Iron as a therapeutic target for Parkinson's disease

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Iron as a therapeutic target for Parkinson's disease

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Introduction

An urgent need for efficacious disease modifying therapies is required to slow down Parkinson's disease (PD) progression. Iron is required as a cofactor in metabolic processes throughout the body and specifically in tissues of high oxygen consumption, such as the central nervous system. The redox chemistry of iron is critical for neurotransmitter regulation as well as mitochondrial oxidative phosphorylation, nitric oxide metabolism and oxygen transport.[1] Iron homeostasis involves the orchestration of systemic and cellular networks for the acquisition, internal distribution and utilization of iron.[2] Disruption of links can lead to abnormal redistribution of iron, causing deleterious consequences (siderosis) either by localized accumulation and/or deficits in specific cellular compartments or tissues. Excessive labile iron in the substantia nigra pars compacta (SNc) has become a pathognomonic hallmark of PD and leads to increased production of noxious reactive oxygen species (ROS), which is also prevalent in PD. Conversely, a deficiency in iron impairs energy production[2] and can also cause dopaminergic neurodegeneration in mice.[3] In mammalian models, chelators that scavenge intracellular iron protect against oxidative neuronal damage. However, these strong iron chelation regimens are designed to treat systemic siderosis and are not suitable for PD patients, as iatrogenic iron depletion and anaemia may ensue. Moderate iron chelation modality that conserves systemic iron offers a novel therapeutic strategy for neuroprotection.

Iron in dopaminergic neurons

Iron is particularly abundant in SNc dopaminergic neurons as an integral component of tyrosine hydroxylase (TH)-dependent dopamine synthesis as well as other enzymatic and non-enzymatic reactions associated with dopamine metabolism.[4] The identification of substantial brain labile non-heme high-spin complexes, which increase with age,[5] might explain the iron catalytic role in the generation of noxious ROS by Fenton chemistry involving hydrogen peroxide. In part, this results from the oxidative deamination of dopamine by monoamine oxidase and the formation of metastable iron-dopamine complexes that in turn lead to dopamine auto-oxidation and quinone formation.

The sequestration of various potentially toxic products sequestered by neuromelanin confer a distinctive pigmentation upon the SNc. However, as the neuromelanin sanctuary for toxins is lost during PD,[4] the labile endogenous autooxidation products of dopamine can strongly impair respiration by the mitochondrial complexes I and IV. A high-energy demand, due to autonomous pace-making activity, might also render the SNc more susceptible to imbalances in labile iron levels and ensuing ROS production.[6]

Iron deposits measured in the SNc in PD

Iron accumulation has been identified in the SNc of post mortem brains of patients[1,2,7,11] as well as all Parkinsonian animal models.[1,2,7-11] This has been confirmed in patients by iron-sensitive high field MRI (3 and 7 Tesla) with a quantitative weighted T2* sequence showing a higher R2* value (**Fig. 1**).[9,12,13] Longitudinal studies as well as a meta-analysis have identified a progressive iron accumulation in the SNc through the course of disease.[12,13,14] Visual assessment of dorsolateral SN hyperintensity by Susceptibility Weighted Imaging can differentiate PD versus controls[15] and these observations have been corroborated by reduced transverse relaxation;[16] another measurement of rate sequence. More recently, a novel MRI approach with Quantitative Susceptibility Mapping (QSM) has demonstrated superior

sensitivity for mapping changes in non-heme iron levels[17,18] and a new sequence of magnetization transfer contrast has identified a local neuromelanin density reduction in the SNc.[19] Transcranial ultrasound that visualizes hyperechogenicity of SNc tissue has also identified increased iron levels relating to alterations in iron metabolism genes.[20,21]

What could be the cellular mechanisms implicated in iron accumulation?

Impaired iron release

Ferroportin is depleted in parkinsonian models including intoxication with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and 6-hydroxydopamine (6-OHDA).[1] Depletion of either amyloid precursor protein (APP) or tau function causes neuronal iron retention as well as iron-dependent nigral cell loss, and both proteins are decreased in the SNc in PD.[7,22] Cellular iron egress by ferroportin may also be assisted by ceruloplasmin and mice deficient of this enzyme develop deferiprone (DFP) rescuable age-dependent iron elevation and parkinsonism.[7] In PD patients a low ceruloplasmin activity has been identified in the SN, cerebrospinal fluid (CSF) and serum.[1] Point mutations in the ceruloplasmin gene also associate with parkinsonism[11] and SN hyperechogenicity in PD.[7,23]

Altered iron storage

The limited capacity of neurons to sequester surplus iron into ferritin molecules[1,2] is complemented by expressing neuromelanin as an alternative "iron sink".[4] However, such capacities might be exceeded in PD,[2] potentially leading to increased ferritin-immunoreactivity in microglia in the SN.[24] Elevated levels of iron loaded ferritin may, over time, contribute to age-related neurodegeneration by acting as a metastable reservoir for iron.[1,2,4]

Increased iron uptake

Single nucleotide polymorphisms in transferrin (Tf) and its receptor (TfR) identified from PD case-control studies may have a protective role via changes to Tf bound iron transport into the cell.[25] Lactoferrin and its receptor may also play a similar role.[26] Finally, iron accumulation in the SNc of patients and MPTP mice correlate with elevation of the iron importer, divalent metal transporter 1 (DMT1).[27]

Is there interplay between iron and α-synuclein?

Iron markedly induces aggregation of α-synuclein into intracellular inclusions (i.e. Lewy bodies).[28] Consistent with iron deposition, QSM measurements follow a pattern with the distributions of α-synuclein in PD pathology.[17] Since the identification that iron can translationally increase protein levels of α-synuclein through its promoter region,[28] more recent evidence has suggested a role for α-synuclein in modulating iron homeostasis. Depletion of α-synuclein in a functional location impairs the capacity for TfR to import iron and indicates that α-synuclein could modulate clatherin-mediated endocytosis.[28] Neonatal iron-feeding of a transgenic mouse model overexpressing human α-synuclein bearing the A53T mutation exacerbates both PD-related motor and non-motor phenotypes.[29] Accordingly, iron chelation reduces the amount of insoluble α-synuclein aggregates[29] and rescues behavioural deficits[30] in murine models of genetic PD.

Ferroptosis – a new iron-dependent cell death pathway in PD that may yield further therapeutic options

A new iron dependent cell death pathway that has recently come to light has strong implications in PD neuropathology. Ferroptosis appears to be selectively triggered by an iron dependent mechanism with key features including lipid peroxidation, specific depletion of glutathione peroxidases-4 to alter glutathione protection, mitochondriopathy and distinct

morphological modifications that are independent from other cell death pathways (e.g. apoptosis, necrosis and autophagy).[31,32] Inhibition of the xCT cystine/glutamate antiporter during ferroptosis consequentially prevents cystine uptake into the cell and leads to lower levels of glutathione synthesis and increases cellular availability of labile iron to catalyse lipid peroxidation (**Fig. 2**).[31] Ferroptosis is associated with pathogenic changes observed in PD, as well as the classical *in vitro* and *in vivo* pro-oxidant models.[33] This includes nigral iron elevation, mitochondriopathy, glutathione depletion, lipid peroxidation, elevated ROS generation and oxidation of dopamine.[33] Ferroptosis can be rescued by iron chelation (e.g. with DFP),[31,33] supporting the requirement for iron in the initiation of this cell death pathway. Importantly, a range of inhibitors with greater specificity to ferroptosis (e.g. liproxstatin-1) have recently been designed with promising future implications in disease modification.

A new therapeutic strategy of conservative chelation based on iron scavenging and redeployment

The implication of siderosis and iron toxicity has largely been based on the protective effects of iron chelation in cell and animal models.[1-3,7-11,34,35] However, for any chelator to be of clinical value in disorders of regional siderosis they ought to be endowed with a requisite accessibility to the relevant sites and differential specificity so as to spare unaffected areas of the organism from scavenging an essential element.[36] Different agents with iron chelating features (e.g. deferoxamine, clioquinol, VK28, M30 and natural plant-derived polyphenol flavonoids) have been assessed but not progressed to clinical trial.

DFP is exceptional among iron chelators in its ability to cross membranes, including the blood brain barrier (BBB), and to chelate components of the cellular labile iron pool in brain tissue.[36] DFP has the remarkable ability of rescuing transfusional hemosiderosis in the heart

of β-thalassemia patients without inducing anemia, largely attributable to the redeployment of captured iron to extracellular iron free Tf and subsequent distribution (**Fig. 3**).[36]

The conservative repositioning strategy has been applied to PD using DFP at the relatively low oral dose of 30 mg/kg/day.[9] An early-stage PD patient pilot study using a delayed start paradigm (6 months DFP or placebo pretreatment followed by 12 months DFP for all) yielded a significant reduction in SNc siderosis, particularly in the group that started early with DFP. Compared to placebo this remained stable until completion (month 18). A concomitant clinical benefit was noted at 6 months with a 3-point improvement in the motor-unified Parkinson's disease rating scale (UPDRS) in the early start group (21.6±8) versus the delayed start group (24±6). Importantly, at 12 months these 'early start' patients retained a significantly lower motor handicap (1 point on the motor UPDRS: 21.3±8) compared to the delayed start group (22.8±6), signifying a disease modifying effect.[9] The conservative mode of chelation was reflected by an absence of systemic iron loss with patients showing normal iron indices that were unaltered after 18 or 24 months DFP treatment (except a mild ferritin reduction in blood and cerebro-spinal fluid). Positive clinical outcomes were recently confirmed by another randomised double-blind, placebo controlled trial. In this smaller sized trial, DFP reduced dentate and caudate nucleus iron content and indicated a trend for improvement in motor-UPDRS scores and quality of life.[37] In both trials DFP had a good safety profile; despite the requirement of weekly blood counts during the first 6 months to monitor reversible neutropenia that may occur in 1-3% of patients treated with DFP.

These promising results have now led to a large phase II, European multicentre, parallel-group, placebo-controlled, randomized clinical trial, of which the aim is to evaluate whether DFP can slow progressive impairment in PD patients (www.fairpark2.eu). 338 patients with *de novo* PD are planned for randomization to either DFP (30 mg/kg/day in two doses a day) or placebo for 9 months. All will then participate in a 1-month post-treatment monitoring

period. To assess the hypothesized disease-modifying effect of DFP the primary efficacy criterion will be the total score on the UPDRS, encompassing motor and non-motor symptoms as well as activities of daily living. Secondary criteria include cognition, quality of life, quantitative continuous motor activity in the home environment and a health economics questionnaire. Potential surrogate and theranostic biomarkers of efficacy and safety will also be analysed (i.e. wide range of iron, dopamine and α-synuclein markers). These include the imaging measurement of iron content (MRI R2*/QSM and transcranial ultrasound) and dopamine content (dopamine transporter SPECT imaging). Since the initiation of the FAIR-PARK-II trial a further phase II trial at sites in both Europe and Canada has begun to analyse different DFP doses ranging from 10 to 40 mg/kg/day to evaluate clinical outcome over the same treatment period.

Conclusions and future directions

Cell iron dyshomeostasis has been implicated in a wide range of neurodegenerative disorders other that PD, such as Alzheimer's disease (AD; cortical iron elevation),[38] Amyotrophic lateral sclerosis (ALS; elevated iron in motor neuron pathways)[39] and the less prevalent Brain Iron Accumulation (NBIA) neurodegenerative disorders. This maldistribution of iron might represent a pathological form of regional siderosis that could be treated by a conservative mode of chelation based on drug-mediated iron redistribution, as currently under clinical evaluation in PD, Pantothenate Kinase-Associated Neurodegeneration type 2, ALS and AD. The aim is to slowdown disease progression, but complementary strategies may also block iron-dependent death pathways such as ferroptosis or stimulate cell repair pathways that promote glutathione formation or inhibit iron dependent prolyl hydroxylases.[9,33,40,] It is hoped that with new biomarker developments in iron sensitive MRI sequencing, clinical trials such as the one presented here for conservative iron chelation may change the future clinical practice of PD.

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2) The manuscript: A: writing of the first draft, B. review and critical comment

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LEGENDS

Figure 1: Iron overload in the Substantia Nigra

Post mortem mesencephalon analysis reveals that the dopaminergic neurons, naturally stained in black by auto-oxidation of dopamine in control (upper left), have disappeared in PD (upper right). Perls staining with a pink colour enhancement shows that SN is normally rich in iron (2nd line left) but is iron overloaded in PD (2nd line right). ***Brain magnetic resonance imaging of the mesencephalon*** The quantitative weighted T2* sequence shows a higher intensity (R2* = 1/T2*) in PD (bottom right) than in control (bottom left). The quantitative susceptibility mapping (3 Tesla) distinguishes the iron in the normal SN (3rd line left) but the same nuclei in the iron overloaded PD case exhibits hyperintensity (3rd line right). Above the SN, the red nucleus can be identified.

Figure 2. Ferroptosis as a therapeutic target in Parkinson's disease. Alterations in the iron regulatory pathway and phospholipid oxidation are implicated in Parkinson's disease pathology. Increased intracellular iron occurs by enhanced import of iron within transferrin (Tf) through Transferrin receptor (TfR) endocytosis that is promoted by α-synuclein (α-syn), and increased import of Fe^{2+} through the divalent metal transporter 1 (DMT1). In addition,

iron export is impaired through the destabilization of ferroportin (Fpn) on the cell surface by β-amyloid precursor protein (APP) or ceruloplasmin (CP). When the storage protein neuromelanin (Nm) and ferritin (Ft) are no longer able to safely store intracellular iron, the labile pool of iron is elevated and catalyzes the formation of phospholipid hydroperoxides. Cystine uptake through the X_{c-} antiporter (in oxidative conditions) or the alanine, serine, cysteine – preferring (ASC) system (in reducing conditions) is required for biosynthesis of glutathione (GSH). Glutathione peroxidase 4 (Gpx4) uses 2 GSH molecules to safely reduce phospholipid hydroperoxides to their corresponding lipid-alcohols, producing H_2O and glutathione disulphide (GSSG) as byproducts. Elevated levels of intracellular iron with depletion of Gpx4, as evidenced in models of PD, promotes the accumulation of phospholipid hydroperoxides leading to a disruption in membrane integrity through a ferroptotic pathway. Reducing the labile iron pool (i.e deferiprone) or depleting the phospholipid hydroperoxides (i.e. liproxstatin-1 or ferrostatin-1) are thus promising targets for inhibiting ferroptosis in PD pathology.

Figure 3: A conservative mode of chelation based on: a. the scavenging of iron that accumulated intracellularly (organelle, cell, or tissue), and b. its redeployment to another cell or tissue compartment, either by the chelator or via iron transferred to circulating transferrin. The presence of apo-transferrin in circulating fluids ensures that iron scavenged from cells is conserved and redeployed primarily to areas of iron deficiency.

Table summarizing main points of interest to a broad readership of general neurologists

PD: Parkinson's disease; SNc: Substantia nigra pars compacta; SNr: Substantia nigra pars reticulate; VTA: Ventral Tegmental Area MRI: Magnetic resonance Imaging; SWI: susceptibility weighted imaging; ROS: reactive oxygen species; 6-OHDA: 6-hydroxydopamine; ALS: amyotrophic lateral sclerosis; PKAN-2: Pantothenate Kinase-

Associated Neurodegeneration type 2; AD: Alzheimer's disease. ***The table does not contain the exhaustive list of references on each topic. Reviews are frequently quoted due to space and references limitation.***

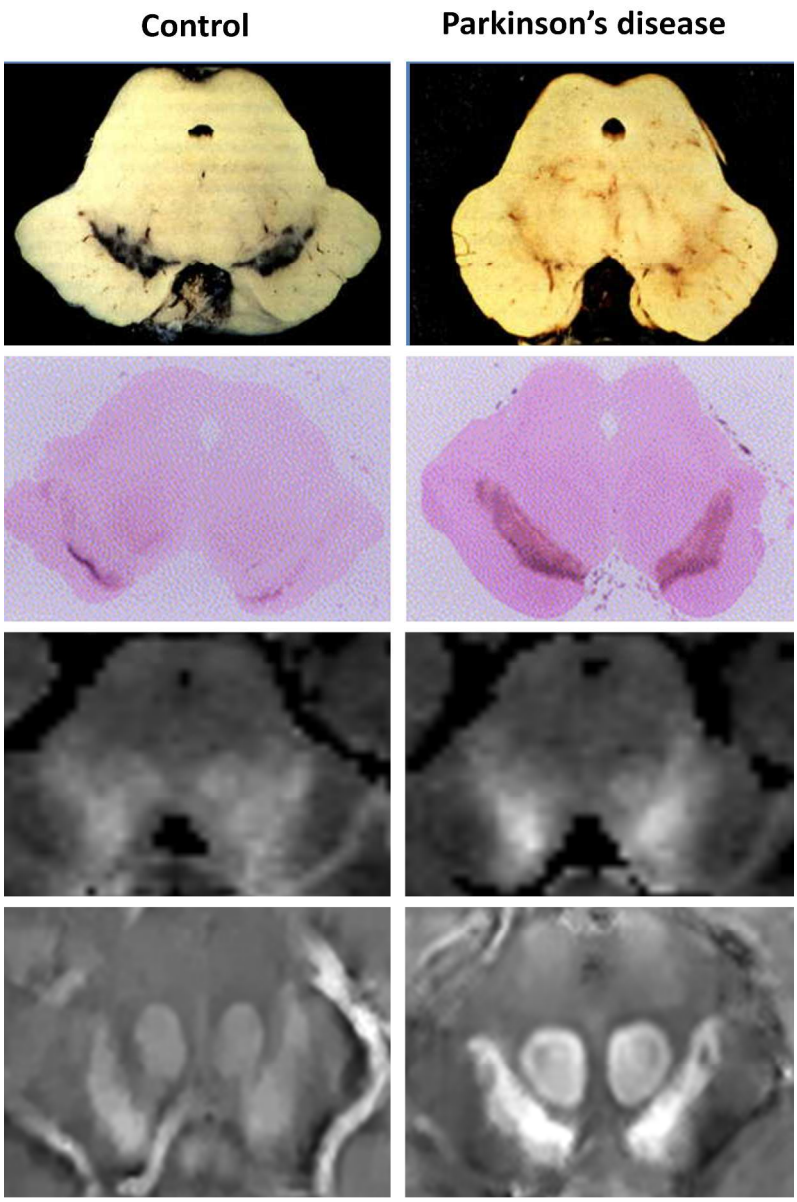

Figure 1 high resolution

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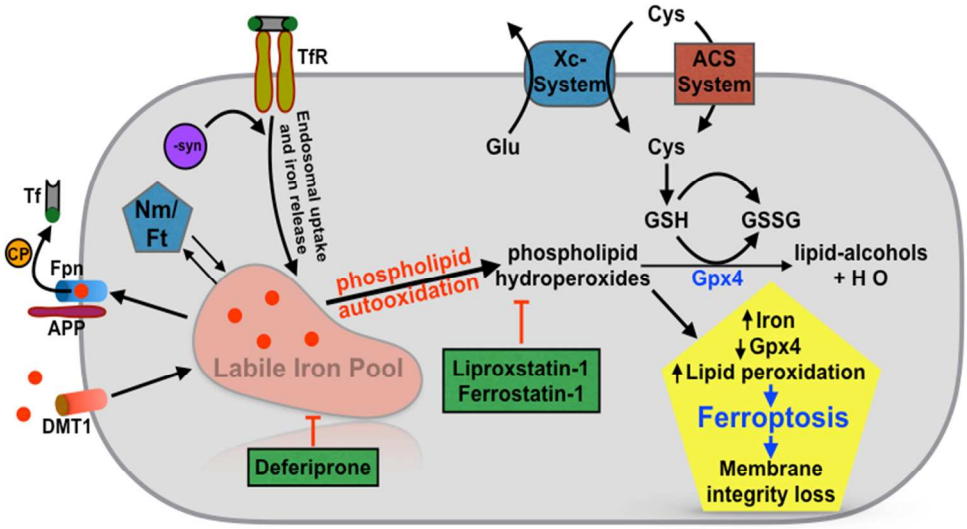

Figure 2

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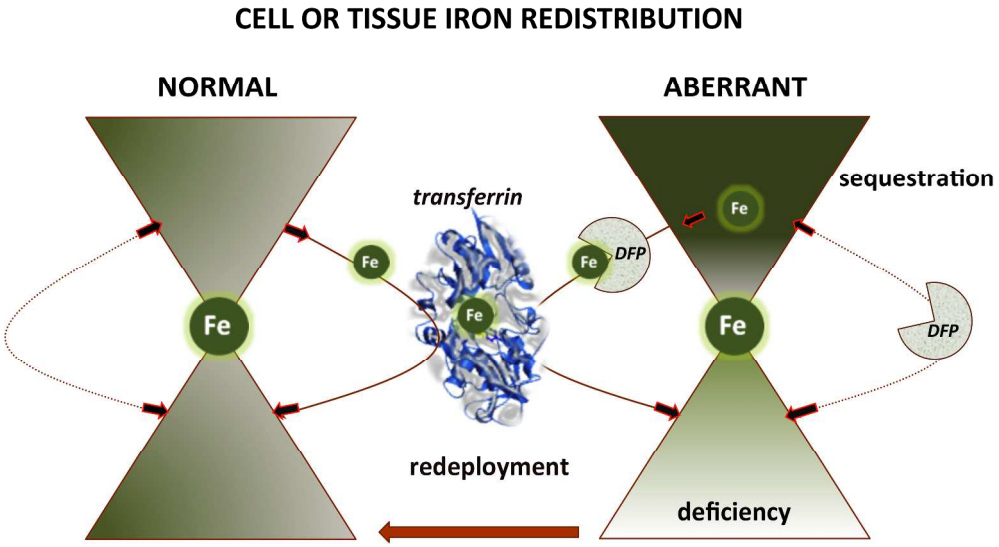

Figure 3

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Table: Frequent asked questions, main findings and clinical implications

Frequent asked questions	What is demonstrated?	Need to be demonstrated	Clinical implications
Iron overload in the SNc?	- First described in 1924, a similar period as Lewy bodies - In models and patients brain analysis,[9-21] MRI and ultrasounds[49,53-68] - Association with motor handicap[9,12,13]	Biomarker for diagnosis and prognosis?	- Identification of iron overload: Hyperintensity of the dorsolateral SNc by MRI (SWI)[15] and hyperechogenicity[20,21] - Quantification of iron by MRI (R2*, QSM) [9,12-19] for follow up[12,13]
Why SNc is vulnerable?	- Naturally rich in iron[1,2] as required for dopamine (synthesis, metabolism)[1,2,4] and higher pace making activity in SNc > VTA[6] - Death by iron related to oxidative stress[1,2,5-10,40,43]	Cell types and factors involved in the excess of oxidative stress and iron redistribution?	- High iron content render SNc vulnerable to oxidative stress without adequate protective measures[1,2,3,4,7-11] - Motor and non motor automatic activity requires high energy demands[6]
What is the role of neuromelanin?	- Sequestration of harmful dopamine quinones (i.e. iron related dopamine auto-oxidation).[1,2,4] - Neuromelanin lost in PD [4] frees 6-OHDA to impair mitochondrial function.	Cellular and brain mechanisms of iron regulation?	- Neuroprotection of neuromelanin by chelating metals (iron) and xenobiotics?[1,2,4] - Neuromelanin measured with specific MRI sequence
Other brain regions overloaded by iron?	- Iron overload is complex, variable and dynamic in the other regions - The main other overloaded region is putamen[9,12,13] (identified by brain analysis and MRI)	The status of the other regions remains to be determined: Nucleus caudatus, red nucleus and globus pallidus, putamen?	- Concept of progressive and extensive brain iron redistribution - Specific correlations between regions, phenotypes and stages of the disease to be determined
Are there different types of iron?	- 1% of ferrous labile iron (Fe^{2+}) required for normal metabolism; harmful in excess (oxidative stress) - 99% of ferric iron (Fe^{3+}) safely stored in equilibrium[1,2,4,36]	In vivo measures of the labile iron (Fe^{2+}) available for patients?	- MRI is an indirect measure of ferric iron - Iron-sensitive MRI evaluation depends on specific situations: safely stored vs. pathological vs. non-active consequence
Is high dietary iron exposure a risk factor of PD?	**No demonstration but reported:** - In adult: no relation or weak association in males - In early life: increase PD mortality rate in Western countries? (Policy of iron fortified food).	Need prospective data	- Iron overload of SNc is not a consequence of adult diet - Policy (after the second world war) of systematic iron fortified food could no longer be necessary or a worsening factor? (To demonstrate)
Is there iron deficiency in PD?	**No demonstration but reported:** - Association between low peripheral iron levels (anaemia, low haemoglobin) and PD risk remains a matter of debate - Requires meta-analytical confirmation	- Need prospective data - Mechanisms of abnormal iron redistribution?	- Abnormal regional iron redistribution; cellular, brain and body overload and deficiency[2] - Care required in iron supplementation for anaemia in PD (Efficiency? Risk?)
What is the relationship between iron and α-synuclein?	**No demonstration but reported:** - Iron induces aggregation of α-synuclein into intracellular inclusions (i.e. Lewy bodies).[28] - Pattern tightly concordant between brain iron deposit and α-synuclein pathology[17]	Pivotal interplay between iron, dopamine and α-synuclein pathology to influence dopaminergic neurotransmission and disease progression?	-Therapeutic targets for neuroprotection and dopasensibility?

	- α-Synuclein pathology involved in iron homeostasis[28] and reduced by iron chelation[29,30]		
What is the therapeutic concept of "conservative" iron chelation	- Neuroprotection in PD models by iron chelation[1-3,7-11,34,35] - Dopaminergic neuronal death by iron deficiency[3] - Concept: low dose of deferiprone redeploys captured iron to extracellular apo-transferrin, and subsequent distribution avoids iron deficit: preclinical efficacy and clinical safety[9,36,37]	Efficacy to be confirmed with large trial in progress? (FAIRPARK-II) - Trials also in progress for PKAN-2, ALS, AD?	Careful therapeutic iron redistribution by conservative chelation based on iron scavenging and redeployment (**Fig. 3**) may afford neuroprotection without anaemia (risk of reversible neutropenia in 1-3%)
Are there several types of cell deaths in PD?	- Apoptosis (mainly with oncogenic cell line): negative trials - Mitophagy in models and genetic causes (Pink-1, Parkin,..) - Ferroptosis in models[31-33] (**Fig.2**)	- Mitophagic modulator - Antiferroptotic drug (both iron chelators and specific inhibitors)	New upcoming therapeutic targets for a combined therapy?
IN TOTAL: Is iron the cause of PD?	**NO but a worsening factor if present 1) in excess and 2) associated with PD physiopathology**	**The interplay between iron and the pathological causes (e.g. synucleinopathy, mitochondriopathy): "double hit theory"?**	**- In specific situations iron overload could be a marker of degeneration and a risk factor for degeneration[1,2,7-21,31-37]** **- A therapeutic strategy**? [9,36,37]