Published in final edited form as:

Neurobiol Dis. 2015 September ; 81: 49–65. doi:10.1016/j.nbd.2015.08.007.

The Relationship between Iron Dyshomeostasis and Amyloidogenesis in Alzheimer's Disease: Two Sides of the Same Coin

Douglas G. Peters, M.S.^{1,2}, James R. Connor, Ph.D.¹, and Mark D. Meadowcroft, Ph.D.^{1,3}

¹Department of Neurosurgery, The Pennsylvania State University – College of Medicine, Milton S. Hershey Medical Center, Hershey, Pennsylvania

²Department of Neural and Behavioral Sciences, The Pennsylvania State University – College of Medicine, Milton S. Hershey Medical Center, Hershey, Pennsylvania

³Department of Radiology (The Center for NMR Research), The Pennsylvania State University – College of Medicine, Milton S. Hershey Medical Center, Hershey, Pennsylvania

Abstract

The dysregulation of iron metabolism in Alzheimer's disease is not accounted for in the current framework of the amyloid cascade hypothesis. Accumulating evidence suggests that impaired iron homeostasis is an early event in Alzheimer's disease progression. Iron dyshomeostasis leads to a loss of function in several enzymes requiring iron as a cofactor, the formation of toxic oxidative species, and the elevated production of beta-amyloid proteins. Several common genetic polymorphisms that cause increased iron levels and dyshomeostasis have been associated with Alzheimer's disease but the pathoetiology is not well understood. A full picture is necessary to explain how heterogeneous circumstances lead to iron loading and amyloid deposition. There is evidence to support a causative interplay between the concerted loss of iron homeostasis and amyloid plaque formation. We hypothesize that iron misregulation and beta-amyloid plaque pathology are synergistic in the process of neurodegeneration and ultimately cause a downward cascade of events that spiral into the manifestation of Alzheimer's disease. In this review, we amalgamate recent findings of brain iron metabolism in healthy versus Alzheimer's disease brains and consider unique mechanisms of iron transport in different brain cells as well as how disturbances in iron regulation lead to disease etiology and propagate Alzheimer's pathology.

Graphical Abstract

Corresponding Author: Mark D. Meadowcroft, Ph.D., The Pennsylvania State University – College of Medicine, Milton S. Hershey Medical Center, Department of Neurosurgery – H110, The Center for NMR Research, 500 University Drive, Hershey, PA 17033, 717-531-6069, markmeadowcroft@psu.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Keywords

Alzheimer's disease; AD; iron; metallosis; dyshomeostasis; beta-amyloid; amyloidogenesis; pathology

Introduction

Alzheimer's disease (AD) is the most common cause of dementia affecting six million people in the United States and forty million people worldwide. The literature base supports that there are two distinct clinical manifestations of Alzheimer's disease; familial and sporadic. Both are characterized by molecular lesions attributed to the aggregation of misfolded proteins, inflammation and metabolic failure leading to neurological dysfunction. Familial Alzheimer's disease (FAD), also characterized as Early Onset Alzheimer's disease (EOAD), has been linked to genetic mutations, affects people under 60 years old, and makes up less than 5% of all Alzheimer's cases. Sporadic Alzheimer's, also referred to as Late Onset (LOAD), is the most common form affecting people over 60 and has no direct pattern of inheritance. Although AD is not a certain outcome of the aging process, age is the primary risk factor as the incidence for Late Onset AD doubles every five years after 65.^{1,2}

The prevalence of AD is expected to triple by 2050 as the average lifespan increases in the future³.

The recognition that iron dyshomeostasis is critical in Alzheimer's disease pathology is based on observations that patients have elevated iron levels in cortical, subcortical, and white matter areas affected by the disease^{4,5}. MRI analysis reveals that increased iron levels in the hippocampus, an important structure perturbed early in Alzheimer's disease, negatively correlates with memory test performance⁶. Increased iron loading in the brain is also associated with beta-amyloid (A β) plaque formation, where it is focally incorporated into the core and halo regions, and hyperphosphorylated tau (pTau) tangles in the brain⁷⁻⁹. Increasing the concentration of iron *in vitro* accelerates A β plaque and pTau tangle aggregation and increases their toxicity¹⁰. Iron dyshomeostasis may lead to toxic pathological features, but the same imbalance can disrupt innate biological systems that depend on iron.

Iron is one of the most abundant elements on Earth and was utilized by early organisms before our current oxygen rich atmosphere was established¹¹. As a transition metal, iron is uniquely involved in reductive and oxidative (redox) cycling reactions and as a cofactor in iron-sulfur clusters within numerous enzymes¹². The majority of the body's iron (70%) is bound to hemoglobin within red blood cells to aid in tissue oxygen transport. The balance of non-hemoglobin bound iron is found within proteins (~6%) facilitating the metabolic energy needs of the body through cellular respiration (ATP synthesis through the TCA cycle, ferredoxin, cytochromes, and aconitase) and those involved in ribosome function, DNA repair, and synthesis¹³⁻¹⁵. The remainder is stored within globular ferritin protein complexes (~24%) for controlled iron sequestration, detoxification, and release¹⁶. Proper iron maintenance is critical for the body and in the brain; thus, there are specialized cells, regions, and organs for storing and releasing iron.

The brain has a large amount of iron, unevenly distributed to the neurons of the basal ganglia, brain capillary endothelial cells (BCECs), and glia. The basal ganglia requires iron for neurotransmitter synthesis, BCECs confer an iron shuttle between the blood and brain, and astrocytes and microglia help to distribute and sequester iron in the perenchyma¹⁷⁻¹⁹. The relative distribution of non-heme iron in the vertebrate body is 55% liver, 20% kidney, 15% heart, and 10% brain²⁰. During vertebrate brain development the brain is highly permeable to iron to facilitate neural growth and intercellular connection²¹. After development, the brain tightly controls circulating non-heme iron entering and exiting the brain. The brain normally acquires approximately 10% of its iron from the diet where it crosses the gut and enters the blood stream, relatively less iron than any other organ: three-fold less than the liver²⁰. Thus, the brain needs more reserve non-heme iron than any other organ to carry out its function. The reason behind this is not well understood, but there are several possibilities. Firstly; the brain is one of the most metabolically active organs in the body, consuming a significant amount of the body's oxygen²². This requires the brain to have an iron reserve to assure that its energy requirements are met during a potential lull in iron status. Secondly; iron influx into and efflux out of the brain is tightly controlled by the blood brain barrier (BBB), brain cerebrospinal fluid interface (BCSFI), and the blood CSF barrier (BCSFB)^{18,23,24}. This regulation resides outside the control of the brain making it

difficult for the brain to finely adjust the influx and efflux of iron. Thirdly; while neuronal growth and division in the brain during adulthood is minimal, limiting the necessity for new exogenous iron to create new synaptic connections, iron rich oligodendrocytes continually require large amounts of the element²⁵.

Brain iron is most prevalent in oligodendrocytes where it is required in the myelination of neuronal axons to form the white matter in the brain facilitating saltatory conduction over longer distances with increased speed²⁶. Humans have proportionally more white to gray matter than any other animal, and we are the only species to have heterochronologic development with brain regions myelinating at different time points^{27,28}. This difference may help to explain our advanced cognitive processes and IQ as well as human brain atrophy later in life^{29,30}. Iron deficiency during infancy causes delayed neurocognitive development, impaired learning and memory, and in some cases, psychiatric disorders; all in part due to the impairment of myelination³¹. Oligodendrocyte precursor cells (OPCs) propagate throughout life to form new oligodendrocytes to myelinate additional structures and to replenish damaged myelin during remyelination. As the brain ages, remyelination is outpaced by myelin breakdown leading to a loss of white matter volume²⁸. The rate and loss of white matter due to normal aging increases free iron, which is toxic to the brain. *In vivo* magnetic resonance imaging (MRI) analysis illustrates that white matter damage precedes gray matter damage in AD compared to age-matched controls³². Exacerbated white matter loss late in life can contribute to increased neuronal loss associated with AD. This damage can be due to increased toxic radical production and impaired molecular systems necessary to ameliorate them.

Alterations in iron load and the proteins responsible for iron metabolism can exacerbate the excess formation and harmful effects of reactive oxygen (ROS) and nitrogen species (RNS), leading to cell death³³. The increased expression of amyloid precursor protein (APP) at the site of axonal damage may underlie the need for amyloid proteins to repair tissue and manage cellular debris³⁴. The response of amyloid and tau to free radicals and hypoxia induced ischemia, as is witnessed in brain trauma, is hypothesized to ameliorate their effects. However, over time APP and cleaved amyloid fragments harbor ferric iron (Fe^{3+}) that cannot be stored or transported adequately^{35,36}. A β bound Fe^{3+} is easily reduced to ferrous iron (Fe^{2+}), escalating the production of ROS species³⁷. This milieu favors the increased pathogenic production of toxic A β oligomers and plaques that propagate from monomeric A β_{42} through β -secretase cleavage^{38,39}. Once formed, tau tangles and aberrant amyloid structures induce synaptic loss and apoptosis independent of ROS^{40,41}.

Dietary ingredients that chelate iron and remove oxidative species are hypothesized to reduce the onset time of Alzheimer's disease^{42–44}. Deferoxamine (DFO), a ferric iron chelator used to treat iron overload, has been shown to decrease tau phosphorylation, slow down amyloidogenesis, and improve behavioral impairment in an Alzheimer's mouse and rabbit models^{45–48}. DFO also improves behavioral performance in AD patients after a 24 month administration period⁴⁹. However, the longitudinal follow-up of these studies have not been adequately performed. Huperzine A (HupA), a natural inhibitor of acetylcholinesterase, is a licensed anti-AD drug in China which may possess other neuro-protective properties that are not well understood. Recent work shows that treatment with

HupA reduces iron in the brain that correlates with a reduction in insoluble and soluble A β , amyloid plaque formation, and hyperphosphorylated tau in the cortex and hippocampus of an AD mouse model. Increasing dietary iron negates any benefit from HupA, suggesting that elevated iron is involved in AD pathoetiology⁵⁰.

The relationship between iron, amyloidogenesis, and AD is multi-faceted. There are four essential questions that are important to understand how iron is associated with Alzheimer's disease. 1) Is iron dyshomeostasis a primary or a secondary factor of Alzheimer's disease pathoetiology? 2) Is the brain iron imbalance in AD caused by a dysfunctional or an overwhelmed iron regulatory system? 3) Is brain iron dyshomeostasis derived from peripheral iron or is it coming from endogenous brain iron? 4) How are iron, amyloidosis, and cognition related; is the etiology of AD one of A β plaque formation, iron dyshomeostasis, or a synergy of both processes?

The Amyloid Cascade Hypothesis

Over the last twenty years the amyloid cascade hypothesis has helped scientists unearth a slew of critical genomic and proteomic data to better characterize amyloid plaque formation as a pathological marker of AD and the mechanism behind amyloidosis. Parallel to this, researchers have accumulated data on iron level regulation in the peripheral and central nervous systems. The developing metallostasis field has emphasized the role of improper metal ion redox cycling during aging and how the improper balance of metals in the brain may lead to amyloid deposition, cell dysfunction, and apoptosis found in AD⁵¹. Iron is not the only transition metal that undergoes dyshomeostasis in the aging and diseased brain state. Copper (Cu) and zinc (Zn) play an important physiological function in the brain, however their concentrations are much lower than that of iron⁵². The role of APP and pathogenic A β derivatives as metalloproteins highlight their complex relationship with iron loading and metabolism in AD^{53,54}. Current findings indicate that metallostasis should be included within the framework of the amyloid cascade hypothesis.

Although the association of APP to Alzheimer's disease has been thoroughly studied there remain a number of uncertainties such as its relationship with iron transportation. APP is processed by several enzymes producing smaller fragments that participate in normal cellular function in an unknown fashion. APP is cleaved by α -secretase (ADAM) or β -secretase (BACE-1) producing the extracellular soluble fragments sAPP α or sAPP β , respectively. The alpha pathway is a non-amyloidogenic process while the beta pathway is amyloidogenic. The membrane bound C-terminal fragments, C83 or C99 produced during α and β cleavage, are cleaved by γ -secretase to generate extracellular fragment p3 or A β _{40/42}, respectively. The remaining C-terminal fragment, amyloid intracellular domain (AICD), is released into the cytoplasm after progressive cleavage by the γ -secretase/presenilin (PSEN) complex, where it is targeted to the nucleus to act as a transcription factor. Increased production of A β ₄₂ leads to the formation of insoluble extracellular amyloid fibrils and amyloid plaques. APP and secretase mutations were initially discovered to be related to Familial Alzheimer's disease because they lead to increased production and aggregation of A β ₄₂. Transgenic mice containing these same mutations express similar hallmark pathological features and memory deficits. The transgenic pathology, however, does not

completely mimic that found in human disease; potentially due to lower brain iron or APP translational regulation^{55,56}. Transgenic plaques formed in mice do not contain the same amount of focal iron and do not bind Pittsburgh Compound B (PIB), a marker for fibrous insoluble A β plaques^{7,57}. Profound A β plaque and pTau tangle formations in non-human vertebrates do not lead to the memory deficit observed in AD pathology. This may be due to reduced plaque toxicity in non-human A β plaques which do not bind iron⁵⁸.

Fe²⁺ and Fe³⁺ interactions with APP and A β facilitates the extent and speed of amyloid beta aggregation into fibrillar forms^{10,59}. Seeded plaques can sequester other metals (Zn, Cu, Al, Mn) and a variety of cellular products, such as lipids, impairing proper cellular function^{60,61}. A β ₄₂ is increased with APP expression or BACE-1 activity or decreased ADAM activity. The A β _{42/40} ratio is dependent on γ -secretase function. A β ₄₂ and A β ₄₀ can both interact with iron; however, the A β ₄₂ fragment forms amyloid aggregates more rapidly. The Alzheimer's brain shifts from a non-amyloidogenic ADAM/A β ₄₀ state to an amyloidogenic BACE-1/A β ₄₂ state. Aggregation of A β ₄₂ initially follows a reversible linear progression of monomers, dimers, misfolded globules, soluble oligomers, seed β -sheet intermediates, protofibrils, fibrils, and senile fibril plaques (Fig. 1). Fe³⁺ has the capacity to bind all amyloid formations but has difficulty disassociating from the late fibrillar forms. The seeding of A β ₄₂ with insoluble Fe³⁺ may allow the initial clearance of excess free iron in the brain through plaque formation and microglial lysosomal degradation. However, some of that Fe³⁺ is lost by reduction to Fe²⁺ facilitated by its interaction with A β ₄₂⁶². Fe²⁺ has also been shown to interact with A β amino acids, and this may confer changes to amyloid formations in a different manner than Cu and Zn^{63,64}. When enough amyloid deposition has occurred, toxic oligomeric formations can propagate in a nonlinear amyloidogenic positive feedback-loop, bypassing the requirement for amyloid monomers to form dimers³⁹. It is currently unknown how iron is involved in nonlinear A β plaque formation. The mature amyloid plaque core contains magnetite in the form of Fe²⁺ and Fe³⁺⁶⁵. The hydrophobic nature of the plaque core is believed to occlude electron sharing within the region, while more easily accessible iron in the outer halo could feasibly be a site of increased redox cycling.

Knock-out removal of endogenous APP in mice increases neuronal iron, while overexpression of the A β containing APP c-terminal fragment reduces neuronal iron^{66,67}. In addition, *in vivo* iron overload in the APP/PS1 AD mouse model leads to increased APP processing, altered neuronal signaling, and cognitive decline with increased amyloid plaque formation⁶⁸. This suggests that brain iron load is directly tied to the initial stages of amyloid processing and plaque formation. While there are findings that suggest total brain content is not altered in the AD brain, regional analysis provides evidence that harmful macroscopic focal deposition of iron is occurring in and around amyloid plaques^{67,69,70}. Illustrating this is the knowledge that *in vivo* MRI imaging of the AD brain demonstrates increased iron in the hippocampus; one of the central brain regions afflicted by amyloidogenesis⁴.

Brain Iron Accumulation with Aging

Iron accumulates in the brain over time, reaching an apparent plateau between thirty and forty years of age⁷¹. While the work of Hallgren and Sourander demonstrates the plateauing

of brain iron levels, it is evident that the distribution between individuals becomes more variable after the age of 60⁷¹. A relative decline may reflect a brain that is decreasing its iron absorption or increasing its release. A relative increase illustrates a brain that is either increasing its iron absorption and/or decreasing its iron release. Increasing brain iron may be due to several factors including, but not limited to, the imbalance in iron transport and storage proteins, mitochondrial dysfunction, neurovascular mechanisms, and myelin breakdown and disrepair^{72–74}. Late onset AD presents after age 65 which chronologically coincides with an increase in brain iron in subjects prone to iron overloading⁷⁵ (Fig. 2). While Alzheimer's disease is generally considered an aging disease it is of interest that early onset AD presents itself as early as 40 years of age, coinciding with the plateauing of iron levels in the brain⁷⁶. This trend also is apparent in Down's syndrome where a trisomy of chromosome 21 results in APP overexpression, a six-fold likelihood of developing amyloid plaque pathology, and brain iron deposition at 35 years old⁷⁷.

Oxidative Stress and Toxic Mechanisms

The free radical theory of aging postulates that reactions with metal generated oxygen free radicals result in a pattern of increased cellular aging⁷⁸. Brain aging involves structural, functional, and chemical changes that occur over time within normal health and neurodegenerative disease. Current research suggests that oxidative stress contributes to brain aging by means of altered DNA, enhanced cell death, lipid peroxidation, protein dysfunction, decreased degradation, and altered metabolism^{79,80}.

Iron facilitates the energy needs of the growing and highly active human brain. Most physiological iron is stored and utilized as insoluble Fe^{3+} and iron homeostasis and cytoplasmic transportation are dependent on soluble Fe^{2+} . Unregulated iron homeostasis leads to an increased $\text{Fe}^{2+}/\text{Fe}^{3+}$ ratio which is harmful to neuronal cells as Fe^{2+} drives oxidative stress through Fenton and Haber-Weiss chemistry⁸¹. In addition, the relative decrease of Fe^{3+} is indirectly harmful as it promotes the reduction of superoxide radicals derived from mitochondrial activity^{82,83}. Consequently, excess superoxide may react with nitric oxide (NO) to produce other toxic species.

NO is produced from nitric oxide synthase (NOS) and neuronal damage caused by excessive NO is termed nitrosative stress⁸⁴. NO is a highly diffusible gas produced by neurons, endothelial cells, and glial cells to optimize blood flow by enhancing vasodilation and VEGF production, as well as synaptic excitability^{85–87}. Inhibition of NO increases APP expression, BACE1 activity, and A β production⁸⁸. NO levels are increased in AD brain tissue and are hypothesized to retard amyloidogenic pathway progression. The apparent increase in NO to combat amyloid production is a double-edged sword as increased levels of NO are harmful to neural cells. Increased NO levels react with superoxide and produce peroxynitrate, which impedes tyrosine residue function⁸⁹. In Alzheimer's disease, nitration of tau proteins prevents them from stabilizing the microtubule lattice and nitrated tau has been observed in tau tangles and amyloid plaques^{90,91}. Inflammation and excessive neuronal activation by glutamate can induce NOS, which increases the production of NO⁸⁴. In addition, NO disrupts the iron-sulfur core within aconitase, altering the production of iron regulatory proteins. Therapies that can mitigate oxidative stress and inflammation may help

mediate the progress of AD. It has been shown that vitamin E and Ibuprofen reduce amyloid and improve memory deficits^{92,93}. The effect of these therapies may augment the innate antioxidant and inflammatory mechanisms that are disturbed in AD pathology.

Healthy neuronal cells have ROS and RNS detection and degradation pathways that buffer toxic molecular species. Nuclear factor E2-related factor 2 (Nrf2) affects the homeostasis of ROS and RNS by initiating the antioxidant response element (ARE) pathway, resulting in catabolism of superoxide and peroxides, regeneration of oxidized cofactors and proteins, synthesis of reducing factors, expression of antioxidants, and metal chelation. Nrf2 is post-transcriptionally activated by several chemicals, including NO⁹⁴. In Alzheimer's disease transcription of Nrf2 is impaired and overexpression in an AD mouse model improves spatial memory^{95,96}

Glutathione (GSH) is an important thiol based antioxidant found in the brain which is primarily produced by astrocytes. GSH plays a prominent role in the detoxification of ROS in the aging brain and is reduced in AD astrocytes and microglia cells. Furthermore, AD associated GSH depletion is observed in the inferior frontal and inferior temporal cortices; primarily areas damaged in AD⁹⁷. Cultured astrocytes, microglia, and oligodendrocytes exposed to brief hypoxia express less GSH, reduced redox capacity, and apoptosis; partially explaining the decrease in AD cortical GSH^{98,99}. Brain iron levels are related to damage induced by GSH depletion as the chelation of iron with DFO blocks cellular death in a reduced GSH milieu¹⁰⁰.

Iron Storage and Transport in the Brain

The homeostatic balance of cellular iron in the brain is orchestrated through six distinct processes: transport, uptake, storage, utilization, redox cycling, and export. The bulk of iron transportation throughout the body is performed by the glycoprotein transferrin (Tf)¹⁰¹. Oligodendrocytes and choroid plexus cells are the only two neural cell types that produce Tf. Tf expression is decreased in AD within white matter and stains diffusely in and around the halo region of cortical A β plaques, suggesting that the AD brain has an impairment in this tissue⁵. It is unknown if the localized iron within the A β plaques offers a binding site for Tf where upon it becomes encapsulated within the plaque mass. In addition to its normal distribution within oligodendrocytes, Tf is also found abnormally associated within astrocytes of the AD brain which suggests a homeostatic iron imbalance in these cells⁹. The exact role of astrocytes in this regard is speculated to be related to the sequestration of oligodendrocyte Tf during demyelination or through the phagocytosis of amyloid with bound Tf.

Brain iron uptake is generally regulated by transferrin receptor 1 (TfR1) and divalent metal transporter 1 (DMT1)^{102,103}. TfR1 is highly expressed on arterial endothelial cells at the BBB and choroid plexus epithelial cells at the BCSFB¹⁰⁴. Expression on the luminal side is tightly controlled, decreasing when brain iron is replete or increasing when brain iron is depleted. TfR1 is also expressed on the surface of neuronal cells and reactive microglia, facilitating neuronal iron import and microglial iron sequestration during inflammation. Transferrin receptor expression is reduced in the Alzheimer's disease hippocampus, a region

heavily effected by early amyloidopathy^{105,106}. The dentate gyrus (DG) of the hippocampus is a distinct region of the brain where neurogenesis occurs and newly divided cells highly express TfR1. The cause for the formation of large punctate A β plaques in the AD DG has not been thoroughly investigated but is hypothesized to be related to the inability of this region to limit its iron intake and a progressive increase in iron content. Iron import during non-Tf-bound-iron (NTBI) transport is facilitated by DMT1, whose gene sequence (SLC11A2) is located adjacent to regions commonly mutated in AD on the long arm of chromosome 12¹⁰⁷. In addition, inflammatory cytokines increase the expression of DMT1 within neurons, astrocytes, and microglia in the AD brain¹⁰⁸.

Cellular iron storage, detoxification, and necessary release is regulated by ferritin, a 450kDa protein complex consisting of 24 subunits of light (L-) and heavy (H-) ferritin¹⁰⁹. H-Ferritin serves as a ferroxidase, producing ferric iron within the core of the ferritin protein. L-ferritin seeds the quaternary structure with H-ferritin to hold up to 4500 ferric iron ions depending on intracellular iron levels¹¹⁰. Ferritin distribution during normal aging remains constant throughout the brain. The majority of ferritin is found heterogeneously in oligodendrocytes, but it is also present in neurons, protoplasmic astrocytes, and microglia. *In vivo* MRI measures reveals that basal ganglia ferritin is greater in younger late onset AD patients compared to controls, with the difference being absent in older late onset AD patients¹¹¹. The AD brain presents with robust microglial ferritin around amyloid plaques and blood vessels¹¹². Elevated levels of CSF ferritin are apparent in AD and it is unclear if ferritin enters the CSF from the blood or brain parenchyma. It is known that CSF ferritin increases after a subarachnoid haemorrhage and can be produced intrathecally following a bleed^{113,114}.

Iron is utilized by mitochondrial proteins, such as frataxin, mitoNEET, and numerous other enzymes in the brain that require iron to act as an electron carrier^{115,116}. Deficiency of frataxin is noted in Friedreich's ataxia, the disease in which it was first described. While the physiological function of frataxin is unclear, it is believed to act as an iron chaperone in the assembly of iron-sulfur clusters for which neurons have high demand. In addition, an extra-mitochondrial frataxin was recently discovered to stabilize the Fe-S cluster in aconitase offering molecular control of cytosolic aconitase/IRP1¹¹⁷. MitoNEET is an integral iron containing protein in the outer leaflet of mitochondria. It is implicated in moderating the maximal respiratory rate by controlling the amount of iron imported into the mitochondria and controls oxidative species produced by mitochondrial respiration¹¹⁶. Reduction of mitoNEET leads to enhanced mitochondrial respiratory capacity through increased matrix incorporation of iron and elevated oxidative stress¹¹⁷. Damage or dysfunction of frataxin and mitoNeet is speculated to be involved in several facets of AD including ROS control, redox control, and heme metabolism; however, no studies have directly connected mitoNeet and frataxin dysfunction to amyloid production.

The reduction and oxidative (redox) cycling of iron is required to meet cellular energy demands through which ROS are generated. Careful compartmentalization during redox cycling by neural proteins facilitates detoxification and shuttling of iron throughout the brain. Extracellular and intracellular H-ferritin detoxifies and stores iron by oxidizing soluble Fe²⁺ to insoluble Fe³⁺. Ferrous iron is oxidized by ceruloplasmin and hephaestin and

is subsequently effluxed as Fe^{2+} by means of ferroportin and Tf. Reduction of Fe^{3+} by an endosomal STEAP3 and peripheral membrane cellular prion protein (PrPc) facilitates the influx of iron by means of TfR and NTBI¹¹⁸. PrPc is present at its highest levels in neurons of the brain, but it is also present at lower levels in neural glial cells^{119,120}. Binding of A β to PrPc impairs ferrireductase activity and the loss of PrPc results in the iron overloading of the brain paired with elevated Tf and TfR1 transcription, as discussed in detail in the next section^{121,122}.

Export of iron is predominantly facilitated by the transmembrane protein ferroportin. Ferroportin transports ferrous intracellular iron in tandem with ferroxidases, such as ceruloplasmin, to oxidize and transport ferric iron into the extracellular domain. It is hypothesized that a region within the E2 domain of sAPP stabilizes ferroportin at the cell surface and leads to increased iron efflux^{123,124}. It is unknown how this function differs before and after APP cleavage by alpha and beta secretase. Once extracellular, apo-transferrin binds ferric iron and is shuttled to a transferrin receptor on a nearby neuron or it is hypothesized to be carried out of the brain via binding to transferrin receptors on the apical surface of an arterial endothelial cell of the BBB or the basal surface of an ependymal cell at the BCSFI. Ferric iron can also be reduced and imported into the cell via NTBI transport. If iron is not oxidized in the ferroportin core, the hormone hepcidin binds to ferroportin and triggers the internalization and degradation of the receptor-ligand complex¹²⁵. Hepcidin is mostly secreted by the liver to control peripheral iron levels, but neural expression is elevated on activated astrocytes and microglia exposed to inflammatory cytokines^{108,126}. Ferroportin reduction in an AD mouse model is associated with heme granular deposits adjacent to damaged blood vessels suggesting that vascular dysfunction is altering iron flow and homeostasis in the AD brain¹²⁷.

Iron protein transcription and translation

Iron balance in all cell types is regulated by the transcription of hypoxia-inducible factor (HIF) 1 and 2. HIF1 and HIF2 are structurally similar, but they have unique glycolytic control, expressed in discrete cell types in the brain^{128–130}. HIF consists of a cytosolic subunit HIF α and the nuclear subunit HIF β . The HIF heterodimer forms in a low iron, low oxygen, or high NO conditions to produce target RNA transcripts with iron regulatory elements (IREs), including TfR1 and DMT1. HIF α degradation is mediated by the ubiquitin-proteasome system when iron and oxygen are available. Ischemic events, iron loading, or disrupted proteasomal function alter HIF function and lower TfR1 and DMT1 levels in the AD brain. Recent work reveals that PSEN1/PSEN2 modulates the functional response to hypoxia by altering HIF α expression¹³¹. Stabilizing HIF with a prolyl-hydroxylase inhibitor in normal aging mice enhances hippocampal memory, providing a potential therapy for AD¹³². Iron chelators, such as M30, upregulate HIF and HIF-activating compounds in cortical neurons¹³³.

Iron regulatory protein 1 (IRP1) or IRP2 bind to hairpin IREs present in the untranslated regions (UTRs) of protein mRNA which are important for iron storage and transport¹³⁴. IRP1 functions as an aconitase in a high Fe, low O_2 , or low NO environment. Inverse to these conditions it binds a UTR IRE at either the 5' or 3' position of the pre-translated RNA

transcripts. When the UTR IRE is in the 5' position, RNA polymerase is blocked from translating the target protein; as is the case for H-/L-ferritin, ferroportin, APP, HIF α , and PrPc. If the UTR IRE is in the 3' position, IRPs stabilize the mRNA and facilitate the translation of the target protein such as TfR1 and DMT1. IRP2 acts similarly to IRP1, except that it is inactivated via ubiquitination under sufficient iron conditions. IRP has an increased affinity to IRE in the AD brain explaining the elevation of iron levels without a rise in ferritin production¹³⁵. The mechanism behind this stability is not understood and is speculated to be related to the rise in endogenous ribonuclease activity observed in AD¹³⁵.

Brain Perfusion

AD and iron dyshomeostasis are hypothesized to correlate to a disruption of brain vasculature. Micro-casting of AD mouse model brains reveals that there are alterations of the microvasculature accompanied by A β attachment and deposition. Aged transgenic mouse brain vessels are occluded and terminate at A β plaques, causing small gaps in the micro-vasculature net around which angiogenesis is occurring¹³⁶. While the relationship between AD, iron homeostasis, and cerebral amyloid angiopathy (CAA) is not fully understood, we hypothesize that brain perfusion and iron dyshomeostasis are related to amyloid generation.

Cerebral amyloid angiopathy (CAA) results in the deposition of A β in the meningeal and intracerebral vessels. CAA presents in over 50% of elderly individuals and is also found in 50% of AD cases highlighting that it is a common occurrence in the aging brain but does not always associate with AD¹³⁷. There is a distinction of iron loading in AD with and without CAA; tissue from AD patients with severe CAA had significantly higher non-heme iron levels especially in the walls of large vessels¹³⁸.

It is hypothesized that improper clearance of A β and the buildup of iron at the BBB can lead to aberrant angiogenesis and senescence of the cerebrovascular system. These environmental conditions begin a process of neurovascular uncoupling, vessel regression with vascular smooth muscle loss, brain hypoperfusion, and neurovascular inflammation. Ultimately, these conditions lead to BBB leakage, improper drainage, chemical imbalance in the neuronal environment, and synaptic dysfunction and loss⁷³. CAA and iron deposition lead to the degradation of cytoskeletal elements and aquaporin-4 channels localized on astrocytic end feet at the BBB¹³⁹. There is also a concomitant loss of glucose transporter-1 and lactate transporter in these astrocytes¹⁴⁰. Further research needs to be conducted to illustrate if the initial ischemia and deposition of A β and iron at the cerebral wall is dependent on brain disease or peripheral dysfunction.

The source of amyloid deposition is heterogeneous; concussion and hemorrhage increase the risk of developing A β in the brain¹⁴¹. A β can bind to red blood cell (RBC) heme, a process that increases with aging, and incubation of erythrocytes with A β decreases NOS synthesis, causing deformity and increased cellular volume^{142–144}. It is unclear how RBC A β levels correlate with CAA; however, RBC dysfunction helps explain the hypoperfusion and heme deposition at occluded vessels in AD.

Recent findings indicate that a paravascular pathway exists in the brain, which facilitates CSF flow through the brain parenchyma and allows clearance of interstitial solutes and A β ¹⁴⁵. This glymphatic system is dependent on aquaporin-4, arterial pulsation, and is enhanced during sleep¹⁴⁶. It is unclear without further work describing extracellular iron clearance if impairment of this process in AD leads to poor A β clearance or if CAA and iron deposition precede these alterations.

Neuron

Neurons display TfR1 more than any other cell type in the brain as its expression mediates iron import into these cells (Fig. 3). Iron bound Tf has a high affinity for TfR1 and is regulated through competitive binding with HFE, a major histocompatibility complex class I-like molecule. HFE mutations (H63D and C282Y) increase Tf/TfR1 binding and lead to hemochromatosis and oxidative stress in the brain¹⁴⁷. H63D neurons have been shown to express higher APP and A β ₄₂ and *in vitro* A β induces apoptosis via mitochondrial dysfunction and caspase activation¹⁴⁸. The knowledge that 29.7% of the Caucasian population are carriers for HFE mutations highlights the importance of this polymorphism¹⁴⁹. Transferrin (C2) and HFE mutations, leading to iron overload, are also strongly correlated to Alzheimer's disease^{107,150,151}. Any known population benefit for these mutations is not well understood, but recent findings suggest that Tf mutations provide a means to counteract bacterial iron piracy in primates during infection¹⁵².

When TfR1 forms a complex with Tf, it is taken into the cell via clathrin mediated endocytosis (Fig. 3a). Within the endosome, Fe³⁺ is released from Tf upon acidification and STEAP3 reduces the iron to Fe²⁺ for export from the endosome into the cytoplasm by DMT1. The endosome is recycled back to the cell surface to redeposit HFE, TfR1, and DMT1. Ferroportin shuttles ferrous iron into its channel and works in tandem with ferroxidases, such as ceruloplasmin, to shuttle ferric iron out. Released ferric iron can bind to Tf or it can be reduced and taken back into the cell via NTBI transport. It is hypothesized that A β modifies this process through ferric iron interaction preventing binding with Tf, hindering iron transport.

Previous work has shown that soluble tau is necessary to shuttle APP to the neuronal surface so that it can interact with ferroportin iron export (Fig. 3b). Blocking soluble tau results in neuronal iron overload, as does knocking out APP^{153,154}. Neuronal cell cultures demonstrate that Fe³⁺ induces hyperphosphorylated tau (pTau) aggregation and Fe²⁺ generated oxidative radicals can induce tau hyperphosphorylation via activation of the extracellular signal regulated kinase1/2 (Erk1/2) pathway or the mitogen-activated protein kinase pathway (MAPK)^{155–157}. In AD, increased iron co-localizes with insoluble tau tangles and facilitates the loss of soluble tau, further perturbing tau-mediate APP shuttling and ferroportin iron export^{141,153}. The usage of iron chelators highlights this relationship as they decrease the phosphorylation of tau and its aggregation⁴⁵. The loss of soluble tau prevents the tau stabilization of microtubules, altering molecular transport important for neurotransmission, synapse stability, and cellular health¹⁵⁸.

Phosphorylated tau tangles develop in the axon hillock, soma, and axon of neurons (Fig. 3h). Recent work has shown that tau aggregates can be transferred to adjacent cells and downstream cells synaptically connected in a prion-like manner¹⁵⁹. This spread leads to sequential cellular degeneration at the synapse, axon, and soma¹⁶⁰. This agrees with the finding that the presence of pTau at the synapse has been shown to increase the synaptic toxicity of amyloid oligomers. There are two potential synergistic mechanisms by which iron-amyloid induced degeneration is related to pTau pathology. 1) Amyloid cannot sequester all of the extraneuronal iron, leading to increased transport of intraneuronal iron via NTBI, increased phosphorylation of tau, and neuronal dysfunction. 2) Elevated intraneuronal iron increases APP processing and the level of extracellular amyloid oligomers, consequentially leading to amyloid-pTau toxicity at the synapse. In addition to inter-neuronal cytoskeletal perturbations, alterations in the cholesterol dynamics modify the cellular membrane fluidity.

AD is positively correlated with increased serum cholesterol¹⁶¹. Cholesterol metabolites traveling in the blood from the liver elevate cholesterol levels in the brain, clarifying the interaction between serum cholesterol and *de novo* brain synthesis. A β levels are decreased in an AD mouse model that synthesizes 50% less sterol. Conversely, induced hypercholesteremia in a rabbit animal model fed a cholesterol rich diet increases amyloidosis and cortical distribution of iron¹⁶². As neuronal cholesterol increases, the fluidity of the membrane is reduced and lipid raft composition is altered, decreasing α -secretase contact with APP¹⁶³. Low cholesterol is linked with increased α -secretase activity and decreased production of toxic A β oligomers^{164,165}. There is evidence that β -secretase translation is upregulated by intracellular cholesterol and downregulated by cholesterol synthesis inhibition¹⁶⁶. In addition, γ -secretase activity is dependent on cholesterol found in lipid rafts; removing cholesterol prevents γ -secretase activity and A β production¹⁶⁷. Human observations reveal that alterations in CNS cholesterol synthesis are related to neurodegeneration and in particular the cerebral accumulation of pTau¹⁶⁸. Taken together, increasing cholesterol pushes the brain into an AD amyloidogenic state (Fig. 3d). The process becomes more complex when observing the effects of cholesterol synthesis byproducts.

Brain derived 24S-hydroxycholesterol downregulates the β -cleavage of APP, decreases cholesterol synthesis, and upregulates APOE; aiding in the efflux of excess cholesterol from the brain. Peripheral 27S-hydroxycholesterol from the liver crosses the BBB and may be linked to hypercholesteremia in the brain where upon APP and A β metabolize cholesterol to oxysterol 7-beta hydroxycholesterol; a neurotoxic byproduct which contributes to oxidative stress¹⁶⁹.

PrPc regulates iron influx through ferrireductase activity at the cell surface and interaction with DMT1 and the Zip Fe/Zn transporter to shuttle ferrous iron across the cellular plasma membrane independently of TfR1¹⁷⁰. PrPc also facilitates normal cell function by modulating molecular pathways, such as stress inducible factor 1, and inducing growth and synaptic function. The binding of PrPc to amyloid oligomers present in the AD brain increases tau expression to help shuttle more APP to the cell surface; decreasing neuronal iron while increasing A β production. This adversely affects neurons as NMDAR expression

is decreased when PrPc is exposed to amyloid oligomers¹⁷¹ (Fig. 3c). The depletion of NMDAR is subsequently followed by synaptic loss and cell death via the FYN pathway¹²¹. The misfolding of PrPc into PrP^{Sc} in Cruetzfeldt-Jakob disease leads to iron dyshomeostasis and elevated PrP^{Sc} has been found in the AD brain^{122,172}.

Long-lasting L-Type voltage gated calcium channels are localized to the soma and dendrites of neurons and glial cells, coupling membrane depolarization in neurons to gene expression, synaptic efficacy, and cell survival. Activation leads to a primary influx of calcium and secondary transport of iron through these channels when extracellular iron increases (Fig. 3f). This process leads to neuronal iron overloading and toxicity^{173,174}. The same channels facilitate iron protection or iron burden depending on the cell type and iron overloading status^{174,175}.

Iron regulatory protein production is tightly regulated by iron and oxygen concentration. Decreased oxygen levels increases TfR and DMT expression and decreased ferroportin production, leading to elevated iron loading within the cell (Fig. 3g). Increased NO may be a compensatory mechanism to improve blood flow in ischemia, underlining vascular pathology as a potential shift in iron homeostasis. Nitric oxide synthase activity is increased in AD leading to amplified free radical production as well as NO which, in turn, disrupts IRP function and reduces iron export¹⁷⁶.

Astrocyte

Astrocytes function within the neuronal network to bridge cells and connect them to the vasculature and ventricular systems (Fig. 4). Astrocyte glia limitans end-foot processes surround endothelial cells to create the BBB and regulate traffic into the brain parenchyma. The small interstitial space separating the glia limitans and endothelial cells must be traversed to allow penetration into the brain. Under healthy conditions, astrocytes acquire iron across the interstitial space using distinct NTBI mechanisms such as citrate, ATP, ascorbic acid, DMT1, and Zip^{177,178} (Fig. 4a). High iron conditions stimulate astrocytes to become reactive where upon they become enlarged with cellular processes. Astrocytes strongly express ferritin upon stimulation with iron and are highly resistant to iron toxicity. They convey a neuroprotective role through the intake of excess extracellular iron via the upregulation of ferritin synthesis, DMT1, L-type Ca^{2+} channels, transient receptor potential (TRP) channels, ATP secretion, endocytosis of debris, and downregulation of ferroportin^{178–180}; allowing astrocytes to act like an iron sink¹⁹. Astrocytes increase APP and β -secretase expression under high iron conditions, illustrating that these cells are similar to neurons in how they utilize APP and A β to regulated excess iron¹⁸¹. Under healthy conditions, astrocytic iron is believed to be released from ferritin via lysosomal degradation and the resulting free iron is exported with ferroportin¹⁸². Evidence suggests that iron efflux from astrocytes plays a role in remyelination through a direct interaction with oligodendrocyte precursor cells or indirectly by activating microglial cells¹⁸³. However, when astrocytes fail to return to a resting state in AD they diminish their metabolic support¹⁸⁴.

Astrocytes are the only cells in the brain that facilitate glycogen and glutamate synthase, which is utilized for glucose metabolism by hypoglycemic neurons and oligodendrocytes¹⁸⁴. Astrocyte-neuron lactate shuttling is shown to be critical for long-term memory formation which becomes weak in AD, preventing the sharing of lactate^{185–189} (Fig. 4c). Blood insulin levels play a role in astrocyte function as *in vitro* exposure to insulin promotes glycogen storage and cell proliferation in primary human astrocytes¹⁹⁰. There is evidence that suggests insulin plays a role in amyloidosis as high levels of physiological insulin infusion increase APP and A β ₄₂ production¹⁹¹. Patients in early stages of type 2 diabetes show brain dysfunction while diabetic patients 55 and older on insulin sensitizer, metformin, were less likely to acquire dementia^{192,193}. The mechanism by which this occurs is hypothesized to be related to either hyperinsulinemia upregulation of BACE1 and APP, decreased A β degradation activity of insulin degrading enzyme (IDE), or decreased mitochondria mitoNEET activity (Fig. 4d)¹⁹⁴.

Apolipoprotein E (APOE), predominantly produced by astrocytes, has been identified as an associated factor with late onset AD¹⁹⁵. APOE's primary role is that of a cholesterol transporter, however it also regulates A β transport, metabolism, aggregation, and deposition¹⁹⁶. APOE comes in three human population polymorphisms: ϵ 2 (8%), ϵ 3 (77%), and ϵ 4 (15%)¹⁹⁷. ϵ 4 is significantly associated with the onset and severity of AD with prevalence in 40% of AD patients^{198,199}. ϵ 2 has been shown to be protective against AD, however it also increases the incidence of cardiovascular disease²⁰⁰. APOE binds A β in an isoform-dependent manner ϵ 2 > ϵ 3 > ϵ 4 and facilitates the clearance of A β by either its lysosomal degradation within astrocytes or shuttling A β out of the brain via the BBB or BCSFI^{201–203}. Under non-amyloidogenic conditions, APOE enables the clearance of A β by either facilitating its degradation in the brain or shuttling it out of the brain via the BBB or BCSFI. APOE binds LRP1, LDLR, and HSPG present on neurons, vascular endothelial cells and neurons. These receptors are responsible for lipid metabolism and are present in all major cellular pathways that clear A β ²⁰⁴. A β not complexed with APOE has been shown to block these receptors, inhibiting APOE metabolism and transport²⁰⁵ (Fig. 4f).

During amyloidosis APOE seeds amyloid deposition independent of fibrillogenesis in an ϵ 4 > ϵ 3 manner and it is unknown how metals interact within this process. There is a strong positive correlation between APOE and CSF ferritin levels in ϵ 4 carriers and patients with low APOE or high ferritin in the CSF showed greater brain atrophy²⁰⁶. It is hypothesized that APOE functions to lower neuronal iron as APOE KO mice exhibit elevated brain iron²⁰⁶. Cells overexpressing APOE have less ferritin and WT cells exposed to iron increased APOE expression²⁰⁶. APOE attenuates A β induced astrogliosis in culture, but it is unknown how astrogliosis alters APOE levels²⁰⁷. Reactive astrocytes elevate the expression of haptoglobin, which increases binding of A β with APOE²⁰⁸ (Fig. 4e). However, under stress conditions, APOE becomes fragmented resulting in increased A β deposition, mitochondrial, and cytoskeletal damage. Decreasing APOE has been shown to reduce amyloid plaque formation in AD mouse models, but the importance of APOE for cholesterol homeostasis and as a A β /Fe shuttle may make it harmful as a long-term treatment²⁰⁹.

Oligodendrocyte

Oligodendrocytes are unique to the brain as they are the only cell with intracellularly synthesized Tf, express the Tim2 ferritin transporter, and contain a large amount of the brain's lipids and cholesterol (Fig. 5). In addition to this, oligodendrocytes contain the majority of the healthy brain's iron in the core of ferritin complexes. High concentrations of iron are required during myelination and levels must be tightly controlled to maintain iron homeostasis. As demyelination and myelin breakdown outpace remyelination during normal aging, ferritin iron and free iron collect in the extracellular space. In the healthy brain, proper iron management by other neural cells eliminates excessive iron toxicity in this domain. Normal brain white matter atrophies and loses 20% of its volume from age 30 to 80, while gray matter atrophies less than 6%²¹⁰. MR imaging of the AD brain reveals that excessive brain atrophy is initiated by myelin degradation followed by GM atrophy; forming the hypothesis that increased myelin loss may contribute to subsequent GM atrophy³². The volume of white matter lesions are inversely related to proper APOE function in middle-aged and elderly subjects, suggesting that impaired cholesterol metabolism and oligodendrocyte damage are related²¹¹. Oligodendrocytes serve as the brain's primary pool of cholesterol and lipids. Lipidation byproducts in the blood are a biomarker for the onset of memory impairment in AD²¹². This is most likely due to the loss of plasma membrane from myelin and neuronal synapses. Impaired lipid and cholesterol metabolism can also lead to hypercholesteremia, which was earlier described as being harmful in AD^{213,214}.

The mechanism of oligodendrocytes Tim2 ferritin receptor endocytosis is unlike neuronal Tf endocytosis as ferritin is not recycled back into the extracellular space (Fig. 5a). Tim2 bound ferritin endocytosis is followed by lysosomal degradation and elevated endogenous ferritin synthesis²¹⁵. Tim2 acts as the iron uptake pathway for oligodendrocytes and is integral to oligodendrocyte health and iron regulation. As A β fragments *in vitro* are observed to be cytotoxic to oligodendrocytes in a dose dependent manner, damage to oligodendrocytes during AD may impair the clearance of ferritin in the brain and leave most of the iron storage to astrocytes²¹⁶.

Oligodendrocytes uniquely synthesize Tf with no known secretion mechanism. While it is unclear why oligodendrocytes synthesize Tf, the current hypothesis suggests that Tf may be necessary to transport Fe along the long stretches of myelin wrappings to and from the soma. Decreased Tf in AD can impair oligodendrocytes from properly transporting iron⁹ (Fig. 5c). Similar to astrocytes, oligodendrocytes express APP to aid in iron export or as an iron binding agent during bouts of iron overload. Increased APP expression in white matter during brain trauma may well indicate the proteins role in the sequestration of iron during myelin damage. The high expression of APP also illustrates the cells role as a potential source for A β aggregation²¹⁷. Myelin breakdown can expose APP to β -secretase on neighboring neurons, facilitating the amyloidogenic process (Fig. 5d).

Microglia

Quiescent microglia in the brain contain highly branched ramified processes in their resting state (Fig. 6). Hypoxia, trauma, and exposure to inflammatory molecules cause microglia to

shrink into an amoeboid form that serves as the brain's macrophage. Microglia respond to a number of molecular signals and are attracted via A β and ATP chemotaxis. As such, amyloidogenesis and astrogliosis mediate microglial movement toward sites of localized inflammation²¹⁸.

Microglia recognize A β via CD36- α 6 β 1-CD47 leading to the phagocytosis of iron bound A β aggregates, lysosomal degradation, and the production of ROS. Active microglia surrounding large A β plaques synthesize elevated levels of ferritin to form a ferritin laden border along the microglial cell membrane²¹⁹. Continued intake and degradation of A β and iron bound to A β leads to increased levels of ROS and RNS with uncontrolled iron uptake. As AD progresses in the brain, A β and ferritin iron overwhelm and slow the efficiency of the microglial lysosome²²⁰. In addition, the acidic environment of the microglial lysosome has been suggested to favor A β aggregation^{202,221}. Improper degradation of ferritin in the microglial lysosome is hypothesized to lead to the accumulation of toxic hemosiderin and magnetite deposits, which cannot be metabolized²²² (Fig. 6d).

Small homogenous microvesicles (exosomes) are shed by all neural cell types via the release of multivesicular bodies along the endocytic pathway, serving as a means of exoplasmic communication between cells (Fig. 6f). Microglial microvesicles interact with soluble A β and initiate the formation of A β oligomers that are toxic to synapses²²³. Macropinocytosis of exosomes produced by damaged oligodendrocytes cause microglia to release inflammatory cytokines²²⁴ (Fig. 6g). CSF microvesicle concentration is correlated with white matter damage in MCI and with hippocampal atrophy in late onset AD²²⁵. This follows the trend of white matter damage prior to gray matter atrophy in AD, as previously described. It has been suggested that hippocampal microglia activation in the presence of excess A β ₄₂ facilitates the formation of toxic A β oligomers that progress brain-wide via white matter damage and microglial activation²²⁵. Hypoxic conditions during AD increase IRP activity and TfR expression of microglial, leading to continued ROS and RNS production and further inflammatory cytokine release (Fig. 6h).

Discussion – The Big Picture

The integration of iron dyshomeostasis and amyloidogenesis denotes that concomitant features of both synergistically amalgamate along related paths towards Alzheimer's pathology (Figs. 7 and 8). Microcapillary lesions contribute to a wide array of problems that may contribute to the AD process and propagate increased extracellular iron. Proper BBB regulation is no longer maintained allowing hemoglobin, transferrin, ferritin, and free iron from the blood to enter the brain contributing to iron overload. Cerebrovascular breakdown is linked to several factors including impaired metabolic transport across the BBB, reactive microglia bypassing the glial sheath, endothelial degeneration, and endfoot separation. Astrocytic endfeet normally contain a high concentration of mitochondria and surround the endothelial vascular tube forming the perivascular endfoot membrane²²⁶. Disruption of the AD BBB correlates with impaired GLUT1 and lactate transporter functionality as well as astrocyte endfoot withdrawal and astrogliosis; all of which precede A β deposition¹⁴⁰. BBB leakage progresses with age, especially with APOE deficiency compounded with brain injury^{227,228}. Astrocytes help distribute iron throughout the brain and clear excess

extracellular iron. AD astrocytes abnormally store increased amounts of ferritin, APP, and A β intracellularly. It is unclear if this process contributes to plaque formation or if it is related to the failure of astrocyte or microglial lysosomal and iron export processes.

We hypothesize that amyloid production increases to ameliorate iron overload and patch the damaged vascular system. The time course of this progression is congruent with the plateauing of brain iron levels as early onset Alzheimer's disease first presents itself during this period. The continued heterogeneity of iron levels in the aged population past this plateau and the incidence of iron incorporation in amyloid plaques further provide indication of a plausible interaction between these processes (Fig. 2). Regions which have relatively lower concentrations of iron, such as the frontal lobe and temporal lobes, form amyloid pathology⁷¹. Whereas regions with high iron content, such as the brainstem, motor cortex and cerebellum, are not affected by amyloidosis. We hypothesize that low iron brain regions are more susceptible to changes in iron homeostasis, elevated free iron, and subsequent amyloidosis compared to endogenous high iron regions which, conceivably, are better equipped to maintain iron equilibrium. Increased free neuronal iron upregulates APP translation and, when paired with aberrant secretase activity, leads to increased extracellular A β ₄₂, tau phosphorylation, neuronal toxicity, dysfunction, destabilization, and intraneuronal tau tangle formations.

Genetic mutations may convey a patient subset to be less suited to manage iron overload while other mutations prompt patients to follow an amyloidogenic path in a high iron milieu. The relationship between these two circumstances represent a crossroad for Alzheimer's progression. The higher incidence of AD in women is also of interest and cannot purely be attributed to increased female longevity. Women are more susceptible to the APOE ϵ 4 variant and female AD mouse models present greater amyloid deposition than their male counterparts^{229,230}. Our iron-amyloid hypothesis of AD helps to explain, in part, why this may be the case. The amount and density of long-term iron stored in the male (600mg) body within ferritin is double that of women (300mg). This suggests that the useable iron reservoir in women is in constant flux between intake and redox cycling. We hypothesize that females utilize short-term iron storage for immediate or intermediate usage compared to men (eg Tf or NTBI). Iron in this state may be more susceptible to interactions with A β and APOE leading to the genesis of toxic amyloid species.

The pathogenesis of Alzheimer's disease is heterogeneous with numerous physiological features correlated to its progression. Brain iron overload does not always lead to amyloidogenesis and Alzheimer's disease and, conversely, amyloidogenesis does not always lead to iron overload²³¹. Current literature supports the hypothesis that there is a link between iron and amyloid dyshomeostasis in the AD brain. However, the exact nature of this relationship remains unclear and encompasses multiple possibilities; the aging AD brain may absorb more iron, transport iron improperly, or insufficiently release iron. Further distinction between cell culture, animal model, and human studies require assessment due to differential endogenous iron levels, variations in oxygen saturation, and contributions from various cell types and pathologies making translation between these systems difficult. The finding that amyloid plaque burden does not correlate well to memory loss in a patient

subset suggests that there is a plaque-independent mechanism that is responsible for AD memory impairment.

Therapies aimed at reducing amyloid have proved problematic in clinical trials as the removal of amyloid from the brain releases free iron and destabilizes microvessels²³². Targeted reduction of A β deposition and amyloidogenesis with secretase inhibitors and immunotherapies leads to iron accumulation and cognitive deficits. These outcomes are not observed in AD model mice perhaps due to lower endogenous iron levels in mice than that found in the human brain or translational modification of humanized sequences. The data support the hypothesis that amyloid in the brain serves as part of an iron maintenance mechanism and is initially protectant against iron-mediated damage. A better understanding of the time course of iron dyshomeostasis and AD onset are needed to help guide therapies that can stabilize iron levels before they can become harmful in at-risk populations. Iron chelating agents, such as clioquinol, given at an appropriate time may be able to curb the onset of AD. HupA and J147 are experimental treatments for AD, which may function to lower amyloid levels and improve cognitive performance by reducing iron levels^{50,233}. In the human brain it may be necessary to prophylactically treat with these types of compounds prior to amyloid formation to have an effect on brain health. To do so, the long lasting effects of such therapies and potential gender differences must be investigated.

The discovery that increased iron levels elevate APP expression along the amyloidogenic secretase pathway to efflux cellular iron reveals that iron management is central to the amyloid hypothesis^{67,234}. An understanding of the iron regulatory systems within neural cells and its link to amyloid production will help in the generation of therapy strategies aimed to curb iron dyshomeostasis and amyloid formation as synergistic contributors to the Alzheimer's disease process.

Acknowledgments

Funding for this project has been made available in part through the NIH-NIA (R03AG047461), The Charleston Conference on Alzheimer's Disease Research Grant, The Barsumian Trust, The Neuroimaging Research Grant, and The George M. Leader Foundation.

References

1. Den Dunnen WFA, et al. No disease in the brain of a 115-year-old woman. *Neurobiol Aging*. 2008; 29:1127–1132. [PubMed: 18534718]
2. Hirtz D, et al. How common are the 'common' neurologic disorders? *Neurology*. 2007; 68:326–337. [PubMed: 17261678]
3. Hebert LE, Scherr PA, Bienias JL, Bennett DA, Evans DA. Alzheimer disease in the US population: prevalence estimates using the 2000 census. *Arch Neurol*. 2003; 60:1119–1122. [PubMed: 12925369]
4. Raven EP, Lu PH, Tishler Ta, Heydari P, Bartzokis G. Increased iron levels and decreased tissue integrity in hippocampus of Alzheimer's disease detected in vivo with magnetic resonance imaging. *J Alzheimer's Dis*. 2013; 37:127–136. [PubMed: 23792695]
5. Connor JR, Snyder BS, Beard JL, Fine RE, Mufson EJ. REGIONAL DISTRIBUTION OF IRON AND IRON-REGULATORY PROTEINS IN THE BRAIN IN AGING AND ALZHEIMERS-DISEASE. *J Neurosci Res*. 1992; 31:327–335. [PubMed: 1573683]
6. Ding B, et al. Correlation of iron in the hippocampus with MMSE in patients with Alzheimer's disease. *J Magn Reson Imaging*. 2009; 29:793–8. [PubMed: 19306401]

7. Meadowcroft MD, Connor JR, Smith MB, Yang QX. MRI and histological analysis of beta-amyloid plaques in both human Alzheimer's disease and APP/PS1 transgenic mice. *J Magn Reson Imaging*. 2009; 29:997–1007. [PubMed: 19388095]
8. Smith MA, Harris PLR, Sayre LM, Perry G. Iron accumulation in Alzheimer disease is a source of redox-generated free radicals. *Proc Natl Acad Sci U S A*. 1997; 94:9866–9868. [PubMed: 9275217]
9. Connor JR, Menzies SL, St Martin SM, Mufson EJ. A histochemical study of iron, transferrin, and ferritin in Alzheimer's diseased brains. *J Neurosci Res*. 1992; 31:75–83. [PubMed: 1613823]
10. Rottkamp CA, et al. Redox-active iron mediates amyloid- β toxicity. *Free Radic Biol Med*. 2001; 30:447–450. [PubMed: 11182300]
11. Coby AJ, Picardal F, Shelobolina E, Xu H, Roden EE. Repeated anaerobic microbial redox cycling of iron. *Appl Environ Microbiol*. 2011; 77:6036–6042. [PubMed: 21742920]
12. Sheftel AD, Mason AB, Ponka P. The long history of iron in the Universe and in health and disease. *Biochim Biophys Acta - Gen Subj*. 2012; 1820:161–187.
13. Shi Y, Ghosh M, Kovtunovych G, Crooks DR, Rouault TA. Both human ferredoxins 1 and 2 and ferredoxin reductase are important for iron-sulfur cluster biogenesis. *Biochim Biophys Acta - Mol Cell Res*. 2012; 1823:484–492.
14. Rovira C, Carloni P. The iron-sulfur bond in cytochrome c. *J Phys Chem B*. 1999; 103:7031–7035.
15. Kennedy MC, Emptage MH, Dreyer JL, Beinert H. The role of iron in the activation-inactivation of aconitase. *J Biol Chem*. 1983; 258:11098–11105. [PubMed: 6309829]
16. Ford GC, et al. Ferritin: design and formation of an iron-storage molecule. *Philos Trans R Soc Lond B Biol Sci*. 1984; 304:551–565. [PubMed: 6142491]
17. Rouault, Ta. Iron metabolism in the CNS: implications for neurodegenerative diseases. *Nat Rev Neurosci*. 2013; 14:551–64. [PubMed: 23820773]
18. Moos T, Rosengren Nielsen T, Skjørringe T, Morgan EH. Iron trafficking inside the brain. *J Neurochem*. 2007; 103:1730–40. [PubMed: 17953660]
19. Hohnholt MC, Dringen R. Uptake and metabolism of iron and iron oxide nanoparticles in brain astrocytes. *Biochem Soc Trans*. 2013; 41:1588–1592. [PubMed: 24256259]
20. Chen JH, Shahnavas S, Singh N, Ong WY, Walczyk T. Stable iron isotope tracing reveals significant brain iron uptake in adult rats. *Metallomics*. 2013; 5:167–73. [PubMed: 23340610]
21. Dallman PR. Brain Iron in the Rat: Extremely Slow Turnover in Normal Rats May Explain Long-Lasting Effects of Early Iron Deficiency. *J Nutr*. 1977; 107:1075–1081. [PubMed: 864518]
22. Sasaki T, et al. Visualization of mitochondrial oxygen fixation in brain slices by gas-tissue autoradiography. *Brain Res*. 1999; 831:263–272. [PubMed: 10412005]
23. Moos T, Morgan EH. Transferrin and transferrin receptor function in brain barrier systems. *Cell Mol Neurobiol*. 2000; 20:77–95. [PubMed: 10690503]
24. Connor JR, Benkovic Sa. Iron regulation in the brain: histochemical, biochemical, and molecular considerations. *Ann Neurol*. 1992; 32(Suppl):S51–S61. [PubMed: 1510381]
25. Bartzokis G. Schizophrenia: Breakdown in the well-regulated lifelong process of brain development and maturation. *Neuropsychopharmacology*. 2002; 27:672–683. [PubMed: 12377404]
26. Connor JR, Menzies SL. Relationship of iron to oligodendrocytes and myelination. *Glia*. 1996; 17:83–93. [PubMed: 8776576]
27. Rakic P, Bourgeois JP, Eckenhoff MF, Zecevic N, Goldman-Rakic PS. Concurrent overproduction of synapses in diverse regions of the primate cerebral cortex. *Science*. 1986; 232:232–235. [PubMed: 3952506]
28. Bartzokis G. Age-related myelin breakdown: a developmental model of cognitive decline and Alzheimer's disease. *Neurobiol Aging*. 2004; 25:5–18. [PubMed: 14675724]
29. Schoenemann PT, Sheehan MJ, Glotzer LD. Prefrontal white matter volume is disproportionately larger in humans than in other primates. *Nat Neurosci*. 2005; 8:242–52. [PubMed: 15665874]
30. Sherwood CC, et al. Aging of the cerebral cortex differs between humans and chimpanzees. *Proc Natl Acad Sci U S A*. 2011; 108:13029–34. [PubMed: 21788499]
31. Radlowski EC, Johnson RW. Perinatal iron deficiency and neurocognitive development. *Front Hum Neurosci*. 2013; 7:11. [PubMed: 23378833]

32. Agosta F, et al. White matter damage in Alzheimer disease and its relationship to gray matter atrophy. *Radiology*. 2011; 258:853–63. [PubMed: 21177393]
33. Jomova K, Vondrakova D, Lawson M, Valko M. Metals, oxidative stress and neurodegenerative disorders. *Mol Cell Biochem*. 2010; 345:91–104. [PubMed: 20730621]
34. Sherriff FE, Bridges LR, Sivaloganathan S. Early detection of axonal injury after human head trauma using immunocytochemistry for beta-amyloid precursor protein. *Acta Neuropathol*. 1994; 87:55–62. [PubMed: 8140894]
35. Corrigan F, et al. Characterisation of the effect of knockout of the amyloid precursor protein on outcome following mild traumatic brain injury. *Brain Res*. 2012; 1451:87–99. [PubMed: 22424792]
36. Ayton S, et al. Ceruloplasmin and β -amyloid precursor protein confer neuroprotection in traumatic brain injury and lower neuronal iron. *Free Radic Biol Med*. 2014; 69:331–7. [PubMed: 24509156]
37. Khan A, Dobson JP, Exley C. Redox cycling of iron by A β 42. *Free Radic Biol Med*. 2006; 40:557–69. [PubMed: 16458186]
38. Zhang X, et al. Hypoxia-inducible factor 1 α (HIF-1 α)-mediated hypoxia increases BACE1 expression and beta-amyloid generation. *J Biol Chem*. 2007; 282:10873–80. [PubMed: 17303576]
39. Cohen SIA, et al. Proliferation of amyloid- β 42 aggregates occurs through a secondary nucleation mechanism. *PNAS*. 2013; 110:9758–63. [PubMed: 23703910]
40. Ittner LM, et al. Dendritic function of tau mediates amyloid-beta toxicity in Alzheimer's disease mouse models. *Cell*. 2010; 142:387–397. [PubMed: 20655099]
41. Lacor PN, et al. Synaptic targeting by Alzheimer's-related amyloid beta oligomers. *J Neurosci*. 2004; 24:10191–10200. [PubMed: 15537891]
42. Jiao Y, et al. Iron chelation in the biological activity of curcumin. *Free Radic Biol Med*. 2006; 40:1152–60. [PubMed: 16545682]
43. Vaquero MP, et al. Mineral and vitamin status in elderly persons from Northwest Spain consuming an Atlantic variant of the Mediterranean diet. *Ann Nutr Metab*. 2004; 48:125–33. [PubMed: 15133316]
44. Scarmeas N, Stern Y, Tang MX, Mayeux R, Luchsinger JA. Mediterranean diet and risk for Alzheimer's disease. *Ann Neurol*. 2006; 59:912–21. [PubMed: 16622828]
45. Guo C, et al. Deferoxamine inhibits iron induced hippocampal tau phosphorylation in the Alzheimer transgenic mouse brain. *Neurochem Int*. 2013; 62:165–172. [PubMed: 23262393]
46. Guo C, et al. Intranasal deferoxamine reverses iron-induced memory deficits and inhibits amyloidogenic APP processing in a transgenic mouse model of Alzheimer's disease. *Neurobiol Aging*. 2013; 34:562–575. [PubMed: 22717236]
47. Prasanthi JRP, et al. Deferiprone reduces amyloid- β and tau phosphorylation levels but not reactive oxygen species generation in hippocampus of rabbits fed a cholesterol-enriched diet. *J Alzheimers Dis*. 2012; 30:167–82. [PubMed: 22406440]
48. Savory J, Huang Y, Wills MR, Herman MM. Reversal by desferrioxamine of tau protein aggregates following two days of treatment in aluminum-induced neurofibrillary degeneration in rabbit: implications for clinical trials in Alzheimer's disease. *Neurotoxicology*. 1998; 19:209–214. [PubMed: 9553957]
49. McLachlan DR, Smith WL, Kruck TP. Desferrioxamine and Alzheimer's disease: video home behavior assessment of clinical course and measures of brain aluminum. *Therapeutic drug monitoring*. 1993; 15
50. Huang XT, et al. Reducing iron in the brain: a novel pharmacologic mechanism of huperzine A in the treatment of Alzheimer's disease. *Neurobiol Aging*. 2014; 35:1045–54. [PubMed: 24332448]
51. Ayton S, Lei P, Bush AI. Metallostatics in Alzheimer's disease. *Free Radic Biol Med*. 2013; 62:76–89. [PubMed: 23142767]
52. Deibel MA, Ehmann WD, Markesbery WR. Copper, iron, and zinc imbalances in severely degenerated brain regions in Alzheimer's disease: Possible relation to oxidative stress. *J Neurol Sci*. 1996; 143:137–142. [PubMed: 8981312]
53. Rogers JT, et al. An iron-responsive element type II in the 5'-untranslated region of the Alzheimer's amyloid precursor protein transcript. *J Biol Chem*. 2002; 277:45518–28. [PubMed: 12198135]

54. Bousejra-ElGarah F, Bijani C, Coppel Y, Faller P, Hureau C. Iron(II) Binding to Amyloid-beta, the Alzheimer's Peptide. *Inorg Chem.* 2011; 50:9024–9030. [PubMed: 21800824]
55. Zecca L, et al. Iron and Other Metals in Neuromelanin, Substantia Nigra, and Putamen of Human Brain. *J Neurochem.* 2008; 62:1097–1101. [PubMed: 8113797]
56. Holmes-Hampton GP, et al. Changing iron content of the mouse brain during development. *Metallomics.* 2012; 4:761–70. [PubMed: 22810488]
57. Manook A, et al. Small-animal PET imaging of amyloid-beta plaques with [¹¹C]PiB and its multi-modal validation in an APP/PS1 mouse model of Alzheimer's disease. *PLoS One.* 2012; 7:e31310. [PubMed: 22427802]
58. Heuer E, Rosen RF, Cintron A, Walker LC. Nonhuman primate models of Alzheimer-like cerebral proteopathy. *Curr Pharm Des.* 2012; 18:1159–69. [PubMed: 22288403]
59. Ha C, Ryu J, Chan BP. Metal ions differentially influence the aggregation and deposition of Alzheimer's β -amyloid on a solid template. *Biochemistry.* 2007; 46:6118–6125. [PubMed: 17455909]
60. Takeda A. Essential trace metals and brain function. *Yakugaku Zasshi.* 2004; 124:577–585. [PubMed: 15340179]
61. Liao CR, et al. Synchrotron FTIR reveals lipid around and within amyloid plaques in transgenic mice and Alzheimer's disease brain. *Analyst.* 2013; 138:3991–7. [PubMed: 23586070]
62. Everett J, et al. Ferrous iron formation following the co-aggregation of ferric iron and the Alzheimer's disease peptide b-amyloid (1–42). *J R Soc Interface.* 2014; 11
63. Dahms SO, et al. Metal binding dictates conformation and function of the amyloid precursor protein (APP) E2 domain. *J Mol Biol.* 2012; 416:438–52. [PubMed: 22245578]
64. Leskovjan, aC; Kretlow, a; Lanzirrotti, a; Miller, LM. Synchrotron-based imaging detects metal and plaques in a mouse model of Alzheimer's disease. *Bioeng Proc Northeast Conf.* 2007:54–55.10.1109/NEBC.2007.4413276
65. Collingwood JF, et al. Three-dimensional tomographic imaging and characterization of iron compounds within Alzheimer's plaque core material. *J Alzheimers Dis.* 2008; 14:235–245. [PubMed: 18560134]
66. Maynard CJ, et al. Overexpression of Alzheimer's disease amyloid- β opposes the age-dependent elevations of brain copper and iron. *J Biol Chem.* 2002; 277:44670–44676. [PubMed: 12215434]
67. Duce JA, et al. Iron-Export Ferroxidase Activity of beta-Amyloid Precursor Protein Is Inhibited by Zinc in Alzheimer's Disease. *Cell.* 2010; 142:857–867. [PubMed: 20817278]
68. Becerril-Ortega J, Bordji K, Freret T, Rush T, Buisson A. Iron overload accelerates neuronal amyloid-beta production and cognitive impairment in transgenic mice model of Alzheimer's disease. *Neurobiol Aging.* 2014; 35:2288–2301. [PubMed: 24863668]
69. White AR, et al. Copper levels are increased in the cerebral cortex and liver of APP and APLP2 knockout mice. *Brain Res.* 1999; 842:439–444. [PubMed: 10526140]
70. Schrag M, Mueller C, Oyoyo U, Smith MA, Kirsch WM. Iron, zinc and copper in the Alzheimer's disease brain: a quantitative meta-analysis. Some insight on the influence of citation bias on scientific opinion. *Prog Neurobiol.* 2011; 94:296–306. [PubMed: 21600264]
71. Hallgren B, Sourander P. The effect of age on the non-haemin iron in the human brain. 1958; 3:41–51.
72. Horowitz MP, Greenamyre JT. Mitochondrial iron metabolism and its role in neurodegeneration. *Journal of Alzheimer's Disease.* 2010; 20
73. Zlokovic BV. Neurovascular mechanisms of Alzheimer's neurodegeneration. *Trends Neurosci.* 2005; 28:202–208. [PubMed: 15808355]
74. Bartzokis G. Alzheimer's disease as homeostatic responses to age-related myelin breakdown. *Neurobiol Aging.* 2011; 32:1341–71. [PubMed: 19775776]
75. Koedam EL, et al. Early-versus late-onset Alzheimer's disease: More than age alone. *J Alzheimer's Dis.* 2010; 19:1401–1408. [PubMed: 20061618]
76. Mullan M, et al. Age of onset in familial early onset Alzheimer's disease correlates with genetic aetiology. *Am J Med Genet.* 1993; 48:129–130. [PubMed: 8291565]

77. Roth GM, Sun B, Greensite FS, Lott IT, Dietrich RB. Premature aging in persons with Down syndrome: MR findings. *Am J Neuroradiol*. 1996; 17:1283–1289. [PubMed: 8871713]
78. Harman D. Aging: a theory based on free radical and radiation chemistry. *J Gerontol*. 1956; 11:298–300. [PubMed: 13332224]
79. Piloni NE, Fernandez V, Videla LA, Puntarulo S. Acute iron overload and oxidative stress in brain. *Toxicology*. 2013; 314:174–82. [PubMed: 24120471]
80. Yakes FM, Van Houten B. Mitochondrial DNA damage is more extensive and persists longer than nuclear DNA damage in human cells following oxidative stress. *Proc Natl Acad Sci*. 1997; 94:514–519. [PubMed: 9012815]
81. Koppenol WH. The Haber-Weiss cycle--70 years later. *Redox Rep*. 2001; 6:229–234. [PubMed: 11642713]
82. Kudin AP, Bimpong-Buta NYB, Vielhaber S, Elger CE, Kunz WS. Characterization of Superoxide-producing Sites in Isolated Brain Mitochondria. *J Biol Chem*. 2004; 279:4127–4135. [PubMed: 14625276]
83. Aliaga ME, Carrasco-Pozo C, López-Alarcón C, Olea-Azar C, Speisky H. Superoxide-dependent reduction of free Fe³⁺ and release of Fe²⁺ from ferritin by the physiologically-occurring Cu(I)-glutathione complex. *Bioorganic Med Chem*. 2011; 19:534–541.
84. Gu Z, Nakamura T, Lipton SA. Redox reactions induced by nitrosative stress mediate protein misfolding and mitochondrial dysfunction in neurodegenerative diseases. *Molecular Neurobiology*. 2010; 41:55–72. [PubMed: 20333559]
85. Lowenstein CJ, Dinerman JL, Snyder SH. Nitric oxide: A physiologic messenger. *Annals of Internal Medicine*. 1994; 120:227–237. [PubMed: 8273987]
86. Kimura H, et al. Hypoxia response element of the human vascular endothelial growth factor gene mediates transcriptional regulation by nitric oxide: control of hypoxia-inducible factor-1 activity by nitric oxide. *Blood*. 2000; 95:189–97. [PubMed: 10607702]
87. Steinert JR, et al. Nitric Oxide Is an Activity-Dependent Regulator of Target Neuron Intrinsic Excitability. *Neuron*. 2011; 71:291–305. [PubMed: 21791288]
88. Austin SA, Santhanam AV, Katusic ZS. Endothelial nitric oxide modulates expression and processing of amyloid precursor protein. *Circ Res*. 2010; 107:1498–502. [PubMed: 21127294]
89. Nakamura T, Lipton SA. S-nitrosylation of critical protein thiols mediates protein misfolding and mitochondrial dysfunction in neurodegenerative diseases. *Antioxid Redox Signal*. 2011; 14:1479–1492. [PubMed: 20812868]
90. Reynolds MR, et al. Tau nitration occurs at tyrosine 29 in the fibrillar lesions of Alzheimer's disease and other tauopathies. *J Neurosci*. 2006; 26:10636–10645. [PubMed: 17050703]
91. Kummer MP, et al. Nitration of tyrosine 10 critically enhances amyloid β aggregation and plaque formation. *Neuron*. 2011; 71:833–844. [PubMed: 21903077]
92. Wilkinson BL, et al. Ibuprofen attenuates oxidative damage through NOX2 inhibition in Alzheimer's disease. *Neurobiol Aging*. 2012; 33
93. Sano M, et al. A Controlled Trial of Selegiline, Alpha-Tocopherol, or Both as Treatment for Alzheimer's Disease. *New England Journal of Medicine*. 1997; 336:1216–1222. [PubMed: 9110909]
94. Um HC, Jang JH, Kim DH, Lee C, Surh YJ. Nitric oxide activates Nrf2 through S-nitrosylation of Keap1 in PC12 cells. *Nitric Oxide - Biology and Chemistry*. 2011; 25:161–168.
95. Mladenka P, Sim nek T, Hübl M, Hrdina R. The role of reactive oxygen and nitrogen species in cellular iron metabolism. *Free Radic Res*. 2006; 40:263–72. [PubMed: 16484042]
96. Caldeira GL, Ferreira IL, Rego AC. Impaired transcription in Alzheimer's disease: key role in mitochondrial dysfunction and oxidative stress. *J Alzheimers Dis*. 2013; 34:115–31. [PubMed: 23364141]
97. Karelson E, et al. The cerebrocortical areas in normal brain aging and in Alzheimer's disease: Noticeable differences in the lipid peroxidation level and in antioxidant defense. *Neurochem Res*. 2001; 26:353–361. [PubMed: 11495345]
98. Makarov P, Kropf S, Wiswedel I, Augustin W, Schild L. Consumption of redox energy by glutathione metabolism contributes to hypoxia/reoxygenation-induced injury in astrocytes. *Mol Cell Biochem*. 2006; 286:95–101. [PubMed: 16583144]

99. Rathnasamy G, Ling EA, Kaur C. Iron and iron regulatory proteins in amoeboid microglial cells are linked to oligodendrocyte death in hypoxic neonatal rat periventricular white matter through production of proinflammatory cytokines and reactive oxygen/nitrogen species. *J Neurosci.* 2011; 31:17982–95. [PubMed: 22159112]
100. Chouraqui E, et al. Deferoxamine blocks death induced by glutathione depletion in PC 12 cells. *Neurotoxicology.* 2013; 37:221–230. [PubMed: 23680049]
101. Kanninen K, et al. Intrahippocampal injection of a lentiviral vector expressing Nrf2 improves spatial learning in a mouse model of Alzheimer's disease. *Proc Natl Acad Sci U S A.* 2009; 106:16505–16510. [PubMed: 19805328]
102. Pardridge WM, Eisenberg J, Yang J. Human blood-brain barrier transferrin receptor. *Metabolism.* 1987; 36:892–895. [PubMed: 3306281]
103. Andrews NC. The iron transporter DMT1. *International Journal of Biochemistry and Cell Biology.* 1999; 31:991–994. [PubMed: 10582331]
104. Connor JR, et al. Profile of altered brain iron acquisition in restless legs syndrome. *Brain.* 2011; 134:959–968. [PubMed: 21398376]
105. Reilly JF, et al. Amyloid deposition in the hippocampus and entorhinal cortex: quantitative analysis of a transgenic mouse model. *Proc Natl Acad Sci U S A.* 2003; 100:4837–42. [PubMed: 12697936]
106. Morris CM, Candy JM, Kerwin JM, Edwardson JA. Transferrin receptors in the normal human hippocampus and in Alzheimer's disease. *Neuropathol Appl Neurobiol.* 1994; 20:473–7. [PubMed: 7845533]
107. Jamieson SE, et al. Candidate gene association study of solute carrier family 11a members 1 (SLC11A1) and 2 (SLC11A2) genes in Alzheimer's disease. *Neurosci Lett.* 2005; 374:124–8. [PubMed: 15644277]
108. Urrutia P, et al. Inflammation alters the expression of DMT1, FPN1 and hepcidin, and it causes iron accumulation in central nervous system cells. *J Neurochem.* 2013; 126:541–549. [PubMed: 23506423]
109. Harrison P. The ferritins: molecular properties, iron storage function and cellular regulation. *Biochim Biophys Acta - Bioenerg.* 1996; 1275:161–203.
110. Macara IG, Hoy TG, Harrison PM. The formation of ferritin from apoferritin. Kinetics and mechanism of iron uptake. *Biochem J.* 1972; 126:151–162. [PubMed: 5075227]
111. Bartzokis G, Tishler TA, Shin IS, Lu PH, Cummings JL. Brain ferritin iron as a risk factor for age at onset in neurodegenerative diseases. *Ann N Y Acad Sci.* 2004; 1012:224–236. [PubMed: 15105269]
112. Jellinger K, Paulus W, Grundke-Iqbal I, Riederer P, Youdim MBH. Brain iron and ferritin in Parkinson's and Alzheimer's diseases. *J Neural Transm - Park Dis Dement Sect.* 1990; 2:327–340. [PubMed: 2078310]
113. Petzold A, et al. Cerebrospinal fluid ferritin level, a sensitive diagnostic test in late-presenting subarachnoid hemorrhage. *J Stroke Cerebrovasc Dis.* 2011; 20:489–93. [PubMed: 20719531]
114. Keir G, Tasdemir N, Thompson EJ. Cerebrospinal fluid ferritin in brain necrosis: evidence for local synthesis. *Clin Chim Acta.* 1993; 216:153–66. [PubMed: 8222266]
115. Busi MV, Gomez-Casati DF. Exploring frataxin function. *IUBMB Life.* 2012; 64:56–63. [PubMed: 22095894]
116. Wiley SE, Murphy AN, Ross SA, van der Geer P, Dixon JE. MitoNEET is an iron-containing outer mitochondrial membrane protein that regulates oxidative capacity. *Proc Natl Acad Sci U S A.* 2007; 104:5318–5323. [PubMed: 17376863]
117. Kusminski CM, et al. MitoNEET-driven alterations in adipocyte mitochondrial activity reveal a crucial adaptive process that preserves insulin sensitivity in obesity. *Nat Med.* 2012; 18:1539–49. [PubMed: 22961109]
118. Singh A, et al. Prion Protein Regulates Iron Transport by Functioning as a Ferrireductase. *J Alzheimers Dis.* 2013; 35:541–552. [PubMed: 23478311]
119. Manson J, et al. The prion protein gene: a role in mouse embryogenesis? *Development.* 1992; 115:117–122. [PubMed: 1353438]

120. Moser M, Colello RJ, Pott U, Oesch B. Developmental expression of the prion protein gene in glial cells. *Neuron*. 1995; 14:509–517. [PubMed: 7695897]
121. Um JW, et al. Alzheimer amyloid- β oligomer bound to postsynaptic prion protein activates Fyn to impair neurons. *Nat Neurosci*. 2012; 15:1227–35. [PubMed: 22820466]
122. Singh A, et al. Abnormal brain iron homeostasis in human and animal prion disorders. *PLoS Pathog*. 2009; 5
123. McCarthy RC, Park YH, Kosman DJ. sAPP modulates iron efflux from brain microvascular endothelial cells by stabilizing the ferrous iron exporter ferroportin. *EMBO Rep*. 2014; 15:809–15. [PubMed: 24867889]
124. Wong BX, et al. β -Amyloid Precursor Protein Does Not Possess Ferroxidase Activity but Does Stabilize the Cell Surface Ferrous Iron Exporter Ferroportin. *PLoS One*. 2014; 9:e114174. [PubMed: 25464026]
125. Nemeth E, et al. Hepcidin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. *Science*. 2004; 306:2090–2093. [PubMed: 15514116]
126. Nicolas G, et al. The gene encoding the iron regulatory peptide hepcidin is regulated by anemia, hypoxia, and inflammation. *J Clin Invest*. 2002; 110:1037–1044. [PubMed: 12370282]
127. Raha AA, Vaishnav RA, Friedland RP, Bomford A, Raha-Chowdhury R. The systemic iron-regulatory proteins hepcidin and ferroportin are reduced in the brain in Alzheimer's disease. *Acta Neuropathol Commun*. 2013; 1:55. [PubMed: 24252754]
128. Hu CJ, Wang LY, Chodosh LA, Keith B, Simon MC. Differential roles of hypoxia-inducible factor 1 α (HIF-1 α) and HIF-2 α in hypoxic gene regulation. *Mol Cell Biol*. 2003; 23:9361–9374. [PubMed: 14645546]
129. Wiesener MS, et al. Widespread hypoxia-inducible expression of HIF-2 α in distinct cell populations of different organs. *FASEB J*. 2003; 17:271–273. [PubMed: 12490539]
130. Chávez JC, Agani F, Pichiule P, LaManna JC. Expression of hypoxia-inducible factor-1 α in the brain of rats during chronic hypoxia. *J Appl Physiol*. 2000; 89:1937–42. [PubMed: 11053346]
131. Kaufmann MR, et al. Dysregulation of hypoxia-inducible factor by presenilin/ γ -secretase loss-of-function mutations. *J Neurosci*. 2013; 33:1915–26. [PubMed: 23365231]
132. Adamcio B, Sperling S, Hagemeyer N, Walkinshaw G, Ehrenreich H. Hypoxia inducible factor stabilization leads to lasting improvement of hippocampal memory in healthy mice. *Behav Brain Res*. 2010; 208:80–4. [PubMed: 19900484]
133. Avramovich-Tirosh Y, Bar-Am O, Amit T, Youdim MBH, Weinreb O. Up-regulation of hypoxia-inducible factor (HIF)-1 α and HIF-target genes in cortical neurons by the novel multifunctional iron chelator anti-Alzheimer drug, M30. *Curr Alzheimer Res*. 2010; 7:300–306. [PubMed: 20043814]
134. Muckenthaler MU, Galy B, Hentze MW. Systemic iron homeostasis and the iron-responsive element/iron-regulatory protein (IRE/IRP) regulatory network. *Annu Rev Nutr*. 2008; 28:197–213. [PubMed: 18489257]
135. Piñero DJ, Hu J, Connor JR. Alterations in the interaction between iron regulatory proteins and their iron responsive element in normal and Alzheimer's diseased brains. *Cell Mol Biol*. 2000; 46:761–776. [PubMed: 10875438]
136. Meyer EP, Ulmann-Schuler A, Staufenbiel M, Krucker T. Altered morphology and 3D architecture of brain vasculature in a mouse model for Alzheimer's disease. *Proc Natl Acad Sci U S A*. 2008; 105:3587–3592. [PubMed: 18305170]
137. Kövari E, Herrmann FR, Hof PR, Bouras C. The relationship between cerebral amyloid angiopathy and cortical microinfarcts in brain ageing and Alzheimer's disease. *Neuropathol Appl Neurobiol*. 2013; 39:498–509. [PubMed: 23163235]
138. Schrag M, et al. Effect of cerebral amyloid angiopathy on brain iron, copper, and zinc in Alzheimer's disease. *J Alzheimers Dis*. 2011; 24:137–149. [PubMed: 21187585]
139. Wilcock DM, Vitek MP, Colton CA. Vascular amyloid alters astrocytic water and potassium channels in mouse models and humans with Alzheimer's disease. *Neuroscience*. 2009; 159:1055–1069. [PubMed: 19356689]

140. Merlini M, Meyer EP, Ulmann-Schuler A, Nitsch RM. Vascular β -amyloid and early astrocyte alterations impair cerebrovascular function and cerebral metabolism in transgenic arcA β mice. *Acta Neuropathol.* 2011; 122:293–311. [PubMed: 21688176]
141. Randolph C, Karantzoulis S, Guskiewicz K. Prevalence and Characterization of Mild Cognitive Impairment in Retired National Football League Players. *J Int Neuropsychol Soc.* 2013; 19:873–880. [PubMed: 23902607]
142. Kiko T, et al. Amyloid β levels in human red blood cells. *PLoS One.* 2012; 7:e49620. [PubMed: 23166730]
143. Misiti F, Carelli-Alinovi C, Sampaiolese B, Giardina B. beta-amyloid decreases detectable endothelial nitric oxide synthase in human erythrocytes: a role for membrane acetylcholinesterase. *Cell Biochem Funct.* 2012; 30:474–479. [PubMed: 22431227]
144. Jayakumar R, et al. Red cell perturbations by amyloid β -protein. *Biochim Biophys Acta - Gen Subj.* 2003; 1622:20–28.
145. Iliff JJ, et al. A paravascular pathway facilitates CSF flow through the brain parenchyma and the clearance of interstitial solutes, including amyloid β . *Sci Transl Med.* 2012; 4:147ra111.
146. Xie L, et al. Sleep drives metabolite clearance from the adult brain. *Science.* 2013; 342:373–7. [PubMed: 24136970]
147. Nandar W, Neely EB, Unger E, Connor JR. A mutation in the HFE gene is associated with altered brain iron profiles and increased oxidative stress in mice. *Biochim Biophys Acta-Molecular Basis Dis.* 2013; 1832:729–741.
148. Mairuae N, Hall EC, Cheepsunthorn P, Lee SY, Connor JR. The H63D HFE gene variant promotes activation of the intrinsic apoptotic pathway via mitochondria dysfunction following β -amyloid peptide exposure. *J Neurosci Res.* 2010; 88:3079–3089. [PubMed: 20734416]
149. Flicek P, et al. Ensembl 2014. *Nucleic Acids Res.* 2014; 42:D749–55. [PubMed: 24316576]
150. Mariani S, et al. Effects of hemochromatosis and transferrin gene mutations on peripheral iron dyshomeostasis in mild cognitive impairment and Alzheimer's and Parkinson's diseases. *Front Aging Neurosci.* 2013; 5:37. [PubMed: 23935582]
151. Loeffler DA, et al. Transferrin and Iron in Normal, Alzheimer's Disease, and Parkinson's Disease Brain Regions. *J Neurochem.* 1995; 65:710–724. [PubMed: 7616227]
152. Barber MF, Elde NC. Nutritional immunity. Escape from bacterial iron piracy through rapid evolution of transferrin. *Science.* 2014; 346:1362–6. [PubMed: 25504720]
153. Lei P, et al. Tau deficiency induces parkinsonism with dementia by impairing APP-mediated iron export. *Nat Med.* 2012; 18:291–5. [PubMed: 22286308]
154. Needham E, Ciccotosto GD, Cappai R. Combined deletions of Amyloid Precursor Protein and Amyloid Precursor-like Protein 2 reveals different effects on mouse brain metal homeostasis. *Metallomics.* 2014:598–603.10.1039/c3mt00358b [PubMed: 24448592]
155. Hanger DP, et al. Tau in Alzheimer's disease and Down's syndrome is insoluble and abnormally phosphorylated. 1991; 275:99–104.
156. Chan A, Shea TB. Dietary and genetically-induced oxidative stress alter tau phosphorylation: influence of folate and apolipoprotein E deficiency. *J Alzheimers Dis.* 2006; 9:399–405. [PubMed: 16917148]
157. Muñoz P, et al. Effect of iron on the activation of the MAPK/ERK pathway in PC12 neuroblastoma cells. *Biological Research.* 2006; 39:189–190. [PubMed: 16629179]
158. Guo JL, Lee VMY. Seeding of normal tau by pathological tau conformers drives pathogenesis of Alzheimer-like tangles. *J Biol Chem.* 2011; 286:15317–15331. [PubMed: 21372138]
159. Clavaguera F, et al. Transmission and spreading of tauopathy in transgenic mouse brain. *Nat Cell Biol.* 2009; 11:909–913. [PubMed: 19503072]
160. De Calignon A, et al. Propagation of Tau Pathology in a Model of Early Alzheimer's Disease. *Neuron.* 2012; 73:685–697. [PubMed: 22365544]
161. Solomon A, Kivipelto M, Wolozin B, Zhou J, Whitmer RA. Midlife serum cholesterol and increased risk of Alzheimer's and vascular dementia three decades later. *Dement Geriatr Cogn Disord.* 2009; 28:75–80. [PubMed: 19648749]

162. Ghribi O, Golovko MY, Larsen B, Schrag M, Murphy EJ. Deposition of iron and β -amyloid plaques is associated with cortical cellular damage in rabbits fed with long-term cholesterol-enriched diets. *J Neurochem*. 2006; 99:438–449. [PubMed: 17029598]
163. Galbete JL, et al. Cholesterol decreases secretion of the secreted form of amyloid precursor protein by interfering with glycosylation in the protein secretory pathway. *Biochem J*. 2000; 348(Pt 2):307–313. [PubMed: 10816423]
164. Raffai RL, Weisgraber KH. Cholesterol: from heart attacks to Alzheimer's disease. *J Lipid Res*. 2003; 44:1423–1430. [PubMed: 12837845]
165. Schneider A, Schulz-Schaeffer W, Hartmann T, Schulz JB, Simons M. Cholesterol depletion reduces aggregation of amyloid-beta peptide in hippocampal neurons. *Neurobiol Dis*. 2006; 23:573–577. [PubMed: 16777421]
166. Sidera C, Parsons R, Austen B. The regulation of beta-secretase by cholesterol and statins in Alzheimer's disease. *J Neurol Sci*. 2005; 229–230:269–273.
167. Wahrle S, et al. Cholesterol-dependent gamma-secretase activity in buoyant cholesterol-rich membrane microdomains. *Neurobiol Dis*. 2002; 9:11–23. [PubMed: 11848681]
168. Popp J, et al. Cerebral and extracerebral cholesterol metabolism and CSF markers of Alzheimer's disease. *Biochem Pharmacol*. 2013; 86:37–42. [PubMed: 23291240]
169. Dasari B, Prasanthi JR, Marwarha G, Singh BB, Ghribi O. The oxysterol 27-hydroxycholesterol increases beta-amyloid and oxidative stress in retinal pigment epithelial cells. *BMC Ophthalmol*. 2010; 10:22. [PubMed: 20836858]
170. Pinilla-Tenas JJ, et al. Zip14 is a complex broad-scope metal-ion transporter whose functional properties support roles in the cellular uptake of zinc and nontransferrin-bound iron. *Am J Physiol Cell Physiol*. 2011; 301:C862–71. [PubMed: 21653899]
171. Singh N. The role of iron in prion disease and other neurodegenerative diseases. *PLoS Pathog*. 2014; 10:e1004335. [PubMed: 25232824]
172. Bandyopadhyay S, et al. Novel 5' Untranslated Region Directed Blockers of Iron-Regulatory Protein-1 Dependent Amyloid Precursor Protein Translation: Implications for Down Syndrome and Alzheimer's Disease. *PLoS One*. 2013; 8:14.
173. Ludwiczek S, et al. Ca²⁺ channel blockers reverse iron overload by a new mechanism via divalent metal transporter-1. *Nat Med*. 2007; 13:448–54. [PubMed: 17293870]
174. Bostanci MO, Bagirici F. Blocking of L-type calcium channels protects hippocampal and nigral neurons against iron neurotoxicity The role of L-type calcium channels in iron-induced neurotoxicity. *Int J Neurosci*. 2013; 123:876–882. [PubMed: 23768064]
175. Pelizzoni I, Zacchetti D, Campanella A, Grohovaz F, Codazzi F. Iron uptake in quiescent and inflammation-activated astrocytes: a potentially neuroprotective control of iron burden. *Biochim Biophys Acta*. 2013; 1832:1326–1333. [PubMed: 23583428]
176. Togo T, Katsuse O, Iseki E. Nitric oxide pathways in Alzheimer's disease and other neurodegenerative dementias. *Neurol Res*. 2004; 26:563–6. [PubMed: 15265275]
177. Lane DJR, Robinson SR, Czerwinska H, Bishop GM, Lawen A. Two routes of iron accumulation in astrocytes: ascorbate-dependent ferrous iron uptake via the divalent metal transporter (DMT1) plus an independent route for ferric iron. *Biochem J*. 2010; 432:123–132. [PubMed: 20819077]
178. Pelizzoni I, Zacchetti D, Campanella A, Grohovaz F, Codazzi F. Iron uptake in quiescent and inflammation-activated astrocytes: A potentially neuroprotective control of iron burden. *Biochim Biophys Acta-Molecular Basis Dis*. 2013; 1832:1326–1333.
179. Fujita T, Tozaki-Saitoh H, Inoue K. P2Y1 receptor signaling enhances neuroprotection by astrocytes against oxidative stress via IL-6 release in hippocampal cultures. *Glia*. 2009; 57:244–257. [PubMed: 18756525]
180. Tasdemir-Yilmaz OE, Freeman MR. Astrocytes engage unique molecular programs to engulf pruned neuronal debris from distinct subsets of neurons. *Genes Dev*. 2014; 28:20–33. [PubMed: 24361692]
181. Zhao J, O'Connor T, Vassar R. The contribution of activated astrocytes to A β production: Implications for Alzheimer's disease pathogenesis. *Journal of Neuroinflammation*. 2011; 8:150. [PubMed: 22047170]

182. Kidane TZ, Sauble E, Linder MC. Release of iron from ferritin requires lysosomal activity. *Am J Physiol Cell Physiol*. 2006; 291:C445–55. [PubMed: 16611735]
183. Schulz K, Kroner A, David S. Iron Efflux from Astrocytes Plays a Role in Remyelination. *Journal of Neuroscience*. 2012; 32:4841–4847. [PubMed: 22492039]
184. Steele ML, Robinson SR. Reactive astrocytes give neurons less support: implications for Alzheimer's disease. *Neurobiol Aging*. 2012; 33:423.e1–13. [PubMed: 21051108]
185. Bélanger M, Allaman I, Magistretti PJ. Brain energy metabolism: focus on astrocyte-neuron metabolic cooperation. *Cell Metab*. 2011; 14:724–38. [PubMed: 22152301]
186. Chih CP, Roberts EL Jr. Energy substrates for neurons during neural activity: a critical review of the astrocyte-neuron lactate shuttle hypothesis. *J Cereb Blood Flow Metab*. 2003; 23:1263–81. [PubMed: 14600433]
187. Parys B, Côté A, Gallo V, De Koninck P, Sfik A. Intercellular calcium signaling between astrocytes and oligodendrocytes via gap junctions in culture. *Neuroscience*. 2010; 167:1032–1043. [PubMed: 20211698]
188. Lundgaard I, Osório MJ, Kress BT, Sanggaard S, Nedergaard M. White matter astrocytes in health and disease. *Neuroscience*. 2014; 276:161–173. [PubMed: 24231735]
189. Suzuki A, et al. Astrocyte-neuron lactate transport is required for long-term memory formation. *Cell*. 2011; 144:810–823. [PubMed: 21376239]
190. Heni M, et al. Insulin promotes glycogen storage and cell proliferation in primary human astrocytes. *PLoS One*. 2011; 6:e21594. [PubMed: 21738722]
191. Karczewska-Kupczewska M, et al. The influence of insulin infusion on the metabolism of amyloid b peptides in plasma. *Alzheimer's Dement*. 2013; 9:400–405. [PubMed: 23110868]
192. Cheng D, et al. Type 2 diabetes and late-onset Alzheimer's disease. *Dement Geriatr Cogn Disord*. 2011; 31:424–430. [PubMed: 21757907]
193. Hsu CC, Wahlqvist ML, Lee MS, Tsai HN. Incidence of dementia is increased in type 2 diabetes and reduced by the use of sulfonylureas and metformin. *J Alzheimer's Dis*. 2011; 24:485–493. [PubMed: 21297276]
194. Farris W, et al. Insulin-degrading enzyme regulates the levels of insulin, amyloid beta-protein, and the beta-amyloid precursor protein intracellular domain in vivo. *Proc Natl Acad Sci U S A*. 2003; 100:4162–4167. [PubMed: 12634421]
195. Morikawa M, et al. Production and characterization of astrocyte-derived human apolipoprotein E isoforms from immortalized astrocytes and their interactions with amyloid- β . *Neurobiol Dis*. 2005; 19:66–76. [PubMed: 15837562]
196. Kanekiyo T, Xu HX, Bu GJ. ApoE and A beta in Alzheimer's Disease: Accidental Encounters or Partners? *Neuron*. 2014; 81:740–754. [PubMed: 24559670]
197. Mahley RW. Apolipoprotein E: cholesterol transport protein with expanding role in cell biology. *Science* (80-). 1988; 240:622–630.
198. Huang Y, Mucke L. Alzheimer mechanisms and therapeutic strategies. *Cell*. 2012; 148:1204–1222. [PubMed: 22424230]
199. Farrer LA, et al. Effects of age, sex, and ethnicity on the association between apolipoprotein E genotype and Alzheimer disease. A meta-analysis APOE and Alzheimer Disease Meta Analysis Consortium. *JAMA*. 1997; 278:1349–1356. [PubMed: 9343467]
200. Conejero-Goldberg C, et al. APOE2 enhances neuroprotection against Alzheimer's disease through multiple molecular mechanisms. *Mol Psychiatry*. 2014; 19:1243–1250. [PubMed: 24492349]
201. Castellano JM, et al. Human apoE isoforms differentially regulate brain amyloid- β peptide clearance. *Sci Transl Med*. 2011; 3:89ra57.
202. Li J, et al. Differential regulation of amyloid- β endocytic trafficking and lysosomal degradation by apolipoprotein E isoforms. *J Biol Chem*. 2012; 287:44593–44601. [PubMed: 23132858]
203. LaDu MJ, et al. Isoform-specific binding of apolipoprotein E to β -amyloid. *J Biol Chem*. 1994; 269:23403–23406. [PubMed: 8089103]
204. Spagnuolo MS, et al. Haptoglobin interacts with apolipoprotein e and Beta-amyloid and influences their crosstalk. *ACS Chem Neurosci*. 2014; 5:837–847. [PubMed: 25058565]

205. Verghese PB, et al. ApoE influences amyloid- β ($A\beta$) clearance despite minimal apoE/ $A\beta$ association in physiological conditions. *Proc Natl Acad Sci U S A*. 2013; 110:E1807–16. [PubMed: 23620513]
206. Bush AI, et al. Evidence for APOE protecting against brain iron overload. *Alzheimer's Dement*. 2014; 10:P878.
207. Saunders AM, et al. Association of apolipoprotein-E allele epsilon-4 with late-onset familial and sporadic alzheimers-disease. *Neurology*. 1993; 43:1467–1472. [PubMed: 8350998]
208. Lee M, et al. Upregulation of haptoglobin in reactive astrocytes after transient forebrain ischemia in rats. *J Cereb Blood Flow Metab*. 2002; 22:1176–1180. [PubMed: 12368655]
209. Kim J, et al. Haploinsufficiency of Human APOE Reduces Amyloid Deposition in a Mouse Model of Amyloid-Amyloidosis. *Journal of Neuroscience*. 2011; 31:18007–18012. [PubMed: 22159114]
210. Meier-Ruge W, Ulrich J, Brühlmann M, Meier E. Age-related white matter atrophy in the human brain. *Ann N Y Acad Sci*. 1992; 673:260–9. [PubMed: 1485724]
211. Raji CA, et al. White matter lesions and brain gray matter volume in cognitively normal elders. *Neurobiol Aging*. 2012; 33:834.e7–16. [PubMed: 21943959]
212. Mapstone M, et al. Plasma phospholipids identify antecedent memory impairment in older adults. *Nat Med*. 2014; 20:415–8. [PubMed: 24608097]
213. Lu F, Li X, Suo AQ, Zhang JW. Inhibition of tau hyperphosphorylation and beta amyloid production in rat brain by oral administration of atorvastatin. *Chin Med J (Engl)*. 2010; 123:1864–1870. [PubMed: 20819569]
214. Kurata T, et al. Atorvastatin and pitavastatin improve cognitive function and reduce senile plaque and phosphorylated tau in aged APP mice. *Brain Res*. 2011; 1371:161–170. [PubMed: 21112317]
215. Han J, et al. Iron uptake mediated by binding of H-ferritin to the TIM-2 receptor in mouse cells. *PLoS One*. 2011; 6:e23800. [PubMed: 21886823]
216. Xu J, et al. Amyloid-beta peptides are cytotoxic to oligodendrocytes. *J Neurosci*. 2001; 21:RC118. [PubMed: 11150354]
217. Skaper SD, et al. Oligodendrocytes are a novel source of amyloid peptide generation. *Neurochem Res*. 2009; 34:2243–50. [PubMed: 19557514]
218. Davalos D, et al. ATP mediates rapid microglial response to local brain injury in vivo. *Nat Neurosci*. 2005; 8:752–758. [PubMed: 15895084]
219. Grundke-Iqbal I, et al. Ferritin is a component of the neuritic (senile) plaque in Alzheimer dementia. *Acta Neuropathol*. 1990; 81:105–110. [PubMed: 2082650]
220. Majumdar A, et al. Activation of microglia acidifies lysosomes and leads to degradation of Alzheimer amyloid fibrils. *Mol Biol Cell*. 2007; 18:1490–1496. [PubMed: 17314396]
221. Su Y, Chang PT. Acidic pH promotes the formation of toxic fibrils from β -amyloid peptide. *Brain Res*. 2001; 893:287–291. [PubMed: 11223020]
222. Quintana C, et al. Study of the localization of iron, ferritin, and hemosiderin in Alzheimer's disease hippocampus by analytical microscopy at the subcellular level. *J Struct Biol*. 2006; 153:42–54. [PubMed: 16364657]
223. Joshi P, et al. Microglia convert aggregated amyloid- β into neurotoxic forms through the shedding of microvesicles. *Cell Death Differ*. 2014; 21:582–93. [PubMed: 24336048]
224. Fitzner D, et al. Selective transfer of exosomes from oligodendrocytes to microglia by macropinocytosis. *J Cell Sci*. 2011; 124:447–58. [PubMed: 21242314]
225. Agosta F, et al. Myeloid microvesicles in cerebrospinal fluid are associated with myelin damage and neuronal loss in mild cognitive impairment and Alzheimer disease. *Ann Neurol*. 2014; 76:813–825. [PubMed: 25087695]
226. Mathiisen TM, Lehre KP, Danbolt NC, Ottersen OP. The perivascular astroglial sheath provides a complete covering of the brain microvessels: An electron microscopic 3D reconstruction. *Glia*. 2010; 58:1094–1103. [PubMed: 20468051]
227. Methia N, et al. ApoE deficiency compromises the blood brain barrier especially after injury. *Mol Med*. 2001; 7:810–815. [PubMed: 11844869]

228. Hafezi-Moghadam A, Thomas KL, Wagner DD. ApoE deficiency leads to a progressive age-dependent blood-brain barrier leakage. *Am J Physiol Cell Physiol.* 2007; 292:C1256–C1262. [PubMed: 16870825]
229. Damoiseaux JS, et al. Gender modulates the APOE ϵ 4 effect in healthy older adults: convergent evidence from functional brain connectivity and spinal fluid tau levels. *J Neurosci.* 2012; 32:8254–62. [PubMed: 22699906]
230. Carroll JC, et al. Sex differences in β -amyloid accumulation in 3xTg-AD mice: role of neonatal sex steroid hormone exposure. *Brain Res.* 2010; 1366:233–45. [PubMed: 20934413]
231. Leskovjan AC, et al. Increased brain iron coincides with early plaque formation in a mouse model of Alzheimer's disease. *Neuroimage.* 2011; 55:32–38. [PubMed: 21126592]
232. Joseph-Mathurin N, et al. Amyloid beta immunization worsens iron deposits in the choroid plexus and cerebral microbleeds. *Neurobiol Aging.* 2013; 34:2613–2622. [PubMed: 23796662]
233. Prior M, Dargusch R, Ehren JL, Chiruta C, Schubert D. The neurotrophic compound J147 reverses cognitive impairment in aged Alzheimer's disease mice. *Alzheimers Res Ther.* 2013; 5:25. [PubMed: 23673233]
234. Bodovitz S, Falduto MT, Frail DE, Klein WL. Iron levels modulate alpha-secretase cleavage of amyloid precursor protein. *J Neurochem.* 1995; 64:307–315. [PubMed: 7798927]
235. Dong J, et al. Engineering metal ion coordination to regulate amyloid fibril assembly and toxicity. *Proc Natl Acad Sci U S A.* 2007; 104:13313–8. [PubMed: 17686982]
236. Hallgren B, Sourander P. The non-haemin iron in the cerebral cortex in Alzheimer's disease. *J Neurochem.* 1960; 5:307–310. [PubMed: 14399117]
237. Jack CR, et al. Hypothetical model of dynamic biomarkers of the Alzheimer's pathological cascade. *Lancet Neurol.* 2010; 9:119–128. [PubMed: 20083042]
238. Jack CR, et al. Tracking pathophysiological processes in Alzheimer's disease: An updated hypothetical model of dynamic biomarkers. *The Lancet Neurology.* 2013; 12:207–216. [PubMed: 23332364]
239. Sperling RA, et al. Toward defining the preclinical stages of Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement.* 2011; 7:280–92. [PubMed: 21514248]
240. Richardson DR. Mysteries of the transferrin-transferrin receptor 1 interaction uncovered. *Cell.* 2004; 116:483–485. [PubMed: 14980214]
241. Urrutia PJ, Mena NP, Núñez MT. The interplay between iron accumulation, mitochondrial dysfunction, and inflammation during the execution step of neurodegenerative disorders. *Frontiers in Pharmacology.* Mar.2014 5
242. Wilkinson N, Pantopoulos K. The IRP/IRE system in vivo: insights from mouse models. *Front Pharmacol.* 2014; 5:176. [PubMed: 25120486]
243. Yamamoto A, et al. Iron (III) induces aggregation of hyperphosphorylated tau and its reduction to iron (II) reverses the aggregation: implications in the formation of neurofibrillary tangles of Alzheimer's disease. *J Neurochem.* 2004; 82:1137–1147. [PubMed: 12358761]
244. Moloney AM, et al. Defects in IGF-1 receptor, insulin receptor and IRS-1/2 in Alzheimer's disease indicate possible resistance to IGF-1 and insulin signalling. *Neurobiol Aging.* 2010; 31:224–243. [PubMed: 18479783]
245. Roth AD, Ramírez G, Alarcón R. Oligodendrocytes damage in Alzheimer's disease: Beta amyloid toxicity and inflammation. 2005:381–387.
246. Desagher S, Glowinski J, Premont J. Astrocytes protect neurons from hydrogen peroxide toxicity. *J Neurosci.* 1996; 16:2553–2562. [PubMed: 8786431]
247. Kristinsson J, Snaedal J, Tórsdóttir G, Jóhannesson T. Ceruloplasmin and iron in Alzheimer's disease and Parkinson's disease: A synopsis of recent studies. *Neuropsychiatric Disease and Treatment.* 2012; 8:515–521. [PubMed: 23144563]
248. Nave KA. Myelination and the trophic support of long axons. *Nat Rev Neurosci.* 2010; 11:275–283. [PubMed: 20216548]
249. Honda S, et al. Extracellular ATP or ADP induce chemotaxis of cultured microglia through Gi/o-coupled P2Y receptors. *J Neurosci.* 2001; 21:1975–1982. [PubMed: 11245682]

250. Doens D, Fernández PL. Microglia receptors and their implications in the response to amyloid β for Alzheimer's disease pathogenesis. *J Neuroinflammation*. 2014; 11:48. [PubMed: 24625061]
251. Khurana V, et al. Lysosomal dysfunction promotes cleavage and neurotoxicity of tau in vivo. *PLoS Genet*. 2010; 6:e1001026. [PubMed: 20664788]

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Figure 1.

The formation of amyloid plaques and the incorporation of iron. Amyloid plaque formation can be characterized by primary and secondary aggregation. During primary formation, ferric iron interacts with beta amyloid as it fluctuates between monomer, dimer, and tetramer formations. Iron-amyloid interactions increase ferrous iron, which can lead to the production of reactive oxygen species. Chronic interaction with iron leads to misfolded globules that can form beta-sheet intermediates and toxic soluble oligomers in the secondary aggregation phase. At a certain threshold of amyloid fibril formation, monomers can propagate the production of toxic soluble, bypassing the primary amyloid formation in a non-linear aggregation phase³⁹. Iron can form coordinated covalent bonds with metal ions²³⁵. Magnetite iron (Fe^{+2} and Fe^{+3}) is believed to become entombed within the dense mature fibril plaque core with hindered redox activity. The diffuse halo of protofibril amyloid beta 40/42 around the core contains ferric and ferrous iron and it is hypothesized that iron in this region is more susceptible to reductive-oxidative cycling. The mature amyloid plaque serves to deplete ferric iron necessary for brain function and propagate ferrous iron that leads to oxidative stress.

Figure 2.

The hypothesized inverse relationship of brain iron elevation and relative cognitive decline in Alzheimer's disease and an aging population. The mean brain iron concentration (black line) sharply increases early in life with little population variance. In midlife brain iron concentration plateaus but population variance expands^{71,236}. Normal mental decline for an aged individual decreases slowly over time. The incidence of Early Onset AD and Late Onset AD correlate with increased iron and variability in the brain^{237–239}.

Figure 3.

The neuron in Alzheimer's disease. **A)** Tf binds to TfR1 with augmentation through HFE interaction. Clathrin coat mediated endocytosis occurs, releasing ferrous iron into the cytoplasm via STEAP-3 reductase and DMT1 present in the endosome. Tf and TfR1 are recycled back the cell surface for further Tf binding²⁴⁰. **B)** Under healthy conditions soluble tau traffics APP to the membrane where it acts to stabilize ferroportin for iron export^{67,124}. **C)** PrPc increases Non-Transferrin-Binding-Iron (NTBI) uptake by acting as a ferrireductase and increasing ferrous iron near the exoplasmic cell membrane¹¹⁸. In AD PrPc binding to amyloid oligomers disinhibits tau expression via the FYN pathway leading to increased APP expression at the cell surface¹²¹. FYN activation also causes reduced NMDA expression, which leads to synapse dysfunction and cell death¹²¹. **D)** Increased cell membrane cholesterol causes elevated β -secretase activity, which promotes $A\beta_{42}$ levels along the amyloidogenic pathway¹⁶⁶. **E)** Mitochondrial proteins help to store and reduce excessive oxidation from the TCA cycle¹¹⁵. Mitochondrial dysfunction is hypothesized to increase the labile iron pool²⁴¹. **F)** High extracellular iron leads to the influx of iron into the neuron via Ca^{2+} channels, leading to toxicity¹⁷⁴. **G)** Ischemia leads to hypoxia mediated HIF and IRP alterations; TfR1 expression is elevated and ferroportin expression is decreased leading to increased iron intake and ROS/RNS production^{99,242}. **H)** Iron induced pTau aggregation throughout the neuron leads to microtubule destabilization and $A\beta$ toxicity^{40,243}.

Figure 4.

The astrocyte in Alzheimer's disease. **A)** Astrocytes acquire iron from NTBI sources, including citrate, ATP and ascorbic acid¹⁷⁷. **B)** Astrocytes are scavenger cells, facilitating the cleanup of high extracellular iron concentrations via Zip, DMT1, VGCC and TRP channels. These importers become more active or upregulated during high extracellular iron conditions in Alzheimer's disease¹⁷⁸. **C)** The astrocyte-neuron shuttle facilitates a lactate energy source for neurons under high activity. This connection is weakened in AD by astrogliosis and microtubule pTau interference¹⁸⁴. **D)** MitoNEET is an iron-containing protein that is an insulin sensor¹¹⁷. Insulin is sensed by the astrocyte, causing it to increase glycogen stores and lactate production. Insulin sensitivity is reduced in AD leading to neuronal dysfunction²⁴⁴. **E)** Astrocytes are a major source of APOE with increased export when exposed to Aβ¹⁹⁶. Haptoglobin secretion is elevated during astrogliosis, facilitating the binding of Aβ to APOE²⁰⁴. **F)** LRP-1, LDLR, and HSPG facilitate APOE endocytosis. Aβ binds to these receptors and blocks APOE endocytosis and metabolism²⁰⁵. The extracellular increase in APOE is hypothesized to be a source of Aβ seeding. **G)** Astroglial cells protect oligodendrocytes exposed to Aβ by reducing ROS or removing Aβ through endocytosis²⁴⁵. **H)** Astrocytes produce the antioxidant glutathione and highly express ferritin. They are known to store high levels of iron while being resilient to ROS/RNS²⁴⁶. Decreased glutathione and ferroportin make astrocytes susceptible to iron toxicity in AD and the aging process. **I)** The ferroxidase ceruloplasmin is primarily expressed by astrocytes which promotes the influx of NTBI. Its activity is decreased in AD leading to an increase in the extracellular ferrous iron pool²⁴⁷. **J)** The astrocytic endfoot serves to regulate iron import

into the healthy brain for which damage to these processes in AD lead to unregulated brain iron import¹⁴⁰.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Figure 5.

The oligodendrocyte in Alzheimer's disease. **A)** Tim2 facilitates ferritin endocytosis and iron release via lysosomal degradation²¹⁵. **B)** Normal breakdown and dysfunction of myelin with age releases iron and impairs trophic support²⁴⁸. Apoptotic oligodendrocytes release ferritin into the extracellular space. Loss of white matter can reduce ferritin storage in AD. **C)** Transferrin serves to transport iron precisely to metabolic regions of myelination. AD oligodendrocytes contain lower transferrin levels and have reduced myelination and remyelination⁹. **D)** Oligodendrocytes express APP and can secrete Aβ₄₂²¹⁷. Production and exposure to these proteins are heightened in demyelination and remyelination pathways. **E)** Loss of white matter in AD leads to neuronal cognitive decline and cell death⁷⁴. **F)** It is likely that ROS from oligodendrocytes can lead to tau destabilization of microtubule mediated axonal trafficking, disrupting axonal transport towards and from synaptic terminals. **G)** Exosome release from damaged oligodendrocytes activate microglia to cause further demyelination²²⁴.

Figure 6.

The microglia in Alzheimer's disease. **A)** Microglia are drawn to the site of amyloidosis and astrogliosis via NTBI ATP and Aβ chemotaxis²⁴⁹. **B)** Microglia recognize Aβ via the CD36-CD47 receptor system leading to the phagocytosis of Aβ fibrils and bound iron²⁵⁰. **C)** Amyloid is normally degraded via the lysosomal pathway and ferrous iron is stored in ferritin²²². In the Alzheimer's disease state, excess iron and amyloid overwhelm the lysosome and the decreased pH provides a suitable environment for amyloid aggregation. **D)** ROS/RNS produced from redox cycling of iron impairs the lysosomal breakdown of Aβ and promotes cleavage and neurotoxicity of tau^{222,251}. **E)** Ferritin can be secreted from microglia through a non-classical secretory pathway. This process is impaired in AD, leading to an imbalance of iron transport to oligodendrocytes. **F)** Microvesicles secreted from activated microglia interact with soluble Aβ to form amyloid oligomers that cause oligodendrotoxicity and neurotoxicity²²³. **G)** Macropinocytosis of exosomes produced by damaged oligodendrocytes causes microglia to facilitate myelin degradation and inflammation²²⁴. **H)** Hypoxic conditions increase IRPs and TFR expression, leading to an increase in ROS and further cytokine release.

Figure 7.

A systemic illustration of cellular iron cycling and amyloid production in the healthy brain.

A) Blood and transferrin bound iron metabolites flow through a microcapillary vessel network. Transferrin receptor is highly expressed on the luminal surface of the vessel endothelial cells and binds to holo-transferrin. Transferrin is transcytosed or endocytosed into the endothelial cell. Ferric iron is released into the interstitial space where it is temporarily bound to astrocyte secreted NTBI factors. It is then reduced by ferrireductases present on astrocyte endfoot processes and imported into the astrocyte via DMT1. **B)** Ferric iron is packed into astrocyte ferritin for temporary storage. **C)** Ferritin is broken down in a late endosome/lysosome to restore ferrous iron into the labile iron pool. **D)** ROS are minimized by the endogenous antioxidant glutathione. **E)** Ferrous iron is transported down astrocyte processes, oxidized to its ferric form by ceruloplasmin, and effluxed via ferroportin. **F)** Transferrin binds to ferric iron and is transported into neurons via clathrin-mediated endocytosis. **G)** Normal neuronal iron causes the post-translational expression of APP to occur via IRP1 inactivity. **H)** APP is cleaved by α -secretase and γ -secretase to produce non-amyloidogenic fragments. β -secretase activity is low due to low cell membrane cholesterol and extracellular iron levels. **I)** APOE is produced from the astrocyte rough endoplasmic reticulum and exported via a secretory pathway. **J)** Amyloid fragments bound to iron complex with APOE and are endocytosed and degraded in the astrocytic or microglial lysosome. **K)** Microglia import iron via transferrin endocytosis and export iron via ferroportin and ferritin exocytosis. **L)** Extracellular ferritin and ferric iron can be endocytosed via oligodendrocyte expressed Tim2 or NTBI import. Oligodendrocytes stabilize axons to provide insulation for action potentials and facilitate myelination-remyelination throughout life. **M)** Iron exits the brain through the ventricular CSF via transferrin, NTBI signaling, or APOE amyloid complexes.

Figure 8.

A systemic illustration of cellular iron cycling and amyloid production in the Alzheimer's disease brain. **A)** Microvascular events in the Alzheimer's brain cause the deposition of A β along the luminal surface. Amyloid deposition occludes vessels and impairs the blood brain barrier allowing red blood cells to penetrate into the parenchyma, causing heme deposits to form near vessel walls. A β in the blood stream binds to and deforms circulating red blood cells causing impaired oxygen saturation. **B)** ROS present at the BBB cause astrocytes to withdraw their endfoot processes while secreting NTBI molecules and upregulating ferrous iron transporters. **C)** The increased influx of ferrous iron in astrocytes upregulate ferritin formation for intracellular iron storage. **D)** Glutathione is reduced in AD, exaggerating the effects of deleterious ROS. Activated astrocytes increase their iron load while expressing cytoplasmic APP. **E)** APOE secretion is elevated facilitating the sequestration of A β_{42} in the extracellular space. **F)** High extracellular iron concentration causes neurons to continually influx iron via NTBI and neuronal iron import. **G)** Neuronal iron export is enhanced through increased post-translational expression of APP which acts to export iron via ferroportin. **H)** Elevated cholesterol in the neuronal cell membrane alters secretase activity to elevate the production of A β_{42} production. **I)** Iron efflux from neuron helps seed A β_{42} progressing into A β fibrils and plaques. Microglia migrate toward activated astrocytes and A β deposits where they phagocytose A β plaques. **J)** Lysosomal degradation of A β plaques increase iron within the microglia which is in turn is incorporated into ferritin along their cytoplasmic border. Glutathione levels are decreased and the ferritin degradation and secretion pathway is impaired. Hemosiderin deposits form inside microglia further impairing their function. **K)**

Reduction of ferritin export impairs oligodendrocytes iron uptake reducing remyelination.

L) In addition, microvesicles communicated between microglia and oligodendrocytes cause myelinotoxicity and increased microglial inflammatory cytokine release. **M)** Demyelination sloughs off high amounts of toxic iron, lipids and cholesterol into the exoplasmic space.

This process also exposes APP present in the myelin sheath, which can propagate amyloidosis. **N)** There is a reciprocal increase of iron proteins and ferrous iron in the CSF and a decrease of amyloid products in the CSF, which may reflect a failure of amyloid clearance out of parenchymal space.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript