Int.J.Curr.Res.Aca.Rev.2020; 8(12): 96-108

EXCELLENT PUBLISHERS

International Journal of Current Research and Academic Review

ISSN: 2347-3215 (Online) Volume 8 Number 12 (December-2020)

Journal homepage: http://www.ijcrar.com

doi: https://doi.org/10.20546/ijcrar.2020.812.009

Early Life Iron Exposure and CSF Levels of Cu/ceruloplasmin Correlated with Parkinson's Disease

Yiglet Mebrat*

Ethiopian Biodiversity Institute, Molecular characterization team, Addis Ababa, Ethiopia

**Corresponding author*

Abstract

PD is the second most NDD and its main pathological hallmark is the loss of dopamine-producing neurons that leads increased nigral iron content as well as the presence of aggregates of misfolded proteins. Brain iron homeostasis is relying on IRP and Fe-S cluster proteins that bind to IREs. Iron cross the BBB through the classic TfR-mediated endocytosis and excess iron in neurons and neuroglia can be exported back to the brain interstitial fluid, and can be released into the CSF in the brain ventricles then transport back to the blood circulation. Excessive iron deposition in the brain may cause a cascade event of oxidative stress and neuroinflammation. Increased nigral iron content in patients with PDs is a prominent pathophysiological feature involved in selective dopaminergic neurodegeneration. Early life iron exposure has been proposed as a possible risk factor for PD, and has been shown to stimulate midbrain neurodegeneration with age. CP is a MCO, the major plasma anti-oxidant. GPI secreted in CSF with ferroxidase activity to play a vital role in iron metabolism, which can be considered as the genuine link between Cu and Fe metabolism. Significant loss of ceruloplasmin-ferroxidase activity has been observed in CSF and substantia nigra of PD patients and leads to cellular iron retention. Oxidative modification in Cp leads to Cu release and increases in the CSF which facilitates Fenton's reaction then amplifies general protein damage in PD.

Article Info

Accepted: 08 November 2020
Available Online: 20 December 2020

Keywords

Iron, Parkinsons disease, Ceruloplasmin, α-synuclein and lewy bodies

Introduction

Neurodegenerative diseases (NDDs) are a heterogeneous group of disorders, represent a major threat to human health characterized by gradual and progressive loss of neurons and synaptic connections, usually occurring later in life and often leading to fatal outcomes (Aaron *et al.*, 2017; Giselle and Majel, 2018).. The NDDs diseases are distinguished by the presence of characteristic symptoms that depend on the location in the brain of the neuronal loss (Aaron *et al.*, 2017). In AD, neuronal loss occurs early in the hippocampus; whereas in PD, the characteristic clinical signs of tremor, bradykinesia, and postural instability become evident only after 70–80% of the dopaminergic neurons in the substantia nigra are lost (Giselle and Majel, 2018). Revolutionary studies demonstrated that proteins with altered physicochemical properties (misfolded protein) deposit in the human brain as a fundamental phenomenon in most NDDs, defined also as conformational diseases (Gabor, 2016). Neurodegenerative diseases have in common the very progressivity of neuronal death, and this contributes to the difficulty of establishing valuable animal models of the diseases (André, 2011).

Parkinson's disease (PD) is the second most common neurodegenerative disorder and in the majority of cases, its cause remains unknown (Masoom *et al.,* 2018). The main pathological hallmark of Parkinson's disease is the loss of dopamine-producing neurons, whose cell bodies are located in the *substantia nigra pars compacta* (SNc), as well as the presence of aggregates of misfolded proteins (mainly α-synuclein) and other materials, known as Lewy bodies, which induces motor symptoms including tremor, rigidity, akinesia, bradykinesia, and postural instability (Wataru *et al.,* 2017). In vivo and post-mortem studies have demonstrated that increased nigral iron content in patients with PD is a prominent pathophysiological feature (Jin *et al.,* 2011). However, the mechanism and risk factors associated with nigral iron deposition in patients with PD have not been identified and represent a key challenge in understanding its pathogenesis and for its diagnosis. But there have been a number of scientific reports to address risk factors for subsequent PD, include genetic and environmental risk factors and having a family member with Parkinson's disease increases the chances that you'll develop the disease (Fei *et al.,* 2015; Yu *et al.,* 2018).

Importance of iron in the nervous system

Iron is the second most abundant metal on earth and has multiple oxidation states makes it a leading candidate as a co-factor for enzyme-catalyzed reactions that require electron transfer (Angelova and Brown, 2015) and essential micronutrient that plays a significant role in critical cellular functions in all organ systems and particularly vital for early brain growth and function in humans (Siddappa *et al.,* 2007). It is involved in a series of very important biochemical functions that include oxygen transport, electron transport, glucose metabolism, and the synthesis of neurotransmitters, myelin, and DNA replication (Belaidi and Bush, 2016). Iron is essential for the biosynthesis of lipids and cholesterol, which are important substrates in the synthesis and metabolism of myelin (Siddappa *et al.,* 2007).

Iron in the brain plays a crucial role in maintaining normal physiological functions through its participation in many cellular activities, since it supports neuronal and glial energy metabolism and the brain requirement for iron is relatively high, due to its high-energy needs and the access of iron to the brain is limited by the presence of a blood– brain barrier (BBB) (Wong and Duce, 2014). Whereas, if there is iron deficiency during the fetal or postnatal periods can alter brain structure, neurochemistry and cognitive functioning, which leads to a long-term cognitive and motor impairment that cannot be corrected by iron supplementation (Siddappa *et al.,* 2007).

Brain iron homeostasis

The full regulatory mechanisms of brain iron homeostasis are likely to include a number of redundant pathways by utilization of several importers and exporters for iron transport, storage, and export in the brain that can be helps to maintain iron homeostasis (Wong and Duce, 2014). Dietary iron is absorbed predominately in duodenum and enters blood circulation in small intestine, iron binds to apotransferrin and forms transferrin (Tf). At the target cell, Tf binds to transferrin receptors (TfR) on the cell membrane, and the TfR-Tf-Fe complex is then endocytosed into the cell, where the iron is released (Gao and Chang, 2014).

Iron needs to pass the blood-brain barrier in order to enter the brain, thus Tf-Fe in the blood circulation is uptaken at the surface of cerebral capillary endothelia, mainly through the classic TfR-mediated endocytosis (Skjørringe *et al.,* 2015). Free iron can also enter the brain barriers by divalent metal transport-1 (DMT1). In endothelia, iron is released and transported across the abluminal membrane of the barriers into the cerebral compartment. This process likely involves iron exporter ferroportin (FPN) and DMT1 on the abluminal membrane, but the exact mechanism remains for further exploration (Mills *et al.,* 2010; Zheng and Monnot, 2012). The elemental iron released into the brain interstitial fluid binds to brain Tf and becomes available for neurons and neuroglia expressing TfR. The excess iron in neurons and neuroglia can be exported back to the brain interstitial fluid, and can be released into the cerebrospinal fluid in the brain ventricles through bulk flow (Zheng and Monnot, 2012). The apical microvilli of choroidal epithelia then capture the free iron by TfR or DMT1 and transport it back to the blood circulation (Mills *et al.,* 2010).

The population of the cells present within the brain is diverse and dynamic in their function and requirement of iron (Wong and Duce, 2014). Regions such as the substantia nigra and the globus pallidus have the highest levels, exceeding that of the liver, the main site of iron storage in the body (Angelova and Brown, 2015). According to cell type, oligodendrocytes have the highest iron content and astrocytes have very low cellular iron because the cells utilize iron for biosynthetic functions (Angelova and Brown, 2015). Glia and

microglia are capable of storing large amounts of iron; whereas most neurons can only use the iron they acquire immediately (Sarah *et al.,* 2008).

Regulation of brain iron through Iron response proteins

The proteins required to regulate cellular iron homeostasis in the brain are very similar to those used in the body's periphery and rely on the two cytosolic labile iron pool sensors; iron response protein (IRP1 and IRP2), cytosolic iron-sulphur (Fe-S) cluster proteins that bind to their respective iron regulatory/responsive elements (IREs) (Skjørringe *et al.,* 2015). The activities of both IRP1 and IRP2 respond to changes in cellular Fe through different mechanisms. IRP1 and 2 binds stem-loop structure of the iron-responsive element (IRE) and regulate posttranscriptional expression of the iron metabolism-related mRNAs (Wong and Duce, 2014). Specifically, when the brain cellular iron concentration is low the active center of IRPs 3Fe-4S cluster binds to the stem-loop structure of the iron-responsive element (IRE) located at the 3'-untranslated region (UTR) of ferritin and TfR mRNA (Satoru *et al.,* 2011). This binding inhibits translation of the iron storage protein, ferritin and also stabilizes TfR mRNA, causing an increase in the mRNA translation of TfR proteins; thereby increasing iron uptake (Leipuviene and Theil, 2007; Satoru *et al.,* 2011). When brain cellular iron levels are high, IRP changes to the alternate 4Fe-4S cluster (the active center of IRP is occupied by four Fe-S); in this form, its mRNA-binding activity is repressed, and IRP is released from IRE. Without the binding of IRP (4Fe-4S), ferritin mRNA undergoes translation then storage protein is synthesized (Satoru *et al.,* 2011). TfR mRNA is rapidly degraded by nucleases, decreasing translation, which results in the production of fewer TfR proteins and reduced iron uptake (Satoru *et al.,* 2011; Wong and Duce, 2014). Furthermore, IRP binds to ferritin mRNA, thus diminishing its translation (Salazar *et al.,* 2006).

Iron accumulation and neurotoxicity

Iron accumulation is evident in the aging brain from a range of animals including humans and whilst a heterogeneous distribution of iron is present within the brain, most regions have a continual increase in iron with lifespan (Wong and Duce, 2014). Iron can induce neurotoxicity by its ability to promote the formation of ROS and excessive iron deposition in the brain may cause a cascade event of oxidative stress and neuroinflammation that destroy neuronal phospholipid membranes, proteins and nucleic acids, leading to the degeneration and death of neurons. Thus, appropriate iron level in brain is vital for maintaining a stable internal environment through a rigorous regulatory mechanism (Yu *et al.,* 2018).

Elevated iron is potentially neurotoxic, indeed the direct injection of iron into the rat brain causes neuro-degeneration, possibly via an oxidative stress pathway which initiates several apoptotic signaling pathways (Hare *et al.,* 2017). In recent study, it was found that iron induced dopaminergic neurodegeneration through neuroinflammatoty mechanism indicated by the over activation of microglia and robust production of neurotoxic factors and correlated with the rapid progression of PD (Yu *et al.,* 2018). It is therefore not surprising that iron elevation observed in a number of neurodegenerative diseases, such as AD and PD, is proposed to be a key mediator in cell loss of these diseases (Hare *et al.,* 2013).

Iron accumulation in PD could be contributed by a number of iron-related proteins that are changed in PD. Ferritin is highly expressed in microglia and its levels have been found to be decreased in post-mortem PD brains (Angelova and Brown, 2015); loss of iron storage capacity potentially makes free iron species more available for toxic interactions (Hare *et al.,* 2013). Inappropriate intracellular iron accumulation potentially damages a number of proteins such as Ca^{2+}-ATPase, glutamate transporter, Na^{+}/K^{+}-ATPase, and *N*-methyl-D-aspartate (NMDA) receptor (Munoz *et al.,* 2011), as well as oxidizes lipid such as cholesterol, ceramides, and sphingomyelin; all of which were proposed to ultimately cause synaptic dysfunction and neuronal cell death (Hare *et al.,* 2013).

Correlation with pathogenesis of Parkinson's disease

Iron overload has been implicated in the pathology and pathogenesis of PD (Wang *et al.,* 2016). In vivo and post-mortem studies have demonstrated that increased nigral iron content in patients with PD is a prominent pathophysiological feature (Jin *et al.,* 2011). In the brain substantia nigra (SN; the most vulnerable region in PD and an oxidative stress-prone structure due to its enrichment of dopaminergic neurons), there is a high iron concentration; accordingly, SN is especially more vulnerable to iron deposition than other brain regions (Olivieri *et al.,* 2011). Moreover, the dopamine metabolism of nigral neurons leads to the production of H_2O_2, which in turn can convert to hydroxyl radical

when ferrous iron co-occurs (Olivieri *et al.,* 2011). Spectroscopic analyses of postmortem brains display an increased iron levels in the substantia nigra, which has been suggested to correlate with the severity of PD (Wang *et al.,* 2016). Iron-induced oxidative stress is particularly dangerous because it can cause further iron release from iron-containing proteins, such as ferritin (Ft), heme proteins and iron–sulfur (Fe–S) clusters, forming a destructive intracellular positive-feedback loop that exacerbates the toxic effects of brain iron overload (Eric *et al.,* 2010). Such iron accumulation is known to be associated with increased ferritin and neuromelanin iron loads, as well as increased expression of divalent metal transporter 1 that may contribute to PD pathogenesis via its capacity of transporting ferrous iron (Wang *et al.,* 2016). Furthermore, iron deposition in the brain can also promote conformational changes in α-synuclein, resulting in its aggregation and contributing to the pathogenesis of PD (Jin *et al.,* 2011). Together, all these scientific evidences suggest that iron deposition contributes to the mechanism of brain damage in patients with PD.

Roles of early iron exposure in Parkinson's disease

Iron fortification and supplementation programs have been a widely successful practice for reducing the incidence of iron deficiency anaemia (IDA), though determining the optimal level of iron exposure to ensure IDA is avoided while also limiting potential negative health outcomes later in life but, the long-term health outcomes of iron overload in the brain during development are poorly understood (Hare *et al.,* 2017). Total iron levels in infant formula are over 20-fold greater than reported iron levels in breast milk. This difference means that infants who are fed formula are exposed to higher levels of iron than are infants who are fed breast milk and the possibility that iron overloading of the brain in early life contributes to age-related neurodegeneration is concerning (Dominic *et al.,* 2015). Early life iron exposure has been proposed as a possible risk factor for PD, and has been shown to stimulate midbrain neurodegeneration with age; neurobehavioral dysfunction, alters expression of proteins involved in iron homeostasis, increases astrogliosis, and leads to motor and cognitive abnormalities in adulthood (Jessica *et al.,* 2016). A case-control studies with retrospective collection of exposure data also indicated supplemental iron intake is associated with an increase in PD risk (Jia *et al.,* 2018). These findings suggest that the infant brain is less protected against systemic iron overload than is the adult brain (Dominic *et al.,* 2015).

Scientific hypothesis indicates that exposure to high levels of iron, such as those found in infant formula, in early life could initiate an impaired neuronal iron metabolism combine to produce a neurotoxic environment which further stresses impaired genetic regulation of this essential metal (Dominic *et al.,* 2015, Hare *et al.,* 2017). Epidemiological evidence indicates that high-income countries that are early adopters of food fortification and iron supplementation programs for infants may now be experiencing increasing rates of PD (Hare *et al.,* 2017). Furthermore, individuals exposed to high dietary iron during critical periods of neural development are at risk of excessive iron accumulation in the brain and several lines of biologic evidence link iron to Parkinson's disease, because higher dietary iron intake alone is associated with a 30% increased risk of the Parkinson's disease (Logroscino *et al.,* 2008).

Biochemical natures of ceruloplasmin and copper metabolism

Copper

Copper (Cu) is an essential trace element whose ability to donate and accept electrons gives it great utility in multicellular life that must be acquired through the diet and trafficked to the organs, cells, and proteins that require copper for health (Gaier *et al.,* 2013). The human organism contains about 70-80mg of copper, about 50% of copper content is stored in bones and muscles, 15% in skin, 15% in bone marrow, 8 to 15% in the liver and 8% in the brain (Angelova *et al.,* 2011). Like iron copper has two oxidation states, cuprous (Cu^{1+}, solubility is in the sub-micromolar range) and cupric (Cu^{2+}, is soluble & found in biological system, in the presence of oxygen or other electron acceptors Cu^{1+} is readily oxidized to Cu^{2+}) (Galhardi *et al.,* 2004).

Copper is involved in a variety of biological processes mainly in embryonic development, mitochondrial respiration, regulation of hemoglobin levels as well as hepatocyte and neuronal functions (Krupanidhi *et al.,* 2008), but its unique role in oxidation-reduction reactions is an association between Cu and dioxygen-utilizing enzymatic reactions accompanied the appearance of Cu in biological systems (Gaier *et al.,* 2013).

Function in the nervous system

Copper participates in a myriad cellular activities and physiological processes such as cellular respiration, iron

metabolism, biosynthesis of neurotransmitter, and free radical detoxification (Krupanidhi *et al.,* 2008). The human brain requires copper for its normal development and function (Gaier *et al.,* 2013). Copper concentration in the brain is one of the highest, second only to the liver, ranges from 2.9 to 10.7 μg Cu/g wet weight (Svetlana *et al.,* 2010, Angelova *et al.,* 2011). In the CNS, copper is utilized for general cellular metabolism such as respiration and radical defense, as well as for specialized processes such as production of neuroendocrine peptides and hormones. The mitochondria function and protection against reactive oxygen species in the cytosol rely on the activities of copper-dependent enzymes cytochrome C oxidase (CCO) and Cu,Zn-dependent superoxide dismutase 1 (SOD1), respectively (Svetlana *et al.,* 2010).

Copper is a functional component of several essential enzymes, known as copper enzymes (cuproenzymes) and affects enzyme activity, both as a cofactor and as an allosteric component of several cupro-enzymes (Voskaki *et al.,* 2010). Many enzymatic reactions, which are essential for the proper functioning of the brain and the nervous system, are catalyzed by copper enzymes (Angelova *et al.,* 2011). Amidation of neuropeptides and the production of norepinephrine is mediated by two homologous cuproenzymes: peptidyl-α-amidating monooxygenase (PAM) and dopamine-β-hydroxylase (DBH), respectively (Svetlana *et al.,* 2010).

Myelin sheath is made of phospholipids whose synthesis depends on the activity of the cytochrome c oxidase copper enzyme and the copper-requiring enzymes, tyrosinase and tyrosinase-related protein, are best known as key players in melanin synthesis in melanocytes. These two proteins have also been found in developing and adult brain with particularly high expression in the cortex, olfactory system, hippocampus, epithalamus and substantia nigra (Angelova *et al.,* 2011).

The abundance of Cu in synaptosomal and endosomal fractions, suggests that Cu release and retrieval are an important part of how Cu is used in the brain (Gaier *et al.,* 2013).

Cu homeostasis in the central nervous system (CNS)

A stable metal ion homeostasis and maintaining adequate copper levels is essential to preserving normal brain functions as many metals are required as cofactors for a variety of enzymes (Sergio *et al.,* 2014). Under normal physiological conditions, the brain barriers are impermeable to Cu and the available data indicate that copper balance in the CNS is maintained under very tight control, and compensatory mechanisms are activated to achieve copper balance in disease situations (Svetlana *et al.,* 2010). Abnormal Cu homeostasis both systemically and subcellularly, is believed to be associated with the pathogenesis of Parkinson's disease (Monnot *et al.,* 2011).

The chemical homeostasis of the CNS is maintained through the coordinated action of two major brain barrier systems, i.e., the blood-brain barrier (BBB) and the blood-CSF barrier (BCB) (Kaler, 2011, Monnot *et al.,* 2011). The BBB appears to determine the influx of Cu to the brain whereas the BCB functions as the regulatory site to maintain Cu homeostasis in brain extracellular fluid (Zheng and Monno, 2012).

Under physiological conditions, most plasma copper ions are bound to ceruloplasmin, with a small proportion of copper being carried by albumin, transcuprein, and other amino acids (Sergio *et al.,* 2014). Cu transport into the brain primarily occurs via the BBB as a free Cu ion and the BCB may serve as a regulatory site for Cu in the CSF (Choi and Zheng, 2009).

The mechanism as to how Cu is transported into and out of the brain at both the BBB and BCB is not fully understood. But, movement of Cu across the brain barriers between two fluid compartments requires specific Cu transport systems and it is clear that all the key copper handling proteins mediating copper homeostasis in peripheral tissues are also present in the brain (Zheng and Monnot, 2012). Cu may enter the brain through various Cu transporters located at the brain barriers such as Cu transporter-1 (CTR1), divalent metal transporter-1 (DMT-1), ATP7A, and ATP7B (Monnot *et al.,* 2011).

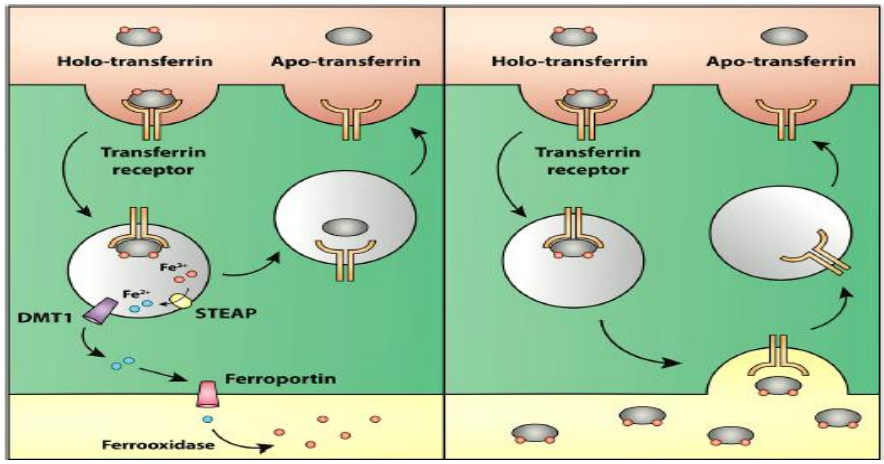
At the BBB, CTR1 (copper transporter-1), ATP7A, and ATOX1 (antioxidant 1 copper chaperone) are all involved in copper transport into the brain (Sergio *et al.,* 2014). The blood-CSF barrier seems to maintain copper at a certain level by sequestering copper from the blood and exporting the excess out of the CNS and back to the blood (Monnot *et al.,* 2011). At the BCB, copper transport is regulated by two major copper transporters: CTR1 and divalent metal transporter-1 (DMT1) (Zheng *et al.,* 2012). These transporters, together with ATP7A, transport copper from CSF to the blood, while ATP7B together with CTR1 achieves this in the opposite direction (Sergio *et al.,* 2014).

`

Table.1 Function of cuproenzymes with oxidation and reduction activity in humans

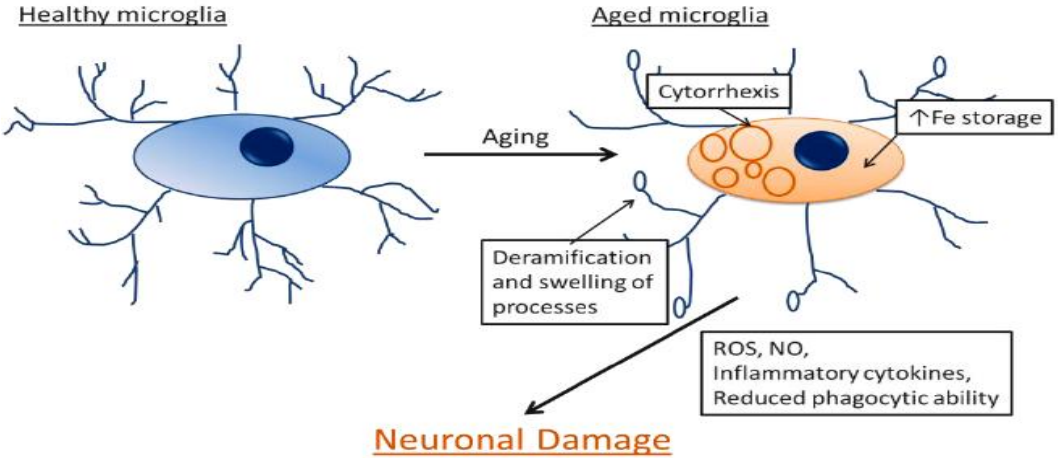
Function	Function
Cytochrome-c oxidase	Electron transport, terminal oxidase
Superoxide dismutase	Superoxide dismutation
Catechol oxidase	Synthesis of melanin
Protein-lysine 6-oxidase	Collagen and elastin cross-linking
Ceruloplasmin	Ferroxidase
Amine oxidases	Deamination of primary amines
Dopamine-b-monooxygenase	Dopamine→norepinephrine
Peptidylglycine monooxygenase	alpha-Amidation of neuropeptides

Fig.1 Iron transport model through the BBB without (left) or with (right) transcytosis of transferrin. (Left)



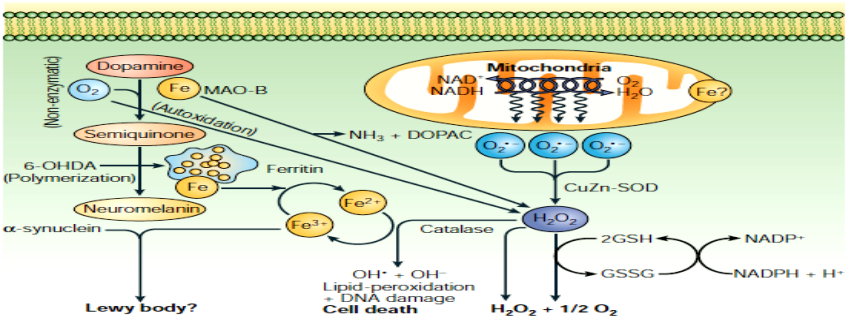
The recent identifications of Steap, DMT1 and ferroportin strongly favor that iron gets detached from transferring inside endosomes, which is then followed by iron efflux in to the brain interstitium mediated by ferroportin. **(Right)** showing the transcytosis of holo-transferin through the brain capillary endothelial cell (Skjørringe *et al*., 2015)

Fig.2 Age-related changes in microglia



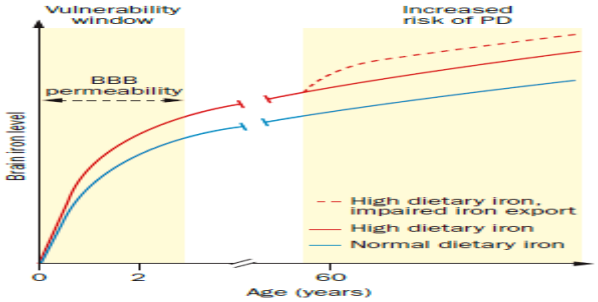
When microglia age they lose some of their processes and develop abnormalities in others. Additionally, they often exhibit cytoplasmic fragmentation. They also store more iron. Their increased release of neurotoxic substances and reduced ability to phagocytose debris and toxic protein aggregates leaves neurons vulnerable (Angelova and Brown, 2015)

Fig.3 Iron and oxidative stress hypothesis of Parkinson's disease



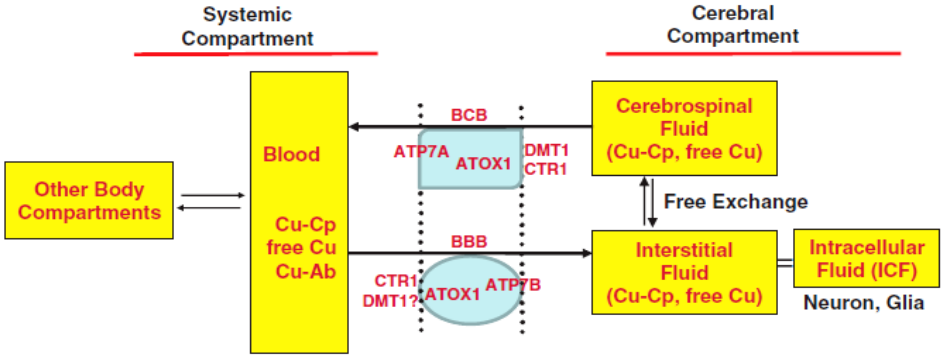
The pathochemical and possible synergistic cascades, such as developing cell-death mechanisms. This indicates reduced mitochondrial complex I activity; loss of GSH; increased iron concentrations; an increase in oxidative stress markers in the substantia nigra; an increase in dopamine turnover and loss of dopamine in the striatum; α- synuclein pathology, and Lewy-body generation and membranal degeneration in neurons. Similar mechanisms involving iron-induced and H_2O_2-induced OS have been put forward for other neurodegenerative diseases. CuZn-SOD, copper and zinc-containing superoxide dismutase; GSH, glutathione; GSH-Px, GSH peroxidase; H_2O_2, hydrogen peroxide, MAO-B, monoamine oxidase B; NAD, nicotinamide adenine dinucleotide; OH., hydroxyl radical;.O_2^-, superoxide radical anion; OH^-, hydroxide anion; 6-OHDA, 6-hydroxydopamine (Luigi *et al*., 2004)

Fig.4 Proposed progression of iron accumulation in the brain when dietary iron levels vary at an early age



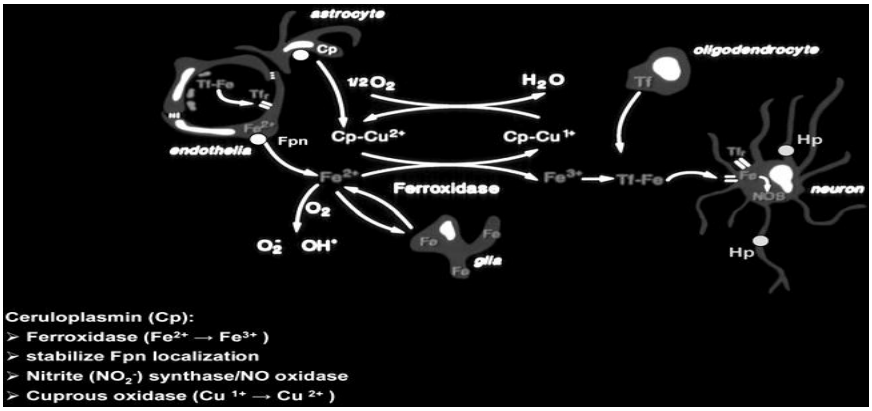
The time during which the BBB is permeable represents a critical window that might contribute to the risk of parkinsonian neurodegeneration. High dietary iron levels during this window cause increased loading of iron into the CNS at an early age, which, following continual accumulation of iron with age, leads to increased iron levels in the brain at the age when the risk of developing PD peaks. Early-life iron overloading in the brain alone is unlikely to increase the risk of PD, but a second hit, such as impaired neuronal export of iron, increases iron retention (dashed red line) and the likelihood of iron mediated oxidative stress. Infants who are breastfed and, therefore, exposed to normal dietary iron levels, will still accumulate iron in the brain with age, but to a lesser extent than individuals who are fed infant formula (Dominic *et al*., 2015)

Fig.5 Cu transport by the BBB and BCB



Most of the Cu molecules in the blood are bound to ceruloplasmin (Cp). At the BBB, free Cu is transported into the cerebral endothelial cells by CTR1; a portion of free Cu ions is transported via DMT1. ATOX1 delivers Cu to either ATP7A or ATP7B to be released into the interstitial fluid, where Cu is utilized by neurons and neuroglial cells. Excess Cu ions in the CSF are taken up by CTR1 or DMT1 in choroidal epithelial microvilli and transported back to the blood (Zheng and Monnot, 2012)

Fig.6 Role of Cp in CNS iron metabolism



GPI-linked Cp on astrocytes most intimately associated with the microvasculature oxidizes Fe^{2+} to Fe^{3+} for binding and subsequent transport to the neuron (Sarah *et al*., 2008)

Ceruloplasmin in the Central Nervous System

Ceruloplasmin is a multi-copper oxidase (MCO), the major plasma anti-oxidant and copper transport protein (Sarah *et al.,* 2008); mainly synthesized and secreted by the liver (Mario, 2014). CP synthesized and secreted by astrocytes as well as predominantly found as a GPI (glycosyl-phosphatidyl-inositol)-linked protein on the astrocyte cell surface (Sarah *et al*., 2008) and secreted as a soluble form in the cerebrospinal fluid (CSF) by choroid plexus epithelial cells (Barbariga *et al.,* 2015) with ferroxidase activity which facilitates the oxidation of ferrous iron (Fe^{2+}) to ferric form (Fe^{3+}) for subsequent binding to transferrin, keeping the level of dangerous ferrous iron within the cell to a minimum, thus playing an important role in iron metabolism (Jin *et al.,* 2011; Barbariga *et al.,* 2015). In the CNS, a glycosyl-phosphatidyl-inositol-linked ceruloplasmin bound to the cell membranes is the major isoform of ceruloplasmin (Mario, 2014). Additionally, Cp is localized to ependymal cells, the choroid plexus, substantia nigra, Purkinje cells and the granule cell layer of the cerebellum (Sarah *et al.,* 2008) and has been demonstrated to antagonize oxidative damage in the CNS (Jin *et al*., 2011). Ceruloplasmin also controls the process of iron oxidation, which allows the clearance of iron from the CNS (Mario, 2014).

Function in Iron and copper homeostasis

As a multicopper oxidase, ceruloplasmin reduces dioxygen, O_2, to two water molecules and a scavenger of reactive oxygen species (ROS) which can be considered that the genuine link between copper and iron metabolism is mediated by ceruloplasmin (Mario, 2014). The mechanism involved, the concept that Cp-Cu is delivered by direct interaction of Cp with the cell plasma membrane is supported by evidence of Cp "receptors" i.e. there is specific binding of Cp to a variety of whole cells (Steinbicker and Muckenthaler, 2013) and plasma membrane fractions (Danny *et al.,* 2016).

Ceruloplasmin plays an important role in cellular iron homeostasis and protects tissues from oxidative damage (Barbariga *et al.,* 2015). Ceruloplasmin is a copper-containing protein with ferroxidase function in under normal conditions; it oxidizes ferrous iron into the ferric form, keeping the level of dangerous ferrous iron within the cell to a minimum, thus playing an important role in iron homeostasis (Jin *et al.,* 2011). Cp ferroxidase activity is essential for iron metabolism because it regulates the expression of Fpn iron transporter on cell membranes (Olivieri *et al.,* 2011) and facilitates cellular iron export by oxidizing Fe^{2+} presented by ferroportin at the cell surface for incorporation into the extracellular iron transporter, transferrine (Ayton *et al.,* 2013). Intracellular iron concentration is controlled both by the storage protein ferritin, which accumulates Fe^{3+}, and by Fe^{2+} efflux through Fpn accompanied by Cp (essential for stabilization of ferroportin) extracellular ferroxidase activity (Olivieri *et al.,* 2011). The absence of extracellular ferroxidase activity as a result of Cp oxidation leads to cellular iron retention (Olivieri *et al.,* 2011). A deficit in copper reduces the ferroxidase activity of ceruloplasmin (Fe^{2+} to Fe^{3+}) because, dietary and recycled iron is in the Fe^{2+} oxidation state, but iron is transported in serum by transferrin only as Fe^{3+} after its export by ferroportin; so the maintenance of the iron balance in the brain is thus closely linked to the metabolism of copper (Mario, 2014).

Copper/Ceruloplasmin level in CSF

Cerebrospinal fluid (CSF) has been widely investigated in PD and in other parkinsonian syndromes with the aim of acquiring knowledge on the pathogenesis of this disease (Jimenez-Jimenez *et al.,* 2014). Scientific report suggests that changes in Cp might be considered as a potential marker for the evaluation of oxidative stress levels in the CNS of PD patients and analysis of CSF Cp oxidation during the progression of neurological disease could reveal a link between gradual CNS oxidative damage and specific pathological symptoms (Olivieri *et al.,* 2011). The endogenous ceruloplasmin in the CSF from PD patients showed higher sensitivity to proteolysis than did that in the CSF from healthy subjects, indicating the structural changes occur in the patients' CSF (Barbariga *et al.,* 2015). Significant loss of ceruloplasmin-ferroxidase activity has been observed in CSF and substantia nigra of patients with PD, suggesting a role of ceruloplasmin dysfunction in neurodegenerative diseases characterized by oxidative stress (Olivieri *et al.*, 2011; Barbariga *et al.,* 2015). The other report indicated that; decreased ceruloplasmin ferroxidase activity in CSF and serum from idiopathic PD patients has been reported, and decreased serum ceruloplasmin levels are associated with earlier age of PD onset (Ayton *et al.,* 2013).

Cp oxidation impairs ferroxidase activity; this impairment induces structural changes that lead in turn to the release of Cp coordinated Cu atoms, which are necessary for enzymatic activity (Olivieri *et al.,* 2011). The provided evidence indicates that the well-documented, moderate decrease of CSF and circulating ceruloplasmin activity in PD may be a reflection of a profound (80%) loss of ceruloplasmin activity in the SN (Ayton *et al.,* 2013). Furthermore, oxidative modification in Cp leads to copper release; a finding that possibly explains why copper increases in the CSF of PD patients (Brewer *et al.,* 2010, Ayton *et al.,* 2013). Copper ions released from oxidized Cp facilitate Fenton's reaction, which amplifies general protein damage (Olivieri *et al.*, 2011). Thus, the changes observed in CSF Cp from PD patients might reflect accelerated protein aging, as induced by oxidative pathological conditions (Grimm *et al.,* 2011). Recent studies have shown increased copper concentrations in the CSF of Parkinson's disease patients (Maass *et al.*, 2018). Therefore, hydrogen peroxide known to be overproduced in neurodegeneration, seems to be an agent crucially involved in the structural and functional modifications of ceruloplasmin in PD-CSF (Barbariga *et al.*, 2015).

Scientific study demonstrated that, in addition to the oxidative modifications and the loss of ferroxidase activity already reported, the ceruloplasmin in the CSF of PD patients undergoes to conformational changes and deamidation. Thus, protein deamidation might foster the α-syn aggregate and favor the formation of stable oligomers formation in the CSF of PD patients (Barbariga *et al.,* 2015). The oligomers in the CSF might contribute to the disease spreading in Parkinson's diseases (Barbariga *et al.,* 2015).

Correlation with iron accumulation in the brain

In the brain substantia nigra (SN), there is a high iron concentration (Olivieri *et al.,* 2011). Iron accumulation in the substantia nigra (SN) is a feature of Parkinson disease (PD), where the iron burden could cause oxidative stress and contribute to Lewy body pathology (Ayton *et al.,* 2013). To date, the risk factors associated with nigral iron deposition in Parkinson's disease have not been identified and represent a key challenge to understand the pathogenesis and to diagnose it (Jin *et al.*, 2011). Cp ferroxidase activity is essential for iron metabolism because it regulates the expression of Fpn iron transporter on cell membranes, thus iron metabolism impairment is the major consequence of the loss of Cp activity (Olivieri *et al.,* 2011). GPI-Cp has been reported to be downmodulated by oxidative stress in astroglial cells, and as such to contribute to intracellular iron deposition (Tapryal *et al.,* 2009). Thus, disrupted ceruloplasmin metabolism probably represents a risk factor for Parkinson's disease by increasing brain iron levels (Jin *et al.,* 2011).

In experimental report on animal model confirmed that, the absence of extracellular ferroxidase activity as a result of Cp oxidation leads to cellular iron retention (Olivieri *et al.,* 2011). Ceruloplasmin's modification fostered by pathological environment might explain the loss of ceruloplasmin-ferroxidase activity reported in CSF and substantia nigra of patients with PD. This might have relevant pathological correlation in the brain cellular iron homeostasis with consequent increase in iron accumulation, oxidative damage and neurodegeneration (Barbariga *et al.,* 2015). It is therefore possible that changes in CSF Cp expression and/or in protein modification(s), which affect enzymatic activity, may contribute to PD neurodegeneration by instigating an increase in ferrous iron, which, in turn, may promote the generation of toxic free radicals (Olivieri *et al.*, 2011). Reduced CSF ferroxidase activity, increased oxidative stress in CNS, and iron overload in SN are PD

features that potentially correlated with Cp oxidation (Boll *et al.*, 2008). Furthermore, impairment of the extracellular ferroxidase activity of GPI-bound membrane Cp has been reported to block iron efflux from cells by downmodulation of Fpn with the ensuing increase of intracellular iron accumulation (De Domenico *et al.*, 2007; Olivieri *et al.*, 2011).

Correlation with Parkinson's disease?

The analysis of CSF Cp oxidation during the progression of neurological disease could reveal a link between gradual CNS oxidative damage and specific pathological symptoms (Olivieri *et al.,* 2011). However, in addition to the Cp prototype, several others proteins might be modified by the pathological pro-oxidant environment in the CSF of PD patients, as inferred by the greater level of total protein carbonylation reported in the CSF of PD patients (Iannaccone *et al.,* 2013). Carbonylation converts side chains of many amino acids to reactive aldehyde and ketone groups, and causes loss of positive charges, which in turn results in protein acidification (Olivieri *et al.,* 2011). These oxidative modifications may have consequences on proteins functions, such as inhibition of the extracellular superoxide dismutase enzymatic activity induced by the hydrogen peroxide (Gottfredsen *et al.,* 2013). Furthermore, the high levels of hydrogen peroxide found in the CSF of PD patients might have additional pathological implications being this molecule able to affect several cellular functions (Dev *et al.,* 2015). A hypothesis is that, the pathological features of PD like aberrant autophagy and mitochondrial dis-functions that have been reported to potentiate the production of hydrogen peroxide might be responsible for the observed concentration increase (Quinlan *et al.*, 2013). This suggests that CSF proteins undergo oxidative modifications during the course of PD pathological events. Collectively, these results suggest that the Cp present in PD CSF is affected by modifications that induce protein acidification. Interestingly, there is a correlation between PD patients' clinical status and Cp acidification (Olivieri *et al.*, 2011).

In conclusion the iron overload in the sensitive part of brain, substantia nigra and loss of dopaminergic neurons has been implicated in the pathology and pathogenesis of Parkinson's disease (PD) without a clear cut of risk factors. But, early life iron exposure is an independent risk factor for the development of PD through age and iron accumulation is purely a consequence of neuronal loss and breakdown of the BBB that allows more iron to access the brain. In PD, the iron regulatory mechanisms in neurons of SNpc are surpass, thus, IRP1-mediated iron dyshomeostasis and sustained DMT1 presence may underlie iron accumulation in this disease. In this context, an evaluation of DMT1 and Ireg1 expression in parkinsonian brain is capital to understand the cause of iron accumulation and to design neuroprotective and therapeutic strategies to prevent progression of PD. Since GPI-anchored Cp has been expressed by astrocytes and secreted as a soluble from in the CSF plays a major role in brain iron homeostasis but it is a targets of pathological oxidative conditions leads to oxidative modifications and results reduced CSF ferroxidase activity, increased oxidative stress in CNS, and iron overload in SN are PD features that potentially correlated with the pathogenesis of PD by contributing to iron dismetabolism. In addition to iron accumulation in the SN, oxidation of Cp releases copper to increase the CSF concentration which participates in the Fentones reaction and plays a role in the oligomerization of alpha-synuclein that implicated in the pathogenesis of PD.

References

Aaron D. Gitler, Paraminder Dhillon and James Shorter. Neurodegenerative disease: models, mechanisms, and a new hope. *Disease Models & Mechanisms*. 2017; 10: 499-502.

André Nieoullon. Neurodegenerative diseases and neuroprotection: current views and prospects. *J Appl Biomed*. 2011; 9: 173–183.

Angelova M., S. Asenova,V. Nedkova, R. Koleva-Kolarova. Copper in the human organism. *Trakia Journal of Sciences*. 2011; 9(1): 88-98.

Angelova M. Dafina and Brown R. David. Iron, Aging, and Neurodegeneration. *Metals.* 2015; 5: 2070-2092.

Ayton Scott, Peng Lei, James A. Duce, Bruce X. W. Wong, Amelia Sedjahtera, BBMS(Hons), Paul A. Adlard, Ashley I. Bush, MBBS, and David I. Finkelstein. Ceruloplasmin Dysfunction and Therapeutic Potential for Parkinson Disease. *American Neurological Association*. 2013; 73(4):554–559.

Barbariga Marco, Flavio Curnis, Annapaola Andolfo, Alan Zanardi, Massimo Lazzaro, Antonio Conti, Giuseppe Magnani, Maria Antonietta Volontè, Laura Ferrari, Giancarlo Comi, Angelo Corti and Massimo Alessio. Ceruloplasmin functional changes in Parkinson's disease-cerebrospinal fluid. *Molecular Neurodegeneration*. 2015; 10(59): 1-12.

Belaidi AA and Bush AI. Iron neurochemistry in Alzheimer's disease and Parkinson's disease: targets

for therapeutics. *J Neurochem.* 2016; 139 Suppl 1: 179-197.

Boll MC, Alcaraz-Zubeldia M, Montes S, Rios C. Free copper, ferroxidase and SOD1 activities, lipid peroxidation and NO(x) content in the CSF. A different marker profile in four neurodegenerative diseases. *Neurochem Res*. 2008; 33:1717–1723.

Brewer GJ, Kanzer SH, Zimmerman EA, Celmins DF, Heckman SM, Dick R. Copper and ceruloplasmin abnormalities in Alzheimer's disease. *Am J Alzheimers Dis Other Demen*. 2010 25:490 –497.

Choi BS, Zheng W. Copper transport to the brain by the blood-brain barrier and blood-CSF barrier. *Brain Res*. 2009;1248:14–21.

Danny Ramos, David Mar, Michael Ishida, Rebecca Vargas, Michaella Gaite, Aaron Montgomery, Maria C. Linder. Mechanism of Copper Uptake from Blood Plasma Ceruloplasmin by Mammalian Cells. *PLoS.ONE*. 2016; 11(3):1-23/e0149516. doi:10.1371/journal.pone.0149516.

De Domenico I, Ward DM, di Patti MC, Jeong SY, David S, Musci G, Kaplan J. Ferroxidase activity is required for the stability of cell surface ferroportin in cells expressing GPI-ceruloplasmin. *EMBO J*. 2007; 26:2823–2831.

Dev S, Kumari S, Singh N, Kumar Bal S, Seth P, Mukhopadhyay CK. Role of extracellular hydrogen peroxide in regulation of iron homeostasis genes in neuronal cells: implication in iron accumulation. *Free Radic Biol Med*. 2015;86:78–89.

Dominic J Hare, Manish Arora, Nicole L. Jenkins, David I. Finkelstein, Philip A. Doble and Ashley I. Bush. Is early-life iron exposure critical in neurodegeneration? *Nat. Rev. Neurol*. 2015; doi:10.1038/nrneurol.2015.100

Eric Mills, Xian-ping Dong, Fudi Wang & Haoxing Xu. Mechanisms of brain iron transport: insight into neurodegeneration and CNS disorders. *Future Med. Chem.* 2010; 2(1): 51–64.

Fei Yang, Ylva Trolle Lagerros, Rino Bellocco, Hans-Olov Adami, Fang Fang, Nancy L. Pedersen and Karin Wirdefeldt. Physical activity and risk of Parkinson's disease in the Swedish National March Cohort. *BRAIN*. 2015; 138: 269–275.

Gabor G. Kovacs. Molecular Pathological Classification of Neurodegenerative Diseases: Turning towards Precision Medicine. *Int. J. Mol. Sci*. 2016; 17(189):1-33.

Gaier ED, BA Eipper, and RE Mains. Copper signaling in the mammalian nervous system: synaptic effects. *J Neurosci Res*. 2013; 91(1): 2–19.

Galhardi CM, Diniz YS, Faine LA, Rodrigues HG, Burneiko CM, Ribas BO, Novelli EL. Toxicity of copper intake: lipid profile, oxidative stress and susceptibility to renal dysfunction. *Food Chem Toxicol*. 2004; 42:2053–2060.

Gao G and Chang Y. Mitochondrial ferritin in the regulation of brain iron homeostasis and neurodegenerative diseases. *Front. Pharmacol.* 2014; 5:19. doi:10.3389/fphar.2014.00019

Giselle Pentón-Rol and Majel Cervantes-Llanos. Report on the Symposium "Molecular Mechanisms Involved in Neurodegeneration". Behav. Sci. 2018; 8(16): 1-11.

Gottfredsen RH, Larsen UG, Enghild JJ, Petersen SV. Hydrogen peroxide induce modifications of human extracellular superoxide dismutase that results in enzyme inhibition. *Redox biology*. 2013;1:24–31

Grimm S, Hoehn A, Davies KJ, Grune T. Protein oxidative modifications in the ageing brain: consequence for the onset of neurodegenerative disease. *Free Radic Res*. 2011; 45:73–88.

Hare J. Dominic, Bárbara Rita Cardoso, Erika P Raven, Kay L Double, David I Finkelstein, Ewa A Szymlek-Gay and Beverley-Ann Biggs. Excessive early-life dietary exposure: a potential source of elevated brain iron and a risk factor for Parkinson's disease. *npj Parkinson's Disease.* 2017; 3:1; doi:10.1038/s41531-016-0004-y

Iannaccone S, Cerami C, Alessio M, Garibotto V, Panzacchi A, Olivieri S, *et al.*, In vivo microglia activation in very early dementia with Lewy bodies, comparison with Parkinson's disease. *Parkinsonism Relat Disord*. 2013;19:47–52.

Jessica L. Billings, Dominic J. Hare, Milawaty Nurjono, Irene Volitakis, Robert A. Cherny, Ashley I. Bush, Paul A. Adlard, and David I. Finkelstein. Effects of Neonatal Iron Feeding and Chronic Clioquinol Administration on the Parkinsonian Human A53T Transgenic Mouse. *ACS Chem. Neurosci*. 2016; 7: 360−366.

Jia Fengju, Ning Song, Weiwei Wang, Xixun Du, Yajing Chi and Hong Jiang. High Dietary Iron Supplement Induces the Nigrostriatal Dopaminergic Neurons Lesion in Transgenic Mice Expressing Mutant A53T Human Alpha-Synuclein. *Frontiers in Aging Neuroscience.* 2018; 10(97): 1-9/10.3389/fnagi.2018.00097.

Jimenez-Jimenez Felix J, Hortensia Alonoso-Navarro, Elena Garcia-Martin and Jose A. G. Agundez. Cerebrospinal fluid biochemical studies in the patients with Parkinson's disease: toward a potential

search for biomarkers for this disease. *Cellular Neuroscience*. 2014; 8(369): 1-31.

Jin L, Wang J, Zhao L, Jin H, Fei G, Zhang Y, Zeng M, Zhong C. Decreased serum ceruloplasmin levels characteristically aggravate nigral iron deposition in Parkinson's disease. *Brain*. 2011; 134:50 –58.

Kaler SG. ATP7A-related copper transport diseases-emerging concepts and future trends. *Nat. Rev. Neurol*. 2011; 7:15–29.

Krupanidhi S, Arun Sreekumar & C.B. Sanjeevi. Copper & biological health. *Indian J Med Res* 2008; 128; 448-461.

Leipuviene, R., and Theil, E.C. The family of iron responsive RNA structures regulated by changes in cellular iron and oxygen. *Cell Mol Life Sci*. 2007; *64*: 2945-2955.

Logroscino Giancarlo, Xiang Gao, Honglei Chen, Al Wing, and Alberto Ascherio. Dietary Iron Intake and Risk of Parkinson's disease. *Am J Epidemiol*. 2008; 168:1381–1388.

Luigi Zecca,Moussa B.H.Youdim, Peter Riederer, James R. Connor, and Robert R. Crichton. Iron, brain ageing and neurodegenerative disorders. *Neuroscience*. 2004; 5: 1-11.

Maass Fabian, Bernhard Michalke, Andrease Leha, Matthias Boergr, Inga Zerr, Jan-Christoph Koch, Lars Tonges, Mathias Bahr and Paul Lingor. Elemental fingerprint as a cerebrospinal fluid biomarker for the diagnosis of Parkinson's disease. *J.Neurochemistry*. 2018; 145: 342-351.

Mario Manto. Abnormal Copper Homeostasis: Mechanisms and Roles in Neurodegeneration. Toxics. 2014; 2: 327-345.

Masoom M. Abbas, Zheyu Xu, Chir, Louis C.S. Tan. Epidemiology of Parkinson's disease—East Versus West. Movement disorders clinical practice. 2018; 5(1): 14–28.

Mills, E., Dong, X.P., Wang, F., and Xu, H. Mechanisms of brain iron transport: insight into neurodegeneration and CNS disorders. *Future Med Chem.* 2010; 2: 51-64.

Monnot A. D., M. Behl, S. Ho, and W. Zheng. Regulation of brain copper homeostasis by the brain barrier systems: effects of Fe-overload and Fe-deficiency. *Toxicology and Applied Pharmacology*. 2011; 256(3): 249–257.

Munoz P., Humeres A., Elgueta C., Kirkwood A., Hidalgo C., and Nunez M.T. Iron mediates N-methyl-D-aspartate receptor-dependent stimulation of calcium- induced pathways and hippocampal synaptic plasticity. *J. Biol. Chem.* 2011; 286: 13382–13392.

Olivieri Stefano, Antonio Conti, Sandro Iannaccone, Carlo V. Cannistraci, Alessandro Campanella, Marco Barbariga, Franca Codazzi, Ilaria Pelizzoni, Giuseppe Magnani, Mariasabina Pesca, Diego Franciotta, Stefano F. Cappa, and Massimo Alessio. Ceruloplasmin Oxidation, a Feature of Parkinson's Disease CSF, Inhibits Ferroxidase Activity and Promotes Cellular Iron Retention. *J.Neuroscience*. 2011; 31(50):18568 –18577.

Quinlan CL, Perevoshchikova IV, Hey-Mogensen M, Orr AL, Brand MD. Sites of reactive oxygen species generation by mitochondria oxidizing different substrates. *Redox biology*. 2013;1:304–12.

Salazar J, N. Mena, M. T. Nu´n˜ez. Iron dyshomeostasis in Parkinson's disease. *J Neural Transm*. 2006; [Suppl] 71: 205–213.

Sarah J. Texel, Xueying Xu and Z. Leah Harris. Ceruloplasmin in neurodegenerative diseases. *Biochem. Soc. Trans*. 2008; 36: 1277–1281.

Satoru Oshiro, Masaki S.MoriokaandMasataka Kikuchi. Dysregulation of IronMetabolism in Alzheimer's Disease, Parkinson's Disease, and Amyotrophic Lateral Sclerosis. *Advances in Pharmacological Sciences*. 2011: 1-8.

Sergio Montes, Susana Rivera-Mancia, Araceli Diaz-Ruiz,
Luis Tristan-Lopez, and Camilo Rios. Copper and Copper Proteins in Parkinson's Disease. *Oxidative Medicine and Cellular Longevity*. 2014; 1-15/http://dx.doi.org/10.1155/2014/147251

Siddappa M. Ashajyothi M., Raghavendra Rao, Jeffrey D. Long, John A. Widness, Michael K. Georgieff. The Assessment of Newborn Iron Stores at Birth: A Review of the Literature and Standards for Ferritin Concentrations. *Neonatology*. 2007; 92:73–82.

Skjørringe Tina, Annette Burkhart, Kasper Bendix Johnsen and Torben Moos. Divalent metal transporter 1 (DMT1) in the brain: implications for a role in iron transport at the blood-brain barrier, and neuronal and glial pathology. *Front. Mol. Neurosci.* 2015; 8(19): 1-13.

Steinbicker AU, Muckenthaler MU. Out of balance-systemic iron homeostasis in iron-related disorders. Nutrients. 2013; 5: 3034–3061.

Svetlana Lutsenko, Ashima Bhattacharjee and Ann L. Hubbard. Copper handling machinery of the brain. *Metallomics*, 2010; 2:596–608.

Tapryal N, Mukhopadhyay C, Das D, Fox PL, Mukhopadhyay CK. Reactive oxygen species regulate ceruloplasmin by a novel mRNA decay mechanism involving its 3 -untranslated region: implications in

neurodegenerative diseases. *J Biol Chem*. 2009; 284:1873–1883.

Voskaki Irene, Arvanitidou Vasiliki, Athanasopoulou Helen, Tzagkaraki Angeliki, Tripsianis Gregory, GiannouliaKarantana Aglaia, Serum copper and zinc levels in healthy greek children and their parents. *Biol Trace Elem Res*. 2010; 134(2): 136-45.

Wang, J.-Y. Qing-Qing Zhuang, Lan-Bing Zhu, Hui Zhu, Ting Li, Rui Li, Song-Fang Chen, Chen-Ping Huang, Xiong Zhang & Jian-Hong Zhu. Meta-analysis of brain iron levels of Parkinson's disease patients determined by postmortem and MRI measurements. *Sci. Rep.* 6. 2016: 1-13.

Wataru Sako, Nagahisa Murakami, Keisuke Motohama, Yuishin Izumi & Ryuji Kaji. The efect of istradefylline for Parkinson's disease: A meta analysis. Nature. 2017; 1-7/ DOI:10.1038/s41598-017-18339-1.

Wong X. Bruce and Duce A. James. The iron regulatory capability of the major protein participants in prevalent neurodegenerative disorders. *Frontier in pharmacology*. 2014; 5(81): 1-11.

Yu Shu-yang, Chen-jie Cao, Li-jun Zuo, Ze-jie Chen, Teng-hong Lian, Fang Wang, Yang Hu, Ying-shan Piao, Li-xia Li, Peng Guo, Li Liu, Qiu-jin Yu, Rui-dan Wang, Piu Chan, Sheng-di Chen, Xiao-min Wang, and Wei Zhang. Clinical features and dysfunctions of iron metabolism in Parkinson disease patients with hyper echogenicity in substantia nigra: a cross-sectional study. *BMC Neurology*. 2018; 18(9): 1-8.

Zheng G., J. Chen, and W. Zheng. Relative contribution of CTR1 and DMT1 in copper transport by the blood-CSF barrier: implication in manganese-induced neurotoxicity. *Toxicology and Applied Pharmacology*. 2012; 260(3): 285–293.

Zheng, W, and Monnot, A.D. Regulation of brain iron and copper homeostasis by brain barrier systems: implication in neurodegenerative diseases. *Pharmacol Ther*. 2012; 133: 177-188.

How to cite this article:

Yiglet Mebrat. 2020. Early Life Iron Exposure and CSF Levels of Cu/ceruloplasmin Correlated with Parkinson's Disease. *Int.J.Curr.Res.Aca.Rev*. 8(12), 96-108. doi: https://doi.org/10.20546/ijcrar.2020.812.009