviewed in Constantinidis and Procyk, 2004; Simpson

et al, 2010). Striatofrontal networks interconnect specific areas of the prefrontal cortex to subregions of the basal ganglia, forming loops that are intricately involved in higher cognitive functions including working memory (Alexander *et al*, 1986; Battig *et al*, 1960; Chorover and Gross, 1963; Landau *et al*, 2009; Lewis *et al*, 2004). In iron gene noncarriers (IRON− group), higher brain iron in the basal ganglia was again significantly associated with worse verbal working memory. A different (opposite, albeit nonstatistically significant) relationship was seen in the gene carrier (IRON+) group. These genes have been associated with brain iron uptake as well as complex interactions between basal ganglia ferritin iron levels, metabolic processes, and cognitive functions (Burdo *et al*, 2004; Lee *et al*, 2007; Li *et al*, 2010; Ma *et al*, 2008; Mitchell *et al*, 2009). The presence of these genes seems to mitigate the detrimental effect of basal ganglia iron accumulation on working memory function observed in the IRON− group and this may help explain, at least in part, the very high prevalence of these genes in the population.

Animal models support the suggestion that iron overload is associated with memory decline (Maaroufi *et al*, 2009), and treatment with iron chelators have been reported to result in improved memory even in healthy aged rodents (de Lima *et al*, 2007). Human studies have also suggested that chelation treatment may be helpful in degenerative brain diseases (reviewed in Kell, 2009). The mechanism of iron toxicity is likely related to promotion of damaging free-radical reactions and associated inflammation (Smith *et al*, 1997) (reviewed in Kell, 2009). It is thus not surprising that invertebrate data (Drosophila) suggest that age-related iron accumulation is proportional to the rate of aging (Massie *et al*, 1985) and inhibition of iron absorption prolongs lifespan (Massie *et al*, 1993). Similarly, human gender differences in longevity have been proposed to relate to reproduction-related iron losses in women (Sullivan, 1989) and life-extending effects of calorie restriction have been associated with reduced dietary iron uptake and lowered iron deposits in tissue (Cook and Yu, 1998; Kastman *et al*, 2010; Valle *et al*, 2008; Xu *et al*, 2008).

Several limitations of the current study also need to be considered. First, strict criteria for inclusion of healthy older individuals and small sample size may underestimate the strength of correlations between tissue iron and cognition by restricting sample variance. Second, interpretation of 'changes' from cross-sectional data must be made with caution (Kraemer *et al*, 2000), and prospective studies and larger samples are needed to further define age-related trajectories in each gender and genetic subgroup. Third, the absence of a sample of younger adults limits the ability to detect quadratic or other nonlinear associations. Fourth, peripheral iron measures and detailed information on blood loss during life, and other environmental influences such as iron supplementation that may affect brain iron were not available. Fifth, although we observed a significant effect of HFE and TfC2 genes on brain iron levels (Bartzokis *et al*, 2010) and the basal ganglia–working memory association, these genes likely account for a minority of the variance, much of the genetic influence on iron levels remains unknown (Njajou *et al*, 2006; Whitfield *et al*, 2000), and future studies may identify additional genetic influences on brain iron and its impact on cognitive performance. Finally, although reproducible (Bartzokis *et al*, 1994; Bartzokis *et al*, 2000) and very highly correlated with postmortem iron levels (Bartzokis *et al*, 2007c), the FDRI measure specifically quantifies ferritin iron load that may be only indirectly related to the amount of free iron or other transition metals that may be more directly associated with toxicity (Lavados *et al*, 2008; Rajendran *et al*, 2009).

The significantly different associations we observed in subgroups suggest that toxic consequences on cognitive function of age-related brain iron increases may differ substantially by gender and genotypes, and that brain iron deposition is a complex multifactorial endophenotype. Treatment efforts to reduce the deleterious effects of brain iron accumulations in old age (Adlard *et al*, 2008; Cahill *et al*, 2008; Chen-Roetling *et al*, 2009; Hider *et al*, 2008; Mandel *et al*, 2008; Zecca *et al*, 2008) (for review see Kell, 2009) should take into consideration that large subgroups of the population may substantially differ in brain iron regulation mechanisms and thus differ in their response to such interventions.

The advent of *in vivo* neuroimaging methods that can assess tissue ferritin iron deposits on a regional basis with high specificity provides the means to prospectively examine the impact of age-related changes in iron on trajectories of cognitive decline into neurodegenerative diseases. These methods could be used to measure iron accumulations as well as target emerging therapeutic interventions (Adlard *et al*, 2008; Cahill *et al*, 2008; Chen-Roetling *et al*, 2009; Hider *et al*, 2008; Mandel *et al*, 2008; Zecca *et al*, 2008) (for review see Kell, 2009) to high-risk groups identified by MRI, genetic, and clinical biomarkers, years before clinical manifestations of disease. Early intervention in higher-risk subgroups may make it possible to increase effectiveness of treatments, decrease the need for more-aggressive approaches at later stages, and may identify heretofore unexplored opportunities for primary prevention for the exponentially increasing burden of age-related neurodegenerative diseases (Bartzokis, 2009; Bartzokis *et al*, 2007c).

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DISCLOSURE

The authors declare no conflict of interest.

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