Minireview *Highlight article*

Overdosing on iron: Elevated iron and degenerative brain disorders

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Impact statement

Brain degenerative disorders, which include some neurodevelopmental disorders and age-associated diseases, cause debilitating neurological deficits and are generally fatal. A large body of emerging evidence indicates that iron accumulation in neurons within specific regions of the brain plays an important role in the pathogenesis of many of these disorders. Iron homeostasis is a highly complex and incompletely understood process involving a large number of regulatory molecules. Our review provides a description of what is known about how iron is obtained by the body and brain and how defects in the homeostatic processes could contribute to the development of brain diseases, focusing on Alzheimer's disease and Parkinson's disease as well as four other disorders belonging to a class of inherited conditions referred to as neurodegeneration based on iron accumulation (NBIA) disorders. A description of potential therapeutic approaches being tested for each of these different disorders is provided.

Abstract

All cells in organisms ranging from yeast to humans utilize iron as a cofactor or structural element of proteins that function in diverse and critical cellular functions. However, deregulation of the homeostatic mechanisms regulating iron metabolism resulting in a reduction or excess of iron within the cell or outside of it can have serious effects to the health of cells and the organism. This review provides a brief overview of the molecular and cellular mechanisms regulating iron physiology, including the molecules and processes regulating iron uptake, its storage and utilization, its recycling, and its release from the cell, such that the cellular iron levels are sufficient to meet metabolic demand but below those that cause permanent damage. The major focus of review is on the pathological consequences of dysregulation of these homeostatic mechanisms, focusing on the brain. Current advances on the role of iron accumulation to the pathogenesis of rare neurological disorders caused by genetic mutations as well as to the more prevalent and age-associated neurodegenerative diseases are described.

Keywords: Alzheimer's diseases, Parkinson's disease, neurodegenerative disease, neurodegeneration with brain iron accumulation, iron transport, iron homeostasis

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Introduction

Iron is a critical metal that acts as an electron donor and acceptor in a plethora of fundamental cellular processes including oxygen transport, cellular respiration, energy metabolism, DNA synthesis, and cell proliferation.[1,2] Not surprisingly, iron deficiency can have serious effects on fetal and postnatal development and through adulthood, particularly on organs such as the brain the proper functioning of which critically dependent on it. On the other hand, a major consequence of elevated iron is impairment of the mitochondrial respiratory chain leading to an abnormal increase in the production of excessive levels of reactive oxygen species (ROS), including hydroxyl radical (OH^-), hydrogen peroxide (H_2O_2), and other highly reactive radicals which damage proteins, lipids, and nucleic acids generally resulting in the death of the cell.[1,2] Although the most abundant metal in the brain, the brain is also the organ that is most vulnerable to an elevation of iron, in part because of its high utilization of oxygen for cellular respiration. A compelling body of evidence suggest that

deregulated iron homeostasis leading to its accumulation is at least a key contributor to disorders of the developing and adult brain. In this review, we summarize the molecules and mechanisms involved in the transport of iron and the homeostatic mechanisms involved in keeping it at physiological levels. We then describe how deregulation of iron accumulation contributes to neurodevelopmental disorders and neurodegenerative diseases.

Iron transport and homeostasis

Iron transport

In humans, all iron is obtained from diet where it exists in a heme or non-heme form. Heme-iron comes mostly from the breakdown of hemoglobin and myoglobin in animal products, whereas non-heme iron is derived from plant as well as animal products. Although heme-iron is more bioavailable than non-heme iron, non-heme iron represents over 85% of dietary iron in humans. Dietary iron exists largely in two valency forms: the reduced, reactive, and toxic ferrous ion (Fe^{2+}) form present in heme-iron and the oxidized and relatively non-toxic ferric ion (Fe^{3+}) form in non-heme iron.[3–5] Both heme and non-heme are taken up by enterocytes lining the intestinal brush border.[3,4] Non-heme iron is transported through the apical membrane of enterocytes through DMT1 (divalent metal transporter-1), a transmembrane transporter (Figure 1).[3,5–7] Transport through DMT1 requires reduction of Fe^{3+} to Fe^{2+} which is performed by duodenal cytochrome B (Dycb), a ferrireductase also localized at the apical membrane of the enterocytes.[8] Because iron absorption in Dycb knockout mice is normal, it is believed that there are other intestinal ferroreductases but these remain to be identified.

The identity of the intestinal transporter for heme-iron has yet to be resolved. One candidate is heme-responsive gene-1 (HRG1), better known for its role in transporting iron within macrophages, but that is also expressed by enterocytes. Following transport into the enterocyte, heme-iron binds to hemeoxygenase (HO) localized to the cytosolic side of smooth ER which catalyzes the release of Fe^{2+} (Figure 1).[9] Whether originating from heme-iron or non-heme iron, in the cytoplasm, Fe^{2+} is bound by iron-binding proteins, of which ferritin is the most abundant.[10] Ferritin is composed of two proteins, ferritin-light chain (L-ferritin) and ferritin-heavy chain (H-ferritin), that assemble into a hollow, spherical protein cage capable of storing Fe^{2+} atoms within it.[10,11] Sequestration in the ferritin cage requires the oxidation of Fe^{2+} to Fe^{3+} which is mediated through ferroxidase activity that H-ferritin possesses.

Fe^{2+} is transported to subcellular locations for use or to the basolateral side for export by cytosolic chaperones, the best characterized of which is PCBP2, a member of the poly (rC)-binding protein (PCBP) family of metallochaperones (Figure 1).[12,13] Export at the basolateral membrane is mediated by ferroportin-1 (FPN1), the only known exporter of iron in all mammals cells.[14,15] Efficient export of Fe^{2+} through FPN1 requires its oxidation back to Fe^{3+} by hephaestin (HEPH), a soluble ferroxidase secreted by the liver[16–18] (Figure 1). Whereas HEPH is the major ferroxidase in enterocytes, another ferroxidase called ceruloplasmin, synthesized and secreted almost exclusively by the liver and abundant in plasma, also participates in the oxidation of Fe^{2+}.[19–21]

Upon export into interstitial fluid, Fe^{3+} is bound by transferrin (Tf).[3–5] Tf-bound Fe^{3+} binds to the transferrin receptor-1 (Tfr1) at surface of endothelial cells lining blood

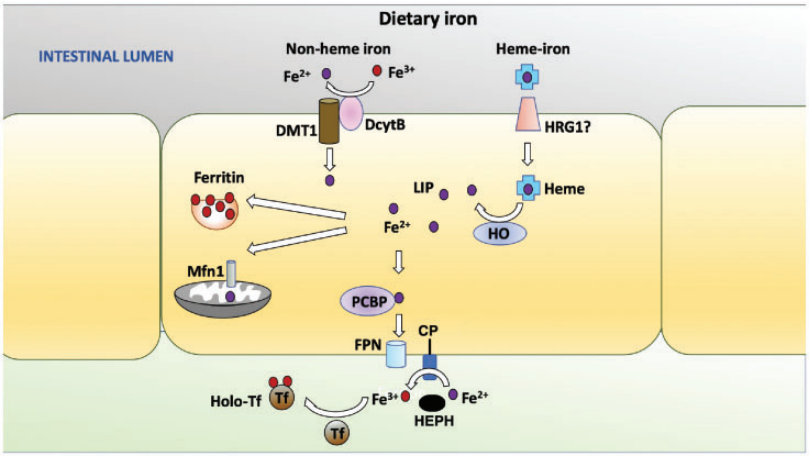

Figure 1. Uptake and release of iron from intestinal enterocytes. Non-heme iron (free Fe^{3+} or loosely bound to small molecules) is transported through DMT1 after its reduction to Fe^{2+}. Heme-iron (composed of Fe^{2+}) is transported across the apical membrane of intestinal enterocytes through a transporter that has not been firmly identified (HRG1is a candidate). Degradation of heme-iron by HO releases Fe^{2+} in the cytosol. Fe^{3+} in non-heme iron is reduced to Fe^{2+} by DcytB localized at the apical membrane and transported through the membrane by DMT1. As in all cell types, Fe^{2+} is stored in the cytosol as a complex with ferritin (as Fe^{3+} following oxidation by H-ferritin), transported into mitochondria through the Mrfn1 transporter, incorporated into iron-requiring enzymes or proteins, or utilized for other cellular purposes. Within enterocytes, transport proteins such as PCBP deliver Fe^{2+} to the basolateral side of the cell where it is released into the interstitial fluid by the FPN1 transporter. Release by FPN1 involves oxidation of Fe^{2+} to Fe^{3+} which is mediated by liver-secreted HEPH or ceruloplasmin (CP). In some cell types, CP is synthesized as a GPI-linked protein as shown in the figure. Fe^{3+} in interstitial fluid is immediately bound by transferrin, a highly abundant iron transport protein. Fe^{3+}-bound Tf is taken by the endothelial cells of blood vessels which vascularize the microvilli and then delivered to cells that need iron. Most Fe^{3+}-bound Tf is used by erythrocyte precursor cells for the production of heme that becomes part of hemoglobin within RBCs upon differentiation of the precursors. In some cell types. (A color version of this figure is available in the online journal.)

vessels after which it is endocytosed as a complex with Tfr1. Fe^{3+} disassociates from transferrin because of endosome acidification and is exported into the cytosol by transmembrane DMT1 after reduction to Fe^{2+} a ferric reductase, STEAP3 (six-transmembrane epithelial antigen of prostrate-3).[3–5] From the cytosolic pool referred to as labile iron pool (LIP), Fe^{2+} is transported to different cellular locations for metabolic functions, with much of it going to the mitochondria through the inner membrane proteins mitoferrin-1 or mitoferrin-2 (Mfrn-1/2).[22,23] Excess Fe^{2+} is pumped out of the cell through FPN1 via oxidation to Fe^{3+} by ceruloplasmin.[24] In addition to its ferroxidase function, ceruloplasmin stabilizes FPN1 at the plasma membrane promoting iron efflux into portal blood and facilitates the loading of Fe^{3+} onto transferrin.[19,25] In contrast to HEPH which is soluble, several cell types produce ceruloplasmin as a GPI-anchored protein that localizes to the plasma membrane where it associates with FPN1.[19,26,27]

Iron exported from blood vessels is taken up by other cell types, an overwhelming majority of which utilize the Tf-Tfr1 system for iron import.

Iron homeostasis

Several mechanisms regulate iron metabolism normally or in response to pathophysiological conditions, such as iron overload, hypoxia, and inflammation.[5] Cells of the intestinal epithelium exfoliate when the body requires low iron reducing the transport of iron to cells of the body.[28] Internalization of the Tf-Tfr1 complex in endosomes is under control of intracellular iron levels and can be regulated.[29,30] Absorption of iron from the intestinal lining or its release is controlled by hepcidin, a small peptide produced in the liver, that acts as the ligand for FPN1.[31,32] Binding of hepcidin to FPN1 at membrane of intestinal enterocytes and macrophages causes the internalization of FPN1 and its degradation resulting in reduced release of iron into body fluids[33–35] (Figure 2(a)). The synthesis of hepcidin decreases during iron deficiency and is increased by elevation of stored iron.[33,34] Downregulation of ceruloplasmin production is another known mechanism of iron homeostasis resulting in reduced cellular iron export.

A well-characterized mechanism of cellular iron homeostasis is the upregulation of ferritin and downregulation of Tfr1 mRNA translation in response to increasing level of intracellular iron (Figure 2(b)). Both effects depend on two iron regulatory proteins (IRP1 and IRP2) that bind to RNA hairpin structures called iron-responsive elements (IREs) in the untranslated regions (UTRs) of target mRNAs resulting in effects on the stability or translation of the mRNA. When intracellular iron is high, IRP-1 adopts a conformation that is incapable of binding the IRE, whereas IRP2 is degraded. Thus, the IRE is unbound under high-iron conditions. When iron is low, however, IRP1 acquires the conformation for IRE binding and IRP2 expression is elevated permitting either of these IRPs to bind the IRE (Figure 3(b)). The ferritin mRNA contains an IRE in the 5′-untranslated region (UTR), whereas in the Tfr1 mRNA, multiple IREs are located within the 3′ UTR.[35–37] Binding of IRPs to ferritin mRNA causes a translational block resulting in reduced synthesis and hence decreased storage, whereas binding of IRP to the TfR1 mRNA protects it from degradation by endonucleases resulting in increased expression of TfR1 and

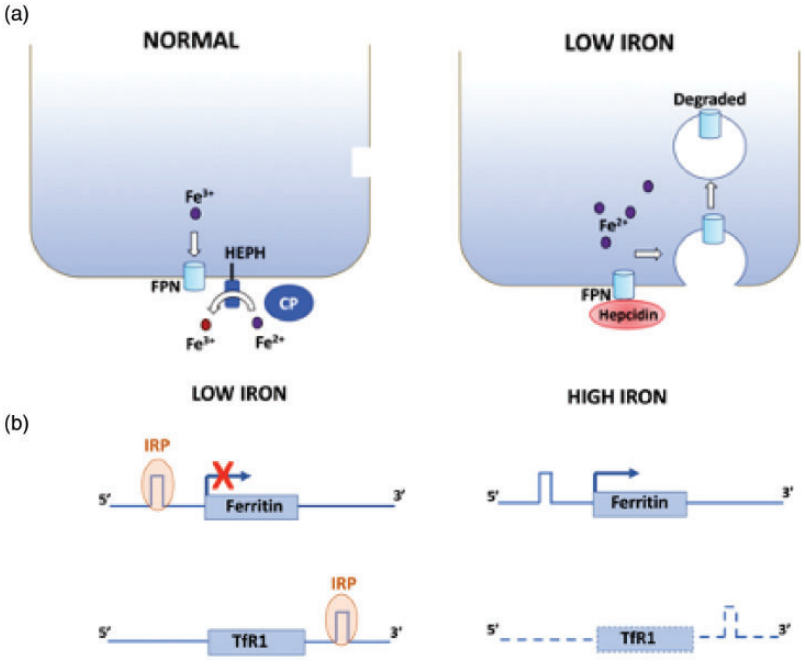

Figure 2. Mechanisms of iron homeostasis. Two major mechanisms are shown. (a) Under normal conditions, iron is exported out of the cell through FPN1, a process that is facilitated by HEPH or ceruloplasmin, which oxidize $Fe^{2}+$. When iron is low, liver-secreted hepcidin binds to FPN1 causing its internalization and degradation. (b) When intracellular iron is low, IRPs bind to the IRE localized at the 5′ UTR of the FPN1 mRNA and at the 3′ UTR of the TfR1 mRNA. Binding to the 5′ UTR IRE of ferritin mRNA results in a block in its translation reducing its intracellular levels when iron storage is not required. In contrast, binding to the 3′ UTR of TfR1 mRNA stabilizes it resulting in increased import of iron into the cell. (A color version of this figure is available in the online journal.)

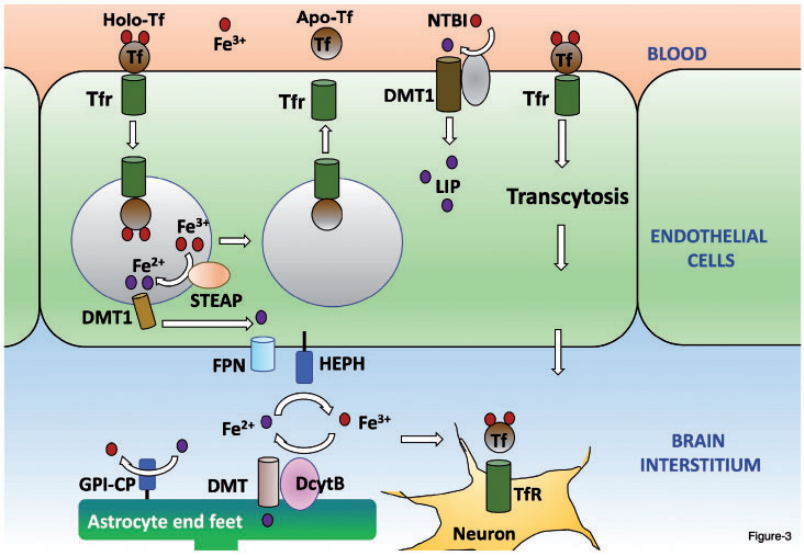

Figure 3. Delivery to iron from blood vessels of the BBB to the brain. Iron-bound transferrin (holo-Tf) binds to the transferrin receptor (Tfr). The holo-Tf-Tfr1 complex is internalized in endosomes and the acidification of the endosome causes the release of Fe^{3+} from Tf. The Tf-Tfr1 complex is recycled to the membrane where Tf dissociates from Tfr1 (apo-Tf) and is available for binding to iron. The Fe^{3+} is reduced to Fe^{2+} within the endosome by STEAP before it is transported out into the cytosol by DMT. Fe^{2+} is oxidized by HEPH at the abluminal membrane before it is transported out of the endothelial cell by the FPN1 transporter into the interstitial fluid of the brain. It has been proposed that the Tf-Tfr1 complex can translocate through the apical membrane and be exported out of the abluminal membrane without being internalized in an endosome. Since transferrin is not present at saturating levels, iron exists in the brain parenchyma as NRBI. Neurons take up Fe^{2+} through the Tf-Tfr1 system, whereas astrocytes, which lack Tfr1 uptake NTBI through DMT1. (A color version of this figure is available in the online journal.)

higher iron uptake. When iron is low however, translation of ferritin is blocked, while the stabilized TfR1 mRNA allows its translation permitting efficient iron entry into the cell.[35–37] The mRNAs of other RNA transport proteins, including FPN1 and DMT1, also contain IREs and are regulated by the IRE/IRP system[35–38] such that when iron is high FPN1 expression is increased due to stabilization of its mRNA, whereas DMT1 expression is reduced via a translation block. These changes increase iron efflux while reducing its influx, respectively.

In response to pathogenic challenge, the expression of hepcidin is induced in macrophage within the site of inflammation resulting in higher uptake of iron. This lowers iron levels in serum depriving pathogens, which depend on iron for their growth, of it.[33,34,39]

Brain iron transport

Iron transport to the brain

After the liver, which serves as a major storehouse of iron within the body, the brain is the organ with the highest content of iron. In the brain iron is essential for a number of important processes, including energy production, myelination, neurotransmitter synthesis and metabolism, and synaptic activity.[40–45] Given its importance to such diverse and important functions, the level of iron in the brain has to be carefully regulated such that there is neither a deficiency nor excess. Iron homeostasis in the brain is regulated separately from the periphery and hence alterations in peripheral iron do not impact the level of iron in the brain. Much of the brain's homeostatic regulation occurs at the level of the blood–brain barrier (BBB), formed by endothelial cells of the capillary wall have tight junctions between them and with pericytes in their basement membrane. Along with the end-feet of astrocytes that cover the endothelial cell membrane, the BBB is semi-permeable barrier separating circulating blood in the brain from the cerebrospinal fluid and brain parenchyma.[46,47] The mechanism by which iron is transported across the endothelial cell lining of BBB vasculature is not well understood. There is some consensus that, as in most other cell types, Tf-bound Fe^{3+} in blood binds to Tfr1 at the apical side of the endothelial cells, is internalized in endosomes, and following conversion to Fe^{2+} is released to the cytosol, after which it is exported on the abluminal side of the endothelial cells by FPN1 (Figure 3). Re-oxidation to Fe^{3+} is performed primarily by soluble ceruloplasmin. Although abundant in serum, liver-secreted ceruloplasmin does not cross the BBB in a healthy brain. Much of the ceruloplasmin in the brain interstitium is synthesized and released by the choroid plexus. In addition to the soluble form, astrocytes (and many cell types outside the brain) synthesize ceruloplasmin in a GPI-anchored form that localizes at their end-feet which is in contact with the FPN1 on the abluminal side of the endothelial cells.[26,48–51]

Another mechanism that has been proposed for transport of iron across the endothelial cells of the BBB is the translocation of the internalized endosome containing the iron-bound Tf-Tfr1 complex across the cytosol and fusion with the abluminal membrane resulting in the release of iron disassociated from Tf-Tfr1 by exocytosis[51,52]. Yet, another model posits that rather than being transported in the endosome, the iron-bound Tf-Tfr1 complex travels from the apical membrane to the abluminal membrane by transcytosis where iron-bound Tf is released[49, 53,54] (Figure 3). Besides its transport out of the blood vessels of BBB, iron

enters the brain interstitium through the epithelial cell lining of the choroid plexus, which has a more permeable than the lining of the BBB.[55,56] The choroid plexus expresses the major components for iron transport including high levels of both Tfr11and DMT1 as well as FPN1, DcytB, STEAP3 and FPN1 and, as described above, ceruloplasmin.[57,58]

Iron transport within the brain

Much of the iron released from endothelial cells of the BBB is taken up by the end-feet of astrocytes and then made available to neurons and other cells types via release in the brain parenchyma[59,60] (Figure 3). Because they express ferritin poorly, astrocytes are unlikely to store much iron.[61–63] Fe^{3+} released by astrocytes or the endothelial cells of the BBB is bound by Tf synthesized and secreted predominantly by the epithelial cells of the choroid plexus.[55,60,64,65] Although oligodendrocytes also synthesize Tf and may represent another potential source, it is suggested that oligodendrocyte-synthesized Tf lacks the signal peptide for secretion.[55,66,67] In contrast to the periphery, however, where Tf is in vast excess, in the brain interstitial fluid, Tf levels are much lower and completely saturated with iron.[50,68] Some iron is bound by ferritin, heme, and albumin. But a substantial portion of the iron is unbound and referred to as non-transferrin-bound iron (NTBI). NTBI is loosely bound to small molecules including citrate, acetate, ascorbate, or ATP released by astrocytes. Therefore, while NTBI is not present in plasma of healthy individuals because of the saturating amounts of Tf, it is a normal component of brain interstitial fluid and CSF and is the source of iron for most brain cell types.[69] While uptake of Tf-bound iron occurs through the Tf-Tfr1 system, as in other most cells of the body, NTBI is transported into brain cells by DMT1 although the ferrireductases involved in reducing Fe^{3+} to Fe^{2+} have not yet been firmly identified. Interestingly, recent studies have shown that the prion protein (PrP), mutations of which causes prion disease, is also involved in transporting NTBI, at least in the liver, kidney, and neuroblastoma cells, by acting as a ferrireductase partner for ZIP14 and DMT1.[70–73] As discussed later in this review, α-synuclein, mutations of which cause Parkinson's disease, also possesses ferrireductase activity and promotes Fe^{2+} influx.[74–77]

Neurons and microglia internalize iron from interstitial fluid through the classic Tf-Tfr1 pathway.[60,78,79] Neurons also take up iron through another protein called lactoferrin (Lf), which besides binding iron (Fe^{3+}) has other functions including antimicrobial and anti-inflammatory activities.[80] In the brain, Lf is synthesized mostly by microglia and generally under conditions when microglia are activated.[81] Fe^{3+}-bound Lf binds to the Lf receptor (LrF) expressed by neurons and astrocytes and is internalized by receptor-mediated endocytosis. Expression of LfR in neurons is elevated in disease states.[82,83] Several other NTBI transporting proteins have been identified and are expressed by different types of neurons, including the Zrt-Irt-like protein 8 (ZIP8) and ZIP14 transporters and L-type, T-type, and TRPC (transient receptor potential canonical) calcium channels. Therefore, for example, while ZIP8 is the predominant transporter of NTBI in hippocampal neurons, in retinal neurons, it is both ZIP8 and ZIP14.[84–86] Ceruloplasmin stabilizes FPN1 in neurons ensuring efflux of excess iron. FPN1 in neurons is also stabilized by amyloid precursor protein (APP) although in contrast to ceruloplasmin and HEPH, it is thought that APP lacks ferroxidase activity.[87]

The mechanism for iron uptake by astrocytes has not been resolved. Although many studies have reported that astrocytes do not express Tfr1, some recent studies have detected Tfr11 in cultured astrocytes and *in vivo*.[88–91] Binding of Tf to Tfr11 and transport of iron has also been described, at least in cultured astrocytes.[31,91,92] It is likely that most iron uptake by astrocytes involves internalization of NTBI through DMT1 which is highly expressed by astrocytes.[43,93–95] Additionally, ZIP14 expressed in astrocytes and has been shown to take up Fe^{3+}.[96] Finally, uptake by astrocytes through TRPC channels has been proposed.[43,97] As in all other cell types in the body, astrocytes release iron through FPN1. However, at least in cultured astrocytes, FPN1 is directly bound by GPI-linked ceruloplasmin and ceruloplasmin is required for the efflux of iron through FPN1.[51]

While having a high iron content, oligodendrocytes do not express Tfr11 and exactly how iron (which is necessary for myelination) is taken up by these cells is not clear. Internalization in the form of NTBI through DMT1 is one likely mechanism. Oligodendrocytes may also take up iron as a complex with ferritin by binding to a receptor called TIM2 (T cell immunoglobulin domain 2 protein) followed by endocytosis of the iron-bound ferritin/TIM2 complex.[98,99] The ferritin/TIM2 system of iron uptake has been described in cell types outside the brain.[100] Another difference between the brain cell types with regard to iron is while astrocytes use ceruloplasmin, oligodendrocytes use HEPH.[51,101] Like ceruloplasmin, HEPH is thought to stabilize FPN1 in oligodendrocytes.

While widely distributed in the brain, the concentrations of iron in various brain regions vary substantially.[102] Most iron is concentrated in the substantia nigra (SN), globus pallidus, and locus coeruleus. At the cellular level, oligodendrocytes contain the highest amount of iron. The level of iron increases in the normal adult brain as a function of age.[103] Indeed, it has been suggested that iron accumulation is directly proportional to aging.[13] Interestingly, the increase is not uniform across brain regions. For example, whereas in individuals over 80 years of age iron deposition increases in the SN and globus pallidus, there is no increase in the LC.[104,105] Besides the increase itself, during aging, iron is converted from its stable and non-toxic ferritin-bound form to hemosiderin and other derivatives in which iron is more reactive.[105,106] At the cellular level, although oligodendrocytes contain more iron that other brain cell types, this does not change during aging.[105,107] The molecular mechanisms underlying the differences in iron concentration and in their age-related changes are unclear but likely reflect differences in expression of uptake and storage proteins as well as of iron uptake and usage mechanisms.

Deregulated iron homeostasis and neurodegenerative disorders

Deregulation of iron metabolism, its redox activity, or its ability to transition in valency between the Fe^{2+} and Fe^{3+} state can have serious consequences to neuronal health and survival, and consequently to brain function.[13] In addition to the well-established damaging effects of free radicals on cellular component, iron accumulation/deposition harm the functioning of neurons and other brain cell types through abnormal interactions with proteins, the facilitation of protein aggregation, and disruption of spatially dependent processes, such as axonal transport, release of neurotransmitters, neurotrophic factors and cytokines, and synaptic activity. There are two categories of neurodegenerative disorders to which elevated brain iron is responsible for, or to which it likely contributes. One of these disease categories is neurodegeneration with brain iron accumulation (NBIA) disorders, a group of monogenic inherited disorders characterized by iron deposition in the brain. The second category is composed of a various age-associated neurodegenerative diseases, including Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), Friedreich's ataxia, ALS, and stroke (for recent reviews, see literature[13,81,108–111]). We review these two types of iron-associated CNS diseases separately.

Age-associated neurodegenerative diseases

Although there is general consensus that brain iron levels are elevated in several age-associated neurodegenerative diseases, the extent to which this abnormality contributes to neurodegeneration has yet to be resolved. Iron accumulation could promote neuronal loss by different mechanisms. For example, a neuropathological hallmark of many neurodegenerative diseases is the misfolding, fibrillization, and aggregation of specific proteins.[112] Iron is often a component of these disease-associated protein aggregates and in the case of some such proteins directly induces their misfolding, fibrillization, and aggregation.[113] Another common denominator of age-associated neurodegenerative diseases is oxidative stress. The brain is particularly vulnerable to mitochondrial dysfunction and the generation of toxic free-radicals. A form of iron-dependent cell death that has been described relatively recently is ferroptosis, which is characterized by lipid peroxidation resulting in membrane rupture.[114–116] Because of their unique structure composed of axons and dendrites and consequently high plasma membrane composition, neurons could be particularly sensitive to ferroptosis.[117,118] Ferroptotic death has been detected in a variety of neurodegenerative diseases.[49,118,119] Ferroptosis is often triggered by reduced activity of glutathione peroxidase (GPX4), an enzyme that utilizes glutathione (GSH) as reducing substrate to detoxify highly reactive hydroxyl radical and peroxyl radicals that are produced through the Fenton reaction catalyzed by Fe^{2+}. GSH synthesis, and hence GPX4 activity, depends on the import of cystine through the system Xc- transporter. With a reduction of GPX4 activity, or when the activity of the system Xc-transporter is reduced or inhibited (which results in decreased GSH), harmful levels of hydroxyl, peroxyl radicals, and other ROS accumulate within the cell. Ultimately, peroxidation of membrane lipids results in the rupture of the plasma membrane killing the cell (Figure 4). Ferroptosis can also be triggered by a large or sustained increase in Fe^{2+} which overwhelms the capacity of GPX4 to reduce ROS and prevent lipid peroxidation. Although GPX4/glutathione activity was believed to be the only defense against ferroptosis, a recent study

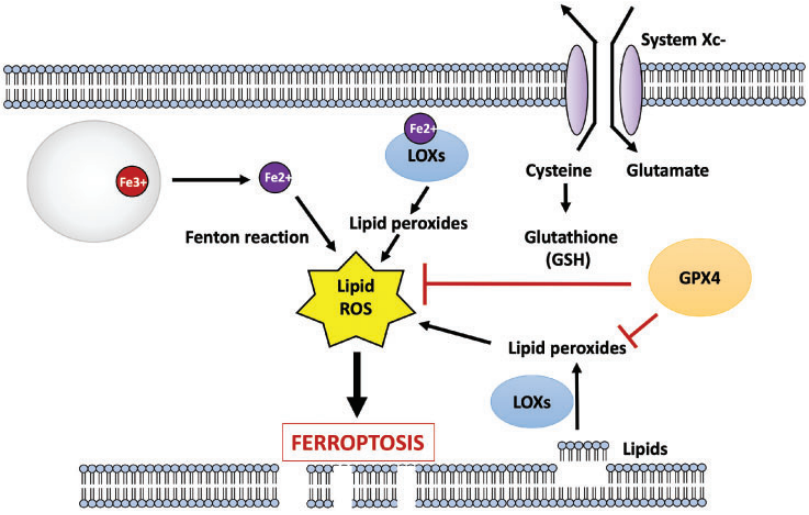

Figure 4. Iron, lipid peroxidation and ferroptosis. Following endocytosis of apo-Tf-Tfr1 complex and the disassociation of Fe^{3+}, the Fe^{3+} is reduced to Fe^{2+} and transported to the cytoplasm. Although not shown in the figure, Fe^{2+} can also accumulate through import of NTBI via DMT1. Fe^{2+} can produce toxic hydroxyl ion and lipid peroxides through the Fenton reaction. Elevated Fe^{2+} can also catalyze lipid peroxidation by combining with cytosolic lipoxygenases (LOXs). Peroxidation of membrane lipids damages the plasma membrane rupturing it. Ferroptosis is normally inhibited by glutathione peroxidase (GPX4), which uses glutathione as its substrate to detoxify lipid peroxides, hydroxyl ions, and other ROS by their reduction. Glutathione (GSH) synthesis is critically dependent on the proper functioning of the transporter, system Xc-, which imports cystine which, after reduction to cysteine, is used to produce glutathione (GSH). Although most often resulting from reduced activity system Xc- and/or GPX4/GSH, disruption of iron homeostasis leading to increased free iron can overwhelm the protective activity of GPX4/GSH to promote ferroptosis. (A color version of this figure is available in the online journal.)

described that reduction in the activity of another protein, ferroptosis suppressor protein-1 (FSP1), can also trigger ferroptosis acting in a GSH-independent manner.[120] This study showed that FSP1 acts downstream of iron to inhibit lipid peroxidation to suppress ferroptosis.[120] Iron can also induce death of neurons through apoptosis, a form of cell death triggered by the release of cytochrome c from mitochondria and subsequent activation of caspase-3 or by the activation of caspase-8 through binding of ligands, such as TNF or FasL, to their receptors at the plasma membrane.[121–123]

Although most effort has been placed on the effects of iron on neurons, it is now well-accepted that dysfunction of glial cells makes key contributions to the pathogenesis of neurodegenerative disorders.[124–127] Iron is required for the proper development of oligodendrocytes and a high amount of ferritin-bound iron is necessary for synthesis of myelin and fatty acid synthesis.[128] Abnormalities in oligodendrocytes, including effects on the production and maintenance of myelination, are a feature of various neurodegenerative diseases.[129–132] Breakdown of myelination has been described in different neurodegenerative disease.[133–136] In addition to a direct effect on the functioning of neurons, breakdown of myelin leads to a leakage of iron that not only damages oligodendrocytes (resulting in further myelin destruction) and other cell types, but also promotes the fibrillization of disease-causing proteins, such as α-synuclein and Aβ.[134,135,137,138] Additionally, the released iron can be taken up by macrophage and microglia leading to their activation. This has been shown to be the case in multiple sclerosis (MS).[133] Neuroinflammation, involving abnormal activation of astrocytes and microglia, is believed to contribute to neuronal loss in several neurodegenerative diseases through the release of toxic cytokines.[139–141]

There is broad consensus that deregulation of iron homeostasis is involved in the pathogenesis of different neurodegenerative diseases, although whether it triggers disease mechanisms, is a contributor to disease pathogenesis, or is a consequence of pathophysiological alterations that trigger the disease remains to be determined for each disease. The finding that healthy rats fed with a high-iron diet display neurodegeneration is consistent with a causal role for elevated iron in neurodegenerative diseases.[142] Moreover, mutations and deregulated expression of specific proteins regulating iron homeostasis can directly cause disorders with neurodegeneration, as observed in NBIA disorders (see below).

Although a role for iron dyshomeostasis has been suggested to be involved in several neurodegenerative diseases, this review focuses on AD and PD where this issue has been most studied, and the results are most convincing. AD and PD are also the two most prevalent neurodegenerative diseases.

Parkinson's disease. PD is the most common movement disorder and the second most prevalent neurodegenerative disease, afflicting ~1% of individuals over 65 years of age. The disorder, clinically characterized by bradykinesia, resting tremor, postural instability, and gait and balance issues, results from the selective and progressive degeneration of dopaminergic neurons in the substantial nigra (SN).[143–146] In addition to the motor deficits, cognitive decline is also observed in a subset of patients and this is believed to be due to neuronal loss in other brain areas, of which the cortex and other basal ganglia areas are predominant.[146–148] Although the reason for selective vulnerability of SN dopaminergic neurons in PD remains unresolved, one widely accepted factor is the normally high level of oxidative stress in these neurons resulting from the degradation of dopamine by monoamine oxidase (MAO) generating H_2O_2, which in the presence of iron generates toxic hydroxyl radicals and other oxidative species which damage cellular macromolecules.[149,150] Dopamine can also be non-enzymatically oxidized in the presence of oxygen to yield harmful metabolites and free radicals.[149,150] Additionally, metabolites of dopamine oxidation form adducts with cysteine and other amino acid residues that can be highly harmful to the cell.[151,152] As explained below, cysteine is necessary for the synthesis of glutathione (GSH), a potent intracellular antioxidant protects against the build-up of ROS and lipid peroxidation. Dopaminergic neurons of the SN also have high levels of iron under normal conditions rendering them particularly vulnerable to harmful levels of oxidative stress. Another unique feature of most dopaminergic neurons of the SN is that they also contain high levels of neuromelanin, a dark granular pigment that binds Fe^{3+} with high affinity.[104,150,153–155] While neuromelanin can sequester iron preventing toxicity, its levels decrease during aging and such an decrease would leave iron unsequestered.[156] In addition to the age-associated reduction, neuromelanin levels are further reduced in the SN of PD patients. While neuromelanin plays a protective role by trapping iron, this same property also concentrates iron in dopaminergic neurons, which can potentiate deregulated oxidative stress resulting from an imbalance of other mechanisms such as dopamine oxidation or GSH synthesis.[157] Other studies have described that when Fe^{3+} is in excess, neuromelanin can itself enhance hydroxyl radical production and oxidative stress, possibly by converting Fe^{3+} to Fe^{2+}.[158–160] These findings provide a plausible explanation for why neuromelanin-containing neurons are selectively lost in PD.

Release of iron from dying neurons is taken up by microglia and astrocytes in the SN and other parts of the basal ganglia activating them resulting in an unwanted inflammatory response.[161] Neuromelanin released from degenerating neurons can also promote inflammation.[157] Indeed, neuroinflammation is a hallmark of PD and there is strong evidence that it is an essential contributor to disease pathogenesis.[162–164]

Over 85% of PD represent a sporadic/idiopathic form caused by a combination of environmental factors and genetic susceptibility. Administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) or 6-hydroxydopamine (6-OHDA) to rodents as well as monkeys are commonly used models of chemically-induced PD that recapitulate core disease features including robust mitochondrial dysfunction, neuronal loss, motor impairment, and neuroinflammation.[165–168] Over the past two decades,

several genes have been identified that cause familial PD, the first and best characterized of which is the SNCA gene.[169,170] The SNCA gene encodes α-synuclein, a protein abundant in the brain and that localizes predominantly to pre-synaptic terminals. In the PD brain, α-synuclein is found in abnormal oligomeric, fibrillary, and aggregated forms.[146,169,170] While oligomeric and fibrillary α-synuclein are neurotoxic, aggregated α-synuclein generally localizes within intracellular inclusions called Lewy bodies. Lewy bodies are distributed in neurons in regions other than the SN and are believed to protect neurons by sequestering oligomeric and fibrillary α-synuclein in an aggregated form. Phosphorylation of α-synuclein at Ser129 enhances its neurotoxicity.[143–145] Interestingly, increased expression of normal α-synuclein, as a result of the duplication or triplication of the SNCA gene, is sufficient to cause PD.[143–145,171] Consistently, elevating α-synuclein levels in the SN in rodents through viral expression results in neurodegeneration and behavior deficits.[172–175] Dysfunction of α-synuclein is widely regarded as central to PD pathogenesis triggering abnormalities, including defective synaptic vesicle fusion and DA release, mitochondrial dysfunction, oxidative stress, endoplasmic reticulum stress, induction of the unfolded protein response, and autophagy-lysosomal pathway impairment.[176,177] It has recently been discovered that misfolded α-synuclein can be transferred from one cell to another spreading disease pathology in a prion-like manner.[178,179]

The normally high level of iron is useful to dopaminergic neurons because it serves as a co-factor for tyrosine hydroxylase, the enzyme that catalyzes the rate-limiting step in dopamine biosynthesis.[180] However, many studies have found that iron content is further increased in the SN of patients with PD and have implicated this increase to disease pathogenesis.[149,153,181–190] Moreover, within the SN, the increase occurs in neurons that selectively degenerate. Recent studies on PD patients with cognitive impairment describe increased iron accumulation in the caudate and cortex.[191–193] Arguing in favor of a causative role for iron in PD are the observations that direct infusion of Fe^{3+} to the SN in rats results in mitochondrial dysfunction, oxidative stress, neuronal loss, striatal dopamine depletion, and impaired motor function, all characteristic features of PD.[194–197] Administration of MPTP or 6-OHDA to rodents and monkeys also increases iron content in the SN and this increase is proportional to the loss of neurons.[198–206] When compared, the iron accumulation generally precedes degeneration of the SN.[198–201] It deserves mention, however, that in at least one of these studies in which the time-course of events was tracked, the increase in iron occurred after Tf levels had fallen and there was significant neuronal loss.[203] Relevant to this point, some human studies have found that the increase of SN iron occurs only in the most severely affected patients.[189–195] At least one study utilizing monkeys and the MPTP model also found that the increase of iron in the SN occurs only with severe PD pathology and behavioral deficits.[207] Given that the clinical symptoms, neuropathological features, and rate of disease progression display a significant level of variability, it is possible that in a subset of PD cases, iron accumulation may be causally involved in disease progression, whereas it may be associated or result from degeneration in some other cases of PD. Consistent with such heterogeneity, aggregated α-synuclein, which is generally regarded as a biochemical hallmark of PD (see below) is also not seen in some familial forms of the disease.[201]

The expression or activity of several proteins involved in regulating iron homeostasis is altered in PD.[153,184,193,208–210] Both the levels of ceruloplasmin and its ferroxidase activity, the stability of FPN1, and the loading of Fe^{3+} onto transferrin are severely reduced in the SN and CSF of PD patients and correlate with disease progression. One study reported ~80% loss of ceruloplasmin in the SN of patients with idiopathic PD. Interestingly, PD patients have a reduced level of copper in the SN and, given the need for copper for the ferroxidase activity of ceruloplasmin, may explain the reduced ceruloplasmin activity and consequently increased iron-accumulation.[207–212] Reduced expression of ceruloplasmin in the SN is also seen in rodent model of PD.[213] Furthermore, ceruloplasmin knockout mice display motor deficits consistent with its reduction being a pivotal alteration in PD.[213,214] FPN1 expression is also reduced in cell and rodent models of chemically-induced PD which would cause accumulation of intracellular iron.[213,215,216] Worsening the situation, expression of Tfr11 and more generally DMT1 is increased in the SN in patients and in a variety of cell culture and mouse PD models, an alteration that would increase iron import.[217–221] DMT1 upregulation may involve the IRE/IRP system as well as reduced degradation.[218] Additionally, the proteasomal degradation of DMT1 by parkin, the E3 ubiquitin ligase parkin mutated in some forms of familial PD, is reduced in PD models. Upregulation of DMT1 in the SN has also been described in PD patients.[219] The expression of Lf and the Lf receptor is increased in cell culture and rodent models of PD as well as in patients, alterations that could contribute to iron elevation.[82,83,210,222,223] While some studies have described no change in ferritin expression in the SN in PD, other studies have reported a reduction.[149,159,189,210,224–227] A reduction in ferritin could increase intracellular free Fe^{2+} which would have damaging effects on the cell. Dopaminergic neurons are also one of the few cell types in the body that express Tfr2, a transporter related to the plasma membrane transporter, Tfr1, but that transports iron into the mitochondria.[228] Additionally, while Tfr1 expression is regulated by intracellular iron and is decreased when iron is high, TfR2 is insensitive to it.[228,229] Furthermore, the expression of both Tfr2 and Mfn-2 is significantly upregulated in cell culture models of PD providing an explanation for mitochondrial iron accumulation in the disease.[229,230] Such an upregulation would explain both the accumulation of iron in the mitochondria and the well-documented mitochondrial dysfunction in PD.

In addition to promoting oxidative stress, iron accumulation promotes another cardinal feature of PD pathogenesis—fibrillization of α-synuclein. Fe^{3+} binds α-synuclein with high affinity and this promotes a change of α-synuclein confirmation, converting it to a fibrillar form.[137,138,231,232] Based on TEM analyses, addition of micromolar concentration of Fe^{3+} to wild type α-synuclein

generates fibrils that resemble fibrils formed from disease-associated mutant forms of α-synuclein.[233] Addition of iron to mutant forms has a stronger effect on fibrillization than on wild type α-synuclein.[185] Consistent with binding to α-synuclein, deposits of iron are found in in the core of Lewy bodies where α-synuclein aggregates localize to. Taken together, these results suggest that iron accumulation in the SN promotes α-synuclein fibrilization and oligomerization, which render it neurotoxic.[232,234,235] Consistent with the need for iron in α-synuclein toxicity, is the finding that iron-induced toxicity can be blocked by siRNA-mediated knockdown of intracellular α-synuclein expression in neuroblastoma cells.[234] As described above, neuroinflammation in the SN is a well-established feature of PD and an occurrence that exacerbates neuronal loss.[236,237] Through release of pro-inflammatory cytokines, activated microglia stimulate the uptake of iron by neurons which then can cause additional neurodegeneration through stimulation of α-synuclein aggregation and oxidative stress.[238,239] Some studies have shown that oxidation at specific N-terminus residues stabilizes α-synuclein in a non-toxic form that is prevented from fibrilizing. However, iron inhibits this conformational block thus promoting the fibrilization of α-synuclein to a neurotoxic form.[187,240–243] The structure of neuromelanin is also altered in the PD brain and in this altered conformation can cross-link with α-synuclein contributing to its aggregation.[244] Such cross-linking may reduce the function neuromelanin as well as of α-synucleins.[244,245] Indeed, it has been suggested that the association between neuromelanin and iron is reduced in the parkinsonian SN resulting in higher levels of free iron.[193]

Iron also promotes α-synuclein phosphorylation and in turn the binding affinity of iron is increased in Ser129-phosphorylated α-synuclein.[235,246] α-synuclein phosphorylation at Ser129 is also enhanced by oxidative stress indirectly through the oxidation of Fe^{2+} to Fe^{3+}.[235,247] Elevated iron upregulates α-synuclein expression likely through the IRE/IRP system. The 5′ UTR of α-synuclein transcript has a region that folds into a functional IRE and its synthesis can therefore be expected to be increased by elevated iron.[248] And lastly, iron-induced oxidative stress promotes cell to cell transmission of pathogenic α-synuclein.[235,247,249] Taken together, these results suggest that elevated iron in dopamine-rich SN neurons binds and promotes the pathological phosphorylation and fibrillization of α-synuclein, which besides exerting intracellular toxicity spreads in a prion-like mechanism promoting toxicity in other neurons with the SN and beyond.

It is noteworthy that while elevated iron promotes α-synuclein oligomerization, phosphorylation, and increasing its expression, α-synuclein also regulates iron homeostasis. Tfr1 and α-synuclein colocalize on the plasma membrane.[250] Through its interaction with dynamin and modulation of clathrin-coated endocytosis, α-synuclein stimulates the endocytic uptake of iron in neurons.[250,251] Depletion of α-synuclein results in an accumulation of Tf/Tfr1 complex within recycling endosomes.[250,251] This would reduce iron levels in dopaminergic neurons which would reduce expression of tyrosine hydroxylase and consequently dopamine production. Additionally, ferritin expression is reduced in cells lacking α-synuclein suggesting multiple effects of α-synuclein on iron homeostasis.[250] α-synuclein can be post translationally modified in a number of ways and at many residues. Oxidative stress induced by iron and dopamine is enhanced by altered oxidation and phosphorylation of α-synuclein at the membrane.[252] As described above, α-synuclein also has ferrireductase activity which, counterintuitively, is reduced in brains of PD patients likely due to its deposition in intracellular aggregates or perhaps a disease-associated post-translational modification of α-synuclein.[74–77] Although much of the ferrireductase activity of α-synuclein is membrane-associated, it is not clear exactly how the reduction in this activity increases intracellular iron. It has been suggested that elevated α-synuclein can change the intracellular distribution of iron.[253] Such a redistribution of iron would disrupt its cellular functions.

Results from recent studies suggest that neuronal death in PD is due to ferroptosis.[116,254,255] Human induced pluripotent stem cell (iPSC)-derived neurons generated from patients with α-synuclein gene triplication undergo ferroptosis accompanied by oxidative stress and lipid peroxidation, which can be prevented by reducing iron-dependent build-up of free radicals.[254] Also supporting the idea that neurodegeneration is due to ferroptosis is the recent finding that DJ-1, loss-of-function mutations of which cause inherited PD, suppresses ferroptosis.[255] Loss-of-function mutations of the gene encoding parkin, a E3 ubiquitin ligase, also cause familial PD. Interestingly, overexpression of parkin stimulates the ubiquitination and degradation of DMT1 reducing iron import.[256,257] This suggests that parkin may normally serve to control iron import into neurons. Another study found that transgenic mice overexpressing DMT1 had not only selective accumulation of iron in the SN.[258] Parkin expression was upregulated in these mice likely as a protective response to the increased DMT1. Surprisingly, the DMT1-overexpressing mice did not display behavioral deficits even when fed with an iron-rich diet.[258] Whether the upregulation of parkin expression contributes to the lack of a neurological phenotype in the transgenic mice is unclear.

Based on broad consensus that iron accumulation in the SN causes mitochondrial dysfunction, elevates oxidative stress, and promotes inflammation, which together cause the neurological deficits of PD, administration of iron chelators has been tested as a treatment strategy.[259,260] Studies using a variety *in vitro* systems and cell culture models of PD have found iron chelation be protective.[231,260–263] Most of the *in vivo* testing of iron chelators has used deferiprone because of its ability to cross the BBB. Testing of iron chelators in both chemical and genetic rodent models of PD has revealed the ability to reduce SN degeneration and improve behavioral performance.[197,198,204,264–270] Astrocyte and microglial activation are also reduced in the MPTP and 6-OHDA models.[204,271] One caveat that deserves noting is that the chelators used in mouse studies and human trials are not specific for iron, instead chelating other metals including copper, aluminum and zinc, dysregulation of which has also been linked to neurodegenerative disease.

Therefore, whether the beneficial effects of these inhibitors are solely due to iron chelation, chelation of another metal (s), or a combination of metals is not clear.

In contrast to the success of iron chelators in PD models, a study using deferiprone in PD patients reported a lowering of SN iron in only a small subset of the patients.[272] A possible explanation suggested by the authors of this study is that in contrast to other brain regions, iron in the SN is bound to neuromelanin rather than to ferritin rendering its chelation less efficient in the SNc.[272] However, results of some other studies suggest possible benefit of iron chelation pointing to the need for additional clinical testing. A potential issue with iron chelators is the depletion of peripheral iron leading to anemia.[81,260,273] Also described is that iron depletion in mice causes degeneration of dopaminergic neuron raising another potential difficulty.[274] A conservative or moderate regimen of iron chelators has therefore been proposed in human studies.[259] One study conducted in a limited number of patients described the ability of moderate doses of deferiprone to reduce iron accumulation in the SN and improve motor performance in PD patients.[260]

Iron chelators attenuate the motor deficits displayed in ceruloplasmin knockout mice indicating that the behavioral impairment in these mice is iron-dependent.[275] Another study that correlated ceruloplasmin activity with efficacy of iron chelation in PD patients described that patients with lower ceruloplasmin activity responded better to iron chelation pointing to reduction in ceruloplasmin activity as being responsible for iron accumulation in the SN that then causes or contributes to disease progression.[273] Therefore, targeting ceruloplasmin could be another attractive approach for PD therapy.[276] Consistent with this possibility, peripheral infusion of ceruloplasmin has been shown to attenuate neurodegeneration and nigral iron elevation in the MPTP-administered mice.[275,276] Exactly how such peripherally-delivered ceruloplasmin crosses the BBB is unclear and whether a similar approach could be effective in PD patients remains to be tested. Tf, which both delivers and removes iron from cells, is depleted in the SN of PD patients.[277] Administration of Tf was found to be beneficial in MPTP-treated mice.[277] However, like iron chelator administration, Tf supplementation also lowered iron levels in peripheral organs reducing its potential as a therapeutic approach in patients.[277] Whether sequestration of elevated iron in the SN by increasing levels of ferritin can reduce neurodegeneration has been examined in the MPTP and 6-OHDA models. Overexpression of ferritin in SN neurons of mice did protect against neurodegeneration in younger animals although prolonged expression resulted in age-associated neurodegeneration.[199,278] This age-associated neuronal loss in older transgenic mice could be due to the chronic depletion of iron from the LIP within the neurons, impacting important cellular functions that require iron. Another study descripted that virally-mediated overexpression of hepcidin reduced cellular and mitochondrial iron accumulation and was protective in chemical models of PD.[279] Peripherally, hepcidin is best known for promoting FPN1 degradation, an action that would elevate intracellular iron. The mechanism by which hepcidin exerts its neuroprotective is likely through its ability to downregulate expression of DMT1, Tfr1, ferritin-L, and ferritin-H levels in neurons and astrocytes thereby reducing iron uptake and intracellular content in these cell types.[31,280,281] Indeed, neuroprotection by elevating brain hepcidin has been described in other models of neurodegenerative disease.[282,283] Some studies have described that N-acetyl-cysteine (NAC), a modified form of cysteine (which increases GSH synthesis) can partially protect mice against neurodegeneration in cell culture and the MPTP model of PD.[284–287] NAC is likely to act downstream of iron accumulation to prevent oxidative stress. A recent trial in a limited number of PD patients showed efficacy with NAC therapy suggesting the need for larger-scale studies.[288]

Alzheimer's disease. AD is the most common neurodegenerative disease, clinically characterized by dementia resulting from the selective loss of neurons in the hippocampus and cortex. One of the two key neuropathological hallmarks in AD is the accumulation of extracellular plaques containing aggregates of the amyloid-β (Aβ) peptide, a cleavage product of the amyloid precursor protein (APP).[289–292] The production of Aβ from APP involves sequential proteolysis by two multi-subunit transmembrane proteases, β-secretase and γ-secretase. Aβ levels are elevated in the AD brain through a combination of increased production and reduced clearance.[289–292] Although normally Aβ is a 40 amino acid peptide ($A\beta_{40}$), much of the Aβ generated in the AD brain is 42 amino acids long ($A\beta_{42}$). $A\beta_{42}$ has a much higher propensity to oligomerize and aggregate than $A\beta_{40}$ and is the predominant form of Aβ in amyloid plaques. A second key neuropathological hallmark of the AD brain is cytoplasmic inclusions within neurons called neurofibrillary tangles (NFTs). NFTs are composed of filaments of hyperphosphorylated tau, a microtubule-associated protein. The kinases involved in the pathological phosphorylation of tau include, but are not limited to, the non-mitotic cyclin-dependent kinase-5 (CDK5) and GSK3β.[289–292] Hyperphosphorylation of tau not only causes it to disassociate from microtubules, but promotes its mislocalization to dendrites.

Although it is not clear if deregulation of Aβ or of tau is the primary trigger, the growing consensus is that both proteins are central to disease pathogenesis likely acting synergistically.[289–292] Also widely accepted is that oligomeric and fibrillar forms of Aβ and tau, rather than the aggregated form in plaques and NFTs, are the neurotoxic species, and that these soluble forms start to form early in disease pathogenesis.[293,294] Indeed, plaques and NFTs serve a protective role by sequestering Aβ and hyperphosphorylated tau as insoluble aggregates.[293,294] In addition to plaques and NFTs, extensive synaptic loss, neuroinflammation, and oxidative stress are consistent features in the AD brain and contribute to neurodegeneration.[289–292] Although the vast majority of AD is sporadic, mutations in the genes encoding APP, presenilin-1 (PS1), and presenilin-2 (PS2) cause familial AD.[295,296] PS1 or PS2 is the catalytic subunit of γ-secretase, which includes three other proteins

(nicastrin, PEN-2 and APH-1). Mutation or polymorphisms in several genes contributes to susceptibility in sporadic AD. Of these, inheritance of the E4 isoform of ApoE and mutations in the TREM2 genes are the best characterized genetic susceptibility factors.[295–297]

A growing body of evidence has implicated elevated brain iron in AD pathogenesis.[109,298–301] Iron levels, and specifically of Fe^{3+}, are elevated in the hippocampus and cortex of AD patients.[109,298,302,303] Iron accumulation in the hippocampus can be observed in early stages of the disease and is considered to be a strong predictor of AD-related cognitive decline.[302,304,305] Expression of FPN1 is reduced in the hippocampus of AD patients and likely causes or contributes to the accumulation of iron.[306] Other studies have described that increased iron in the cortex correlates with the amount of Aβ plaques, tau pathology, and cognitive decline suggesting that cortical iron accumulation could be used to evaluate AD progression and severity.[305,307–311] Histological analyses of the cortex of AD patients reveal that the accumulation of iron deposits is also associated with alterations in the pattern of cortical lamination and in myelination changes.[311,312]

Consistent with elevated brain iron being an early event in AD pathogenesis, secreted ferritin, which reflects intracellular iron load, is elevated in the CSF of cognitively normal individuals with mild cognitive impairment (MCI). In these pre-AD individuals, the increase either precedes or correlates with Aβ pathology, reduced fluorodeoxyglucose utilization, and most importantly, progression to AD thereafter.[313–315] Thus, CSF ferritin could be used as a biomarker to predict near-term risk for disease progression.[313–315] Longitudinal imaging studies performed in patients suggest that the increase in CSF ferritin is regulated by ApoE.[315,316] The ApoE4 genotype may mediate the negative effects of iron accumulation on cognitive function in AD, although exactly how is unclear.[315,317]

Although much evidence points to elevated iron triggering Aβ and tau pathology, injection of Aβ oligomers in the hippocampus, which produces Aβ plaques, tau pathology and cognitive decline, causes iron accumulation.[318] It is possible that iron and Aβ function in a positive-feedback loop to promote disease pathology. Interestingly, the accumulation of iron as well as the other pathological and cognitive features of AD that are produced by oligomeric Aβ injection does not occur in tau knockout mice raising the possibility that tau plays an essential role in iron accumulation and its effects on AD pathogenesis.[318]

In contrast to brain tissue, iron level in the CSF of AD patents is not higher from control groups with most studies finding lower levels in AD CSF.[319–323] This may be due to an elevation of ceruloplasmin in the CSF of patients in the early stages of AD, which could be expected to inhibit iron export.[324] Surprisingly, however, the elevation of CSF ceruloplasmin is associated with accelerated cognitive decline and ventricular volume enlargement.[324] It has been suggested that the elevation in ceruloplasmin might be part of a neuroinflammatory response, which would have detrimental effects on neurons.[324]

Consistent with a causal role for iron in AD, normal rats fed with a high-iron diet display altered expression of DMT1, FPN1, and Tfr1, followed by neuronal loss in the hippocampus and cortex.[142] Elevated neuronal iron increases synthesis of APP through IPR binding to an IRE that localizes to the 5-UTR of the APP mRNA.[325] In addition to elevated synthesis, processing of APP to generate Aβ is also increased by iron.[142,326] This involves the binding of L-ferritin to PEN-2, a component of γ-secretase, stabilizing it.[142] However, APP also regulates iron homeostasis as evidenced by the finding that APP knockout mice display an enhancement of age-dependent iron accumulation.[327] This suggests that one function of APP is to suppress iron build-up in the brain during normal aging.[327] The ability of APP to prevent iron build-up can be explained by its ability to interact with FPN1 at the cell surface and stabilize it consequently facilitating efficient iron efflux.[87,328–330] FPN1 expression is reduced and DMT1 expression is increased in mouse models of AD.[331] Another study described that reduced FPN1 and increased ferritin protein levels correlated with iron accumulation in the brains of APP knockout mice.[327] Altered posttranslational modifications of APP also disrupt iron homeostasis and this has been suggested to be due to reduced association with FPN1.[332] A recently-conducted comprehensive study combining mass spectrometry-based proteomics and integrated multi-omics using samples from AD patients and 5XFAD AD mice described the upregulation of four proteins involved in iron homeostasis.[333] Although none of the upregulated proteins described above were identified in this particular study, the expression of Lf, the iron-binding protein, and STEAP3, the endosomal ferroreductase that converts insoluble Fe^{3+} to soluble Fe^{2+} were among the genes that are substantially elevated in both AD mice and patients.[333] Upregulation of these genes would increase cytosolic accumulation of Fe^{2}.

In contrast to APP, which stabilizes FPN1, the amyloidogenic processing of APP to $Aβ_{42}$ destabilizes FPN1 in neurons and impairs iron export.[334] Interestingly, this is not observed in non-amyloidogenic processing of APP, which also stabilizes FPN1.[334] Also in contrast to APP, which promotes iron efflux, $Aβ_{42}$-exposure increases ferritin production in astrocytes suggesting that $Aβ_{42}$ may promote iron accumulation in astrocytes.[335] Such accumulation could be expected to affect the functioning of astrocytes, which would affect the health and functioning of neurons. Indeed, astrocytic dysfunction has been implicated in AD pathogenesis.[336–338] Iron accumulation also occurs in microglia in the vicinity of Aβ plaques in mice, a finding that is consistent with the finding of iron accumulation in the vicinity of plaques in the brains of patients.[339,340] Experiments in AD mice have found that although promoting neuroinflammation, microglia have a reduced ability to phagocytose Aβ, thus potentiating damage to the brain.[339] Intracerebral injection of hepcidin, which, as described above, can reduce intracellular iron in the brain, prevents astrocyte and microglia activation and oxidative damage triggered by Aβ injection supporting a causal role for disrupted iron homeostasis in AD-associated neuroinflammation.[341]

Iron binds directly to Aβ and tau and promotes their oligomerization/aggregation.[342–346] Not surprisingly, iron

(and specifically Fe^{3+}) accumulates in Aβ plaques and NFT in the brains of AD patients.[224,344,347–352] The colocalization of iron with Aβ is also seen in asymptomatic individuals with the ApoE4 allele and in elderly subjects with MCI suggesting that this interaction occurs early in the pathogenic process causing cognitive decline in AD.[353] In cell culture systems the binding of Fe^{3+} to Aβ increases the neurotoxicity of cell culture.[353] In the case of tau, only Fe^{3+} and not Fe^{2+}, stimulates aggregation.[347,354] Indeed, reduction of Fe^{3+} to Fe^{2+} reduces tau aggregation rendering it soluble, whereas aggregation increases with chemically-induced transition of Fe^{2+} to Fe^{3+}.[347] It may be noted that this study was performed prior to the realization that oligomeric or fibrillary tau rather than insoluble aggregates were the neurotoxic tau species and therefore the effects of Fe^{2+} to Fe^{3+} on soluble oligomeric tau forms were not evaluated. However, these results suggest that Fe^{3+} is the more dangerous of the two ions with regard to tau neurotoxicity.[347] As observed with tau, the addition of Fe^{3+} to $A\beta_{42}$ *in vitro* enhances its aggregation.[344,355] Recent studies have shown that oxidative stress, which can be triggered by iron, stimulates formation of soluble Aβ oligomers via Cys–Cys binding between tau molecules.[356,357] Besides promoting oligomerization, Fe^{3+} hyperphosphorylates tau in cultured neurons through activation of specific kinase pathways.[358,359] While iron can regulate tau, tau oligomerization and aggregation can also affect iron homeostasis. Tau plays a critical role in the transport of APP to the membrane in neurons where APP stabilizes FPN1.[84,360] Depletion of cellular soluble tau decreases FPN1 levels resulting in intracellular iron accumulation.[360] Based on all of these findings, it is likely that the dysregulation of iron homeostasis causing its accumulation in neurons can promote pathogenic changes in Aβ and tau, but that Aβ and tau can also disrupt iron homeostasis raising the possibility of a positive-feedback pathogenic process in AD.

As described above, a well-studied mechanism by which elevated iron can cause neuronal death is through oxidative stress, which is a characteristic feature of the AD brain.[301,361–363] Like the accumulation of iron, oxidative stress is also an early event in AD pathogenesis.[364] Besides iron accumulation due to deregulation, other mechanisms have been proposed to promote oxidative stress. Recent studies have demonstrated that $A\beta_{42}$ interacts with iron-bound ferritin within plaques.[365,366] Both in *vitro* and *in vivo* (including within plaques) $A\beta_{42}$ coverts stored Fe^{3+} within ferritin to the more unstable and reactive Fe^{2+}, which through Fenton chemistry drives ROS production and oxidative stress.[344,365–367] The molecular consequences of uncontrolled oxidative stress, such as abnormal protein oxidation, DNA damage, impaired DNA repair, and lipid peroxidation have all been well-documented in the AD brain. As described above, these alterations are also features of ferroptosis.[298,362,368,369] Indeed, ferroptosis has been described in rodent models of AD, and strongly implicated in humans with the disease.[370–374] A recent study performed on postmortem human brain tissue found that besides iron dyshomeostasis, the expression of X_c^- (the cystine/glutamate transporter) was reduced.[374] White matter loss is often among the earliest brain changes in AD, preceding the tangles and plaques that characterize neuronal deficits.[375] Loss of oligodendrocytes is preceded by DNA-damage, which occurs in aging and is elevated in AD.[376] DNA damage-associated oligodendrocyte degeneration precedes amyloid pathology in AD patients and, through its consequences on neuronal functioning, likely contributes to cognitive impairment.[377]

A recent hypothesis is that besides causing oxidative stress, iron dyshomeostasis reactivates dormant microbes in the gut and other tissues, which results in systemic inflammation as well as shedding of potent inflammagens, such as lipopolysaccharides, which along with genetic susceptibilities play a key role in the pathogenesis of AD (as well as other neurodegenerative diseases, such as PD).[378–382] Inflammation resulting from microbial products causes cell damage releasing ferritin, which may provide an explanation for elevated serum ferritin in AD. Therefore, while serum ferritin can represent a measure of liver iron and intracellular iron content, it may also a be a marker of inflammation.[383] While plausible, the Iron Dysregulation and Dormant Microbes (IDDM) hypothesis remains to be rigorously tested.

Increasing hepcidin levels in the brain has been suggested as a potential therapeutic approach for AD and other neurodegenerative diseases.[282,283] Consistently, hepcidin expression is reduced in the AD brain.[282,306] Administration of iron chelators has been tested as a the therapeutic strategies for AD.[326,360,384,385] Iron chelation reduces Fe^{3+}-induced $A\beta_{42}$ aggregation *in vitro*.[386] Iron chelators also disassociate tau aggregates obtained from the brains of AD patients both *in vitro* and in AD brain slices.[387,388] A number of studies testing the efficacy of iron chelator administration in genetic mouse models of AD have described amelioration of cognitive impairment along with reductions in GSK3β activity, tau phosphorylation, generation of Aβ, and aggregation of Aβ in the hippocampus of AD mice.[389–393] Additionally, oxidative stress and microglial activation are reduced with iron chelators.[389,391–393] Reduction of cognitive impairment with iron chelation has also been described in a rat model of sporadic AD.[394] As observed in rodent models, treatment with iron chelators reduces oxidative stress and increases survival and locomotor activity in an $A\beta_{42}$-overexpressing fly model of AD.[395] Sustained administration of iron chelators to a limited number of patients with early AD has been reported to slow the progression of dementia.[396] One study that tested iron chelation in AD patients reported a significant reduction in the rate of decline of daily living skills with intramuscular deferoxamine administration.[396] A study using iodochlorhydroxyquin (clioquinol) which blocks metal binding to Aβ lowered the level of $A\beta_{42}$ in CSF.[397] Another pilot study utilizing a more recently developed chelator, PBT2, reported improvement of cognition and decreased $A\beta_{42}$ in CSF.[398]

Although not examined in adequate detail, it is possible that the accumulation of iron in the mitochondria is particularly significant in AD pathogenesis. Knockdown of the *C. Elegans* ortholog of the mitochondrial iron transporter,

Table 1. List of NBIA disorders.

Gene	Protein	Protein localization	Disorder
AP4M1	Adaptor protein complex-4-Subunit M1	Endosome	
ATP13A2	Cation-transporting ATPase 13A2	Lysosome, mitochondria	Kubor-Raken disease (KRS)
C19orf12	C19orf12	Mitochondrial membrane, ER	Mitochondrial membrane protein-associated neurodegeneration (MPAN)
CPL	Ceruloplasmin	Plasma membrane	Aceruloplasminaemia
CoASY	Coenzyme A synthase	Mitochondria, cytosol	COASY-protein-associated neurodegeneration (CoPAN)
CRAT	Carnitine acetyltransferase	Mitochondria	
DCAF17	DDB1- and CUL4-associated factor-17	Nucleolus	Woodhouse-Sakati syndrome (WSS)
GTPBP2	GTP-binding protein-2	Cytoplasm	
FA2H	Fatty acid 2-hydroxylase	ER	Fatty acid hydroxylase-associated neurodegeneration
FTL	Ferritin light chain	Cytoplasm	Neuroferritinopathy (NF)
PANK2	Panthothenate kinase 2	Mitochondria	
PLA2G6	Calcium-independent phospholipase A2 group VIa (iPLA2VIa)	Mitochondria, ER, cytosol	PLA2G6-associated neurodegeneration
REPS1	RalBP-associated Eps15-homology domain protein	Cytoplasm endosome	
SCP2	Sterol carrier protein 2	Peroxisomes	
WDR45	WD40-repeat protein 45	ER	β-propeller protein-associated neurodegeneration (BPAN)

mitoferrin-1/2, in a worm model of AD reduced mitochondrial iron and mitochondrial ROS and is protective against disease progression.[399] Targeting mitoferrin and other molecules involved in mitochondrial iron transport could be another therapeutic avenue for AD.

Neurodegeneration with brain accumulation disorders

NBIAs are a group of at least 12 very rare, clinically and genetically inherited neurodegenerative disorders characterized by deposition of iron generally (but not in all NBIAs) within the basal ganglia, and most specifically, in the globus pallidus and SN (see Table 1).[400–403] These are regions of the brain that normally have a high iron content and are therefore selectively vulnerable to any further elevation of iron. Neuropathologically, NBIAs are often associated with cerebral, cerebellar and optic atrophy, and retinal degeneration.[400–403] The major clinical manifestations are progressive dystonia, spasticity, parkinsonism, and neuropsychiatric abnormalities. Cognitive impairment is displayed in some NBIAs, but not others. Depending on the disorder, onset ranges from infancy to adulthood. While iron accumulates in the brain in all NBIAs, in most of these disorders the level of systemic iron is not elevated.

While brain iron accumulation is the common denominator, only two NBIA disorders are caused by mutations in proteins that directly regulate iron homeostasis (Table 1). These are ceruloplasmin and L-ferritin, which cause aceruloplasminemia and neuroferritinopathy, respectively. A recent case report of iron accumulation, brain atrophy, and severe neurological impairment in a patient with biallelic loss of the IRP2 gene suggests the possibility of a third NBIA resulting from mutation of a gene regulating iron homeostasis. However, this remains to be confirmed.[404] Of the other known NBIAs, mutations in the PANK2 (pantothenate kinase-2) gene and the PLA2G6 (calcium-independent phospholipase A2, Group VIa) gene together account for a majority of all cases. NBIA disorders caused by mutations in PANK2 and PLA2G6 are referred to as PKAN (PANK-associated neurodegeneration) and PLAN (PLA2-associated neurodegeneration), respectively. Because aceruloplasminemia and neuroferritinopathy are caused by direct disruption of iron homeostasis and because PKAN and PLAN are the best studied of the iron dyshomeostasis-unrelated NBIA disorders, this review will be limited to these four NBIA disorders. For a more comprehensive description of NBIA disorders, their underlying disruptions, and a description of mechanistic studies, the reader is referred to other excellent and recent reviews.[400–403]

Besides elevated iron and abnormal neuronal loss, NBIA disorders share important clinical and neuropathological features with age-associated neurodegenerative diseases. It is therefore likely that the pathogenesis of these two categories of neurodegenerative disorders share molecular and cellular mechanisms. This issue remains to be systematically investigated, however.

Aceruloplasminemia. Aceruloplasminemia is an autosomal recessive disease caused by mutations in the gene encoding ceruloplasmin.[405,406] As described above, ceruloplasmin facilitates export of iron through the FPN1 transporter by oxidizing Fe^{2+}. Indeed, it is the predominant ferroxidase in plasma. Ceruloplasmin also promotes iron export by stabilizing FPN1. Clinical manifestations of aceruloplasminemia, which start at around 50 years of age, are heterogeneous and include ataxia, involuntary movement, dysarthria, retinal degeneration, psychiatric issues, parkinsonism, and cognitive impairment, including dementia.[405–409] Retinal degeneration, diabetes, and dementia are three consistent disease features.[405,406] Diabetes mellitus precedes brain abnormalities by decades. Because of iron accumulation in tissues, the level of blood iron is lower which is

potentiated by microcytosis.[13] Microcytic anemia, low transferrin saturation, and paradoxically hyperferritinemia are also seen decades prior to neurological symptoms.[405] In most patients, brain iron accumulation is seen in the basal ganglia and cerebellum.[405,406,408,409] The extent of brain overload does not correlate with the severity of neurological symptoms suggesting contribution from other genetic or environmental factors. A distinguishing feature of aceruloplasminemia is that it is the only known NBIA in which systemic iron is also elevated.[406,409] This is likely to be because ceruloplasmin plays a key role in iron homeostasis at the systemic level, which cannot be fully compensated for by HEPH. Additionally, and for reasons that are not known, patients with aceruloplasminemia exhibit low serum HEPH levels and decreased FPN1 protein expression in the liver.[410] Outside the brain, iron accumulates in the liver.[405,406,408,409] Individuals heterozygous for the disease mutations have reduced ceruloplasmin activity, but usually display normal iron metabolism and no clinical symptoms.[405,406]

Within the brain of patients, large iron deposits form first in the epithelial cells of the choroid plexus, which likely represents an important event in the disruption of brain iron homeostasis.[411] Depositions are also observed in astrocytes and to a lesser degree in neurons. Oligodendrocytes are unaffected likely because they utilize HEPH and not ceruloplasmin for FPN1-associated ferroxidase activity.

The mechanism by which neurodegeneration occurs in aceruloplasminemia is not known. Unoxidized Fe^{2+} released into the brain interstitium is taken up through unregulated internalization pathways and can have toxic effects. It has been reported that astrocyte loss precedes neuronal loss suggesting that neurodegeneration could be secondary to the loss of astrocytes.[412–414] It is also possible, and perhaps likely, that initial neuronal loss is due to iron starvation resulting from the inability of dysfunctional astrocytes to release iron through FPN1.[408] Eventually, and following astrocytic dysfunction or degeneration, neurons might take up excessive amounts of NTBI resulting in oxidative stress and neurotoxicity.[405,415] Neuronal loss could also result, in part, from the deprivation of astrocytic neurotrophic factors. Since astrocytes play a key role in glutamate uptake, excitotoxic death of neurons may represent yet another possibility. Finally, several studies have described ceruloplasmin itself has neuroprotective activity and defends against different neurodegenerative conditions.[275,416–420] Indeed, antioxidant effects and an ability to inhibit lipid peroxidation have been described.[419,421–423] Loss of such neuroprotective activity could also contribute to the loss of neurons in aceruloplasminemia.[416]

Cultured glial cells overexpressing disease-causing mutant forms of ceruloplasmin display iron overloading and reduced FPN1 stability.[410,424] Some mutant forms of ceruloplasmin form aggregates in astrocytes, which likely cause dysfunction and perhaps death in line with the idea that astrocytic dysfunction and degeneration play a key role in disease pathogenesis.[409] Mice lacking ceruloplasmin have been analyzed and found to have increased iron levels in the brainstem, cerebellum, and spinal cord as they age. As expected, while iron import is unaffected, iron efflux is severely reduced in ceruloplasmin KO mice.[425] Within the cerebellum, DMT1 and ferritin mRNA and protein expression are increased, whereas expression of Tfr1 is reduced in the KO mice.[426] Within the cerebellum, astrocytes and Bergman glia display highly elevated iron content and about 60% of astrocytes are lost as the mutant mice age.[426] About 50% of the Purkinje neurons also die in an age-dependent manner ceruloplasmin$^{-/-}$ mice, although surprisingly there is no increase of iron in these cells.[426] Also somewhat surprisingly, in view of its stabilizing effect on FPN1, the expression of FPN1 is not reduced in the cerebellum of mice lacking ceruloplasmin.[426] Active caspase-3 staining, which is used as a marker of apoptosis, is not elevated indicating that cell death in the cerebellum is not apoptotic, pointing to ferroptosis as a likely mechanism.[426]

Administration of iron chelators to patients with aceruloplasminemia reduces systemic iron overload but convincing evidence that it reduces brain iron accumulation is lacking. Prolonged treatment with iron chelators have not had success because of the anemia resulting from peripheral iron deficiency. Most importantly, neurological symptoms are not reduced by iron chelation treatment.[406,427] Some mitigation of neurological symptoms has been described when iron-chelation with deferiprone is used in combination with phlebotomy.[427] Intravenous administration of fresh-frozen plasma (FFP) partially and temporarily restores circulating ceruloplasmin, reduces brain iron deposition and reduces neurological symptoms, although exactly how the ceruloplasmin crosses the BBB is not clear.[428] Given the heavy iron overload in the epithelial cells of the choroid plexus, one possibility is that ceruloplasmin enters the brain through a damaged choroid plexus lining or of the BBB. In the small number of patients in which iron chelators were administered along with FFP early during the disease, iron deposition and neurological symptoms were reduced raising the possibility of a potential treatment strategy.[429]

Neuroferritinopathy. Neuroferritinopathy is an autosomal dominant disorder caused by a number of different mutations in the L-ferritin gene that reduce the ability of ferritin to sequester iron resulting in increased free, unbound iron in the cytosol.[115,430] The mutated forms of L-ferritin have less stability and act dominant-negatively thus impacting the ability of normal ferritin to sequester iron, explaining the dominant transmission of the disorder.[431,432] While variable, symptoms generally appear in adulthood at 35–45 years of age. Extrapyramidal symptoms, including chorea and dystonia, are the major clinical features during early stages of the disease, whereas progressive aphonia, dysphagia, severe motor disability, and minor cognitive impairment are late features.[430,433] Patients with some types of mutations display parkinsonism, ataxia, and eventually dementia.[434] Iron deposition and lesions are observed in the globus pallidus, putamen, and cerebellum. A small proportion of patients exhibit the "eye of the tiger" sign (low MRI signal in the globus pallidus due to abnormal accumulation of iron that resembles eyes with a vertical

stripe of high MRI signal due to gliosis) a feature seen in a few other brain degenerative disorders including PANK and atypical PD.[435] Serum levels of ferritin are significantly lower than normal (except in pre-menopausal women) and a key feature of neuroferritinopathy.[401,436] Synthesis of ferritin in cells of affected brain areas is paradoxically increased possibly in a failed effort to manage the elevation in intracellular iron.[115] A characteristic neuropathological feature of neuroferritinopathy is the presence of swollen and vacuolated nuclei and cytoplasmic bodies in neurons and glia that contain ferritin and iron in an insoluble form, which precede iron deposition.[435] Most deposition is in astrocytes and oligodendrocytes.[401,436] In the putamen, the increase in iron content is about 4-fold and contains both Fe^{3+} and Fe^{2+}.[435] Abnormalities in mitochondria, lipid peroxidation, and apoptotic death are observed in the putamen but also in the globus pallidus and SN. Iron accumulation along with the presence of lipid peroxidation suggests the possibility of ferroptotic death also. In the cerebellum, degeneration of Purkinje cells and vacuolated Bergmann glia are observed.[435] As the animals age, they display accumulation of lipofuscin (lipid-containing granules) in cells with iron aggregates, particularly in the cerebellum and striatum.[437] It is possible that rupture of lysosomes releases lipofuscin which seeds iron accumulation.[401] This along with the hydrolytic lysosomal enzymes that are also released through rupture likely promotes cell degeneration.[401]

In vitro analyses of disease-causing mutant forms of L-ferritin indicate an inability to sequester iron. When such mutant forms are overexpressed in cells, they cause the formation of ferritin-iron inclusion bodies (the neuropathological hallmark), increase in free iron, oxidative stress, and cell death.[115,438–441] Transgenic mice overexpressing mutant L-ferritin display neurodegeneration and motor deficits.[437] Analyses of these mice have revealed decreased expression of Tfr1 protein mRNA and increased iron levels, and increased lipid peroxidation in the brain.[115,437,442–444] Several genes regulating iron metabolism are also deregulated. Iron deposits are seen in the brain, which localize mostly in the stratum and cerebellum.[115,437,442] IPSCs from patient fibroblasts and differentiated into neuronal precursors and neurons display altered iron homeostasis with increased Fe^{2+}, ferritin aggregates, lipid-containing granules, oxidative stress, and cell death, providing an excellent cellular model to study disease mechanisms and test therapeutic agents.[445] Based on their results, the authors of this study proposed that increased Fe^2 in these ferritinopathic neurons induces ferritin translation, causing ferritin-iron aggregates which promotes ROS accumulation, lipid peroxidation, and cell death. NAC was found to have protective effects in this paradigm.[445]

Treatment of cells overexpressing mutant L-ferritin with the iron chelator deferiprone increased cell viability and reduced iron content.[444] In mice, deferiprone administration reduced systemic iron substantially. While ferritin deposition was also reduced, overall brain pathology in not affected by iron chelation.[444] Likewise, patients administered with iron chelators displayed reduced systemic iron but had no clinical benefit.[401,436] Neurons derived from iPSCs obtained from patients exhibit increased cytosolic iron, iron and ferritin aggregates, oxidative damage and reduced viability.[445] Death of these iPSC-derived neurons was determined to be by ferroptosis.[445] NAC administration has shown efficacy in IPSC-derived ferritinopathic neurons and may represent another treatment approach.

PANK2-associated neurodegeneration. PKAN (previously called Hallervorden-Spatz syndrome) is caused by a number of different autosomal recessive mutations in the PANK2 gene and is the most common of the NBIA disorders accounting for about 50% of all cases.[446–448] PANK2 is one of the four isoforms of PANK, the first enzyme in the synthesis of coenzyme A (CoA) from pantothenate (vitamin B_5). PANK2 is the only one of the four isoforms that localizes to the mitochondria where it exists as a homodimer in the intermembrane space.[449] Onset of symptoms in PKAN can display at as early as three years of age or in adulthood.[403] Clinical manifestations include progressive dystonia, dysarthria, and rigidity. Retinal degeneration is often seen in early-onset PKAN, whereas psychiatric disturbances are often observed in the adult-onset form of the disorder.[402,450] PKAN is characterized by iron overload in the globus pallidus along with the accumulation of axonal spheroids, axonal degeneration, and neuronal loss.[403,447,450,451] Axonal swelling and neurodegeneration are also observed in the SN (where increased iron content is often described in PKAN), the cerebellum, and the dentate nucleus. Although prior to the identification of the gene there were reports of α-synuclein and tau aggregation in the brains of PKAN patients, more recent studies have failed to find α-synuclein aggregation.[451] Mild tau pathology was observed in a small proportion of patients.[451] In addition to iron, a disruption of calcium homeostasis has been described in iPSC-derived glutaminergic neurons as well as in the basal ganglia of PKAN patients.[452] Specifically, elevated calcium influx due to leakage from stores in the ER or mitochondria has been reported and this disruption has been suggested to be due to elevated iron possibly resulting from modification of calcium homeostatic proteins by iron-induced oxidative stress.[452] It is well known that intracellular calcium plays key roles in neuronal survival and function and is under tight regulation.

PANK2 knockout mice have been generated but fail to display iron accumulation in the brain or the neurological impairments seen in patients even after 18 months of age.[453,454] While normally asymptomatic, when fed with a ketogenic diet PANK2$^{-/-}$ mice display severe weight loss, locomotor impairment reminiscent of patients[455] (although another group failed to reproduce this finding[456]). These abnormalities induced by ketogenic diet were found to be substantially reduced by administration of the compound pantetheine, which is hypothesized to activate an alternative CoA biosynthesis pathway that bypasses the PANK2 defect to restore cellular CoA levels.[455] A subsequent study of the PANK2$^{-/-}$ mice confirmed lack of abnormalities in the cerebellum and SN but found reduced CoA, disrupted

expression of genes regulating iron homeostasis, mitochondrial dysfunction, and defective dopamine metabolism in the globus pallidus, which displays highest iron accumulation in PKAN patients.[457] Feeding of PANK$^{-/-}$ mice with 4′-phosphopantetheine, which lies downstream of pantothenate/vitamin B5 in the CoA biosynthetic pathway restored CoA levels and corrected the other abnormalities in the globus pallidus.[457]

The lack of a disease phenotype in the PANK2$^{-/-}$ mice along with restriction of cellular abnormalities to the globus pallidus is likely due to functional compensation by PANK1, which is also expressed in the brain in mice. Consistent with this, double-knockout mice lacking PANK2 in all cells along with PANK1-deficiency in neurons and glial cells do develop a severe disease phenotype and die within two to three weeks.[456] As observed in patients, CoA levels were substantially reduced in the brains of the mutant mice. Surprisingly given the severe phenotype, both heme levels and total iron levels in the brain of the mutant mice were lower than that in control mice.[456] *Drosophila* heterozygous for *fumble (fbl)* deletion, the only PANK2 gene in flies, display neurodegeneration, impaired motor function, defective CoA metabolism, increased protein oxidation, disrupted lipid homeostasis, sensitivity to ROS, impaired DNA integrity, and reduced lifespan.[458,459] However, iron deposits are not found. Knockdown of PANK2 in zebrafish results in severe impairment of neurodevelopment, particularly in the anterior part of the CNS.[460] Neurons have been generated from iPSCs derived from PKAN patients.[461] Although iron deposits were not seen, these neurons exhibited elevated free cytosolic iron, highly increased ROS levels, impairment of mitochondrial iron-dependent biosynthesis, impaired energy production, and premature death of the neurons.[461] Expression of Tfr1 is increased, while heme and ferritin levels are reduced in patient-derived neurons explaining the elevated free iron content.[461] This may reflect the fact that iron deposition occurs mostly in the globus pallidus and not most other neuronal types.[402] Furthermore, iron deposition might require much more time and thus difficult to recapitulate in cultured neurons. These possibilities notwithstanding, none of the *in vivo* or *in vitro* models of PKAN recapitulate iron deposition, the cardinal feature of the disease.

There is no treatment for PKAN. Although a pilot studies in patients with deferiprone indicated efficacy, a larger study conducted recently failed to show significant improvement even after 18 months of drug administration.[462–464] It is possible that treatment may have begun too late in the pathogenic process for it to be stopped. A recently identified blood-permeable, allosteric activator of PANK2 was found to increase CoA levels in the brain and livers.[465] Administration of this compound, PZ-2891, reduced weight loss and locomotor impairment and extended life span in PKAN$^{-/-}$ mice.[465] Clinical trials testing CoA as a target has yet to be conducted in PKAN patients. In the *Drosophila* model of PKAN, all abnormalities including the oxidative damage, impaired DNA integrity, and reduced lifespan are prevented by pantetheine or CoA.[458] As described above, administration of 4-phosphopantetheine normalized CoA and corrected the iron and other abnormalities in the globus pallidus. Treatment with 4-phosphopantetheine also corrected abnormalities in PKAN patient-derived fibroblasts and may hence represent an effective treatment strategy for this disorder.[457]

PLA2G6-associated neurodegeneration (PLAN). PLAN (formerly called Seitelberger disease) is the second-most studied of the iron dyshomeostasis-unrelated NBIA disorders. In this disorder, iron accumulation occurs in the globus pallidum and cerebellum.[446,466,467] It is a complex disorder caused by mutations in the PLA2G6 gene, which encodes iPLA2-β, an intracellular Ca2+-independent PLA2 that metabolizes membrane phospholipids and that regulates cellular processes, including vascular relaxation, secretion, inflammation, and apoptosis.[468] In the brain, iPLA2-β localizes mainly in dendritic and axon terminals. PLAN-causing mutations in PLA2G6 display genetic heterogeneity and include missense mutations, truncation mutations, and copy number differences. It is believed that the clinical heterogeneity depends on the genetic heterogeneity. Based on age of onset and clinical features, PLAN has been subclassified into three groups: infantile neuroaxonal dystrophy (INAD), atypical neuroaxonal dystrophy (atypical NAD), and *PLA2G6*-related dystonia-parkinsonism (DP) also referred to as adult-onset dystonia-parkinsonism. INAD usually begins between ages six months and three years with motor deterioration, dystonia, spasticity, and optic atrophy. Iron deposition is seen in the globus pallidus and SN along with cerebellar atrophy. Disease progression is rapid with increased spasticity, ataxia, cognitive impairment and visual impairment, and death generally occurs within the first decade. Similar clinical features are seen in atypical NAD although the symptoms are mild during early childhood with neurological deterioration beginning slightly later. The neuropathological hallmark is neuroaxonal dystrophy and the presence of intracellular spheroids comprised of tubulovesicular structures. Additionally, extensive phosphorylated α-synuclein-positive Lewy bodies, and tau-positive neurofibrillary tangles are found in INAD and atypical NAD.[117,469,470] Cerebellar atrophy is a common feature in INAD and atypical NAD. In contrast to INAD and DP, Lewy bodies and NFTs are not always seen in DP brains. DP patients also do not display cerebellar atrophy. The diversity in clinical phenotype may be due to differences in the effect of the various mutations on PLA2G6 enzyme activity. Although not a perfect overlap, the commonalities in clinical and neuropathological features between PLAN and PD suggest common downstream pathogenic mechanisms. In this context it is interesting that compound mutations in the PLA2G6 gene have been suggested to cause autosomal recessive early-onset PD.[471,472]

PLA2G6 knockout mice develop progressive motor impairment in their second year of life.[473–476] Like patients, these mice display neuropathological features including damaged mitochondria, axonal degeneration, cerebellar

atrophy, accumulation of α-synuclein-containing and ubiquitin-positive spheroids in axons in nearly all brain regions.[473–476] However, there is no iron accumulation in the basal ganglia or any other brain region in PLA2G6 knockout mice generated so far. Although the mechanism is unclear, a-synuclein expression is highly elevated in neurons cultured from PLA2G6 knockout mice as well as in wild type neurons in which is knocked down.[477] In these neurons, α-synuclein is contained in granules that localized to the membrane of mitochondria that are damaged.[477] The α-synuclein-positive granules are also seen in the brain close to Lewy bodies and prior to the onset of behavioral abnormalities.[477] Flies in which iPLA2-VIA (the fly homolog of PLA2G6) is inactivated have a reduced lifespan, display locomotor defects, and exhibit mitochondrial abnormalities, impaired synaptic transmission, and neurodegeneration.[478,479] Again, no iron deposition has been found. Interestingly, in flies iPLA2-VIA binds vacuolar protein sorting protein 35 (VPS35), a component of the retromer. Loss of iPLA2-VIA activity resulted in impaired retromer function, a defect also resulting from loss of Vps35 activity or overexpression of a-synuclein, alterations that cause or contribute to PD.[479–481] Although best known in the context of PD, results of several studies have implicated impaired retromer in AD pathogenesis also.[482–488]

Studies conducted using patient-derived iPSCs have revealed that the death of dopaminergic neurons is associated with oxidative stress, ER stress, defective mitophagy, and transcriptional dysregulation.[471] Death of these iPSC-generated dopaminergic neurons can be prevented by azoramide, a modulator of the unfolded protein response (UPR) as well as an inhibitor of the CREB transcription factor.[489] Treatment with azorimide also reduced ER stress, ROS accumulation and mitochondrial dysfunction, and restored proper gene expression.[489] Impaired UPR function and other core cellular perturbations in PLAN are similar to those in PD as well as other age-associated neurodegenerative diseases.[490,491] Whether azoramide or other UPR modulators will be useful in the treatment of age-associated neurodegenerative disorders remains to be tested, but has been suggested.[490,491]

Conclusions

The iron content within a cell has to be carefully regulated to permit the normal functioning of the cell while preventing an elevation that could result in its damage or death through mechanisms including, but not restricted to, the generation of highly-reactive free radicals. Research conducted over the past two decades has identified a large number of important regulators of iron homeostasis. Nowhere in the body is precise regulation of iron homeostasis more critical than in the brain. Elevated iron and its accumulation and depositions within cells of the brain are a defining feature of NBIAs and a common feature in a variety to age-associated neurodegenerative diseases. Besides elevated iron, recent studies have revealed a remarkable overlap in the cellular perturbations between NBIAs and age-associated neurodegeneration, including oxidative stress, lipid peroxidation, mitochondrial dysfunction, ER stress, and transcriptional deregulation. A better understanding of the molecular and cellular mechanisms that cause iron accumulation is necessary and the extent to which iron accumulation contributes to the cellular abnormalities as well as disease onset, severity, and progression deserves careful study. Accumulating evidence for a key role for ferroptosis in neuronal loss in both NBIAs and age-associated neurodegenerative disease point to a causative role for iron dyshomeostasis in disease pathogenesis. Future work will provide additional and much-needed information on how iron interfaces normally with disease-relevant molecules, such as $A\beta_{42}$, tau, and α-synuclein as well as ceruloplasmin, PLA2G6, and PANK2, and how these relationships are affected by iron accumulation and/or the dysfunction of the disease-relevant molecules. Together, this knowledge will facilitate the development of effective strategies to treat or cure these devastating brain disorders.

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