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Brain Iron Metabolism Dysfunction in Parkinson's Disease

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Abstract Dysfunction of iron metabolism, which includes its uptake, storage, and release, plays a key role in neurodegenerative disorders, including Parkinson's disease (PD), Alzheimer's disease, and Huntington's disease. Understanding how iron accumulates in the substantia nigra (SN) and why it specifically targets dopaminergic (DAergic) neurons is particularly warranted for PD, as this knowledge may provide new therapeutic avenues for a more targeted neurotherapeutic strategy for this disease. In this review, we begin with a brief introduction describing brain iron metabolism and its regulation. We then provide a detailed description of how iron accumulates specifically in the SN and why DAergic neurons are especially vulnerable to iron in PD. Furthermore, we focus on the possible mechanisms involved in iron-induced cell death of DAergic neurons in the SN. Finally, we present evidence in support that iron chelation represents a plausable therapeutic strategy for PD.

Keywords Parkinson's disease · Brain iron metabolism · Iron transporters · Iron regulatory protein · Iron chelation

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Introduction

Dysfunction of iron metabolism plays a key role in the pathogenesis of Parkinson's disease (PD) [1–5]. Elevated iron levels were first found in neuromelanin (NM)-containing neurons of the substantia nigra pars compacta (SNpc) in PD patients [6], suggesting that an iron-melanin interaction contributed to dopaminergic (DAergic) neurodegeneration in PD [7]. Although the role of iron in the pathogenesis of PD has been extensively studied over the past 2 decades, two key questions are still puzzling: First, why are iron levels elevated only in select brain regions? Second, is excessive iron accumulation in the brain an early event that causes neurodegeneration or a consequence of the disease process? Whether iron accumulation is the initial cause for DAergic neuron degeneration or a subsequent event tied to cell death has been under debate for many years.

However, a growing body of evidence shows that elevated iron levels are one of the initial events leading to DAergic neuron degeneration in PD [8–13]. Direct evidence for high levels of iron in individual DAergic neurons of substantia nigra (SN) from postmortem tissue of PD patients was established using sensitive and specific wavelength dispersive electron probe X-ray microanalysis coupled with cathodoluminescence spectroscopy [10]. Although the mechanisms underlying iron accumulation in the SN are fully undetermined, it is proposed that iron accumulation in the SN results from a combination of increased import, decreased export, and redistribution of intracellular iron. In summary, it is certain that iron accumulation may be due to a general malfunction in iron metabolism in the central nervous system (CNS), which may involve both genetic and environmental factors, centering on divalent metal transporter 1 (DMT1) and transferrin receptor (TfR) for iron uptake, ferroportin (Fpn), and amyloid precursor protein (APP) for iron efflux

and ferritin for iron storage. In this article, we describe brain iron metabolism and its regulation. We then provide a detailed description of iron metabolism dysfunction in PD with a primary focus on how iron specifically accumulates in DAergic neurons in the SN, making them particularly vulnerable.

Brain Iron Metabolism and Its Regulation

Iron enters the brain primarily through the blood-brain barrier (BBB) and ventricular system [14, 15]. A recent study revealed that the expression of iron transporters is exceptionally high in the choroid plexus, suggesting a prominent role of the choroid plexus in brain iron metabolism [16]. Levels of iron in the brain also have developmental, regional, and cellular distribution patterns. In humans, iron concentrations increase with aging in the striatum (and related basal ganglia structures) and the brain stem [17], whereas its levels are decreased in cortical white matter and thalamus in the elderly [18]. Iron is also present in most cell types in the CNS, including neurons, oligodendrocytes, microglia, and astrocytes. Interestingly, iron staining is more abundant in oligodendrocytes than in any other cell types in the brain [19], indicating an important role of oligodendrocytes in brain iron metabolism.

Iron metabolism is important for brain development and brain function. Iron deficiency negatively impacts a variety of neurodevelopmental processes including hippocampal neuronal development and spatial memory behavior [20, 21]. Iron deficiency is also implicated in a number of psychiatric and neurological conditions, including learning disabilities, attention deficit hyperactivity disorder (ADHD) and pediatric restless leg syndrome (RLS) [22, 23]. In fact, iron supplementation was shown to improve RLS symptoms [24]. In addition, iron overload also led to a variety of neurodegenerative diseases including PD, Alzheimer's disease, and Huntington's disease due to iron-induced oxidant stress [8–13]. These reports support the idea that brain iron metabolism is essential for developmental aspects of normal brain function and that both insufficient or high levels of brain iron are associated with neurological disorders.

Cellular Iron Transport

Iron Uptake

There are two pathways responsible for cellular iron uptake, the transferrin (Tf)-transferrin receptor (TfR) pathway, and the non-transferrin-bound iron (NTBI) transport pathway (see Fig. 1). Traditionally, Tf-TfR is considered a major pathway for cellular iron uptake in the brain. The primary receptor responsible for this pathway is TfR1. The Tf-TfR1 complex is internalized into the cell via receptor-mediated endocytosis. In endosomes, where the pH is lower relative to the cytosol, Tf-bound iron is released and then transported across the endosomal membrane by a divalent metal transporter 1 (DMT1)-mediated process. Although Tf can be synthesized by oligodendrocytes and choroid plexus epithelial cells, the primary source for Tf in the brain is its diffusion from the ventricles [25, 26]. TfR1 expression is abundant throughout the CNS, especially in neurons, indicating that these cells can acquire iron through the classic Tf-TfR1 pathway. The transport of iron across these barriers is most likely the result of receptor-mediated endocytosis of Tf-bound iron by capillary endothelial cells and choroid plexus epithelial cells [27].

Another type of TfR, named TfR2, is a homologue of TfR1 and shares about 45 % homology with TfR1 [28, 29]. Unlike TfR1, TfR2 messenger RNA (mRNA) does not contain an iron responsive element (IRE) RNA stem loop in its 3′-untranslated region to iron-dependent control message stability. Therefore, it is not regulated by intracellular iron levels [30]. Although TfR2 has a lower affinity for Tf binding than TfR1 [30], it is involved in Tf-bound iron uptake, which is enhanced in the presence of hemochromatosis (HFE) [28]. HFE is an MHC class I molecule and requires interaction with β2-microglobulin (β2M) for normal cell surface localization. It can also form a stable complex on the cell membrane with TfR2 to regulate iron homeostasis [28, 31]. This TfR2/HFE complex forms the sensor for extracellular iron levels and can regulate the expression of hepcidin to maintain iron homeostasis [32–35].

The main NTBI pathway is DMT1, which is responsible for ferrous iron uptake. Several other metal transport systems such as the ferritin receptor, lactoferrin (Lf)/lactoferrin receptor (LfR), and melanotransferrin (MTf) are also involved in the NTBI pathway [24, 36–39] (Fig. 1). However, some studies also demonstrate that MTf might not be essential for iron metabolism [40–43]. Thus, further studies regarding the role of MTf in iron transport are needed. Previous work has demonstrated that DMT1 is present in endothelial cells of the brain microvasculature, which is responsible for iron release from endosomes to the endothelial cell cytoplasm [44]. It is also present in astrocytes lining the blood vessels, in the choroid plexus, and in ependymal cells, and therefore relevant to the pathways by which iron crosses the blood-brain barrier. DMT1 was first discovered to be responsible for the initial uptake of iron from the duodenal lumen and iron translocation from the endosome. However, we showed that in rat SN, DMT1 is expressed in neurons, astrocytes, and microglia, but not in oligodendrocytes [45], providing evidence that DMT1 might participate in iron influx of these cells.

There are at least four distinct isoforms of DMT1, which differ in both the C-terminus and the N-terminus. At the N-terminus of the gene, alternative promoter usage leads to different transcription start sites at either exon 1A or exon 1B. Of the two C-terminal splice variants, one possesses a stem-loop

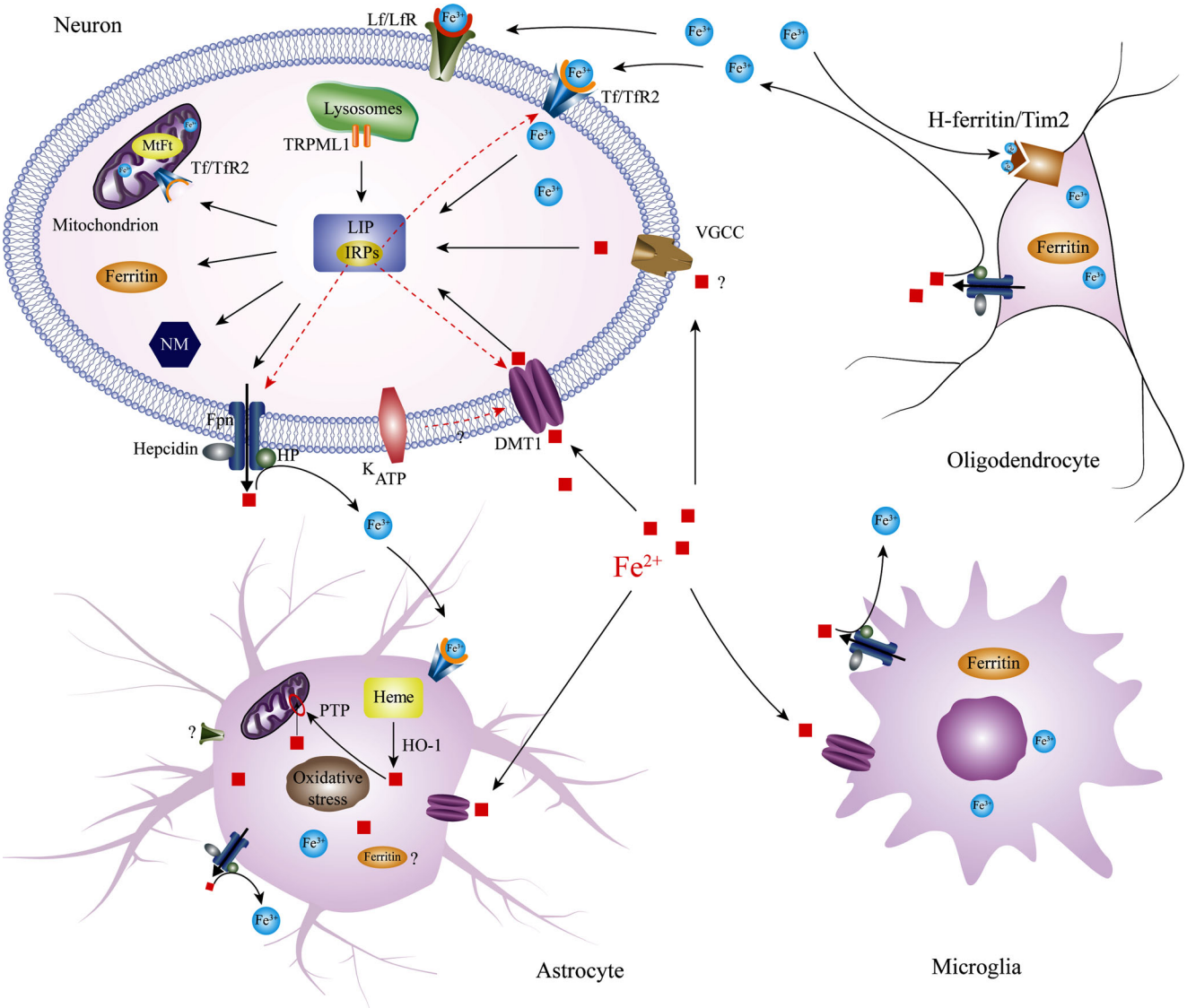

Fig. 1 A hypothetical scheme for iron transport in neuronal and glial cells. There are two pathways responsible for cellular iron uptake, the Tf-TfR1 pathway and the NTBI transporters. In neurons, Tf-Fe is taken up via a TfR1-mediated process and NTBI is acquired probably via a DMT1 or LfR-mediated process. TRPML1, acting as an endolysosomal iron release channel, might be involved in iron release from lysosomes into the cytoplasm. Iron can be transported out of the cell by Fpn with the help of HP and GPI-CP. Hepcidin can also regulate intracellular iron levels by action on Fpn. TfR2 is expressed within the mitochondria of DAergic neurons in the SN and mediates iron sequestration into mitochondria. NM is present in DAergic neurons of the SN and noradrenaline neurons of the locus coeruleus. In astrocytes, iron is taken up via a TfR1-mediated process, as well as DMT1 and LfR-mediated processes. Upregulated HO-1 in astrocytes liberates heme-derived carbon monoxide and free ferrous iron. The latter generates intra-glial oxidative stress that promotes opening of mitochondrial PTP and influx of (non-transferrin derived) iron into the mitochondrial matrix. Iron is taken up via a DMT1-mediated process in microglia and via H-ferritin/Tim2-mediated process in oligodendrocytes. The *red dotted lines* indicate that IRPs can regulate the expression of DMT1, Fpn, and TfR1. (?: representing the potential role of these proteins in brain iron metabolism). *DMT1* divalent metal transporter 1, *Fpn* ferroportin, *GPI-CP* glycosylphosphatidylinositol-anchored ceruloplasmin, *HO-1* heme oxygenase-1, *HP* hephaestin, *IRPs* iron regulatory proteins, *Lf* lactoferrin, *LfR* lactoferrin receptor, *MtFt* mitochondria ferritin, *NM* neuromelanin, *NTBI* non-transferrin-bound iron, *PTP* permeability transition pore, *SN* substantia nigra, *Tf* transferrin, *Tf-Fe* transferrin-bound iron, *TfR* transferrin receptor, *Tim2* T cell immunoglobulin and mucin domain-containing protein-2, *TRPML1* transient receptor potential mucolipin 1 (color figure online)

IRE in the 3′-untranslated region (UTR) of the mRNA (termed +IRE), whereas the other does not (termed −IRE) [46, 47]. The expression of DMT1 can be regulated posttranscriptionally through IRE and posttranslationally through the ubiquitin-proteasome system (UPS) [48]. Parkin is the E3 ligase responsible for ubiquitination of the 1B species of DMT1 [49]. Moreover, Ndifip1 binding to DMT1 is instrumental for its ubiquitination and its downregulation. Nedd4-2 is also a ubiquitin ligase for the polyubiquitination of DMT1 under metal-induced stress [48]. DMT1 expression increases with age, which is in line with increased levels of iron in the aging brain [50]. Decreased levels of brain iron in

Belgrade rats, which have a defect in DMT1, further suggest a critical role for this protein in brain iron uptake [51].

Autoradiographic studies using ^{125}I-recombinant human ferritin demonstrate that ferritin binding sites exist in the brain [52], indicating a ferritin-receptor-dependent iron delivery mechanism. A receptor for heavy-chain ferritin (H-ferritin) has been identified as T cell immunoglobulin and mucin domain-containing protein-2 (Tim2) [53], which can bind and internalize H-ferritin. It is present in oligodendrocytes both in vivo and in vitro, which express neither TfR [54] nor DMT1 [44, 45] (Fig. 1). Since oligodendrocytes contain more iron than any other cells in the brain, Tim 2 is thought to be the primary mechanism for iron acquisition by these cells [54]. For example, ferritin receptors are capable of delivering 2000 times more iron per mole of protein than transferrin. Thus, iron delivered via H-ferritin could make this pathway a predominant player in cellular iron delivery [55]. Receptors for light-chain ferritin (L-ferritin) have not been well documented until a recent study demonstrated Scara5 (scavenger receptor, member 5) as a receptor for L-ferritin in murine embryonic and adult kidney cells. Scara5 could bind L-ferritin but not H-ferritin or Tf and thereby mediate its endocytosis [56]. The discovery of receptors for H-ferritin and L-ferritin provides new insights into iron acquisition and raise the possibility that they might mediate iron accumulation in neurodegenerative diseases.

Iron Release

To our knowledge, Fpn is the only known iron transporter responsible for cellular iron export [57]. Fpn was first found to transport Fe^{2+} across the basolateral membrane of enterocytes with the auxiliary ferroxidase activity of ceruloplasmin (CP) or hephaestin (HP) [58–60] (Fig. 1). In the brain, Fpn is found in endothelial cells of the BBB, neurons, oligodendrocytes, astrocytes, the choroid plexus, and ependymal cells [44, 61, 62]. Using immunohistochemistry, we discovered that expression of Fpn and HP is co-localized in neurons, astrocytes, oligodendrocytes, and microglia, raising the possibility that HP could facilitate Fpn-mediated iron export from a variety of brain cells [63]. The cellular overlap of Fpn and HP further suggests a dynamic flux of iron out of brain cells, just as established in enterocytes of the gut tract. CP is a ferroxidase that converts highly toxic ferrous iron to its nontoxic ferric form and cooperates with Fpn to facilitate iron export. Direct evidence for the role of CP in iron metabolism comes from studies of patients with aceruloplasminemia, a hereditary disease caused by deficiency of CP [64, 65]. These individuals have very little or undetectable levels of CP, which causes severe intracellular iron accumulation in a number of organs, including the brain, particularly in the deep extrapyramidal motor nuclei, where it is associated with neurodegeneration. It is reported recently possible that CP might contribute to the pathology of PD [66]. HP is the homologue of CP, and both are expressed in the brain. However, it is uncertain which one performs the dominant role in the brain iron metabolism. It is certain that mice lacking both CP and HP (double knock-out) show elevated brain iron accumulation relative to single mutant in the cerebellum, substantia nigra, and hippocampus [67].

In addition, APP was shown to promote iron export from DAergic neurons by stabilizing Fpn [68–70]. Lei et al. has demonstrated that Tau deficiency induced Parkinsonism with dementia by impairing APP-mediated iron export from DAergic neurons in the SN [68]. This novel mechanism of iron accumulation may be attributable to APP's role in iron export from neuron [69, 71]. The E2 domain for APP encodes an REXXE motif that, embedded in a peptide, can stabilize the central iron exporter ferroportin (Fpn), thus to facilitate iron export [70].

Iron Storage

The majority of iron is bound and inactivated by ferritin inside brain cells [72]. L-Ferritin is required for the long-term storage of iron [73], whereas H-ferritin has ferroxidase activity [74]. Cellular distribution of the two isoforms of ferritin in the brain is different, varying with iron status, age, and disease state [75, 76]. Immunohistochemical analysis of the cytosolic ferritins in the brain of adult nonhuman primates indicates that neurons express predominantly H-ferritin, while oligodendrocytes express both H- and L-ferritin, and microglia only express L-ferritin [76, 77]. Thus, oligodendrocytes and microglia are capable of storing large amounts of iron. With regard to astrocytes, one study showed that neither forms of ferritin are detectable [76], while another found L-ferritin expression [78]. However, in DAergic neurons of the SNpc, H-ferritin and L-ferritin are barely detectable compared to neurons in other brain regions [79]. The system relevant in iron storage in DAergic neurons is the polymer NM [77, 80]. However, a targeted proteomics approach has revealed L-ferritin in NM granules of postmortem human brain tissue and implicates iron storage and release from iron binding sites in these neurons [81]. Recent findings showed that extracellular ferritin in CSF was elevated in PD and lowering of iron by deferiprone lowered CSF ferritin levels [82], indicating that elevated ferritin might be involved in PD.

Iron Homeostasis Regulation

Iron homeostasis is regulated systemically by hepcidin and cellularly by iron regulatory proteins (IRPs). Hepcidin, originally found in hepatocytes, is proposed to maintain body iron homeostasis through regulating intestinal iron absorption [83, 84]. Binding of hepcidin to Fpn causes the internalization and degradation of Fpn, which is reported to be a hepcidin-

regulated iron efflux protein and hepcidin receptor [57], resulting in decreased iron efflux into the plasma [85, 86] (Fig. 1).

Hepcidin is widely expressed in mouse brain, and the mRNA levels of hepcidin increase with aging [87, 88]. Injection of hepcidin into the mouse lateral cerebral ventricle results in decreased Fpn protein levels in cerebral cortex, hippocampus, and striatum. Treatment of primary cultured rat neurons with hepcidin also causes decreased Fpn expression and results in the subsequent release of iron [87], indicating that local hepcidin might be involved in brain iron metabolism by regulating Fpn protein levels. More recently, it is demonstrated that hepcidin significantly reduces brain iron in iron-overloaded rats by downregulating iron transport proteins including TfR1, DMT1, and Fpn [89].

Cytosolic iron metabolism is primarily controlled by IRPs, which are critical components of a sensory and regulatory system that coordinates mRNA-encoding proteins to maintain cellular iron homeostasis. Through binding to IREs, IRPs are able to posttranscriptionally regulate mRNAs that have IREs in the 3′- or 5′-UTRs, control iron uptake (TfR1, DMT1), store (ferritin), and export (Fpn). There are two distinct forms of IRPs, IRP1 and IRP2, which share 44 transcripts including the transcripts that had been confirmed in literature, i.e., FTL, FTH, TfR1, DMT1, and Fpn [90]. IRP1 is a bifunctional protein with IRE-binding activity and enzymatic activity as cytosolic isoform of aconitase (c-acon). The action (regulation) of IRP1 is achieved in the presence or absence of the [4Fe-4S] cluster within the protein. Unlike IRP1, IRP2 lacks enzymatic activity and is devoid of Fe-S clusters. A notable difference from IRP1, relative to IRP2, is the presence of a unique 73-amino-acid sequence. This motif is an iron-dependent degradation signal for IRP2 and facilitates iron-dependent oxidation, ubiquitination, and proteasomal degradation [91, 92]. IRP1 has a critical role in the pulmonary and cardiovascular systems, while IRP2 function predominates in the nervous system and erythropoietic homeostasis [90]. Signals other than iron levels are capable of regulating IRPs. Oxidative stress, for example, can activate IRPs by other, not yet fully elucidated, pathways. For IRP1, oxidative stress induces the inactivation of its aconitase activity and causes rapid stimulation of IRE binding in several mammalian cell lines. Interestingly, increased IRP1 activity and IRP2 accumulation mediated by oxidative stress are largely abolished by the antioxidant *N*-acetyl-L-cysteine [11, 93].

Iron Metabolism in Lysosomes

There is increasing recognition that lysosomes participate in cellular iron metabolism [89, 94–98]. The lysosomal compartment provides cells with the ability to recycle organelles and long-lived proteins by a process termed autophagy, which is the main mechanism that delivers substrates to the lysosomal compartment for degradation. The lysosomal compartment is rich in iron because of autophagic degradation of iron-containing proteins (e.g. ferritin, mitochondrial electron-transport complexes) [89, 96, 98]. In addition, mitochondrial iron accumulation also results in lysosomal iron loading due to autophagic degradation in lysosomes [99].

It is not clear how iron is transported from lysosomes to the cytosol. A recent report found that, by measuring the difference in iron levels between the cytosol and lysosome, the transient receptor protein mucolipin 1 (TRPML 1, also known as MCOLN1) functions as an iron permeable channel and mediates iron release from late endosomes and lysosomes [100] (Fig. 1). TRPML 1 is also a Ca^{2+}, K^{+}, and Na^{+} permeable cation channel sensitive to pH changes and capable of regulating a critical step in the maturation of late endosomes to lysosomes [101, 102]. Moreover, TRPML 1 is ubiquitously expressed in cells of every tissue [103] (Fig. 1). Interestingly, mucolipidosis type IV (ML4), which is an autosomal recessive, neurodegenerative disorder characterized by severe psychomotor retardation and vision impairment, is caused by mutations in the human *TRPML1* gene [101]. Indeed, ML4 results in a lysosomal storage problem [101]. In fact, ML4 mutations are shown to impair iron permeability of TRPML1 to various degrees, which correlates well with the disease severity, indicating that impaired iron transport might contribute to both the hematological and degenerative symptoms of ML4 patients [100]. There are no reports regarding TRPML1 and PD.

Iron Metabolism in Mitochondria

Mitochondria play a critical role in cellular iron metabolism. Iron is transported into the mitochondria for heme synthesis and iron sulfur cluster synthesis that is involved in the electron transport and oxidative phosphorylation [94, 104, 105]. However, the mechanism by which iron is transported from the cytosol labile iron pool (LIP) to neuronal mitochondria is not well understood. Recently, a novel Tf/TfR2-mediated iron transport pathway in the mitochondria of DAergic neurons in the SN has been reported [106] (Fig. 1). This Tf/TfR2 pathway can deliver Tf-bound iron to mitochondria and also to the respiratory complex I. Disruptions to this Tf/TfR2-dependent system have been associated with PD, and this finding highlights the role for iron accumulation in this movement disorder [106]. Recently, a protective association between PD and a haplotype in Tf and TfR2 was reported, suggesting that Tf or a Tf-TfR2 complex may play a role in the etiology of PD [107].

The discovery of mitochondrial ferritin (MtFt) has enhanced our understanding of mitochondrial iron metabolism. MtFt has a similar structure to ferritins in cytoplasm and incorporates iron in a similar manner with cytosolic H-ferritin [108]. However, there are some differences between

mitochondrial and cytoplasmic ferritin. First, with regard to the quaternary structure, the cytosolic ferritins are heteropolymers, while those in the mitochondria are homopolymers and are assembled after processing within the mitochondria [108]. Second, there is no apparent IRE in the gene for MtFt, suggesting that other mechanisms might be involved in its regulation [109]. Another difference is that MtFt appears to have a limited tissue distribution compared with the ubiquitous H- and L-ferritin. Although MtFt has relatively high expression levels in the testis and erythroblasts of sideroblastic anemia patients, it is also identified in other organs including the brain, spinal cord, heart, kidney, and pancreatic islet of Langerhans and other high oxygen-consumption tissues [110–112]. It is also reported that MtFt participates in brain iron metabolism [111]. Santambrogio et al. [113] demonstrated that MtFt antibodies stain the majority of neurons in the cortex and spinal cord, whereas glial cells were largely immunonegative. In addition, the cerebellum shows a strong signal for MtFt only in Purkinje cells and in scattered glial cells of the molecular and granular layers. Moreover, Shi et al. [111] confirmed the endogenous expression of MtFt in the cerebellum and striatum of mice. The function of MtFt has not been fully elucidated. Its expression levels increase in the face of mitochondrial iron overload, suggesting that it might play an important role in iron trafficking and storage in the mitochondria [108]. In addition, it has ferroxidase activity similar to the ferritins in cytoplasm, detoxifying potentially harmful free ferrous iron to the less soluble ferric iron [114]. Overexpression of MtFt results in an increase in IRP/IRE interaction, accompanied by an increase in TfR1 levels and a decrease in cytoplasmic ferritin synthesis [115]. This indicates that alterations in mitochondrial iron homeostasis lead to changes in cellular iron metabolism.

Pertaining to pathogenic consequences of disruption to the function of MtFt on neuronal cell iron metabolism, its possible role in PD has been investigated. For example, it was demonstrated that MtFt could affect neuronal iron metabolism and prevent neuronal cell damage induced by 6-OHDA [111]. More recently, it was reported that MtFt could protect SH-SY5Y cells against β-amyloid-induced neurotoxicity, implying a potential protective role of MtFt in AD. This provides a new neuroprotective strategy, as a therapy to PD may be persued to regulate MtFt expression in neuronal cells as has been reported for the treatment of AD [116].

Heme oxygenase-1 (HO-1), a rate-limiting enzyme involved in the degradation of heme and a factor that promotes the accumulation of nontransferrin iron in astroglial mitochondria, is upregulated in AD and PD brains [117]. Thus, HO-1 has a prominent role in iron metabolism of astroglial mitochondria. In rat astroglial cultures, HO-1 mRNA, protein, and activity levels are markedly increased after exposure to stressors, including cysteamine, DA, β-amyloid 40/42, TNF-α, and IL-1-β [118]. This causes increased uptake of non-transferrin-derived $^{59/55}$Fe (but not diferric transferrin-derived iron) by the mitochondrial compartment relative to untreated control cultures [118]. The net result of this adaptation is iron sequestration into the mitochondria of cultured astrocytes, an event that is largely abolished by co-incubation with a competitive inhibitor of heme oxygenase activity (mesoporphyrin) or a transcriptional suppressor of the HO-1 gene (dexamethasone) [119]. These findings indicate that astroglial HO-1 induction mediates iron sequestration in degenerating neural tissues. Further studies have revealed that the mitochondrial permeability transition pore inhibitors cyclosporin A and trifluoperazine can also attenuate mitochondrial iron trapping in HO-1-transfected astroglia, as well as cells exposed to DA, TNF-α, or IL-1 [118]. These results suggest that intracellular oxidative stress resulting from HO-1 hyperactivity promotes pore opening [120, 121] and the influx of cytosolic iron into the mitochondrial matrix [117] (Fig. 1). Furthermore, this glial mitochondrial iron deposition significantly enhanced the vulnerability of nearby neuronal constituents to oxidative injury [122].

Iron and PD

There are three lines of evidence that indicate disruption of iron metabolism as a key mechanism involved in neuronal death in PD: (1) Iron levels are increased in the SN, but not in other brain regions of PD patients (see Table 1). (2) Postmortem studies have shown increased iron levels specifically in DAergic neurons in PD, but not in other movement disorders such as HD [10]. (3) Neuroprotection is achieved by pharmacological or genetic chelation of iron in animal models of PD [128–131]. (4) Genetic disorders that resulted in brain iron accumulation often manifested as PD (e.g., aceruloplasminemia) [132].

Iron Metabolism Dysfunction in Patients with PD

The role of iron as an etiological factor for PD was first suggested when it was found that levels of total iron were increased by 176 %, and levels of ferric iron were increased by 225 % in the SNpc of PD patients relative to age-matched controls [133]. In contrast, there were no significant differences in the levels of total iron or ferric iron in the cortex (Brodmann area 21), hippocampus, putamen, and globus pallidus [133]. Other studies also showed increased iron levels in the SNpc of PD brains using a spectrophotometric method and Perl's staining [134], inductively coupled plasma spectroscopy [135], magnetic resonance imaging (MRI) [136, 137], laser microprobe mass analysis [138], susceptibility-weighted imaging (SWI) [139, 140], and enhanced T2 star-weighted angiography (ESWAN) [141]. Recently, it was reported that quantitative susceptibility mapping might be the

Table 1 The iron content in the SN and cerebrospinal fluid (CSF) of normal and PD patients

Author	Methods	Normal			PD		
		SN	Globus pallidus	CSF	SN	Globus pallidus	CSF
Sofic et al. [133],; Riederer et al. [123]	The spectrophotometric method (μg/g fresh weight)	48	81	/	Increase by 77 %	No significance	/
Dexter et al. [6],, [135]	The sensitive technique of inductively coupled plasma spectroscopy (nmol/g dry weight)	About 11000	Lat: About 14500 Med:About 13500	/	Increase by 35 %	Decrease by 29 %	/
Mann et al. [124]	Inductively coupled plasma spectrophotometer (ng/mg protein)	1159	/	/	Increase by 56 %	/	/
Griffiths et al. [125]	Absorption spectrophotometry (g/g wet weight tissue)	139.8	Lat: 207.0 Med:163.8	/	Increase by 101 %	Increase by 43 % Decrease by 31 %	/
Salazar et al. [175],	Atomic absorbance spectroscopy analysis (ng/mg protein)	SNpc 203.8	/	/	SNpc Increase by 44 %	/	/
Good et al. [138],	The Laser Microprobe Mass Analyzer (LAMMA)	/	/	/	Increase	/	/
Jellinger et al. [126]	Energy disperse X-ray analysis	/	/	/	SNpc (neuromelanin-containing neurons) Increase	/	/
Ryvlin et al. [137],	Magnetic resonance imaging (MRI)	/	/	/	SNpc Increase	Decrease	/
Berg et al. [127]	Transcranial sonograph	/	/	/	Increase	/	/
Martin et al. [146],	High field strength MRI	/	/	/	SNpc increase	No significance	/
Popescu et al. [215],	Rapid-scanning x-ray fluorescence (RS-XRF)	/	/	/	Increase by 40 %	Decrease	/
Zhang et al [96, 97]	Susceptibility-weighted imaging (SWI)	/	/	/	Increase	No significance	/
Jiménez- et al. [213],	Atomic absorption spectrophotometry (mg/l)	/	/	0.21	/	/	No significance
Rossi et al. [140]	R2* and susceptibility-weighted imaging (SWI)	/	/	/	Increase	Increase in anterior GP by SWI	/
Wang et al. [141]	Enhanced T2 star weighted angiography (ESWAN)	/	/	/	Increase	/	/
Wu et al. [148]	Susceptibility-weighted imaging (SWI)	/	/	/	Increase	Increase	/

/ nondetective

most sensitive quantitative technique to detect a significant increase of nigral iron for PD [142]. Using a variety of methodologies, it was demonstrated that nigral iron levels are elevated in early-stage PD patients, a phenomenon that is linked to the severity of PD motor symptoms [143–148]. Iron elevation is also reported with familial PD, demonstrating that PD-associated proteins including alpha synuclein, LRRK2, PINK1, Parkin, and DJ-1 are associated with iron accumulation in the SN by TCS [1–4]. We have observed and reported that the iron content is significantly lower in the temporal cortex of patients with PD when compared with age-matched healthy controls. In the same study, the levels of DMT1+IRE, TfR1, FPN1, and IRP1 were shown to be decreased in the temporal cortex [149]. These findings suggest that iron homeostasis might be disrupted through different mechanisms in the SN and temporal cortex.

Epidemiological studies also revealed that an occupational exposure to iron or a high intake of iron can increase the risk for developing PD [150, 151]. A prospective study shows that dietary nonheme iron intake, but not total iron intake (dietary and supplemental), from food is associated with a 30 % increased risk for developing PD [152]. A low intake of cholesterol in combination with iron intake also increases the risk of developing PD in both genders [153]. We noted, however, that these epidemiological studies are restricted to specific populations restricted to the USA.

Iron Metabolism Dysfunction in Animal Models of PD

Treatment of rodents with either 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) or 6-hydroxydopamine (6-OHDA) is a well-established method for generating PD animal models. Neurotoxin-treated animals exhibit the major hallmarks of PD pathology, including the loss of DAergic neurons in the SN. Moreover, the enhanced iron level was observed in dopaminergic neurons in humans [10], and iron accumulation has also been shown in the SN of both MPTP- and 6-OHDA-induced animal models [63, 154–159]. In fact, in 6-OHDA-treated rats, both increased iron levels and neuronal loss are apparent in as short a time as the SN 1 day following 6-OHDA injection [155].

Other animal models of PD also suggested a role for dysfunctional iron metabolism in PD pathogenesis. For example lipopolysaccharide-induced inflammation models were linked to PD by demonstration of increased iron deposits, as well as ferritin accumulation, in the SN [160]. Iron was also considered to have synergistic effects with paraquat in PD animal models, which is a key environmental factor in sporadic PD [161, 162]. The relationship between iron and PD pathogenesis is further illustrated in iron-overloaded animal models, which develop degeneration of DAergic neurons. Moreover, direct injection of ferric iron into the SN decreases both DA content and release in the striatum [42, 163, 164]. In addition, peripheral iron overload leads to iron deposits in the SN and DAergic neuron loss [165, 166]. In fact, in iron-fed rodent models, elevated levels of iron give rise to motor dysfunction, DAergic neuron loss, and reduced DA content in the striatum [167, 168].

Role of Iron Transporters in Nigral Iron Accumulation in PD

The altered expression of iron-related proteins in the SN may be responsible for the nigral iron accumulation in PD. For example, Faucheux et al. [169] reported that the regional density of transferrin binding sites is low in the substantia nigra (SN) but is not different in PD patient brains compared with controls. However, in 1997, the same group revealed a reduced density of transferrin binding sites in the SN of PD patients [170]. More recent studies have shown that 1-methyl-4-phenylpyridinium (MPP^+) treatment enhances the cell surface expression of TfR. Therefore, upregulation of TfR1-dependent iron influx could be involved in elevated iron levels in PD [171, 172]. These discrepant reports might reflect differences in results derived from acute insults in vitro and the chronic lasting status in vivo. Thus, it is pertinent that further work aims to explore TfR1's contribution to iron accumulation in PD. That the levels of Tf and TfR2, as well as their physical interaction, are increased after exposure to rotenone [106] implies that this system may be responsible for the uptake of iron into mitochondria following toxicant exposure [106]. The selective localization of TfR2 in DAergic neurons and the accumulation of Tf in parallel with oxidative damage suggests that the Tf/TfR2 system might lead to iron deposition in PD [106, 173] (see Fig. 2).

In addition to the TfRs, it has been shown that DMT1 plays a key role in brain iron metabolism [44, 174] and is highly expressed in neurons of the SN in PD, which correlates with the abnormally deposited iron in the same area [175] (Fig. 2). This supports that nigral iron accumulation by DMT1 could account for some of the increased iron import in PD, although we discuss the role of FPn and decreased export in section 2 of this review. The presence of IRE in mRNA of DMT1+IRE and Fpn implies that posttranscriptional regulation might be controlled by changes in intracellular iron levels [176]. For example, evidence derived from MPTP- or 6-OHDA-induced PD models indicates that increased expression levels of DMT1+IRE/DMT1-IRE [177] and decreased expression levels of Fpn are prevalent in both animal and cell PD models [63, 178–180] (Fig. 2). The upregulation of DMT1+IRE and the concomitant downregulation of Fpn responsible for iron accumulation in the SN are due to an IRP-IRE-dependent pathway in 6-OHDA-induced PD models [11, 178]. However, the upregulation of DMT1-IRE in MPTP-induced PD models is not in an IRP/IRE-dependent pathway. A reactive oxygen species (ROS)-nuclear factor kappaB (NF-κB) activation pathway might also be involved in DMT1-IRE upregulation [181, 182]. Although PD animal models do not fully recapitulate the neuropathology of human PD patients, they do provide a powerful tool to explore changes in iron transporter expression in the early stage of this disease. Indeed, dysfunction of iron transport may be the primary factor for iron accumulation in PD, and not a secondary event of the disease pathology [45, 63, 179, 182, 183]. Further studies should focus on direct evidence for iron transport dysregulation in PD patients. Notably, a study revealed that DMT1+IRE levels are significantly increased in PD subjects compared with control subjects [175]. These data provide direct evidence that increased intracellular iron import leads to iron accumulation in the SN and ultimately cell death. In addition, the mutation (G185R), which impairs DMT1-mediated iron transport, decreases the susceptibility of microcytic mice (mk/mk) and Belgrade rats to MPTP- and 6-OHDA-induced neurotoxicity, respectively [175]. Therefore, it appears that DMT1-dependent increases in iron levels play a prominent role in the degeneration of DAergic neurons in PD.

Lf is an 80-kDa iron-binding glycoprotein of the Tf family which is present in most biological fluids and is a major component of the mammalian innate immune system. Lf can bind Fe^{2+} or Fe^{3+} ions, as well as Cu^{2+}, Zn^{2+}, and Mn^{2+} ions [184]. In fact, Lf is involved in processes as diverse as regulation of iron absorption in the bowel, immune responses, antioxidant and anticarcinogenic activity, and anti-inflammatory control.

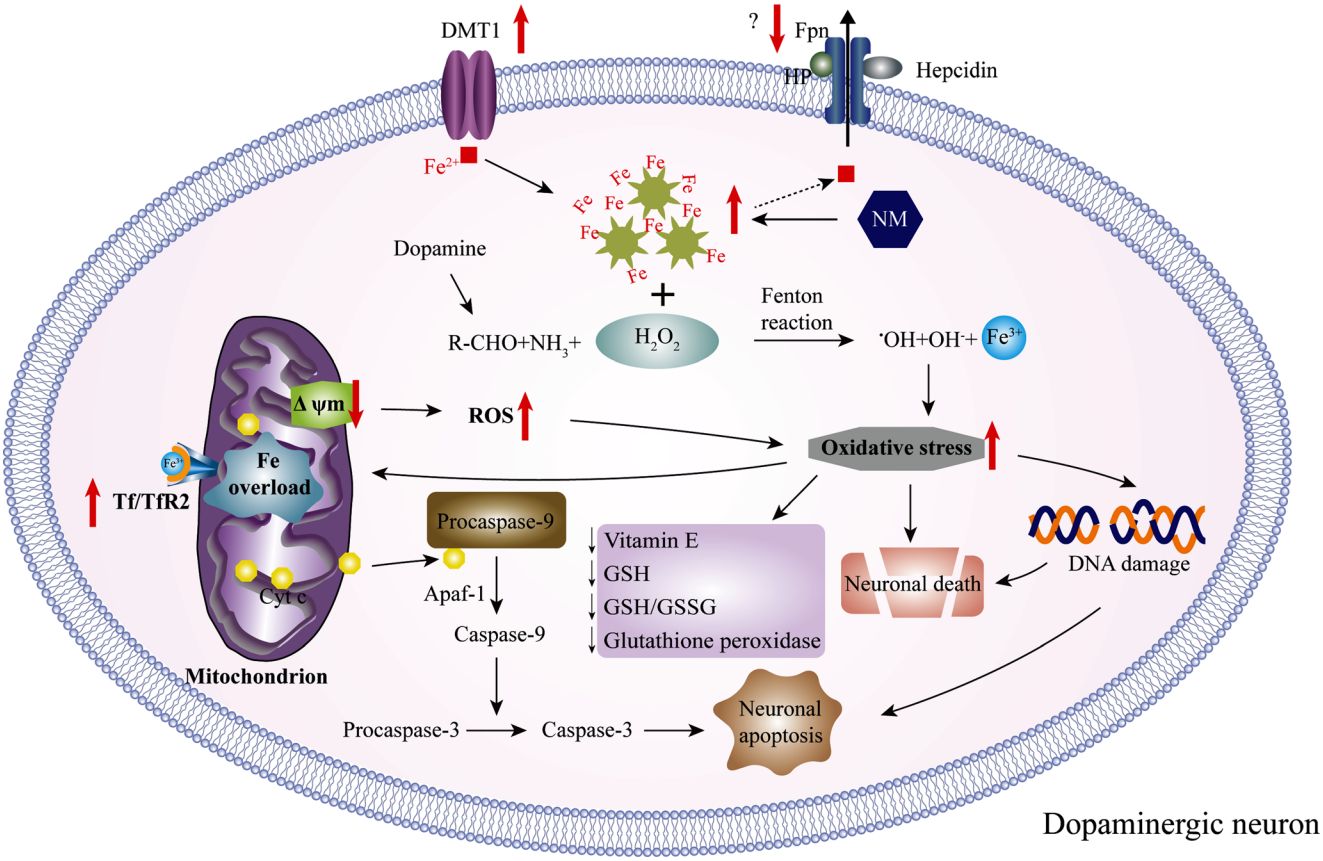

Fig. 2 Iron-induced oxidative stress in nigral DAergic neurons in PD. In PD, elevated iron levels in DAergic neurons can be caused by increased iron import by DMT1, decreased iron export by Fpn (?: decreased Fpn in DAergic neurons was shown in PD models but not in PD patients) and increased release from NM in the presence of H_2O_2 and iron overload. Increased intracellular iron may catalyze the conversion of H_2O_2 via the Fenton reaction to highly reactive ·OH, thus resulting in increased oxidative damage. Depletion of cellular antioxidants (vitamin E, GSH), reduction of GSH/GSSG, possible activation of GSHPx, and DNA damage are also observed. The oxidative stress causes the dysfunction of mitochondria, leading to an increased ROS formation and Cyt C release. This in turn exaggerates oxidative stress and activates caspase-mediated apoptosis and ultimate cell demise. The disrupted Tf/TfR2-dependent system in PD also leads to iron accumulation in mitochondria, which may enhance the dysfunction of mitochondria. *Red arrows* show the increased or decreased status of the components whose levels are altered in PD. *Cyt C* cytochrome C, *DAergic* dopaminergic, *DMT1* divalent metal transporter 1, Fe^{2+} ferrous iron, Fe^{3+} ferric iron, *Fpn* ferroportin, *GSH* reduced glutathione, *GSHPx* glutathione peroxidase, *GSSG* oxidized glutathione, H_2O_2 hydrogen peroxide, *NM* neuromelanin, *·OH* hydroxyl radical, *PD* Parkinson's disease, *ROS* reactive oxygen species, *Tf* transferrin, *TfR2* transferrin receptor 2 (color figure online)

Lf may have a role similar to that of Tf in iron transport in the brain, ensuring that an adequate amount of iron is present under normal circumstances. Lf has a high affinity for iron. In the brain, Lf is synthesized by activated microglia [19] and detected in DAergic neurons by immunostaining [185]. The level of Lf expression is markedly increased in surviving DAergic neurons from high-dose MPTP-treated mice, which is accompanied with a pronounced depletion of DA [186]. In PD patients, an increase in Lf has also been linked with increased iron levels in the SN and degeneration of nigral DAergic neurons [187]. LfR (also called LactoTf receptor), a monomeric glycoprotein of 105 kDa expressed in the brain microvasculature and in the SN, and primarily expressed in neurons and occasionally in astrocytes, is increased in the mesencephalon of patients with PD, indicating a role similar to that of TfR in iron influx in neurons of the brain [188].

CP is the strongest ferroxidase in human plasma, and its ferroxidase activity is required to stabilize the only iron exporter, Fpn [189]. In mice deficient in either CP or HP, no iron deposits have been observed in the retina. However, mice deficient in both CP and HP have a striking, age-dependent increase in retinal iron, as well as the iron storage protein ferritin [190]. Similarly, in CP-only-knockout mice, iron overload is present in several tissues, including the brain. Moreover, these animals exhibit enhanced vulnerability to rotenone-induced neurotoxicity [191]. It was reported that younger-onset PD patients have significantly lower levels of serum CP, indicating that inadequate circulating CP levels may contribute to rapid brain iron accumulation and an earlier-onset of PD [192]. Decreased levels of serum CP may also exacerbate nigral iron deposition in patients with PD [109, 193]. Therefore, ferroxidase dysfunction is a likely

mechanism for iron deposition in neurodegenerative diseases [194]. Recently, it was reported that the activity of Cp in PD brains was reduced in SN, indicating the therapeutic potential in treatment of PD [195].

Effects of LTCC on Iron Accumulation

The proteome analysis of human SN in PD demonstrated that the δ subunit of L-type voltage-gated calcium channel (LTCC) was significantly increased in PD [196], which indicated that LTCC might be involved in the pathogenesis of PD. Several studies demonstrated that LTCC mediate iron import into cardiomyocytes under iron-overload conditions [197, 198]. The functional properties of LTCC in heart, secretory cells, and neurons are quite similar [199]. In addition to the cardiomyocytes, Fe^{2+} uptake through LTCC is common in other excitable cells such as neurons and pancreatic β cells. It was reported that iron could compete with calcium via LTCC for entry into NGF-treated rat PC12 cells and murine N-2α cells, which may be exacerbated in iron-overloaded conditions. This suggests that LTCC provide an alternative route for iron entering into neuronal cells under pathophysiologic conditions of iron-overloaded [200]. Based on the effects of LTCC on the iron accumulation in cardiomyocytes and cell lines, we proposed that LTCC at least partly mediates the iron overload in the SN in the pathogenesis of PD. A preliminary study in our laboratory also showed that the LTCC blocker nifedipine may protect against iron overload-induced DA neuron degeneration and iron accumulation in the SN of rats. These studies further suggest that LTCC might be involved in the selective iron accumulation in the SN in PD and potentially provides a new therapeutic target to reduce the iron accumulation in iron overload diseases, including PD.

Effects of K_{ATP} Channels on Iron Accumulation

ATP-sensitive potassium (K_{ATP}) channels are octameric proteins consisting of two different types of subunit: members of the Kir6 inwardly rectifying potassium channel family (Kir6.1 and Kir6.2) and sulfonylurea receptor (SUR) subunits (SUR1, SUR2A and SUR2B), which are members of the ATP-binding cassette transporter superfamily. Four pore-forming Kir6 subunits are joined together with four regulatory SUR subunits to make a functional channel. Different subunit combinations contribute to different biophysical, pharmacological, and metabolic properties of K_{ATP} channels [201]. It was reported that the selective activation of K_{ATP} channels of dopaminergic neurons in the SN was a potential mechanism for the selective degeneration in this region in PD [202]. Kir6.2 and SUR1 are the relevant K_{ATP} channel subunits in both SN and ventral tegmental area (VTA) dopaminergic neurons in adult mice. However, when treated with MPP^+, electrophysiological activities of dopaminergic neurons in the SN were lost due to the activation of K_{ATP} channels. This was not the case for the dopaminergic neurons in VTA, since K_{ATP} channels were not activated due to the MPP^+ treatment [202]. Genetic inactivation of Kir6.2 resulted in a selective rescue of dopaminergic neurons in the SN but not those in the VTA in the MPTP model and the mutant weaver mouse [202]. This provides some evidence that activation of K_{ATP} channels plays an important role in the selective degeneration of dopaminergic neurons in the SN.

It was reported that higher mRNA levels of the regulatory subunit SUR1 were found in SN DA neurons from PD patients compared with neurons from controls. Otherwise, mRNA expression of SUR2 and the pore-forming subunit Kir6.2 were not altered. The selective upregulation of SUR1 may be consistent with increased burst firing [203]. The activation of K_{ATP} channels hyperpolarizes the membrane potential, which is critical for the generation of membrane oscillations that underlie bursting firing [204]. K_{ATP} channels-gated burst firing in already metabolically challenged SN DA neurons could promote excitotoxicity and increase calcium loading synergistically with NMDA receptors and L-type Ca^{2+} channels [205]. This led to the reduction of mitochondrial calcium buffering capacities and acceleration of calcium-triggered reactive oxygen species production [206], which in turn activated K_{ATP} channels in highly vulnerable SN DA neurons [202]. This positive feedback might lock SN DA neurons in a highly stressful bursting state in vivo, potentially accelerating their degeneration.

It is not known whether the selective activation of K_{ATP} channels in the SN contributes to the selective iron accumulation in this area. The activation of K_{ATP} channels induces hyperpolarization of the membrane potential of dopaminergic neurons in the SN following MPP^+ treatment. The transport function of DMT1 is proton-coupled and depends on the cell membrane potential. It was reported that iron transport via DMT1 was driven at higher rates of hyperpolarized potentials [207]. This leads to the hypothesis that the hyperpolarization of cell membrane induced by activation of K_{ATP} channels might increase DMT1-mediated iron transport, with more iron influx into the dopaminergic neurons. Our preliminary data demonstrated that the selective K_{ATP} channel opener diazoxide could increase intracellular iron levels after ferrous iron incubation. This provides initiatory evidence that K_{ATP} channel activation might contribute to the iron accumulation selectively in the SN. Further investigation should be conducted in the future to reveal the underlying mechanisms.

Vulnerability of DAergic Neurons in the SN

DAergic neurons are particularly vulnerable to oxidative stress, a process that is largely intensified in the excess of iron [94, 208]. In fact, DA itself could be an endogenous source of ROS [209, 210]. A defective sequestration of DA leads to

more ROS generation in the cytoplasm and DA-dependent oxidative stress [211, 212]. Hydrogen peroxide (H_2O_2), which is produced in the process of DA auto-oxidation, is inert and nontoxic, but in the presence of excessive free iron, it interacts with the reduced form of iron and decomposes to the highly reactive hydroxyl radical (·OH). This provides the basis of heterogeneous iron-induced neuronal degeneration in the SN. Recently, it has been reported that a proportion of SN neurons were more vulnerable to iron toxicity induced by loss of CP, although the precise underlying mechanisms are not known [132]. NM also contributes to the vulnerability of DAergic neurons to iron. NM could be considered as a double-edged sword [213–215]. For example, NM is a strong iron chelator and accumulates with aging, which has high or low affinity binding sites for iron [80, 216]. At lower iron concentrations, NM normally exerts protective action on DAergic neurons, but the protective effects are eliminated at higher iron concentrations, at which NM acts as a source of oxidative load rather than an iron chelator [180, 217, 218]. In vitro experiments also demonstrate that NM can inhibit iron-induced ·OH production generated by Fenton's reaction and play a protective role. However, in the presence of H_2O_2 and iron overload, NM shows a time-course degradation and subsequent iron release [219]. This may explain why NM concentration is significantly decreased in PD [220]. Although the NM concentration is the same in the SN and locus coeruleus (LC), LC neurons are not as vulnerable compared to those in the SN because the amount of iron in the LC is 7.9 % of that found in the SN [19]. This suggests that there is less iron mobilization in LC neurons. More recently, a targeted proteomics approach has revealed L-ferritin in NM granules and implicates iron storage and release from iron binding sites in neurons [81]. In addition, the extra-neuronal NM released by dying neurons is a strong candidate for causing chronic inflammation and cell death in the SN in PD [13, 210, 221].

The vulnerability of DAergic neurons, but not glia, to iron is also illustrated by degeneration of DAergic neurons, yet proliferation of astroglia in PD [222]. For example, in vitro studies have shown that when astrocytes, microglia, and neurons are isolated and subsequently treated with iron citrate, cell degeneration and death occur only in neurons and microglia, whereas astrocytes proliferate under the same conditions [223]. Although iron accumulates more in glial cells, iron-induced ROS levels in glia are less pronounced than in neurons [224]. Similarly, it was shown that, although iron accumulated to the same extent in primary cultured neurons, oligodendrocytes and astrocytes, only neurons and oligodendrocytes are affected by elevated intracellular iron [225]. Indeed, in primary astrocyte cultures, iron induces a transient increase in the intracellular ROS level before sufficient quantities of ferritin synthesis are available to store the elevated iron in a redox-inactive form [226]. In addition, HO-1, which is predominately expressed in astrocytes, is considered an antioxidant enzyme because of its ability to reduce intracellular stress. The binding capacity of ferritin also influences levels of unbound cytosolic iron. High levels of ferritin and the presence of strong antioxidants might contribute to the resistance of astrocytes to iron [139]. Somewhat surprisingly, iron may protect astrocytes from 6-OHDA-induced cytotoxicity [227], although the underlying mechanisms remain to be defined.

Biology of Iron in Pathogenic Mechanisms of PD

Iron and Oxidative Stress

Iron has strong redox activity in both its ferrous and ferric states. Indeed, it participates in Fenton chemistry, leading to a cycle between its 2 redox states and the generation of ·OH. ROS, in turn, act as secondary messengers, gene regulators, and cellular activation mediators. However, an imbalance between ROS production and antioxidant defense induces "oxidative stress," causing cell dysfunction and ultimately cell death [211]. The brain requires a high concentration of iron due to its role in energy metabolism, myelin formation, and neurotransmitter synthesis, including DA, norepinephrine, and serotonin [228]. Furthermore, brain cells are particularly sensitive to ROS assault because of their intense oxidative metabolism, high consumption of oxygen, and their propensity to generating large amounts of ROS.

The molecular mechanisms that lead to the degeneration of DAergic neurons in the SN of PD patients are still not fully elucidated. However, some evidence has revealed high levels of basal oxidative stress in the SN in normal brain, a phenomenon which is markedly increased in PD. In fact, DA metabolism, as described above, might be responsible for the high level of basal oxidative stress in the SN. In PD, as illustrated in Fig. 2, oxidative stress is thought to contribute to the cascade of neuronal degeneration, resulting from enhanced levels of redox-active iron within the SN [229, 230]. Increased brain iron likely induces a vicious cycle of oxidative stress, by increasing the levels of free iron, a process that involves releasing iron from ferritin, heme proteins (e.g., hemoglobin, cytochrome c), and iron-sulfur proteins [231]. Furthermore, DAergic neurons in the SN of PD patients have impaired mechanisms of eliminating free radicals (e.g., superoxide dismutase, glutathione peroxidase, catalase).

Mitochondria are the main site for ROS production and also the primary target of oxidative damage. Increased intracellular iron results in the subsequent formation of ROS, which leads to further damage to mitochondria. Elevated ROS and enhanced oxidative damage are key events in the pathogenic cascade that leads to cellular apoptosis and necrosis [232, 233]. In fact, mitochondrial disruption is thought to

be a common feature of many types of familial PD, as diverse PD-related genetic modifications disrupt mitochondrial function [234]. Indeed, a combination of an antioxidant and a mitochondrial complex II activator rescues mitochondrial disruption induced by genetic modification of α-synuclein, Parkin, and DJ-1 in *Caenorhabditis elegans* [234]. The disrupted Tf/TfR2-dependent system in PD results in iron accumulation in the mitochondria and subsequent enhanced oxidative stress [106] (Fig. 2).

The involvement of iron in oxidative stress is demonstrated in the 6-OHDA-induced PD rat model. It has been reported that 6-OHDA induces nigrostriatal DAergic lesions via the generation of oxidative stress both in vivo and in vitro [235, 236]. The mechanisms underlying 6-OHDA neurotoxicity come from its ability to carry out auto-oxidation. H_2O_2, the product of 6-OHDA auto-oxidation, participates in a Fenton reaction in the presence of iron. 6-OHDA can also release iron from its binding sites, similar to the action of ferritin [237]. This explains the protective effects of antioxidants and iron chelators against 6-OHDA toxicity.

Iron and UPS

Impairment of UPS, along with iron accumulation, in the SN has been implicated in the pathogenesis of PD. Excessive iron could indeed impair UPS [130]. Microinjection of the proteasome inhibitor lactacystin causes significant DAergic cell loss in the SN [5, 238, 239]. Desferrioxamine, as well as the brain-permeable iron chelators VK-28 and M30, are shown to exert not only neuroprotective but also neurorestorative effects against nigrostriatal degeneration induced by lactacystin [130, 238]. Moreover, genetic iron chelation protects against proteasome inhibition-induced DAergic neuron degeneration [240]. Thus, iron chelation may act through UPS to protect DAergic neurons.

Impairment of UPS, which is responsible for the degradation of IRPs, may also cause the accumulation of IRPs and iron accumulation [241]. DMT1 is also regulated posttranslationally by degradation via the proteasomal pathway, in which Parkin is responsible for the ubiquitination of the 1B species of DMT1 [49]. Loss of Parkin gene function results in elevated expression of DMT1 [49]. Evidence also showed that Ndfip1 could bind to DMT1 and mediate their ubiquitination in CHO cells [90]. However, the exact targeting specificity of Ndfip1 remains to be obtained until now [98]. Our results showed that Ndfip1 attenuated 6-OHDA-induced iron accumulation via regulating the degradation of DMT1 [242]. Thus, dysregulation of UPS and/or accumulation of iron may induce a vicious cycle in which UPS impairment causes an iron overload, while increased iron levels further aggravate UPS dysfunction.

Iron and α-Synuclein Aggregation

Aggregation of α-synuclein is a key event in PD. The misfolding and aggregation of α-synuclein, which may be resistant to ubiquitination, is proposed to contribute to the neurodegeneration of PD [243]. Mutations in the α-synuclein gene, A53T and A30P, have been identified in autosomal-dominant, early onset PD [244, 245]. Intriguingly, analysis of Lewy bodies in the parkinsonian SN reveals high levels of iron [246]. There is also accumulating evidence suggesting that iron plays an important role in modulating structural transformations, aggregation, and fibrillation of α-synuclein [247–249]. In fact, iron-induced α-synuclein oligomers form toxic species. These toxic α-synuclein oligomers, moreover, can form ion-permeable pores in planar lipid bilayers and give rise to α-synuclein-dependent toxicity in neuronal cells [247]. Interestingly, iron accumulates in high concentrations in the SN, which is the same region where aggregated α-synuclein accumulation occurs. Moreover, in in vitro studies, it was found that purified α-synuclein aggregates rapidly with application of ferrous [250] or ferric iron. Thus, the interaction between iron and α-synuclein might have important biological relevance to PD etiology. Recently, it has been indicated that human α-synuclein also acts as a cellular ferrireductase, responsible for reducing Fe^{3+} to bioavailable Fe^{2+} [251]. This provides the possibility that increased expression ofα-synuclein in PD could lead to the excess generation of Fe^{2+} and initiate oxidative damage through Fenton reaction [252]. Additionally, aggregation of α-synuclein would result in loss of ferrireductase activity and then enhance the accumulation of Fe^{3+} [252]. Another study showed that overexpression of human α-synuclein led to increased intracellular iron levels in neurons exposed to iron [253]. This provides evidence that α-synuclein might be involved in the iron accumulation that occurred in PD. Therefore, further studies should be elucidated to investigate the ferrireductase activity of α-synuclein and iron accumulation in PD in the future research. Analysis of the secondary structure of the α-synuclein transcript using computer-generated folding has revealed a single RNA stem loop formed by 46 nucleotides in the 5′-UTR. This stem loop is similar to that of the H-ferritin IRE, as well as the IREs in the 5′-UTRs of mRNAs encoding L-ferritin, Fpn, erythroid 5-aminolevulinate (eALAS), and mitochondrial aconitase. Interestingly, only human α-synuclein, rather than β- and γ-synuclein transcripts, has this loop motif [254]. One study has shown that the α-synuclein IRE from the shorter α-synuclein transcript confers desferrioxamine-dependent repression of a luciferase reporter gene in response to iron chelation in SH-SY5Y neuroblastoma cell lines [255]. However, this sequence has not yet been tested for the longer α-synuclein alternative spliced transcript, which deems it questionable as to whether it is functional. If, and how, the IRE-like structure in α-synuclein mRNA functions as a

posttranscriptional regulator will be important to determine. Our preliminary data show increased α-synuclein expression in cells containing an IRP knockdown, which is likely due to suppressed binding activity of α-synuclein IRE and IRP. This indicates that this particular IRE might be a functional one to bind with IRP. Recently, it was reported that iron overload could induce distinctive neuropathology and disease phenotypes in mutant alpha-synuclein expressing flies. They showed that iron treatment could induce a more severe motor decline in A53T and A30P mutant alpha-synuclein expressing flies than WT alpha-synuclein expressing flies. This suggested the possible interaction between mutant alpha-synuclein and iron [256].

Iron and Gene Mutation

Parkin, α-synuclein, LRRK2, PINK1, and DJ-1 are among several genes known to be linked to PD. In fact, it has been shown that there are relationships between genes involved in monogenetic PD and iron (see the discussion of iron and α-synuclein above). For example, mutations in Parkin, which mediates the degradation of a subset of cellular proteins, are currently recognized as the most common cause of familial Parkinsonism [257–260]. Iron induces alterations in Parkin solubility and results in its intracellular aggregation. The depletion of soluble, functional forms of Parkin is associated with reduced proteasomal activities and increased cell death [261]. In autosomal recessive juvenile parkinsonism (ARJP), of which the Parkin mutation was first identified in 1998, it is reported that iron staining in the SN is more intense than that of controls, as well as sporadic PD patients [262]. Thus, loss of the Parkin gene might lead to cellular iron accumulation. More recently, it was reported that Parkin is responsible for ubiquitination of DMT1+IRE. It is also relevant that overexpression of Parkin in SH-SY5Y cells results in decreased expression of 1B-DMT1 isoforms. Expression of DMT1+IRE is also shown to be elevated in human lymphocytes containing a homozygous deletion of exon 4 of Parkin and in the brains of Parkin knockout animals [49]. Thus, loss of Parkin gene function may cause intracellular iron accumulation. Therefore, the protective function of Parkin is compromised by iron (see Fig. 3). Hyperechogenicity of the SN has also been found to

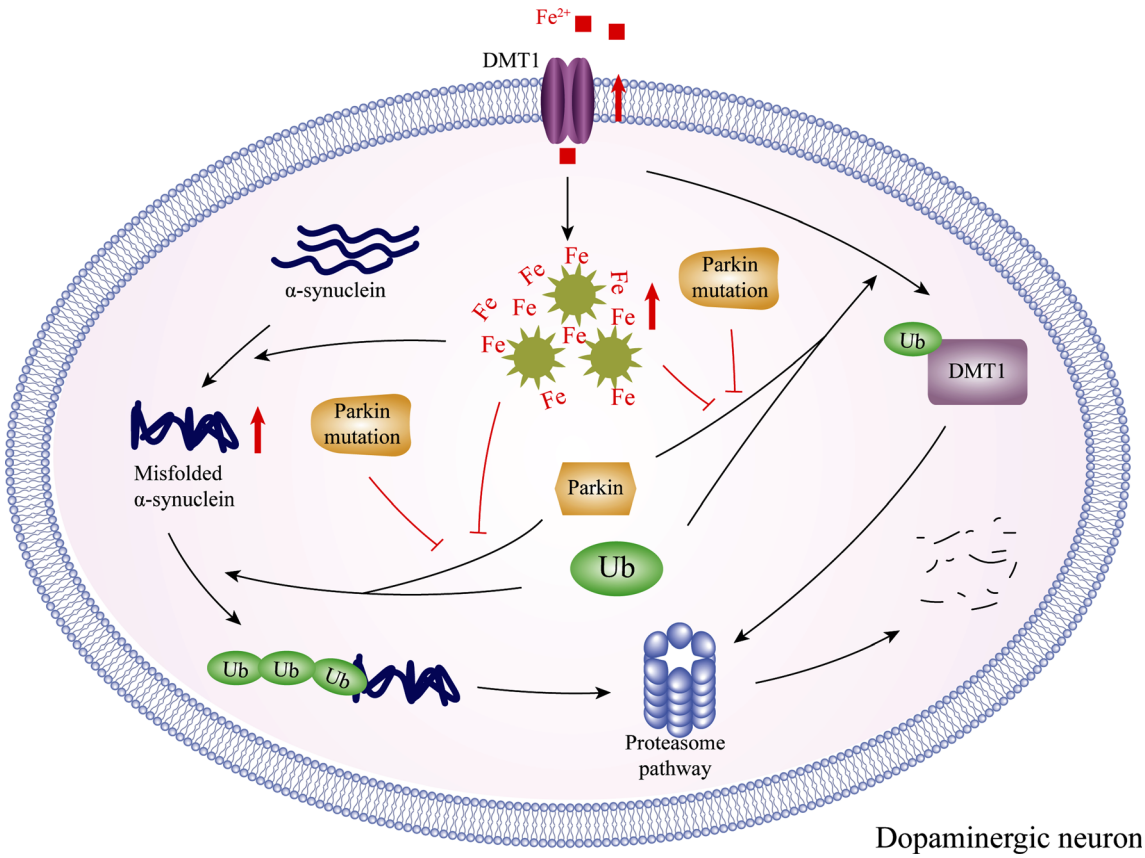

Fig. 3 Parkin mutation-related failure of protein ubiquitination in nigral DAergic neurons in PD. Parkin acts as an E3 ubiquitin ligase, which could tag specific proteins with ubiquitin and target them for destruction in the proteasome. α-Synuclein and DMT1 are the candidate targets of Parkin. Ubiquitin-tagged α-synuclein and DMT1 are then directed to the proteasome for degradation. Failure of Parkin-mediated degradation of α-synuclein and DMT1 may cause intracellular α-synuclein aggregation and increased DMT1 levels. The subsequent increased intracellular iron results in Parkin aggregation and function loss. *DAergic* dopaminergic, *DMT1* divalent metal transporter 1, *PD* Parkinson's disease

be a typical sign in idiopathic PD, and SN hyperechogenicity has been shown to be, at least in part, due to an increased iron level in this brain area [263, 264]. In a screen with PD patients having either α-synuclein, LRRK2, Parkin, PINK1, or DJ-1 mutations, it was found, using transcranial sonography, that all patients showed significantly larger echogenicity in the SN relative to healthy controls [173], indicating increased nigral iron levels. It will be important for future studies to clarify the role of iron in monogenetic PD.

We summarize the possible mechanisms underlying iron accumulation in nigral DAergic neurons and the subsequent neurodegeneration of these neurons in PD in Fig. 4.

Iron Chelation as a Therapy for PD

The role of iron in the pathology of neurodegenerative disorders points to iron chelation as a promising therapeutic strategy. Indeed, chelation of iron in vivo via ferritin, clioquinol, or restriction of dietary iron prevents iron-dependent oxidative insult, thereby protecting mice from MPTP-induced neurotoxicity [128, 265]. Similarly, animals deficient in iron, and those administered iron chelators, are resistant to MPTP, 6-OHDA, and kainate-induced toxicity [265–267]. Brain permeable iron chelators, which exert neuroprotective and neurorestorative effects, such as VK-28, M30, and VAR10303 (VAR), have been shown to treat PD animal models [130, 268]. More importantly, recent phase II clinical trial data showed that the iron chelator deferiprone improved motor symptoms of PD subjects. This provided strongest evidence that iron contributed to the pathogenesis of PD [82].

In recent years, extracts from natural foods have been shown to have iron-chelating effects. Examples of natural iron chelators include (-)-epigallocatechin-3-gallate (EGCG), the main polyphenol constituent of green tea, the polyphenolic flavonoid curcumin found in the rhizomes of *Curcuma longa* (zingiberaceae), myricetin (a type of flavonoid ubiquitous in fruits, vegetables, and herbs), ginsenosides, the principal active components of ginseng, and ginkgetin, a natural biflavonoid isolated from leaves of *Ginkgo biloba* L. In fact, these compounds have been known to have neuroprotective effects due to their iron-chelating properties [154, 156, 269–271]. Compared with agents that produce iron-chelating effects, only these natural compounds typically exert multiple effects against neurotoxicity. Therefore, it will be imperative to explore the possible use of low toxic natural compounds, particularly iron chelators, for use in the prevention and treatment of PD.

Despite these promising findings, severe iron restriction can lead to p53 (a tumor suppressor gene)-mediated neuronal apoptosis [265]. It also appears that the midbrain may be more susceptible to iron deficiency than other brain regions

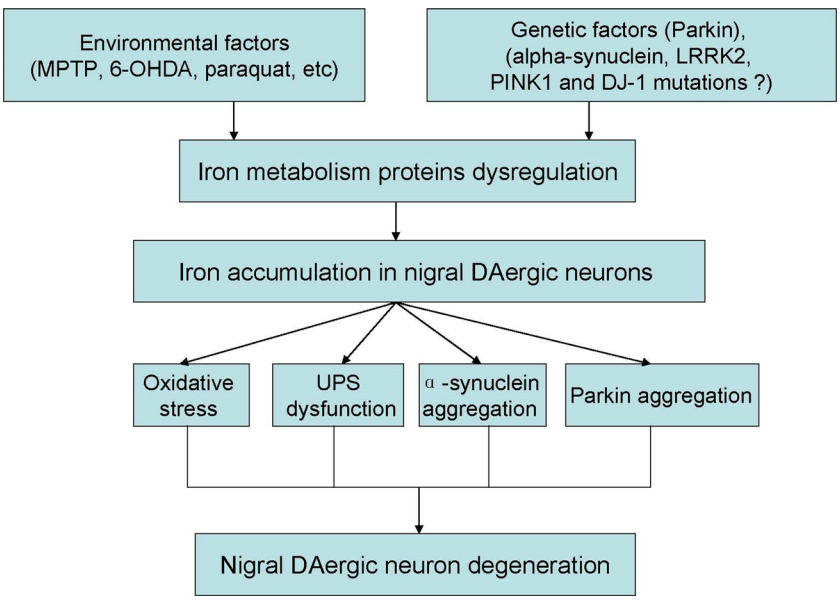

Fig. 4 A schematic diagram showing how iron accumulation in nigral DAergic neurons causes neuron degeneration in PD. Environmental and genetic factors act together to cause to a dysregulation of iron metabolism proteins, which results in iron accumulation in nigral DAergic neurons. Increased intracellular iron further promotes oxidative stress, UPS dysfunction, α-synuclein aggregation, and Parkin aggregation, which all lead to ultimate DAergic neuron degeneration (?: PD patients having either α-synuclein, LRRK2, Parkin, PINK1, or DJ-1 mutations showed significantly larger echogenicity in the SN relative to healthy controls, indicating increased nigral iron levels; it was speculated that this increased nigral iron levels might be due to dysregulation of iron metabolism proteins). *DAergic* dopaminergic, *PD* Parkinson's disease, *UPS* ubiquitin-proteasome system

[272–274]. For example, iron deficiency results in the impairment of tyrosine hydroxylase activity and reduces D1 and D2 receptors [275, 276]. Thus, while iron chelation may provide protection against PD, the impact of iron chelation on DA synthesis and neuronal survival should be considered to avoid toxic side effects [277], particularly when developing iron-chelating agents for chronic clinical application.

Conclusion and Perspectives

Brain iron metabolism dysfunction in PD has been extensively studied and is beginning to gain increasing interest as a novel clinical approach. Although several transporters and iron-related proteins are involved in iron-related neurodegeneration (as summarized in Fig. 4), it should be noted that many of the conclusions described herein are considered tentative at this point. We have recently reported a specific increase of iron levels in the SN of PD animal models [11]. Moreover, we showed that DMT1 expression is increased and expression of Fpn and HP is decreased, which may be responsible for nigral-specific iron accumulation [11, 63, 278]. The mechanisms for these changes are either IRE/IRP-dependent or IRE/IRP-independent due to different neurotoxin treatment. Moreover, some iron chelators, such as desferrioxamine, curcumin, myricetin, and Rg1, can protect DAergic neurons against neurotoxins.

The following is a list of research topics that may, in our opinion, represent future research directions:

1. It is worth exploring the changes in iron metabolism in patients with PD. It will be important to correlate iron metabolism with the onset, progression, and prognosis of PD.
2. To investigate the association of SNPs and possible mutations of genes involved in iron metabolism in patients with PD.
3. To develop noninvasive high-resolution imaging techniques to help detect early intracellular iron deposits in patients with PD and to track at-risk individuals.
4. Detailed research on the molecular regulation of iron accumulation and its toxicity is highly needed.
5. To identify targeted therapeutic strategies that prevent brain iron metabolism dysfunction in PD.

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Compliance with Ethical Standards

Competing Interests The authors declare that they have no competing interests.

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