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The Azalea Hypothesis of Alzheimer's Disease: A Functional Iron Deficiency Promotes Neurodegeneration

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Abstract

Chlorosis in azaleas is characterized by an interveinal yellowing of leaves that is typically caused by a deficiency of iron. This condition is usually due to the inability of cells to properly acquire iron as a consequence of unfavorable conditions, such as an elevated pH, rather than insufficient iron levels. The causes and effects of chlorosis were found to have similarities with those pertaining to a recently presented hypothesis that describes a pathogenic process in Alzheimer's disease. This hypothesis states that iron becomes sequestered, e.g., by amyloid β and tau, causing a functional deficiency of iron that disrupts biochemical processes leading to neurodegeneration. Additional mechanisms that contribute to iron becoming unavailable include iron-containing structures not undergoing proper recycling (e.g., disrupted mitophagy and altered ferritinophagy) and failure to successfully translocate iron from one compartment to another (e.g., due to impaired lysosomal acidification). Other contributors to a functional deficiency of iron in patients with Alzheimer's disease include altered metabolism of heme or altered production of iron-containing proteins and their partners (e.g., subunits, upstream proteins). A review of the evidence supporting this hypothesis is presented. Also, parallels between the mechanisms underlying a functional iron deficient state in Alzheimer's disease and those occurring for chlorosis in plants are discussed. Finally, a model describing the generation of a functional iron deficiency in Alzheimer's disease is put forward.

Keywords

anemia; Alzheimer's disease; chlorosis; ferritinophagy; heme; iron; lysosome; mitochondria; mitophagy

Introduction

Iron is essential for nearly all forms of life and is used by every living cell within the human body (Cairo and others, 2006; Kaplan and Ward, 2013; Andreini and others, 2018). Numerous biochemical reactions rely on iron's ability to undergo redox reactions, i.e., acquire and lose electrons, in response to interactions with ligands (Kaplan and Ward, 2013; Andreini and others, 2018). Within cells, iron-binding proteins can be present in numerous

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subcellular locations (in descending order based on the relative number of iron-containing proteins): 1) within mitochondria and the endoplasmic reticulum, 2) endosomes and nucleus, and 3) Golgi apparatus, cell membrane, and cytoplasm, as well as in the extracellular space (Andreini and others, 2018). Iron is used by proteins containing an iron-sulfur cluster, heme, or independent of these, and many reactions involve oxygen-based chemistry (Kaplan and Ward, 2013; Andreini and others, 2018). Iron containing proteins are involved with numerous functions including mitochondrial respiration, lipid metabolism, regulation of angiogenesis, hypoxia sensing, extracellular matrix formation, production of nitric oxide, inflammation, antioxidative responses, regulation of transcription and translation, DNA synthesis and repair, etc. (Hare and others, 2013; Alnuwaysir and others, 2021; Liu and others, 2022). In addition to these functions, iron is essential for myelination and neurotransmitter synthesis, and synaptic activity is linked to iron uptake by mitochondria in order to address energy demands (Hare and others, 2013; Tena-Morraja and others, 2023).

Mitochondrial dysfunction has been linked to synaptic impairment and neurodegeneration in Alzheimer's disease (Akhter and others, 2017; Swerdlow and others, 2017), which is a progressive condition that impairs memory and cognition. Besides being essential for mitochondrial respiration, iron is involved with numerous other biochemical activities that have the potential to impact the course of Alzheimer's disease. Iron can partake in reactions that can cause cellular injury, such as oxidative damage and ferroptosis, while a deficiency of iron can impair dendritic growth, synaptic activity and neuronal survival. Thus, it is imperative to examine the various roles of iron in the pathogenesis of Alzheimer's disease. Others have thoroughly covered the pro-pathogenic effects of excess or mishandled iron in Alzheimer's disease (Ashraf and others, 2020; Wu and others, 2023). Here the focus will be on the role of a functional deficiency of iron in promoting disease activity.

A hypothesis was recently presented that describes a new interpretation of pathological processes in Alzheimer's disease (LeVine and others, 2023). It states that iron becomes sequestered, e.g., by amyloid β and tau, resulting in a functional iron deficient state that contributes to neurodegeneration (LeVine and others, 2023) (Table 1). Besides being bound to these proteins, in the central nervous system of patients with Alzheimer's disease, iron can become trapped within proteins and subcellular structures, e.g., due to impaired ferritinophagy and mitophagy, respectively, as well as within lysosomes, due to an elevated lysosomal pH (Yambire and others, 2019) (Table 1). Additionally, decreased production (or decreased catabolism) of heme and iron-containing proteins can promote a functional iron deficient state (Table 1). Thus, despite the presence of iron, under the right conditions an iron deficient state can develop where iron does not adequately supply cellular and molecular demands resulting in the disruption of biochemical processes, such as mitochondrial respiration, that leads to degeneration.

Many features of this pathological process were found to have similarities with those occurring during chlorosis in azaleas. Chlorosis is an iron-deficient state that impairs biochemical processes, such as chlorophyll synthesis and photosynthesis. This condition in plants often occurs despite the presence of iron. Thus, both Alzheimer's disease and chlorosis in azaleas share a functional iron deficient state. A description of the mechanisms and consequences of a functional iron deficient state that occur in Alzheimer's disease

is presented first followed by a description of chlorosis in plants, and then the parallels between these processes are highlighted. Finally, a model for the development of a functional iron deficiency and considerations for therapeutic interventions are presented.

A functional deficiency of iron in Alzheimer's disease

The premise for the hypothesis describing a functional iron deficiency in Alzheimer's disease was based on new analyses of gene expression data sets, iron histochemical staining results, and a reexamination of the scientific literature (LeVine and others, 2023). A GeneCards database search exemplifies the extensive involvement of iron within Alzheimer's disease. Using the search term Alzheimer, and then matching the results with their relationship to iron, reveals that more than two-thirds of the top 21 search results have a connection with iron (Table 2).

To illustrate the scope of these interactions, the top gene from this search (APP for amyloid precursor protein) has multiple connections with iron (Table 2). These include having an iron response element (IRE) in its transcript, potentially influencing the function of ferroportin which facilitates cellular iron export, and its proteolytic product, amyloid β, directly binding iron.

Amyloid precursor protein and iron metabolism

The transcript for amyloid precursor protein has an IRE at the 5'-untranslated region that is responsive to iron regulatory protein (IRP) 1 (Rogers and others, 2008; Cho and others, 2010; Khan and others, 2023). Thus, the binding of IRP1 to the transcript reduces translation, but when ferrous iron interacts with the IRP1, it is released from the transcript allowing for increased translation of amyloid precursor protein (Cho and others, 2010; Rogers and Cahill, 2020).

Amyloid precursor protein is thought to help stabilize ferroportin, which is involved with iron export from the cell, but this role in facilitating ferroportin has been questioned (Hin and others, 2021). Ferroportin has also been found in presynaptic vesicles indicating that iron may be released into the synaptic cleft (Wu and others, 2004), and iron might influence NMDA receptor function including facilitating ERK1/2 activation and supporting basal synaptic activity (Muñoz and others, 2011) (Table 3). During Alzheimer's disease, the amyloidogenic processing of amyloid precursor protein by β and γ secretases can destabilize ferroportin leading to less efflux resulting in the accumulation of iron (Tsatsanis and others, 2020; Hin and others, 2021; Jakaria and others, 2021). Consistent with this process, less ferroportin was present in the brains of patients with Alzheimer's disease as well as in an animal model (APP/PS1) (Raha and others, 2013; Xian-hui and others, 2015; Bao and others, 2021). Iron that is unable to be exported from the cell, e.g., due to impaired ferroportin, could go into storage.

Ferritin is responsible for iron storage, and it undergoes similar translational control to that for amyloid precursor protein (Rogers and others, 2008; 2019). Ferritin is increased in the CNS of patients with Alzheimer's disease (Ashraf and others, 2020; Tran and others, 2022) and in the APP/PS1 mouse model of Alzheimer's disease (Svobodová and others,

2019). Normally, ferritin undergoes recycling via ferritinophagy in the lysosome where the acidic environment is necessary for the release of iron (Kidane and others, 2006; Asano and others, 2011; Mancias and others, 2014). In senescent cells, ferritinophagy is impaired (Masaldan and others, 2018). Senescent cells are a feature of Alzheimer's disease as is disruption to autophagy and lysosomal function (Kritsilis and others, 2018; Liu, 2022) (also discussed below). Mitochondrial ferritin is also increased (Wang and others, 2011; Yang and others, 2013) and mitophagy is disrupted (Kerr and others, 2017) in the CNS of patients with Alzheimer's disease. Incomplete ferritinophagy or mitophagy could lead to iron accumulation, which occurs in Alzheimer's disease (discussed below), and iron becoming trapped. In other words, less iron would be available due to decreased recycling. Under normal conditions, iron depletion would result in an increase of mitophagy (Hara and others, 2020).

In addition to these alterations, amyloid β, the proteolytic processing product of amyloid precursor protein, binds iron (and copper and zinc), which facilitates the aggregation of amyloid β (Boopathi and Kolandaivel, 2016; LeVine and others, 2023). The affinity for iron is increased by the aggregation of amyloid β (Garzon-Rodriguez and others, 1999). In addition, amyloid β binds heme (Atamna and Frey, 2004; Atamna, 2006). Numerous studies have shown that iron is co-localized with amyloid plaques (Fig. 1, Fig. 2), and that iron is more abundant within the cortex, hippocampus, and some deep gray matter structures of patients with Alzheimer's disease compared to control subjects (LeVine, 1997; Langkammer and others, 2014; Hare and others, 2016; Sands and others, 2016; van Duijn and others, 2013; 2017; Du and others, 2018; Yang and others, 2022). In addition, iron is bound to tangles (Smith and others, 1997), and ferric iron promotes aggregation and phosphorylation of tau (Yamamoto and others, 2002; Bader and others, 2011; Guo and others, 2013; Rao and Adlard, 2018). Iron is more tightly bound in cerebral tissue in patients with Alzheimer's disease than in patients with other conditions, likely due to its interactions with amyloid β and tau (LeVine and others, 2023). The tightly bound state would make iron less available for use in biochemical reactions, creating the conditions for a functional iron-deficient state.

The carboxy cleaved fragment of amyloid precursor protein, also known as C99, is elevated in Alzheimer's disease (Delport and Hewer, 2022). This fragment has been shown to impair lysosomal function resulting in an increase in lysosomal pH (Jiang and others, 2019; Delport and Hewer, 2022). This elevated pH impairs the delivery of iron from the lysosome to the cytosol as discussed below.

Presenilin 1 and the lysosome

The second top ranked result from the database search (Table 2), presenilin 1, is a core component of the γ secretase complex, which is a protease that cleaves proteins into smaller pieces. Amyloid precursor protein is cleaved by γ secretase generating smaller peptides including amyloid β. Since amyloid β binds and sequesters iron (discussed above), presenilin 1 is associated with biochemical processes that affect the availability of iron.

Presenilin 1 is also connected to iron by other mechanisms. Mutations in presenilin 1, which is a cause of familial Alzheimer's disease, can negatively affect lysosomal performance.

Presenilin 1 has multiple functions, but one effect of impaired presenilin 1 is a reduction of the glycosylation of a subunit of the vATPase proton pump (ATPase H+ transporting V0 subunit a1; gene ATP6V0A1), and without proper glycosylation the subunit cannot get properly directed to the lysosome (Lee and others, 2010; 2015; Lumsden and others, 2018; Yambire and others, 2019; Hin and others, 2021). The resulting impaired vATPase, disrupts the pumping of protons into the lysosome causing a higher lysosomal pH. Defective lysosomal acidification has been observed in various models of Alzheimer's disease (Wolfe and others, 2013). Also, an excess of amyloid precursor protein, which occurs in Down's syndrome, causes an elevation of lysosomal pH, which may be mediated by a carboxy terminal fragment of amyloid precursor protein disrupting the vATPase (Jiang and others, 2019).

In lysosomes with diminished vATPase activity, ferric iron derived from endocytosis of transferrin, ferritinophagy or mitophagy is unable to undergo reduction to ferrous iron (Yambire and others, 2019) since presumably the ideal acidic pH is not achieved for ferrireductase STEAP3, which catalyzes this reaction. Since ferric iron cannot get transported to the cytosol by divalent metal transporter 1, iron ends up accumulating in the lysosome while cytoplasmic iron levels drop (Yambire and others, 2019). This leads to adjustments by the cell to acquire more iron. Eventually a functional iron deficient state could develop resulting in disruption of biochemical processes, including impaired mitochondrial function, and cell death (Lee and others, 2010; 2015; Lumsden and others, 2018; Yambire and others, 2019; Hin and others, 2021) (Table 3).

Apolipoprotein E influences iron deposition in the CNS

The third ranked result from the database search, apolipoprotein E (Table 2), can also influence brain iron homeostasis. The ε4 allele, which increases the risk for sporadic Alzheimer's disease, is associated with reduced clearance and greater deposition of amyloid β and iron (Schmechel and others, 1993; Patel and others, 2021; Belaidi and others, 2022; Uchida and others, 2022). Some of the amyloid β deposition occurs within neurons (Dafnis and others, 2016) as does some iron accumulation (Smith and others, 1997; LeVine and others, 2023). In astrocytes, the ε4 allele caused a substantial elevation in the lysosomal pH (pH 4.08 to 5.20) compared to the ε3 allele and a decreased clearance of amyloid β (Prasad and Rao, 2018). As discussed above, the increased lysosomal pH could impair iron transport into the cytosol.

In patients with mild cognitive impairment, who are at risk for developing Alzheimer's disease, the ε4 allele was also associated with greater cortical iron accumulation and amyloid plaque formation (van Bergen and others, 2016). In the brains of older individuals, the ε4 allele was associated with elevated subcortical iron (i.e., hippocampus and amygdala) (Nir and others, 2022), increased cortical iron, and greater default mode network activity (Kagerer and others, 2020), the latter of which occurs at the earliest stages of amyloid β accumulation, perhaps functioning as a compensation mechanism (Hahn and others, 2019). Thus, the ε4 allele is associated with greater amyloid β accumulation, which is a pathological feature of Alzheimer's disease; and as mentioned earlier, amyloid β can bind and sequester iron potentially leading to a functional iron deficiency.

In addition to the three examples covered above, other proteins associated with Alzheimer's disease have interactions with iron and can participate in the cause or consequence of a functional iron deficient state that advances pathology (Table 2).

Cellular adjustments to an iron-deficient state

In response to less iron being available, cells attempt to adjust accordingly to increase the uptake and availability of iron. Thus, elevated levels of iron that are observed in cortical and subcortical regions of the CNS of patients with Alzheimer's disease would not be unexpected if a substantial amount of iron is unavailable, e.g., due to iron being bound to amyloid β or tau or sequestered in lysosomes. Thus, Occam's razor would apply, i.e., the brain of a patient with Alzheimer's disease has more iron because it needs more iron.

Cells adjust to an iron-deficient state via IRE/IRP for the regulation of translation of proteins involved in iron homeostasis (Rouault, 2006). Additionally, post-translational modifications, in response to low iron levels, can lead to altered expression of numerous genes. For instance, prolyl 4-hydroxylases, which are part of the Fe(II)/2-oxoglutarate-dependent (Fe/2OG) oxygenases superfamily, are involved in the stress response that detects low levels of oxygen, and they may also respond to levels of iron or 2-oxoglutarte (Mole, 2010; Karuppagounder and others, 2015; Strowitzki and others, 2019). Under normoxia conditions, prolyl 4-hydroxylases-1–3 hydroxylate two prolines on hypoxia-inducible factor (HIF)-1α subunit, which allows it to be detected by von Hippel-Lindau tumor suppressor protein, a component of the E3 ubiquitin ligase complex, to cause proteasomal degradation. During hypoxia, the HIF-1α subunit doesn't become hydroxylated which allows it to enter the nucleus, and together with HIF-1β, it binds DNA target genes (Karuppagounder and others, 2015; Strowitzki and others, 2019). In addition, under normal conditions, another protein that utilizes iron, factor inhibiting HIF, which is also a member of the Fe/2OG oxygenases superfamily, will hydroxylate an asparaginyl residue on HIF-1α resulting in the decrease of transcriptional activity, and during hypoxia, the hydroxylation is reduced allowing for increased transcription (Barrett and others, 2015; Strowitzki and others, 2019). The nature of the response to HIF stabilization will vary depending on the cell type (e.g., neuron vs astrocyte) or the stimulus, and may be directed to a pro-survival or a pro-death pathway (Karuppagounder and others, 2015). Interestingly, inhibition of prolyl 4-hydroxylases is linked with neuroprotection (Karuppagounder and others, 2015). Others have suggested that prolyl 4-hydroxylases serve as sensor for excess iron during neurological diseases where increased activity of prolyl 4-hydroxylases, due to excess available iron, is related to stimulation of activating transcription factor 4-regulated gene expression associated with ferroptosis (Ratan, 2019).

Besides detecting hypoxia, prolyl 4-hydroxylases have been proposed to be sensors of iron deficiencies (Mole, 2010). Thus, analogous to hypoxic conditions, a similar mechanism may also occur during iron deficiency, but this response is less established (Karuppagounder and others, 2015). During iron chelation, and possibly in response to iron deficiency that is present over a period of time, the hydroxylation via prolyl 4-hydroxylases will be reduced allowing for activation of genes by HIF (Karuppagounder and others, 2015). In heart tissue from iron-deficient mice, there was an upregulation of the expression for HIF-1α and

altered expressions of genes downstream from induction by this factor (Chung and others, 2021). It was suggested that altered prolyl 4-hydroxylases activity was in response to lower oxygen levels rather than less available iron since the iron content in cardiac cells was not significantly decreased and there was a discrepancy of the predicted expressions of transcripts for divalent metal transporter-1 and hepcidin, which did not change (Chung and others, 2021). However, the expression of transcripts for the transferrin receptor and ferroportin did go in the predicted directions (Chung and others, 2021). Furthermore, in a similar study, the protein but not the transcript for divalent metal transporter-1 was substantially elevated in the heart of iron-deficient rats, indicating there was a response to less available iron by divalent metal transporter-1, but it was at the protein level (Nam and others, 2013). Additionally, protein levels for transferrin receptor 1 were elevated in the heart of iron-deficient rats further indicating a response by cardiac cells sensing an iron deficient state (Nam and others, 2013). Thus, during iron deficiency, it is possible that there is not sufficient *accessible* iron to meet energy/cellular demands by cardiac cells. This is supported by cardiac cells from iron-deficient animals having a decreased activity of complex I and IV (Chung and others, 2021), which both require iron. Furthermore, links between iron deficiency and cardiac disease have been established (Alnuwaysir and others, 2021; Massaiu and others, 2023).

Jumonji domain-containing (Jmjd) 6, whose gene expression and protein levels are increased in Alzheimer's disease (Nativio and others, 2020; Bai and others, 2021), is a member of the Fe/2OG oxygenases superfamily, and it has also been linked to iron-sensing possibly via alternative splicing, e.g., for ferrochelatase, which catalyzes the final step of heme synthesis (Barman-Aksözen and others, 2013; Kwok and others, 2017; Yi and others, 2017). Presumably, during iron deficiency, the transcript for ferrochelatase undergoes aberrant splicing as a consequence of decreased Jmjd6 hydroxylation of U2 snRNP auxiliary factor 65-kDa subunit, which is a necessary factor for splicing, resulting in lower transcript and protein levels of ferrochelatase (Barman-Aksözen and others, 2013) (Table 3). In a meta-analysis of seven proteomic datasets, ferrochelatase was significantly reduced in patients with Alzheimer's disease (Bai and others, 2021), which is in contrast to one earlier study which found an increase (Atamna and Frey, 2004). Iron deficiency leads to disruption of heme synthesis and the inability of ferrochelatase to add iron to heme (Atamna and others, 2002A; Atamna, 2004). Heme levels are generally thought to be decreased in Alzheimer's disease and linked with impaired formation of complex IV (Atamna and others, 2002B; Atamna, 2006; Dwyer and others, 2006).

Jmjd6 also regulates transcriptional activity. It is involved with controlling promoter-proximal Pol II pausing release and is an arginine demethylase of both histone (H3 and H4) and non-histone proteins (RNA helicase A and heat shock protein 70) (Yi and others, 2017). In cell culture experiments, decreased iron levels result in lowered transcription for mitochondrial proteins, and these transcriptional changes corresponded with changes in methylation and acetylation of histones (Rensvold and others, 2016) (Table 3). Interestingly, prolyl 4-hydroxylases have been proposed to be sensors for 2-oxoglutarate, besides sensing oxygen and iron levels (Mole, 2010; Karuppagounder and others, 2015; Strowitzki and others, 2019). 2-Oxoglutarate, which is also known as α ketoglutarate, is an intermediate of the tricarboxylic acid cycle and used for nitrogen-assimilatory reactions, and its levels may

reflect nutrient status (Huergo and Dixon, 2015). Of note, the mitochondrial 2-oxoglutarate/malate carrier protein (SLC25A11) has lower expression in patients with Alzheimer's disease (Bai et al, 2021). Other Jmjd proteins affect the function of the synapse, as well as other processes in the CNS, and are likely impacted during Alzheimer's disease (Liu and others, 2022).

To ascertain whether transcriptional adjustments in response to Alzheimer's disease are in line with changes that occur during an iron-deficient state, such as anemia, an analysis of protein-coding transcripts from the olfactory bulb of patients divided into different stages of Alzheimer's disease vs control subjects (GEO dataset GSE93885; Lachen-Montes and others, 2017) was performed (LeVine and others, 2023). The olfactory bulb shares many pathological features seen elsewhere in affected CNS areas, and the initial stage of disease was emphasized in the analysis in order to capture early events related to neurodegeneration rather than later events covering more extensive pathological changes (LeVine and others, 2023). This analysis of early disease revealed that the number of genes with altered expressions were significantly associated with anemia-related processes, and this association was even greater than that observed for Alzheimer's disease-related genes (LeVine and others, 2023). Significant associations were also observed for additional categories involving iron: mitochondria, 3'- IREs, 5'- IREs, iron transport, heme-binding proteins, and iron-sulfur proteins (LeVine and others, 2023).

One gene within the mitochondria category that was downregulated was PITRM1, which encodes for pitrilysin metallopeptidase 1. Normally, this protein digests peptides, including amyloid β, as well as precursor proteins within mitochondria and chloroplasts, and a decrease in its function is linked with both tau and amyloid deposits (Alikhani and others, 2011; Kmiec and others, 2014; Pinho and others, 2014; Brunetti and others, 2021). Mice deficient in pitrilysin metallopeptidase 1 accumulate both amyloid β and amyloid precursor protein (Brunetti and others, 2016). Also, pitrilysin metallopeptidase 1 deficient human organoids mimic several features seen in Alzheimer's disease such as mitochondrial dysfunction, protein aggregates and elevated levels of phosphorylated tau (Pérez and others, 2021). Like the analysis of the dataset from patients with initial Alzheimer's disease, the analysis of single cell RNA sequencing from pitrilysin metallopeptidase 1 deficient human organoids (Pérez and others, 2021) revealed a significant association with anemia-related genes, and these were most often observed within neuronal clusters, followed by progenitor cell clusters, and then glia together with astrocyte clusters (LeVine and others, 2023).

Consequences of an iron-deficient state

Metabolic processes within mitochondria are sensitive to iron-deficient states (Atamna and others, 2002A). In primary mouse hippocampal cultures, iron deficiency impaired dendritic branching and growth, largely attributable to reduced mitochondrial respiratory capacity (Bastian and others, 2019) (Table 3). In iron-deficient animals, the function of mitochondrial complexes I and IV in the heart were less active, despite the amount of enzyme being unchanged (Chung and others, 2021). Similarly, numerous studies have observed that the activity of complex IV is reduced in the CNS of patients with Alzheimer's disease (Mutisya and others, 1994; Ojaimi and others, 1999; LeVine and others, 2023) (Table 3).

Furthermore, amyloid β binds heme which could cause a deficiency of heme, and heme deficiency can reduce the formation of complex IV (Atamna and others, 2002A; Atamna and Frey, 2004; Atamna, 2004; 2006) (Table 1). Amyloid β also disrupts the degradation of heme contributing to a functional deficiency of heme (Vidal and others, 2019) (Table 1). Interestingly, besides iron deficiency disrupting heme biosynthesis, the accumulation of unavailable iron within brain cells, and perhaps within mitochondria, has been proposed to be due to disrupted metabolism of heme (Atamna and others, 2002B).

In addition to impairing metabolic processes, iron deficiency can lead to pro-oxidative conditions by disrupting the function of the electron transport chain, resulting in mitochondrial damage (Atamna and others, 2002A; Walter and others, 2002). Impaired mitochondrial activity can affect neuronal and synaptic performance that could impede learning and memory and even lead to neurodegeneration (Li and others, 2006; Cavallucci and others, 2013; Todorova and Blokland, 2017). Besides disruptions to mitochondrial function, any biochemical process that utilizes iron or heme, or is associated with an iron-catalyzed reaction, has the potential to be negatively affected. For example, Fe/2OG oxygenases have been proposed to be involved with Alzheimer's disease progression (Liu and others, 2022). Approximately 60 to 70 reactions are catalyzed by these oxygenases (Liu and others, 2022), including hydroxylation by prolyl hydroxylases as discussed above. In the CNS of patients with Alzheimer's disease, there is an overall downregulation of Fe/2OG oxygenases, particularly those affecting nucleic acids (discussed above), and altered catalytic activity has been proposed to facilitate the development of Alzheimer's disease (Liu and others, 2022).

Other processes that could be adversely affected in response to an iron-deficient state include reduced uptake of neurotransmitters, impaired dopaminergic signaling, decreased myelin production, etc. (Atamna and others, 2002A; Puig and others, 2017; Andreini and others, 2018). The biochemical changes that result from deficits of iron can affect learning, memory, behavior, motor performance, etc. (Zhukovskaya and others, 2019; Ferreira and others, 2019). Similar to iron-deficiency in Alzheimer's disease, iron deficiency in plants disrupts biochemical processes leading to impaired function, i.e., photosynthesis and growth. In fact, upon close examination, there are many potential parallels between iron-deficiency in plants and iron deficiency in Alzheimer's disease.

Chlorosis in plants

In Greek, chlōrós means "greenish yellow" or "light green" referring to new growth in plants. In botany, chlorosis refers to a yellowing of normally green leaves. This is caused by a lack of chlorophyll, which can be due to a variety of causes including a lack of nutrients. Chlorosis is frequently a result of a deficiency of iron, which is normally used to support the synthesis of chlorophyll. Thus, when iron is depleted, chlorophyll production is decreased (Li and others, 2021), which is due, in part, to impaired production of gamma-aminolevulinic acid, a precursor used for chlorophyll synthesis (Marschner, 1995). Iron is also used for photosynthesis and respiration. In fact, the great majority of iron, ~80–90%, is located within chloroplasts (Kobayashi and Nishizawa, 2012), which is where photosynthesis occurs. During a shortage of iron, photosynthesis becomes impaired causing

a reduction in electron transfer between the two photosystems resulting in photooxidative injury (Kobayashi and Nishizawa, 2012). Depletion of iron often occurs to a greater degree in young leaves compared to old leaves (Brown, 1956; Demasi and others, 2017) and impairment of these biochemical activities causes diminished growth, yellowing and browning of leaves, and chloroplast degeneration (Demasi and others, 2017; Li and others, 2021). In response to iron deficiency, many genes can become induced including FRO2, the gene for ferric-chelate reductase, which reduces ferric iron into soluble ferrous iron so that it can be taken up by root cells via iron-regulated transporter 1 (Robinson and others, 1999; Vert and others, 2002; Jeong and Connolly, 2009; Kobayashi and Nishizawa, 2012).

In azaleas, chlorosis typically results in an interveinal yellow presentation with green concentrated in veins, in contrast to leaves without chlorosis, which display a more uniform green appearance (Fig. 3, Fig. 4). Interestingly, chlorosis is not necessarily due to insufficient levels of iron in the soil, but rather the inability to acquire the iron due to suboptimal conditions. For instance, azaleas have an ideal soil pH requirement of 4.5 to 6.0 for iron uptake. At these pH conditions, a ferric chelate reductase at the epidermal cell membrane reduces ferric iron, a process that is a prerequisite for the transport of iron into the root cell cytosol (Mengel, 1994; Tagliavini and Rombola, 2001). At the leaf mesophyll cell, a ferric chelate reductase at the plasma membrane also reduces ferric iron to facilitate the uptake of ferrous iron by a zinc-regulated transporter/iron-regulated transporter related protein and/or by natural resistance-associated macrophage proteins (Mengel, 1994; Tagliavini and Rombola, 2001; Jeong and Guerinot, 2009). A ferric chelate reductase is also present at the chloroplast as is the enzyme called permease in chloroplasts 1, which is thought to facilitate iron uptake by the chloroplast (Jeong and Guerinot, 2009).

When the pH of the soil becomes elevated, the activity of ferric chelate reductase is decreased and iron uptake is diminished (Mengel, 1994; Susin et al 1996; Tagliavini and Rombola, 2001; Zhao and others, 2023). In the case of lime induced chlorosis in azaleas, calcium carbonate in the soil adjacent to a cement wall (such as a basement) or concrete structures (such as a driveway) in association with limestone can be the culprit that increases the pH (University of Illinois at Urbana Champaign, 1996). Even though a proton pump at the root plasmalemma attempts to acidify the soil, bicarbonate can neutralize this H+ (Mengel, 1994). Furthermore, the uptake of nitrogen by a H+/nitrate cotransporter removes protons thereby contributing to the elevation of the pH (Mengel, 1994). Thus, under the right conditions, such as an elevation of pH, azaleas can become deficient for iron even when sufficient iron levels are present in the soil.

A similar mechanism of impaired iron uptake has been hypothesized to occur at the leaves in chlorotic plants, i.e., bicarbonate and nitrate raise the pH and impair the activity of a ferric chelate reductase limiting the movement of iron into the cytosol of the mesophyll (Mengel, 1994; Zhao and others, 2023). In support of this mechanism at leaves, iron can be present at greater concentration in chlorotic leaves than in green leaves and spraying the chlorotic leaves with a mildly acidic solution results in them becoming green again (Mengel, 1994). However, subsequent experimentation led to a reevaluation of the early predictions (Nikolic and Römheld, 2007). In particular, bicarbonate and nitrate taken up from the soil did not affect the pH at the leaves; iron was not usually observed to be at a higher concentration in

chlorotic leaves, and if it was it was due to restriction of leaf expansion; and treatment of leaves with a dilute acidic solution was thought to cause regreening of leaves by extracting iron that was bound to the cell wall (Nikolic and Römheld, 2007). But more recent analyses offer a somewhat different explanation. Chlorosis in leaves of sensitive plants or trees was postulated to be due to poor transfer of iron from the xylem to the mesophyll cells, since the concentration of iron was greater in the veins than in the mesophyll cells of chlorotic leaves as a result of a high apoplastic pH (the apoplast is the intercellular space between membranes used for transport of water and solutes) (Zhao and others, 2023). The elevated apoplastic pH was correlated with reduced activity of the ferric chelate reductase at the plasma membrane of mesophyll cells, which is needed to convert ferric iron into ferrous iron so that it can be transported across the membrane into the cytoplasm (Jeong and Guerinot, 2009; Zhao and others, 2023). Regardless of the exact mechanism leading to yellowing of leaves during chlorosis, the underlying problem is that iron is unable to reach the destination in the chloroplast where it is required for chlorophyll production and photosynthesis.

Parallels between a functional iron deficiency in Alzheimer's disease and chlorosis in azaleas.

Chlorosis in plants and a functional iron deficiency in Alzheimer's disease have multiple similarities. For instance, iron is present in sufficient quantities but is unavailable for use. In chlorosis, iron can be trapped in the soil as ferric hydroxide, which is insoluble, or possibly bound to the cell wall and/or at veins, while in Alzheimer's disease iron can be bound to amyloid β and tau or trapped due to impaired autophagic processes. The failure to achieve an optimal acidic pH has similar effects in both conditions. Without a proper acidic pH, reductases are unable to convert ferric iron into ferrous iron, which is a prerequisite for subsequent transport of iron across a membrane. In the plant, this can occur at the roots, e.g., in response to elevated levels of calcium carbonate in calcareous soils, and possibly in the leaves. In Alzheimer's disease, this can occur at the lysosome where mutated presenilin 1 or the carboxy terminal fragment of amyloid precursor protein disrupt vATPase activity preventing acidification of the lysosome.

Similar critical mechanisms are disrupted during iron deficiency in both plants and Alzheimer's disease. For instance, iron is utilized for electron transport chains to generate ATP. In chloroplasts, the electron transport chain is used for photophosphorylation while in mitochondria it is used for oxidative phosphorylation. Disruption to these processes not only impair ATP production but can lead to prooxidant conditions which can cause further damage including injury to both the chloroplast and mitochondria (Atamna and others, 2002A; Walter and others, 2002; Kobayashi and Nishizawa, 2012; Li and others, 2021).

Conclusions

In the initial presentation of the hypothesis describing a functional deficiency of iron in Alzheimer's disease, the sequestration of iron was largely attributed to it binding amyloid β and tau (LeVine and others, 2023). In addition to these mechanisms, the availability of iron can also be restricted due to impaired recycling of iron, e.g., as a consequence of disruptions to ferritinophagy and mitophagy. Furthermore, an elevated lysosomal pH during

disease can disrupt the translocation of iron into the cytosol after uptake via the transferrin receptor or following recycling, and a functional iron deficiency can develop if biosynthetic or metabolic pathways, e.g., for heme, are disrupted. Thus, iron can become unavailable for utilization in biochemical reactions by multiple mechanisms (Table 1). Besides iron, similar processes could cause other essential metals, e.g., copper and zinc, to be unavailable leading to their functional deficiency.

Neurons and other brain cells would likely respond to unavailable iron by trying to take up more iron and/or limiting its exodus from the cell. Over time, the net result would be an increase in iron levels in affected brain regions, which occurs in Alzheimer's disease; and as total iron levels increase, the portion that is attributable to unavailable iron would account for an increasing percentage (Fig. 5).

It will be difficult to capture the complete dynamics of this system by experimental measurements. For instance, changes in response to a functional iron deficient state could occur at different points in time among various neurons, or some neurons might be more resistant than others to less available iron. Additionally, there could be effects of iron deficiency occurring concurrently with pro-oxidative effects by iron. Other histopathological changes (e.g., amyloid plaques, microgliosis) in the CNS could also influence the availability of iron, and non-neuronal cells could be impacted by a functional iron-deficient state themselves, thereby being less able to support neuronal function. Thus, carefully designed experiments that consider an extensive number of variables will be needed to accurately assess iron's altered homeostasis during ongoing pathology.

Iron deficiency in other organisms can provide insights into possible pathological mechanisms occurring in Alzheimer's disease. Indeed, there are overlapping mechanisms between a functional iron deficiency in Alzheimer's disease and chlorosis in azaleas. Thus, the pathogenic processes in Alzheimer's disease may not be unique among those found in nature. A tenet is the unavailability of iron despite its presence at sufficient or elevated levels. Given these similarities, the hypothesis accounting for a functional iron deficiency has been named The Azalea Hypothesis of Alzheimer's Disease.

The above hypothesis describes a potentially important pathogenic process in Alzheimer's disease, but a relevant question is, what starts the abnormal decline in available iron? I describe a model where conditions that allow HIF-1α to be active (such as low levels of oxygen, 2-oxoglutarate, and/or iron) result in the amyloidogenic processing of amyloid precursor protein generating increased production of amyloid β, which can trap iron making it less available (Fig. 6). A feature of this model is that HIF-1α is one of the various events that can modulate γ secretase activity (Gertsik and others, 2015). Thus, active HIF results in activation of γ secretase, and subsequently, the production of amyloid β (Gertsik and others, 2015; Alexander and others, 2022). In addition, HIF-1α also increases the transcription and activity of β secretase (Alexander and others, 2022). Thus, the lower availability of iron, 2-oxoglutarate, or oxygen can trigger events that lead to a pathogenic loop where iron becomes progressively less available (Fig. 6). The use of iron chelators, which is a means to deplete iron, has been shown to increase the activity of HIF-1 (Soucek and others, 2003), which has been linked with pro-death or anti-death mechanisms (Piret and others,

2002; Karuppagounder and others, 2015). As pathology develops, more mechanisms can contribute to a functional iron deficient state (Table 1). Failure to adequately supply iron for biochemical reactions can result in decreased neuronal performance and eventually neuronal death (Table 3).

If a functional iron deficiency is a relevant pathogenic mechanism, then the natural question is how could it be alleviated? Iron chelators have been tested in both preclinical settings and in clinical trials for neurodegenerative diseases, but the rationale for their use was usually to prevent the toxic effects of iron due to its excessive accumulation thought to cause oxidative damage and/or ferroptosis, not to alleviate a functional iron deficiency. A close examination of iron chelators is warranted, because under the right conditions their characteristic feature of interacting with iron may be employed to help alleviate a functional iron deficiency.

First, if the drug is to act directly within the CNS, then it is necessary that the iron chelator be able to cross the blood-brain barrier, with the possible exception of a disease which has extensive disruption to the blood-brain barrier, such as multiple sclerosis. Furthermore, the chelator would need to be able to access the relevant cells and subcellular compartments (e.g., mitochondrial matrix, inner and outer mitochondrial membrane, etc.) where iron sequestration can occur, e.g., due to the presence of amyloid β (Pagani and Eckert, 2011). Deferiprone (molecular weight 139.15) is a chelator that can cross the blood-brain barrier, while other typically utilized chelators, i.e., deferoxamine and deferasirox, are less prone to do so (Weigel and others, 2014).

Another important consideration is the dosage. For Friedreich's ataxia, a low dose of deferiprone (20 mg/kg/day) may have provided some benefit, but this is not established, while higher doses (40 or 60 mg/kg/day) worsened disease activity, e.g., Friedreich Ataxia Rating Scale or ataxia (Pandolfo and others, 2014; Elincx-Benizri and others, 2016). For Parkinson's disease, a dose of 30 mg/kg/day (i.e., 15 mg/kg/day twice daily) worsened disease activity (Devos and others, 2022). If a functional iron deficiency is present in Friedreich's ataxia and Parkinson's disease, then deferiprone, especially at higher doses, would have exacerbated this deficiency resulting in a worsening of disease. However, a low dosage may function differently than a high dose. When chelating iron, deferiprone forms a complex with iron in a 3:1 ratio. Lower doses would therefore provide less capacity to fully chelate iron, but it may still compete with other molecules, e.g., amyloid β and tau, that sequester iron.

Besides competing with proteins that sequester iron, methods that reduce these proteins, such as amyloid β, would be another potential approach to alleviate a functional iron deficiency. The goal would be to make iron more readily available for use without increasing the toxic burden, i.e., oxidative damage. Interestingly, deferiprone may help redistribute iron within cells (Sohn and others, 2008; Kakhlon and others, 2010) but it is unknown if this will make iron more available for use in the context of Alzheimer's disease. This is a tall order since increased availability and usage of iron would likely need to apply to multiple classes of cells and various sites within cells including organelles (e.g., mitochondria) and subcellular compartments (e.g., mitochondrial matrix).

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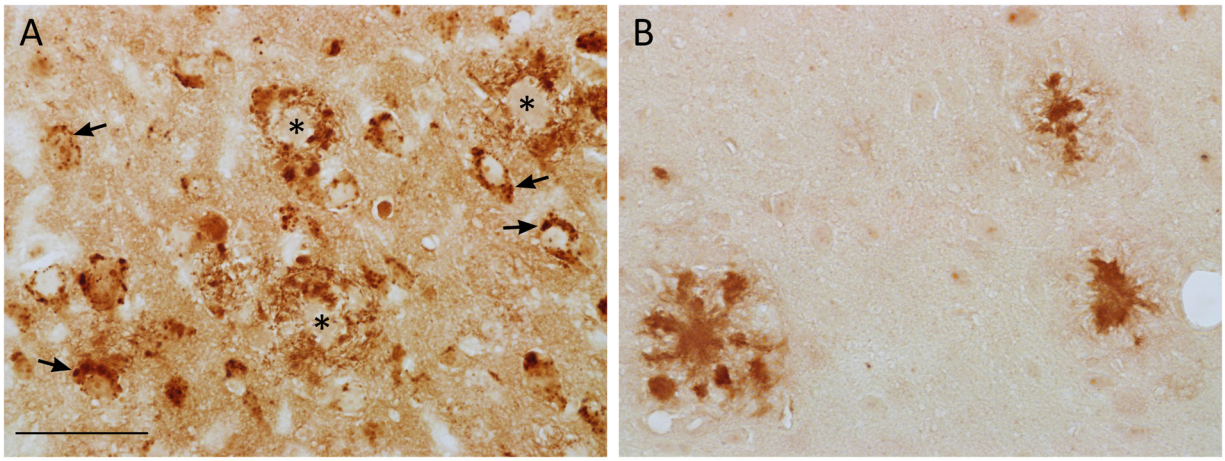

Fig. 1.

Iron histochemical staining of CNS tissue from APP/PS1 mice (Sands and others, 2016), an animal model of Alzheimer's disease. **A)** Extensive labeling of cytoplasmic structures (arrows) and plaque rims, but plaque cores are unstained (*). Some nucleoli are stained while nuclei in this field of view are generally unstained or lightly stained (see cell at bottom left arrow). Background staining is also apparent. Sections were briefly deparaffinized, air dried and stained following a variation of the procedures described in Sands and others (2016). **B)** Partial tissue digestion with proteinase K reveals extensive labeling of iron in plaques cores similar to early observations in human Alzheimer's disease CNS tissue (LeVine, 1997). Sections were deparaffinized, rehydrated and treated with proteinase K following a variation of the procedure described in Sands and others (2016). Bar = ~50 μm

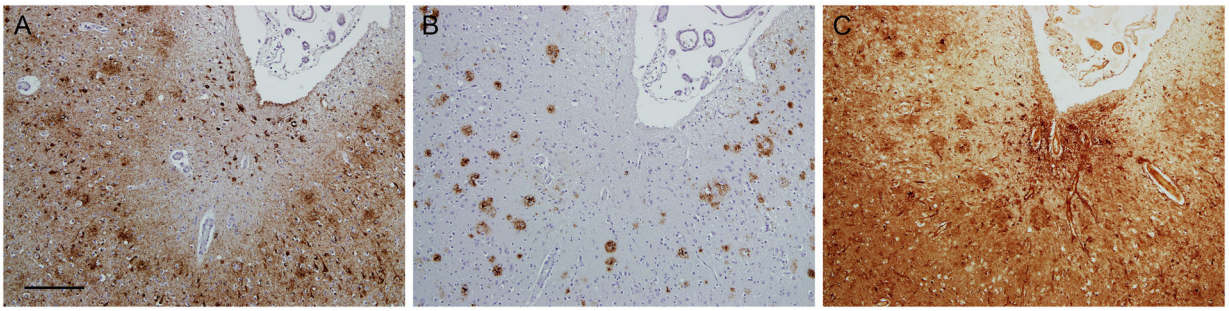

Fig. 2.

Colocalization of iron with neuropathological features of Alzheimer's disease. Paraffin sections of the entorhinal cortex of a patient with Alzheimer's disease stained by **A)** immunohistochemistry against phosphorylated tau reveals labeling of tangles and neuritic plaques, **B)** immunohistochemistry against amyloid β depicting amyloid plaques, and **C)** iron histochemical staining of plaques, neurons (some which may include tangles), glia, and vessels using a variation of the procedures described in Sands and others (2016). Note, the densely stained cells near the bottom left of the sulcus which correlate with the location of some dense staining of phosphorylated tau seen in panel A. The stained slides used for panels A and B, and paraffin section used for panel C, were provided by the Neuropathology Core of the University of Kansas Alzheimer's Disease Research Center. The region shown in panel C is from the same cortical sulcus as shown in panels A and B, but the section was collected further in the paraffin block. Bar = ~200 μm

Fig. 3.

Chlorosis in azaleas. These azaleas were adjacent to the foundation (basement wall) of a house, a gutter downspout, and a stone and mortar splash block. **A, B)** Azaleas displaying signs of chlorosis; note leaves with interveinal yellowing that were amongst leaves with a more uniform dark green appearance (pictures taken on 12/11/2021, ~12:37 p.m.). Based on memory, azaleas displaying chlorotic features had received a couple of treatments of Ferti-lome Chelated Liquid Iron (3.25%) that also contained lower amounts of manganese (0.15%), zinc (0.16%), and copper (0.05%); then later (after the picture date) received treatments believed to be of Ferti-lome Azalea, Camellia Rhododendron food (Voluntary Purchasing Groups, Inc., Bonham, TX). At some point following these treatments, it is likely that the plants were exposed to some Hi Yield Muriate of Potash (Voluntary Purchasing Groups, Inc., Bonham, TX). In hindsight, this latter treatment was not suitable because it could potentially elevate the pH of the soil, which would worsen the conditions leading to chlorosis. **C)** The following spring the azaleas displayed brilliant fuchsia or red flowers as usual, however signs of chlorosis are still present (note yellow area at upper left of the fuchsia azalea) (picture taken on 05/07/2022, 10:16 a.m.). **D)** Treatments were not maintained and, in the spring of 2023, yellowing and very few flowers are present in the azalea that normally displayed fuchsia flowers while the azalea that had displayed red flowers had substantially died back (picture taken on 04/29/2023, 11:29 a.m.). **E)** One of only a few flowers, which was among leaves with signs of chlorosis, i.e., interveinal light green or yellowing (picture taken on 05/05/2023 at 7:33 p.m.). Note, the absence of dark green leaves observed together with chlorotic leaves in B. **F)** Light green chlorotic leaves and yellow chlorotic leaves from the fuchsia azalea (picture taken on 05/05/2023 at 7:32

p.m.). **G)** A greater magnification view of chlorotic leaves from the fuchsia azalea (picture taken on 05/011/2023 at 2:20 p.m.). **H)** Chlorotic leaves from the few remining live branches from the azalea that normally has red flowers are seen poking through the green blades from an iris (blade width below lower group of azalea leaves is ~4.4 cm) (picture taken on 05/011/2023 at 2:22 p.m.). Note, the yellow chlorotic leaves near the top compared to the light green chlorotic leaves located lower in the picture. The neighboring irises are apparently tolerant of the environmental conditions and do not display signs of chlorosis (i.e., yellowing and brown) at this point in time suggesting that iron levels in the soil are sufficient, and that the chlorosis in the azaleas is due to a pH higher and outside of their optimal range. However, approximately 5 weeks later, it was noted that a small percentage of iris leaves did display yellow and brown on their leaves particularly those closer to the azaleas.

Fig. 4.

Normal appearing, non-chlorotic, leaves and flowers from a healthy azalea. This azalea is adjacent to a foundation (basement wall), but on a different side of the same house as for the azaleas depicted in Fig. 3, and not adjacent to a downspout and stone and mortar splash block. In contrast to the azaleas with chlorosis depicted in Fig. 3, note **A)** the plentiful flowers (picture taken on 04/16/2023 at 2:57 p.m.), and the absence of interveinal pattern in **B)** new leaf growth (light green leaves, center) (picture taken on 04/25/2023 at 11:35 a.m.) as well as in **C)** young leaves 16 days later (medium dark green leaves in contrast to dark green leaves) (picture taken on 05/11/2023 at 2:24 p.m.). The healthy profile from this azalea points to the downspout (perhaps delivering roof runoff) and/or stone and mortar splash block promoting the development (at least initially) of chlorosis for the azaleas in Fig. 3, although other factors (e.g., plant age, soil drainage) could have also contributed.

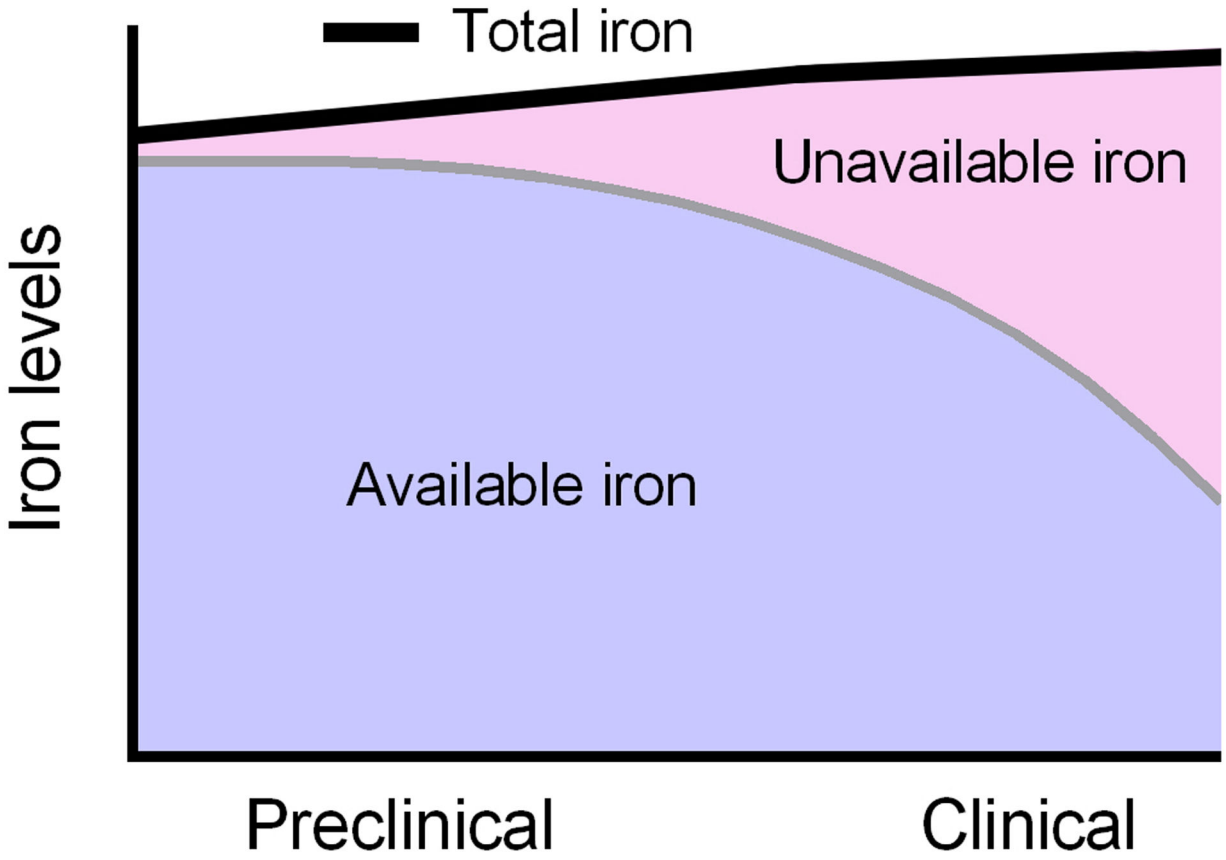

Fig. 5.

Available vs unavailable iron within the CNS over the course of Alzheimer's disease. During ongoing pathogenic events in Alzheimer's disease, multiple mechanisms contribute to iron becoming unavailable (see Table 1), which in turn lowers the level of available iron. The amount of unavailable iron becomes progressively more (worse) as the disease progresses (shift to the right on the x axis). Cells respond to less available iron by taking up additional iron resulting in the total level of iron (thick black line) increasing during disease, but as cell die off the rate of increase in total iron is not as steep. Similar mechanisms may occur during normal aging, but to a lesser degree.

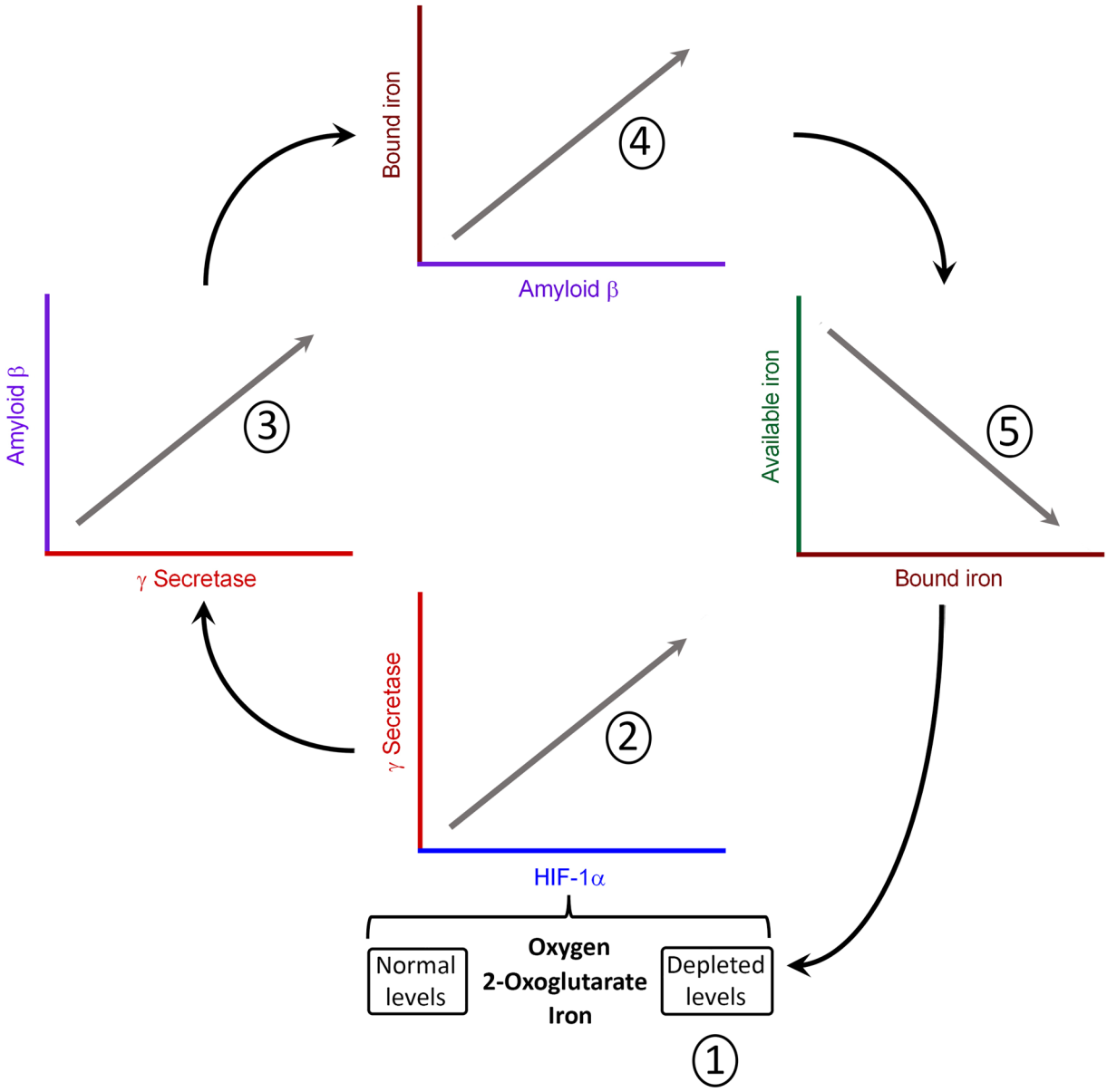

Fig. 6.

A model for aiding the initiation and progression of pathology. **1)** When oxygen, 2-oxoglutarate, and/or iron levels decline, the activity of prolyl 4-hydroxylases (not shown) are reduced allowing HIF-1α to become active (shift right on the x-axis). **2)** Increased activity of HIF-1α increases the activation of γ secretase (and β secretase, not shown). **3)** Increased activity of γ secretase results in increased production of amyloid β. **4)** Increased levels of amyloid β lead to increased amounts of iron being bound. **5)** Increased levels of bound iron lead to reduced levels of available iron. Reduced levels of available iron result in increased activity of HIF-1α (circling back to 1), which perpetuates the cycle. Note, besides binding to amyloid β, iron can become unavailable via multiple mechanisms (see Table 1) which can amplify the progression to a functional iron deficient state.

Table 1:

Processes potentially contributing to a functional deficiency of iron

Affected protein, peptide or process	Change	Effect	Reference
Amyloid β	Accumulates	Binds and sequesters iron and heme	Atamna and Frey, 2004; Atamna, 2006; Boopathi and Kolandaivel, 2016
Amyloid plaques	Accumulate	Binds and sequesters iron	LeVine, 1997; Langkammer and others, 2014; Hare and others, 2016; van Duijn and others, 2013; 2017; Du and others, 2018; Yang and others, 2022
Ferritinophagy	Disrupted	Prevents recycling of iron	Masaldan and others, 2018; Yambire and others, 2019
Ferrochelatase	Decreased	Reduced production of heme	Bai and others, 2021; Barman-Aksözen and others, 2013
Ferroportin	Decreased	Iron accumulation	Tsatsanis and others, 2020; Hin and others, 2021; Jakaria and others, 2021
Heme	Decreased usable level due to decreased production and/or metabolism	Reduced activity of heme containing proteins such as complex IV	Atamna and others, 2002B; Atamna and Frey, 2004; Dwyer and others, 2006; Vidal and others, 2019
Mitophagy	Disrupted	Prevents recycling of iron	Kerr and others, 2017; Yambire and others, 2019
Pitrm1	Decreased activity	Lead to amyloid β and tau accumulation, which can sequester iron	Alikhani and others, 2011; Pinho and others, 2014; Brunetti and others, 2021
Tau	Accumulates	Binds and sequesters iron	Rao and Adlard, 2018; Madsen and others, 2020
Tangles	Accumulate	Binds and sequesters iron	Smith and others, 1997
vATPase	Blockage	Less acidic lysosomal pH impairs transport of iron from endosome to the cytosol	Yambire and others, 2019; Weber and others, 2020

Table 2:

Associations with iron for the top 21 results out of 14,351 entries using the search term Alzheimer (with no quotation marks) for the GeneCards Database [*]

Rank	Gene	Name	Relevance Score [**]	Interactions with Iron and Iron-Related Processes
1	APP	Amyloid Beta Precursor Protein	141.18	Interacts with ferroportin to mediate iron export; mRNA has an IRE (Hin and others, 2021); translational regulation shares features with the iron storage protein ferritin (Rogers and others, 2008; 2019); amyloid β binds iron (LeVine and others, 2023); carboxy fragment can increase lysosomal pH (Jiang and others, 2019; Delport and Hewer, 2022), which could reduce the transport of iron into the cytosol
2	PSEN1	Presenilin 1	131.83	PSEN1 mutations may lead to less acidic lysosomes affecting iron uptake leading to a deficiency of iron (Lee and others, 2010; 2015; Lumsden and others, 2018; Yambire and others, 2019)
3	APOE	Apolipoprotein E	100.97	Iron increases intracellular levels of ApoE in neurons and astrocytes and may reduce its secretion (Xu and others, 2016); CSF ApoE levels are correlated with CSF ferritin levels, which are more pronounced in individuals with ApoE ε4 (Ayton and others, 2015); ApoE ε4 may interact with iron to influence default mode network activity prior to cognitive decline (Kagerer and others, 2020); Apo ε4 may elevated lysosomal pH in astrocytes (Prasad and Rao, 2018) which could reduce the transport of iron into the cytosol
4	PSEN2	Presenilin 2	75.79	Mutations may disrupt autophagy (Pizzo and others, 2020), which could decrease the recycling of iron
5	MAPT	Microtubule Associated Protein Tau	68.13	Iron facilitates aggregation of tau; iron increases activity of kinases that facilitate hyperphosphorylation of tau; iron accumulates in neurons with tangles (Smith and others, 1997; Rao and Adlard, 2018)
6	BACE1[b]	Beta-Secretase 1	50.59	Interactions with APP leading to amyloid beta 1–42 formation might be affected by iron concentrations (Gong and others, 2019)
7	HFE	Homeostatic Iron Regulator	44.97	Controls brain iron levels (Kim and Connor, 2020); regulates iron levels and can compete with transferrin to bind transferrin receptor (Giannetti and Bjorkman, 2004)
8	ABCA7[b]	ATP Binding Cassette Subfamily A Member 7	44.87	N.A.
9	SORL1[b]	Sortilin Related Receptor 1	43.58	May have a role in iron homeostasis (Barthelson and others, 2020)
10	TNF	Tumor Necrosis Factor	42.03	TNF increases iron concentration within endothelial cells (Nanami and others, 2005); iron that accumulates in macrophages induces expression of TNF (Kroner and others, 2014)
11	MPO[b]	Myeloperoxidase	41.44	A heme containing enzyme (Nauseef, 2018); activity is decreased during anemia (Turgeon-O'Brien and others, 1985); has a role in iron-induced damage (Xiao and others, 2018).
12	NOS3	Nitric Oxide Synthase 3	40.52	Heme is associated with the oxygenase domain (Oliveira-Paula and others, 2016)
13	GRN[b]	Granulin Precursor	38.47	N.A.
14	MT-ND1[b]	Mitochondrially Encoded NADH:Ubiquinone Oxidoreductase Core Subunit 1	38.17	Encodes for one of many subunits of Complex 1, which has 8 iron-sulfur clusters (Andrews and others, 2013)
15	PLAU[b]	Plasminogen Activator, Urokinase	36.18	N.A.

Rank	Gene	Name	Relevance Score**	Interactions with Iron and Iron-Related Processes
16	VCP	Valosin Containing Protein	34.66	N.A.
17	TF[b]	Transferrin	34.34	Delivers iron to the blood-brain barrier and cells of the brain via the transferrin receptor (Qian and Ke, 2019).
18	HFE-AS1[b]	HFE Antisense RNA 1	33.35	May affect HFE levels (see above)
19	CSF1R[a,b]	Colony Stimulating Factor 1 Receptor	33.27	N.A.
20	LOC106694315[b]	MPO Proximal Enhancer and Promoter Region	32.10	See MPO above
21	AD5[a,b]	Alzheimer Disease 5 (Genetic Locus)	31.50	N.A.

* https://www.genecards.org/Search/Keyword?queryString=Alzheimer accessed 4-5-2023

** The search platform was Elasticsearch 7.11

N.A. – not applicable. IRE – iron response element. IRP – iron response protein.

[a] Not contained within the top 21 results out of 14,351 entries using the search term: Alzheimer's (with no quotation marks). Additional top 21 results with this search term were: LOC106694316 (Enhancer Region in Introns 7–9 of MPO); MIR146A (MicroRNA 146a)

[b] Not contained within the top 21 results out of 14,351 entries using the search term: Alzheimer's disease (with no quotation marks). Additional top 21 results with this search term were: PKHD1 (PKHD1 Ciliary IPT Domain Containing Fibrocystin/Polyductin); NPC1 (NPC Intracellular Cholesterol Transporter 1); PKD1 (Polycystin 1, transient Receptor Potential Channel Interacting); AGL (Amylo-Alpha-1, 6-Glucosidase, 4-Alpha-Gucanotransferase); GBA1 (Glucosylceramidase Beta 1); LMNA (Lamin A/C); NF1 (Neurofibromin 1); GAA (Alpha Glucosidase); SNCA (Synuclein Alpha); MFN2 (Mitofusin 2); LRRK2 (Leucine Rich Repeat Kinase 2); VWF (Von Willebrand Factor)

Table 3.

Potential consequences of a functional iron deficiency in the CNS*

Affected process	Change	Effect	Reference
Alternative splicing	Altered	Decreased transcript and protein for ferrochelatase, which catalyzed the final step of heme synthesis	Barman-Aksözen and others, 2013
Dendrites	Diminished branching and growth	Tied together with impaired mitochondrial function	Bastian and others, 2019
Epigenetic changes	Altered	Lower transcription of genes for mitochondrial proteins	Rensvold and others, 2016; Liu and others, 2022
Heme biosynthesis	Decreased	Impair activity of heme containing proteins and diminish mitochondrial function	Atamna and others, 2002B; Atamna, 2004; 2006; Barman-Aksözen and others, 2013
Mitochondrial activity	Impaired	Decreased complex IV activity; decreased ATP synthesis; increased production of reactive oxygen species	Atamna and others, 2002A; 2002B; Walter and others, 2002; LeVine and others, 2023
NMDA receptor activity	Decreased	Decreased calcium signaling and ERK1/2 activation; inhibited basal synaptic activity	Muñoz and others, 2011
Synaptic mitochondrial activity	Diminished	Decrease of oxygen consumption (mitochondrial activity) for both basal and elevated levels of synaptic activity	Tena-Morraja and others, 2023

* Higher level consequences of a functional iron deficiency in the CNS include alterations in memory, cognition, and neuronal performance and even neuronal death (see section on Consequences…).