Pooled Analysis of Iron-related Genes in Parkinson's Disease: Association with Transferrin

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Abstract

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 five 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: 7036). 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.

Keywords

Epidemiology; genetics; pooled-analysis; iron homeostasis; transferrin; transferrin receptor 2

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 postmortem brain tissue have found increased levels of iron deposits in the substantia nigra (SN) of parkinsonian brains (Dexter et al., 1987, Sofic et al., 1991) and specifically in neurons of the SN (Oakley et al., 2007). These pathologic findings are further supported by magnetic resonance imaging (Gorell et al., 1995, Bartzokis et al., 1999, Michaeli et al., 2007, Martin et al., 2008) and transcranial ultrasound (Becker et al., 1995, Walter et al., 2002, Mehnert et al., 2010) 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 (Moos et al., 1998, LaVaute et al., 2001) than other cell types in the brain. Iron is also hypothesized to aggravate some key pathogenic processes related to PD including alphasynuclein fibril formation (Uversky et al., 2001, Olivares et al., 2009) and mitochondrial dysfunction (Lin et al., 2001, Horowitz and Greenamyre, 2010). 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, Patel et al., 2002, Moos and Morgan, 2004). Iron administration to unaltered animals has resulted in brain iron accumulation (Sengstock et al., 1993) or iron accumulation with a decrease in dopamine levels (Wesemann et al., 1994, Sziraki et al., 1998). 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 cells 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 predominantly 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 ironrelated 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 disorders 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 (Elbaz et al., 2004, Elbaz et al., 2009, Dutheil et al., 2010). 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 were 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 Haploview (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.

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 and rs7786877 in *TFR2*, rs11709714 in *CP*, rs1535921 in *HFE2*, and rs4428180 in *TF* failed genotyping and rs2858996 in *HFE* was homozygous in the US Study population. Therefore, we evaluated 84 SNPs across 15 of 16 genes (only one SNP was genotyped in *HFE2* and that SNP failed) in this first phase. We limited the first phase analyses to those subjects with a genotyping success rate of at least 90% resulting in 347 cases and 360 controls contributing to this investigation.

Phase 2, AU and FR Studies—We genotyped 20 SNPs in 1035 cases and 774 controls from the AU Study and genotyped 17 SNPs in 209 cases and 501 controls from the FR Study selected based on results from phase 1. Discrepancies in the number of tagSNPs

genotyped were due to budget limitations requiring priority given to different candidate genes by the respective Principle Investigators. Genotyping was performed using the Sequenom iPLEX platform (AU Study) and the Illumina Goldengate assay (FR Study). We limited the second phase analyses to those subjects with a genotyping success rate of at least 96% resulting in 739 cases and 538 controls from the AU Study and 203 cases and 493 controls from the FR Study contributing to this investigation.

Statistical Methods

The 84 SNPs genotyped in the US study that passed quality assessment were screened for differences in allele frequency between case and controls. These 84 SNPs within 15 genes captured the genetic variation across 31 regions or haplotype blocks (Daly et al., 2001, Wall and Pritchard, 2003, Crawford and Nickerson, 2005). The number of regions exceeds the number of genes because many genes contained more than one region of high linkage disequilibrium; for example, *transferrin* (*TF*) contains three regions. The regions that contained one or more SNPs with a screening p-value less than 0.20 were further assessed in the US study using haplotypes constructed in PLINK (Purcell et al., 2007). We then evaluated association with PD for these haplotype block regions using an unconditional logistic regression model adjusted for age (continuous), sex (male/female), and race (CA/non-CA) for association with PD. Finally, the block regions that contained at least one haplotype with a p-value less than 0.15 were considered for investigation in all three studies combined. The pooled analysis was performed using unconditional logistic regression adjusted for age (continuous), sex (male/female), race (Caucasian/non-Caucasian), and study (US/AU/FR). Two sensitivity analyses were performed. First, the pooled analysis was performed excluding non-Caucasian subjects from the US Study. Second, the pooled analysis was performed using the haplo.stats R package (version 1.5.0) which implements probability weighting to account for ambiguity in the inferred haplotype (Schaid et al., 2002, Sinnwell and Schaid, 2011).

RESULTS

Phase 1, Screening in US Study

From the 84 SNPs screened in the US Study (phase 1; Supplementary Table 2), 12 out of 31 regions (haplotype blocks) contained one or more SNPs with a sufficient screening p-value for consideration in the phase 1 haplotype analysis. These 12 regions were in 10 of the 15 genes evaluated in the US Study: *FRRS1*, *SLC40A1*, *TF* (2 regions), *CP* (2 regions), *HFE*, *TFR2*, *IREB2*, *CYB561*, *FECH*, and *HAMP* (no SNPs in *LTF*, *TFRC*, *FTMT*, *SLC11A2*, and *HEPH* met the screening criterion). In the haplotype analysis of the US Study (Supplementary Table 3), seven of those 12 regions met the p-value criteria for investigation in the combined US/AU/FR sample.

The seven regions, identified by the screening analysis in the US study, were located in seven independent genes and were defined by 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 anlaysis (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. 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 46kb 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 (Lee et al., 2001, Aisen, 2003, Delanghe et al., 2006, 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 serum transferrin) 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, Benyamin et al., 2009b, Blanco-Rojo et al., 2011, McLaren et al., 2011, Traglia et al., 2011, McLaren et al., 2012). 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, Benyamin et al., 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 (Ezquerra et al., 2005, Benyamin et al., 2009a, Drakesmith and Prentice, 2012), 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 rs1880669-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 (Zecca et al., 2004, Rouault et al., 2009) and the location of most brain iron due to its use in mylenation (Todorich 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 (Faucheux et al., 1993, Morris and Edwardson, 1994, Mastroberardino et al., 2009) 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 models 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 (Febbraro 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 (Kaur et al., 2003, Jimenez-Del-Rio et al., 2010).

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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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HIGHLIGHTS

- 16 iron homeostasis genes were considered for association with Parkinson's disease

- Data from 3 case-control studies (United States, Australia, and France) were pooled

- 3' region of transferrin (TF) was associated with PD (OR=0.83 [0.71–0.96])

- Transferrin receptor 2 (TFR2) genetic variation may also be associated with PD

- TF-TFR2 findings suggest roles for iron misregulation or mitochondrial dysfunction

Table 1

Iron-related Genes Investigated

Gene id	Location	Symbol	Gene Name
391059	1p21.2	FRRS1	ferric-chelate reductase 1
			function: ferric reductase
148738	1q21.1	HFE2	hemochromatosis type 2
			function: hepcidin regulation
30061	2q32	SLC40A1	solute carrier family 40, member 1
			function: iron export from cells
4057	3p21.31	LTF	lactotransferrin
			function: iron binding and transport
7018	3q22.1	TF	transferrin
			function: iron binding and transport
1356	3q23-q25	CP	ceruloplasmin
			function: peroxidation of Fe^{2+}transferrin to Fe^{3+} transferrin
7037	3q29	TFRC	transferrin receptor
			function: transferrin endocytosis and recycling
94033	5q21.3	FTMT	mitochondrial ferritin
			function: iron binding and oxidation
3077	6p21.3	HFE	hemochromatosis
			function: regulate iron absorption
7036	7q22	TFR2	transferrin receptor 2
			function: mediates cellular uptake of transferrin-bound iron
4891	12q13	SLC11A2	solute carrier family 11, member 2
			function: iron absorption and transport of divalent metals
3658	15q25.1	IREB2	iron-responsive element binding protein 2
			function: protein regulation via iron-responsive elements
1534	17q23.3	CYB561	cytochrome b561
			function: ferric reductase
2235	18q21.3	FECH	ferrochelatase
			function: catalyze the insertion of iron into heme
57817	19q13.1	HAMP	hepcidin antimicrobial peptide
			function: regulation of iron storage
9843	Xq11-q12	HEPH	hephaestin
			function: iron transport

Table 2

Pooled analysis and study-specific of iron gene haplotypes: frequencies, odds ratios, and 95% confidence intervals

Gene: Haplotype[4]	Pooled Sample[1] (1286 cases, 1391 ctrls)			US study[2] (347 cases, 360 ctrls)		AU study[3] (739 cases, 538 ctrls)		FRs study[3] (203 cases, 493 ctrls)	
	case/ct rf[5]	OR (95% CI)	p-value	case/ct rf[5]	OR (95% CI)	case/ct rf[5]	OR (95% CI)	case/ct rf[5]	OR (95% CI)
TF, Transferrin: rs1880669, rs1049296									
GC	56.0/55.1	1.00 (reference)		60.3/55.9	1.00 (reference)	53.5/54.4	1.00 (reference)	57.9/55.4	1.00 (reference)
AC	27.7/26.2	1.00(0.88,1.14)	0.97	24.6/27.3	0.83(0.64,1.06)	29.8/26.2	1.1(0.91,1.33)	25.6/25.4	0.92(0.69,1.22)
AT	16.2/18.7	0.83(0.71,0.96)	0.01	15.0/16.6	0.82(0.61,1.10)	16.7/19.4	0.85(0.68,1.05)	16.5/19.3	0.83(0.60,1.15)
TFR2, Transferrin Receptor 2: rs 10247962, rs4434553									
AG	48.9/48.3	1.00(reference)		47.7/43.2	1.00(reference)	48.2/50.6	1.00(reference)	53.4/49.5	1.00(reference)
AA	36.9/36.0	0.99(0.87,1.12)	0.84	37.9/40.0	0.88(0.69,1.10)	37.6/34.2	1.15(0.96,1.37)	32.8/35.1	0.87(0.67,1.14)
GA	14.2/15.7	0.87(0.73,1.02)	0.09	14.4/16.8	0.75(0.55,1.03)	14.2/15.2	1.00(0.78,1.27)	13.8/15.3	0.88(0.62,1.26)
HFE, Hemochromatosis: rs1572982, rs17596719, rs198855									
GGT	55.1/52.2	1.00(reference)		53.7/51.4	1.00(reference)	56.1/55.8	1.00(reference)	53.4/48.8	1.00(reference)
AGT	17.9/18.3	0.92(0.79,1.07)	0.29	18.0/22.8	0.74(0.56,0.98)	19.0/16.3	1.18(0.94,1.47)	14.0/17.2	0.81(0.57,1.15)
AGA	15.2/17.7	0.91(0.78,1.06)	0.24	14.8/15.1	0.94(0.69,1.28)	13.5/15.3	0.88(0.69,1.11)	21.9/22.1	0.92(0.68,1.25)
AAA	11.8/11.9	0.95(0.80,1.14)	0.58	13.4/10.7	1.16(0.83,1.62)	11.4/12.6	0.93(0.72,1.20)	10.6/11.9	0.80(0.54,1.18)
CYB561, Cytochrome b-561: rs2015356, rs4968647									
CT	49.3/50.2	1.00(reference)		48.4/47.5	1.00(reference)	49.4/51.5	1.00(reference)	50.5/50.8	1.00(reference)
CC	27.1/27.9	0.99(0.87,1.13)	0.91	25.4/29.7	0.83(0.64,1.06)	27.3/25.1	1.11(0.91,1.35)	29.3/29.6	1.04(0.79,1.37)
TC	23.6/21.8	1.08(0.94,1.24)	0.29	26.2/22.7	1.14(0.88,1.48)	23.3/23.2	1.06(0.87,1.30)	20.2/19.5	1.10(0.81,1.51)

SLC40A1, Solute carrier family 40, member 1:

rs7596205, rs4145237

Gene: Haplotype[4]	Pooled Sample[1] (1286 cases, 1391 ctrls)			US study[2] (347 cases, 360 ctrls)		AU study[3] (739 cases, 538 ctrls)		FRs study[3] (203 cases, 493 ctrls)	
	case/ct rl[5]	OR (95% CI)	p-value	case/ct rl[5]	OR (95% CI)	case/ct rl[5]	OR (95% CI)	case/ct rl[5]	OR (95% CI)
GG	62.3/63.2	1.00(reference)		63.6/66.3	1.00(reference)	62.3/60.0	1.00(reference)	60.1/64.4	1.00(reference)
GC	27.5/27.3	0.99(0.87,1.12)	0.85	27.7/27.7	1.05(0.83,1.33)	27.3/28.7	0.89(0.74,1.07)	27.8/25.4	1.18(0.90,1.55)
AC	10.2/9.5	1.10(0.91,1.33)	0.33	8.7/6.0	1.53(1.01,2.32)	10.4/11.2	0.88(0.68,1.15)	12.1/10.2	1.26(0.86,1.84)

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

[1] Pooled odds ratios adjusted for age (continuous), sex (male/female), race (Caucasian/non-Caucasian), and study.

[2] US study odd ratios adjusted for age (continuous), sex (male/female), and race (Caucasian/non-Caucasian).

[3] AU and FR study odds ratios adjusted for age (continuous) and sex (male/female).

[4] SNPs that compose the haplotypes are listed in order below the gene name.

[5] Case and control percentages may not add to 100 due to very rare haplotypes not analyzed or missing genotype data.