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Metallostasis in Alzheimer's disease

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Abstract

2012 has been another year where multiple large scale clinical trials for Alzheimer's disease (AD) have failed to meet their clinical endpoints. With the social and financial burden of this disease increasing every year, the onus is now on the field of AD researchers to investigate alternative ideas in order to deliver outcomes for patients. While several major clinical trials targeting Aβ have failed, three smaller clinical trials targeting metal interactions with Aβ have all shown benefit for patients. Here we review the genetic, pathological, biochemical and pharmacological evidence that underlie the metal hypothesis of AD. The AD-affected brain suffers from metallostasis, or, fatigue of metal trafficking resulting in redistribution of metals into inappropriate compartments. The metal hypothesis is built upon the triad of transition elements: iron, copper, and zinc. The hypothesis has matured from early investigations showing amyloidogenic and oxidative stress consequences of these metals; recently, disease related proteins: APP, tau and presenilin, have been shown to have major roles in metal regulation, which provides insight into the pathway of neurodegeneration in AD and illuminates potential new therapeutic avenues.

Key Words: Alzheimer's disease; Amyloid; Tau; Copper; Zinc; Iron

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Introduction

Alzheimer's disease: an overview

AD is a slowly progressive neurological disorder, where neurodegeneration is believed to progress 20-30 years before clinical onset [1]. The predominant symptoms of AD are impairment to cognition and profound memory loss, which reflects neuropathology observed in the frontal cortex and hippocampus of the brain. Neurofibrillary tangles (NFT) and neuritic senile plaques (SP) are the two pathological hallmarks that define AD. Tau protein is the major component of NFT [2-8]. β-amyloid (Aβ) was shown to be the major component of SP [9-11]; this peptide is produced by cleavage of amyloid precursor protein (APP) [12-14].

APP processing occurs via two different pathways; the nonamyloidogenic and amyloidogenic pathways. The first cleavage event, by either α-secretase or β-secretase, determines whether Aβ is generated; the proceeding cleavage by γ-secretase determines the species of Aβ to be generated. β-secretase activity is contributed mostly by an integral membrane aspartic protease named β-site APP-cleaving enzyme 1(BACE1) [15-17]. γ-secretase is a protease complex consisting of several proteins including presenilin (encoded by *PSEN1* and *2*) which harbours the active site [18-20]. Certain mutations in *APP*, *PSEN1*, and *PSEN2* genes can cause familial AD [23], and the fact that they are involved with Aβ production highlights the importance of this peptide to AD pathogenesis. For sporadic AD, the most significant risk factor (other than age) is the allelic variation of the APOE gene; individuals harbouring the *APOE* ε4 allele have the greatest risk of AD [24]. It is still unknown how ApoE participates in AD pathogenesis, but one possibility is through interaction with Aβ [25-27].

Historic and emerging evidence linking Aβ to AD undoubtedly places this peptide at the centre of AD etiopathology [28]. However, as discussed below, Aβ alone is not sufficient, and is not necessary, to progress the disease.

-Age

The biggest risk factor for AD is age[29]. The disease is never evident in the young, even in the young with disease-causing mutations whom over-express Aβ from birth. Aging is also required to observe cognitive loss, pathology and degeneration in transgenic mice engineered to over-express multiple disease causing genes[30-32]. This means that some phenomenon in the process of aging is required to induce Aβ-mediated neurodegeneration and that Aβ expression alone is not a sufficient cause of disease.

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-Regional specific degeneration

Aβ is produced in all brain regions, and indeed is ubiquitously expressed in the body [33-35]. AD is, however, characterized by discrete and predictable neuronal loss in the temporal lobe, parietal lobe and parts of the frontal cortex and cingulated gyrus [36] while areas such as the visual cortex and cerebellum are spared. It remains unknown why some areas of brain are unaffected by the disease since these areas also produce Aβ. Since Aβ is not sufficient to cause disease in anatomical areas preserved in AD, it is likely that there are factors in the affected areas which are able to either induce Aβ toxicity, or make distinct areas vulnerable to disease.

-Failures of Aβ-targeting drugs in large clinical trials

Owing to the extensive evidence linking Aβ to AD, this peptide has been the target of multiple large-scale clinical trials. Drugs have been used to prevent Aβ production (in multiple ways) and antibodies have targeted Aβ for clearance. No drug that aims to lower Aβ has been effective in preventing cognitive decline in patients with AD [37-40]. Some have argued that we have targeted this disease too late in its progression, and we need to start treatment years before symptoms present. Although this maybe the case, the evidence from these trials suggests that Aβ is not causing continued progression of the disease once symptoms begin, and degeneration from this point onward is likely to be resultant from other factors. Targeting Aβ, therefore, is unlikely to be beneficial to symptomatic patients. Degeneration however, continues in patients with symptoms of disease and there is underexplored opportunity to target other factors that are causing degeneration to continue. Our research group has long argued that fatigue of metal homeostasis in the brain during the aging process causes deleterious metallic reactions in the brain, including the aggregation of Aβ, and may be a major contributor to the expression of AD, and, more importantly, provide a more tractable therapeutic target to ameliorate disease progression.

The involvement of metals in AD: a historic perspective

Aluminium was the first metal linked with neurodegenerative disease. Injecting aluminium into the brain was shown to induce NFTs in animals [41]. Aluminium was shown later to be enriched in AD brains, and specifically in NFTs [42-45] and SPs [46]. Considerable interest in the aluminium hypothesis of AD was generated by a study which showed that aluminium contamination in drinking water increase the risk of AD by 1.5 fold [47]. However, with the

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advance in our knowledge of biochemistry of metals and of AD, the aluminium hypothesis was sidelined. Aluminium is a common contaminant of glassware which could have exaggerated the levels measured in NFTs [43]. Furthermore, aluminium has no known biological function and its concentration within the brain is orders of magnitude less than other bonafide biometals that are essential for brain function, and can also induce Aβ and tau aggregation. Therefore, the metal hypothesis of AD now focuses on a triad of transition metals: zinc [48-50], copper [51-53] and iron [54-57]. This review will discuss the biological and pathological roles of these metals and present a hypothesis that metallostasis in AD, or fatigue in how the brain regulates metals, participates in degeneration.

Zinc

Zinc in neurobiology and its homeostasis in the brain

The brain contains the highest concentrations of zinc within the body [58]. Zinc is an essential component of hundreds of enzymes and transcription factors. Within the healthy brain, the majority of zinc is compartmentalized in membrane-bound metalloproteins (predominantly metallothioneins: MTI,II,III), loosely bound zinc within the cytoplasm, and vesicular zinc enriched in synapses. Synaptic transmission releases high concentrations (1-100μM [59-61]) of zinc into the synapse, which represents a major zinc-efflux mechanism of neurons. Synaptic zinc acts as an antagonist to GABAa [62, 63] and NMDA receptors [59, 64, 65], and activates GPR39 [66], which underlies its functional roles in signal transmission.

Although the uptake of zinc in neurons is not completely defined, the Zrt-, Irt-like Protein (ZIP) members (ZIP1-5, 7-15) are thought to be the major uptake mechanism. Zinc uptake can also be mediated by N-methylD aspartate (NMDA) receptor dependent, volatage gated L-type Ca^{2+} [67], Ca^{2+} permeable AMPA/kainate channels [68] and Na+/Zn^{2+} exchangers [69]. Approximately 50% of zinc uptake requires the AD-linked presenilin protein, however the mechanism of how it contributes to zinc uptake is unknown [70].

Intracellular zinc is sequestered or exported by the ZnT protein family. There are eight known ZnT proteins, however ZnT-2,5,7,8 have low expression within the brain. ZnT-1 is found on the plasma membrane and thus differs from the other ZnT proteins which are expressed on intracellular organelles. ZnT-1 exports zinc from neurons and other cells. ZnT-3 is found in granule, pyramidal, and interneuron cells of the hippocampus and this transports zinc into glutamatergic vesicles [71]. ZnT-4 sequesters cytosolic zinc into acidic vesicles [72] while ZnT-6 sequesters zinc in the trans-Golgi network and vesicular compartments [73].

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Zinc levels in AD brains

Sustained elevation of zinc is potentially dangerous [74] and has been investigated for its role in AD. However, reporting of zinc levels in AD-affected brains has been inconsistent. Early surveys of brain tissue found no difference in zinc levels between AD and controls [75, 76]. Later studies showed a decrease in zinc levels in the neocortex [77] and in the superior frontal and parietal gyri, the medial temporal gyrus and thalamus [78], and the hippocampus [78, 79]. Conflicting reports have however shown elevated zinc in Alzheimer's-affected amygdala [77, 80-82], hippocampus [77, 82], cerebellum [77], olfactory areas [81] and superior temporal gyrus [83]. It is unclear why zinc levels have been reported inconsistently over the years, this may relate to methodological issues, for example, tissue fixation which can affect zinc measurement [84]. Simple bulk tissue analyses, however, is unlikely to capture the full evolvement of zinc in AD. More nuanced analysis of the AD-affected brain has shown altered compartmentalization of zinc.

Zinc mislocalization and dysregulation in AD

Our seminal work regarding zinc in the pathogensis of AD demonstrated enriched zinc levels in amyloid plaque [49]. Further evidence for mis-compartmentalization of zinc in the AD brain was obtained with microparticle-induced x-ray emission tomography which surveyed zinc status in plaque and surrounding neuropil in the amygdala [85]. Using this technique zinc levels in AD neuropil were found to be elevated 2-3 times above that of controls, while plaque contained 3-4 times the amount of zinc compared to the adjacent neuropil. This finding was later corroborated by histochemistry [86], autometallographic tracing [87] and synchrotron-based infrared and X-ray imaging [88]. While there is still debate regarding zinc levels derived from bulk tissue analysis, there is increasing evidence of inappropriate compartmentalization of zinc in AD brains; this is likely to occur by a failure of proteins that control zinc homeostasis.

The cause of this zinc dysregulation in AD remains unknown, but likely involves failure of one or more of these proteins involved in zinc homeostasis. Metallothionein III, which is found on neurons is reduced in AD brains [89, 90], while metallothionein I/II is increased in astrocytes of post mortem AD brains, and pre-clinical AD brains [91]. ZnT-1 levels are elevated in the amygdala, hippocampus, and inferior parietal lobule and decreased in the superior and middle temporal gyrus of AD affect post mortem brains compared to controls [92]. ZnT-4 and ZnT-6 are both increased in the hippocampus of AD post mortem brains

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while ZnT-3 is decreased in AD-affected medial temporal gyrus, superior occipital gyrus, superior parietal gyrus, superior frontal gyrus [93].

The role of zinc in AD pathology

Aβ binds zinc at residues 6-28 [49, 94-96], with up to three zinc ions bound to histidines 6, 13 and 14 [97]. Zinc binding rapidly induces the aggregation of Aβ into insoluble precipitates, which typify AD pathology [49]. The rat and mouse Aβ sequence differs to that of human Aβ by three amino acids which attenuates the affinity for zinc and may underlie why rodents do not develop amyloid pathology [49].

Zinc dysregulation may offer an explanation as to why Aβ deposition is only apparent in AD-affected neocortex but not other areas of the brain, despite its ubiquitous expression. As previously discussed, ZnT-3 loads zinc into glutamatergic synaptic vesicles, and release of high concentrations of labile zinc in the glutamatergic synapse makes it available for interactions with Aβ. To examine the role of synaptic zinc in Aβ deposition, Tg2576 mice (AD mouse model which overexpresses the Swedish mutation of APP) were crossed with ZnT-3 knockout animals. The double mutant exhibited decreased Aβ burden, supporting a role for synaptic zinc in Aβ toxicity [98].

Zinc, therefore, accentuates Aβ toxicity and zinc sequestration into amyloid deposits [49] induces loss of functional zinc in the synapse. Synaptic zinc deficiency is exacerbated in AD by concomitant loss of ZnT-3 expression [85]. Therefore, by two mechanisms labile zinc is made deficient in the brain neuropil in AD. Loss of synaptic zinc likely contributes to cognitive decline in AD since genetic ablation of ZnT-3, which causes loss of zinc in the synaptic compartment, engenders age-dependent cognitive decline in mice [71]. Mis-compartmentilization of zinc in AD likely interferes with signal transduction by NMDA and GABA receptors which may underlie why ZnT-3 knockout mice have cognitive dysfunction. A similar loss of labile synaptic zinc in AD, therefore, could exaggerate cognitive decline.

Zinc may also interfere with APP processing and function. Aβ is produced from proteolytic processing, coordinated by a number of secretases which are regulated by zinc. ADAM requires zinc binding for proteolysis [99]. Zinc also increases the synthesis of presenilin 1 [100], however the activity of the γ-secretase complex is inhibited by zinc [101]. Zinc binding directly to Aβ can mask the proteolytic cleavage site [96] thus inhibiting degradation of Aβ by matrix metalloproteases [102].

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Zinc may also be involved in tau pathology since the metal is enriched in tangle-bearing neurons [86]. Zinc moderates translation of tau and also modulate its phosphoyrlation by affecting the activities of glycogen synthase kinase (GSK)-3β, protein kinase B, ERK1/2 and c-Jun N-terminal kinase [103-105]. Zinc can also directly bind to tau monomers with moderate affinity, altering its conformation [106], and can induce both the fibrillization and aggregation of the protein [107].

Copper

Copper in neurobiology

Copper can undergo redox cycling between Cu^{1+} and Cu^{2+}; this ability has made it useful for many biological functions. Some important copper-containing enzymes include:

- Superoxide dismutase: breaks down superoxide radicals into hydrogen peroxide and oxygen
- Cytochrome c oxidase: Complex IV of the mitochondrial electron transport chain involved in electron transport
- Ceruloplasmin (Cp): oxidises Fe^{2+} to Fe^{3+} which is required for iron export from cells
- Tyrosinase: catalyses melanin and pigment formation

However, the same chemistry that makes copper useful for biology also allows for free radical formation by the Fenton reaction. In this reaction, free copper catalyses the formation of the toxic hydroxyl radical, from physiologically available hydrogen peroxide [108].

The two classic disorders of copper metabolism, Menkes and Willson's diseases, highlight copper as an essential element required for brain health. Menkes disease is caused by mutations in the ATP7A gene which is expressed in all cells except hepatic cells [109]. In the intestine, ATP7A allows for copper absorption, and in other organs, ATP7A facilitates copper influx into the Golgi apparatus for copper incorporation into proteins as required [110]. Individuals with Menkes disease are copper deficient and exhibit developmental delay, seizures and neurodegeneration [111]. On the other hand, Wilson's disease is caused by mutations to the ATP7B gene [112]. ATP7B, like ATP7A, is a p-type ATPase that transports copper into the Golgi apparatus; however unlike ATP7A, ATP7B is predominantly expressed in liver. Liver excretes copper in bile; loss of ATP7B function causes copper accumulation in liver [113]. Liver also secrets Cp, the dominant copper binding protein of plasma, and dysfunction of ATP7B causes loss of Cp and copper in plasma, and a build-up of copper in

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liver [114]. Over time, excess copper in liver is excreted into the blood where it accumulates in other tissue. The neurological complications of Wilson's disease include parkinsonism, dementia and psychosis [115]. Too much or too little copper, therefore, is deleterious to brain health.

Copper dyshomeostasis in AD

As is the case for zinc, copper is mis-localised in AD brains. Copper levels in the affected regions of AD brains are decreased [82, 116] with enrichment in plaques and tangles [117]. Copper is released into the glutamatergic synaptic cleft, facilitated by ATP7A, at concentration of around 15μM[118, 119]. Within the synaptic cleft copper causes s-nitrosylation of NMDA receptors which inhibits their activation [120, 121]. It is hypothesised that loss of neuronal copper in AD occurs by sequestration of copper into plaques by Aβ.

It is now well established that Aβ interacts with copper. Copper directly binds to the Aβ peptide [122, 123], but the specific binding sites are still a subject of investigation. $A\beta_{1-16}$ was shown to be the minimal sequence required for copper binding [124] and has often been studied as a model peptide. The binding between $A\beta_{1-16}$ and copper is pH dependent [125-128]. Between pH 6-7, $A\beta_{1-16}$ binds to copper at Asp1, His6, and His13/14; when at pH 8, the binding sites of $A\beta_{1-16}$ shift to His6, His13 and His14 [126]. At pH10 and higher, Asp1, Ala2, Glu3, and Phe4 can also form a complex with copper [127, 129]. Investigations on longer fragments of Aβ, such as $A\beta_{1-28}$, and full length Aβ, also support the involvement of N-terminal residues, His6, His13 and His14 for Aβ-copper binding [123, 125]. In addition, Tyr10 and Met35 are also suggested to participate in Aβ-copper binding, but with a lower affinity [130, 131]. $A\beta_{1-40}$ and $A\beta_{1-42}$ appear to have different binding affinity to Cu(II) when measured by competitive metal capture analysis. The copper binding site of $A\beta_{1-42}$ has an affinity of $\log K_{app}$=17.2 in contrast to $\log K_{app}$=10.3 for $A\beta_{1-40}$ [132]. In contrast, mouse Aβ lacks a histidine which significantly reduced its ability to bind to copper [133]. Amylin, a protein implicated in type 2 diabetes, shares 80% homology with Aβ [134]. Amylin interacts with insulin [135], and has also been shown to bind to copper with N-terminal residues (Cys2, Cys7,His18) [136].

Copper modifies Aβ and accelerates its aggregation. Aβ purified from human plaques contains less histidine residues and tyrosine residues, which has been explained by copper-mediated oxidation [137]. Copper promotes dityrosine cross-linking of Aβ, which is

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suggested to act as a seed to accelerate Aβ aggregation [138, 139]. The copper induced Aβ oligomer was found to contain a membrane-penetrating structure with histidine bridging[140, 141], highlighting the importance of histidine in the copper-Aβ interaction[141, 142]. Copper binding initiates [143], and induces rapid Aβ aggregation [52]. Unlike zinc, the copper-mediated Aβ aggregation forms oligomers rather than fibrils, which is not recognized by ThT[144-146].

The copper-Aβ complex has been shown to exhibit cytotoxic properties. Aβ toxicity in cell culture is partially dependent on copper [147]. Copper chelation, or competitive binding, ameliorates Aβ– induced cell death in cell culture [148, 149]. Others showed that copper-mediated Aβ toxicity is more pronounced with oligomers prepared from the $A\beta_{1-42}$ peptide, rather than the $A\beta_{1-40}$ species [150]. The mechanism for copper-Aβ toxicity is still a matter of debate, but one possibility involves inhibition of human cytochrome c oxidase [151]. In addition, oxidative stress is also implicated in copper-Aβ toxicity since *in vitro* studies showed that copper and Aβ can generate hydrogen peroxide via a catalytic cycle[53, 152, 153]. In accordance with copper-Aβ binding, histidine and methionine residues of Aβ are suggested to be crucial for exerting Aβ's toxic effects [139, 141, 142].

APP also interacts with copper. In addition to the Aβ segment of the protein, copper binds to APP between residues 142-166 [154-156] and it catalytically reduces copper (II) to copper (I) [51]. APP knockout primary cortical neurons are susceptible to copper-induced toxicity, but copper uptake is not affected [154]. Copper promotes APP internalization [157], and copper deficiency promotes Aβ secretion, but not APP cleavage [157]. Therefore, APP may not directly influence copper homeostasis, but inappropriate interaction with copper may be neurotoxic.

Tau phosphorylation and aggregation can also be induced by copper. Certain fragments in the four-repeat microtubule-binding domain of tau (residues 256-273, 287-304, and 306-336) were shown to aggregate in the presence of copper *in vitro* [158-160]. Microtubule-binding domain residues 287-293 and 310-324 of a 198 amino acid fragment of tau have been shown by NMR to bind copper [161]. Copper binding to tau induces hydrogen peroxide production *in vitro* [162], while NFTs have also been shown to bind copper in a redox-dependent manner, acting as a source for ROS within the neuron [163]. Chronic copper exposure induces tau hyperphosphorylation and promotes tau pathology in a mouse model of AD [164]. The copper-induced phosphorylation of tau is suggested to be mediated by abnormal Cdk5

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activation. However, drugs that deliver copper to the cell have been shown to reduce tau phosphorylation in the APP/PS1 mouse model of AD, possibly through the inhibition of GSK-3 [165].

Iron

Iron biology

Iron is the most abundant element on the Earth [166]. It is hypothesized that the early chemistry of life occurred on mineral sources rich in ferrous iron, sulphide and hydrogen [167], which are fundamental for energy metabolism in biology. Iron is a transition element that can exist in oxidation states from -2 to +8, but in biological systems only ferrous (Fe^{2+}) and ferric (Fe^{3+}) states exist. The cycling between Fe^{3+} to Fe^{2+} is utilized in biology for various electron transfer (redox) reactions essential to life. However, this same chemistry can allow for deleterious reactions with oxygen such as the Fenton reaction where H_2O_2 is catalysed by iron to $OH^{\cdot}$[168] which is a source of oxidative stress [169].

A number of biological processes require iron, including electron transfer reactions [170, 171], transport of oxygen [172], regulation of protein expression [173, 174], cell growth [175] and differentiation [176]. In the brain, iron is involved in development [177], neurotransmitter systems [178], myelination [179] and is required for a number of iron-dependent enzymes [180-182]. Iron is therefore tightly regulated by multiple proteins in the cell, to utilise the element for various functions and to protect against toxicity.

Iron levels in AD

Elevated iron in AD brains was first reported in 1953 [183] and shown to be associated with SP. This initial finding has later confirmed by multiple techniques [57, 184-188]. Iron is enriched in both NFT [54] and SP [183], with the concentration in the latter estimated to be three times that of the normal neuropil level [117]. Iron accumulation occurs in AD cortex, but not cerebellum [56, 189], consistent with the anatomical profile of neurodegeneration in AD. The iron storage protein ferritin binds most iron within the brain [185], and this protein is increases with age and in AD [190]. The iron accumulation in AD could result from a failure of the iron regulatory system, or dysfunctional iron regulating proteins; which is explored below.

Iron regulation in the brain

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Transferrin (Tf), which is produced within the brain by oligodendrocytes [191], is the extracellular iron transporting protein which exchanges iron between cells. Iron-bound Tf (holo-Tf) binds to the transferrin receptor (Tfr) expressed on the cell surface [192]. This Tf/Tfr complex is endocytosed [193], where iron is reduced to its ferrous state by an unknown ferrireductase. Ferrous iron is then able to pass through the endosomal membrane via DMT1 and the imported iron assimilates with a labile iron pool (LIP) in the cytosol [194, 195]. Iron within the LIP is available for incorporation into iron-binding proteins, notably ferritin, the iron storage protein [196]. Excess iron is able to exit the cell by ferroportin (Fpn)[197, 198]. Fpn channels ferrous iron though the plasma membrane [199], however, a ferroxidase partner enzyme is required for iron to be released from the cell. Ferrous iron presented on the cell surface by Fpn is available for uptake by extracellular Tf. Tf, however, has a low affinity for ferrous iron and thus iron requires oxidation by a ferroxidase for removal from the cell by Tf. Ceruloplasmin is the classic ferroxidase [200, 201], but this protein it is not expressed in neurons [202]. Recently, AD-associated APP was identified as the analogous neuronal ferroxidase [56]. APP knockout mice exhibit iron accumulation in brain and peripheral tissues and loss of APP ferroxidase activity in AD brain is coincident with iron retention in the tissue [56].

We recently showed that iron-export capability of APP requires tau [203]. Tau has been implicated in axonal transport [204], and binds APP [205]; loss of tau in mice causes age-dependent iron accumulation [203]. In tau knockout primary cortical neurons, APP was inappropriately trafficked and not present on the extracellular surface where it acts as a ferroxidase. Furthermore, the iron accumulation seen in tau knockout neurons can be restored by supplement of sAPP695α.

Iron dysregulation in AD

Iron elevation in AD brains could be caused by disturbance of the iron regulatory system; indeed a number of iron associated proteins have an altered expression profile in AD. Ferritin has been reported to be elevated in AD and co-localizes with SP [57]. Tf, normally expressed solely by oligodendrocytes, is also expressed in astrocytes in the AD brain [57], and was found to be increased in frontal cortex of AD [206]. DMT1 levels are is increased in the cortex and hippocampus of an AD transgenic model, but its status in AD brains is unknown [207]. Lower Cp expression was found in AD brains [208] as well as its ferroxidase activity in plasma [209]. The amount of APP protein isn't significantly changed in AD cortex, but its ferroxidase activity is significantly reduced [56]. Soluble tau protein level is also reduced in

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AD brains compared to control brains [210-215]. Taken together, it is likely that the iron accumulation observed in AD is a result of multiple failures in its regulatory proteins.

Genetic factors could also increase the susceptibility to iron burden in AD. Tf variant C2 is weakly associated with AD-risk [216-221]. Mutations in the hemochromatosis gene (H63D and C82Y; which cause iron retention in peripheral tissues in affected patients), were also found to be associated with AD-risk [222-224], possibly by interacting with the ApoE gene [225-227] to synergistically increase AD risk [218, 228-230]. These two genes were also found to modify the brain iron levels and memory in a healthy aging study [231]. However, the links between Tf and the hemochromatosis gene with AD was not confirmed in a recent large-scale GWAS [232, 233].

A role for iron in AD pathogenesis

It is hypothesized that the iron burden in AD contributes to oxidative stress in the tissue and can also cause the aggregation of neurodegenerative disease-linked proteins. Iron content is enriched around the SP region [187, 234] and iron promotes Aβ aggregation *in vitro* [235], which is prevented by iron chelation [236]. Iron aggregated Aβ is toxic to cultured cells [237, 238] which was suggested to be mediated by ROS [55], by Fenton chemistry [239], or the activation of Bcl-2 related apoptosis pathway [240]. The redox activity of the iron- Aβ interaction is suggested to facilitate iron binding to His6, His13, and His14 of Aβ [142, 241].

Iron also binds to tau. In fact, an early purification procedure of tau employs an iron-chelating affinity chromatography column [242, 243], clearly indicating binding between tau and iron. Binding of Fe(III), but not Fe(II), induces an aggregation of hyperphosphorylated tau that can be reversed by reducing Fe(III) to Fe(II) [244] or with iron chelators [245]. Within the brain, iron enrichment co-localizes with NFTs in AD and in neurodegeneration with brain iron accumulation (NBIA) [54, 246]. NFTs bind iron which acts as a source for ROS generation within neurons [54, 163]; this can be abolished by iron chelation [247].

Iron has also been shown to affect the phosphorylation status of tau. Treatment of Fe(III) to hippocampal neuron culture resulted in a decrease in tau phosphorylation [248], which corresponded with a decrease CDK5 activity. Conversely, Fe(II) treatment was shown to induce tau phosphorylation [249, 250], without inducing aggregation of the protein. Iron-mediated tau phosphorylation may be caused by up-stream activation of the Erk1/2, MAPK pathway [251, 252].

Metal hypothesis based AD therapeutics

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A disease modifying therapy for AD has been the subject of extensive research and investment. Unfortunately for patients and families, several large-scale phase III trials have failed to reach their clinical endpoints [37-40]. Reducing Aβ levels within the brain, by inhibiting its production or by removing it with immunotherapy, have been the approaches most extensively investigated so far. It is possible that Aβ is too up-stream in the disease process for a drug to alter disease progression in symptomatic patients, and these same drugs might be more efficacious if treatment commenced years before disease-onset. However, there is of course some process occurring within the AD-afflicted brain that is causing it to deteriorate after symptoms present; but evidence from these failed drug trials would suggest that this process does not involve Aβ. Given that targeting Aβ in multiple ways has so far failed to confer clinical benefits, there is a need to target other, more tractable, pathways in the disease.

Clioquinol and PBT2

5-chloro-7-iodo-quinolin-8-ol (Clioquinol) is a derivative of 8-hydroxyquinoline that was wildly used as an anti-parasitic agent for intestinal amebiasis in 1930-1970s. The drug was withdrawn from the worldwide market in 1985, due to a speculated severe side-effect, subacute myelo-optico-neuropathy (SMON), only observed in Japan[253, 254], although this association has been disputed [255]. Later, clioquinol was found to act as moderate chelator of iron, copper and zinc, and it is this activity for which the drug is now best characterized. Oral treatment of clioquinol for 9 weeks in an AD mouse model reduced brain Aβ deposition by 40% [255], while another study showed that clioquinol rescued memory impairment [256]. Clioquinol has been shown to modulate copper efflux activities of APP [257], inhibit Aβ oligomer formation [258, 259], and prevent Aβ-injection induced cell loss [260]. A Phase II clinical trial [261] and a case study [262] reported improvement in cognitive outcomes for patients with AD, however complications with large scale manufacturing of the compound have hindered further exploration for its use in AD.

Clioquinol has also been explored in animal models of other neurodegenerative diseases. Clioquinol protected against dopamine depletion and nigral neuron loss in mice administered the PD toxin, MPTP [263] and tau knockout mice which exhibit an age-dependent PD phenotype [203]. Chronic treatment of clioquinol to transgenic Huntington's mice (R6/2) reduced huntingtin expression and aggregation, prevented striatal atrophy and motor impairment of the mice, and extended their lifespan [264]. Clioquinol was also shown to

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attenuate the effects of general aging. CLK-1 (also known as COQ7), a mitochondrial hydroxylase required for biosynthesis of ubiquinone [265, 266], was shown to be involved in a molecularly defined, and evolutionarily conserved, aging pathway [267-270]. Treatment with clioquinol in cell culture, nematodes, and mice, showed reductions in CLK-1 protein levels and delayed aging in these models, indicating a protective effect of clioquinol against general aging [271]. This effect is possibly mediated by iron since addition of ferrous iron blocked the protective effect of clioquinol [271]. However, toxic effects of clioquinol were reported by some groups [272-274].

Clioquinol has been well studied, but the precise mechanism of action of the drug is unknown. Therapeutic effects of clioquinol have often been attributed to its chelation of copper and zinc [257, 275-277]. However, the affinity of clioquinol for copper and zinc is moderate (Kd_{Cu} is 1.2 x10^{-10} M, Kd_{Zn} is 7x 10^{-8}M) [278], and no *in vivo* data has demonstrated a reduction in copper and zinc levels in the brain of clioquinol-treated animals [255, 279, 280]. More recently, clioquinol has been shown to act as an ionophore which redistributes copper and zinc into the cell [255, 280-284]. In addition, clioquinol is hypothesized to confer neuroprotection by iron chelation, as iron binds to clioquinol [285-287], and a number of the reported beneficial effects of clioquinol are iron-dependent [239, 263, 267, 288, 289]. In addition, clioquinol can affect the PI3K pathway [290-292] which up-regulates matrix metalloprotease activity to promote Aβ degradation [290].

A second generation 8-hydroxyquinoline derivative, PBT2, showed even greater therapeutic effects on an AD mouse model [281] as well as a Phase II clinical trial [293, 294]. The proposed mechanism for neuroprotection of this drug is, by acting as a copper-zinc ionophore, redistributing copper and zinc inside the cell which induces inhibitory phosphorylation of the α- and β-isoforms of glycogen synthase kinase 3 and subsequently lowering Aβ levels [284].

bis(thiosemicarbazone) ligands

Given that the mechanisms of action for both clioquinol and PBT2 likely involve its copper ionophore activity, copper-containing bis(thiosemicarbazone) ligands have been explored for their potential to treat AD. One prototype, Cu^{II}GTSM, delivers copper into neurons [295] and has been show to lower Aβ levels in cell culture [295], restore cognitive performance of APP/PS1 mice in the Y-maze, which reflected changes in GSK3β activity, phosphorylated tau, and Aβ levels within the brain [296]. Another prototype, Cu^{II}ATSM, is more stable and does not release copper within the cell; this compound has not been shown to be beneficial in

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the APP/PS1 mouse model [296]. However, Cu^{II}ATSM has been shown to confer protection in four independent mouse models of PD, possibly by inhibiting peroxynitrite-driven toxicity [297].

Iron chelators

APP has an iron responsive element that induces translation of the protein in high iron environments [173]. Therefore, containing pro-oxidant iron accumulated in the AD afflicted brain by iron chelation likely has the added benefit of reducing Aβ production. Indeed, the iron chelators, (-)-epigallocatechine-3-gallate and M-30 have been shown to reduce APP expression in cultured cells [298, 299]. The classic iron chelator, deferoxamine, inhibits amyloidogenic APP processing in cultured cells and in APP/PS1 mice, which attenuated Aβ burden within the brain and reversed spatial memory impairment. Intramuscular injection of deferoxamine was tested in a single-blind clinical trial of 48 probable AD patients over a 24 month period [300]. The trial was enacted because deferoxamine also binds aluminium, which the researchers were attempting to target. Over the 24 month period, treatment reduced the rate of decline by half compared to the non-treatment group. Although this trial was published in 1991, iron chelation remains an underexplored avenue to prevent decline in AD patients.

Conclusions

Over the past 25 years the AD field at large has understood the disease to be manifestation of abnormally generated toxic species of Aβ and tau, however targeting these species pharmacologically has not conferred benefit to patients so far [37-40]. After well publicized failures of large scale clinical trials, it seems that our field needs an alternative direction. As reviewed here, the role of metals in AD has received extensive investigation. Pathological, biochemical, pharmacological and genetic evidence point to a role for loss of metalostasis in the pathogenesis of disease; however where in the pathway of neurodegeneration metal fatigue occurs is only beginning to be elucidated. It has long been known that metals modulate the aggregation properties of degenerative disease-linked proteins. More recently, the same disease-linked proteins: APP[56], tau[203], and presenilin[70], have been shown to be major players in brain metal homeostasis. It is not of coincidence then, that loss of function of these disease-associated (and now metal-associated) proteins coincides with altered metal compartmentalization in the AD-affected brain [49, 85]. This new data might signify a role for metals more upstream in the disease process than previously thought, and

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might provide new targets for pharmacotherapy. In three small scale clinical trials attempted so far, drugs that target metals have shown positive outcomes for patients[261, 293, 294, 300]. Regardless of where metal fatigue occurs in the pathway of AD pathogenesis, targeting metals likely represents a tractable avenue for an AD disease modifying therapy and approaches targeting metals warrant investigation in large scale clinical trials.

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Figure Legend

Figure 1. Metallostasis in AD. Alzheimer's disease is complicated by fatigue of metal transport mechanisms, which causes redistribution of metals into inappropriate compartments, leading to neurodegeneration. **Zinc**: In health, zinc is transported by ZIP and ZnT proteins and is required for the function of metallothionein and SOD. ZnT3 loads zinc into exosomes where it modulates synaptic neurotransmission by inhibiting NMDAR. In AD, exchangeable synaptic zinc is limited because of reduced ZnT3 levels, and zinc is sequestered into amyloid plaque. **Copper:** In health, copper is transported by CtR1 and ATP7a proteins and is required for the functioning of SOD and metallothionein. In AD, copper is depleted intraneuronally while it is sequestered into amyloid deposits. **Iron**: In health iron is imported by TfR-DMT1 endocytic pathway and is exported via ferroportin. APP is a ferroxidase which is required for iron release from neurons and tau is involved in the trafficking of APP to the neuronal surface. In AD, tau is phosphorylated and APP is inhibited, leading to intraneuronal iron accumulation.

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References

[1] Davies, L.; Wolska, B.; Hilbich, C.; Multhaup, G.; Martins, R.; Simms, G.; Beyreuther, K.; Masters, C. L. A4 amyloid protein deposition and the diagnosis of Alzheimer's disease: prevalence in aged brains determined by immunocytochemistry compared with conventional neuropathologic techniques. *Neurology* **38:**1688-1693; 1988.
[2] Lee, V. M.; Balin, B. J.; Otvos, L., Jr.; Trojanowski, J. Q. A68: a major subunit of paired helical filaments and derivatized forms of normal Tau. *Science* **251:**675-678; 1991.
[3] Goedert, M.; Wischik, C. M.; Crowther, R. A.; Walker, J. E.; Klug, A. Cloning and sequencing of the cDNA encoding a core protein of the paired helical filament of Alzheimer disease: identification as the microtubule-associated protein tau. *Proceedings of the National Academy of Sciences of the United States of America* **85:**4051-4055; 1988.
[4] Wischik, C. M.; Novak, M.; Thogersen, H. C.; Edwards, P. C.; Runswick, M. J.; Jakes, R.; Walker, J. E.; Milstein, C.; Roth, M.; Klug, A. Isolation of a fragment of tau derived from the core of the paired helical filament of Alzheimer disease. *Proceedings of the National Academy of Sciences of the United States of America* **85:**4506-4510; 1988.
[5] Grundke-Iqbal, I.; Iqbal, K.; Tung, Y. C.; Quinlan, M.; Wisniewski, H. M.; Binder, L. I. Abnormal phosphorylation of the microtubule-associated protein tau (tau) in Alzheimer cytoskeletal pathology. *Proceedings of the National Academy of Sciences of the United States of America* **83:**4913-4917; 1986.
[6] Grundke-Iqbal, I.; Iqbal, K.; Quinlan, M.; Tung, Y. C.; Zaidi, M. S.; Wisniewski, H. M. Microtubule-associated protein tau. A component of Alzheimer paired helical filaments. *The Journal of biological chemistry* **261:**6084-6089; 1986.
[7] Delacourte, A.; Defossez, A. Alzheimer's disease: Tau proteins, the promoting factors of microtubule assembly, are major components of paired helical filaments. *Journal of the neurological sciences* **76:**173-186; 1986.
[8] Kosik, K. S.; Joachim, C. L.; Selkoe, D. J. Microtubule-associated protein tau (tau) is a major antigenic component of paired helical filaments in Alzheimer disease. *Proceedings of the National Academy of Sciences of the United States of America* **83:**4044-4048; 1986.
[9] Glenner, G. G.; Wong, C. W. Alzheimer's disease and Down's syndrome: sharing of a unique cerebrovascular amyloid fibril protein. *Biochemical and Biophysical Research Communications* **122:**1131-1135; 1984.
[10] Glenner, G. G.; Wong, C. W. Alzheimer's disease: initial report of the purification and characterization of a novel cerebrovascular amyloid protein. *Biochem. Biophys. Res. Commun.* **120:**885-890; 1984.
[11] Masters, C. L.; Simms, G.; Weinman, N. A.; Multhaup, G.; McDonald, B. L.; Beyreuther, K. Amyloid Plaque Core Protein in Alzheimer Disease and Down Syndrome. *Proceedings of the National Academy of Sciences of the United States of America* **82:**4245-4249; 1985.
[12] Shoji, M.; Golde, T. E.; Ghiso, J.; Cheung, T. T.; Estus, S.; Shaffer, L. M.; Cai, X. D.; McKay, D. M.; Tintner, R.; Frangione, B. Production of the Alzheimer amyloid beta protein by normal proteolytic processing. *Science* **258:**126-129; 1992.
[13] Tanzi, R. E.; Vaula, G.; Romano, D. M.; Mortilla, M.; Huang, T. L.; Tupler, R. G.; Wasco, W.; Hyman, B. T.; Haines, J. L.; Jenkins, B. J.; et al. Assessment of amyloid beta-protein precursor gene mutations in a large set of familial and sporadic Alzheimer disease cases. *American Journal of Human Genetics* **51:**273-282; 1992.
[14] Tanzi, R. E.; Gusella, J. F.; Watkins, P. C.; Bruns, G. A.; St George-Hyslop, P.; Van Keuren, M. L.; Patterson, D.; Pagan, S.; Kurnit, D. M.; Neve, R. L. Amyloid beta protein gene: cDNA, mRNA distribution, and genetic linkage near the Alzheimer locus. *Science* **235:**880-884; 1987.
[15] Vassar, R.; Bennett, B. D.; Babu-Khan, S.; Kahn, S.; Mendiaz, E. A.; Denis, P.; Teplow, D. B.; Ross, S.; Amarante, P.; Loeloff, R.; Luo, Y.; Fisher, S.; Fuller, J.; Edenson, S.; Lile, J.; Jarosinski, M. A.; Biere, A. L.; Curran, E.; Burgess, T.; Louis, J. C.; Collins, F.; Treanor, J.; Rogers, G.; Citron, M. Beta-secretase cleavage of Alzheimer's amyloid precursor protein by the transmembrane aspartic protease BACE. *Science* **286:**735-741; 1999.

Accepted Manuscript

ACCEPTED MANUSCRIPT

[16] Sinha, S.; Anderson, J. P.; Barbour, R.; Basi, G. S.; Caccavello, R.; Davis, D.; Doan, M.; Dovey, H. F.; Frigon, N.; Hong, J.; Jacobson-Croak, K.; Jewett, N.; Keim, P.; Knops, J.; Lieberburg, I.; Power, M.; Tan, H.; Tatsuno, G.; Tung, J.; Schenk, D.; Seubert, P.; Suomensaari, S. M.; Wang, S.; Walker, D.; Zhao, J.; McConlogue, L.; John, V. Purification and cloning of amyloid precursor protein beta-secretase from human brain. *Nature* **402:**537-540; 1999.
[17] Yan, R.; Bienkowski, M. J.; Shuck, M. E.; Miao, H.; Tory, M. C.; Pauley, A. M.; Brashier, J. R.; Stratman, N. C.; Mathews, W. R.; Buhl, A. E.; Carter, D. B.; Tomasselli, A. G.; Parodi, L. A.; Heinrikson, R. L.; Gurney, M. E. Membrane-anchored aspartyl protease with Alzheimer's disease beta-secretase activity. *Nature* **402:**533-537; 1999.
[18] De Strooper, B.; Annaert, W.; Cupers, P.; Saftig, P.; Craessaerts, K.; Mumm, J. S.; Schroeter, E. H.; Schrijvers, V.; Wolfe, M. S.; Ray, W. J.; Goate, A.; Kopan, R. A presenilin-1-dependent gamma-secretase-like protease mediates release of Notch intracellular domain. *Nature* **398:**518-522; 1999.
[19] Wolfe, M. S.; Xia, W.; Ostaszewski, B. L.; Diehl, T. S.; Kimberly, W. T.; Selkoe, D. J. Two transmembrane aspartates in presenilin-1 required for presenilin endoproteolysis and gamma-secretase activity. *Nature* **398:**513-517; 1999.
[20] Li, Y.-m.; Xu, M.; Lai, M. T.; Huang, Q.; Castro, J. L.; DiMuzio-Mower, J.; Harrison, T.; Lellis, C.; Nadin, A.; Neduvelil, J. G.; Register, R. B.; Sardana, M. K.; Shearman, M. S.; Smith, A. L.; Shi, X. P.; Yin, K. C.; Shafer, J. A.; Gardell, S. J. Photoactivated gamma-secretase inhibitors directed to the active site covalently label presenilin 1. *Nature* **405:**689-694; 2000.
[21] Asai, M.; Hattori, C.; Szabo, B.; Sasagawa, N.; Maruyama, K.; Tanuma, S.; Ishiura, S. Putative function of ADAM9, ADAM10, and ADAM17 as APP alpha-secretase. *Biochemical and Biophysical Research Communications* **301:**231-235; 2003.
[22] Lammich, S.; Kojro, E.; Postina, R.; Gilbert, S.; Pfeiffer, R.; Jasionowski, M.; Haass, C.; Fahrenholz, F. Constitutive and regulated alpha-secretase cleavage of Alzheimer's amyloid precursor protein by a disintegrin metalloprotease. *Proceedings of the National Academy of Sciences of the United States of America* **96:**3922-3927; 1999.
[23] Bertram, L.; Lill, C. M.; Tanzi, R. E. The genetics of Alzheimer disease: back to the future. *Neuron* **68:**270-281; 2010.
[24] Farrer, L. A.; Cupples, L. A.; Haines, J. L.; Hyman, B.; Kukull, W. A.; Mayeux, R.; Myers, R. H.; Pericak-Vance, M. A.; Risch, N.; van Duijn, C. M. 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 : the journal of the American Medical Association* **278:**1349-1356; 1997.
[25] Castellano, J. M.; Kim, J.; Stewart, F. R.; Jiang, H.; Demattos, R. B.; Patterson, B. W.; Fagan, A. M.; Morris, J. C.; Mawuenyega, K. G.; Cruchaga, C.; Goate, A. M.; Bales, K. R.; Paul, S. M.; Bateman, R. J.; Holtzman, D. M. Human apoE Isoforms Differentially Regulate Brain Amyloid-{beta} Peptide Clearance. *Science Translational Medicine* **3:**89ra57; 2011.
[26] Cramer, P. E.; Cirrito, J. R.; Wesson, D. W.; Lee, C. Y.; Karlo, J. C.; Zinn, A. E.; Casali, B. T.; Restivo, J. L.; Goebel, W. D.; James, M. J.; Brunden, K. R.; Wilson, D. A.; Landreth, G. E. ApoE-Directed Therapeutics Rapidly Clear beta-Amyloid and Reverse Deficits in AD Mouse Models. *Science*; 2012.
[27] Liu, Q.; Wu, W. H.; Fang, C. L.; Li, R. W.; Liu, P.; Lei, P.; Hu, J.; Sun, X.; Zheng, Y. Z.; Zhao, Y. F.; Li, Y. M. Mapping ApoE/Abeta binding regions to guide inhibitor discovery. *Molecular bioSystems* **7:**1693-1700; 2011.
[28] Hardy, J.; Selkoe, D. J. The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science* **297:**353-356; 2002.
[29] Ferri, C. P.; Prince, M.; Brayne, C.; Brodaty, H.; Fratiglioni, L.; Ganguli, M.; Hall, K.; Hasegawa, K.; Hendrie, H.; Huang, Y.; Jorm, A.; Mathers, C.; Menezes, P. R.; Rimmer, E.; Scazufca, M. Global prevalence of dementia: a Delphi consensus study. *Lancet* **366:**2112-2117; 2005.
[30] Mueggler, T.; Baumann, D.; Rausch, M.; Staufenbiel, M.; Rudin, M. Age-dependent impairment of somatosensory response in the amyloid precursor protein 23 transgenic mouse model of Alzheimer's disease. *J Neurosci* **23:**8231-8236; 2003.

Accepted manuscript

ACCEPTED MANUSCRIPT

[31] Grienberger, C.; Rochefort, N. L.; Adelsberger, H.; Henning, H. A.; Hill, D. N.; Reichwald, J.; Staufenbiel, M.; Konnerth, A. Staged decline of neuronal function in vivo in an animal model of Alzheimer's disease. *Nat Commun* **3:**774; 2012.
[32] Holcomb, L.; Gordon, M. N.; McGowan, E.; Yu, X.; Benkovic, S.; Jantzen, P.; Wright, K.; Saad, I.; Mueller, R.; Morgan, D.; Sanders, S.; Zehr, C.; O'Campo, K.; Hardy, J.; Prada, C. M.; Eckman, C.; Younkin, S.; Hsiao, K.; Duff, K. Accelerated Alzheimer-type phenotype in transgenic mice carrying both mutant amyloid precursor protein and presenilin 1 transgenes. *Nature medicine* **4:**97-100; 1998.
[33] Neve, R. L.; Finch, E. A.; Dawes, L. R. Expression of the Alzheimer amyloid precursor gene transcripts in the human brain. *Neuron* **1:**669-677; 1988.
[34] Spillantini, M. G.; Hunt, S. P.; Ulrich, J.; Goedert, M. Expression and cellular localization of amyloid beta-protein precursor transcripts in normal human brain and in Alzheimer's disease. *Brain Res Mol Brain Res* **6:**143-150; 1989.
[35] Arai, H.; Lee, V. M.; Messinger, M. L.; Greenberg, B. D.; Lowery, D. E.; Trojanowski, J. Q. Expression patterns of beta-amyloid precursor protein (beta-APP) in neural and nonneural human tissues from Alzheimer's disease and control subjects. *Ann Neurol* **30:**686-693; 1991.
[36] Wenk, G. L. Neuropathologic changes in Alzheimer's disease. *The Journal of clinical psychiatry* **64 Suppl 9:**7-10; 2003.
[37] Karran, E.; Mercken, M.; De Strooper, B. The amyloid cascade hypothesis for Alzheimer's disease: an appraisal for the development of therapeutics. *Nature reviews. Drug discovery* **10:**698-712; 2011.
[38] Pfizer Pfizer Announces Co-Primary Clinical Endpoints Not Met In Second Phase 3 Bapineuzumab Study In Mild-To-Moderate Alzheimer's Disease Patients Who Do Not Carry The Apoe4 Genotype. Business Wire; 2012.
[39] Lilly, E. Eli Lilly and Company Announces Top-Line Results on Solanezumab Phase 3 Clinical Trials in Patients with Alzheimer's Disease. Acquire Media; 2012.
[40] Johnson, J. Johnson & Johnson Announces Discontinuation Of Phase 3 Development of Bapineuzumab Intravenous (IV) In Mild-To-Moderate Alzheimer's Disease. Johnson & Johnson; 2012.
[41] Terry, R. D.; Pena, C. Experimental Production of Neurofibrillary Degeneration 2. Electron Microscopy, Phosphatase Histochemistry and Electron Probe Analysis. *Journal of neuropathology and experimental neurology* **24:**200-210; 1965.
[42] Perl, D. P.; Brody, A. R. Alzheimer's disease: X-ray spectrometric evidence of aluminum accumulation in neurofibrillary tangle-bearing neurons. *Science* **208:**297-299; 1980.
[43] Trapp, G. A.; Miner, G. D.; Zimmerman, R. L.; Mastri, A. R.; Heston, L. L. Aluminum levels in brain in Alzheimer's disease. *Biological psychiatry* **13:**709-718; 1978.
[44] McDermott, J. R.; Smith, A. I.; Iqbal, K.; Wisniewski, H. M. Aluminium and Alzheimer's disease. *Lancet* **2:**710-711; 1977.
[45] Crapper, D. R.; Krishnan, S. S.; Dalton, A. J. Brain aluminum distribution in Alzheimer's disease and experimental neurofibrillary degeneration. *Science* **180:**511-513; 1973.
[46] Candy, J. M.; Oakley, A. E.; Klinowski, J.; Carpenter, T. A.; Perry, R. H.; Atack, J. R.; Perry, E. K.; Blessed, G.; Fairbairn, A.; Edwardson, J. A. Aluminosilicates and senile plaque formation in Alzheimer's disease. *Lancet* **1:**354-357; 1986.
[47] Martyn, C. N.; Barker, D. J.; Osmond, C.; Harris, E. C.; Edwardson, J. A.; Lacey, R. F. Geographical relation between Alzheimer's disease and aluminum in drinking water. *Lancet* **1:**59-62; 1989.
[48] Bush, A. I.; Pettingell, W. H.; Deparadis, M.; Tanzi, R. E.; Wasco, W. The Amyloid Beta-Protein Precursor And Its Mammalian Homologs - Evidence For A Zinc-Modulated Heparin-Binding Superfamily. *The Journal of biological chemistry* **269:**26618-26621; 1994.
[49] Bush, A. I.; Pettingell, W. H.; Multhaup, G.; d Paradis, M.; Vonsattel, J. P.; Gusella, J. F.; Beyreuther, K.; Masters, C. L.; Tanzi, R. E. Rapid induction of Alzheimer A beta amyloid formation by zinc. *Science* **265:**1464-1467; 1994.
[50] Bush, A. I.; Pettingell, W. H.; Paradis, M. D.; Tanzi, R. E. Modulation of A beta adhesiveness and secretase site cleavage by zinc. *The Journal of biological chemistry* **269:**12152-12158; 1994.

Accepted manuscript

ACCEPTED MANUSCRIPT

[51] Multhaup, G.; Schlicksupp, A.; Hesse, L.; Beher, D.; Ruppert, T.; Masters, C. L.; Beyreuther, K. The amyloid precursor protein of Alzheimer's disease in the reduction of copper(II) to copper(I). *Science* **271:**1406-1409; 1996.
[52] Atwood, C. S.; Moir, R. D.; Huang, X.; Scarpa, R. C.; Bacarra, N. M.; Romano, D. M.; Hartshorn, M. A.; Tanzi, R. E.; Bush, A. I. Dramatic aggregation of Alzheimer abeta by Cu(II) is induced by conditions representing physiological acidosis. *The Journal of biological chemistry* **273:**12817-12826; 1998.
[53] Huang, X.; Cuajungco, M. P.; Atwood, C. S.; Hartshorn, M. A.; Tyndall, J. D.; Hanson, G. R.; Stokes, K. C.; Leopold, M.; Multhaup, G.; Goldstein, L. E.; Scarpa, R. C.; Saunders, A. J.; Lim, J.; Moir, R. D.; Glabe, C. G.; Bowden, E. F.; Masters, C. L.; Fairlie, D. P.; Tanzi, R. E.; Bush, A. I. Cu(II) potentiation of alzheimer abeta neurotoxicity. Correlation with cell-free hydrogen peroxide production and metal reduction. *The Journal of biological chemistry* **274:**37111-37116; 1999.
[54] Smith, M. A.; Harris, P. L.; Sayre, L. M.; Perry, G. Iron accumulation in Alzheimer disease is a source of redox-generated free radicals. *Proceedings of the National Academy of Sciences of the United States of America* **94:**9866-9868; 1997.
[55] Rottkamp, C. A.; Raina, A. K.; Zhu, X. W.; Gaier, E.; Bush, A. I.; Atwood, C. S.; Chevion, M.; Perry, G.; Smith, M. A. Redox-active iron mediates amyloid-beta toxicity. *Free Radic. Biol. Med.* **30:**447-450; 2001.
[56] Duce, J. A.; Tsatsanis, A.; Cater, M. A.; James, S. A.; Robb, E.; Wikhe, K.; Leong, S. L.; Perez, K. A.; Johanssen, T. J.; Greenough, M. A.; Cho, H.-H.; Galatis, D.; Moir, R. D.; Masters, C. L.; Mclean, C.; Tanzi, R. E.; Cappai, R.; Barnham, K. J.; Ciccotosto, G. D.; Rogers, J. T.; Bush, A. I. Iron-export ferroxidase activity of β-amyloid precursor protein is inhibited by zinc in Alzheimer's disease. *Cell* **142:**857-867; 2010.
[57] Connor, J. R.; Menzies, S. L.; St Martin, S. M.; Mufson, E. J. A histochemical study of iron, transferrin, and ferritin in Alzheimer's diseased brains. *Journal of neuroscience research* **31:**75-83; 1992.
[58] Frederickson, C. J. Neurobiology of zinc and zinc-containing neurons. *International review of neurobiology* **31:**145-238; 1989.
[59] Vogt, K.; Mellor, J.; Tong, G.; Nicoll, R. The actions of synaptically released zinc at hippocampal mossy fiber synapses. *Neuron* **26:**187-196; 2000.
[60] Qian, J.; Noebels, J. L. Visualization of transmitter release with zinc fluorescence detection at the mouse hippocampal mossy fibre synapse. *The Journal of physiology* **566:**747-758; 2005.
[61] Frederickson, C. J.; Giblin, L. J., 3rd; Balaji, R. V.; Masalha, R.; Zeng, Y.; Lopez, E. V.; Koh, J. Y.; Chorin, U.; Besser, L.; Hershfinkel, M.; Li, Y.; Thompson, R. B.; Krezel, A. Synaptic release of zinc from brain slices: factors governing release, imaging, and accurate calculation of concentration. *Journal of neuroscience methods* **154:**19-29; 2006.
[62] Molnar, P.; Nadler, J. V. Lack of effect of mossy fiber-released zinc on granule cell GABA(A) receptors in the pilocarpine model of epilepsy. *Journal of neurophysiology* **85:**1932-1940; 2001.
[63] Ruiz, A.; Walker, M. C.; Fabian-Fine, R.; Kullmann, D. M. Endogenous zinc inhibits GABA(A) receptors in a hippocampal pathway. *Journal of neurophysiology* **91:**1091-1096; 2004.
[64] Paoletti, P.; Ascher, P.; Neyton, J. High-affinity zinc inhibition of NMDA NR1-NR2A receptors. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **17:**5711-5725; 1997.
[65] Traynelis, S. F.; Burgess, M. F.; Zheng, F.; Lyuboslavsky, P.; Powers, J. L. Control of voltage-independent zinc inhibition of NMDA receptors by the NR1 subunit. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **18:**6163-6175; 1998.
[66] Besser, L.; Chorin, E.; Sekler, I.; Silverman, W. F.; Atkin, S.; Russell, J. T.; Hershfinkel, M. Synaptically released zinc triggers metabotropic signaling via a zinc-sensing receptor in the hippocampus. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **29:**2890-2901; 2009.
[67] Koh, J. Y.; Choi, D. W. Zinc toxicity on cultured cortical neurons: involvement of N-methyl-D-aspartate receptors. *Neuroscience* **60:**1049-1057; 1994.
[68] Jia, Y.; Jeng, J. M.; Sensi, S. L.; Weiss, J. H. Zn2+ currents are mediated by calcium-permeable AMPA/kainate channels in cultured murine hippocampal neurones. *The Journal of physiology* **543:**35-48; 2002.

Accepted manuscript

ACCEPTED MANUSCRIPT

[69] Cheng, C.; Reynolds, I. J. Calcium-sensitive fluorescent dyes can report increases in intracellular free zinc concentration in cultured forebrain neurons. *Journal of neurochemistry* **71:**2401-2410; 1998.
[70] Greenough, M. A.; Volitakis, I.; Li, Q. X.; Laughton, K.; Evin, G.; Ho, M.; Dalziel, A. H.; Camakaris, J.; Bush, A. I. Presenilins promote the cellular uptake of copper and zinc and maintain copper chaperone of SOD1-dependent copper/zinc superoxide dismutase activity. *J Biol Chem* **286:**9776-9786; 2011.
[71] Adlard, P. A.; Parncutt, J. M.; Finkelstein, D. I.; Bush, A. I. Cognitive loss in zinc transporter-3 knock-out mice: a phenocopy for the synaptic and memory deficits of Alzheimer's disease? *J Neurosci* **30:**1631-1636; 2010.
[72] Kelleher, S. L.; Lonnerdal, B. Zinc transporters in the rat mammary gland respond to marginal zinc and vitamin A intakes during lactation. *The Journal of nutrition* **132:**3280-3285; 2002.
[73] Huang, L.; Kirschke, C. P.; Gitschier, J. Functional characterization of a novel mammalian zinc transporter, ZnT6. *The Journal of biological chemistry* **277:**26389-26395; 2002.
[74] Koh, J. Y.; Suh, S. W.; Gwag, B. J.; He, Y. Y.; Hsu, C. Y.; Choi, D. W. The role of zinc in selective neuronal death after transient global cerebral ischemia. *Science* **272:**1013-1016; 1996.
[75] Ehmann, W. D.; Markesbery, W.; Alauddin, M. Quantitation, localization and variations of brain zinc with aging by instrumental neutron activation analysis. In: Frederickson, C. J.; Howell, G. A.; Kasarskis, E. J., eds. *Neurobiology of zinc*. New York: Alan R. Liss; 1984: 329-342.
[76] Hershey, L. A.; Hershey, C. O.; Varnes, A.; Songmongkolrit, T.; Strain, W. H. Quantitation, localization and variations of brain zinc with aging by instrumental neutron activation analysis. In: Frederickson, C. J.; Howell, G. A.; Kasarskis, E. J., eds. *The neurobiology of zinc, Part B: Deficiency, toxicity and pathology*. New York: Alan R. Liss; 1984: 325-334.
[77] Danscher, G.; Jensen, K. B.; Frederickson, C. J.; Kemp, K.; Andreasen, A.; Juhl, S.; Stoltenberg, M.; Ravid, R. Increased amount of zinc in the hippocampus and amygdala of Alzheimer's diseased brains: a proton-induced X-ray emission spectroscopic analysis of cryostat sections from autopsy material. *Journal of neuroscience methods* **76:**53-59; 1997.
[78] Panayi, A. E.; Spyrou, N. M.; Iversen, B. S.; White, M. A.; Part, P. Determination of cadmium and zinc in Alzheimer's brain tissue using inductively coupled plasma mass spectrometry. *Journal of the neurological sciences* **195:**1-10; 2002.
[79] Corrigan, F. M.; Reynolds, G. P.; Ward, N. I. Hippocampal tin, aluminum and zinc in Alzheimer's disease. *Biometals* **6:**149-154; 1993.
[80] Thompson, C. M.; Markesbery, W. R.; Ehmann, W. D.; Mao, Y. X.; Vance, D. E. Regional brain trace-element studies in Alzheimer's disease. *Neurotoxicology* **9:**1-7; 1988.
[81] Samudralwar, D. L.; Diprete, C. C.; Ni, B. F.; Ehmann, W. D.; Markesbery, W. R. Elemental imbalances in the olfactory pathway in Alzheimer's disease. *Journal of the neurological sciences* **130:**139-145; 1995.
[82] Deibel, M. A.; Ehmann, W. D.; Markesbery, W. R. Copper, iron, and zinc imbalances in severely degenerated brain regions in Alzheimer's disease: possible relation to oxidative stress. *Journal of the neurological sciences* **143:**137-142; 1996.
[83] Religa, D.; Strozyk, D.; Cherny, R. A.; Volitakis, I.; Haroutunian, V.; Winblad, B.; Naslund, J.; Bush, A. I. Elevated cortical zinc in Alzheimer disease. *Neurology* **67:**69-75; 2006.
[84] Schrag, M.; Dickson, A.; Jiffry, A.; Kirsch, D.; Vinters, H. V.; Kirsch, W. The effect of formalin fixation on the levels of brain transition metals in archived samples. *Biometals* **23:**1123-1127; 2010.
[85] Lovell, M. A.; Robertson, J. D.; Teesdale, W. J.; Campbell, J. L.; Markesbery, W. R. Copper, iron and zinc in Alzheimer's disease senile plaques. *J Neurol Sci* **158:**47-52; 1998.
[86] Suh, S. W.; Jensen, K. B.; Jensen, M. S.; Silva, D. S.; Kesslak, P. J.; Danscher, G.; Frederickson, C. J. Histochemically-reactive zinc in amyloid plaques, angiopathy, and degenerating neurons of Alzheimer's diseased brains. *Brain Research* **852:**274-278; 2000.
[87] Stoltenberg, M.; Bruhn, M.; Sondergaard, C.; Doering, P.; West, M. J.; Larsen, A.; Troncoso, J. C.; Danscher, G. Immersion autometallographic tracing of zinc ions in Alzheimer beta-amyloid plaques. *Histochem Cell Biol* **123:**605-611; 2005.

Accepted manuscript

ACCEPTED MANUSCRIPT

[88] Miller, L. M.; Wang, Q.; Telivala, T. P.; Smith, R. J.; Lanzirotti, A.; Miklossy, J. Synchrotron-based infrared and X-ray imaging shows focalized accumulation of Cu and Zn co-localized with beta-amyloid deposits in Alzheimer's disease. *J Struct Biol* **155:**30-37; 2006.
[89] Uchida, Y.; Takio, K.; Titani, K.; Ihara, Y.; Tomonaga, M. The growth inhibitory factor that is deficient in the Alzheimer's disease brain is a 68 amino acid metallothionein-like protein. *Neuron* **7:**337-347; 1991.
[90] Yu, W. H.; Lukiw, W. J.; Bergeron, C.; Niznik, H. B.; Fraser, P. E. Metallothionein III is reduced in Alzheimer's disease. *Brain Research* **894:**37-45; 2001.
[91] Adlard, P. A.; West, A. K.; Vickers, J. C. Increased density of metallothionein I/II-immunopositive cortical glial cells in the early stages of Alzheimer's disease. *Neurobiol Dis* **5:**349-356; 1998.
[92] Lovell, M. A.; Smith, J. L.; Xiong, S.; Markesbery, W. R. Alterations in zinc transporter protein-1 (ZnT-1) in the brain of subjects with mild cognitive impairment, early, and late-stage Alzheimer's disease. *Neurotox Res* **7:**265-271; 2005.
[93] Beyer, N.; Coulson, D. T.; Heggarty, S.; Ravid, R.; Irvine, G. B.; Hellemans, J.; Johnston, J. A. ZnT3 mRNA levels are reduced in Alzheimer's disease post-mortem brain. *Molecular neurodegeneration* **4:**53; 2009.
[94] Bush, A. I.; Multhaup, G.; Moir, R. D.; Williamson, T. G.; Small, D. H.; Rumble, B.; Pollwein, P.; Beyreuther, K.; Masters, C. L. A novel zinc(II) binding site modulates the function of the beta A4 amyloid protein precursor of Alzheimer's disease. *The Journal of biological chemistry* **268:**16109-16112; 1993.
[95] Bush, A. I.; Pettingell, W. H., Jr.; de Paradis, M.; Tanzi, R. E.; Wasco, W. The amyloid beta-protein precursor and its mammalian homologues. Evidence for a zinc-modulated heparin-binding superfamily. *The Journal of biological chemistry* **269:**26618-26621; 1994.
[96] Bush, A. I.; Pettingell, W. H., Jr.; Paradis, M. D.; Tanzi, R. E. Modulation of A beta adhesiveness and secretase site cleavage by zinc. *J Biol Chem* **269:**12152-12158; 1994.
[97] Damante, C. A.; Osz, K.; Nagy, Z.; Pappalardo, G.; Grasso, G.; Impellizzeri, G.; Rizzarelli, E.; Sovago, I. Metal loading capacity of Abeta N-terminus: a combined potentiometric and spectroscopic study of zinc(II) complexes with Abeta(1-16), its short or mutated peptide fragments and its polyethylene glycol-ylated analogue. *Inorganic Chemistry* **48:**10405-10415; 2009.
[98] Lee, J. Y.; Cole, T. B.; Palmiter, R. D.; Suh, S. W.; Koh, J. Y. Contribution by synaptic zinc to the gender-disparate plaque formation in human Swedish mutant APP transgenic mice. *Proceedings of the National Academy of Sciences of the United States of America* **99:**7705-7710; 2002.
[99] Lammich, S.; Kojro, E.; Postina, R.; Gilbert, S.; Pfeiffer, R.; Jasionowski, M.; Haass, C.; Fahrenholz, F. Constitutive and regulated alpha-secretase cleavage of Alzheimer's amyloid precursor protein by a disintegrin metalloprotease. *Proc Natl Acad Sci U S A* **96:**3922-3927; 1999.
[100] Park, I. H.; Jung, M. W.; Mori, H.; Mook-Jung, I. Zinc enhances synthesis of presenilin 1 in mouse primary cortical culture. *Biochemical and Biophysical Research Communications* **285:**680-688; 2001.
[101] Hoke, D. E.; Tan, J. L.; Ilaya, N. T.; Culvenor, J. G.; Smith, S. J.; White, A. R.; Masters, C. L.; Evin, G. M. In vitro gamma-secretase cleavage of the Alzheimer's amyloid precursor protein correlates to a subset of presenilin complexes and is inhibited by zinc. *Febs J* **272:**5544-5557; 2005.
[102] Crouch, P. J.; Tew, D. J.; Du, T.; Nguyen, D. N.; Caragounis, A.; Filiz, G.; Blake, R. E.; Trounce, I. A.; Soon, C. P.; Laughton, K.; Perez, K. A.; Li, Q. X.; Cherny, R. A.; Masters, C. L.; Barnham, K. J.; White, A. R. Restored degradation of the Alzheimer's amyloid-beta peptide by targeting amyloid formation. *Journal of neurochemistry* **108:**1198-1207; 2009.
[103] An, W. L.; Bjorkdahl, C.; Liu, R.; Cowburn, R. F.; Winblad, B.; Pei, J. J. Mechanism of zinc-induced phosphorylation of p70 S6 kinase and glycogen synthase kinase 3beta in SH-SY5Y neuroblastoma cells. *Journal of neurochemistry* **92:**1104-1115; 2005.
[104] Pei, J. J.; An, W. L.; Zhou, X. W.; Nishimura, T.; Norberg, J.; Benedikz, E.; Gotz, J.; Winblad, B. P70 S6 kinase mediates tau phosphorylation and synthesis. *FEBS Letters* **580:**107-114; 2006.
[105] Lei, P.; Ayton, S.; Bush, A. I.; Adlard, P. A. GSK-3 in Neurodegenerative Diseases. *Int J Alz Dis* **2011:**189246; 2011.

Accepted manuscript

[106] Boom, A.; Authelet, M.; Dedecker, R.; Frederick, C.; Van Heurck, R.; Daubie, V.; Leroy, K.; Pochet, R.; Brion, J. P. Bimodal modulation of tau protein phosphorylation and conformation by extracellular Zn2+ in human-tau transfected cells. *Biochimica et Biophysica Acta* **1793:**1058-1067; 2009.
[107] Mo, Z. Y.; Zhu, Y. Z.; Zhu, H. L.; Fan, J. B.; Chen, J.; Liang, Y. Low micromolar zinc accelerates the fibrillization of human tau via bridging of Cys-291 and Cys-322. *The Journal of biological chemistry* **284:**34648-34657; 2009.
[108] Barnham, K. J.; Masters, C. L.; Bush, A. I. Neurodegenerative diseases and oxidative stress. *Nature reviews. Drug discovery* **3:**205-214; 2004.
[109] Vulpe, C.; Levinson, B.; Whitney, S.; Packman, S.; Gitschier, J. Isolation of a candidate gene for Menkes disease and evidence that it encodes a copper-transporting ATPase. *Nature genetics* **3:**7-13; 1993.
[110] Nyasae, L.; Bustos, R.; Braiterman, L.; Eipper, B.; Hubbard, A. Dynamics of endogenous ATP7A (Menkes protein) in intestinal epithelial cells: copper-dependent redistribution between two intracellular sites. *American journal of physiology. Gastrointestinal and liver physiology* **292:**G1181-1194; 2007.
[111] Kodama, H.; Murata, Y.; Kobayashi, M. Clinical manifestations and treatment of Menkes disease and its variants. *Pediatrics international : official journal of the Japan Pediatric Society* **41:**423-429; 1999.
[112] Bull, P. C.; Thomas, G. R.; Rommens, J. M.; Forbes, J. R.; Cox, D. W. The Wilson disease gene is a putative copper transporting P-type ATPase similar to the Menkes gene. *Nature genetics* **5:**327-337; 1993.
[113] Schilsky, M. L.; Irani, A. N.; Gorla, G. R.; Volenberg, I.; Gupta, S. Biliary copper excretion capacity in intact animals: correlation between ATP7B function, hepatic mass, and biliary copper excretion. *Journal of biochemical and molecular toxicology* **14:**210-214; 2000.
[114] Merle, U.; Weiss, K. H.; Eisenbach, C.; Tuma, S.; Ferenci, P.; Stremmel, W. Truncating mutations in the Wilson disease gene ATP7B are associated with very low serum ceruloplasmin oxidase activity and an early onset of Wilson disease. *BMC gastroenterology* **10:**8; 2010.
[115] Merle, U.; Schaefer, M.; Ferenci, P.; Stremmel, W. Clinical presentation, diagnosis and long-term outcome of Wilson's disease: a cohort study. *Gut* **56:**115-120; 2007.
[116] Magaki, S.; Raghavan, R.; Mueller, C.; Oberg, K. C.; Vinters, H. V.; Kirsch, W. M. Iron, copper, and iron regulatory protein 2 in Alzheimer's disease and related dementias. *Neuroscience letters* **418:**72-76; 2007.
[117] Lovell, M. A.; Robertson, J. D.; Teesdale, W. J.; Campbell, J. L.; Markesbery, W. R. Copper, iron and zinc in Alzheimer's disease senile plaques. *J Neurol Sci* **158:**47-52; 1998.
[118] Hopt, A.; Korte, S.; Fink, H.; Panne, U.; Niessner, R.; Jahn, R.; Kretzschmar, H.; Herms, J. Methods for studying synaptosomal copper release. *Journal of neuroscience methods* **128:**159-172; 2003.
[119] Hartter, D. E.; Barnea, A. Evidence for release of copper in the brain: depolarization-induced release of newly taken-up 67copper. *Synapse (New York)* **2:**412-415; 1988.
[120] Schlief, M. L.; Craig, A. M.; Gitlin, J. D. NMDA receptor activation mediates copper homeostasis in hippocampal neurons. *J Neurosci* **25:**239-246; 2005.
[121] Schlief, M. L.; West, T.; Craig, A. M.; Holtzman, D. M.; Gitlin, J. D. Role of the Menkes copper-transporting ATPase in NMDA receptor-mediated neuronal toxicity. *Proceedings of the National Academy of Sciences of the United States of America* **103:**14919-14924; 2006.
[122] Tõugu, V.; Karafin, A.; Palumaa, P. Binding of zinc(II) and copper(II) to the full-length Alzheimer's amyloid-beta peptide. *Journal of neurochemistry* **104:**1249-1259; 2008.
[123] Hou, L.; Zagorski, M. G. NMR reveals anomalous copper(II) binding to the amyloid Abeta peptide of Alzheimer's disease. *Journal of the American Chemical Society* **128:**9260-9261; 2006.
[124] Minicozzi, V.; Stellato, F.; Comai, M.; Serra, M. D.; Potrich, C.; Meyer-Klaucke, W.; Morante, S. Identifying the minimal copper- and zinc-binding site sequence in amyloid-beta peptides. *The Journal of biological chemistry* **283:**10784-10792; 2008.
[125] Syme, C. D.; Nadal, R. C.; Rigby, S. E. J.; Viles, J. H. Copper binding to the amyloid-beta (Abeta) peptide associated with Alzheimer's disease: folding, coordination geometry, pH dependence,

Accepted manuscript

ACCEPTED MANUSCRIPT

stoichiometry, and affinity of Abeta-(1-28): insights from a range of complementary spectroscopic techniques. *The Journal of biological chemistry* **279:**18169-18177; 2004.
[126] Drew, S. C.; Noble, C. J.; Masters, C. L.; Hanson, G. R.; Barnham, K. J. Pleomorphic copper coordination by Alzheimer's disease amyloid-beta peptide. *Journal of the American Chemical Society* **131:**1195-1207; 2009.
[127] Alies, B.; Eury, H.; Bijani, C.; Rechignat, L.; Faller, P.; Hureau, C. pH-Dependent Cu(II) coordination to amyloid-beta peptide: impact of sequence alterations, including the H6R and D7N familial mutations. *Inorganic Chemistry* **50:**11192-11201; 2011.
[128] Karr, J. W.; Szalai, V. A. Role of aspartate-1 in Cu(II) binding to the amyloid-beta peptide of Alzheimer's disease. *Journal of the American Chemical Society* **129:**3796-3797; 2007.
[129] Dorlet, P.; Gambarelli, S.; Faller, P.; Hureau, C. Pulse EPR spectroscopy reveals the coordination sphere of copper(II) ions in the 1-16 amyloid-beta peptide: a key role of the first two N-terminus residues. *Angewandte Chemie …* **48:**9273-9276; 2009.
[130] Ma, Q. F.; Hu, J.; Wu, W. H.; Liu, H. D.; Du, J. T.; Fu, Y.; Wu, Y. W.; Lei, P.; Zhao, Y. F.; Li, Y. M. Characterization of copper binding to the peptide amyloid-beta(1-16) associated with Alzheimer's disease. *Biopolymers* **83:**20-31; 2006.
[131] Gómez-Balderas, R.; Raffa, D. F.; Rickard, G. A.; Brunelle, P.; Rauk, A. Computational studies of Cu(II)/Met and Cu(I)/Met binding motifs relevant for the chemistry of Alzheimer's disease. *J Phys Chem A* **109:**5498-5508; 2005.
[132] Atwood, C. S.; Scarpa, R. C.; Huang, X.; Moir, R. D.; Jones, W. D.; Fairlie, D. P.; Tanzi, R. E.; Bush, A. I. Characterization of copper interactions with alzheimer amyloid beta peptides: identification of an attomolar-affinity copper binding site on amyloid beta1-42. *Journal of neurochemistry* **75:**1219-1233; 2000.
[133] Eury, H.; Bijani, C.; Faller, P.; Hureau, C. Copper(II) coordination to amyloid beta: murine versus human peptide. *Angew Chem Int Ed Engl* **50:**901-905; 2011.
[134] Janson, J.; Laedtke, T.; Parisi, J. E.; O'Brien, P.; Petersen, R. C.; Butler, P. C. Increased risk of type 2 diabetes in Alzheimer disease. *Diabetes* **53:**474-481; 2004.
[135] Cui, W.; Ma, J.-w.; Lei, P.; Wu, W.-h.; Yu, Y.-p.; Xiang, Y.; Tong, A.-J.; Zhao, Y.-f.; Li, Y.-m. Insulin is a kinetic but not a thermodynamic inhibitor of amylin aggregation. *Febs J* **276:**3365-3371; 2009.
[136] Yu, Y.-p.; Lei, P.; Hu, J.; Wu, W.-h.; Zhao, Y.-f.; Li, Y.-M. Copper-induced cytotoxicity: reactive oxygen species or islet amyloid polypeptide oligomer formation. *Chem Commun* **46:**6909-6911; 2010.
[137] Atwood, C. S.; Huang, X. D.; Khatri, A.; Scarpa, R. C.; Kim, Y. S.; Moir, R. D.; Tanzi, R. E.; Roher, A. E.; Bush, A. I. Copper catalyzed oxidation of alzheimer A beta. *Cell. Mol. Biol.* **46:**777-783; 2000.
[138] Atwood, C. S.; Perry, G.; Zeng, H.; Kato, Y.; Jones, W. D.; Ling, K.-Q.; Huang, X.; Moir, R. D.; Wang, D.; Sayre, L. M.; Smith, M. A.; Chen, S. G.; Bush, A. I. Copper mediates dityrosine cross-linking of Alzheimer's amyloid-beta. *Biochemistry* **43:**560-568; 2004.
[139] Ali, F. E.; Separovic, F.; Barrow, C. J.; Cherny, R. A.; Fraser, F.; Bush, A. I.; Masters, C. L.; Barnham, K. J. Methionine regulates copper/hydrogen peroxide oxidation products of Abeta. *J Pept Sci* **11:**353-360; 2005.
[140] Curtain, C. C.; Ali, F. E.; Volitaskis, I.; Cherny, R. A.; Norton, R. S.; Beyreuther, K.; Barrow, C. J.; Masters, C. L.; Bush, A. I.; Barnham, K. J. Alzheimer's disease amyloid-beta binds copper and zinc to generate an allosterically ordered membrane-penetrating structure containing superoxide dismutase-like subunits. *The Journal of biological chemistry* **276:**20466-20473; 2001.
[141] Smith, D. P.; Smith, D. G.; Curtain, C. C.; Boas, J. F.; Pilbrow, J. R.; Ciccotosto, G. D.; Lau, T.-L.; Tew, D. J.; Perez, K. A.; Wade, J. D.; Bush, A. I.; Drew, S. C.; Separovic, F.; Masters, C. L.; Cappai, R.; Barnham, K. J. Copper-mediated amyloid-beta toxicity is associated with an intermolecular histidine bridge. *The Journal of biological chemistry* **281:**15145-15154; 2006.
[142] Nakamura, M.; Shishido, N.; Nunomura, A.; Smith, M. A.; Perry, G.; Hayashi, Y.; Nakayama, K.; Hayashi, T. Three histidine residues of amyloid-beta peptide control the redox activity of copper and iron. *Biochemistry* **46:**12737-12743; 2007.
[143] Talmard, C.; Guilloreau, L.; Coppel, Y.; Mazarguil, H.; Faller, P. Amyloid-beta peptide forms monomeric complexes with Cu(II) and Zn(II) prior to aggregation. *Chembiochem* **8:**163-165; 2007.

Accepted manuscript

ACCEPTED MANUSCRIPT

[144] Jiao, Y.; Han, D. X.; Yang, P. Molecular modeling of the inhibitory mechanism of copper(II) on aggregation of amyloid beta-peptide. *Sci China Ser B* **48:**580-590; 2005.
[145] Jiao, Y.; Yang, P. Mechanism of copper(II) inhibiting Alzheimer's amyloid beta-peptide from aggregation: a molecular dynamics investigation. *Journal of Physical Chemistry B* **111:**7646-7655; 2007.
[146] Tõugu, V.; Karafin, A.; Zovo, K.; Chung, R. S.; Howells, C.; West, A. K.; Palumaa, P. Zn(II)- and Cu(II)-induced non-fibrillar aggregates of amyloid-beta (1-42) peptide are transformed to amyloid fibrils, both spontaneously and under the influence of metal chelators. *Journal of neurochemistry* **110:**1784-1795; 2009.
[147] You, H.; Tsutsui, S.; Hameed, S.; Kannanayakal, T. J.; Chen, L.; Xia, P.; Engbers, J. D. T.; Lipton, S. A.; Stys, P. K.; Zamponi, G. W. Aβ neurotoxicity depends on interactions between copper ions, prion protein, and N-methyl-D-aspartate receptors. *Proc Natl Acad Sci USA* **109:**1737-1742; 2012.
[148] Wu, W.-h.; Lei, P.; Liu, Q.; Hu, J.; Gunn, A. P.; Chen, M.-s.; Rui, Y.-f.; Su, X.-y.; Xie, Z.-p.; Zhao, Y.-f.; Bush, A. I.; Li, Y.-m. Sequestration of copper from beta-amyloid promotes selective lysis by cyclen-hybrid cleavage agents. *The Journal of biological chemistry* **283:**31657-31664; 2008.
[149] Perrone, L.; Mothes, E.; Vignes, M.; Mockel, A.; Figueroa, C.; Miquel, M.-C.; Maddelein, M.-L.; Faller, P. Copper transfer from Cu-Abeta to human serum albumin inhibits aggregation, radical production and reduces Abeta toxicity. *Chembiochem* **11:**110-118; 2010.
[150] Jin, L.; Wu, W. H.; Li, Q. Y.; Zhao, Y. F.; Li, Y. M. Copper inducing Abeta42 rather than Abeta40 nanoscale oligomer formation is the key process for Abeta neurotoxicity. *Nanoscale* **3:**4746-4751; 2011.
[151] Crouch, P. J.; Blake, R.; Duce, J. A.; Ciccotosto, G. D.; Li, Q. X.; Barnham, K. J.; Curtain, C. C.; Cherny, R. A.; Cappai, R.; Dyrks, T.; Masters, C. L.; Trounce, I. A. Copper-dependent inhibition of human cytochrome c oxidase by a dimeric conformer of amyloid-beta(1-42). *The Journal of neuroscience : the official journal of the Society for Neuroscience* **25:**672-679; 2005.
[152] Huang, X.; Atwood, C. S.; Hartshorn, M. A.; Multhaup, G.; Goldstein, L. E.; Scarpa, R. C.; Cuajungco, M. P.; Gray, D. N.; Lim, J.; Moir, R. D.; Tanzi, R. E.; Bush, A. I. The A beta peptide of Alzheimer's disease directly produces hydrogen peroxide through metal ion reduction. *Biochemistry* **38:**7609-7616; 1999.
[153] Jiang, D.; Men, L.; Wang, J.; Zhang, Y.; Chickenyen, S.; Wang, Y.; Zhou, F. Redox reactions of copper complexes formed with different beta-amyloid peptides and their neuropathological [correction of neuropathalogical] relevance. *Biochemistry* **46:**9270-9282; 2007.
[154] White, A. R.; Multhaup, G.; Maher, F.; Bellingham, S.; Camakaris, J.; Zheng, H.; Bush, A. I.; Beyreuther, K.; Masters, C. L.; Cappai, R. The Alzheimer's disease amyloid precursor protein modulates copper-induced toxicity and oxidative stress in primary neuronal cultures. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **19:**9170-9179; 1999.
[155] Barnham, K. J.; McKinstry, W. J.; Multhaup, G.; Galatis, D.; Morton, C. J.; Curtain, C. C.; Williamson, N. A.; White, A. R.; Hinds, M. G.; Norton, R. S.; Beyreuther, K.; Masters, C. L.; Parker, M. W.; Cappai, R. Structure of the Alzheimer's disease amyloid precursor protein copper binding domain. A regulator of neuronal copper homeostasis. *The Journal of biological chemistry* **278:**17401-17407; 2003.
[156] Cappai, R.; Kong, G.; McKinstry, W.; Galatis, D.; Adams, J.; Bellingham, S.; Camakaris, J.; Curtain, C.; Williamson, N.; White, A. Structural and functional analysis of the Alzheimer's disease amyloid precursor protein copper binding domain. *Neurobiology Of Aging* **25:**S60-S60; 2004.
[157] Acevedo, K. M.; Hung, Y. H.; Dalziel, A. H.; Li, Q. X.; Laughton, K.; Wikhe, K.; Rembach, A.; Roberts, B.; Masters, C. L.; Bush, A. I.; Camakaris, J. Copper promotes the trafficking of the amyloid precursor protein. *The Journal of biological chemistry* **286:**8252-8262; 2011.
[158] Ma, Q. F.; Li, Y. M.; Du, J. T.; Kanazawa, K.; Nemoto, T.; Nakanishi, H.; Zhao, Y. F. Binding of copper (II) ion to an Alzheimer's tau peptide as revealed by MALDI-TOF MS, CD, and NMR. *Biopolymers* **79:**74-85; 2005.
[159] Ma, Q.; Li, Y.; Du, J.; Liu, H.; Kanazawa, K.; Nemoto, T.; Nakanishi, H.; Zhao, Y. Copper binding properties of a tau peptide associated with Alzheimer's disease studied by CD, NMR, and MALDI-TOF MS. *Peptides* **27:**841-849; 2006.

Accepted manuscript

ACCEPTED MANUSCRIPT

[160] Zhou, L. X.; Du, J. T.; Zeng, Z. Y.; Wu, W. H.; Zhao, Y. F.; Kanazawa, K.; Ishizuka, Y.; Nemoto, T.; Nakanishi, H.; Li, Y. M. Copper (II) modulates in vitro aggregation of a tau peptide. *Peptides* **28:**2229-2234; 2007.
[161] Soragni, A.; Zambelli, B.; Mukrasch, M. D.; Biernat, J.; Jeganathan, S.; Griesinger, C.; Ciurli, S.; Mandelkow, E.; Zweckstetter, M. Structural characterization of binding of Cu(II) to tau protein. *Biochemistry* **47:**10841-10851; 2008.
[162] Su, X. Y.; Wu, W. H.; Huang, Z. P.; Hu, J.; Lei, P.; Yu, C. H.; Zhao, Y. F.; Li, Y. M. Hydrogen peroxide can be generated by tau in the presence of Cu(II). *Biochemical and Biophysical Research Communications* **358:**661-665; 2007.
[163] Sayre, L. M.; Perry, G.; Harris, P. L.; Liu, Y.; Schubert, K. A.; Smith, M. A. In situ oxidative catalysis by neurofibrillary tangles and senile plaques in Alzheimer's disease: a central role for bound transition metals. *Journal of neurochemistry* **74:**270-279; 2000.
[164] Kitazawa, M.; Cheng, D.; Laferla, F. M. Chronic copper exposure exacerbates both amyloid and tau pathology and selectively dysregulates cdk5 in a mouse model of AD. *Journal of neurochemistry* **108:**1550-1560; 2009.
[165] Crouch, P. J.; Hung, L. W.; Adlard, P. A.; Cortes, M.; Lal, V.; Filiz, G.; Perez, K. A.; Nurjono, M.; Caragounis, A.; Du, T.; Laughton, K. M.; Volitaskis, I.; Bush, A. I.; Li, Q.-X.; Masters, C. L.; Cappai, R.; Cherny, R. A.; Donnelly, P. S.; White, A. R.; Barnham, K. J. Increasing Cu bioavailability inhibits Abeta oligomers and tau phosphorylation. *Proc Natl Acad Sci USA* **106:**381-386; 2009.
[166] Sheftel, A. D.; Mason, A. B.; Ponka, P. The long history of iron in the Universe and in health and disease. *Biochimica et Biophysica Acta* **1820:**161-187; 2012.
[167] Cody, G. D.; Boctor, N. Z.; Filley, T. R.; Hazen, R. M.; Scott, J. H.; Sharma, A.; Yoder, H. S., Jr. Primordial carbonylated iron-sulfur compounds and the synthesis of pyruvate. *Science* **289:**1337-1340; 2000.
[168] Fenton, J. M.; Pellin, M. J.; Govindjee; kaufmann, K. J. Primary photochemistry of the reaction center of photosystem I. *FEBS Letters* **100:**1-4; 1979.
[169] Zigler, J. S., Jr.; Jernigan, H. M., Jr.; Garland, D.; Reddy, V. N. The effects of "oxygen radicals" generated in the medium on lenses in organ culture: inhibition of damage by chelated iron. *Archives of biochemistry and biophysics* **241:**163-172; 1985.
[170] Lenhoff, H. M.; Nicholas, D. J.; Kaplan, N. O. Effects of oxygen, iron, and molybdenum on routes of electron transfer in Pseudomonas fluorescens. *The Journal of biological chemistry* **220:**983-995; 1956.
[171] Malkin, R.; Rabinowitz, J. C. Nonheme iron electron-transfer proteins. *Annual Review of Biochemistry* **36:**113-148; 1967.
[172] Sanders-Loehr, J. Involvement of oxo-bridged binuclear iron centers in oxygen transport, oxygen reduction, and oxygenation. *Progress in Clinical and Biological Research* **274:**193-209; 1988.
[173] Rogers, J. T.; Randall, J. D.; Cahill, C. M.; Eder, P. S.; Huang, X.; Gunshin, H.; Leiter, L.; McPhee, J.; Sarang, S. S.; Utsuki, T.; Greig, N. H.; Lahiri, D. K.; Tanzi, R. E.; Bush, A. I.; Giordano, T.; Gullans, S. R. An iron-responsive element type II in the 5'-untranslated region of the Alzheimer's amyloid precursor protein transcript. *The Journal of biological chemistry* **277:**45518-45528; 2002.
[174] Friedlich, A. L.; Tanzi, R. E.; Rogers, J. T. The 5'-untranslated region of Parkinson's disease alpha-synuclein messengerRNA contains a predicted iron responsive element. *Mol Psychiatry* **12:**222-223; 2007.
[175] Verger, C.; Imbenotte, J. Decreased heme content and cessation of cell growth in cultured chick embryo fibroblasts in the presence of horse serum: stimulation of heme synthesis and cell growth by iron. *Journal of Cellular Physiology* **113:**193-196; 1982.
[176] Landschulz, W.; Thesleff, I.; Ekblom, P. A lipophilic iron chelator can replace transferrin as a stimulator of cell proliferation and differentiation. *Journal of Cell Biology* **98:**596-601; 1984.
[177] Beard, J. Iron deficiency alters brain development and functioning. *The Journal of nutrition* **133:**1468S-1472S; 2003.
[178] Agarwal, K. N. Iron and the brain: neurotransmitter receptors and magnetic resonance spectroscopy. *British Journal of Nutrition* **85 Suppl 2:**S147-150; 2001.
[179] Schulz, K.; Kroner, A.; David, S. Iron Efflux from Astrocytes Plays a Role in Remyelination. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **32:**4841-4847; 2012.

Accepted manuscript

ACCEPTED MANUSCRIPT

[180] King, M. M.; Huang, C. Y. The calmodulin-dependent activation and deactivation of the phosphoprotein phosphatase, calcineurin, and the effect of nucleotides, pyrophosphate, and divalent metal ions. Identification of calcineurin as a Zn and Fe metalloenzyme. *The Journal of biological chemistry* **259:**8847-8856; 1984.
[181] Wang, X.; Culotta, V. C.; Klee, C. B. Superoxide dismutase protects calcineurin from inactivation. *Nature* **383:**434-437; 1996.
[182] Namgaladze, D.; Hofer, H. W.; Ullrich, V. Redox control of calcineurin by targeting the binuclear Fe(2+)-Zn(2+) center at the enzyme active site. *The Journal of biological chemistry* **277:**5962-5969; 2002.
[183] Goodman, L. Alzheimer's disease; a clinico-pathologic analysis of twenty-three cases with a theory on pathogenesis. *The Journal of nervous and mental disease* **118:**97-130; 1953.
[184] Connor, J. R.; Snyder, B. S.; Beard, J. L.; Fine, R. E.; Mufson, E. J. Regional distribution of iron and iron-regulatory proteins in the brain in aging and Alzheimer's disease. *Journal of neuroscience research* **31:**327-335; 1992.
[185] Collingwood, J. F.; Mikhaylova, A.; Davidson, M.; Batich, C.; Streit, W. J.; Terry, J.; Dobson, J. In situ characterization and mapping of iron compounds in Alzheimer's disease tissue. *Journal of Alzheimer's disease : JAD* **7:**267-272; 2005.
[186] Baltes, C.; Princz-Kranz, F.; Rudin, M.; Mueggler, T. Detecting amyloid-beta plaques in Alzheimer's disease. *Methods Mol Biol* **711:**511-533; 2011.
[187] Meadowcroft, M. D.; Connor, J. R.; Smith, M. B.; Yang, Q. X. MRI and histological analysis of beta-amyloid plaques in both human Alzheimer's disease and APP/PS1 transgenic mice. *Journal of magnetic resonance imaging : JMRI* **29:**997-1007; 2009.
[188] Collingwood, J. F.; Chong, R. K.; Kasama, T.; Cervera-Gontard, L.; Dunin-Borkowski, R. E.; Perry, G.; Posfai, M.; Siedlak, S. L.; Simpson, E. T.; Smith, M. A.; Dobson, J. Three-dimensional tomographic imaging and characterization of iron compounds within Alzheimer's plaque core material. *Journal of Alzheimer's disease : JAD* **14:**235-245; 2008.
[189] Andrasi, E.; Farkas, E.; Scheibler, H.; Reffy, A.; Bezur, L. Al, Zn, Cu, Mn and Fe levels in brain in Alzheimer's disease. *Archives of gerontology and geriatrics* **21:**89-97; 1995.
[190] Bartzokis, G.; Tishler, T. A. MRI evaluation of basal ganglia ferritin iron and neurotoxicity in Alzheimer's and Huntingon's disease. *Cell Mol Biol (Noisy-le-grand)* **46:**821-833; 2000.
[191] Swaiman, K. F.; Machen, V. L. Iron uptake by mammalian cortical neurons. *Ann Neurol* **16:**66-70; 1984.
[192] Aisen, P. Entry of iron into cells: a new role for the transferrin receptor in modulating iron release from transferrin. *Ann Neurol* **32 Suppl:**S62-68; 1992.
[193] Eckenroth, B. E.; Steere, A. N.; Chasteen, N. D.; Everse, S. J.; Mason, A. B. How the binding of human transferrin primes the transferrin receptor potentiating iron release at endosomal pH. *Proceedings of the National Academy of Sciences of the United States of America* **108:**13089-13094; 2011.
[194] Fleming, M. D.; Trenor, C. C., 3rd; Su, M. A.; Foernzler, D.; Beier, D. R.; Dietrich, W. F.; Andrews, N. C. Microcytic anaemia mice have a mutation in Nramp2, a candidate iron transporter gene. *Nature genetics* **16:**383-386; 1997.
[195] Gunshin, H.; Mackenzie, B.; Berger, U. V.; Gunshin, Y.; Romero, M. F.; Boron, W. F.; Nussberger, S.; Gollan, J. L.; Hediger, M. A. Cloning and characterization of a mammalian proton-coupled metal-ion transporter. *Nature* **388:**482-488; 1997.
[196] Granick, S.; Michaelis, L. Ferritin and Apoferritin. *Science* **95:**439-440; 1942.
[197] Donovan, A.; Lima, C. A.; Pinkus, J. L.; Pinkus, G. S.; Zon, L. I.; Robine, S.; Andrews, N. C. The iron exporter ferroportin/Slc40a1 is essential for iron homeostasis. *Cell Metabolism* **1:**191-200; 2005.
[198] Ganz, T. Cellular iron: ferroportin is the only way out. *Cell Metabolism* **1:**155-157; 2005.
[199] Rice, A. E.; Mendez, M. J.; Hokanson, C. A.; Rees, D. C.; Björkman, P. J. Investigation of the biophysical and cell biological properties of ferroportin, a multipass integral membrane protein iron exporter. *Journal of Molecular Biology* **386:**717-732; 2009.
[200] Osaki, S. Kinetic studies of ferrous ion oxidation with crystalline human ferroxidase (ceruloplasmin). *The Journal of biological chemistry* **241:**5053-5059; 1966.

Accepted manuscript

ACCEPTED MANUSCRIPT

[201] Harris, Z. L.; Takahashi, Y.; Miyajima, H.; Serizawa, M.; MacGillivray, R. T.; Gitlin, J. D. Aceruloplasminemia: molecular characterization of this disorder of iron metabolism. *Proceedings of the National Academy of Sciences of the United States of America* **92:**2539-2543; 1995.
[202] Klomp, L. W.; Farhangrazi, Z. S.; Dugan, L. L.; Gitlin, J. D. Ceruloplasmin gene expression in the murine central nervous system. *Journal of Clinical Investigation* **98:**207-215; 1996.
[203] Lei, P.; Ayton, S.; Finkelstein, D. I.; Spoerri, L.; Ciccotosto, G. D.; Wright, D. K.; Wong, B. X.; Adlard, P. A.; Cherny, R. A.; Lam, L. Q.; Roberts, B. R.; Volitakis, I.; Egan, G. F.; McLean, C. A.; Cappai, R.; Duce, J. A.; Bush, A. I. Tau deficiency induces parkinsonism with dementia by impairing APP-mediated iron export. *Nature medicine* **18:**291-295; 2012.
[204] Lei, P.; Ayton, S.; Finkelstein, D. I.; Adlard, P. A.; Masters, C. L.; Bush, A. I. Tau protein: relevance to Parkinson's disease. *The international journal of biochemistry & cell biology* **42:**1775-1778; 2010.
[205] Islam, K.; Levy, E. Carboxyl-terminal fragments of beta-amyloid precursor protein bind to microtubules and the associated protein tau. *The American journal of pathology* **151:**265-271; 1997.
[206] Loeffler, D. A.; Connor, J. R.; Juneau, P. L.; Snyder, B. S.; Kanaley, L.; DeMaggio, A. J.; Nguyen, H.; Brickman, C. M.; LeWitt, P. A. Transferrin and iron in normal, Alzheimer's disease, and Parkinson's disease brain regions. *Journal of neurochemistry* **65:**710-724; 1995.
[207] Zheng, W.; Xin, N.; Chi, Z.-H.; Zhao, B.-L.; Zhang, J.; Li, J.-Y.; Wang, Z.-Y. Divalent metal transporter 1 is involved in amyloid precursor protein processing and Abeta generation. *FASEB Journal* **23:**4207-4217; 2009.
[208] Connor, J. R.; Tucker, P.; Johnson, M.; Snyder, B. Ceruloplasmin levels in the human superior temporal gyrus in aging and Alzheimer's disease. *Neuroscience letters* **159:**88-90; 1993.
[209] Torsdottir, G.; Kristinsson, J.; Snaedal, J.; Sveinbjornsdottir, S.; Gudmundsson, G.; Hreidarsson, S.; Johannesson, T. Case-control studies on ceruloplasmin and superoxide dismutase (SOD1) in neurodegenerative diseases: a short review. *Journal of the neurological sciences* **299:**51-54; 2010.
[210] Shin, R. W.; Iwaki, T.; Kitamoto, T.; Sato, Y.; Tateishi, J. Massive accumulation of modified tau and severe depletion of normal tau characterize the cerebral cortex and white matter of Alzheimer's disease. Demonstration using the hydrated autoclaving method. *American Journal of Pathology* **140:**937-945; 1992.
[211] van Eersel, J.; Bi, M.; Ke, Y. D.; Hodges, J. R.; Xuereb, J. H.; Gregory, G. C.; Halliday, G. M.; Gotz, J.; Kril, J. J.; Ittner, L. M. Phosphorylation of soluble tau differs in Pick's disease and Alzheimer's disease brains. *J Neural Transm* **116:**1243-1251; 2009.
[212] Ksiezak-Reding, H.; Binder, L. I.; Yen, S.-H. C. Immunochemical and biochemical characterization of tau proteins in normal and Alzheimer's disease brains with Alz 50 and Tau-1. *The Journal of biological chemistry* **263:**7948-7953; 1988.
[213] Zhukareva, V.; Vogelsberg-Ragaglia, V.; Van Deerlin, V. M.; Bruce, J.; Shuck, T.; Grossman, M.; Clark, C. M.; Arnold, S. E.; Masliah, E.; Galasko, D. R.; Trojanowski, J. Q.; Lee, V. M.-Y. Loss of brain tau defines novel sporadic and familial tauopathies with frontotemporal dementia. *Ann Neurol.* **49:**165-175; 2001.
[214] Zhukareva, V.; Sundarraj, S.; Mann, D.; Sjogren, M.; Blenow, K.; Clark, C. M.; McKeel, D. W.; Goate, A.; Lippa, C. F.; Vonsattel, J. P.; Growdon, J. H.; Trojanowski, J. Q.; Lee, V. M. Selective reduction of soluble tau proteins in sporadic and familial frontotemporal dementias: an international follow-up study. *Acta Neuropathol* **105:**469-476; 2003.
[215] Khatoon, S.; Grundke-Iqbal, I.; Iqbal, K. Levels of normal and abnormally phosphorylated tau in different cellular and regional compartments of Alzheimer disease and control brains. *FEBS Letters* **351:**80-84; 1994.
[216] Bertram, L.; Tanzi, R. E. Thirty years of Alzheimer's disease genetics: the implications of systematic meta-analyses. *Nature reviews* **9:**768-778; 2008.
[217] Van Landeghem, G. F.; Sikstrom, C.; Beckman, L. E.; Adolfsson, R.; Beckman, L. Transferrin C2, metal binding and Alzheimer's disease. *Neuroreport* **9:**177-179; 1998.
[218] Kauwe, J. S.; Bertelsen, S.; Mayo, K.; Cruchaga, C.; Abraham, R.; Hollingworth, P.; Harold, D.; Owen, M. J.; Williams, J.; Lovestone, S.; Morris, J. C.; Goate, A. M. Suggestive synergy between genetic variants in TF and HFE as risk factors for Alzheimer's disease. *American journal of medical*

Accepted manuscript

ACCEPTED MANUSCRIPT

genetics. Part B, Neuropsychiatric genetics : the official publication of the International Society of Psychiatric Genetics **153B:**955-959; 2010.
[219] Namekata, K.; Imagawa, M.; Terashi, A.; Ohta, S.; Oyama, F.; Ihara, Y. Association of transferrin C2 allele with late-onset Alzheimer's disease. *Human genetics* **101:**126-129; 1997.
[220] van Rensburg, S. J.; Carstens, M. E.; Potocnik, F. C.; Aucamp, A. K.; Taljaard, J. J. Increased frequency of the transferrin C2 subtype in Alzheimer's disease. *Neuroreport* **4:**1269-1271; 1993.
[221] Schjeide, B. M.; McQueen, M. B.; Mullin, K.; DiVito, J.; Hogan, M. F.; Parkinson, M.; Hooli, B.; Lange, C.; Blacker, D.; Tanzi, R. E.; Bertram, L. Assessment of Alzheimer's disease case-control associations using family-based methods. *Neurogenetics* **10:**19-25; 2009.
[222] Blazquez, L.; De Juan, D.; Ruiz-Martinez, J.; Emparanza, J. I.; Saenz, A.; Otaegui, D.; Sistiaga, A.; Martinez-Lage, P.; Lamet, I.; Samaranch, L.; Buiza, C.; Etxeberria, I.; Arriola, E.; Cuadrado, E.; Urdaneta, E.; Yanguas, J.; Lopez de Munain, A. Genes related to iron metabolism and susceptibility to Alzheimer's disease in Basque population. *Neurobiol Aging* **28:**1941-1943; 2007.
[223] Pulliam, J. F.; Jennings, C. D.; Kryscio, R. J.; Davis, D. G.; Wilson, D.; Montine, T. J.; Schmitt, F. A.; Markesbery, W. R. Association of HFE mutations with neurodegeneration and oxidative stress in Alzheimer's disease and correlation with APOE. *American journal of medical genetics. Part B, Neuropsychiatric genetics : the official publication of the International Society of Psychiatric Genetics* **119B:**48-53; 2003.
[224] Sampietro, M.; Caputo, L.; Casatta, A.; Meregalli, M.; Pellagatti, A.; Tagliabue, J.; Annoni, G.; Vergani, C. The hemochromatosis gene affects the age of onset of sporadic Alzheimer's disease. *Neurobiol Aging* **22:**563-568; 2001.
[225] Percy, M.; Moalem, S.; Garcia, A.; Somerville, M. J.; Hicks, M.; Andrews, D.; Azad, A.; Schwarz, P.; Beheshti Zavareh, R.; Birkan, R.; Choo, C.; Chow, V.; Dhaliwal, S.; Duda, V.; Kupferschmidt, A. L.; Lam, K.; Lightman, D.; Machalek, K.; Mar, W.; Nguyen, F.; Rytwinski, P. J.; Svara, E.; Tran, M.; Wheeler, K.; Yeung, L.; Zanibbi, K.; Zener, R.; Ziraldo, M.; Freedman, M. Involvement of ApoE E4 and H63D in sporadic Alzheimer's disease in a folate-supplemented Ontario population. *Journal of Alzheimer's disease : JAD* **14:**69-84; 2008.
[226] Alizadeh, B. Z.; Njajou, O. T.; Millan, M. R.; Hofman, A.; Breteler, M. M.; van Duijn, C. M. HFE variants, APOE and Alzheimer's disease: findings from the population-based Rotterdam study. *Neurobiol Aging* **30:**330-332; 2009.
[227] Connor, J. R.; Lee, S. Y. HFE mutations and Alzheimer's disease. *Journal of Alzheimer's disease : JAD* **10:**267-276; 2006.
[228] Robson, K. J.; Lehmann, D. J.; Wimhurst, V. L.; Livesey, K. J.; Combrinck, M.; Merryweather-Clarke, A. T.; Warden, D. R.; Smith, A. D. Synergy between the C2 allele of transferrin and the C282Y allele of the haemochromatosis gene (HFE) as risk factors for developing Alzheimer's disease. *Journal of medical genetics* **41:**261-265; 2004.
[229] Lehmann, D. J.; Schuur, M.; Warden, D. R.; Hammond, N.; Belbin, O.; Kolsch, H.; Lehmann, M. G.; Wilcock, G. K.; Brown, K.; Kehoe, P. G.; Morris, C. M.; Barker, R.; Coto, E.; Alvarez, V.; Deloukas, P.; Mateo, I.; Gwilliam, R.; Combarros, O.; Arias-Vasquez, A.; Aulchenko, Y. S.; Ikram, M. A.; Breteler, M. M.; van Duijn, C. M.; Oulhaj, A.; Heun, R.; Cortina-Borja, M.; Morgan, K.; Robson, K.; Smith, A. D. Transferrin and HFE genes interact in Alzheimer's disease risk: the Epistasis Project. *Neurobiol Aging* **33:**202 e201-213; 2012.
[230] Giambattistelli, F.; Bucossi, S.; Salustri, C.; Panetta, V.; Mariani, S.; Siotto, M.; Ventriglia, M.; Vernieri, F.; Dell'acqua, M. L.; Cassetta, E.; Rossini, P. M.; Squitti, R. Effects of hemochromatosis and transferrin gene mutations on iron dyshomeostasis, liver dysfunction and on the risk of Alzheimer's disease. *Neurobiol Aging*; 2011.
[231] Bartzokis, G.; Lu, P. H.; Tingus, K.; Peters, D. G.; Amar, C. P.; Tishler, T. A.; Finn, J. P.; Villablanca, P.; Altshuler, L. L.; Mintz, J.; Neely, E.; Connor, J. R. Gender and iron genes may modify associations between brain iron and memory in healthy aging. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology* **36:**1375-1384; 2011.
[232] Hollingworth, P.; Harold, D.; Sims, R.; Gerrish, A.; Lambert, J. C.; Carrasquillo, M. M.; Abraham, R.; Hamshere, M. L.; Pahwa, J. S.; Moskvina, V.; Dowzell, K.; Jones, N.; Stretton, A.; Thomas, C.; Richards, A.; Ivanov, D.; Widdowson, C.; Chapman, J.; Lovestone, S.; Powell, J.; Proitsi, P.; Lupton, M. K.; Brayne, C.; Rubinsztein, D. C.; Gill, M.; Lawlor, B.; Lynch, A.; Brown, K. S.; Passmore, P. A.; Craig, D.; McGuinness, B.; Todd, S.; Holmes, C.; Mann, D.; Smith, A. D.;

Accepted manuscript

ACCEPTED MANUSCRIPT

Beaumont, H.; Warden, D.; Wilcock, G.; Love, S.; Kehoe, P. G.; Hooper, N. M.; Vardy, E. R.; Hardy, J.; Mead, S.; Fox, N. C.; Rossor, M.; Collinge, J.; Maier, W.; Jessen, F.; Ruther, E.; Schurmann, B.; Heun, R.; Kolsch, H.; van den Bussche, H.; Heuser, I.; Kornhuber, J.; Wiltfang, J.; Dichgans, M.; Frolich, L.; Hampel, H.; Gallacher, J.; Hull, M.; Rujescu, D.; Giegling, I.; Goate, A. M.; Kauwe, J. S.; Cruchaga, C.; Nowotny, P.; Morris, J. C.; Mayo, K.; Sleegers, K.; Bettens, K.; Engelborghs, S.; De Deyn, P. P.; Van Broeckhoven, C.; Livingston, G.; Bass, N. J.; Gurling, H.; McQuillin, A.; Gwilliam, R.; Deloukas, P.; Al-Chalabi, A.; Shaw, C. E.; Tsolaki, M.; Singleton, A. B.; Guerreiro, R.; Muhleisen, T. W.; Nothen, M. M.; Moebus, S.; Jockel, K. H.; Klopp, N.; Wichmann, H. E.; Pankratz, V. S.; Sando, S. B.; Aasly, J. O.; Barcikowska, M.; Wszolek, Z. K.; Dickson, D. W.; Graff-Radford, N. R.; Petersen, R. C.; van Duijn, C. M.; Breteler, M. M.; Ikram, M. A.; DeStefano, A. L.; Fitzpatrick, A. L.; Lopez, O.; Launer, L. J.; Seshadri, S.; Berr, C.; Campion, D.; Epelbaum, J.; Dartigues, J. F.; Tzourio, C.; Alperovitch, A.; Lathrop, M.; Feulner, T. M.; Friedrich, P.; Riehle, C.; Krawczak, M.; Schreiber, S.; Mayhaus, M.; Nicolhaus, S.; Wagenpfeil, S.; Steinberg, S.; Stefansson, H.; Stefansson, K.; Snaedal, J.; Bjornsson, S.; Jonsson, P. V.; Chouraki, V.; Genier-Boley, B.; Hiltunen, M.; Soininen, H.; Combarros, O.; Zelenika, D.; Delepine, M.; Bullido, M. J.; Pasquier, F.; Mateo, I.; Frank-Garcia, A.; Porcellini, E.; Hanon, O.; Coto, E.; Alvarez, V.; Bosco, P.; Siciliano, G.; Mancuso, M.; Panza, F.; Solfrizzi, V.; Nacmias, B.; Sorbi, S.; Bossu, P.; Piccardi, P.; Arosio, B.; Annoni, G.; Seripa, D.; Pilotto, A.; Scarpini, E.; Galimberti, D.; Brice, A.; Hannequin, D.; Licastro, F.; Jones, L.; Holmans, P. A.; Jonsson, T.; Riemenschneider, M.; Morgan, K.; Younkin, S. G.; Owen, M. J.; O'Donovan, M.; Amouyel, P.; Williams, J. Common variants at ABCA7, MS4A6A/MS4A4E, EPHA1, CD33 and CD2AP are associated with Alzheimer's disease. *Nature genetics* **43:**429-435; 2011.

[233] Naj, A. C.; Jun, G.; Beecham, G. W.; Wang, L. S.; Vardarajan, B. N.; Buros, J.; Gallins, P. J.; Buxbaum, J. D.; Jarvik, G. P.; Crane, P. K.; Larson, E. B.; Bird, T. D.; Boeve, B. F.; Graff-Radford, N. R.; De Jager, P. L.; Evans, D.; Schneider, J. A.; Carrasquillo, M. M.; Ertekin-Taner, N.; Younkin, S. G.; Cruchaga, C.; Kauwe, J. S.; Nowotny, P.; Kramer, P.; Hardy, J.; Huentelman, M. J.; Myers, A. J.; Barmada, M. M.; Demirci, F. Y.; Baldwin, C. T.; Green, R. C.; Rogaeva, E.; St George-Hyslop, P.; Arnold, S. E.; Barber, R.; Beach, T.; Bigio, E. H.; Bowen, J. D.; Boxer, A.; Burke, J. R.; Cairns, N. J.; Carlson, C. S.; Carney, R. M.; Carroll, S. L.; Chui, H. C.; Clark, D. G.; Corneveaux, J.; Cotman, C. W.; Cummings, J. L.; DeCarli, C.; DeKosky, S. T.; Diaz-Arrastia, R.; Dick, M.; Dickson, D. W.; Ellis, W. G.; Faber, K. M.; Fallon, K. B.; Farlow, M. R.; Ferris, S.; Frosch, M. P.; Galasko, D. R.; Ganguli, M.; Gearing, M.; Geschwind, D. H.; Ghetti, B.; Gilbert, J. R.; Gilman, S.; Giordani, B.; Glass, J. D.; Growdon, J. H.; Hamilton, R. L.; Harrell, L. E.; Head, E.; Honig, L. S.; Hulette, C. M.; Hyman, B. T.; Jicha, G. A.; Jin, L. W.; Johnson, N.; Karlawish, J.; Karydas, A.; Kaye, J. A.; Kim, R.; Koo, E. H.; Kowall, N. W.; Lah, J. J.; Levey, A. I.; Lieberman, A. P.; Lopez, O. L.; Mack, W. J.; Marson, D. C.; Martiniuk, F.; Mash, D. C.; Masliah, E.; McCormick, W. C.; McCurry, S. M.; McDavid, A. N.; McKee, A. C.; Mesulam, M.; Miller, B. L.; Miller, C. A.; Miller, J. W.; Parisi, J. E.; Perl, D. P.; Peskind, E.; Petersen, R. C.; Poon, W. W.; Quinn, J. F.; Rajbhandary, R. A.; Raskind, M.; Reisberg, B.; Ringman, J. M.; Roberson, E. D.; Rosenberg, R. N.; Sano, M.; Schneider, L. S.; Seeley, W.; Shelanski, M. L.; Slifer, M. A.; Smith, C. D.; Sonnen, J. A.; Spina, S.; Stern, R. A.; Tanzi, R. E.; Trojanowski, J. Q.; Troncoso, J. C.; Van Deerlin, V. M.; Vinters, H. V.; Vonsattel, J. P.; Weintraub, S.; Welsh-Bohmer, K. A.; Williamson, J.; Woltjer, R. L.; Cantwell, L. B.; Dombroski, B. A.; Beekly, D.; Lunetta, K. L.; Martin, E. R.; Kamboh, M. I.; Saykin, A. J.; Reiman, E. M.; Bennett, D. A.; Morris, J. C.; Montine, T. J.; Goate, A. M.; Blacker, D.; Tsuang, D. W.; Hakonarson, H.; Kukull, W. A.; Foroud, T. M.; Haines, J. L.; Mayeux, R.; Pericak-Vance, M. A.; Farrer, L. A.; Schellenberg, G. D. Common variants at MS4A4/MS4A6E, CD2AP, CD33 and EPHA1 are associated with late-onset Alzheimer's disease. *Nature genetics* **43:**436-441; 2011.

[234] Gallagher, J. J.; Finnegan, M. E.; Grehan, B.; Dobson, J.; Collingwood, J. F.; Lynch, M. A. Modest amyloid deposition is associated with iron dysregulation, microglial activation, and oxidative stress. *Journal of Alzheimer's disease : JAD* **28:**147-161; 2012.

[235] Mantyh, P. W.; Ghilardi, J. R.; Rogers, S.; DeMaster, E.; Allen, C. J.; Stimson, E. R.; Maggio, J. E. Aluminum, iron, and zinc ions promote aggregation of physiological concentrations of beta-amyloid peptide. *Journal of neurochemistry* **61:**1171-1174; 1993.

[236] Huang, X.; Atwood, C. S.; Moir, R. D.; Hartshorn, M. A.; Tanzi, R. E.; Bush, A. I. Trace metal contamination initiates the apparent auto-aggregation, amyloidosis, and oligomerization of Alzheimer's Abeta peptides. *J Biol Inorg Chem* **9:**954-960; 2004.

Accepted manuscript

ACCEPTED MANUSCRIPT

[237] Schubert, D.; Chevion, M. The role of iron in beta amyloid toxicity. *Biochemical and Biophysical Research Communications* **216:**702-707; 1995.
[238] Liu, B.; Moloney, A.; Meehan, S.; Morris, K.; Thomas, S. E.; Serpell, L. C.; Hider, R.; Marciniak, S. J.; Lomas, D. A.; Crowther, D. C. Iron promotes the toxicity of amyloid beta peptide by impeding its ordered aggregation. *The Journal of biological chemistry* **286:**4248-4256; 2011.
[239] Rival, T.; Page, R. M.; Chandraratna, D. S.; Sendall, T. J.; Ryder, E.; Liu, B.; Lewis, H.; Rosahl, T.; Hider, R.; Camargo, L. M.; Shearman, M. S.; Crowther, D. C.; Lomas, D. A. Fenton chemistry and oxidative stress mediate the toxicity of the beta-amyloid peptide in a Drosophila model of Alzheimer's disease. *Eur J Neurosci* **29:**1335-1347; 2009.
[240] Kuperstein, F.; Yavin, E. Pro-apoptotic signaling in neuronal cells following iron and amyloid beta peptide neurotoxicity. *Journal of neurochemistry* **86:**114-125; 2003.
[241] Bousejra-ElGarah, F.; Bijani, C.; Coppel, Y.; Faller, P.; Hureau, C. Iron(II) binding to amyloid-beta, the Alzheimer's peptide. *Inorganic Chemistry* **50:**9024-9030; 2011.
[242] García de Ancos, J.; Correas, I.; Avila, J. Differences in microtubule binding and self-association abilities of bovine brain tau isoforms. *The Journal of biological chemistry* **268:**7976-7982; 1993.
[243] Ledesma, M. D.; Avila, J.; Correas, I. Isolation of a phosphorylated soluble tau fraction from Alzheimer's disease brain. *Neurobiology Of Aging* **16:**515-522; 1995.
[244] Yamamoto, A.; Shin, R.-W.; Hasegawa, K.; Naiki, H.; Sato, H.; Yoshimasu, F.; Kitamoto, T. 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. *Journal of neurochemistry* **82:**1137-1147; 2002.
[245] Amit, T.; Avramovich-Tirosh, Y.; Youdim, M. B. H.; Mandel, S. Targeting multiple Alzheimer's disease etiologies with multimodal neuroprotective and neurorestorative iron chelators. *FASEB Journal* **22:**1296-1305; 2008.
[246] Tofaris, G. K.; Revesz, T.; Jacques, T. S.; Papacostas, S.; Chataway, J. Adult-onset neurodegeneration with brain iron accumulation and cortical alpha-synuclein and tau pathology: a distinct clinicopathological entity. *Archives of neurology* **64:**280-282; 2007.
[247] Shin, R.-W.; Kruck, T. P. A.; Murayama, H.; Kitamoto, T. A novel trivalent cation chelator Feralex dissociates binding of aluminum and iron associated with hyperphosphorylated tau of Alzheimer's disease. *Brain Research* **961:**139-146; 2003.
[248] Egaña, J. T.; Zambrano, C.; Nuñez, M. T.; Gonzalez-Billault, C.; Maccioni, R. B. Iron-induced oxidative stress modify tau phosphorylation patterns in hippocampal cell cultures. *Biometals* **16:**215-223; 2003.
[249] Lovell, M. A.; Xiong, S.; Xie, C.; Davies, P. L.; Markesbery, W. R. Induction of hyperphosphorylated tau in primary rat cortical neuron cultures mediated by oxidative stress and glycogen synthase kinase-3. *Journal of Alzheimer's disease : JAD* **6:**659-671; discussion 673-681; 2004.
[250] Chan, A.; Shea, T. B. Dietary and genetically-induced oxidative stress alter tau phosphorylation: influence of folate and apolipoprotein E deficiency. *Journal of Alzheimer's disease : JAD* **9:**399-405; 2006.
[251] Huang, X.; Dai, J.; Huang, C.; Zhang, Q.; Bhanot, O.; Pelle, E. Deferoxamine synergistically enhances iron-mediated AP-1 activation: a showcase of the interplay between extracellular-signal-regulated kinase and tyrosine phosphatase. *Free radical research* **41:**1135-1142; 2007.
[252] Muñoz, P.; Zavala, G.; Castillo, K.; Aguirre, P.; Hidalgo, C.; Nuñez, M. T. Effect of iron on the activation of the MAPK/ERK pathway in PC12 neuroblastoma cells. *Biol Res* **39:**189-190; 2006.
[253] Tsubaki, T.; Honma, Y.; Hoshi, M. Neurological syndrome associated with clioquinol. *Lancet* **1:**696-697; 1971.
[254] Tateishi, J. Subacute myelo-optico-neuropathy: clioquinol intoxication in humans and animals. *Neuropathology* **20 Suppl:**S20-24; 2000.
[255] Cherny, R. A.; Atwood, C. S.; Xilinas, M. E.; Gray, D. N.; Jones, W. D.; McLean, C. A.; Barnham, K. J.; Volitaskis, I.; Fraser, F.; Kim, Y.-H.; Huang, X.; Goldstein, L. E.; Moir, R. D.; Lim, J. T.; Beyreuther, K.; Zheng, H.; Tanzi, R. E.; Masters, C. L.; Bush, A. I. Treatment with a copper-zinc chelator markedly and rapidly inhibits beta-amyloid accumulation in Alzheimer's disease transgenic mice. *Neuron* **30:**665-676; 2001.

Accepted Manuscript

ACCEPTED MANUSCRIPT

[256] Grossi, C.; Francese, S.; Casini, A.; Rosi, M. C.; Luccarini, I.; Fiorentini, A.; Gabbiani, C.; Messori, L.; Moneti, G.; Casamenti, F. Clioquinol decreases amyloid-beta burden and reduces working memory impairment in a transgenic mouse model of Alzheimer's disease. *Journal of Alzheimer's disease : JAD* **17:**423-440; 2009.
[257] Treiber, C.; Simons, A.; Strauss, M.; Hafner, M.; Cappai, R.; Bayer, T. A.; Multhaup, G. Clioquinol mediates copper uptake and counteracts copper efflux activities of the amyloid precursor protein of Alzheimer's disease. *The Journal of biological chemistry* **279:**51958-51964; 2004.
[258] LeVine, H., 3rd; Ding, Q.; Walker, J. A.; Voss, R. S.; Augelli-Szafran, C. E. Clioquinol and other hydroxyquinoline derivatives inhibit Abeta(1-42) oligomer assembly. *Neuroscience letters* **465:**99-103; 2009.
[259] Mancino, A. M.; Hindo, S. S.; Kochi, A.; Lim, M. H. Effects of clioquinol on metal-triggered amyloid-beta aggregation revisited. *Inorganic Chemistry* **48:**9596-9598; 2009.
[260] Stoppelkamp, S.; Bell, H. S.; Palacios-Filardo, J.; Shewan, D. A.; Riedel, G.; Platt, B. In vitro modelling of Alzheimer's disease: degeneration and cell death induced by viral delivery of amyloid and tau. *Experimental Neurology* **229:**226-237; 2011.
[261] Ritchie, C. W.; Bush, A. I.; Mackinnon, A.; Macfarlane, S.; Mastwyk, M.; MacGregor, L.; Kiers, L.; Cherny, R.; Li, Q. X.; Tammer, A.; Carrington, D.; Mavros, C.; Volitakis, I.; Xilinas, M.; Ames, D.; Davis, S.; Beyreuther, K.; Tanzi, R. E.; Masters, C. L. Metal-protein attenuation with iodochlorhydroxyquin (clioquinol) targeting Abeta amyloid deposition and toxicity in Alzheimer disease: a pilot phase 2 clinical trial. *Archives of neurology* **60:**1685-1691; 2003.
[262] Ibach, B.; Haen, E.; Marienhagen, J.; Hajak, G. Clioquinol treatment in familiar early onset of Alzheimer's disease: a case report. *Pharmacopsychiatry* **38:**178-179; 2005.
[263] Kaur, D.; Yantiri, F.; Rajagopalan, S.; Kumar, J.; Mo, J. Q.; Boonplueang, R.; Viswanath, V.; Jacobs, R.; Yang, L.; Beal, M. F.; DiMonte, D.; Volitaskis, I.; Ellerby, L.; Cherny, R. A.; Bush, A. I.; Andersen, J. K. Genetic or pharmacological iron chelation prevents MPTP-induced neurotoxicity in vivo: a novel therapy for Parkinson's disease. *Neuron* **37:**899-909; 2003.
[264] Nguyen, T.; Hamby, A.; Massa, S. M. Clioquinol down-regulates mutant huntingtin expression in vitro and mitigates pathology in a Huntington's disease mouse model. *Proceedings of the National Academy of Sciences of the United States of America* **102:**11840-11845; 2005.
[265] Ewbank, J. J.; Barnes, T. M.; Lakowski, B.; Lussier, M.; Bussey, H.; Hekimi, S. Structural and functional conservation of the Caenorhabditis elegans timing gene clk-1. *Science* **275:**980-983; 1997.
[266] Jonassen, T.; Larsen, P. L.; Clarke, C. F. A dietary source of coenzyme Q is essential for growth of long-lived Caenorhabditis elegans clk-1 mutants. *Proceedings of the National Academy of Sciences of the United States of America* **98:**421-426; 2001.
[267] Felkai, S.; Ewbank, J. J.; Lemieux, J.; Labbe, J. C.; Brown, G. G.; Hekimi, S. CLK-1 controls respiration, behavior and aging in the nematode Caenorhabditis elegans. *EMBO Journal* **18:**1783-1792; 1999.
[268] Kenyon, C. The plasticity of aging: insights from long-lived mutants. *Cell* **120:**449-460; 2005.
[269] Hekimi, S. How genetic analysis tests theories of animal aging. *Nature genetics* **38:**985-991; 2006.
[270] Taguchi, A.; Wartschow, L. M.; White, M. F. Brain IRS2 signaling coordinates life span and nutrient homeostasis. *Science* **317:**369-372; 2007.
[271] Wang, Y.; Branicky, R.; Stepanyan, Z.; Carroll, M.; Guimond, M.-P.; Hihi, A.; Hayes, S.; McBride, K.; Hekimi, S. The anti-neurodegeneration drug clioquinol inhibits the aging-associated protein CLK-1. *The Journal of biological chemistry* **284:**314-323; 2009.
[272] Ismail, T.; Mauerhofer, E.; Slomianka, L. The hippocampal region of rats and mice after a single i.p. dose of clioquinol: loss of synaptic zinc, cell death and c-Fos induction. *Neuroscience* **157:**697-707; 2008.
[273] Asakura, K.; Ueda, A.; Kawamura, N.; Ueda, M.; Mihara, T.; Mutoh, T. Clioquinol inhibits NGF-induced Trk autophosphorylation and neurite outgrowth in PC12 cells. *Brain Research* **1301:**110-115; 2009.
[274] Takeda, A.; Takada, S.; Ando, M.; Itagaki, K.; Tamano, H.; Suzuki, M.; Iwaki, H.; Oku, N. Impairment of recognition memory and hippocampal long-term potentiation after acute exposure to clioquinol. *Neuroscience* **171:**443-450; 2010.

Accepted manuscript

ACCEPTED MANUSCRIPT

[275] Raman, B.; Ban, T.; Yamaguchi, K.-i.; Sakai, M.; Kawai, T.; Naiki, H.; Goto, Y. Metal Ion-dependent Effects of Clioquinol on the Fibril Growth of an Amyloid {beta} Peptide. *The Journal of biological chemistry* **280:**16157-16162; 2005.
[276] Priel, T.; Aricha-Tamir, B.; Sekler, I. Clioquinol attenuates zinc-dependent beta-cell death and the onset of insulitis and hyperglycemia associated with experimental type I diabetes in mice. *European journal of pharmacology* **565:**232-239; 2007.
[277] Barrea, R. A.; Chen, D.; Irving, T. C.; Dou, Q. P. Synchrotron X-ray imaging reveals a correlation of tumor copper speciation with Clioquinol's anticancer activity. *Journal of Cellular Biochemistry* **108:**96-105; 2009.
[278] Di Vaira, M.; Bazzicalupi, C.; Orioli, P.; Messori, L.; Bruni, B.; Zatta, P. Clioquinol, a drug for Alzheimer's disease specifically interfering with brain metal metabolism: structural characterization of its zinc(II) and copper(II) complexes. *Inorganic Chemistry* **43:**3795-3797; 2004.
[279] Yassin, M. S.; Ekblom, J.; Xilinas, M.; Gottfries, C. G.; Oreland, L. Changes in uptake of vitamin B(12) and trace metals in brains of mice treated with clioquinol. *Journal of the neurological sciences* **173:**40-44; 2000.
[280] Nitzan, Y. B.; Sekler, I.; Frederickson, C. J.; Coulter, D. A.; Balaji, R. V.; Liang, S. L.; Margulis, A.; Hershfinkel, M.; Silverman, W. F. Clioquinol effects on tissue chelatable zinc in mice. *J Mol Med (Berl)* **81:**637-644; 2003.
[281] Adlard, P. A.; Cherny, R. A.; Finkelstein, D. I.; Gautier, E.; Robb, E.; Cortes, M.; Volitaskis, I.; Liu, X.; Smith, J. P.; Perez, K. A.; Laughton, K. M.; Li, Q.-X.; Charman, S. A.; Nicolazzo, J. A.; Wilkins, S.; Deleva, K.; Lynch, T.; Kok, G.; Ritchie, C. W.; Tanzi, R. E.; Cappai, R.; Masters, C. L.; Barnham, K. J.; Bush, A. I. Rapid restoration of cognition in Alzheimer's transgenic mice with 8-hydroxy quinoline analogs is associated with decreased interstitial Abeta. *Neuron* **59:**43-55; 2008.
[282] Li, C.; Wang, J.; Zhou, B. The metal chelating and chaperoning effects of clioquinol: insights from yeast studies. *Journal of Alzheimer's disease : JAD* **21:**1249-1262; 2010.
[283] Park, M. H.; Lee, S. J.; Byun, H. R.; Kim, Y.; Oh, Y. J.; Koh, J. Y.; Hwang, J. J. Clioquinol induces autophagy in cultured astrocytes and neurons by acting as a zinc ionophore. *Neurobiol Dis* **42:**242-251; 2011.
[284] Crouch, P. J.; Savva, M. S.; Hung, L. W.; Donnelly, P. S.; Mot, A. I.; Parker, S. J.; Greenough, M. A.; Volitakis, I.; Adlard, P. A.; Cherny, R. A.; Masters, C. L.; Bush, A. I.; Barnham, K. J.; White, A. R. The Alzheimer's therapeutic PBT2 promotes amyloid-beta degradation and GSK3 phosphorylation via a metal chaperone activity. *Journal of neurochemistry* **119:**220-230; 2011.
[285] Tamura, Z.; Yoshioka, M.; Imanari, T.; Fukaya, J.; Kusaka, J. Identification of green pigment and analysis of clioquinol in specimens from patients with subacute myelo-optico-neuropathy. *Clinica Chimica Acta* **47:**13-20; 1973.
[286] Kidani, Y., Naga, S., Koike, H. Mass spectrophotometry of 5-chloro-7iodo-8-quinolinol metal chelates. *Jap. Analyst* **23:**1375-1378; 1974.
[287] Ohtsuka, K.; Ohishi, N.; Eguchi, G.; Yagi, K. Degeneration of retinal neuroblasts by chinoform-ferric chelate. *Experientia* **38:**120-122; 1982.
[288] Atamna, H.; Frey, W. H. A role for heme in Alzheimer's disease: heme binds amyloid beta and has altered metabolism. *Proc Natl Acad Sci USA* **101:**11153-11158; 2004.
[289] Choi, S. M.; Choi, K. O.; Park, Y. K.; Cho, H.; Yang, E. G.; Park, H. Clioquinol, a Cu(II)/Zn(II) chelator, inhibits both ubiquitination and asparagine hydroxylation of hypoxia-inducible factor-1alpha, leading to expression of vascular endothelial growth factor and erythropoietin in normoxic cells. *The Journal of biological chemistry* **281:**34056-34063; 2006.
[290] White, A. R.; Du, T.; Laughton, K. M.; Volitaskis, I.; Sharples, R. A.; Xilinas, M. E.; Hoke, D. E.; Holsinger, R. M. D.; Evin, G.; Cherny, R. A.; Hill, A. F.; Barnham, K. J.; Li, Q.-X.; Bush, A. I.; Masters, C. L. Degradation of the Alzheimer disease amyloid beta-peptide by metal-dependent up-regulation of metalloprotease activity. *The Journal of biological chemistry* **281:**17670-17680; 2006.
[291] Filiz, G.; Caragounis, A.; Bica, L.; Du, T.; Masters, C. L.; Crouch, P. J.; White, A. R. Clioquinol inhibits peroxide-mediated toxicity through up-regulation of phosphoinositol-3-kinase and inhibition of p53 activity. *The international journal of biochemistry & cell biology* **40:**1030-1042; 2008.
[292] Filiz, G.; Price, K. A.; Caragounis, A.; Du, T.; Crouch, P. J.; White, A. R. The role of metals in modulating metalloprotease activity in the AD brain. *Eur Biophys J* **37:**315-321; 2008.

Accepted manuscript

ACCEPTED MANUSCRIPT

[293] Lannfelt, L.; Blennow, K.; Zetterberg, H.; Batsman, S.; Ames, D.; Harrison, J.; Masters, C. L.; Targum, S.; Bush, A. I.; Murdoch, R.; Wilson, J.; Ritchie, C. W.; group, P.-.-E. s. Safety, efficacy, and biomarker findings of PBT2 in targeting Abeta as a modifying therapy for Alzheimer's disease: a phase IIa, double-blind, randomised, placebo-controlled trial. *Lancet Neurol* **7:**779-786; 2008.
[294] Faux, N. G.; Ritchie, C. W.; Gunn, A. P.; Rembach, A.; Tsatsanis, A.; Bedo, J.; Harrison, J.; Lannfelt, L.; Blennow, K.; Zetterberg, H.; Ingelsson, M.; Masters, C. L.; Tanzi, R. E.; Cummings, J. L.; Herd, C. M.; Bush, A. I. PBT2 rapidly improves cognition in Alzheimer's Disease: additional phase II analyses. *Journal of Alzheimer's disease : JAD* **20:**509-516; 2010.
[295] Donnelly, P. S.; Caragounis, A.; Du, T.; Laughton, K. M.; Volitakis, I.; Cherny, R. A.; Sharples, R. A.; Hill, A. F.; Li, Q. X.; Masters, C. L.; Barnham, K. J.; White, A. R. Selective intracellular release of copper and zinc ions from bis(thiosemicarbazonato) complexes reduces levels of Alzheimer disease amyloid-beta peptide. *The Journal of biological chemistry* **283:**4568-4577; 2008.
[296] Crouch, P. J.; Hung, L. W.; Adlard, P. A.; Cortes, M.; Lal, V.; Filiz, G.; Perez, K. A.; Nurjono, M.; Caragounis, A.; Du, T.; Laughton, K.; Volitakis, I.; Bush, A. I.; Li, Q. X.; Masters, C. L.; Cappai, R.; Cherny, R. A.; Donnelly, P. S.; White, A. R.; Barnham, K. J. Increasing Cu bioavailability inhibits Abeta oligomers and tau phosphorylation. *Proceedings of the National Academy of Sciences of the United States of America* **106:**381-386; 2009.
[297] Hung, L. W.; Villemagne, V. L.; Cheng, L.; Sherratt, N. A.; Ayton, S.; White, A. R.; Crouch, P. J.; Lim, S.; Leong, S. L.; Wilkins, S.; George, J.; Roberts, B. R.; Pham, C. L.; Liu, X.; Chiu, F. C.; Shackleford, D. M.; Powell, A. K.; Masters, C. L.; Bush, A. I.; O'Keefe, G.; Culvenor, J. G.; Cappai, R.; Cherny, R. A.; Donnelly, P. S.; Hill, A. F.; Finkelstein, D. I.; Barnham, K. J. The hypoxia imaging agent CuII(atsm) is neuroprotective and improves motor and cognitive functions in multiple animal models of Parkinson's disease. *The Journal of experimental medicine* **209:**837-854; 2012.
[298] Reznichenko, L.; Amit, T.; Zheng, H.; Avramovich-Tirosh, Y.; Youdim, M. B.; Weinreb, O.; Mandel, S. Reduction of iron-regulated amyloid precursor protein and beta-amyloid peptide by (-)-epigallocatechin-3-gallate in cell cultures: implications for iron chelation in Alzheimer's disease. *Journal of neurochemistry* **97:**527-536; 2006.
[299] Avramovich-Tirosh, Y.; Amit, T.; Bar-Am, O.; Zheng, H.; Fridkin, M.; Youdim, M. B. Therapeutic targets and potential of the novel brain- permeable multifunctional iron chelator-monoamine oxidase inhibitor drug, M-30, for the treatment of Alzheimer's disease. *Journal of neurochemistry* **100:**490-502; 2007.
[300] Crapper McLachlan, D. R.; Dalton, A. J.; Kruck, T. P.; Bell, M. Y.; Smith, W. L.; Kalow, W.; Andrews, D. F. Intramuscular desferrioxamine in patients with Alzheimer's disease. *Lancet* **337:**1304-1308; 1991.

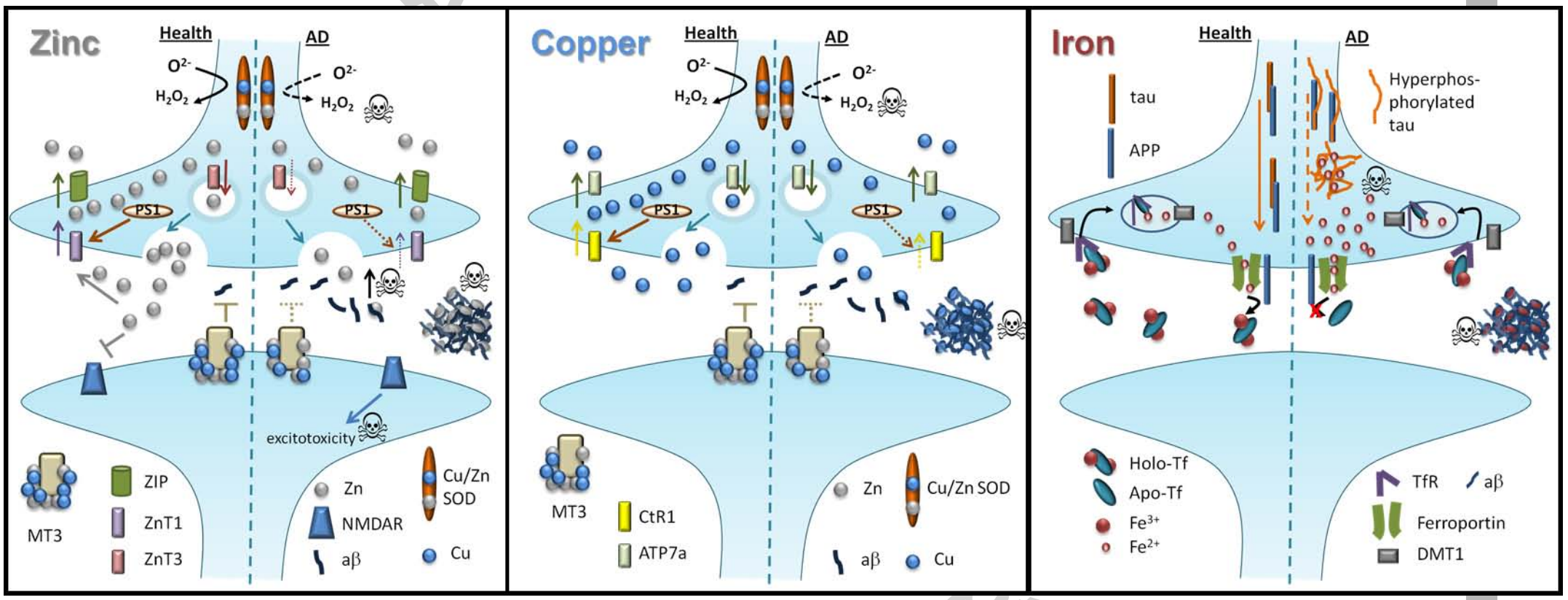
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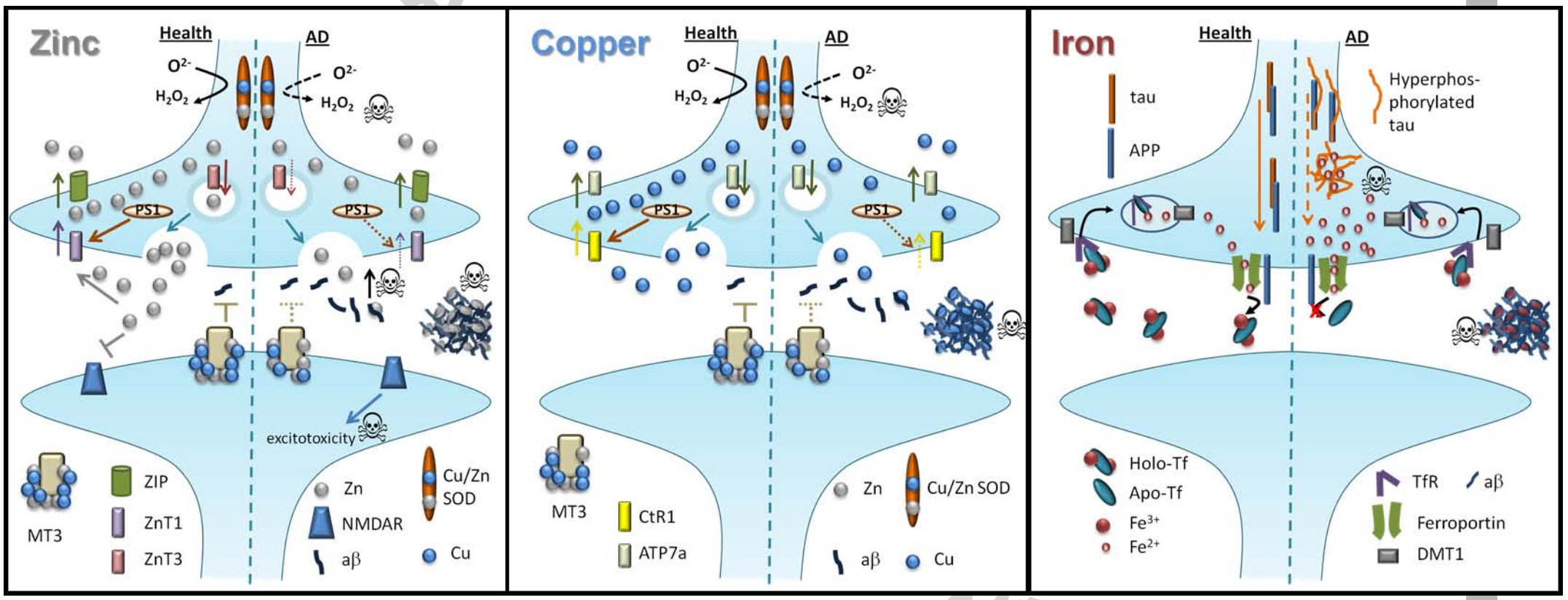
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Highlights

- Alzheimer's disease is complicated by inappropriate compartmentalization of metals
- Zn and Cu are sequestered into plaque while intraneunally these metals are depleted
- Dysfunction of APP and tau engender intraneuronal iron accumulation
- Drugs that modulate metals have shown promise in 3 clinical trials for AD

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Zinc
Health
AD
O^{2-}
H_2O_2
PS1
excitotoxicity
MT3
ZIP
ZnT1
ZnT3
Zn
NMDAR
aβ
Cu/Zn SOD
Cu
Copper
CtR1
ATP7a
Iron
tau
APP
Hyperphos-phorylated tau
Holo-Tf
Apo-Tf
Fe^{3+}
Fe^{2+}
TfR
Ferroportin
DMT1


Zinc
Health
AD
O^{2-}
H_2O_2
PS1
excitotoxicity
MT3
ZIP
ZnT1
ZnT3
Zn
NMDAR
aβ
Cu/Zn SOD
Cu
Copper
CtR1
ATP7a
Iron
tau
APP
Hyperphos-phorylated tau
Holo-Tf
Apo-Tf
Fe^{3+}
Fe^{2+}
TfR
Ferroportin
DMT1