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Iron-related gene variants and brain iron in multiple sclerosis and healthy individuals

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ABSTRACT

Brain iron homeostasis is known to be disturbed in multiple sclerosis (MS), yet little is known about the association of common gene variants linked to iron regulation and pathological tissue changes in the brain. In this study, we investigated the association of genetic determinants linked to iron regulation with deep gray matter (GM) magnetic susceptibility in both healthy controls (HC) and MS patients. Four hundred (400) patients with MS and 150 age- and sex-matched HCs were enrolled and obtained 3 T MRI examination. Three (3) single nucleotide polymorphisms (SNPs) associated with iron regulation were genotyped: two SNPs in the human hereditary hemochromatosis protein gene *HFE*: rs1800562 (C282Y mutation) and rs1799945 (H63D mutation), as well as the rs1049296 SNP in the transferrin gene (C2 mutation). The effects of disease and genetic status were studied using quantitative susceptibility mapping (QSM) voxel-based analysis (VBA) and region-of-interest (ROI) analysis of the deep GM. The general linear model framework was used to compare groups. Analyses were corrected for age and sex, and adjusted for false discovery rate. We found moderate increases in susceptibility in the right putamen of participants with the C282Y (+6.1 ppb) and H63D (+6.9 ppb) gene variants vs. non-carriers, as well as a decrease in thalamic susceptibility of progressive MS patients with the C282Y mutation (left: −5.3 ppb, right: −6.7 ppb, $p < 0.05$). Female MS patients had lower susceptibility in the caudate (−6.0 ppb) and putamen (left: −3.9 ppb, right: −4.6 ppb) than men, but only when they had a wild-type allele ($p < 0.05$). Iron-gene linked increases in putamen susceptibility (in HC and relapsing remitting MS) and decreases in thalamus susceptibility (in progressive MS), coupled with apparent sex interactions, indicate that brain iron in healthy and disease states may be influenced by genetic factors.

1. Introduction

In the brain, iron is an abundant element mostly stored in ferritin clusters. It is essential for brain development and is involved in many biochemical processes, including myelin synthesis (Hagemeier et al., 2012a; Rouault, 2013). In several neurological disorders such as multiple sclerosis (MS), brain iron is dysregulated and often present around plaques as activated iron-laden macrophages (Mehta et al., 2013; Haider et al., 2014). Excessive free iron in the deep gray matter (GM) may induce (e.g. due to oligodendrocyte dysfunction) or amplify neurodegeneration through generation of reactive oxygen species (Farina et al., 2013).

Brain iron concentrations have long been known to increase with normal aging (Hallgren and Sourander, 1958; Hagemeier et al., 2017). In most deep GM regions, the increase appears to slow down and level with age, while in the thalamus a reduction in iron concentration is

Abbreviations: MS, multiple sclerosis; GM, gray matter; HFE, human hemochromatosis gene; QSM, quantitative susceptibility mapping; HC, healthy control; RRMS, relapsing-remitting multiple sclerosis; EDSS, Expanded Disability Status Scale; TF, transferrin; SNP, single nucleotide polymorphism; GRE, gradient recalled echo; T1w, T1-weighted; ppb, parts per billion; VBA, voxel-based analysis; TFCE, threshold-free cluster enhancement; FWE, family-wise error rate; GLM, general linear model; FDR, false discovery rate; ROI, region of interest; MSSS, multiple sclerosis severity scale

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often observed after mid-life. Also, sex differences have been reported (Bartzokis et al., 2010), with men having higher brain iron loads. For years, many post-mortem and in vivo studies using iron-sensitive imaging techniques have described changes in brain iron homeostasis in an array of neurologic disorders (Hagemeier et al., 2012a; Ward et al., 2014), including Alzheimer's disease (Jellinger et al., 1990; Connor et al., 1992; Acosta-Cabronero et al., 2013), Parkinson's disease (Jellinger et al., 1990; Costa-Mallen et al., 2017), and MS (Craelius et al., 1982; Zivadinov et al., 2012; Hagemeier et al., 2017).

Genetic variations in iron-regulating genes, e.g., H63A and C282Y variants of the human hemochromatosis (*HFE*) gene, can influence peripheral iron load (Burt et al., 1998). However, it remains unclear to what extent specific iron-regulating genes influence brain iron homeostasis, and whether there would be effects specific to neurological diseases. A recent R2* study by Pirpamer et al. (Pirpamer et al., 2016) did not observe iron regulatory gene effects (nor any effect of sex) on brain iron levels of healthy subjects. Another study, however, reported that healthy men carrying either the *HFE* or Transferrin C2 genetic variant did have increased caudal iron levels, as compared to non-carriers (Bartzokis et al., 2010), suggesting that a genetic effect on brain iron may be sex dependent. Iron gene status may also be linked to the association between brain iron and cognition (Bartzokis et al., 2011). In addition, the presence of common gene variants involved in iron metabolism and transport appear to influence the age of onset and disease severity, and are more prevalent in neurological diseases associated with iron dysregulation, such as Alzheimer's disease (van Rensburg et al., 1993; Moalem et al., 2000; Sampietro et al., 2001), amyotrophic lateral sclerosis (Wang et al., 2004), and Parkinson's disease (Dekker et al., 2003). In two European MS studies, the frequency of *HFE* H63A and C282Y variants were comparable to controls. However, *HFE* C282Y mutant allele carriers did report earlier disease onset (Ristic et al., 2005) and more rapid disability progression (Bettencourt et al., 2011). An Australian study reported increased prevalence of the *HFE* C282Y mutation in MS (10.2% vs. controls: 6.7%) (Rubio et al., 2004).

In MS, studies have shown that brain iron levels are disturbed especially in the deep GM (Bakshi et al., 2002; Zivadinov et al., 2012; Stankiewicz et al., 2014), and around plaques (Craelius et al., 1982). Several recent MRI studies have utilized the novel quantitative susceptibility mapping (QSM) technique in MS to investigate in vivo tissue changes (Zhang et al., 2016; Hagemeier et al., 2017). QSM is an advanced MR imaging method (Reichenbach et al., 2015; Schweser et al., 2016) allowing the measurement of subtle changes of the magnetic susceptibility, reflecting tissue concentrations of paramagnetic iron complexes (Langkammer et al., 2012; Stuber et al., 2014), but also calcium (Schweser et al., 2010; Chen et al., 2014; Stuber et al., 2014), and myelin (Schweser et al., 2011; Stuber et al., 2014; Groeschel et al., 2016). It is currently regarded as one of the most sensitive and specific techniques for studying tissue iron in vivo (Langkammer et al., 2013; Stuber et al., 2015).

To date, gene variants linked to iron regulation have not been investigated in the context of in vivo brain iron measurements in MS patients. As such, the present study investigated the effect of genetic variants linked to iron regulation in relation to deep GM tissue changes as measured by QSM in both healthy individuals and MS patients.

2. Methods

2.1. Subjects

In this substudy of the cardiovascular, genetic and environmental study in MS (Kappus et al., 2016; Zivadinov et al., 2016), healthy controls (HC) and MS patients were prospectively enrolled and group-matched by age and sex: 150 HC and 400 MS patients (relapsing-remitting [RRMS]: 261, progressive MS: 139). Subjects needed to have 3 T MRI examination with the sequences suitable for QSM and brain volumetry examination having been applied successfully, as well as genetic determination for iron-related gene variants. MS patients were included if they had a relapsing or progressive (secondary- or primary-progressive) disease course. Patients with a relapse and/or steroid treatment within 30 days prior to MRI were excluded. Additional exclusion criteria were: pregnancy, presence of pre-existing medical conditions known to be associated with brain pathology (e.g., cerebrovascular disease, positive history of alcohol dependence). MS patients were diagnosed using the revised McDonald criteria (Polman et al., 2011) and clinical disease severity was measured using the Expanded Disability Status Scale (EDSS) (Kurtzke, 1983). HC were recruited from volunteers with a normal neurological examination and no history of neurologic disorders or chronic psychiatric disorders. The study was approved by the local Institutional Review Board at the University at Buffalo. Written informed consent was obtained from all participants.

2.2. Genotyping

Anti-coagulated peripheral blood was obtained by venipuncture for all participants. DNA was extracted from peripheral blood mononuclear cells preserved in TRI reagent (Molecular Research Center Inc., Cincinnati, OH, USA). A panel of candidate single nucleotide polymorphisms (SNPs) linked to iron regulation pathways were genotyped using the OpenArray platform (Applied Biosystems, Life Technologies, Foster City, CA). The genotyped polymorphisms were: rs1800562, rs1799945, and rs1049296. The SNP rs1800562 is found in the majority of cases with hemochromatosis (risk allele A: C282Y). The SNP rs1799945 is also a gene associated with hereditary hemochromatosis (risk allele G: H63D). rs1049296 is associated with transferrin (TF), the main iron transport protein (risk allele T: C2-subtype). The homozygous and heterozygous minor allele-containing genotypes (classified as ‘carriers’) were pooled for statistical comparison to the homozygous major allele genotype (classified as ‘non-carriers’).

2.3. MRI image acquisition

All participants were imaged with the same clinical 3 T GE Signa Excite HD 12.0 scanner (General Electric, Milwaukee, WI, USA) using an eight-channel head-and-neck coil. A detailed description of pulse sequences and image reconstruction steps has been reported previously (Hagemeier et al., 2017). Briefly, acquired sequences included a 3D single-echo spoiled gradient recalled echo (GRE) sequence for QSM (512x192x64 matrix and a nominal resolution of $0.5 \times 1 \times 2\ mm^3$ (FOV = $256 \times 192 \times 128\ mm^3$), flip angle = 12°, TE/TR = 22 ms/40 ms, and bandwidth = 13.89 kHz) and a 3D T1-weighted (T1w) fast spoiled GRE sequence for the determination of brain volume measures (TE/TI/TR = 2.8/900/5.9 ms, flip angle = 10°, isotropic 1 mm resolution).

2.4. QSM

QSM processing was performed by a fully automated pipeline with in-house developed MATLAB programs (2013b, The MathWorks, Natick, MA). Processing and susceptibility map reconstruction details were provided previously (Hagemeier et al., 2017). Magnitude and phase GRE images were reconstructed offline on a $512 \times 512 \times 64$ spatial matrix. *K*-space was zero-padded in phase-encode direction prior to the processing to achieve isotropic in-plane resolution. Distortions due to imaging gradient non-linearity were compensated (Polak et al., 2015). Phase images were unwrapped with a best-path algorithm (Abdul-Rahman et al., 2007), background-field corrected with V-SHARP (Schweser et al., 2011; Wu et al., 2012) (radius 5 mm; TSVD threshold 0.05), and converted to magnetic susceptibility maps using the HEIDI algorithm (Schweser et al., 2012). Magnetic susceptibility maps were referenced (0 ppb) to the average susceptibility of the brain. This was done to minimize potential confounding bias from