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Pooled analysis of iron-related genes in Parkinson’s disease: Association with transferrin

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Pathologic features of Parkinson’s disease (PD) include death of dopaminergic neurons in the substantia nigra, presence of α-synuclein containing Lewy bodies, and iron accumulation in PD-related brain regions. The observed iron accumulation may be contributing to PD etiology but it also may be a byproduct of cell death or cellular dysfunction. To elucidate the possible role of iron accumulation in PD, we investigated genetic variation in 16 genes related to iron homeostasis in three case-control studies from the United States, Australia, and France. After screening 90 haplotype tagging single nucleotide polymorphisms (SNPs) within the genes of interest in the US study population, we investigated the most promising gene regions in two additional independent case-control studies. For the pooled data set (1289 cases, 1391 controls) we observed a protective association (OR = 0.83, 95% CI: 0.71–0.96) between PD and a haplotype composed of the A allele at rs1880669 and the T allele at rs1049296 in transferrin (TF; GeneID: 7018). Additionally, we observed a suggestive protective association (OR = 0.87, 95% CI: 0.74–1.02) between PD and a haplotype composed of the G allele at rs10247962 and the A allele at rs4434553 in transferrin receptor 2 (TFR2; GeneID: 7026). We observed no associations in our pooled sample for haplotypes in SLC40A1, CYB561, or HFE. Taken together with previous findings in model systems, our results suggest that TF or a TF-TFR2 complex may have a role in the etiology of PD, possibly through iron misregulation or mitochondrial dysfunction within dopaminergic neurons.

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Introduction

Brain iron accumulation, beyond that seen in age-matched controls, is frequently observed in Parkinson’s disease (PD) affected brains (reviewed in (Gerlach et al., 2006)). Histochemical comparisons of post-mortem brain tissue have found increased levels of iron deposits in the substantia nigra (SN) of Parkinsonian brains (Dexter et al., 1987; Sofic...
intronic, and exonic SNPs specifically in neurons of the SN (Oakley et al., 2007). These pathologic findings are further supported by magnetic resonance imaging (Bartzokis et al., 1999; Gorgell et al., 1995; Martin et al., 2008; Michaeli et al., 2007) and transcranial ultrasound (Becker et al., 1995; Mehnert et al., 2010; Walter et al., 2002) studies that observed iron deposits in the SN in living idiopathic PD patients.

It is debatable whether this accumulation of iron is a cause, co-factor, or consequence of the dopaminergic (DA) cell death in PD. Iron is a very potent oxidation–reduction agent that can create oxidative stress in cells and prior work suggests that neurons may be more sensitive to alterations in iron (LaVaute et al., 2001; Moos et al., 1998) than other cell types in the brain. Iron is also hypothesized to aggravate some key pathogenic processes related to PD including alpha-synuclein fibril formation (Oliwares et al., 2009; Uversky et al., 2001) and mitochondrial dysfunction (Horowitz and Greenamyre, 2010; Lin et al., 2001). Finally, iron may simply be a remnant of neuronal cell death (He et al., 2003).

Animal models provide some potential insight. Genetic knockouts of some iron-related genes, when not lethal, produce altered brain iron levels (LaVaute et al., 2001; Moos and Morgan, 2004; Patel et al., 2002). Iron administration to unaltered animals has resulted in brain iron accumulation (Sengstock et al., 1993) or iron accumulation with a decrease in dopamine levels (Sziraki et al., 1998; Wesemann et al., 1994). Iron feeding studies in animals have also observed decreased dopamine levels with a large excess of dietary iron (Kaur et al., 2007) and with iron in combination with toxins (Levenson et al., 2004; Peng et al., 2007). These observations suggest that iron is either a cause or a co-factor, not a consequence, of dopaminergic cell damage, possibly due to the role iron plays in the synthesis of tyrosine hydroxylase (Snyder and Connor, 2009). Therefore, we hypothesize that small imbalances in ferrous or ferric brain iron contribute to one or more of the pathogenic processes contributing to neurodegeneration in PD and propose to investigate this hypothesis by evaluating the associations between PD and iron-related genes.

To date, epidemiologic studies investigating associations between occupational or dietary iron exposure and PD have been unpersuasive, and the few reports of iron-related genes and PD have been predominately in small studies, investigating rare exonic SNPs, and inconclusive (reviewed in (Rhodes and Ritz, 2008)). Nevertheless, studies in experimental models of PD support a role for iron in the etiology or progression of PD (e.g. (Ben-Shachar and Youdim, 1991; Fredriksson et al., 2001; Kaur et al., 2003)). To facilitate the investigation of the iron-PD hypothesis, we pooled data from three independent case–control studies: one each from the United States (US), Australia (AU), and France (FR). All studies were designed for the investigation of genetic variation; the US and FR studies were also designed to investigate the influence of pesticide exposure on PD etiology and, therefore, include subjects with an increased likelihood of exposure to pesticides. In this report, we present the results from our two-phase study of promoter region, intronic, and exonic SNPs specifically selected to span each of 16 iron-related candidate genes and their associations with PD in a pooled sample of 1286 idiopathic PD cases and 1391 controls.

Methods

For our two-phase design we screened a larger number of genetic variants in an initial study, the US Study described below, followed by genotyping of selected variants in two additional studies based on findings from the initial study. All three studies (Supplemental Table 1) were pooled for the final analysis.

Studies and subjects

US Study

The Parkinson’s Environment and Genes (PEG) Study enrolled incident PD patients with movement disorder specialist confirmed idiopathic PD and population-based controls between 2001 and 2007 from three counties in the highly agricultural central California valley of the United States. The recruitment strategy and case definition criteria have been described in detail elsewhere (Costello et al., 2009; Jacob et al., 2010). Participants provided either a blood or saliva sample and DNA was extracted by standard methods. This study was approved by the UCLA Institutional Review Board. At the time of this investigation, the US Study had enrolled a total of 373 PD cases, but reclassified 13 PD cases as not idiopathic PD during a follow-up study (Ritz et al., 2012), resulting in a total of 360 idiopathic PD cases and 403 population-based controls available for this investigation.

AU Study

The Australian case–control sample is a subset of the larger Queensland Parkinson’s Project (QPP) Cohort of over 4000 community dwelling individuals recruited to participate in research into Parkinsonism and related disorders. The recruitment strategy and inclusion criteria are detailed elsewhere (Sutherland et al., 2009). Only Caucasian subjects were included in the study. Patients with PD meeting standard criteria (Gelb et al., 1999) were recruited from two private and two public movement disorder clinics in Brisbane, Australia. Control subjects consisted of unaffected electoral roll volunteers, patient spouses, and community-dwelling unaffected volunteers collected from community groups and patient neighborhoods. Participants provided blood samples and genomic DNA was extracted according to standard methods. The study was approved by human research ethics committees at the Princess Alexandra Hospital, University of Queensland, Royal Brisbane and Women’s Hospital and Griffith University. At the time of this investigation, the AU Study had enrolled 1035 PD cases and 774 controls.

FR Study

This French population-based case–control study was conducted among subjects enrolled in the Mutualité Sociale Agricole (MSA), the organization responsible for the reimbursement of health-related expenses to workers in agriculture and has been described previously (Dutheil et al., 2010; Elbaz et al., 2004, 2009). Patients in 62 French districts fulfilling standard criteria (Bower et al., 1999), applying for free health care for PD for the first time between February 1998 and August 1999, and aged 18–75 years old were enrolled in the study. Population-based controls were recruited among all the MSA affiliates who requested reimbursement of health expenses between February 1998 and February 2000. A maximum of three controls was matched to each case on age (±2 years), sex, and region of residency. Participants provided blood samples and genomic DNA was extracted from peripheral blood leukocytes. The research protocol was approved by the ethics committee of Hôpital du Kremlin-Bicêtre, and all subjects signed an informed consent. At the time of this investigation, the FR Study had enrolled a total of 209 PD cases and 501 population-based controls.

Selection of genes and SNPs

Candidate genes were selected based on (i) prior reports in studies of PD, (ii) reported associations from iron-related disorders, and (iii) biologic support for a role in brain iron homeostasis. Genes were selected prior to publication of the genome-wide association studies (GWAS) so certain new candidate genes for iron metabolism (e.g. TMPRSS6 (Tanaka et al., 2010)) were not investigated. The 16 selected genes and their role in iron homeostasis are listed in Table 1. Candidate SNPs were selected from the literature and haplotype tagging SNPs sufficient to capture the majority of genetic variation in each gene were selected using Haploviev (Barrett et al., 2005). Some candidate SNPs, e.g. H63D in HFE, were not available on the platform used for the initial phase of genotyping and therefore were not investigated. In total 90 SNPs in 16 genes were genotyped in the US Study.
Table 1
Iron-related genes investigated.

<table>
<thead>
<tr>
<th>Gene_id</th>
<th>Location</th>
<th>Symbol</th>
<th>Gene name</th>
</tr>
</thead>
<tbody>
<tr>
<td>391059</td>
<td>1p21.2</td>
<td>FRRS1</td>
<td>Ferric-chelate reductase 1 function: ferric reductase</td>
</tr>
<tr>
<td>148738</td>
<td>1q21.1</td>
<td>HFE2</td>
<td>Hemochromatosis type 2 function: hepcidin regulation</td>
</tr>
<tr>
<td>30061</td>
<td>2q32</td>
<td>SLC40A1</td>
<td>Solute carrier family 40, member 1 function: iron export from cells</td>
</tr>
<tr>
<td>4057</td>
<td>3p21.31</td>
<td>LF</td>
<td>Lactoferelin function: iron binding and transport</td>
</tr>
<tr>
<td>7018</td>
<td>3q22.1</td>
<td>TF</td>
<td>Transferrin function: iron binding and transport</td>
</tr>
<tr>
<td>1356</td>
<td>3q23-q25</td>
<td>CP</td>
<td>Ceruloplasmin function: peroxidation of Fe2+ transferrin to Fe3+ transferrin</td>
</tr>
<tr>
<td>7037</td>
<td>3q29</td>
<td>TTRC</td>
<td>Transferrin receptor function: transferrin endocytosis and recycling</td>
</tr>
<tr>
<td>94033</td>
<td>5q12.3</td>
<td>FTMT</td>
<td>Mitochondrial ferritin function: iron binding and oxidation</td>
</tr>
<tr>
<td>3077</td>
<td>6p21.1</td>
<td>HFE</td>
<td>Hemochromatosis function: regulate iron absorption</td>
</tr>
<tr>
<td>7036</td>
<td>7q22</td>
<td>TTR2</td>
<td>Transferrin receptor 2 function: mediates cellular uptake of transferrin-bound iron</td>
</tr>
<tr>
<td>4891</td>
<td>12q13</td>
<td>SLC11A2</td>
<td>Solute carrier family 11, member 2 function: iron absorption and transport of divalent metals</td>
</tr>
<tr>
<td>3658</td>
<td>15q25.1</td>
<td>IREB2</td>
<td>Iron-responsive element binding protein 2 function: protein regulation via iron-responsive elements</td>
</tr>
<tr>
<td>1534</td>
<td>17q23.3</td>
<td>CYB561</td>
<td>Cytochrome b561 function: ferric reductase</td>
</tr>
<tr>
<td>2235</td>
<td>18q21.3</td>
<td>FECH</td>
<td>Ferrochelatase function: catalyzes the insertion of iron into heme</td>
</tr>
<tr>
<td>57817</td>
<td>19q13.1</td>
<td>HAMP</td>
<td>Hepcidin antimicrobial peptide function: regulation of iron storage</td>
</tr>
<tr>
<td>9843</td>
<td>Xq11-q12</td>
<td>HEPH</td>
<td>Hephaestin function: iron transport</td>
</tr>
</tbody>
</table>

Genotyping methods

Phase 1, US Study

Of the 360 PD cases and 403 population-based controls in the US Study, we genotyped 356 cases and 375 controls with available DNA for 90 SNPs using the Illumina Goldengate assay. In total 6 of 90 SNPs failed genotyping or were homozygous in the population: rs2075674 (HFE, 19 SNPs), a priori, the FECH region was not chosen to be of interest in the two phase 2 study samples (AU/FR) and so we are unable to follow up on it. Additionally, we could only consider a single SNP for the region located in CP (rs701754, which tags the risk haplotype observed in the US study) and only using the US and FR studies; we found no association for that single SNP in the two studies combined (OR = 1.09, 95%CI: 0.88–1.35). Overall, we evaluated five regions located in five independent genes and defined by 11 SNPs in the combined US/AU/FR Study population.

Phase 2, pooled analysis in US/AU/FR studies

We observed a protective association (OR = 0.83, 95% CI: 0.71–0.96) between PD and a haplotype in the transferrin gene (TF; GeneID: 7018) comprised of the A allele at rs1880669 and the T allele at rs1049296 in the pooled analysis (Table 2). When considering each study individually, we observed very similar magnitude odd ratios in all three populations. Analyses excluding the non-Caucasians from the US study did not alter the pooled odds ratio or the individual US study association.

Additionally, in the pooled sample, we observed a suggestive protective association (OR = 0.87, 95% CI: 0.73–1.02) between PD and a haplotype in the transferrin receptor 2 gene (TFR2; GeneID: 7036) comprised of the G allele at rs10247962 and the A allele at rs4434553. When considering each study individually, we observed this association predominantly in the US study; the FR study demonstrated only a weakly suggestive association (OR = 0.88) and the AU study demonstrated no association (OR = 1.00).

Analyses excluding the non-Caucasians from the US study did not alter the pooled odds ratio or the individual US study association.
Table 2
Pooled analysis and study specific iron gene haplotypes: frequencies, odds ratios, and 95% confidence intervals.

<table>
<thead>
<tr>
<th>Gene: haplotype</th>
<th>Case/ctrl</th>
<th>OR (95% CI)</th>
<th>p-Value</th>
<th>Case/ctrl</th>
<th>OR (95% CI)</th>
<th>Case/ctrl</th>
<th>OR (95% CI)</th>
<th>Case/ctrl</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TF, transferrin: rs1880669, rs1049296</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GC</td>
<td>56.0/55.1</td>
<td>1.00 (Reference)</td>
<td></td>
<td>60.3/55.9</td>
<td>1.00 (Reference)</td>
<td></td>
<td>53.5/54.4</td>
<td>1.00 (Reference)</td>
<td></td>
</tr>
<tr>
<td>AT</td>
<td>16.2/18.7</td>
<td>0.83 (0.71, 0.96)</td>
<td>0.01</td>
<td>15.0/16.6</td>
<td>0.82 (0.61, 1.10)</td>
<td></td>
<td>16.7/19.4</td>
<td>0.85 (0.68, 1.05)</td>
<td></td>
</tr>
<tr>
<td>TF receptor 2: rs10247962, rs4434553</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AG</td>
<td>48.9/48.3</td>
<td>1.00 (Reference)</td>
<td></td>
<td>47.7/43.2</td>
<td>1.00 (Reference)</td>
<td></td>
<td>48.2/50.6</td>
<td>1.00 (Reference)</td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>36.9/36.0</td>
<td>0.99 (0.87, 1.12)</td>
<td>0.84</td>
<td>37.9/40.0</td>
<td>0.88 (0.69, 1.10)</td>
<td></td>
<td>37.6/34.2</td>
<td>1.15 (0.96, 1.37)</td>
<td></td>
</tr>
<tr>
<td>GA</td>
<td>14.2/15.7</td>
<td>0.87 (0.73, 1.02)</td>
<td>0.09</td>
<td>14.4/16.8</td>
<td>0.75 (0.55, 1.03)</td>
<td></td>
<td>14.2/15.2</td>
<td>1.00 (0.78, 1.27)</td>
<td></td>
</tr>
<tr>
<td>HFE, hemochromatosis: rs1572982, rs17596719, rs198855</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>GGT</td>
<td>55.1/52.2</td>
<td>1.00 (Reference)</td>
<td></td>
<td>53.7/51.4</td>
<td>1.00 (Reference)</td>
<td></td>
<td>56.1/55.8</td>
<td>1.00 (Reference)</td>
<td></td>
</tr>
<tr>
<td>AGT</td>
<td>17.9/18.3</td>
<td>0.92 (0.79, 1.07)</td>
<td>0.29</td>
<td>18.0/22.8</td>
<td>0.74 (0.56, 0.98)</td>
<td></td>
<td>19.0/16.3</td>
<td>1.18 (0.94, 1.47)</td>
<td></td>
</tr>
<tr>
<td>AGA</td>
<td>15.2/17.7</td>
<td>0.91 (0.78, 1.06)</td>
<td>0.24</td>
<td>14.8/15.1</td>
<td>0.94 (0.69, 1.28)</td>
<td></td>
<td>13.5/15.3</td>
<td>0.88 (0.69, 1.11)</td>
<td></td>
</tr>
<tr>
<td>AAA</td>
<td>11.8/11.9</td>
<td>0.95 (0.80, 1.14)</td>
<td>0.38</td>
<td>13.4/10.7</td>
<td>1.16 (0.83, 1.62)</td>
<td></td>
<td>11.4/12.6</td>
<td>0.93 (0.72, 1.20)</td>
<td></td>
</tr>
<tr>
<td>CYB561, cytochrome b-561: rs2015356, rs4968647</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CT</td>
<td>49.3/56.2</td>
<td>1.00 (Reference)</td>
<td></td>
<td>48.4/47.5</td>
<td>1.00 (Reference)</td>
<td></td>
<td>49.4/51.5</td>
<td>1.00 (Reference)</td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>27.1/27.9</td>
<td>0.99 (0.87, 1.13)</td>
<td>0.91</td>
<td>25.4/29.7</td>
<td>0.83 (0.64, 1.06)</td>
<td></td>
<td>27.3/25.1</td>
<td>1.11 (0.91, 1.35)</td>
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<tr>
<td>TC</td>
<td>23.6/21.8</td>
<td>1.08 (0.94, 1.24)</td>
<td>0.29</td>
<td>26.2/22.7</td>
<td>1.14 (0.88, 1.48)</td>
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<td>23.3/23.2</td>
<td>1.06 (0.87, 1.30)</td>
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<td>SLC40A1, solute carrier family 40, member 1: rs7596205, rs4145237</td>
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</tr>
<tr>
<td>GG</td>
<td>63.3/63.2</td>
<td>1.00 (Reference)</td>
<td></td>
<td>63.6/66.3</td>
<td>1.00 (Reference)</td>
<td></td>
<td>62.3/60.0</td>
<td>1.00 (Reference)</td>
<td></td>
</tr>
<tr>
<td>GC</td>
<td>27.5/27.3</td>
<td>0.99 (0.87, 1.12)</td>
<td>0.85</td>
<td>27.7/27.7</td>
<td>1.05 (0.83, 1.33)</td>
<td></td>
<td>27.3/28.7</td>
<td>0.89 (0.74, 1.07)</td>
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<tr>
<td>AC</td>
<td>10.2/9.5</td>
<td>1.10 (0.91, 1.33)</td>
<td>0.33</td>
<td>8.7/6.0</td>
<td>1.53 (1.01, 2.32)</td>
<td></td>
<td>10.4/11.2</td>
<td>0.88 (0.68, 1.15)</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: ctrl, control; OR, odds ratio; CI, 95% confidence interval.

- a Pooled odds ratios adjusted for age (continuous), sex (male/female), race (Caucasian/non-Caucasian), and study.
- b US study odds ratios adjusted for age (continuous), sex (male/female), and race (Caucasian/non-Caucasian).
- c AU study odds ratios adjusted for age (continuous) and sex (male/female).
- d SNPs that compose the haplotypes are listed in order below the gene name.
- e Case and control percentages may not add to 100 due to very rare haplotypes not analyzed or missing genotype data.
Analysis accounting for the posterior probability of the inferred haplotypes resulted in nearly identical effect estimates and confidence intervals in the pooled sample (Supplementary Table 4).

Discussion

Based on the well-documented accumulation of iron in the substantia nigra (SN) of Parkinson’s disease (PD) brains we pursued a hypothesis generating effort investigating genetic variants of iron-related genes for association with PD. Our results are intriguing and suggest of a role for genetic variants in transferrin and transferrin receptor 2 in the etiology or pathology of PD. While these findings must be considered exploratory, we observed a 15–20% decrease in risk of PD for subjects with genetic variation in the TF gene suggesting that if variation in the TF gene impacts iron import, storage, transport, or export in dopaminergic neurons then iron accumulation in PD brains may be a cause, not a consequence of neuronal cell death.

The functional consequences of any causal variant(s) captured by our haplotype block within the TF gene (a 46 kb length including exons 9–17 and the 3’ UTR) are unknown. Genetic variants in the promoter and G277S (rs1799899) in exon 7 have been investigated for association with iron-related measures with equivocal results (Aisen, 2003; Delanghe et al., 2006; Lee et al., 2001; Sarria et al., 2007). In contrast, recent GWAS findings consistently report an increase in serum transferrin levels (or total iron binding capacity (TIBC), a proxy for iron transport in to SNpc DA neurons) associated with the minor allele of rs3811647 in intron 11 of TF. This association appears to be independent of race/ethnicity, gender, menstruation status, or diet (Benyamin et al., 2009a, 2009b; Blanco-Rojo et al., 2011; McLaren et al., 2011, 2012; Traglia et al., 2011). Benyamin et al. (2009a) and Blanco-Rojo (2011) report borderline associations between rs3811647 and transferrin saturation, and none of the studies observed an association between rs3811647 and serum iron or serum ferritin. The association between TF rs3811647 and serum transferrin, but lack of association with other measures of iron status, suggests a role for TF in serum transferrin levels independent of total body iron metabolism (McLaren et al., 2011).

We observed no association between genetic variation in the HFE gene and PD in our pooled analysis. Considering the C282Y (rs1800562) variant alone, the US study observed no association (OR = 0.91, 95%CI: 0.59–1.41) and the FR Study has not published on this variant. The AU Study had previously published an association between C282Y and PD (Buchanan et al., 2002) but this was on an early, smaller set of subjects. The C282Y variant of HFE is associated with serum transferrin levels (Benyamin et al., 2009a, 2009b; Blanco-Rojo et al., 2011; McLaren et al., 2011; Traglia et al., 2011), and associated with transferrin saturation and serum iron levels in some studies (Benyamin et al., 2009b; Traglia et al., 2011) but not others (Blanco-Rojo et al., 2011; McLaren et al., 2011). The H63D (rs1799945) variant, which we were unable to assess in our study populations and is not investigated in most of the GWAS, may (Blanco-Rojo et al., 2011) or may not (Whitfield et al., 2000) be associated with serum transferrin levels, but is associated with serum iron and transferrin saturation in the one study reporting on that variant (Whitfield et al., 2000). Our lack of observed associations between PD and genetic variation in hemochromatosis (HFE) or hepcidin (HAMP), both proteins with recognized roles in systemic iron regulation (Benyamin et al., 2009a; Drakesmith and Prentice, 2012; Ezquerra et al., 2005), support a hypothesis that the pathologic role of iron in PD could be unrelated to systemic iron homeostasis.

The TF haplotype associated with a decreased risk of PD in this pooled analysis does not contain the minor (A) allele of rs3811647 observed to be associated with increased serum transferrin/TIBC levels in GWAS; our associated haplotype contains the major allele (G) at this locus. This could suggest that the protection against PD conferred by the AT haplotype of rs1800669-rs1049296 is related to non-elevated serum transferrin/TIBC levels, an unlikely hypothesis given that PD affects fewer than 1% of the population and normal serum transferrin/TIBC levels are observed in the vast majority of the general population. Alternatively, our protective TF haplotype could be influencing PD etiology through alterations in regulation, structural changes in the protein, modifications to the iron binding affinity of the transferrin protein, or biologic interactions of the transferrin protein with other cellular proteins.

The transferrin protein is a glycoprotein with homologous carboxyl and amino domains, each binding one ion of ferric iron (MacGillivray et al., 1982) that transports iron through the extracellular environment. Transferrin is the primary receptor-mediated transporter of iron across the blood brain barrier (Moos et al., 2007) and a transporter of iron throughout the brain. Oligodendrocytes are the primary source of transferrin protein for the brain (Rouault et al., 2009; Zecca et al., 2004) and the location of most brain iron due to its use in myelination (Todoric et al., 2009), but iron has been detected in dopaminergic neurons and even co-localizes with dopamine in vesicles (Ortega et al., 2007). Transferrin receptor, the primary binding site for transferrin and facilitator of iron transport into most cells, is found at very low concentrations in the substantia nigra pars compacta (SNpc) and on SN dopaminergic (DA) neurons (Fauchex et al., 1993; Mastroberardino et al., 2009; Morris and Edvardson, 1994) suggesting an alternative method of iron import into dopaminergic neurons, which may further suggest that our association between PD and TF is due to actions of transferrin unrelated to the transport of iron into dopaminergic neurons.

We observed a suggestive association between PD and transferrin receptor 2 (TFR2), a protein that has been detected on SNpc DA neurons, but not on SN pars reticulata neurons or astrocytes and microglia in the SN brain region (Mastroberardino et al., 2009). This suggests that iron transport in to SNpc DA neurons might be mediated through a TF-TFR2 complex. Furthermore, TFR2 proteins have been detected on mitochondria of DA neurons implying a possible link between iron accumulation, oxidative stress, and mitochondrial dysfunction.

This TF-TFR2 mediated mitochondrial mechanism of pathology is likely to be only one of a number of factors contributing to PD pathology. Given that iron excess (or iron depletion) in model systems aggravates (or attenuates) pesticide toxicity (e.g., Kaur et al., 2003; Peng et al., 2007), it is possible that genetic susceptibility to iron mishandling combined with pesticide exposure contributes to the etiology of PD, for example through increased oxidative stress and mitochondrial dysfunction. Of note, two of the three studies in this report were designed to investigate the contributions of pesticides to PD and therefore enrolled subjects with a higher likelihood of pesticide exposure than many other studies of PD. Our heightened level of pesticide exposure might explain why prior studies of PD and TF genetic variation, particularly the GWAS studies of PD, do not detect associations. The lack of pesticide exposure in the AU study might also explain why the TFR2 finding is closer to null in that study population.

Mitochondrial dysfunction and oxidative stress are two highly interrelated pathogenic hypotheses implicated in PD etiology. Our epidemiologic research and the basic science research of others (e.g., Kaur et al., 2007; Mastroberardino et al., 2009)) suggest that iron accumulation may also be a pathogenic mechanism pertinent to PD etiology. In addition to the role iron might have in mitochondrial dysfunction and oxidative stress, iron may aggravate inhibition of the ubiquitin–proteasome system (Zhang et al., 2005) and may contribute to alpha-synuclein aggregation (Uversky et al., 2001) or overexpression (Febraro et al., 2012). The toxicity of paraquat, a pesticide that has been associated with PD (Costello et al., 2009), is increased by excess iron (Andersen, 2003) and iron chelation can mediate toxin induced cell death (Jimenez-Del-Rio et al., 2010; Kaur et al., 2003).

PD is recognized as a disease of complex etiology (Vance et al., 2010) and our findings lend credence to the continued investigation of iron accumulation as a causal contributor to PD. In particular, our findings of an association between PD and transferrin genetic variation, as well as the suggestive association with transferrin receptor 2 genetic variation,
encourage further elucidation of iron transport mechanisms in dopaminergic neurons and their mitochondria.

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Appendix A. Supplementary data

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References


