Iron metabolism in the CNS: implications for neurodegenerative diseases

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Abstract | Abnormal accumulation of brain iron has been detected in various neurodegenerative diseases, but the contribution of iron overload to pathology remains unclear. In a group of distinctive brain iron overload diseases known as 'neurodegeneration with brain iron accumulation' (NBIA) diseases, nine disease genes have been identified. Brain iron accumulation is observed in the globus pallidus and other brain regions in NBIA diseases, which are often associated with severe dystonia and gait abnormalities. Only two of these diseases, aceruloplasminaemia and neuroferritinopathy, are directly caused by abnormalities in iron metabolism, mainly in astrocytes and neurons, respectively. Understanding the early molecular pathophysiology of these diseases should aid insights into the role of iron and the design of specific therapeutic approaches.

Iron is indispensable in mammalian metabolism because it is integral to the formation of haem and iron–sulphur clusters and functions as a cofactor in numerous metabolic reactions. Without iron, erythrocytes would be unable to transport haemoglobin-bound oxygen to tissues, and oxidative phosphorylation by the mitochondrial respiratory chain complexes, which contain twelve iron–sulphur clusters and seven haems, would not be possible. Understanding iron metabolism in the CNS is becoming increasingly important, as there is growing evidence that abnormalities in brain iron metabolism are involved in the pathogenesis of several degenerative brain diseases. However, owing to technological obstacles (BOX 1), our understanding of brain iron metabolism continues to lag behind our knowledge about systemic iron metabolism. For example, iron metabolism probably differs between neurons, astrocytes, oligodendrocytes and microglia, as each of these cell types has distinct metabolic and architectural features, but the low resolution of non-invasive techniques such as MRI do not permit the identification of specific cell types. In addition, much remains unknown about how iron crosses the blood–brain barrier (BBB) or the brain–cerebrospinal fluid (CSF) barrier from the systemic circulation and how it traffics in the brain parenchyma to supply neurons, oligodendrocytes, microglia and astrocytes with sufficient iron.

Brain imaging techniques such as MRI, which detects iron when certain magnetic pulse sequences are used during imaging[1], have enabled investigators to detect abnormal brain iron accumulations in several previously known and newly described diseases, and this has led to the identification of several disease genes. These discoveries have been both exciting and frustrating, because they have not yet revealed insights into disease pathogenesis. For example, it is often not known whether iron accumulation contributes to disease progression or whether accumulation of iron occurs only after widespread neuronal death. In general, when iron accumulation is detected by MRI, it is often assumed to have a causal role in disease by enhancing free radical formation and contributing to oxidative stress and neuronal death in iron-overloaded cells[2,3]. However, it may not be correct to assume that any iron overload detected in specific brain regions on MRI scans reflects iron overload in neurons. Importantly, a better understanding of iron accumulation in neurodegenerative diseases has therapeutic implications, as iron chelators are currently being used in the treatment of numerous diseases that affect the CNS, including Parkinson's disease[2] and Friedreich's ataxia[4], even though the value of such chelation therapy remains unclear in most cases.

In this Review, I will first discuss basic aspects of brain iron metabolism, including how iron enters the CNS, how iron contents of the brain change during normal

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Box 1 | **Detecting iron in the brain**

Although the blood–brain and blood–cerebrospinal fluid barriers limit iron uptake in the CNS, the brain was recognized as a substantial repository of iron as long ago as 1886, when whole-brain iron determinations and specific stains revealed that most brain iron existed in a non-haem, protein-bound, ferric-iron state[42]. Subsequently, high concentrations of non-haem iron were discovered in specific regions of the brain. These include the globus pallidus, where brain iron concentrations of up to 450 μg per g wet weight of brain were comparable to liver iron concentrations and, to lesser degrees, the substantia nigra, the red nucleus and the dentate nucleus of the cerebellum[136].

Standard procedures for staining for brain iron were developed. Perhaps the best known is the reaction of ferric iron with potassium ferrocyanide to form an intense blue precipitate, known as the Prussian blue reaction. To increase the sensitivity of the assay, the Prussian blue reaction was later modified by addition of diaminobenzidine tetrahydrochloride (DAB) and hydrogen peroxide[137], which leads to the formation of a brown precipitate. This addition greatly enhanced the sensitivity of the staining procedure and allowed detection of iron (primarily ferritin iron) in neurons, astrocytes, microglia and oligodendrocytes throughout the brain. Correct preparation of samples is important, as iron can readily contaminate or leach out of tissues, yielding misleading results[51].

Experiments using brain iron staining have shown that iron concentrations increase gradually with age in the globus pallidus and other iron-rich areas but that this accumulation levels off in early adulthood, after which brain iron concentrations remain fairly constant[40]. Interestingly, brain iron content remains constant even in adult individuals who experience iron deficiency that is sufficient to deplete the liver of iron[17], suggesting that the CNS can retain the iron it has already acquired through efficient re-use to protect the brain from depletion. Radioactive uptake studies have demonstrated that iron fluxes through the brain, entering as transferrin-bound iron through the blood–brain barrier and actively contributing to the metabolism of CNS cells, before exiting through the venous circulation[17,28].

MRI detects iron in the brain mainly as iron sequestered in ferritin, in ferritin degradation products or perhaps in other high-molecular-weight iron aggregates[1,51] such as those that develop in mitochondrial iron overload diseases[112]. As MRI studies have revealed brain iron accumulation in patients in whom such overload was not suspected, MRI studies are now an integral part of the early evaluation of patients with motor problems and movement disorders. MRI does not substitute for pathological samples, because the resolution is not high enough to distinguish individual cells, but it provides a way to detect abnormal iron accumulations non-invasively in patients, and distinctive patterns of iron deposition aid physicians to diagnose patients in the early stages of a neurodegeneration with brain iron accumulation disease.

ageing and how brain iron overload might develop in diseases in which it has been observed. I will also briefly discuss several common diseases, such as Parkinson's disease and Alzheimer's disease, in which iron overload often correlates with pathology[5], and then focus on a newer group of diseases, termed 'neurodegeneration with brain iron overload accumulation' (NBIA) diseases. In NBIA, iron overload is clearly present in discrete brain regions as judged by MRI and iron stains, and causal mutations have been identified in multiple, unrelated *bona fide* disease genes.

Systemic iron metabolism

Numerous proteins are involved in the uptake and distribution of iron in mammals. Iron is acquired from food in the form of iron salts and haem, and dedicated transporters — divalent metal transporter 1 (DMT1; also known as SLC11A2) and ferroportin 1 (FPN1; also known as SLC40A1) — aid iron to cross the duodenal mucosa and enter the systemic circulation[6]. Ferroportin-mediated export is aided by the membrane-bound ferroxidase hephaestin, which oxidizes exported ferrous iron (Fe^{2+}) to ferric iron (Fe^{3+}) and facilitates binding to transferrin, an abundant ferric iron-binding protein that circulates in the systemic bloodstream[7] (FIG. 1). Transferrin usually binds two atoms of ferric iron per molecule to generate holo-transferrin. Duodenal iron uptake is regulated in several ways. Hepcidin, a peptide hormone secreted by iron-loaded hepatocytes, can bind to duodenal ferroportin, induce its intracellular degradation and thereby diminish duodenal iron uptake[8]. In addition, transcriptional expression of *DMT1* is regulated by hypoxia-inducible factor 2α (HIF2α; initially named EPAS1), which coordinately regulates the transcriptional response to hypoxia and iron deficiency in the duodenal mucosa[9]. Most cells in tissues perfused by the systemic circulation acquire iron from holo-transferrin. Holo-transferrin binds to the surface cellular transferrin receptor 1 (TFRC; also known as TFR1), after which the complex undergoes endocytosis. Ferric iron is then reduced to ferrous iron by metalloreductase STEAP3 (REF. 10) and is transported across the membrane by DMT1 (FIG. 1a).

In the cytosol, iron may be transported by binding to chaperones that donate iron to specific target proteins[11], or it may traffic to mitochondria and enter the mitochondrial matrix through the dedicated mitochondrial iron transporters mitoferrin 1 or mitoferrin 2 (REF. 12). Iron is also stored by cytosolic ferritin, a 24-subunit heteropolymer composed of ferritin heavy and light chains, which co-assemble in varying ratios to form a hollow sphere into which iron atoms are deposited (FIG. 1). Storing up to 4,500 iron atoms as an insoluble precipitate, ferritin effectively compartmentalizes iron in a non-reactive form without the aid of membranes, and it thereby prevents free iron from reaching high concentrations in the cytosolic and nuclear compartments[13]. Iron stored within cytosolic ferritin can be released during lysosomal degradation[14] or perhaps is released as needed from intact ferritin[15]. Within the CNS, the iron cycle involves the same proteins and mechanisms, with a few exceptions (see below).

Iron entry into the CNS

Cells in the CNS do not have direct access to nutrients, including iron, in the systemic circulation because the BBB and the blood–CSF barrier separate the CNS from the systemic circulation[16–18]. Therefore, receptors and transporters mediate uptake of iron across the luminal barrier membrane of polarized endothelial cells of the BBB and across the abluminal membrane into the CNS. On the luminal side of the BBB, TFRC mediates endothelial iron uptake[19]. FPN1, which transports iron out of the cell, has been detected in endothelia of the BBB in some studies[20,21] but not others[22] and may enable iron to cross the abluminal membrane to enter the interstitial fluid. Endothelial cells are ensheathed by astrocytic foot processes, which express a membrane-linked form of the ferroxidase ceruloplasmin. This protein may facilitate export activity of ferroportin on adjacent cells[23] (FIG. 1b). Interestingly, like in duodenal mucosal epithelial cells, HIF2α is highly expressed in brain endothelial cells[24]. HIF proteins are oxygen- and iron-sensitive because of their degradation through the von Hippel–Lindau disease tumour suppressor (VHL)–prolyl hydroxylase pathway[25]. The presence of HIF2α in the brain vasculature

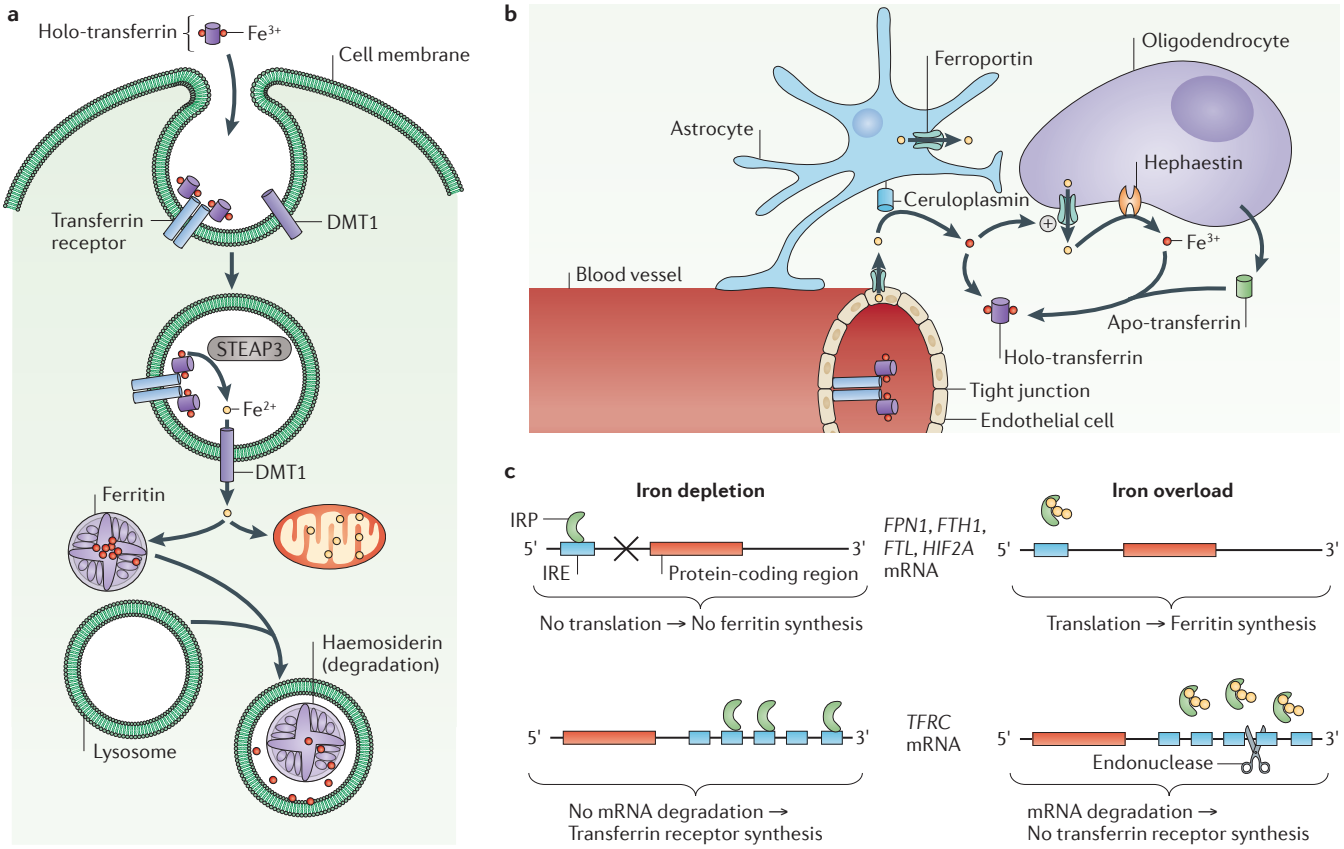

Figure 1 | **Cellular iron metabolism and transport in mammalian cells.** **a** | Ferric iron (Fe^{3+}) in the bloodstream binds to transferrin to form holo-transferrin, with one or two atoms of ferric iron per transferrin. Holo-transferrin binds to transferrin receptors on the cell surface. The transferrin receptor–holo-transferrin complex undergoes endocytosis through clathrin pit formation. The endosome then acidifies, and the endosomal metalloreductase STEAP3 reduces ferric iron to ferrous iron (Fe^{2+})[10], allowing iron, now released from transferrin, to be transported into the cytosol by divalent metal transporter 1 (DMT1). Iron can then bind to chaperones that donate iron to specific target proteins[11] (not shown) or enter mitochondria through the dedicated mitochondrial iron transporters mitoferrin 1 or mitoferrin 2 (REF. 12), where it is used for the synthesis of iron–sulphur clusters and haem. Iron can also be stored in cytosolic proteins such as ferritin, which can sequester up to 4,500 iron atoms. Ferritin sequestration of iron prevents free iron from reaching high concentrations in the cytosolic and nuclear compartments[13]. Degradation of ferritin in lysosomes leads to the formation of disorganized iron-rich deposits known as haemosiderin[14]. **b** | Mechanism for iron to cross the blood–brain barrier. Holo-transferrin circulates through brain capillaries, and luminal transferrin receptors internalize iron using the same mechanisms as described for non-polarized cells. The iron exporter ferroportin may allow iron to cross the abluminal membrane of the endothelial cell to enter the interstitial fluid. Most transferrin in the brain interstitial fluid is synthesized and secreted by oligodendrocytes as apo-transferrin. Astrocytes express the ferroxidase ceruloplasmin, which is linked to the membrane by glycosyl phosphatidylinisotol (GPI). Ceruloplasmin and hephaestin, another ferroxidase, are expressed differentially throughout the CNS. Neurons and glia probably acquire most iron through the transferrin receptor and holo-transferrin that is present in interstitial fluid and cerebrospinal fluid. **c** | In the cytosol, iron regulatory protein 1 (IRP1) and IRP2 affect post-transcriptional regulation of cellular iron metabolism by sensing iron levels in the cytosol and then binding to iron-responsive elements (IREs) when iron levels are low. About nine genes contain functional IREs. Most transcripts contain IREs near the 5′ end of the transcript, where IRP binding interferes with the assembly of the translational machinery and prevents synthesis of the encoded protein. In the gene encoding transferrin receptor 1 (*TFRC*), and possibly in an alternatively spliced *DMT1* transcript, IRP binding stabilizes the mRNA, leading to increased synthesis of the normally encoded protein. *FPN1*, ferroportin 1; *FTH1*, ferritin, heavy polypeptide 1; *FTL*, ferritin light polypeptide; *HIF2A*, hypoxia-inducible factor 2α.

raises the possibility that regional blood flow is regulated both by hypoxia and by iron deficiency (in addition to its regulation by other metabolic factors that link perivascular astrocytes to brain chemosensing and its regulation in response to neuronal activity (reviewed in REF 26)). In the CNS, transferrin is mainly synthesized and secreted by oligodendrocytes and cells of the choroid plexus[27].

It is likely that iron enters the CNS not only through the endothelial cells of the BBB[22,28] but also through the epithelial cells of the choroid plexus, using many of the same transporters that have been identified in the duodenum[28]. Unlike the capillaries of the BBB, the capillaries of the choroid plexus are fenestrated, and holo-transferrin can readily cross endothelial cells to reach the basolateral

membrane of the polarized choroidal epithelium, where tight junctions between cells make it necessary for iron to traverse the epithelial cell.

Upon entry into the interstitial fluid or CSF in ventricles, iron binds to transferrin synthesized in the CNS and diffuses through the CSF and interstitial fluid of the brain parenchyma as holo-transferrin[17], delivering iron to cells within the CNS that express TFRC. Ultimately, apo-transferrin (that is, iron-free transferrin) and holo-transferrin probably return to the systemic circulation by passing through the arachnoid villi into veins that return blood to the systemic circulation[17,19].

Iron metabolism in the brain

Most cells in the body, including in the brain, express a full complement of iron proteins, including TFRC and DMT1 for iron uptake, ferritin heavy and light chains for iron sequestration, the mitochondrial iron importers mitoferrin 1 and/or mitoferrin 2 to supply iron to mitochondria, the cytosolic iron exporter FPN1 to export iron and iron regulatory protein 1 (IRP1; also known as ACO1) and IRP2 (also known as IREB2) to regulate intracellular iron homeostasis (reviewed in REF. 29). However, the amount of each protein expressed varies greatly depending on the cell type and its iron status. In the brain, oligodendrocytes stain strongly for transferrin, whereas microglia stain most strongly for ferritin, and neurons stain quite strongly for TFRC; on the basis of expression levels, stains for each of these proteins were initially used as markers to distinguish different cell types[30]. The inability to identify TFRC on mature oligodendrocytes has led to the assertion that oligodendrocytes acquire iron from the ferritin heavy chain[31] rather than from circulating holo-transferrin[28], although immature oligodendrocytes do express TFRC[31]. Most brain cells acquire iron through TFRC and endosomal DMT1, store iron in ferritin and export iron through ferroportin.

The iron ferroxidases ceruloplasmin and hephaestin, which are almost 50% identical, facilitate ferroportin export activity and are widely expressed throughout the CNS[32]. Hephaestin encodes a transmembrane peptide sequence that attaches the ferroxidase component of the protein to the plasma membrane of cells. Soluble ceruloplasmin is detectable in the CSF at a low concentration (1.5 μg per ml), which is about 100-fold less than the concentration of serum ceruloplasmin[33]. In the CNS, most ceruloplasmin is synthesized by astrocytes, and alternative splicing of the ceruloplasmin transcript in astrocytes leads to attachment of glycosyl phosphatidylinisotol (GPI)[34], which links ceruloplasmin to the outer leaflet of the plasma membrane. Soluble ceruloplasmin in the CSF may be released mainly by cleavage of the GPI linkage on astrocytes.

IRP1 and IRP2 regulate post-transcriptional iron homeostasis in most known cell types[29,35,36] and both are expressed throughout the brain. Virtually all cells express both proteins, although IRP2 is more highly expressed in many brain cells[37]. Two of the most important targets for regulation by IRPs are transcripts that encode ferritin and TFRC. In iron-depleted cells, IRPs bind to stem-loop structures in transcripts to prevent translation of ferritin or to prevent degradation of the *TFRC* transcript. Through these actions, they increase iron availability by simultaneously increasing iron uptake through TFRC and preventing sequestration of iron in ferritin (FIG. 1c). In iron-replete cells, the iron-responsive element (IRE)-binding form of IRP1 converts to an active cytosolic aconitase that interconverts cytosolic citrate and isocitrate, whereas IRP2 is degraded by the ubiquitin proteasome system[38]. Thus, it appears likely that each resident CNS cell type regulates its own iron homeostasis, distributing and storing iron according to the particular needs and functions of each cell type[28]. Nevertheless, there is some specialization of iron metabolism within the brain: astrocytes synthesize GPI-linked ceruloplasmin, and oligodendrocytes synthesize mainly hephaestin[39]. In addition, transferrin is synthesized and secreted into CNS interstitial fluid primarily by oligodendrocytes. The secreted transferrin binds iron that has been imported into the CNS and newly constituted holo-transferrin redistributes iron to cells exposed to CSF and interstitial fluid. This is presumably aided by diffusion and bulk flow of the CSF through the ventricles and into interstitial spaces to flow back through the venous system into the systemic circulation each day.

Brain iron accumulation

Iron accumulation occurs in the brain in ageing animals, including humans, in areas primarily associated with motor activity, including the globus pallidus, red nucleus, dentate nucleus and substantia nigra[40–43], but the factors that favour regional iron accumulation remain unknown. These brain regions become rich with ferritin iron (that is, iron stored inside ferritin), which tends to accumulate in humans by the third decade of life and to colocalize with iron, as detected by histopathology and immunohistochemistry (reviewed in REFS 44,45). Levels of iron in the globus pallidus are as high as levels in the human liver, a known repository for iron[46]. It is not known why so much iron is stored in the globus pallidus and other basal ganglia, but it is possible that some specialized neurons in the globus pallidus and basal ganglia are programmed to transcribe high amounts of ferritin and thereby create a ferritin-rich iron repository in the CNS — analogous to the liver iron repository created by hepatocytes that serves the systemic circulation. Perhaps previously unknown cells types, such as those recently discovered in the globus pallidus[47], are programmed to store iron and create a CNS iron reservoir.

The iron accumulation associated with ageing is not generally associated with pathology (most ageing individuals do not develop neurodegenerative disease). This suggests that the iron observed is contained in healthy ferritin-rich cells, which may include unique types of neurons and/or oligodendrocytes, astroglia and microglia in the iron-rich brain regions. Some cells can become ferritin-rich through high expression of ferritin transcripts; when ferritin transcription increases, ferritin protein synthesis also increases, even though the IRE–IRP regulatory system superimposes its regulatory activity on the translation of target transcripts.

The composition of cells and iron content of a brain region may change when an iron-rich area begins to degenerate. When a cell dies, microglia and/or macrophages that invade from the peripheral circulation phagocytose debris released by degenerating cells[48]. When many cells die in an iron-rich brain area, these scavenger cells become iron-rich by virtue of having phagocytosed iron-rich cellular debris. Moreover, microglia express high amounts of ferritin upon activation[49]. Some diseases, including Parkinson's disease, seem to specifically affect iron-rich areas such as the substantia nigra[50,51]. This makes it difficult to ascertain whether the iron accumulation often observed in Parkinson's disease is a cause or a consequence of the degeneration of substantia nigra neurons associated with this disease[2]. Similarly, in Huntington's disease, microglial ferritin and iron accumulation occur early, raising the question of whether these changes result from or cause neuronal loss[52]. Whether iron overload occurs in Alzheimer's disease is a subject of debate, as it is not detected in many studies[53], but iron accumulation as a cause has been proposed by many[54,55] and is suggested by MRI results[56]. Careful analysis of the temporal progression and distribution of iron accumulation in diseases complicated by abnormal brain iron accumulation may help to determine whether iron misregulation has a primary role in the pathogenesis of some of the subtypes of these common diseases.

Brain iron accumulation in disease. Many papers have been written about iron overload in the brain and the possible contribution of brain iron overload to various human diseases. It has been proposed that iron overload develops in certain brain regions that are predisposed to mishandle iron because they are already very iron-rich and that diseases such as Parkinson's disease, Friedreich's ataxia and various other diseases associated with brain iron accumulation could be ameliorated by treatment with iron chelators, which would reduce harmful iron-dependent oxidations of DNA, proteins and lipids[4,57,58] (reviewed in REF. 45). This issue has been much discussed in the context of Parkinson's disease and Alzheimer's disease without clear resolution (see REFS 2,54,55 for reviews). Here, I will focus mainly on a defined group of diseases termed NBIA diseases. These diseases are associated with mutations in identified genes and are characterized by movement dysfunction, gait problems, spasticity and motor and cognitive problems, as well as with iron accumulation in specific brain regions, as observed initially in autopsy studies and more recently in MRI studies. In NBIA diseases, iron accumulation occurs in defined areas of the brain, usually including the globus pallidus but sometimes extending to other areas such as cerebellum or substantia nigra, depending on the disease. Nine different NBIA diseases have so far been recognized (listed in TABLE 1), and more will probably be identified. However, of the mutated genes that cause NBIA diseases, only two encode proteins that are specifically involved in iron metabolism — ceruloplasmin and ferritin light chain. The other NBIA disease genes encode proteins with other functions, some of which are related to fatty acid metabolism or lysosomal activity[59–61].

NBIA caused by defective iron metabolism

Aceruloplasminaemia. Aceruloplasminaemia was originally described in 1987. Up to 35 affected families have now been described in multiple Japanese kindreds[62–64], and patients have been identified all over the world[23]. It is caused by mutations in *CP*, the gene that encodes ceruloplasmin, which result in loss of the functional protein. Symptoms of aceruloplasminaemia include a triad of adult-onset neurological disease, diabetes mellitus and retinal degeneration (reviewed in REF. 23), and its pathology has been characterized in human autopsy samples[64] and in mice lacking *Cp*, which develop ataxia[65,66].

Marked iron overload has been observed in astrocytes in the affected brain regions of patients with aceruloplasminaemia, particularly in the basal ganglia[34]. These brain regions usually show loss of neurons and accumulation of large iron-rich globular structures that appear to represent astrocytic remnants[67]. The events that lead to neuronal loss remain somewhat unclear[23]. There is evidence for markedly increased levels of oxidative stress, as indicated by increased lipid peroxidation and protein carbonylation[67]. Loss of Purkinje and deep cerebellar neurons has been observed, but these cells do not manifest iron overload[66]. How might astrocytic iron overload occur in aceruloplasminaemia? As described above, ceruloplasmin is a ferroxidase that facilitates ferroportin-mediated cellular iron export and oxidizes ferrous iron (Fe^{2+}) to ferric iron (Fe^{3+}), generating the oxidized form of iron that can bind to extracellular transferrin (FIG. 1c), and astrocytic ceruloplasmin is required for iron export from astrocytes[34]. Thus, in aceruloplasminaemia, iron entering the CNS as ferrous iron might not undergo oxidation, and cells exposed to the resulting excess ferrous iron could readily become iron-loaded through an unregulated pathway of non-transferrin-bound iron uptake[68]. The unregulated uptake of ferrous iron coupled with an inability to export iron could produce the marked astrocytic iron overload that has been observed in this disease. It is possible that iron does not reach neurons, causing them to die as a result of both iron deficiency and exposure to toxins released from nearby astrocytes that are dying from iron overload. Marked astrocytic iron overload in conjunction with neuronal loss was recently observed not only in the basal ganglia but also in the cerebrum in a human case report[69]. Some mutant forms of ceruloplasmin may also accumulate in aggresomes and cause cell death through a non-iron related mechanism[67,70].

Although astrocytes depend on GPI-linked ceruloplasmin for ferroxidase activity, other cells in the CNS, including oligodendrocytes, depend on hephaestin[39]. Mice lacking both ceruloplasmin and hephaestin develop abnormalities reminiscent of age-related macular degeneration and show retinal iron overload, increased retinal oxidative stress (probably caused by the iron overload in retinal pigment cells) and retinal degeneration[32]. The oral iron chelator deferiprone was reported to protect against

Table 1 | **Neurodegeneration with brain iron accumulation diseases**

Disease	Disease gene	Brain iron features	Clinical features
Pantothenate kinase-associated neurodegeneration (PKAN)	*PANK2*	Iron overload in the globus pallidus in >90% of patients; 'eye of the tiger' sign common	Childhood-onset dystonia and spasticity[99]
PLA2G6-associated neurodegeneration (PLAN)	*PLA2G6*	Iron overload in the globus pallidus in <50% of patients[138]; iron overload observed in the substantia nigra	Infantile neuronal dystrophy associated with hypotonia, gait disturbances and cerebellar atrophy; onset in childhood and adulthood is associated with dystonia, spasticity and parkinsonism[138]
Mitochondrial membrane-associated neurodegeneration (MPAN)	*C19orf12*	Iron in the globus pallidus and substantia nigra; abundant Lewy bodies[113]	Global developmental delay, cognitive and motor delay, dystonia, dementia and parkinsonism[84]
Fatty acid hydroxylase-associated neurodegeneration (FAHN)	*FA2H*	Leukodystrophy; iron deposition in the globus pallidus and, in some cases, in the substantia nigra	Dysarthria, gait abnormalities, dystonia and parkinsonism[115]
Neuroferritinopathy	*FTL*	Iron deposition in the dentate nuclei, globus pallidus, putamen, caudate, thalamus and red nuclei	Onset of dystonic gait late in disease, with preservation of cognition[72]
Aceruloplasminaemia	*CP*	Iron deposition in the dentate nuclei, globus pallidus, putamen, caudate, thalamus and red nuclei	Onset of cognitive impairment late in disease, with cerebellar ataxia, retinal degeneration and craniofacial dyskinesia[23]
Kufor–Rakeb disease	*ATP13A2* (also known as *PARK9*)	Often no brain iron accumulation[86,139,140]	Dystonia and dystonia and/or parkinsonism[86]
Woodhouse–Sakati syndrome	*C2orf37* (also known as *DCAF17*)	Often no brain iron (iron in the globus pallidus and substantia nigra in some patients)[141]	Dystonia and deafness[141]
β-propeller protein-associated neurodegeneration	*WDR45*	Iron deposition in the substantia nigra and globus pallidus[85]	Parkinsonism, dystonia, dementia[85] and global developmental delay

ATP13A2, ATPase type 13A2; *C2orf37*, chromosome 2 open reading frame 37; *C19orf12*, chromosome 19 open reading frame 12; *CP*, ceruloplasmin; *FA2H*, fatty acid 2-hydroxylase; *FTL*, ferritin light polypeptide; *PANK2*, pantothenate kinase 2; *PLA2G6*, phospholipase A2, group VI; *WDR45*, WD repeat domain 45.

the iron overload-induced retinal oxidative stress and degeneration, to prevent accumulation of the fluorescent and reactive pigment lipofuscin (a product of protein degradation in iron-overloaded cells)[71], to reduce ataxia and to increase the lifespan of these mice[71]. This may suggest that iron chelation could be beneficial in patients with aceruloplasminaemia. However, in one recent report, iron chelation appeared to worsen symptoms in the patient[69], which is consistent with the possibility that neurons are in fact iron-deficient in aceruloplasminaemia.

Neuroferritinopathy. A second form of iron metabolism-related NBIA disease is neuroferritinopathy, an autosomal dominant neurodegenerative disease that affects the globus pallidus and other deep brain structures[72]. Seven distinct disease-causing mutations have been reported in this disease, but it is most commonly caused by the insertion of an extra nucleotide in the fourth exon of the gene encoding the ferritin light chain, which causes a frameshift and synthesis of an abnormal C terminus[73]. When the ferritin light chain with this abnormal C terminus (which may constitute an important part of the iron entry pore[72]) is incorporated into the ferritin heteropolymer, the spherical ferritin no longer has an intact proteinaceous shell to protect sequestered iron, and as a result, it is likely that iron 'leaks' out of ferritin[74] (FIG. 2). This could cause iron-dependent oxidation, particularly in cells that normally express high amounts of ferritin light chains[73]. The ferritin heavy and light chain gene promoters contain binding sites for nuclear factor erythroid 2-related factor 2 (NRF2; also known as NFE2L2), and its binding is induced by oxidative stress[75,76]; transcription of the abnormal ferritin light chain may therefore further increase in response to the oxidative stress caused by iron leakage. Moreover, the failure to sequester iron and thereby reduce cytosolic iron levels may lead to further increased production of abnormal ferritin, because IRE-binding proteins do not switch to the IRE-binding form when cytosolic iron levels remain high, and ferritin translation therefore continues unabated[77]. Thus, the initial failure to successfully sequester iron may lead to increased expression of the abnormal protein both through increased transcription and reduced translational repression of the ferritin light chain transcript. The presence of one abnormal allele that encodes a truncated form of ferritin is sufficient to cause neuroferritinopathy associated with cognitive and behavioural impairments, which generally manifest between the third and sixth decades of life[77].

A mouse model of neuroferritinopathy recapitulates many aspects of the disease[78] and therefore can be used as a model for testing therapies. Crystal structures of ferritin containing the mutant light chain have revealed that

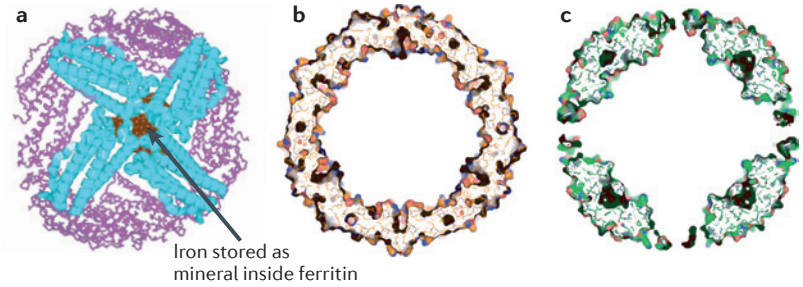

Figure 2 | **The proteinaceous ferritin shell is porous in neuroferritinopathy.** **a** | A three-dimensional view of ferritin demonstrates the spherical protein surface composed of two different ferritin peptides (the heavy subunit and the light subunit) shown in blue and purple, respectively, and the stored iron within, which is oxidized and insoluble. Normal ferritin stores thousands of iron atoms inside the central cavity. **b** | The crystal structure of normal ferritin is shown in cross-section, and there are no discontinuities present on the surface. **c** | In neuroferritinopathy, the spherical protein shell of ferritin (shown in cross-section and coloured green) has discontinuities that allow iron to leak out, perhaps in the form of oxidized iron oxide aggregates. The crystal structure of ferritin from patients with neuroferritinopathy revealed that the truncated C terminus of the light ferritin peptide leaves an opening to the cytosol[77]. Images courtesy of R. Vidal, Indiana University, Bloomington, USA.

the initial iron sequestration is impaired and that iron-induced ferritin precipitation is enhanced[77]. It is not yet clear whether iron chelator treatments confer any therapeutic benefit in this disease[79]. A possible future treatment might involve degradation of the abnormal ferritin light chain transcript using RNAi[80] or, potentially, antisense oligonucleotides that bind specifically to the abnormal transcript (which contains the adenine insertion) to prevent its use as a template for new protein synthesis[81].

Other NBIA diseases

A second group of NBIA diseases consists of rare genetic diseases in which the disease gene has been identified but is not involved in iron metabolism, and the molecular causes of brain iron overload and neuronal death in these diseases therefore remain unclear. They include pantothenate (also known as vitamin B_5) kinase-associated neurodegeneration (PKAN)[82], phospholipase A2, group VI (PLA2G6)-associated neurodegeneration (PLAN)[83], fatty acid hydroxylase-associated neurodegeneration (FAHN)[83], mitochondrial membrane-associated neurodegeneration (MPAN)[84] and several new or rare diseases, including the recently described β-propeller disease[85] and several diseases in which iron accumulation is not always present, such as Kufor–Rakeb disease[86] and Woodhouse–Sakati syndrome[87] (TABLE 1).

PKAN. PKAN (previously known as Hallervorden–Spatz syndrome) is the best-known example of neuronal brain iron accumulation associated with neurological impairments, including childhood-onset dystonia and spasticity[82]. The gene associated with the disease was identified in 2001 as the gene that encodes pantothenate kinase 2 (PANK2)[88], an enzyme that occupies the mitochondrial inter-membrane space[89,90] and is important for biosynthesis of coenzyme A from pantothenate. Coenzyme A, a high-energy carrier of acetyl and fatty acid groups, is important in multiple metabolic pathways, including the citric acid cycle, fatty acid oxidation and synthesis, and cholesterol and sphingolipid synthesis.

PANK2 dysfunction is compatible with life, and two functional homologues, *PANK1* and *PANK3* (REF. 91), encode isoforms that are located in the cytosol and may compensate for the loss of PANK2. There is also a PANK4 protein, which is fairly dissimilar from PANK1, PANK2 and PANK3 and apparently lacks enzymatic activity[92].

Mice lacking *Pank2* (*Pank2*$^{-/-}$ mice)[93] did not develop brain iron accumulation or apparent neurological difficulties as expected, but they developed retinal problems and had a lack of viable sperm[93]. A subsequent study showed that neurological symptoms occurred only when *Pank2*$^{-/-}$ mice were deprived of dietary pantothenic acid[94]. Fly *PANK2* homologue (*fumble*) hypomorphs did not show CNS iron overload, although they developed neurodegeneration and had diminished coenzyme A levels[95,96]. Interestingly, both effects could be prevented by providing pantethine in the diet, which may allow bypass of the mutant enzyme and may be a possible approach for the treatment of human subjects[97–99].

Notably, patients with PKAN have increased serum levels of lactic acid and pantothenate, and an analysis of fibroblasts from these patients suggested that cholesterol levels and fatty acid synthesis were decreased[100]. Moreover, an analysis of mitochondria from *Pank2*$^{-/-}$ mice found that there were reductions in mitochondrial potential, oxygen consumption and ATP generation, and that numerous mitochondria were swollen and contained disrupted cristae[90].

In the absence of an animal model that develops overt brain iron overload that is attributable to a loss of PANK2, it is difficult to explore why PANK2 deficiency is associated with profound iron overload specifically in the globus pallidus. Abnormal ferroportin expression has been described in PANK2-deficient cell lines[101]. Perhaps the globus pallidus is particularly affected in PKAN because the expression levels of PANK1 and/or PANK3 are too low to compensate for the loss of PANK2. At present, the relative PANK expression profiles of the various cells of the globus pallidus in healthy individuals are not known. If PANK2 deficiency causes frank PANK deficiency in the globus pallidus, then cellular energy failure might be expected to be a primary problem in PKAN pathogenesis. Specialized MRI techniques[102] might reveal diminished mitochondrial respiration in the globus pallidus of patients with PKAN, even at very early stages of disease.

Some early descriptions of pathological changes in PKAN were based on subjects who were not genetically classified, but more recent studies of genetically confirmed patients with PKAN have shown diffuse iron staining of the neuropile in the globus pallidal areas[103]. Here, increased deposits of iron were detected in perivascular regions, where astrocytic processes are usually found. Importantly, iron was also increased in the cytoplasm of degenerating neurons, implying that neurons manifest iron overload before their demise and that iron overload may therefore contribute to neuronal loss in PKAN[103]. Astrocytes showed marked iron overload in the globus pallidus of patients with PKAN, but there was no substantial iron accumulation in microglia or oligodendrocytes[103].

These findings provided a histological explanation for a characteristic MRI finding known as the 'eye of the tiger' — a region of hyperintensity surrounded by an area of hypointensity (characteristic of iron accumulation). In a recent pathological study, the 'eye of the tiger' was identified as an ovoid region in the globus pallidus that was markedly depleted of viable neurons but rich in large spheroids that consisted of degenerating neurons, and smaller spheroids composed of dystrophic axons[103]. This region was also remarkably devoid of intact synapses, which probably contributed to the diminished tissue density (known as rarefaction). Serial MRI studies of presympomatic patients with PKAN[104] may support the possibility that neuronal loss precedes iron accumulation and that iron accumulation may be a secondary effect[83], although earlier diagnosis and further serial MRI studies will be needed to clarify the precise order of events (FIG. 3).

PLAN. After *PANK2* sequencing became available, many patients who had previously been grouped together diagnostically turned out to have mutations in different genes. The first disease to be recognized as a distinctive non-PKAN type of NBIA disease was PLAN[83]. Some older patients with PLAN develop dystonia and spasticity, but (unlike in PKAN) infantile neuronal dystrophy and cerebellar atrophy with hypotonia and gait abnormalities are common presentations of PLAN, and abnormal iron accumulation in the globus pallidus is observed only in approximately 50% of cases[83]. The *PLA2G6* gene encodes a calcium-independent phospholipase that may play a critical part in cell membrane homeostasis[105] and may thereby contribute to the axonal pathology of these patients. Mice that lack PLA2G6 have disorganized mitochondrial inner membranes[106], which are distinctive because they contain large amounts of cardiolipin, a unique phospholipid that consists of two phosphatidyl groups bridged by a glycerol[107]. The flexible structure of cardiolipins enables them to envelop respiratory chain complexes, tether these complexes to the inner mitochondrial membrane and protect proteins from free radical damage by donating electrons from the unsaturated fatty acid side-chains of cardiolipin. The role of PLA2G6 may be to remove saturated fatty acid side-chains from newly synthesized cardiolipins so that they can be replaced with unsaturated linoleic fatty acids (which are characteristic of cardiolipins[108]) and also to remove fatty acids damaged by oxidative stress. Although PLA2G6 may be important in deacylation (removal of fatty acid side-chains from phospholipids), the pathway for reacylation in the brain is not yet known[106]. However, a study in mice revealed that one of the first pathological signs of PLA2G6 deficiency is disorganization of the mitochondrial inner membrane cristae, followed by mitochondrial rupture, release of cytochrome *c* and formation of swollen axons as the disease progresses. Presynaptic membranes were also abnormal in PLA2G6-deficient mice, perhaps because mitochondria at these sites require PLA2G6-mediated phospholipid remodelling to maintain integrity[106]. Because some patients with PLAN develop parkinsonism, the gene has been categorized as a Parkinson's disease gene and is also known as *PARK14* (REF. 109). Iron accumulation is found in macrophages and in aggregates in the globus pallidus in patients with the parkinsonian presentation[110].

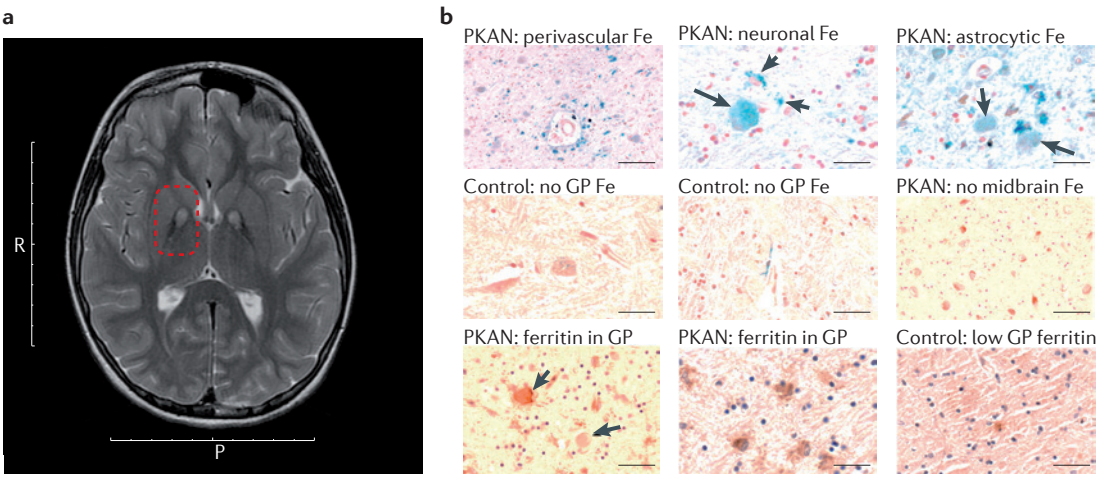

Figure 3 | **MRI, pathology and potential molecular basis of PKAN.** **a** | MRI of a patient with pantothenate kinase deficiency-associated neurodegeneration (PKAN) shows the classical 'eye of the tiger' sign on axial T2 fast spin-echo imaging. Hyperintense (white) signals indicative of tissue rarefaction are seen (centre of red rectangle) surrounded by areas of hypointensity (black areas) attributable to iron accumulation. **b** | Pathological changes in the globus pallidus (GP) of patients with PKAN showing iron (Fe) accumulation and increased ferritin that are not present in the GP of a healthy control subject. Prussian blue staining in a patient with PKAN to detect ferric iron (top row) demonstrates that iron accumulates in perivascular areas, diffuse neuropile, some intact neurons (arrows) and some astrocytes (short arrows in middle panel and intensely blue stained accumulations in right panel, where arrows point to degenerating neurons). Normal controls do not show iron accumulation (middle row). High ferritin was detected in neuronal and astrocytic remnants in patients with PKAN (bottom row, left and middle) but not in controls (bottom row, right)[103]. Part **b** is reproduced, with permission, from REF. 103 © (2011) Oxford University Press. Image in part **a** courtesy of S. Hayflick, Oregon Health & Science University, Portland, USA.

The fact that the phenotypes of PKAN and PLAN have common features may be related to the possibility that the enzymes affected in the two diseases may contribute to the synthesis of cardiolipin in the mitochondrial inner membrane, either by synthesizing coenzyme A to activate fatty acids required for cardiolipin remodelling and repair (in the case of PKAN), or by supplying an enzyme that facilitates deacylation and remodelling of mature cardiolipin (in the case of PLAN) (FIG. 4a). Notably, a recent study showed that mitochondrial inner membranes are highly disorganized in *Pank2*$^{-/-}$ mice[90], similar to the mitochondrial inner membrane abnormalities observed in mouse models of PLAN[106] (FIG. 4b). Moreover, as the mitochondrial inner membrane is the anchor site of respiratory chain complexes, energy failure has been observed in PKAN[90] and has been suggested to occur in PLAN[106]. Notably, recent studies in *Saccharomyces cerevisiae* demonstrated that loss of cardiolipin synthase results in diminished synthesis of iron–sulphur clusters and mitochondrial iron overload[111], a complication that is frequently observed in human diseases caused by dysfunctional assembly of iron–sulphur clusters (reviewed in REF. 112). Thus, it is worth examining whether mitochondrial iron overload occurs in the early stages of PKAN and PLAN — that is, before cells die — and whether mitochondrial iron accumulation contributes to the iron overload observed on MRI scans and histological stains. In PKAN and PLAN, astrocytes might increase iron uptake through the BBB (FIG. 1) to compensate for deficient iron–sulphur cluster assembly, a possibility that is consistent with the build-up of iron in perivascular regions and within intact astrocytes of the globus pallidus in both diseases.

MPAN and FAHN. Another common form of NBIA syndromes is the recently described MPAN caused by mutations in chromosome 19 open reading frame 12 (*C19orf12*)[84,113,114], which encodes a mitochondrial protein of unknown function that is co-expressed with genes involved in fatty acid metabolism and branched

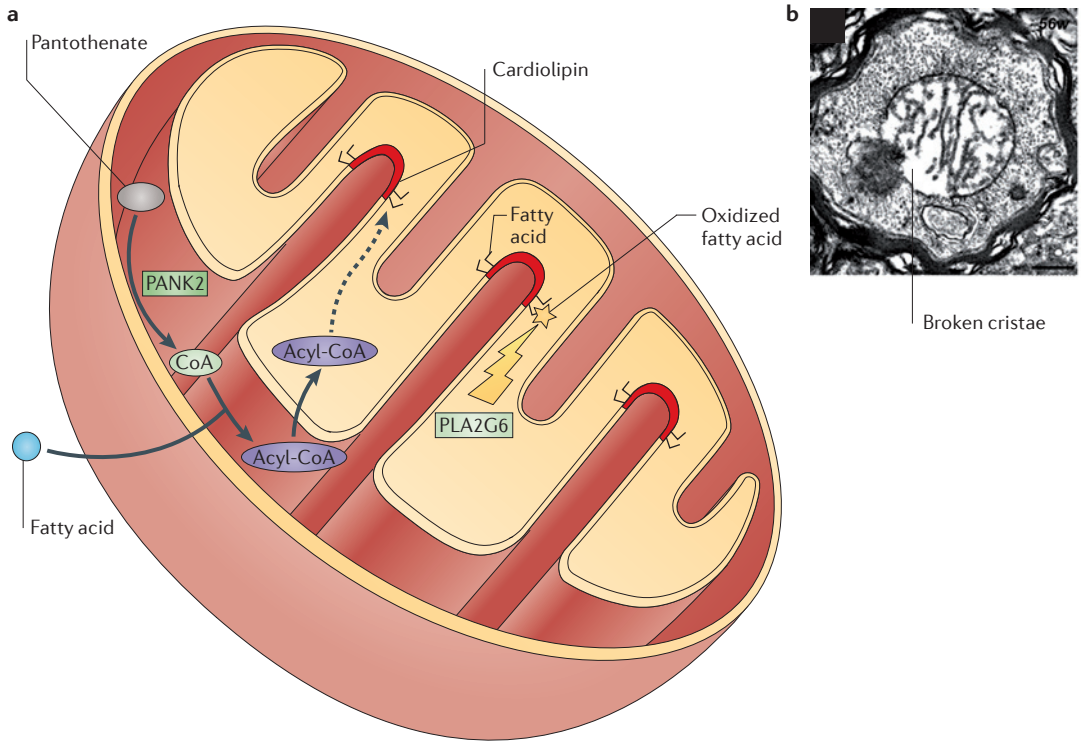

Figure 4 | **Potential effects of *PANK2* and *PLA2G6* mutations on mitochondria.** Mutations in pantothenate kinase 2 (*PANK2*) and phospholipase A2, group VI (*PLA2G6*) may interfere with synthesis and remodelling of the mitochondrial inner membrane lipid cardiolipin (panel **a**), giving rise to characteristic loss of cristae as seen in electron micrographs of *Pank2*-knockout mice[90] and *Pla2g6*-knockout mice[106] (panel **b**). **a** | PANK2 is needed for the formation of coenzyme A (CoA) from pantothenate . CoA condenses with fatty acids to form acyl-CoA, which crosses into the mitochondrial matrix using the carnitine carrier system (not shown). Acyl-CoA in the mitochondrial matrix either delivers fatty acids for incorporation into complex intra-mitochondrial lipids such as cardiolipin or may alternatively undergo oxidation by the mitochondrial respiratory chain to generate ATP (not shown). The matrix membrane contains cardiolipin, a flexible molecule in which two fatty acid-bearing glycerol molecules are bridged by a single glycerol. Cardiolipins enable the inner membrane to bend and turn. PLA2G6 may remove damaged fatty acids to allow incorporation of flexible unsaturated fatty acids, such as linoleic acid, from the acyl-CoA pool in the matrix. **b** | The mitochondrial inner membranes form convoluted structures known as cristae, and loss of cristae is characteristic of mouse models of PANK2 and PLA2G6 loss. Enzymes involved in maturation of mammalian cardiolipins are incompletely characterized, and 'new players and functions' are needed to understand how cardiolipins are synthesized[107]. Part **b** reproduced, with permission, from REF. 106 © (2011) Society for Neuroscience.

chain amino acid degradation[113]. Interestingly, patients with MPAN have Lewy bodies containing abnormal α-synuclein in the globus pallidus and midbrain areas, whereas Lewy bodies do not occur in PKAN[84]. In addition, iron deposits were increased in perivascular regions of patients with MPAN, similar to a distribution previously observed in patients with PKAN[84].

Another NBIA syndrome, FAHN, is caused by mutations in the gene encoding fatty acid-2-hydroxylase (FA2H)[115]. FAHN is characterized initially by spasticity, a mixed movement disorder, ataxia, dystonia, optic atrophy and oculomotor abnormalities, and later by progressive intellectual impairment and seizures[83,116]. As FA2H produces the 2-hydroxylated fatty acids that are incorporated into sphingolipids[117], FA2H deficiency leads to abnormal myelination (up to 15% of myelin lipids contain the product of FA2H[118]). Indeed, axonal function is compromised in mice lacking FA2H[119]. Interestingly, in mouse models of FAHN, regions of pronounced white matter degeneration showed accumulation of microglia-like cells[119]. MRI studies in patients with FAHN have detected iron accumulation not only in the globus pallidus but also in the substantia nigra and subcortical and periventricular regions[83], a distribution that readily distinguishes FAHN from PKAN. Migration of microglial cells into areas of tissue degeneration has been observed in mouse models[119], and these microglia may accumulate iron-loaded ferritin. Thus, it would be interesting to correlate iron overload detected by MRI with iron stains of pathological samples obtained at autopsy from patients with FAHN.

New forms of NBIA are being discovered every year, and characteristics of three diseases that are not discussed here are summarized in TABLE 1. Notably, the cause and clinical significance of the iron accumulation may differ in each case.

Diseases characterized by mitochondrial iron overload. In some diseases, such as Friedreich's ataxia, mitochondrial iron misregulation contributes to neuronal death[112,120]. In Friedreich's ataxia, a defect in iron–sulphur cluster assembly leads to mitochondrial failure and mitochondrial iron overload in a specific subset of neurons, including the large neurons of the dentate nucleus of the cerebellum and the sensory neurons of the dorsal root ganglia[121]. Friedreich's ataxia has also occasionally been classified as a brain iron overload syndrome based on the discovery of high iron levels in the cerebellar dentate nucleus with enhanced Prussian blue staining[122]. However, further analysis of autopsy material suggested that in Friedreich's ataxia iron-rich neurons in the dentate nucleus die, and this is followed by the appearance of iron-rich glial cells that do not occupy the same anatomical position as the intact neurons occupied before the onset of disease[123,124]. Thus, it seems that neuronal death precedes the accumulation of iron that has been reported in this disease, and Friedreich's ataxia therefore does not fit well into the NBIA classification scheme. However, it raises an interesting question about whether mitochondrial iron overload occurs in the NBIA syndromes discussed above. Although ultrastructural pictures do not show the characteristic black iron deposits associated with mitochondrial iron overload in Friedreich's ataxia and several related diseases[112], dense particles of unknown composition are reported in the mitochondria of *Pla2g6*-deficient mice[106], and highly sensitive iron-staining procedures such as Perls' DAB (diaminobenzidine tetrahydrochloride) could reveal subtle iron accumulations in the PKAN and PLAN mouse models. Specialized microscopic techniques for high-resolution iron detection[125] might also yield interesting findings about mitochondrial iron overload in the PANK and PLAN mouse models.

Brain iron overload is attributable to the accumulation of iron-rich microglia. Another category of neurodegenerative disease associated with abnormal iron metabolism involves diseases in which the primary cause of neuronal death is unknown but in which visible iron overload occurs as a secondary phenomenon — namely, when microglia catabolize cell debris and express large amounts of iron-rich ferritin. This type of iron-related

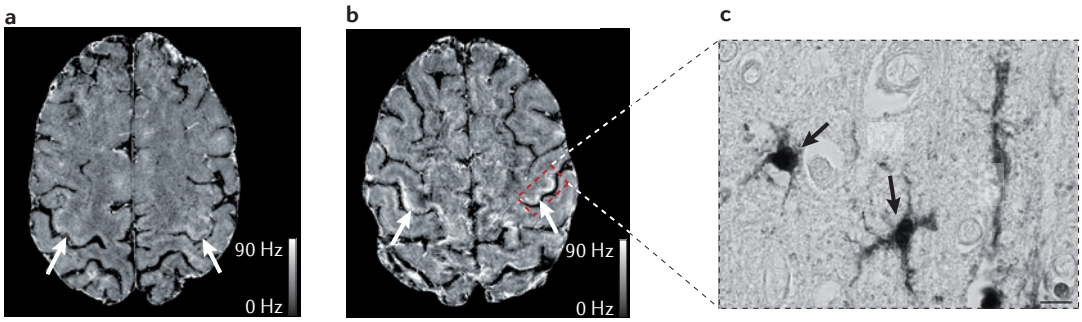

Figure 5 | **MRI detection of iron — is iron accumulation a cause or consequence of disease?** MRI has revealed iron overload in the motor cortices of patients with amyotrophic lateral sclerosis (ALS) on R2* maps of 7T MRI scans, raising the possibility that iron overload is important in disease pathogenesis. In a recent MRI and autopsy study, an iron overload signal was detected by MRI in the motor cortex (white arrows) of a patient with ALS (panel **b**, red box), whereas the signal was absent in the motor cortex of a normal control (panel **a**). Histochemical staining of the iron-rich area obtained at autopsy revealed that motor neurons were absent or shrunken in the cortical motor strip (panel **b**), and the iron signals were caused by infiltration of iron-rich microglia (panel **c**, arrowheads)[126]. Figure is reproduced from REF. 126.

brain disease is exemplified by amyotrophic lateral sclerosis (ALS). A recent combined MRI and pathological study of ALS detected iron accumulation in activated microglia in the motor cortex area of several autopsied patients, specifically in the region occupied by upper motor neuron cell bodies before death[126] (FIG. 5). This finding may be relevant in light of studies that have suggested that primary iron overload is an important factor in the pathogenesis of Parkinson's disease and related diseases of the basal ganglia. Chelator therapy is unlikely to be valuable after neurons have died and been replaced by microglia.

A cautionary tale from a mouse model. Recent studies in *Irp2*$^{-/-}$ mice demonstrated that the main cause of neuronal dysfunction and neuron death in these mice is probably neuronal iron deficiency and concomitant failure of the mitochondrial respiratory chain rather than iron overload[127]. Neuronal iron deficiency develops concurrently with iron overload in glial cells, such as oligodendrocytes and microglia, as detected by histopathology[128,129] (although these changes were disputed in a comparable mouse model[130]). The iron 'overload' reported in earlier studies that was visible in *Irp1*$^{-/-}$ animals in many brain regions reflected increased ferritin — the storage form of iron that can be readily detected by both MRI and immunostaining. As loss of IRP2 de-represses ferritin translation[29] (FIG. 1c), *Irp2*-deficient animals have high levels of ferritin and therefore misallocate their iron to a storage protein rather than to the many metabolic proteins that need iron for their functioning, including proteins of the mitochondrial respiratory chain[131]. Moreover, loss of *Irp2* leads to reduced expression of TRFC (FIG. 1c) and the iron transporter DMT1, which further exacerbates intracellular iron deficiency[29]. In the *Irp2*$^{-/-}$ animals, it appears that the death of motor neurons is the most prominent cause of disability and that motor neuron dysfunction and death probably stem from intracellular iron deficiency and mitochondrial dysfunction[127].

Although no human counterpart of *Irp2* loss has yet been discovered, the phenomenon that staining methods can implicate iron overload under certain conditions, even when neurons are prone to death from iron deficiency may also apply to human patients in whom cellular iron levels have so far only been assessed using traditional histochemical methods.

Conclusions and future directions

Abnormal iron metabolism in the brain has been unequivocally identified as a heritable cause of two rare diseases, aceruloplasminaemia and neuroferritinopathy. Iron accumulation may also have a causal role in many other diseases (TABLE 1). Interestingly, among the NBIA diseases, *PLA2G6* is also classified as *PARK14* (REF. 109) because mutations cause some patients to present with Parkinson's disease, and parkinsonian features are frequently present in the other NBIA diseases (TABLE 1).

Importantly, iron overload detected in specific brain regions in MRI scans does not necessarily reflect iron overload in neurons, as the current scale of imaging does not permit identification of specific cell types. In human diseases, few studies have matched MRI scans with pathology specimens to identify iron-overloaded cells or to determine whether the iron is extracellular. Serial MRI may help to define whether iron overload becomes a problem early in the course of disease. It is possible that much of the iron overload detected in a region of diseased brain is sequestered within reactive microglia, which express high amounts of the iron-rich protein ferritin[132] and its degradation product, haemosiderin, when they are activated[133]. Thus, in some NBIA diseases, iron accumulation may be the result of a microglial response to neuronal death and may not have a causal role in disease.

If iron overload is a primary cause of NBIA diseases, as appears to be the case in aceruloplasminaemia, it would be reasonable to expect that iron chelation might ameliorate symptoms. Indeed, iron chelation therapy prevented retinal degeneration in mice lacking both ceruloplasmin and hephaestin[71]. Even if the iron overload is not a primary driving force in pathophysiology, secondary iron overload can have serious consequences, which might be mitigated by iron chelation with deferiprone, which crosses the BBB (summarized in REF. 134). Chelation therapy has received considerable attention as a potential treatment for human diseases in which iron accumulation has been observed, such as Parkinson's disease and Alzheimer's disease, which are not classified as NBIA diseases (reviewed in REF. 45). However, these therapies have not been tested in a large enough number of patients to generate statistically meaningful results[135].

In the future, clinical studies will determine whether iron chelation therapy is helpful in PKAN and other NBIA diseases. As MRI has revolutionized diagnostics and gene discovery, further imaging combined with DNA sequencing and gene identification in familial diseases will probably reveal more types of rare and distinctive brain iron overload diseases. The relationship between movement disorders and abnormal CNS iron metabolism probably relates to a basic aspect of brain iron physiology in the basal ganglia that is not yet understood. It is possible that some neurons in the basal ganglia are programmed to transcribe high amounts of ferritin, which in turn leads to high sequestration of iron, and this may enable these cells to serve as a brain iron reservoir and to buffer the CNS against iron deficiency. In many cases of brain iron detection, iron accumulation may be a consequence of the disease process, and iron chelation may not help because it is a secondary phenomenon that develops after the disease is well under way. More work on the primary pathophysiology of these apparently disparate diseases may generate more treatments that focus on correcting dysfunction early in the course of disease. Identification of primary events may reveal whether antisense RNA directed at an abnormal allele (such as the ferritin light polypeptide allele of neuroferritinopathy), replacement in loss-of-function diseases such as PKAN and PLAN through gene therapy, dietary intervention in PKAN or other disease-specific interventions will benefit patients.

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