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Genetics of iron regulation and the possible role of iron in Parkinson's disease

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Abstract

Parkinson's disease (PD) is acknowledged as the second most common neurodegenerative disorder after Alzheimer's Disease. Older age may be the only unequivocal risk factor for PD although the male to female ratio is consistently greater than 1 in populations of European ancestry. Characteristic features of PD include dopaminergic neuron death in the substantia nigra (SN) pars compacta, accumulation of α -synuclein inclusions known as Lewy bodies in the SN, and brain iron accumulation beyond that observed in non-PD brains of a similar age. In this review article, we will provide an overview of human and animal studies investigating the contributions of iron in PD, a summary of human studies of iron-related genes in PD, a review of the literature on the genetics of iron metabolism, and some hypotheses on possible roles for iron in the pathogenic processes of PD including potential interactions between iron and other factors associated with Parkinson's disease.

Keywords

Parkinson's disease; Iron regulatory proteins; Genetics; Pathogenesis

Introduction

Parkinson's disease (PD) is generally acknowledged as the second most common neurodegenerative disorder after Alzheimer's disease. Older age may be the only unequivocal risk factor for PD (Tanner and Goldman, 1996) and the male to female ratio appears to be greater than 1 in populations of European ancestry (de Lau and Breteler, 2006). The most consistently observed environmental factors associated with decreased risk of PD are tobacco smoking and coffee/caffeine intake, while rural living, well-water use, and pesticide exposures possibly contribute to an increase in risk. Characteristic features of PD include death of dopaminergic neurons (DA) of the substantia nigra (SN) pars compacta (Forno, 1996), accumulation of α -synuclein inclusions known as Lewy neurites and Lewy bodies in the SN (Spillantini et al., 1997), and brain iron accumulation beyond that observed in non-PD brains of a similar age (Sofic et al., 1988). Both postmortem and imaging studies have described increased levels of iron deposits in relevant brain regions, particularly in the substantia nigra (Dexter et al., 1987) and globus pallidus (Loeffler et al., 1995). Non-

invasive imaging studies of PD patients not only confirmed increased iron deposits in the SN but also linked the extent of deposits to the severity of disease (Gorell et al., 1995). For these and other reasons, exposure to iron through diet, occupation, or other means has been investigated for its association with PD.

In the following pages, we will provide a brief overview of human and animal studies that have investigated iron's possible role in Parkinson's disease and of human studies investigating iron-related genes for associations with PD. Then we will review the literature on the genetics of iron metabolism and homeostasis, beginning with those protein products found to participate in systemic iron regulation, storage, and transport; followed by those identified or thought to participate in neuronal iron metabolism. Finally, we will summarize prior work that suggests a number of possible roles for iron in the pathogenic processes of PD, and potential interactions between iron and other risk or protective factors associated with Parkinson's disease.

Human studies of iron and PD

Iron concentrations in the brain change over a lifetime: they are highest at birth, decrease during the first two postnatal weeks, then increase again until death (Hallgren and Sourander, 1958; Höck et al., 1975; Aoki et al., 1989; Connor, 1994). Despite the natural increase in brain iron concentrations, reports have regularly described PD brains as having extensive iron deposits, beyond that of non-PD brains of a similar age. A number of postmortem studies (reviewed by Gotz et al., 2004) histochemically showed increased levels of iron deposits in the substantia nigra and globus pallidus in parkinsonian compared with control brains. Imaging studies of living PD patients have confirmed the presence of increased iron deposits in the SN (Gorell et al., 1995; Michaeli et al., 2007; Martin et al., 2008) and have linked the extent of deposits to the severity of disease (Ryvlin et al., 1995; Ye et al., 1996). More recent studies using transcranial ultrasound identified increased echogenicity in the SN of PD patients (Berg et al., 2001; Walter et al., 2002; Berg, 2007) which appears to be associated with increased iron and decreased neuromelanin content (Zecca et al., 2005).

Given the observation that males are at an approximately 50% increased risk of developing Parkinson's disease compared to females (e.g. Elbaz et al., 2002; meta-analysis in Wooten et al., 2004), a hypothesis can be constructed that menstruation is protective for women because it might decrease their body's iron. Investigations of parity, age at menarche, age at menopause, and other indicators of duration of fertile life have been inconclusive but suggest that a shorter fertile life, i.e. fewer menses or greater lifetime exposure to iron, is accompanied by an increased risk of PD (Benedetti et al., 2001; Ragonese et al., 2004; Popat et al., 2005; Haaxma et al., 2007) and/or later onset (Ragonese et al., 2006a; Haaxma et al., 2007). This observation is complicated by the possibly neuroprotective effects of estrogens (reviewed in Ragonese et al., 2006b).

The small number of epidemiologic studies investigating associations between occupational or dietary iron exposure and Parkinson's disease have been inconclusive. Studies of occupational metal exposure typically reported odds ratios greater than 2.0; however, these studies were largely uninformative with wide confidence intervals that frequently included the null (reviewed in Lai et al., 2002). Recently, the Geoparkinson Study (Dick et al., 2007) calculated an odds ratio (OR) of 1.07 (95% CI: 0.82–1.40) for iron exposure and Parkinson's disease; i.e. found no evidence for an association, even when they evaluated high levels of occupational (and hobby-related) iron exposures. Furthermore, occupational exposure to iron has mostly occurred in conjunction with co-exposures to other metals such as lead, copper, or manganese (Zayed et al., 1990; Gorell et al., 1997; Gorell et al., 2004) making it

hard to distinguish independent iron effects from those possibly due to other metals or the combination.

In epidemiologic studies relying on recall of diet patterns to assess past exposure to dietary iron, one report (Anderson et al., 1999) found no association (OR=0.71, 95%CI: 0.34–1.48); whereas two other studies reported possible increased risks of PD with increasing levels of dietary iron — Johnson et al. (1999): 1st quartile of intake vs. 4th quartile of intake OR=1.88, 95%CI: 1.05–3.38; Powers et al. (2003): 1st quartile of intake vs. 4th quartile of intake OR=1.7 95%CI: 1.0–2.7. One study (Logroscino et al., 1997) identified lower levels of circulating iron and ferritin in patients compared to controls; a finding associated with decreased iron stores and normally accompanied by increased total iron binding capacity (TIBC), increased transferrin levels, and increased transferrin receptor levels. In this study, however, the PD cases had lower TIBC and transferrin concentrations compared to normal controls, possibly indicating abnormal regulation of iron or iron-related proteins.

Iron in animal models of neurodegeneration

Animal models have been developed that mimic one or more of the pathologic components of PD, such as DA cell death, disturbed motor function, or increased oxidative stress. These models and the findings associated with them have been criticized, and therefore considered with caution when extrapolating to humans. Regardless, animal models are a valid and relevant access point to biological processes unobservable at the same level of detail in humans.

A number of studies have detected reduction in striatal (Ben-Shachar and Youdim, 1991; Sengstock et al., 1993; Wesemann et al., 1994) or caudate nucleus (Sziraki et al., 1998) dopamine levels in rats with increased brain iron concentrations resulting from injected or infused iron compounds. Additionally, excess brain iron resulting from either infusion (Sziraki et al., 1998) or a high iron diet (Lan and Jiang, 1997) has been correlated with elevated measures of oxidative stress in mouse models; although the latter (Lan and Jiang, 1997) did not detect any changes in dopamine concentration with the high iron diet. In contrast, another study (Levenson et al., 2004) observed a 35% decrease in striatal dopamine and impaired motor function in mice fed a low iron (4 parts per million, ppm) diet compared to mice fed an adequate diet (48 ppm). Iron chelation experiments have also provided some insight into the role of iron in degeneration of dopaminergic neurons. The iron chelator desferrioxamine has been shown to protect against oxidative stress induced by 6-hydroxydopamine and against dopamine depletion, and prevent impaired motor function in treated animals (Ben-Shachar and Youdim, 1991; Youdim et al., 2004).

Fredriksson et al. (1999) demonstrated that iron feeding in murine neonates resulted in motor dysfunctions in the adult animals and a higher basal iron content in the SN at 3–4 months. In a similar model, Kaur et al. (2007), observed the increased SN iron concentration at 3 months, as well as elevated markers of oxidative stress and decreased striatum dopamine content at 12, 16, and 24 months, but not at 2 months, when compared to saline fed animals. They also observed a significant decrease in TH+ dopaminergic neurons in the iron-fed compared to saline fed mice at 24 months, but not at 2, 12 or 16 months. These studies suggest that elevated iron concentrations alone, given enough time, might cause some pathologic features of PD.

In Peng et al. (2007), the introduction of a toxin, in this case the pesticide paraquat, into adult animal produced significant decreases in TH+ DA neurons compared to control animals within 7 days of toxin administration. Furthermore, this is observed regardless of age (2, 6, 12, or 24 months). Yet, the decrease in neurons was significantly greater at 24 months in those animals fed iron as neonates and then treated with paraquat compared to

either those animals fed iron and untreated with paraquat or those animals on a normal diet and treated with paraquat. This and other studies (Ben-Shachar et al., 1991; Glinka et al., 1996; Fredriksson et al., 2001; Kaur et al., 2003) support the hypothesis that iron accumulation is an event that precedes neurodegeneration and may increase vulnerability to later toxic exposures.

On the whole, animal model studies thus suggest a role for iron in the biology of neurodegeneration.

Human studies of iron-associated genes and PD

Fourteen studies (Table 1) have reported the potential association between Parkinson's disease and various genes involved in iron metabolism or homeostasis. The genes investigated include *ferritin light chain*, *ferritin heavy chain*, *transferrin*, *transferrin receptor*, *iron responsive element binding protein 2*, *lactotransferrin*, *ceruloplasmin*, *frataxin*, and the *hemochromatosis* gene. Six studies (Chen et al., 2002; Lee et al., 2002; Felleitschin et al., 2003; Deplazes et al., 2004/Hochstrasser et al., 2004; Akbas et al., 2006; Foglieni et al., 2007) specifically sequenced the exons of iron-related genes in their PD cases searching for previously unknown mutations and subsequently genotyped controls for any identified variants. The remaining studies tested specific known variants.

Felleitschin et al. (2003) and Foglieni et al. (2007) sequenced the *ferritin light* (*FTL*) and the *ferritin heavy chain* (*FTH1*) genes. Felleitschin reported identifying two silent mutations in *FTL* and one nonsynonymous amino acid change in *FTH1*, but only in a single subject each; Foglieni identified the same three variants — K54R of *FTH1* in 2 control subjects, L55L of *FTL* in 82 cases and 60 controls, and H174H in *FTL* in 1 case. An insertion at positions 460–461 in *FTL* had been previously reported in familial basal ganglia disease, which manifests with clinical symptoms similar to PD (Curtis et al., 2001). This specific variant was thus investigated by Felleitschin et al. and by Chen et al. (2002), but neither found the insertion polymorphism in any study subject, PD patient or control. Foglieni identified one new variation in *FTH1* in 5 cases and 16 controls and two new variations in *FTL*, each in only one case.

Specific variants in *transferrin* (*TF*), *transferrin receptor* (*TFRC*), *lactotransferrin* (*LTF*; also known as *lactoferrin*), and *frataxin* (*FXN*) were investigated by Borie et al. (2002). Only one variant, a nonsynonymous G to A change in exon 7 of *TF* (G258S), appeared to be associated with PD; particularly in the subset of subjects older than 60 years of age. These findings were not replicated in a second study with a different population (Ezquerria et al., 2005).

Both Lee et al. (2002) and Deplazes et al. (2004) sequenced the *iron responsive element binding protein 2* (*IREB2*); Lee et al. sequenced the entire gene and Deplazes et al. sequenced only the promoter and exons. Neither report identified the same set of variants; although this was not surprising as all but four of the nineteen variants identified were found in a single subject only. No variant was associated with PD.

Hochstrasser et al. (2004) sequenced the exons of the *ceruloplasmin* (*CP*) gene and reported six missense variations, one known and five novel. They investigated the association of these variants with PD occurrence and with hyperechogenicity, a feature that has been related to increased iron levels in the SN. Only one variant, rs701753, an A to T change in exon 9 that results in a glutamic acid to aspartic acid amino acid change at position 544, was associated with PD, hyperechogenicity, and by extension possibly increased brain iron levels. The T allele is found at a population frequency of 7.5% in Caucasians, the majority

of which contributes to a heterozygote genotype (86.7% AA, 11.7% AT, 1.7% TT; NCBI dbSNP, accessed Jan. 21, 2008).

Eight studies have investigated the association between variants in the *hemochromatosis* (*HFE*) gene and PD, particularly the variants (C282Y and H63D) associated with familial hemochromatosis disease. Akbas et al. (2006) sequenced *HFE* and identified two novel intragenic mutations but only in a single case. Although the findings from individual studies are somewhat inconsistent, meta-analysis of studies investigating the C282Y variant calculated an odds ratio of 0.85 (95% CI: 0.66–1.09) in Caucasians; whereas the odds ratio for H63D was estimated at 0.99 (95% CI: 0.84–1.16) for all studies (Bagade et al., 2006), i.e. the data available for these meta-analyses argued against a role for these two variants in PD susceptibility.

Although sequencing has the possibility of identifying new mutations, such an approach is likely to only identify very rare variants, e.g. found in only one or two subjects out of 400–600. Additionally, testing only for select known variants, usually identified because of their involvement in other disorders, is likely to overlook other regions of possible variation in the gene. Despite the unimpressive findings of this small number of studies that investigated iron genes in PD case-control studies to date, reasonable biological support for a role for iron in the etiology and/or progression of PD exists. In an effort to more comprehensively delineate the genetics of iron biology, we have reviewed the literature for articles discussing genes involved in systemic and cerebral iron balance.

Genetics of iron metabolism and homeostasis

The majority of iron in the human body is utilized in heme, a component of hemoglobin in erythroid cells and myoglobin in muscle cells. The human body contains 3–5 g of iron, and generally the iron level is lower in women than in men. There are no specific mechanisms for iron secretion from the body; most iron is recycled when erythrocytes are phagocytosed. Iron loss only occurs through bleeding and the sloughing-off of epithelial cells from the gut. This minimal iron loss (less than 0.1% of the total body iron) is replaced by dietary iron which is absorbed in the duodenum from foods such as meat, poultry, and fish (heme and non-heme iron) or legumes, spinach, and fortified cereal products (non-heme iron). Heme iron is more absorbable, although vitamin C can enhance the absorption of non-heme iron. Dietary factors that inhibit the absorption of iron include phytates found in legumes, rice, and whole grains; polyphenols found in the tannins of tea and coffee; vegetable protein; and calcium.

Iron is essential for the adequate development and functioning of the brain (reviewed in Lozoff et al., 2006). Iron is a cofactor in cell proliferation/DNA synthesis, the mitochondrial electron transport chain, and in myelin and neurotransmitter synthesis. Of the cells in the SN and adjacent regions, oligodendrocytes have large stores of iron for myelin synthesis (Connor and Menzies, 1996); astrocytes maintain a lower physiologic level of iron (Hoepken et al., 2004), have considerable unutilized iron storage capacity (Regan et al., 2002; Hoepken et al., 2004), and may provide neuroprotection from oxidative stress (Oshiro et al., 2000; Oshiro et al., 2008); and microglia, which act as the brain's macrophages, have been shown to accumulate iron (Oshiro et al., 2008) and may contribute to protection from neuronal cell death (Toku et al., 1998). Research indicates that neurons, when compared to astrocytes, oligodendrocytes, and microglia, are more sensitive to both iron deficiency (Moos et al., 1998) and excess (Sengstock et al., 1992; LaVaute et al., 2001; Kress et al., 2002).

Nomenclature for the genes and protein products discussed in the following sections is listed in Table 2 and the Supplementary Table available at the website.

Systemic iron

Dietary heme iron is transported from the intestine into enterocytes via the heme carrier protein 1 (HCP1; Shayeghi et al., 2005), encoded by the gene *SLC46A1*. In the enterocytes the heme is disassembled by one of two heme-oxygenases (HMOX1, HMOX2 formerly HO-1, HO-2; Elbirt and Bonkovsky, 1999) into biliverdin, carbon monoxide, and ferrous iron (Fe^{2+}), which becomes part of the intracellular calcein-chelatable iron pool (ICIP). Non-heme or ferric iron (Fe^{3+}) undergoes reduction at the apical surface, probably by duodenal cytochrome b (CYBRD1, formerly DCYTB; McKie et al., 2001), a ferric reductase that localizes to the brush-border membrane of the duodenum; although this is unlikely to be the only reductase acting on non-heme iron. Fe^{2+} is then transported across the apical membrane to the ICIP via the divalent metal transporter 1 (DMT1; Gunshin et al., 1997), a transmembrane protein encoded by the *SLC11A2* gene and capable of transporting a number of transition metals including iron. *SLC11A2* is ubiquitously expressed (though most extensively in the duodenum) and up-regulated under iron deficient conditions (Collins et al., 2005). Studies have suggested that other transporters exist on the placenta (maternal transfer of iron to the fetus), in the liver, and the brain (Gunshin et al., 2005).

Ferrous iron within the ICIP of the enterocytes can be utilized for cellular reactions, stored in ferritin, or transported across the basolateral membrane and into the circulatory system. The cellular component most likely responsible for this export of iron is ferroportin (FPN; McKie et al., 2000), encoded by the *SLC40A1* gene. Once in the portal vein, ferrous iron must be oxidized ($\text{Fe}^{2+} \rightarrow \text{Fe}^{3+}$) for binding with transferrin. There are two known intestinal proteins with ferroxidase activity, ceruloplasmin (CP; Attieh et al., 1999) and hephaestin (HEPH; Griffiths et al., 2005). The ferric iron is then rapidly bound to transferrin (TF) and transported to the liver, erythroid bone marrow, and other sites. Cellular import of transferrin-bound iron occurs most frequently via transferrin receptor mediated endocytosis (Fig. 1; reviewed in Hentze et al., 2004). Briefly, the transferrin-Fe complex binds to the transferrin receptor (TFR) and is endocytosed. The endosome is acidified via a proton pump, which results in release of the transferrin-Fe complex from the transferrin receptor and release of Fe^{3+} from transferrin. Before the iron can be transported out of the endosome via DMT1, it must be reduced from Fe^{3+} to Fe^{2+} , probably by Steap family member 3 (STEAP3; Ohgami et al., 2005) or possibly another ferric reductase such as CYBRD1. Both the apotransferrin and the transferrin receptor are returned to the cell surface and reused. A homologous transferrin receptor, TFR2, is found in hepatocytes, duodenal crypt cells, and erythroid cells; in addition, TFR2 has been identified in dopaminergic neurons and appears to localize to the mitochondria (Mastroberardino et al., in press). TFR2 binds to the transferrin-Fe complex with a 30-fold lower affinity when compared to TFR (Kawabata et al., 2000). Research supports the likelihood of transferrin-independent cellular import mechanisms for iron transport into non-erythroid cells, and some of these are discussed below in relevance to neuronal import.

Regulation of systemic iron balance—Systemic iron balance is achieved predominately through macrophage iron recycling and hepatocyte iron mobilization according to certain stimuli including erythroid iron requirements, oxygen status/hypoxia, iron deficiency or overload, and inflammation. Research indicates that hepcidin (HAMP; Krause et al., 2000), a protein produced predominately by hepatocytes, is at the center of the iron recycling-mobilization balance (reviewed in Ganz, 2005), and that expression of the gene encoding for hepcidin is influenced by anemia, hypoxia, and inflammation (Nicolas et al., 2002a).

In iron-normal cell cultures and murine models, *hepcidin* expression appears to be influenced by a number of proteins from different pathways. Inactivation of the bone

morphogenetic protein (BMP/SMAD) pathway cofactor hemochromatosis type 2 (HFE2, formerly hemojuvelin, HJV) or the BMP/SMAD protein SMAD4 results in decreased *hepcidin* expression; whereas, treatment with BMPs increases *hepcidin* expression (Babitt et al., 2006; Wang et al., 2005). Additionally, mRNA expression appears to be influenced by inflammatory conditions in that interleukin-6 (IL6), an inflammatory cytokine, has been found to induce transcription of *hepcidin* (Nemeth et al., 2004b), probably through binding of signal transducer and activator of transcription 3 (STAT3) protein to the promoter region of *hepcidin* (Wrighting and Andrews, 2006). Furthermore, it appears that TFR2, with expression apparently regulated by TF (Johnson and Enns, 2004), influences *hepcidin* expression independent of the inflammatory pathway (Nemeth et al., 2005; Kawabata et al., 2005). Finally, studies of humans with the genetic disorder hemochromatosis and mouse models mimicking the disorder suggest that many forms of the condition are associated with decreased hepcidin production (Bridle et al., 2003); therefore, the hemochromatosis protein (HFE) is probably also involved in *hepcidin* regulation.

In animal models, researchers have demonstrated that inactivation (Nicolas et al., 2001) or removal of the *hepcidin* gene (Viatte et al., 2005) results in an iron overload phenotype, and over expression of *hepcidin* results in an anemia phenotype (Nicolas et al., 2002b). Furthermore, iron overload appears to induce over-expression of *hepcidin* (Pigeon et al., 2001), and anemia appears to decrease expression (Vokurka et al., 2006). Hecpudin binds to FPN, the cellular iron export protein, which results in the internalization and degradation of ferroportin (Nemeth et al., 2004a) and discontinuation of iron transport from the enterocytes into circulation. In this way, *hepcidin*, which is regulated by iron concentrations, in turn regulates iron absorption by and export from cells.

Regulation of cellular iron balance—Cellular iron balance is accomplished through the interplay of ferritin (FT), the iron storage protein; FPN, the iron export protein; DMT1, the iron transporter; and TFRC, the primary iron import mechanism. (ALAS2, a heme synthase enzyme, is also involved in cellular iron balance but is found almost exclusively in red blood cells.) The mRNA for each of these proteins contains at least one stem-loop structure known as an iron responsive element (IRE) in their untranslated regions (UTR) that can be bound by iron responsive element binding proteins (IREBs or IRPs; Rouault et al., 1990; Rouault et al., 1992) of which there are two, ACO1 (formerly IREB1 or IRP1) and IREB2 (or IRP2). Production and degradation of these proteins are regulated by cellular iron concentrations, though this is better understood for ACO1 than IREB2 (reviewed in Pantopoulos, 2004). The IRE–IRP mechanism (Fig. 2) provides for posttranscriptional iron regulation in response to intracellular iron concentrations. The locations of the IRE on the respective mRNA vary depending upon the regulatory action needed in response to iron concentrations. Both the *ferritin light chain (FTL)* and the *ferritin heavy chain (FTH1)* mRNAs contain a single IRE each in their 5' UTR (Hentze et al., 1987; Rouault et al., 1988). *TFRC* mRNA contains five IREs in the 3' UTR (Koeller et al., 1989). *SLC40A1*, encoding FPN, and *ALAS2* both have IREs 5' (McKie et al., 2000; Dandekar et al., 1991); whereas *SLC11A2*, encoding DMT1, has an IRE 3' (Gunshin et al., 2001).

Under low cellular iron conditions, the IREBs bind to the IREs. In the case of the *TFRC* IREs located in the 3' UTR, the mRNA is stabilized and translation enabled, resulting in increased iron uptake from the circulation in the form of holo-transferrin. As DMT1 is required for transport of iron from the transferrin receptor-containing endosome into the cytosol, it is logical for a similar stabilization and translation to occur given the presence of a 3' UTR IRE in the mRNA of *SLC11A2*. For IREs located in the 5' UTR, as in both *FTL* and *FTH1* mRNAs, the IRE–IRP complex prevents 43S pre-initiation complex binding and hence translation is inhibited. Without additional ferritin molecules the iron imported into the cytosol cannot be stored, thereby increasing the cellular iron concentration. It appears

that the same regulation mechanism acts on *SLC40A1* to prevent production of FPN protein and export of iron from the cell, and on *ALAS2* in red blood cells to prevent heme synthesis. Under high cellular iron conditions, the IREBs/IRPs disassociate from the IREs which allows for *TFRC* and *SLC11A2* degradation and *SLC40A1*, *ALAS2*, and *ferritin* subunit translation, decreasing iron uptake and increasing iron storage, respectively.

Neuronal iron

The iron transport mechanisms across the blood brain barrier (BBB) and into neurons are thought to be similar to, although probably more complex than, those found in other cells (Qian et al., 2002; Fig. 3). The luminal membrane of the BBB endothelial cells is known to contain transferrin receptors (Jefferies et al., 1984) that, when bound to a transferrin-Fe complex, either perform endocytosis (Roberts et al., 1993; Visser et al., 2004) or transcytosis (Fishman et al., 1987; Descamps et al., 1996). Endocytosis, release of iron, and recycling of apo-transferrin and the transferrin receptor are thought to occur through the same steps in the brain endothelial cell as elsewhere (Fig. 1), although this has not been confirmed. Both DMT1 and FPN have been localized to brain endothelial cells (Burdo et al., 2001) and probably participate in the export of iron (Wu et al., 2004) from the endosome (DMT1; Fig. 1) and into the parenchymal space (FPN; Fig. 3), although the function and importance of DMT1 has recently been questioned (Moos et al., 2006). Once in the parenchymal space/brain interstitial fluid, iron is bound to apo-transferrin, present as a large interstitial pool (Visser et al., 2004). The oxidation of Fe^{2+} to Fe^{3+} prior to its binding to apo-transferrin is probably accomplished by ceruloplasmin, which is known to be expressed in the central nervous system (Qian and Wang, 1998), although some other ferroxidase such as hephaestin (Hahn et al., 2004) might be present as well.

A number of methods of non-transferrin mediated transport of iron across the BBB have been documented including lactotransferrin- (Fillebeen et al., 1999), melanotransferrin- (Demeule et al., 2002), and ferritin (Fisher et al., 2007) endo- and/or transcytosis (Fig. 3). Lactotransferrin (LTF) is an iron-containing molecule involved in response to infection and inflammation that is thought to be transported through the endothelial cells bound to an unknown receptor, possibly of the low density lipoprotein receptor-related protein family LDLR; (Fillebeen et al., 1999). Two members of this family, *LRP1* and *LRP2* (Pan et al., 2004), appear to be expressed at the blood brain barrier and their protein products might participate in the transport of lactotransferrin-bound iron. Melanotransferrin (MFI2 or p97) occurs as a membrane-bound, glycosylphosphatidylinositol-anchored molecule in brain capillary endothelium alongside transferrin receptor molecules (Rothenberger et al., 1996) and appears to transcytose the blood brain barrier more rapidly than holo-transferrin (Moroo et al., 2003) or endocytose in a receptor-mediated fashion, possibly using a member of the LRP family as a receptor (Demeule et al., 2002). Subsequent studies, however, indicated that MFI2 may contribute minimally, compared to transferrin, in the provision of iron to the brain (Richardson and Morgan, 2004). Iron bound to H-ferritin, but not α -ferritin has been observed to transcytose in a cell culture model of the blood-brain barrier (Fisher et al., 2007), but the receptor for such action at the BBB is yet unconfirmed; possible receptors have been observed on other organs (Mack et al., 1983; Chen et al., 2005).

Once in the interstitial fluid of the brain, iron could be transported into neurons through many of the same mechanisms that are used outside the brain including transferrin-receptor complex or lactotransferrin-receptor complex (Moos and Morgan, 2004), although many of these mechanisms and the proteins involved have not been explicitly demonstrated in neurons. For example, the ferric reductase used to convert Fe^{3+} released from transferrin to Fe^{2+} might be cytochrome b-561 (CYB561) or ferric-chelate reductase 1 (FRRS1, formerly SDR2), both of which belong to the same family of molecules as CYBRD1 (Ponting, 2001) and have been detected in the choroid plexus and in the ependymal cells (Vargas et al.,

2003). Additionally, a gene of the ubiquitin-conjugating E2 family, *UBE2D1* or *SFT* (*stimulator of Fe transport*), is highly expressed in the corpus callosum and cerebellum tissues (Knutson et al., 2004), and the protein is thought to participate in both transferrin and non-transferrin associated transport of iron into neurons. There are indications that not all cells in the brain (neurons versus oligodendrocytes versus astro- or microglia) use the same mechanisms for iron transport and that iron exists in the interstitial fluid in both the ferrous and ferric (transferrin-bound) states (Ke and Ming Qian, 2003).

Neuronal iron storage—Iron storage in the brain is similar to and different from systemic iron storage in that ferritin is found in the brain and thought to be an important iron storage protein, and yet other iron storage mechanisms exist including neuromelanin. The ferritin complex (Casiday and Frey, 2000) is composed of 24 ferritin light and heavy chain subunits in varying ratios resulting in a hollow ball that can bind up to 4500 hydrated (ferric) iron oxide molecules at a time. *Ferritin* is expressed predominately in oligodendrocytes, astrocytes, and microglia but less in neurons (Connor et al., 1990). The majority of brain iron is stored in ferritin molecules located in the microglia, and only 10–20% of brain iron is stored in the SN (Zecca et al., 2001). Disruptions in the structure of the ferritin complex can result in brain iron accumulation and subsequent increased oxidation and mitochondrial dysfunction as documented for neuroferritinopathy (OMIM #606159), also known as adult-onset basal ganglia disease or neurodegeneration with brain iron accumulation type 2, which appears to be caused by an insertion and subsequently altered reading frame in the *ferritin light chain* gene (Curtis et al., 2001; Chinnery et al., 2003; Burn and Chinnery, 2006).

Iron storage in the neurons of the SN is thought to occur through binding of iron within neuromelanin (Youdim et al., 1994; Zecca et al., 2001), but the physiological function, overall composition, and molecular structure of neuromelanin are still under investigation. Neuromelanin is likely an auto-oxidation product of dopamine (Double et al., 2000), although enzymatic production of neuromelanin has not been ruled out despite the absence from the brain of tyrosinase, the enzyme used for melanin synthesis elsewhere in the body (Fedorow et al., 2005). Also, iron might be involved in the reaction to form neuromelanin from dopamine (Sulzer et al., 2000). A second form of neuromelanin appears to be a by-product of auto-oxidation of noradrenaline and occurs in the locus coeruleus. Some researchers have suggested that neuromelanin might be a polymer constructed from two different dopamine oxidation products that interleave to create interconnected layers of planar sheets (Gaeta and Hider, 2005).

Binding and coordination studies have demonstrated that there are both high and low affinity iron binding sites within the neuromelanin structure (Double et al., 2003) and that under low or normal iron concentrations, only the high affinity sites are utilized. Double et al. (2002) have suggested that iron bound to the lower affinity sites, usually under high iron concentration conditions, may remain redox-active rather than being adequately sequestered. Studies of normal brains suggest that neuromelanin is only 50% saturated with iron (Shima et al., 1997), enabling additional chelating capacity should neuronal iron concentrations increase. In a synthetic model of neuromelanin, Zareba et al. (1995) demonstrated that at low iron concentrations most iron is bound to the melanin polymer and no free radicals are detectable, at intermediate iron concentrations iron was still bound to the polymer but free radicals became detectable, and at high iron concentrations the iron binding capacity of the neuromelanin was exhausted and free radical activity increased proportional to increasing iron concentration. Q-band electron paramagnetic spectroscopic studies (Aime et al., 1997; Lopiano et al., 2000) have indicated that there might be structural differences and differential iron binding abilities in the neuromelanin extracted from midbrains of patients who died of non-neurological diseases compared to neuromelanin extractions from brains of patients affected by Parkinson's disease. It appears that under physiological conditions

neuromelanin may play a protective role and minimize the redox activity of iron in the neuron, whereas at increased iron concentrations neuromelanin might facilitate, or at least no longer prevent, the redox activity of cellular iron.

Biology of iron in pathogenic mechanisms of PD

Iron may play a role in some aspect of many cellular processes hypothesized to be dysfunctional or disturbed in PD; examples include α -synuclein aggregation and Lewy body formation, ubiquitin–proteasome system dysfunction, and oxidative stress damage.

α -synuclein and Lewy bodies

α -synuclein is a 140 amino acid protein that consists of an N-terminal amphipathic region containing six imperfect repeats, a hydrophobic central region, and an acidic C-terminal region. The protein appears to possess considerable conformational plasticity and, depending on the environment, can remain unfolded, form alpha-helices or beta-sheets, or exist as a monomer or oligomer (Uversky, 2003); as well as cross link with itself, other proteins via advanced glycation end-products (Munch et al., 2000), and neuromelanin (Fasano et al., 2003). α -synuclein has been identified as one of the primary components of Lewy bodies (Spillantini et al., 1998) and Lewy neurites (Braak et al., 1999) in PD. Iron and other metals appear to induce α -synuclein fibril formation (Uversky et al., 2001; Golts et al., 2002) and may contribute to or aggravate existing Lewy body aggregation. Furthermore, the 5' UTR of α -synuclein mRNA contains a predicted IRE (Friedlich et al., 2007), which implies that the same proteins that influence iron metabolism might influence α -synuclein concentrations.

Ubiquitin–proteasome system

One of the primary pathways for the degradation of cellular, non-membrane-bound proteins is the ubiquitin–proteasome system (UPS), which functions to maintain appropriate cellular concentration of short-lived regulatory/functional proteins and destroy abnormal, misfolded, damaged, or toxic proteins reviewed in (Betarbet et al., 2005). Defects in, or overload of, the UPS might result in accumulation of toxic proteins and cellular dysfunction or death. This UPS overload or dysfunction appears to occur under experimental conditions of increased oxidative stress and/or mitochondrial complex I inhibition (e.g., the rotenone rat model of PD). Additionally, there is some indication that altered UPS function is at least a component of PD pathogenesis because Lewy bodies and Lewy neurites contain not only α -synuclein aggregates, but also ubiquitin (Forno, 1996), parkin (an E3 ligase; Shimura et al., 1999), torsin A (an ATPase; Shashidharan et al., 2000), and other proteasomal pathway proteins (Ii et al., 1997). A possible role for iron in UPS dysfunction was suggested by the observation that the iron chelator desferrioxamine attenuates drug-induced proteasome inhibition and subsequent dopamine neuron loss and reduces the presence of ubiquitin-positive intracellular inclusions in the SN of mice (Zhang et al., 2005). Additionally, the proteins that regulate iron levels (IREBs) appear to also regulate the mRNA of a 75 kDa mitochondrial complex I protein (Lin et al., 2001) and by extension might influence complex I function, widely acknowledged for its pathogenic role in PD.

Oxidative stress

Hydrogen peroxide (H_2O_2) is a by-product of dopamine oxidation (Graham, 1978). Iron is a very potent oxidation-reduction agent, and in the presence of H_2O_2 , ferrous iron (Fe^{2+}) catalyzes the Fenton reaction to produce reactive oxygen species (ROS) that increase the oxidative stress of the cell (Gerlach et al., 1994). In fact, rat neuron cultures treated with a combination of Fe^{2+} and H_2O_2 demonstrated decreased cell survival and had increased levels of free radicals compared to culture treatment with a control vehicle (Xiong et al.,

2007). Protein damage, misfolding, and aggregation are known to result from the presence of free-radicals (Davies, 1987).

The relationships between ROS and the human brain have been reviewed in detail by numerous specialists in this field including Andersen (2004), Sayre et al. (2005), and Mancuso et al. (2007). Briefly, the brain is thought to be particularly susceptible to free-radial accumulation, mainly ROS, because of its high utilization of oxygen for metabolic processes and its relative paucity of antioxidant and regenerative properties compared to other organs. In addition to the Fenton reaction, ROS arise from any number of normal or dysfunctional cellular mechanisms including auto-oxidation of dopamine, disruption of mitochondrial complex I, inappropriate incorporation of exogenous toxins, inadequate availability of glutathione, or improperly stored or excess concentrations of iron. Ramifications of uncompensated ROS production potentially include damaged nuclear and/or mitochondrial DNA, lipid peroxidation, damaged or misfolded proteins, and aggregated proteins unrecognized by the UPS because of incorrect ubiquitination. Iron might be contributing at many levels to oxidative stress both through direct ROS production and through its roles in α -synuclein aggregation, interference with the function of the UPS system, and other biological mechanisms in the brain.

Iron in combination with other predictors of PD

Tobacco smoking and iron

The association most consistently observed in epidemiologic studies is between PD and tobacco smoking where smoking appears to be a protective factor. Ritz et al. (2007) performed a pooled analysis of patient-level data from eleven studies and found a strong, consistent protective association between PD and smoking, as well as a dose response association for pack-years. Many theories have been proposed to explain this observation including a potential confounding by selective mortality or recall bias, a neuroprotective compound in cigarette smoke/tobacco, or a personality trait, pre-clinical disease, or genetic factor that predisposes subjects to both non-smoking and PD. In addition to smoking (or a compound in cigarettes) possibly acting as a neuroprotective factor, there is some evidence that smoking, or more particularly exposure to nicotine, might have an effect on iron concentrations in the body. Through traditional chemistry techniques, nicotine has been identified as a strong complexing agent for iron (Linert et al., 1996; Bridge et al., 2004) and appears to decrease the formation of 6-hydroxydopamine resulting from Fe^{2+} and H_2O_2 reacting with dopamine, implying that nicotine affects the Fenton reaction (Linert et al., 1999). No studies have identified the specific *in vitro* or *in vivo* mechanisms of nicotine and iron interaction, but the possibility exists that such an interaction may play a role in PD by decreasing the availability of redox-active iron and thereby decreasing oxidative stress in the DA neurons.

Coffee drinking and iron

Apart from smoking, coffee drinking has been studied most frequently in epidemiologic studies. A meta-analysis (Hernan et al., 2002) of thirteen studies compared coffee drinkers with non-coffee drinkers and calculated an odds ratio of 0.69 (95% CI: 0.59–0.80), which remained when analyses were limited to studies that adjusted for smoking status. Coffee and tea (or more particularly components of coffee and/or tea) have been identified to possess antioxidant activity (Daglia et al., 2000; Natella et al., 2002; Yanagimoto et al., 2004) and, more specifically, hydroxyl radical scavenging activity (Shi et al., 1991; Daglia et al., 2004); but also appear to exhibit pro-oxidant activity in the presence of high concentrations of metal ions and caffeine (Azam et al., 2003), specifically the caffeine appears to bind and reduce copper ions resulting in oxygen radical production. Pigments in coffee have been

identified as potential iron chelators (Morck et al., 1983), as have flavanoids (Morel et al., 1994). Maillard reaction products (responsible for the aroma and brown color of roasted coffee beans) when incubated with DNA and Fe^{2+} protected the DNA from strand breakage (Wijewickreme and Kitts, 1998), possibly indicating that these components might provide protection from genotoxicity, in particular oxidative damage from the Fenton reaction. Additionally, both coffee and tea (or components thereof) are known to inhibit the intestinal absorption of iron (Morck et al., 1983; Hurrell et al., 1999; Zijp et al., 2000), possibly inducing a minimal and non-clinically relevant iron deficiency when consumed together. As is the case for smoking, no studies have identified the specific *in vitro* or *in vivo* mechanisms of coffee/tea and iron interaction, although both antioxidant activity and iron chelation have been identified as possible mechanisms and such an interaction may play a role in PD.

Pesticides and iron

The most recent meta-analysis reporting on pesticide exposure and Parkinson's disease (Priyadarshi et al., 2001) calculated an OR of 1.85 (95% CI: 1.31–2.60) when including all studies and an OR of 2.16 (95% CI: 1.95–2.39) when including only studies conducted in the US. Categories of pesticides commonly investigated in association with PD include fungicides, herbicides, and insecticides which can be subdivided into organochlorines, organophosphates, carbamates, pyrethroids, and plant-derived toxins. Human studies, as opposed to animal studies, investigating the association of individual pesticides with PD have been infrequent, with the possible exception of studies evaluating paraquat (1,1'-dimethyl-4,4'-bipyridium). Paraquat, an herbicide, is chemically similar to MPP⁺, a conversion product of 1-methyl 4-phenyl 1,2,3,6-tetrahydropyridine (MPTP). MPTP was first identified in association with PD because it is a toxic by-product of synthetic opioid production that produced Parkinson-like symptoms when injected (Langston et al., 1983).

In mouse studies, paraquat has been shown to cross the blood brain barrier and produce cell death in dopaminergic neurons (McCormack et al., 2002; McCormack and Di Monte, 2003; Peng et al., 2007). An *in vitro* study (Chun et al., 2001) investigating MPP⁺, paraquat, and other neurotoxins indicated that the initial insult from exposure is increased production of H_2O_2 , which can react with neuronal iron via the Fenton reaction to produce reactive oxygen species. Additionally, elevated iron levels might contribute to an increased toxicity of paraquat, as has been observed in model systems for the structurally and biochemically similar MPTP (Poirier et al., 1985; Lan and Jiang, 1997; Kaur et al., 2007). Finally, there is some indication, as seen in animal and *in vitro* studies of MPTP and paraquat, that high iron levels and toxicant administration might individually and together contribute to increased α -synuclein aggregation (Uversky et al., 2001; Manning-Bog et al., 2002; Kalivendi et al., 2004). The specific mechanisms for interactions between iron and paraquat or other pesticides in the pathogenesis or neurodegenerative pathology associated with PD in humans are not known, although iron chelation when administered prior to the exogenous toxin appears neuroprotective in mice and non-human primates (Kaur et al., 2003).

Concluding remarks

Iron is an essential element in normal biological processes and yet it has the potential, if out of balance, to severely disrupt those processes. When the protein products involved in iron homeostasis are incorrectly formed, either due to a genetic mutation or an error in protein processing, the resulting system dysfunction can be detrimental, as in hemochromatosis (OMIM #235200) resulting from error in the HFE gene, neurodegeneration with brain iron accumulation (NBIA; OMIM #610217) associated with PLA2G6, and aceruloplasminemia (OMIM #604290) caused by a mutation in the CP gene. Disturbances in brain iron

concentrations have not only been described for Parkinson's disease but also been identified in conjunction with Alzheimer's disease (Ong and Farooqui, 2005).

The role of iron in neurodegenerative diseases, particularly PD, is unlikely to be as straightforward as observed in animal models, although the observation that neonatal iron uptake may cause neurodegeneration that only becomes apparent in older age animals is intriguing considering the increasing risk of Parkinson's disease with increasing age. Perhaps age is a proxy measure for the gradual accumulation of iron in the normal human brain; and, for a subset of PD cases, oxidative stress events or other putative risk factors associated with PD are causative only in combination with or after sufficient iron accumulation. This might indicate that some of the conditions currently defined as idiopathic Parkinson's disease could be subdivided by a measure of iron concentration in the brain's substantia nigra.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

AA	amino acid
BBB	blood–brain barrier
BMP	bone morphogenetic protein
CA	California
CI	confidence interval
DA	dopaminergic neurons
dbSNP	NCBI SNP database
DNA	deoxyribonucleic acid
Fe	iron
Fe²⁺	ferrous iron
Fe³⁺	ferric iron
FT	ferritin
H₂O₂	hydrogen peroxide
ICIP	intracellular calcein-chelatable iron pool
IRE	iron responsive element
IREBs	iron responsive element binding proteins
IRP	iron regulatory protein
kDa	kilo Daltons
LDLR	low density lipoprotein receptor-related protein
MPP⁺	1-methyl-4-phenylpyridinium
MPTP	1-methyl 4-phenyl 1,2,3,6-tetrahydropyridine
mRNA	messenger ribonucleic acid
NBIA	neurodegeneration with brain iron accumulation
NCBI	National Center for Biotechnology Information
OMIM	NCBI Online Mendelian Inheritance in Man
OR	odds ratio
PD	Parkinson's disease
ppm	parts per million
PS	parkinsonism
ROS	reactive oxygen species
SMAD	SMAD family
SN	substantia nigra
TH⁺	tyrosine hydroxylase positive
TIBC	total iron binding capacity
UPS	ubiquitin–proteasome system

UTR untranslated region

List of abbreviations for iron-related genes and proteins

ACO1/IREB1	aconitase 1, soluble/iron responsive element binding protein 1
ALAS2	aminolevulinate delta-, synthase 2
CP	ceruloplasmin
CYBRD1/DCYTB	cytochrome <i>b</i> reductase 1/duodenal cytochrome <i>b</i>
CYB561	cytochrome <i>b</i> -561
DCYTB/CYBRD1	duodenal cytochrome <i>b</i> /cytochrome <i>b</i> reductase 1
DMT1/SLC11A2	divalent metal ion transporter 1/solute carrier family 11 member 2
FPN/SLC40A1	ferroportin/solute carrier family 40 member 1
FRRS1	ferric-chelate reductase 1
FTH1	ferritin heavy chain
FTL	ferritin light chain
FXN	frataxin
HAMP	hepcidin
HCP1/SLC46A1	heme carrier protein/solute carrier family 46 member 1
HEPH	hephaestin
HFE	hemochromatosis
HFE2/HVJ	hemochromatosis type 2/hemojuvelin
HJV/HFE2	hemojuvelin/hemochromatosis type 2
HMOX1/HO-1	heme oxygenase 1
HMOX2/HO-2	heme oxygenase 2
IL6	interleukin-6
IREB1/ACO1	iron responsive element binding protein 1/aconitase 1 soluble
IREB2	iron responsive element binding protein 2
LRP1	low density lipoprotein-related protein 1
LRP2	low density lipoprotein-related protein 2
LTF	lactotransferrin or lactoferrin
MF12	antigen p97 (melanoma associated)
SFT/UBE2D1	stimulator of Fe transport/ubiquitin-conjugating enzyme E2D 1
SLC11A2/DMT1	solute carrier family 11 member 2/divalent metal ion transporter 1
SLC40A1/FPN	solute carrier family 40 member 1/ferroportin
SLC46A1/HCP1	solute carrier family 46 member 1/heme carrier protein
SMAD4	SMAD family member 4
STAT3	signal transducer and activator of transcription 3

STEAP3	Steap family member 3
TF	transferrin
TFR2	transferrin receptor 2
TFRC	transferrin receptor
UBE2D1/SFT	ubiquitin-conjugating enzyme E2D 1/stimulator of Fe transport

Fig. 1. Transferrin Endocytosis. The transferrin-Fe complex (TF) binds to the transferrin receptor (TFRC) and is endocytosed. The endosome is acidified via a proton pump (H⁺), which results in release of the transferrin-Fe complex from the transferrin receptor and release of Fe³⁺ from transferrin. The iron is reduced from Fe³⁺ to Fe²⁺, probably by Steap family member 3 (STEAP3) or possibly another ferric reductase such as CYBRD1, then transported out of the endosome via DMT1. Both the apo-transferrin and the transferrin receptor are returned to the cell surface and reused. The Fe²⁺ can become part of the intracellular calcein-chelatable iron pool (ICIP), stored in ferritin, or utilized by the cell.

Fig. 2. IRE-IRP Regulation of Iron Concentrations. The IRE-IRP mechanism provides for posttranscriptional iron regulation in response to intracellular iron concentrations. Under low cellular iron conditions, the IRPs bind to the IREs. In the case of TFRC and DMT1, the IREs are located in the 3' UTR and IRP binding stabilizes the mRNA and translation is enabled, resulting in increased iron uptake from the circulation and incorporation of iron into the cell. For FTL, FTH1, FPN, and ALAS2 mRNAs, the IRE-IRP complex prevents 43S pre-initiation complex binding and hence translation is inhibited. Without additional ferritin molecules iron imported into the cytosol cannot be stored and without FPN iron export is limited, thereby increasing the cellular iron concentration. Under high cellular iron conditions, the IRPs disassociate from the IREs which allows for TFRC and DMT1 degradation and FPN, ALAS, and ferritin subunit translation, decreasing iron uptake and increasing iron storage, respectively.

Fig. 3.

Neuronal Iron Transport. Transferrin receptors (TFRC) on the blood brain barrier (BBB), when bound to a transferrin–iron (TF) complex, either perform endocytosis or transcytosis into the endothelial cells. Other mechanisms of iron transport across the BBB include endo- or transcytosis of lactotransferrin (LTF) likely bound to a member of the low density lipoprotein receptor (LDLR) family; endo- or transcytosis of the melanotransferrin–glycosylphosphatidylinositol complex (p97-GPI) possibly also mediated by an LDLR family member; and transcytosis of H-ferritin, though the receptor is unknown. Iron is likely exported from the endothelial cell into the parenchymal space/brain interstitial fluid by FPN and then oxidized, probably by ceruloplasmin or some other ferroxidase such as hephaestin, for binding to apo-transferrin or some other molecule for transport to the neuron. Iron might be transported into neurons through the transferrin–receptor complex, lactotransferrin–receptor complex, or the UBE2D1/stimulator of Fe transport (SFT) protein. Once in the neuron iron may be reduced by cytochrome b-561 (CYB561) or ferric-chelate reductase 1 (FRRS1).

Table 1

Human studies investigating iron-related genes in PD cases

Author, Date	Cases/Controls	Methods	Genes ^a : Markers ^b
Borie et al., 2002	216/193	PCR-RFLP	<i>TF</i> : G258S, P570S <i>TFRC</i> : S82G <i>HFE</i> : H63D, C282Y <i>FXN</i> : +81 ins/del, +1386 rpt <i>LTF</i> : N534N
Buchanan et al., 2002	179/226 ¹	PCR-RFLP	<i>HFE</i> : C228Y
Buchanan et al., 2002	259/259 ²	PCR-RFLP	<i>HFE</i> : C228Y
Chen et al., 2002	253/ND	Sequencing; PCR-RFLP	<i>FTL</i> : 460–461 ins
Lee et al., 2002	58/24/1417 ³	Sequencing; ASOH	<i>IRP2</i> : –89 C>T, –35 G>A, L159V, F272L, T410T, T560I, A852A, IVS1 –127 A>G, IVS5+22 C>A, IVS5+73 C del, IVS6 –68 T>C, IVS7+19 A>G, IVS7+58 G>A, IVS12+50 A>G, IVS21+23 T>C
Dekker et al., 2003	137/47 ⁴	N.D.	<i>HFE</i> : H63D, C282Y
Dekker et al., 2003	60 (25)/ 2914 ^{5,6}	N.D.	<i>HFE</i> : H63D, C282Y
Felltschin et al., 2003	186/186	Sequencing; PCR dHPLC; PCR-RFLP	<i>FTL</i> : L55L, 460–461 ins, H174H <i>FTH1</i> : K54R
Deplazes et al., 2004	176/180 ⁷	Sequencing; PCR dHPLC; restriction, pyro-sequencing	<i>IRP2</i> : –74C>T, –88C>T, I888V, I580T, g.58863/C>T, g.59019/G>A
Hochstrasser et al., 2004	176/180 ⁸	Sequencing; PCR dHPLC; restriction, pyro-sequencing	<i>CP</i> : I63T, P477L, D544E, T551I, R793H, T841R
Ezquerro et al., 2005	210/102	PCR-RFLP	<i>TF</i> : G258S
Guerreiro et al., 2006	132/115	PCR-RFLP	<i>HFE</i> : H63D, C282Y
Akbas et al., 2006	278/280	Sequencing; PCR dHPLC; PCR-RFLP	<i>HFE</i> : H63D, K92N, I217T, IVS2 c.3889, C282Y
Aamodt et al., 2007	388/505	PCR; OLA	<i>HFE</i> : H63D, C282Y
Foglieni et al., 2007	124/180	PCR dHPLC	<i>FTL</i> : 249+60T>G, L55L, 249 +50C>T, H174H, H133P <i>FTH1</i> : 261+29 Ins 7 bp, K54R

Abbreviations: ASOH, allele-specific oligomer hybridization; dHPLC, denaturing high performance liquid chromatography; OLA, oligo ligation assay; N.D., not described; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism.

^aGene abbreviations are defined in the text and in the List of Iron-Related Genes at the beginning of this article.

^bMarker designations are of one of two forms and are as defined by the original author. Form 1: wild type amino acid (AA) followed by AA position followed by variant AA. Form 2: base position followed by base change.

¹Cohort 1, published in the same article as cohort 2.

²Cohort 2, published in the same article as cohort 1.

³Additional anonymous controls.

⁴Rotterdam Study source, published in the same article as Southwestern Netherlands source.

⁵Southwestern Netherlands source, published in the same article as Rotterdam Study source.

⁶60 PD cases, 25 non-PD PS cases, and 2914 controls.

⁷ Same study subjects as Hochstrasser et al., 2004.

⁸ Same study subjects as Deplazes et al., 2004.

Table 2

Genes involved in iron metabolism and homeostasis

NCBI GeneID	Symbol	Full name	Alternate symbol
48	ACO1	Aconitase 1, soluble	IREB1
212	ALAS2	Aminolevulinate, delta-, synthase 2 (sideroblastic/hypochromic anemia)	
1356	CP	Ceruloplasmin (ferroxidase)	
79901	CYBRD1	Cytochrome <i>b</i> reductase 1	DCYTB
1534	CYB561	Cytochrome <i>b</i> -561	
391059	FRRS1	Ferric-chelate reductase 1	
2495	FTH1	Ferritin, heavy polypeptide 1	
2512	FTL	Ferritin, light polypeptide	
2395	FXN	Frataxin	
57817	HAMP	Hepcidin antimicrobial peptide	
9843	HEPH	Hephaestin	
3077	HFE	Hemochromatosis	
148738	HFE2	Hemochromatosis type 2 (juvenile)	HJV
3162	HMOX1	Heme oxygenase (decycling) 1	HO-1
3163	HMOX2	Heme oxygenase (decycling) 2	HO-2
3569	IL6	Interleukin 6 (interferon, beta 2)	
3658	IREB2	Iron-responsive element binding protein 2	
4035	LRP1	Low density lipoprotein-related protein 1 (alpha-2-macroglobulin receptor)	
4036	LRP2	Low density lipoprotein-related protein 2	
4057	LTF	Lactotransferrin	
4241	MFI2	Antigen p97 (melanoma associated) identified by monoclonal antibodies 133.2 and 96.5	
4891	SLC11A2	Solute carrier family 11 (proton-coupled divalent metal ion transporters), member 2	DMT1
30061	SLC40A1	Solute carrier family 40 (iron-regulated transporter), member 1	FPN
113235	SLC46A1	Solute carrier family 46, member 1	HCP1
4089	SMAD4	SMAD family member 4	
6774	STAT3	Signal transducer and activator of transcription 3 (acute-phase response factor)	
55240	STEAP3	STEAP family member 3	
7018	TF	Transferrin	
7036	TFR2	Transferrin receptor 2	
7037	TFRC	Transferrin receptor (p90, CD71)	
7321	UBE2D1	Ubiquitin-conjugating enzyme E2D 1 (UBC4/5 homolog, yeast)	SFT