Published in final edited form as:

J Alzheimers Dis. 2009 March ; 16(3): 471–483. doi:10.3233/JAD-2009-0992.

A Potential Role for Alterations of Zinc and Zinc Transport Proteins in the Progression of Alzheimer's Disease

M.A. Lovell^{1,2}

¹ Department of Chemistry, University of Kentucky, Lexington, KY 40536

² Sanders-Brown Center on Aging, University of Kentucky, Lexington, KY 40536

Abstract

Although multiple studies have suggested a role for alterations of zinc (Zn) and zinc transport (ZnT) proteins in the pathogenesis of Alzheimer's disease (AD), the exact role of this essential trace element in the progression of AD remains unclear. The following review discusses the normal role of Zn and ZnT proteins in brain and the potential effects of their alteration in the pathogenesis of AD particularly in the processing of the amyloid precursor protein and amyloid beta peptide generation and aggregation.

Keywords

zinc; zinc transport proteins; mild cognitive impairment; early Alzheimer's disease

AD and MCI

Alzheimer's disease (AD) is the fourth leading cause of death in the United States and in 2000 affected 4.5 million Americans [87]. Estimates indicate that ~3% of Americans between ages 65 and 74, 19% ages 75 – 84, and 47% over age 85 are victims of the disease [67] and that ~60% of nursing home patients over age 65 suffer from AD. Clinically AD is characterized by a progressive decline in multiple cognitive functions and is thought to begin with amnesic mild cognitive impairment (MCI), widely considered to be a transition between normal aging and dementia. Current data suggest that conversion from MCI to dementia occurs at a rate of 10 to 15% per year [156] with ~80% conversion by the sixth year of followup; although ~5% of MCI subjects remain stable or convert back to normal [13,52]. Progression from MCI leads to early AD (EAD) which is clinically characterized by a) a decline in cognitive function from a previous higher level, b). decline in one or more areas of cognition in addition to memory, c) a clinical dementia rating scale score of 0.5 to 1, d) impaired ADLs, and e) a clinical evaluation that excludes other causes of dementia. The disease eventually progresses to late stage AD (LAD) characterized by severe dementia with disorientation, profound memory impairment, global cognitive deficits and immobility. Without preventive strategies, there may be 14 million Americans with AD by the year 2040 [87].

Pathologically AD is characterized by an abundance of neurofibrillary tangles (NFT), senile plaques (SP), neuropil thread formation, and A β deposition; neuron and synapse loss; and proliferation of reactive astrocytes, particularly in the hippocampus, amygdala, entorhinal cortex, and neocortex. NFT are composed of intracellular deposits of paired helical filaments

composed of hyperphosphorylated tau. Senile plaques are present in two forms: a) diffuse plaques (DP) composed of amorphous extracellular deposits of A β that lack neurites, and b) neuritic plaques (NP) composed of extracellular deposits of insoluble A β surrounded by dystrophic neurites, reactive astrocytes, and activated microglia. Recent studies suggest that, in addition to insoluble A β present in SP, soluble A β oligomers are present in the AD brain and may represent the main toxic form of A β , thus implicating them in the disease process [79,109,192].

Risk factors identified for AD include age [67], genetic factors including presenilin 1 (PS1) and 2 (PS2) and APP mutations [125] [180], single nucleotide polymorphisms in the gene that codes for ubiquilin-1 [16], a genetic locus on chromosome 10 that includes the insulin-degrading enzyme [15,65,145] that may interact with and degrade A β , inherited variants in SORL1 [163], and the presence of apolipoprotein E4 alleles [43]. Additional risk factors for AD include head injury [142], low educational attainment and low linguistic ability early in life [176,177], diabetes [33,124,153], hyperlipidemia [99], hypertension [174], heart disease [110], smoking [136,137], elevated plasma homocysteine [169], and obesity [83].

The major barrier to treating and eventually preventing AD is a lack of understanding of the etiology and pathogenesis of neuron degeneration and loss. Clearly, AD is a disease of multiple, probably interrelated, etiologic/pathogenic factors. Numerous etiologic/pathogenic mechanisms have been suggested for the cause of AD including genetic defects [179,180], the amyloid cascade hypothesis (reviewed in [178]), trace element toxicity (reviewed in [135]), the oxidative stress hypothesis [46], mitochondrial defects [190], or a combination of the above. One hypothesis receiving renewed interest is the potential role of altered zinc (Zn) homeostasis in the pathogenesis of neuron degeneration mediated by A β processing and oligomerization and SP formation in AD.

Zinc and Zinc Homeostasis in Brain

Zinc is an essential trace element [159] that is redox inert with structural, catalytic, and regulatory roles [17,80,186] and functions as a crucial component in over 300 enzymes and transcription factors where it serves as an essential cofactor for catalytic activity [70] or by conferring structural stability to Zn finger domains of DNA binding proteins [42] including stimulating protein-1 (sp-1), a transcription factor responsible for ~30% of APP transcription [18,48,49]. In addition to its structural and catalytic roles, recent studies suggest free Zn has important signaling functions including inhibition of gamma aminobutyric acid (GABA-ergic) neurotransmission [84], modulation of protein kinase C (PKC) signaling pathways [116], modulation of p53 mediated DNA repair through stabilization of p53/genomic DNA interactions [139], and modulation of glycogen synthase kinase 3 β [2,95].

In the brain, Zn is distributed in 3 pools including: a) a membrane-bound metalloprotein, or protein-metal complex pool involved in metabolic reactions and nonmetabolic functions such as biomembrane structure and support; b) a vesicular pool localized in nerve terminal synaptic vesicles; and c) an ionic pool of free or loosely bound ions in the cytoplasm [70]. Of these sources, the vesicular pool, which is easily chelated, is thought to be the most important [50, 69,86,155] because it is released during neurotransmission and may reach concentrations of 300 μ M in the synapse. Unless these Zn gradients are immediately sequestered, they could potentially induce neurodegeneration. Overall, mean brain Zn concentrations are ~ 10 times serum Zn levels [184] and range between 150 and 200 μ M [62,160]. Transport of Zn into the brain occurs via the blood/brain and blood/cerebrospinal fluid (CSF) barriers [147] where brain capillary endothelial cells respond to changes in Zn status by increasing or decreasing Zn uptake [123]. Regionally, Zn concentrations, particularly vesicular Zn, are highest in the hippocampus, amygdala, and neocortex and are relatively low in cerebellum [51,72], a pattern that mirrors

the distribution of pathologic features in AD. Zinc concentrations range from $< 10^{-9}$ M in the cytoplasm in most neurons to $> 10^{-3}$ M in vesicles of mossy fiber terminals [69,196]. Peak extracellular concentrations may reach 300 μ M during seizure activity or during neurotransmission [7,89]. Therefore, it is imperative that cells regulate Zn levels through control of influx and efflux and through chelation to Zn sequestering proteins.

In general, Zn homeostasis is maintained by three families of proteins: a) metallothioneins (MT), b) Zrt-Irt like (ZIP) proteins, and c) zinc transporter (ZnT) proteins. Metallothioneins are a group of low molecular weight (6 – 7 kDa) single polypeptide chains of 60 or more amino acids, 25% – 30% of which are cysteines. These proteins display high Zn binding affinity ($K_{Zn} = 3.2 \times 10^{-13} \text{ M}^{-1}$ at pH 7.4), can bind 7 atoms of Zn per molecule, and function to sequester Zn immediately after uptake by cells to prevent toxicity [152]. Metallothioneins are ubiquitous and can be induced by metals, glucocorticoids, pro-inflammatory cytokines, oxidative stress, electrophilic compounds, and xenobiotics [100,152,187]. In brain, MT is expressed as 3 functional isoforms including MT-I and II, which are expressed in astrocytes, the perivascular space and pia mater [154] in a Zn-dependent manner [8,60] and MT-III, which is most abundant in neurons that sequester Zn in synaptic vesicles [25]. The low redox potential of MT (–366 mV) allows mild oxidation to decrease Zn binding and facilitates release of Zn for binding to Zn finger and other transcription factors to modulate DNA binding efficiency and expression of genes involved in antioxidant responses during periods of oxidative stress [143].

Although the transport of Zn from brain extracellular environments to intracellular compartments in neurons and glia is not completely understood, it is thought to involve members of the ZIP family of proteins [39]. ZIP proteins are predicted to have 8 transmembrane domains with a histidine-rich intracellular loop between domains 3 and 4 [92] and are part of the plasma membrane or membranes of intracellular organelles. To date, 14 mammalian ZIP proteins that function to increase intracellular Zn by increasing Zn uptake (ZIP 1- 5; 7 – 15) or by releasing Zn from intracellular stores when Zn is deficient (ZIP 6 and 7) have been identified using mouse and human sequence analysis. In addition to ZIP proteins, neuronal Zn uptake may also be mediated by Zn-permeable membrane spanning channels including voltage gated L-type Ca^{2+} [42], N-methyl-D aspartate (NMDA) receptor gated [113], Ca^{2+} permeable AMPA/kainate channels [98] and $\text{Na}^+/\text{Zn}^{2+}$ exchangers [34].

Export and sequestration of Zn are carried out by the ZnT family of proteins that are predicted to have 6 transmembrane domains with a histidine-rich loop between transmembrane domains 4 and 5. To date, 8 ZnT proteins have been described (reviewed [64]). ZnT-1 is located at the plasma membrane and is expressed in the brain and other organs [150], whereas the other ZnT proteins are expressed at the membrane of intracellular organelles. ZnT-1 expression is induced in the presence of elevated cytoplasmic Zn through direct binding of Zn to the Zn-finger domain of metal response element-binding transcription factor-1 (MTF-1) (reviewed [6]). After binding Zn, MTF-1 adopts the appropriate DNA binding conformation and translocates to the nucleus where it binds the metal response element (MRE) in genes for ZnT-1, MT and gamma glutamylcysteine synthetase heavy chain which controls the rate-limiting step in glutathione synthesis (reviewed [6]). Overexpression of ZnT-1 in baby hamster kidney cells conferred resistance to increased Zn with the rate of Zn efflux increasing as extracellular Zn concentrations increased leading to the conclusion that Zn efflux mediated by ZnT-1 is an energy dependent process and argues against ZnT-1 being a channel or facilitated transporter [150]. In contrast, Chohanadisa et al. [38] reported that rats provided a Zn-deficient diet demonstrated decreased brain ZnT-1, suggesting low systemic Zn could decrease ZnT-1 to maintain brain Zn stores. These studies are consistent with those of Takeda et al. [147] who found rats on a Zn-deficient diet showed increased brain Zn. Studies from our laboratory show ZnT-1 protein expression and function can be inactivated by HNE [132], a neurotoxic aldehydic

marker of lipid peroxidation present in MCI and LAD brain [129,197]. Additionally, in the only study of ZnT-1 in AD, our Western blot analyses show significantly decreased ZnT-1 in the HPG of MCI, but significant elevations in EAD and LAD [131]. More recent studies demonstrated that ZnT-1 reduces Zn influx through the L-type calcium channels (LTCC) without increasing Zn efflux [146,149,167].

ZnT-2 is a component of vesicular acid intracellular compartments, and in mice, is predominately expressed in intestine, kidney, and testis and is scarcely detected in brain [151]. In *in vitro* experiments using baby hamster kidney cells, ZnT-2 overexpression conferred resistance to elevated Zn with sequestration into acidic compartments at higher concentrations [151]. Additionally, co-expression of ZnT-1 suppressed ZnT-2 mediated transport into acidic vesicles suggesting ZnT-2 has a relatively low affinity for Zn and functions only under excessive elevations of Zn as a second line of defense when other ZnTs fail to function properly [151]. ZnT-3 sequesters Zn in vesicles and is expressed only in brain and testis [151]. In mouse brain, ZnT-3 is associated with hippocampal dentate granule cells, pyramidal and interneurons as evidenced by levels of mRNA [151]. A potential role for ZnT-3 and synaptic Zn in SP formation in transgenic mice was suggested by recent studies that showed crossing mice expressing mutant APP with ZnT-3 null mice led to diminished A β deposition [81]. More recently, Friedlich et al. [74] showed that these mice also demonstrate reduced cerebral amyloid angiopathy that is hypothesized to be due to diminished Zn concentrations in the perivascular space of ZnT-3 null mice. In our studies of LAD and control brain, we find no significant alterations of ZnT-3 in LAD.

ZnT-4 exhibits considerable homology with ZnT-2 and 3 and is present in mammary gland and brain [90]. ZnT-4 functions to sequester Zn in acidic vesicles and is best characterized in its involvement in the transport of Zn²⁺ into milk during lactation [103]. Our study of ZnT-4 in MCI, EAD, and LAD brain is the first study of this protein in human brain and shows significant elevations of the protein in the HPG and SMTG of EAD and LAD subjects compared to age-matched controls [127]. Although ZnT-5 mRNA is found in most organs in mice, the highest protein expression is in pancreas where it is associated with Zn-enriched secretory granules in insulin containing β cells and is scarcely detected in brain [101]. In contrast to other ZnT proteins, ZnT-5 is predicted to have 15 membrane spanning domains and is ~ twice the size of other ZnT proteins [42].

ZnT-6 functions to sequester cytoplasmic Zn in the TGN and vesicular compartments [91] and in mice, ZnT-6 mRNA is present in multiple organs including brain. In the only studies of ZnT-6 in human brain, we show significantly increased ZnT-6 levels in the HPG of EAD and LAD subjects compared to NC and a trend toward a significant elevation in MCI [175]. We also observed a striking association of ZnT-6 with NFT-bearing neurons identified using the modified Bielschowsky stain in LAD and in neurons positive for MC-1, a marker of early NFT formation in MCI [127]. Similar to ZnT-6, ZnT-7 functions to sequester Zn in the TGN but has expression limited to lung and small intestine [108]. Most recently, ZnT-8 has been characterized and is primarily associated with secretory granules of pancreatic β cells [110, 162] where it likely plays a role in insulin transport. In mice ZnT-8 has limited expression in brain.

Although Zn is essential for normal brain function, experimental studies show high concentrations of Zn are toxic to neurons *in vitro* [37,59,203] and *in vivo* [40,47,114] with increased oxidative stress, and necrotic and apoptotic cell death occurring in as little as 30 min [37,76,133] [106]. Unfortunately, the exact mechanism of Zn-induced cell death remains unclear. One possible mechanism is through the potentiation of glutamate [12,20,50,69,105, 181], AMPA [23,37,73,112] or kainic acid [37,170,202] toxicity. Zinc may play a role in mitochondrial dysfunction through inhibition of the cell respiratory chain by blocking the initial

step of respiration – the transfer of an electron between coenzyme Q and cytochrome b of the bc₁ complex [19,93,111]. At high concentrations, Zn inhibits levels of complex I and II and cytochrome oxidase [173], although Yamaguchi et al. [199] demonstrated increased mitochondrial function in rat liver after a single low dose of Zn. More recent studies [32,88, 118] show that nM – μM concentrations of Zn inhibit a number of enzymes required for mitochondrial respiration and glycolysis and that exposure to 100 μM Zn caused a 50% loss of intracellular phosphate [88]. A dysfunction in oxidative phosphorylation, coupled with the presence of free radicals, could in turn lead to release of Zn from MT and increased intracellular concentrations of Zn [68]. At relatively low concentrations, Zn inhibits sodium/potassium ATPase (Na⁺K⁺ ATPase) activity in isolated protein, inhibits glutamate and GABA uptake in mice synaptosomes [75] and glutamate transport by human excitatory amino acid transporter (EAAT) 1 in *Xenopus laevis* oocytes [188].

Zinc is hypothesized to induce structural abnormalities by influencing assembly and disassembly of tubulin [61,76,77] and several microtubule associated proteins *in vitro* [9,76, 77,117]. In addition, Zn has been shown to mediate tau phosphorylation through modulation of P13/AKT, ERK1/2 and p38/MAPK signaling cascades [2]. Zinc influx into cells by NMDA receptor channels may lead to a depolarization of the cells resulting in a rise of calcium (Ca) that could activate second messenger systems via PKC-mediated phosphorylation of receptor ion channels or voltage-dependent gene expression [8,144,164]. Zinc may also disrupt Ca homeostasis by binding to calmodulin [11] and through the inhibition of calmodulin-complexed Ca ATPase [21]. Chelatable Zn accumulates in the cell perikarya of apoptotic neurons before and during degeneration after ischemic insult [117,185] and seizure activity [70], and is suggested to play a pathological role in neuron death. A more recent study using Zinquin, a Zn-specific fluorophore, demonstrated an increase in intracellular Zn levels as an early event in apoptosis that occurs in the absence of exogenous Zn and is consistent with a release of Zn from intracellular stores [205].

Zinc and Alzheimer's Disease

Interest in Zn and its possible role in the pathogenesis of AD was initiated in 1981 when Burnet proposed that Zn deficiencies led to dementia [22]. Although initial studies of AD and control brain showed significantly decreased Zn in the hippocampus, inferior parietal lobule and occipital cortex of LAD subjects [3,4,45,55] later studies using short postmortem interval (PMI) tissue specimens from well characterized LAD and control subjects showed significant elevations of Zn in LAD hippocampus, amygdala, and multiple neocortical areas [44,51,53, 63,166,194]. The differences observed between the studies may lie in the fact that formalin-fixed tissues were used in some of the earlier studies that also included control subjects that were not prospectively evaluated. Although multiple studies show alterations of Zn in late stage AD, there have been no studies of Zn concentrations in brain in MCI.

Despite considerable study of Zn at the bulk level, the cellular localization of Zn alterations in the progression of AD is unclear. Studies of Zn distribution in AD have primarily focused on the association of Zn with SP. Using micro-particle induced x-ray emission (micro-PIXE), we initially showed increased Zn in SP compared to adjacent neuropil and an elevation of Zn in LAD neuropil compared to age-matched NC [130]. Several subsequent studies have confirmed those findings in AD [35,71,138,182] and in amyloid plaques in Tg2576 transgenic mice expressing mutant APP [74,122]. Recent studies using Raman microscopy to evaluate the structure and composition of isolated senile plaques Dong et al. [56] showed Zn²⁺ and Cu²⁺ are specifically coordinated with histidine residues and that chelation of Cu²⁺ led to a disruption of the β-structure of isolated plaques. Despite considerable study of Zn in SP, there have been relatively few studies that measure Zn in individual neurons in AD.

Although the subject of extensive study over the past 25 years, the reasons for elevated brain Zn in AD are unclear. Several studies have attempted to relate changes in peripheral Zn to elevated brain levels, although these studies have been contradictory. Haines et al. [85], Molina et al. [141], and Shore et al. [170] showed no significant differences between AD and control serum Zn, whereas Jeandel et al. [97] showed a significant decrease in Zn and other nutrients and antioxidant properties in AD serum, although the AD group may have contained malnourished subjects. The study of Haines et al. [85] may also be questioned because it included control subjects whose Mini Mental Status Examination (MMSE) scores were considered cognitively impaired. In contrast, Rulon et al. [165] and Gonzales et al. [30] showed significant elevations of Zn in AD serum. Additionally, Gonzales et al. [30] showed that serum Zn correlated with the presence of APOE4 alleles and concluded that of the parameters analyzed in their study, only serum Zn appeared to be an independent risk factor associated with the development of AD. In more recent studies of serum Zn in the progression of AD we observed a statistically significant decrease of serum Zn in men with MCI compared to women with MCI or age-matched normal control men [58]. In contrast, we found no significant differences in serum Zn between well characterized LAD subjects and cognitively normal control subjects. Our observation of decreased serum Zn in MCI is of interest in light of previous *in vivo* rat studies in which systemic Zn deficiencies led to diminished ZnT-1 levels and increased brain Zn [38,147]. Our data support the hypothesis that elevated brain Zn in AD may be due to increased Zn uptake by brain under conditions of diminished extraparenchymal Zn in MCI.

Studies of CSF Zn levels have also been inconsistent with Molina et al. [141] showing decreased Zn in AD CSF compared to age matched control subjects whereas Basun et al. [10] showed no significant changes in AD CSF Zn. In addition, recent studies [78,183] show there is an inverse relationship between Zn and copper concentrations and levels of A β ₁₋₄₂ in CSF of LAD subjects and that degradation of soluble A β is normally promoted by physiologic concentrations of both Cu and Zn [183]. Although the potential variation of Zn through the progression of AD is of interest there have been no published studies of CSF levels of Zn in MCI subjects.

Zinc and Amyloid Beta (A β) Peptide Processing and Aggregation

Although several studies indicate alterations in Zn levels in the AD brain, the reason for its elevation and direct evidence for its role in the pathogenesis of AD has been lacking. Although Zn may play a role in multiple pathways relevant to AD, the most widely studied has been the possible role of Zn in processing of APP and aggregation of A β . APP synthesis is regulated by Zn-containing transcription factors (NF- κ B and Sp1) and although Zn is essential for their activity [200,204,206], it is unclear whether the activity *in vivo* is regulated by Zn availability. In addition to the potential influence of Zn on APP expression, it also influences processing of the protein. Normal processing of APP by α -secretase cleavage in the Golgi complex leads to formation of sAPP, a neurotrophic factor [198]. Additional proteolytic processing of APP by β -secretase (BACE) at the β -cleavage site [5,31,94,171,189] occurs in endosomes [107, 115], where acidic pH necessary for β -secretase activity is possible [198]. Further processing by the γ -secretase complex at the plasma membrane (reviewed [172]) leads to formation of A β , a 40 or 42 amino acid peptide that is the major component of SP in AD [168]. Additionally, APP contains a ligand-binding site for Zn spanning the α -secretase position [24,25]. Zn concentrations less than 50 μ M inhibit α -secretase mediated sAPP formation and increase generation of A β [25] perhaps through altered protein conformation. Additionally, high Zn concentrations can inhibit matrix metalloproteinase-2 (MMP-2) [9], which is able to partially degrade soluble A β ₁₋₄₂ *in vitro* [14]. This inhibition of MMP-2 could lead to increased amyloidogenic A β levels. Recent studies suggest that most APP molecules are transported through the TGN where α -secretase cleavage likely occurs. Because Zn can significantly

modulate APP processing leading to increased A β production, ZnT-6 mediated accumulation of Zn in the TGN could initially diminish α -secretase cleavage of APP. In addition, the presence of elevated Zn in endosomes mediated by ZnT-2 and/or ZnT-4 could further enhance β -secretase activity through modulation of pH.

In addition to its effect on APP processing, several reports indicate that Zn at low physiological concentrations [25–27,134] induces A β aggregation, although later studies indicate that higher Zn concentrations are required [41,66] for significant aggregation (fibril formation). More recent studies using atomic force and transmission electron microscopy and A β ₁₃₋₂₁ show Zn²⁺ specifically controls the rate of fibril assembly and regulates fibril morphology via specific coordination sites [57].

Multiple studies show that treatment of cortical neuron cultures with A β leads to increased levels of reactive oxygen species, increased lipid peroxidation, protein oxidation, mitochondrial dysfunction, caspase activation, and neuron death [29,32,104,201]. Additionally, several transgenic models of AD [82,126,148] including those with mutant APP, mutant APP/PS1 or mutant APP/PS1 and tau show increased A β deposition. Although A β deposits are associated with AD, the specific A β species responsible for neurodegeneration are unclear. Fibrillar A β , the predominant component of insoluble amyloid plaques, is neurotoxic [128,158]. However, *in vivo*, insoluble A β deposits do not accurately predict the severity of dementia in AD subjects [35]. In addition, studies of transgenic mice including those with APP mutations show cognitive dysfunction and synaptic damage that precede amyloid plaque deposition and neuron loss [96,119,140,143,195], leading to the suggestion that soluble oligomeric or protofibril A β species may be the most toxic.

In vitro studies of synthetic A β show monomeric A β aggregates in a time-dependent manner that may be accelerated by Zn leading to oligomeric species, which may eventually form fibrils [39,157,191]. Increasing evidence suggests that it is these soluble oligomeric species that are the predominant neurotoxic species for neurons [54,109], leading to inhibition of long term potentiation in synaptic hippocampal slices [120,193] and calcium dysregulation and membrane dysfunction [54,102]. Although the exact A β species responsible for mediating neurodegeneration in AD are unclear, several lines of evidence support a role for oxidative damage mediated by A β or other oxidative species in the pathogenesis of AD including lipid peroxidation, protein oxidation, and DNA/RNA oxidation.

Zinc as a Therapeutic Target in AD

Based on the potential role of Zn and Cu in the deposition of A β in AD brain there has been considerable interest in the use of metal chelators to decrease amyloid pathology [28]. *In vitro* studies show clioquinol (CQ), an 8-OH quinoline can inhibit aggregation of A β by Cu and Zn [36]. In *in vivo* studies treatment of transgenic mouse models of amyloid deposition (Tg 2576) with CQ for 9 weeks significantly reduced amyloid plaque burden while simultaneously increasing brain Zn and Cu [36]. In pilot, phase 2 double blind placebo controlled clinical trials CQ was shown to significantly slow cognitive decline in AD patients compared to placebo controls [161]. More recently, PBT2, an 8-hydroxy quinoline with increased blood brain barrier permeability has been developed [1] and in a 12 week phase IIa clinical trial of AD subjects reversed frontal lobe functional deficits and significantly decreased A β ₁₋₄₂ levels in cerebrospinal fluid [121]. Together, these data suggest modulation of Zn may be an effective potential therapeutic target in AD.

Conclusions and Future Directions

Although considerable evidence suggests a link between alterations in Zn and the proteins responsible for its uptake and sequestration in the progression of AD, there is a need for further

in-depth study of Zn, ZIP and ZnT proteins, particularly early in the progression of AD (MCI) when therapeutic interventions would have greater efficacy. In particular there is a need for quantification of Zn levels in CSF of subjects with MCI and EAD and the correlation of those concentrations with brain ZnT, ZIP and Zn levels and the factors that regulate regional and cellular Zn concentrations. Based on *in vivo* studies, it is tempting to hypothesize that low extra-parenchymal Zn early in disease progression may lead to increased levels of brain Zn. These elevations of Zn could then become concentrated in subcellular organelles in which A β processing occurs leading to increased generation of oligomeric A β species and the promotion of oxidative damage associated with AD. As the disease progresses and extra-parenchymal Zn levels normalize, the resulting alterations in multiple ZnT proteins could further promote A β aggregation and SP formation.

Acknowledgments

Supported by NIH grants 1R01-AG16269, 5-P01-AG05119 and 1P30-AG028383, and by a grant from the Abercrombie Foundation. The author thanks Ms. Paula Thomason for editorial assistance.

References

- Adlard PA, Cherny RA, Finkelstein DI, Gautier E, Robb E, Cortes M, Volitakis I, Liu X, Smith JP, Perez K, Laughton K, Li QX, Charman SA, Nicolazzo JA, Wilkins S, Deleva K, Lynch T, Kok G, Ritchie CW, Tanzi RE, Cappai R, Masters CL, Barnham KJ, Bush AI. Rapid restoration of cognition in Alzheimer's transgenic mice with 8-hydroxy quinoline analogs is associated with decreased interstitial A β . *Neuron* 2008;59:43–55. [PubMed: 18614028]
- An WL, Bjorkdahl C, Liu R, Cowburn RF, Winblad B, Pei JJ. Mechanism of zinc-induced phosphorylation of p70 S6 kinase and glycogen synthase kinase 3 β in SH-SY5Y neuroblastoma cells. *J Neurochem* 2005;92:1104–1115. [PubMed: 15715661]
- Andrasi E, Nadasdi J, Molnar Z, Bezur L, Ernyei L. Determination of main and trace element contents in human brain by NAA and ICP-AES methods. *Biol Trace Elem Res* 1990;26–27:691–698.
- Andrasi E, Suhajda M, Saray I, Bezur L, Ernyei L, Reffy A. Concentration of elements in human brain: glioblastoma multiforme. *Sci Total Environ* 1993;139–140:399–402.
- Andrasi E, Farkas E, Gawlik D, Rosick U, Bratter P. Brain Iron and Zinc Contents of German Patients with Alzheimer Disease. *J Alzheimers Dis* 2000;2:17–26. [PubMed: 12214107]
- Andrews GK. Cellular zinc sensors: MTF-1 regulation of gene expression. *Biomaterials* 2001;14:223–237. [PubMed: 11831458]
- Assaf SY, Chung SH. Release of endogenous Zn²⁺ from brain tissue during activity. *Nature* 1984;308:734–736. [PubMed: 6717566]
- Atar D, Backx PH, Appel MM, Gao WD, Marban E. Excitation-transcription coupling mediated by zinc influx through voltage-dependent calcium channels. *J Biol Chem* 1995;270:2473–2477. [PubMed: 7852308]
- Backstrom JR, Miller CA, Tokes ZA. Characterization of neutral proteinases from Alzheimer-affected and control brain specimens: identification of calcium-dependent metalloproteinases from the hippocampus. *J Neurochem* 1992;58:983–992. [PubMed: 1738004]
- Basun H, Forsell LG, Wetterberg L, Winblad B. Metals and trace elements in plasma and cerebrospinal fluid in normal aging and Alzheimer's disease. *J Neural Transm Park Dis Dement Sect* 1991;3:231–258. [PubMed: 1772577]
- Baudier J, Haglid K, Haiech J, Gerard D. Zinc ion binding to human brain calcium binding proteins, calmodulin and S100b protein. *Biochem Biophys Res Commun* 1983;114:1138–1146. [PubMed: 6615509]
- Beaulieu C, Dyck R, Cynader M. Enrichment of glutamate in zinc-containing terminals of the cat visual cortex. *Neuroreport* 1992;3:861–864. [PubMed: 1358251]
- Bennett DA, Wilson RS, Schneider JA, Evans DA, Beckett LA, Aggarwal NT, Barnes LL, Fox JH, Bach J. Natural history of mild cognitive impairment in older persons. *Neurology* 2002;59:198–205. [PubMed: 12136057]

14. Bergeron C, Petrunka C, Weyer L. Copper/zinc superoxide dismutase expression in the human central nervous system. Correlation with selective neuronal vulnerability. *Am J Pathol* 1996;148:273–279. [PubMed: 8546216]
15. Bertram L, Blacker D, Mullin K, Keeney D, Jones J, Basu S, Yhu S, McInnis MG, Go RC, Vekrellis K, Selkoe DJ, Saunders AJ, Tanzi RE. Evidence for genetic linkage of Alzheimer's disease to chromosome 10q. *Science* 2000;290:2302–2303. [PubMed: 11125142]
16. Bertram L, Hiltunen M, Parkinson M, Ingelsson M, Lange C, Ramasamy K, Mullin K, Menon R, Sampson AJ, Hsiao MY, Elliott KJ, Velicelebi G, Moscarillo T, Hyman BT, Wagner SL, Becker KD, Blacker D, Tanzi RE. Family-based association between Alzheimer's disease and variants in UBQLN1. *N Engl J Med* 2005;352:884–894. [PubMed: 15745979]
17. Bettger WJ, O'Dell BL. A critical physiological role of zinc in the structure and function of biomembranes. *Life Sci* 1981;28:1425–1438. [PubMed: 7017326]
18. Bittel D, Dalton T, Samson SL, Gedamu L, Andrews GK. The DNA binding activity of metal response element-binding transcription factor-1 is activated in vivo and in vitro by zinc, but not by other transition metals. *J Biol Chem* 1998;273:7127–7133. [PubMed: 9507026]
19. Blennow K, Wallin A, Agren H, Spenger C, Siegfried J, Vanmechelen E. Tau protein in cerebrospinal fluid: a biochemical marker for axonal degeneration in Alzheimer disease? *Mol Chem Neuropathol* 1995;26:231–245. [PubMed: 8748926]
20. Bramham CR, Torp R, Zhang N, Storm-Mathisen J, Ottersen OP. Distribution of glutamate-like immunoreactivity in excitatory hippocampal pathways: a semiquantitative electron microscopic study in rats. *Neuroscience* 1990;39:405–417. [PubMed: 2087264]
21. Brewer GJ, Aster JC, Knutsen CA, Kruckeberg WC. Zinc inhibition of calmodulin: a proposed molecular mechanism of zinc action on cellular functions. *Am J Hematol* 1979;7:53–60. [PubMed: 159621]
22. Burnet FM. A possible role of zinc in the pathology of dementia. *Lancet* 1981;1:186–188. [PubMed: 6162062]
23. Buschke H, Kuslansky G, Katz M, Stewart WF, Sliwinski MJ, Eckholdt HM, Lipton RB. Screening for dementia with the memory impairment screen. *Neurology* 1999;52:231–238. [PubMed: 9932936]
24. Bush AI, Multhaup G, Moir RD, Williamson TG, Small DH, Rumble B, Pollwein P, Beyreuther K, Masters CL. A novel zinc(II) binding site modulates the function of the beta A4 amyloid protein precursor of Alzheimer's disease. *J Biol Chem* 1993;268:16109–16112. [PubMed: 8344894]
25. Bush AI, Pettingell WH, Multhaup G, Paradis M d, Vonsattel JP, Gusella JF, Beyreuther K, Masters CL, Tanzi RE. Rapid induction of Alzheimer A beta amyloid formation by zinc. *Science* 1994;265:1464–1467. [PubMed: 8073293]
26. Bush AI, Pettingell WH, Paradis MD, Tanzi R. Zinc and Alzheimer's disease. *Science* 1995;268:1921–1923. [PubMed: 17797535]
27. Bush AI, Atwood CS, Huang L. Abnormal homeostasis of ph1 and zinc: the prelunde for cerebral Aβ amyloid formation. *Eur Neuropsychopharmacol* 1996;6(suppl 3):S2–4.
28. Bush AI. The metallobiology of Alzheimer's disease. *Trends Neurosci* 2003;26:207–214. [PubMed: 12689772]
29. Butterfield DA. Amyloid beta-peptide [1-42]-associated free radical-induced oxidative stress and neurodegeneration in Alzheimer's disease brain: mechanisms and consequences. *Curr Med Chem* 2003;10:2651–2659. [PubMed: 14529455]
30. Martin GCT, Cacho J, Brenea MT. Serum zinc, copper, insulin and lipids in Alzheimer's disease epsilon 4 apolipoprotein E allele carriers. *Eur J Clin Invest* 1999;29:637–642. [PubMed: 10411671]
31. Calingasan NY, Uchida K, Gibson GE. Protein-bound acrolein: a novel marker of oxidative stress in Alzheimer's disease. *J Neurochem* 1999;72:751–756. [PubMed: 9930749]
32. Canzoniero LM, Turetsky DM, Choi DW. Measurement of intracellular free zinc concentrations accompanying zinc-induced neuronal death. *J Neurosci* 1999;19:RC31. [PubMed: 10493776]
33. Chan SL, Griffin WS, Mattson MP. Evidence for caspase-mediated cleavage of AMPA receptor subunits in neuronal apoptosis and Alzheimer's disease. *J Neurosci Res* 1999;57:315–323. [PubMed: 10412022]

34. Cheng C, Reynolds IJ. Calcium-sensitive fluorescent dyes can report increases in intracellular free zinc concentration in cultured forebrain neurons. *J Neurochem* 1998;71:2401–2410. [PubMed: 9832138]
35. Cherny RA, Legg JT, McLean CA, Fairlie DP, Huang X, Atwood CS, Beyreuther K, Tanzi RE, Masters CL, Bush AI. Aqueous dissolution of Alzheimer's disease Abeta amyloid deposits by biometal depletion. *J Biol Chem* 1999;274:23223–23228. [PubMed: 10438495]
36. Cherny RA, Atwood CS, Xilinas ME, Gray DN, Jones WD, McLean CA, Barnham KJ, Volitakis I, Fraser FW, Kim Y, Huang X, Goldstein LE, Moir RD, Lim JT, Beyreuther K, Zheng H, Tanzi RE, Masters CL, Bush AI. Treatment with a copper-zinc chelator markedly and rapidly inhibits beta-amyloid accumulation in Alzheimer's disease transgenic mice. *Neuron* 2001;30:665–676. [PubMed: 11430801]
37. Choi DW, Yokoyama M, Koh J. Zinc neurotoxicity in cortical cell culture. *Neuroscience* 1988;24:67–79. [PubMed: 3368058]
38. Chohanadisai W, Kelleher SL, Lonnerdal B. Zinc deficiency is associated with increased brain zinc import and LIV-1 expression and decreased ZnT-1 expression in neonatal rats. *J Nutr* 2005;135:1002–1007. [PubMed: 15867272]
39. Chromy BA, Nowak RJ, Lambert MP, Viola KL, Chang L, Velasco PT, Jones BW, Fernandez SJ, Lacor PN, Horowitz P, Finch CE, Krafft GA, Klein WL. Self-assembly of Abeta(1-42) into globular neurotoxins. *Biochemistry* 2003;42:12749–12760. [PubMed: 14596589]
40. Chuah MI, Tennent R, Jacobs I. Response of olfactory Schwann cells to intranasal zinc sulfate irrigation. *J Neurosci Res* 1995;42:470–478. [PubMed: 8568933]
41. Clements A, Allsop D, Walsh DM, Williams CH. Aggregation and metal-binding properties of mutant forms of the amyloid A beta peptide of Alzheimer's disease. *J Neurochem* 1996;66:740–747. [PubMed: 8592147]
42. Colvin RA, Fontaine CP, Laskowski M, Thomas D. Zn²⁺ transporters and Zn²⁺ homeostasis in neurons. *Eur J Pharmacol* 2003;479:171–185. [PubMed: 14612148]
43. Corder EH, Saunders AM, Strittmatter WJ, Schmechel DE, Gaskell PC, Small GW, Roses AD, Haines JL, Pericak-Vance MA. Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. *Science* 1993;261:921–923. [PubMed: 8346443]
44. Cornett CR, Markesbery WR, Ehmann WD. Imbalances of trace elements related to oxidative damage in Alzheimer's disease brain. *Neurotoxicology* 1998;19:339–345. [PubMed: 9621340]
45. Corrigan FM, Reynolds GP, Ward NI. Hippocampal tin, aluminum and zinc in Alzheimer's disease. *Biomaterials* 1993;6:149–154. [PubMed: 8400761]
46. Coyle JT, Puttfarcken P. Oxidative stress, glutamate, and neurodegenerative disorders. *Science* 1993;262:689–695. [PubMed: 7901908]
47. Cuajungco MP, Lees GJ. Prevention of zinc neurotoxicity in vivo by N,N,N',N'-tetrakis (2-pyridylmethyl) ethylene-diamine (TPEN). *Neuroreport* 1996;7:1301–1304. [PubMed: 8817554]
48. Dalton TP, Li Q, Bittel D, Liang L, Andrews GK. Oxidative stress activates metal-responsive transcription factor-1 binding activity. Occupancy in vivo of metal response elements in the metallothionein-I gene promoter. *J Biol Chem* 1996;271:26233–26241. [PubMed: 8824273]
49. Dalton TP, Bittel D, Andrews GK. Reversible activation of mouse metal response element-binding transcription factor 1 DNA binding involves zinc interaction with the zinc finger domain. *Mol Cell Biol* 1997;17:2781–2789. [PubMed: 9111349]
50. Danscher G, Howell G, Perez-Clausell J, Hertel N. The dithizone, Timm's sulphide silver and the selenium methods demonstrate a chelatable pool of zinc in CNS. A proton activation (PIXE) analysis of carbon tetrachloride extracts from rat brains and spinal cords intravitaly treated with dithizone. *Histochemistry* 1985;83:419–422. [PubMed: 3000994]
51. Danscher G, Jensen KB, Frederickson CJ, 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. *J Neurosci Methods* 1997;76:53–59. [PubMed: 9334939]
52. DeCarli C. Mild cognitive impairment: prevalence, prognosis, aetiology, and treatment. *Lancet Neurol* 2003;2:15–21. [PubMed: 12849297]

53. Deibel MA, Ehmann WD, Markesbery WR. Copper, iron, and zinc imbalances in severely degenerated brain regions in Alzheimer's disease: possible relation to oxidative stress. *J Neurol Sci* 1996;143:137–142. [PubMed: 8981312]
54. Demuro A, Mina E, Kaye R, Milton SC, Parker I, Glabe CG. Calcium dysregulation and membrane disruption as a ubiquitous neurotoxic mechanism of soluble amyloid oligomers. *J Biol Chem* 2005;280:17294–17300. [PubMed: 15722360]
55. Deng QS, Turk GC, Brady DR, Smith QR. Evaluation of brain element composition in Alzheimer's disease using inductively coupled plasma mass spectrometry. *Neurobiol Aging* 1994;15:464.
56. Dong J, Atwood CS, Anderson VE, Siedlak SL, Smith MA, Perry G, Carey PR. Metal binding and oxidation of amyloid-beta within isolated senile plaque cores: Raman microscopic evidence. *Biochemistry* 2003;42:2768–2773. [PubMed: 12627941]
57. Dong J, Shokes JE, Scott RA, Lynn DG. Modulating amyloid self-assembly and fibril morphology with Zn(II). *J Am Chem Soc* 2006;128:3540–3542. [PubMed: 16536526]
58. Dong J, Robertson JD, Markesbery WR, Lovell MA. Serum Zinc in the Progression of Alzheimer's Disease. *J Alzheimers Dis*. 2008 In press.
59. Duncan MW, Marini AM, Watters R, Kopin IJ, Markey SP. Zinc, a neurotoxin to cultured neurons, contaminates cycad flour prepared by traditional guamanian methods. *J Neurosci* 1992;12:1523–1537. [PubMed: 1556606]
60. Durnam DM, Palmiter RD. Transcriptional regulation of the mouse metallothionein-I gene by heavy metals. *J Biol Chem* 1981;256:5712–5716. [PubMed: 7240167]
61. Eagle GR, Zombola RR, Himes RH. Tubulin-zinc interactions: binding and polymerization studies. *Biochemistry* 1983;22:221–228. [PubMed: 6403030]
62. Ebadi M, Iversen PL, Hao R, Ceruti DR, Rojas P, Happe HK, Murrin LC, Pfeiffer RF. Expression and regulation of brain metallothionein. *Neurochem Int* 1995;27:1–22. [PubMed: 7655341]
63. Ehmann WD, Markesbery WR, Alauddin M, Hossain TI, Brubaker EH. Brain trace elements in Alzheimer's disease. *Neurotoxicology* 1986;7:195–206. [PubMed: 3714121]
64. Eide DJ. Zinc transporters and the cellular trafficking of zinc. *Biochim Biophys Acta* 2006;1763:711–722. [PubMed: 16675045]
65. Ertekin-Taner N, Graff-Radford N, Younkin LH, Eckman C, Baker M, Adamson J, Ronald J, Blangero J, Hutton M, Younkin SG. Linkage of plasma Aβ42 to a quantitative locus on chromosome 10 in late-onset Alzheimer's disease pedigrees. *Science* 2000;290:2303–2304. [PubMed: 11125143]
66. Esler WP, Stimson ER, Jennings JM, Ghilardi JR, Mantyh PW, Maggio JE. Zinc-induced aggregation of human and rat beta-amyloid peptides in vitro. *J Neurochem* 1996;66:723–732. [PubMed: 8592145]
67. Evans DA, Funkenstein HH, Albert MS, Scherr PA, Cook NR, Chown MJ, Hebert LE, Hennekens CH, Taylor JO. Prevalence of Alzheimer's disease in a community population of older persons. Higher than previously reported. *Jama* 1989;262:2551–2556. [PubMed: 2810583]
68. Fliss H, Menard M. Oxidant-induced mobilization of zinc from metallothionein. *Arch Biochem Biophys* 1992;293:195–199. [PubMed: 1309984]
69. Frederickson CJ, Klitenick MA, Manton WI, Kirkpatrick JB. Cytoarchitectonic distribution of zinc in the hippocampus of man and the rat. *Brain Res* 1983;273:335–339. [PubMed: 6616240]
70. Frederickson CJ. Neurobiology of zinc and zinc-containing neurons. *Int Rev Neurobiol* 1989;31:145–238. [PubMed: 2689380]
71. Frederickson CJ, Suh SW, Silva D, Thompson RB. Importance of zinc in the central nervous system: the zinc-containing neuron. *J Nutr* 2000;130:1471S–1483S. [PubMed: 10801962]
72. Frederickson CJ, Koh JY, Bush AI. The neurobiology of zinc in health and disease. *Nat Rev Neurosci* 2005;6:449–462. [PubMed: 15891778]
73. Freund WD, Reddig S. AMPA/Zn(2+)-induced neurotoxicity in rat primary cortical cultures: involvement of L-type calcium channels. *Brain Res* 1994;654:257–264. [PubMed: 7527288]
74. Friedlich AL, Lee JY, van Groen T, Cherny RA, Volitakis I, Cole TB, Palmiter RD, Koh JY, Bush AI. Neuronal zinc exchange with the blood vessel wall promotes cerebral amyloid angiopathy in an animal model of Alzheimer's disease. *J Neurosci* 2004;24:3453–3459. [PubMed: 15056725]

75. Gabriëlsson B, Robson T, Norris D, Chung SH. Effects of divalent metal ions on the uptake of glutamate and GABA from synaptosomal fractions. *Brain Res* 1986;384:218–223. [PubMed: 2877711]
76. Gaskin F, Kress Y. Zinc ion-induced assembly of tubulin. *J Biol Chem* 1977;252:6918–6924. [PubMed: 893451]
77. Gaskin F, Kress Y, Brosnan C, Bornstein M. Abnormal tubulin aggregates induced by zinc sulfate in organotypic cultures of nerve tissue. *Neuroscience* 1978;3:1117–1128. [PubMed: 745786]
78. Gerhardtsson L, Lundh T, Minthon L, Londos E. Metal Concentrations in Plasma and Cerebrospinal Fluid in Patients with Alzheimer's Disease. *Dement Geriatr Cogn Disord* 2008;25:508–515. [PubMed: 18463412]
79. Glabe CG. Common mechanisms of amyloid oligomer pathogenesis in degenerative disease. *Neurobiol Aging* 2006;27:570–575. [PubMed: 16481071]
80. Golden, BE. Zinc in cell division and tissue growth: Physiological aspects. In: Mills, CF., editor. *Zinc in Human Biology*. Springer-Verlag; London: 1989. p. 119-127.
81. Gosavi N, Lee HJ, Lee JS, Patel S, Lee SJ. Golgi fragmentation occurs in the cells with prefibrillar alpha-synuclein aggregates and precedes the formation of fibrillar inclusion. *J Biol Chem* 2002;277:48984–48992. [PubMed: 12351643]
82. Gotz J, Chen F, van Dorpe J, Nitsch RM. Formation of neurofibrillary tangles in P3011 tau transgenic mice induced by Aβeta 42 fibrils. *Science* 2001;293:1491–1495. [PubMed: 11520988]
83. Gustafson D, Rothenberg E, Blennow K, Steen B, Skoog I. An 18-year follow-up of overweight and risk of Alzheimer disease. *Arch Intern Med* 2003;163:1524–1528. [PubMed: 12860573]
84. Haase H, Beyersmann D. Intracellular zinc distribution and transport in C6 rat glioma cells. *Biochem Biophys Res Commun* 2002;296:923–928. [PubMed: 12200136]
85. Haines A, Iliffe S, Morgan P, Dormandy T, Wood B. Serum aluminium and zinc and other variables in patients with and without cognitive impairment in the community. *Clin Chim Acta* 1991;198:261–266. [PubMed: 1889125]
86. Haug FM. Electron microscopical localization of the zinc in hippocampal mossy fibre synapses by a modified sulfide silver procedure. *Histochemie* 1967;8:355–368. [PubMed: 4876575]
87. Hebert LE, Scherr PA, Bienias JL, Bennett DA, Evans DA. Alzheimer disease in the US population: prevalence estimates using the 2000 census. *Arch Neurol* 2003;60:1119–1122. [PubMed: 12925369]
88. Ho LH, Ratnaik RN, Zalewski PD. Involvement of intracellular labile zinc in suppression of DEVD-caspase activity in human neuroblastoma cells. *Biochem Biophys Res Commun* 2000;268:148–154. [PubMed: 10652229]
89. Howell GA, Welch MG, Frederickson CJ. Stimulation-induced uptake and release of zinc in hippocampal slices. *Nature* 1984;308:736–738. [PubMed: 6717567]
90. Huang L, Gitschier J. A novel gene involved in zinc transport is deficient in the lethal milk mouse. *Nat Genet* 1997;17:292–297. [PubMed: 9354792]
91. Huang L, Kirschke CP, Gitschier J. Functional characterization of a novel mammalian zinc transporter, ZnT6. *J Biol Chem* 2002;277:26389–26395. [PubMed: 11997387]
92. Huang L, Kirschke CP, Zhang Y, Yu YY. The ZIP7 gene (Slc39a7) encodes a zinc transporter involved in zinc homeostasis of the Golgi apparatus. *J Biol Chem* 2005;280:15456–15463. [PubMed: 15705588]
93. Hunter FEJ, Ford L. Inactivation of oxidative and phosphorylative systems in mitochondria by preincubation with phosphate and other. *J Biol Chem* 1955;216:357–369. [PubMed: 13252035]
94. Hussain I, Powell D, Howlett DR, Tew DG, Meek TD, Chapman C, Gloger IS, Murphy KE, Southan CD, Ryan DM, Smith TS, Simmons DL, Walsh FS, Dingwall C, Christie G. Identification of a novel aspartic protease (Asp 2) as beta-secretase. *Mol Cell Neurosci* 1999;14:419–427. [PubMed: 10656250]
95. Ilouz R, Kaidanovich O, Gurwitz D, Eldar-Finkelman H. Inhibition of glycogen synthase kinase-3β by bivalent zinc ions: insight into the insulin-mimetic action of zinc. *Biochem Biophys Res Commun* 2002;295:102–106. [PubMed: 12083774]
96. Irizarry MC, McNamara M, Fedorchak K, Hsiao K, Hyman BT. APPSw transgenic mice develop age-related Aβeta deposits and neuropil abnormalities, but no neuronal loss in CA1. *J Neuropathol Exp Neurol* 1997;56:965–973. [PubMed: 9291938]

97. Jeandel C, Nicolas MB, Dubois F, Nabet-Belleville F, Penin F, Cuny G. Lipid peroxidation and free radical scavengers in Alzheimer's disease. *Gerontology* 1989;35:275–282. [PubMed: 2630382]
98. Jia Y, Jeng JM, Sensi SL, Weiss JH. Zn²⁺ currents are mediated by calcium-permeable AMPA/kainate channels in cultured murine hippocampal neurones. *J Physiol* 2002;543:35–48. [PubMed: 12181280]
99. Jick H, Zornberg GL, Jick SS, Seshadri S, Drachman DA. Statins and the risk of dementia. *Lancet* 2000;356:1627–1631. [PubMed: 11089820]
100. Kagi JH, Schaffer A. Biochemistry of metallothionein. *Biochemistry* 1988;27:8509–8515. [PubMed: 3064814]
101. Kambe T, Yamaguchi-Iwai Y, Sasaki R, Nagao M. Overview of mammalian zinc transporters. *Cell Mol Life Sci* 2004;61:49–68. [PubMed: 14704853]
102. Kaye R, Sokolov Y, Edmonds B, McIntire TM, Milton SC, Hall JE, Glabe CG. Permeabilization of lipid bilayers is a common conformation-dependent activity of soluble amyloid oligomers in protein misfolding diseases. *J Biol Chem* 2004;279:46363–46366. [PubMed: 15385542]
103. Kelleher SL, Lonnerdal B. Zinc transporters in the rat mammary gland respond to marginal zinc and vitamin A intakes during lactation. *J Nutr* 2002;132:3280–3285. [PubMed: 12421840]
104. Keller JN, Schmitt FA, Scheff SW, Ding Q, Chen Q, Butterfield DA, Markesbery WR. Evidence of increased oxidative damage in subjects with mild cognitive impairment. *Neurology* 2005;64:1152–1156. [PubMed: 15824339]
105. Kesslak JP, Frederickson CJ, Gage FH. Quantification of hippocampal noradrenaline and zinc changes after selective cell destruction. *Exp Brain Res* 1987;67:77–84. [PubMed: 3622685]
106. Kim EY, Koh JY, Kim YH, Sohn S, Joe E, Gwag BJ. Zn²⁺ entry produces oxidative neuronal necrosis in cortical cell cultures. *Eur J Neurosci* 1999;11:327–334. [PubMed: 9987035]
107. Kinoshita A, Fukumoto H, Shah T, Whelan CM, Irizarry MC, Hyman BT. Demonstration by FRET of BACE interaction with the amyloid precursor protein at the cell surface and in early endosomes. *J Cell Sci* 2003;116:3339–3346. [PubMed: 12829747]
108. Kirschke CP, Huang L. ZnT7, a novel mammalian zinc transporter, accumulates zinc in the Golgi apparatus. *J Biol Chem* 2003;278:4096–4102. [PubMed: 12446736]
109. Klein WL. Abeta toxicity in Alzheimer's disease: globular oligomers (ADDLs) as new vaccine and drug targets. *Neurochem Int* 2002;41:345–352. [PubMed: 12176077]
110. Kleiweke JW, Brand IA. Rapid changes in intracellular Zn²⁺ in rat hepatocytes. *J Pharmacol Toxicol Methods* 1997;38:181–187. [PubMed: 9566441]
111. Kleiner D, von Jagow G. On the inhibition of mitochondrial electron transport by Zn(2+) ions. *FEBS Lett* 1972;20:229–232. [PubMed: 11946424]
112. Koh JY, Choi DW. Quantitative determination of glutamate mediated cortical neuronal injury in cell culture by lactate dehydrogenase efflux assay. *J Neurosci Methods* 1987;20:83–90. [PubMed: 2884353]
113. Koh JY, Choi DW. Zinc toxicity on cultured cortical neurons: involvement of N-methyl-D-aspartate receptors. *Neuroscience* 1994;60:1049–1057. [PubMed: 7936205]
114. Koh JY, Suh SW, Gwag BJ, He YY, Hsu CY, Choi DW. The role of zinc in selective neuronal death after transient global cerebral ischemia. *Science* 1996;272:1013–1016. [PubMed: 8638123]
115. Koo EH, Squazzo SL. Evidence that production and release of amyloid beta-protein involves the endocytic pathway. *J Biol Chem* 1994;269:17386–17389. [PubMed: 8021238]
116. Korichneva I, Hoyos B, Chua R, Levi E, Hammerling U. Zinc release from protein kinase C as the common event during activation by lipid second messenger or reactive oxygen. *J Biol Chem* 2002;277:44327–44331. [PubMed: 12213816]
117. Kress Y, Gaskin F, Brosnan CF, Levine S. Effects of zinc on the cytoskeletal proteins in the central nervous system of the rat. *Brain Res* 1981;220:139–149. [PubMed: 6974032]
118. Krotkiewska B, Banas T. Interaction of Zn²⁺ and Cu²⁺ ions with glyceraldehyde-3-phosphate dehydrogenase from bovine heart and rabbit muscle. *Int J Biochem* 1992;24:1501–1505. [PubMed: 1426532]
119. Kumar-Singh S, Dewachter I, Moechars D, Lubke U, De Jonghe C, Ceuterick C, Checler F, Naidu A, Cordell B, Cras P, Van Broeckhoven C, Van Leuven F. Behavioral disturbances without amyloid

- deposits in mice overexpressing human amyloid precursor protein with Flemish (A692G) or Dutch (E693Q) mutation. *Neurobiol Dis* 2000;7:9–22. [PubMed: 10671319]
120. Lambert MP, Barlow AK, Chromy BA, Edwards C, Freed R, Liosatos M, Morgan TE, Rozovsky I, Trommer B, Viola KL, Wals P, Zhang C, Finch CE, Krafft GA, Klein WL. Diffusible, nonfibrillar ligands derived from A β 1–42 are potent central nervous system neurotoxins. *Proc Natl Acad Sci U S A* 1998;95:6448–6453. [PubMed: 9600986]
 121. Lannfelt L, Blennow K, Zetterberg H, Batsman S, Ames D, Harrison J, Masters CL, Targum S, Bush AI, Murdoch R, Wilson J, Ritchie CW. Safety, efficacy, and biomarker findings of PBT2 in targeting A β as a modifying therapy for Alzheimer's disease: a phase IIa, double-blind, randomised, placebo-controlled trial. *Lancet Neurol*. 2008 Epub ahead of print. 10.1016/S1474-4422(08)70167-4
 122. Lee JY, Mook-Jung I, Koh JY. Histochemically reactive zinc in plaques of the Swedish mutant beta-amyloid precursor protein transgenic mice. *J Neurosci* 1999;19:RC10. [PubMed: 10341271]
 123. Lehmann HM, Brothwell BB, Volak LP, Bobilya DJ. Zinc status influences zinc transport by porcine brain capillary endothelial cells. *J Nutr* 2002;132:2763–2768. [PubMed: 12221242]
 124. Leibson CL, Rocca WA, Hanson VA, Cha R, Kokmen E, O'Brien PC, Palumbo PJ. Risk of dementia among persons with diabetes mellitus: a population-based cohort study. *Am J Epidemiol* 1997;145:301–308. [PubMed: 9054233]
 125. Levy-Lahad E, Wasco W, Poorkaj P, Romano DM, Oshima J, Pettingell WH, Yu CE, Jondro PD, Schmidt SD, Wang K, et al. Candidate gene for the chromosome 1 familial Alzheimer's disease locus. *Science* 1995;269:973–977. [PubMed: 7638622]
 126. Lewis J, Dickson DW, Lin WL, Chisholm L, Corral A, Jones G, Yen SH, Sahara N, Skipper L, Yager D, Eckman C, Hardy J, Hutton M, McGowan E. Enhanced neurofibrillary degeneration in transgenic mice expressing mutant tau and APP. *Science* 2001;293:1487–1491. [PubMed: 11520987]
 127. Liu X, Lovell MA, Lynn BC. Detection and quantification of endogenous cyclic DNA adducts derived from trans-4-hydroxy-2-nonenal in human brain tissue by isotope dilution capillary liquid chromatography nanoelectrospray tandem mass spectrometry. *Chem Res Toxicol* 2006;19:710–718. [PubMed: 16696574]
 128. Lorenzo A, Yankner BA. Beta-amyloid neurotoxicity requires fibril formation and is inhibited by congo red. *Proc Natl Acad Sci U S A* 1994;91:12243–12247. [PubMed: 7991613]
 129. Lovell MA, Ehmann WD, Mattson MP, Markesbery WR. Elevated 4-hydroxynonenal in ventricular fluid in Alzheimer's disease. *Neurobiol Aging* 1997;18:457–461. [PubMed: 9390770]
 130. Lovell MA, Robertson JD, Teesdale WJ, Campbell JL, Markesbery WR. Copper, iron and zinc in Alzheimer's disease senile plaques. *J Neurol Sci* 1998;158:47–52. [PubMed: 9667777]
 131. Lovell MA, Markesbery WR. Ectopic expression of Musashi-1 in Alzheimer disease and Pick disease. *J Neuropathol Exp Neurol* 2005;64:675–680. [PubMed: 16106215]
 132. Lovell MA, Smith JL, Markesbery WR. Elevated zinc transporter-6 in mild cognitive impairment, Alzheimer disease, and pick disease. *J Neuropathol Exp Neurol* 2006;65:489–498. [PubMed: 16772872]
 133. Manev H, Kharlamov E, Uz T, Mason RP, Cagnoli CM. Characterization of zinc-induced neuronal death in primary cultures of rat cerebellar granule cells. *Exp Neurol* 1997;146:171–178. [PubMed: 9225750]
 134. Mantyh PW, Ghilardi JR, Rogers S, DeMaster E, Allen CJ, Stimson ER, Maggio JE. Aluminum, iron, and zinc ions promote aggregation of physiological concentrations of beta-amyloid peptide. *J Neurochem* 1993;61:1171–1174. [PubMed: 8360682]
 135. Markesbery, WR.; Ehmann, WD. Trace element alterations in Alzheimer's disease. In: Terry, RD.; Katzman, R., editors. *Alzheimer's disease*. Raven Press; New York: 1994.
 136. McMahon RJ, Cousins RJ. Regulation of the zinc transporter ZnT-1 by dietary zinc. *Proc Natl Acad Sci U S A* 1998;95:4841–4846. [PubMed: 9560190]
 137. Merchant C, Tang MX, Albert S, Manly J, Stern Y, Mayeux R. The influence of smoking on the risk of Alzheimer's disease. *Neurology* 1999;52:1408–1412. [PubMed: 10227626]

138. Miller LM, Wang Q, Telivala TP, Smith RJ, 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* 2006;155:30–37. [PubMed: 16325427]
139. Mocchegiani E, Bertoni-Freddari C, Marcellini F, Malavolta M. Brain, aging and neurodegeneration: role of zinc ion availability. *Prog Neurobiol* 2005;75:367–390. [PubMed: 15927345]
140. Moechars D, Lorent K, De Strooper B, Dewachter I, Van Leuven F. Expression in brain of amyloid precursor protein mutated in the alpha-secretase site causes disturbed behavior, neuronal degeneration and premature death in transgenic mice. *Embo J* 1996;15:1265–1274. [PubMed: 8635459]
141. Molina JA, Jimenez-Jimenez FJ, Aguilar MV, Meseguer I, Mateos-Vega CJ, Gonzalez-Munoz MJ, de Bustos F, Porta J, Orti-Pareja M, Zurdo M, Barrios E, Martinez-Para MC. Cerebrospinal fluid levels of transition metals in patients with Alzheimer's disease. *J Neural Transm* 1998;105:479–488. [PubMed: 9720975]
142. Mortimer JA, van Duijn CM, Chandra V, Fratiglioni L, Graves AB, Heyman A, Jorm AF, Kokmen E, Kondo K, Rocca WA, et al. Head trauma as a risk factor for Alzheimer's disease: a collaborative re-analysis of case-control studies. EURODEM Risk Factors Research Group. *Int J Epidemiol* 1991;20(Suppl 2):S28–35. [PubMed: 1833351]
143. Mucke L, Yu GQ, McConlogue L, Rockenstein EM, Abraham CR, Masliah E. Astroglial expression of human alpha(1)-antichymotrypsin enhances alzheimer-like pathology in amyloid protein precursor transgenic mice. *Am J Pathol* 2000;157:2003–2010. [PubMed: 11106573]
144. Murakami K, Whiteley MK, Routtenberg A. Regulation of protein kinase C activity by cooperative interaction of Zn²⁺ and Ca²⁺ *J Biol Chem* 1987;262:13902–13906. [PubMed: 3654645]
145. Myers A, Holmans P, Marshall H, Kwon J, Meyer D, Ramic D, Shears S, Booth J, DeVrieze FW, Crook R, Hamshire M, Abraham R, Tunstall N, Rice F, Carty S, Lillystone S, Kehoe P, Rudrasingham V, Jones L, Lovestone S, Perez-Tur J, Williams J, Owen MJ, Hardy J, Goate AM. Susceptibility locus for Alzheimer's disease on chromosome 10. *Science* 2000;290:2304–2305. [PubMed: 11125144]
146. Nolte C, Gore A, Sekler I, Kresse W, Hershfinkel M, Hoffmann A, Kettenmann H, Moran A. ZnT-1 expression in astroglial cells protects against zinc toxicity and slows the accumulation of intracellular zinc. *GLIA* 2004;48:145–155. [PubMed: 15378655]
147. Nunomura A, Perry G, Aliev G, Hirai K, Takeda A, Balraj EK, Jones PK, Ghanbari H, Wataya T, Shimohama S, Chiba S, Atwood CS, Petersen RB, Smith MA. Oxidative damage is the earliest event in Alzheimer disease. *J Neuropathol Exp Neurol* 2001;60:759–767. [PubMed: 11487050]
148. Oddo S, Billings L, Kesslak JP, Cribbs DH, LaFerla FM. Abeta immunotherapy leads to clearance of early, but not late, hyperphosphorylated tau aggregates via the proteasome. *Neuron* 2004;43:321–332. [PubMed: 15294141]
149. Ohana E, Sekler I, Kaisman T, Kahn N, Cove J, Silverman WF, Amsterdam A, Hershfinkel M. Silencing of ZnT-1 expression enhances heavy metal influx and toxicity. *Journal of Molecular Medicine* 2006;84:753–763. [PubMed: 16741752]
150. Palmiter RD. Constitutive expression of metallothionein-III (MT-III), but not MT-I, inhibits growth when cells become zinc deficient. *Toxicol Appl Pharmacol* 1995;135:139–146. [PubMed: 7482533]
151. Palmiter RD, Cole TB, Findley SD. ZnT-2, a mammalian protein that confers resistance to zinc by facilitating vesicular sequestration. *Embo J* 1996;15:1784–1791. [PubMed: 8617223]
152. Palmiter RD. The elusive function of metallothioneins. *Proc Natl Acad Sci U S A* 1998;95:8428–8430. [PubMed: 9671693]
153. Peila R, Rodriguez BL, Launer LJ. Type 2 diabetes, APOE gene, and the risk for dementia and related pathologies: The Honolulu-Asia Aging Study. *Diabetes* 2002;51:1256–1262. [PubMed: 11916953]
154. Penkowa M, Nielsen H, Hidalgo J, Bernth N, Moos T. Distribution of metallothionein I + II and vesicular zinc in the developing central nervous system: correlative study in the rat. *J Comp Neurol* 1999;412:303–318. [PubMed: 10441758]
155. Perez-Clausell J, Danscher G. Release of zinc sulphide accumulations into synaptic clefts after in vivo injection of sodium sulphide. *Brain Res* 1986;362:358–361. [PubMed: 3942885]

156. Petersen, RC.; Morris, JC. Clinical features. In: Petersen, RC., editor. *Mild Cognitive Impairment*. Oxford University; New York: 2003. p. 15-39.
157. Pike CJ, Walencewicz AJ, Glabe CG, Cotman CW. In vitro aging of beta-amyloid protein causes peptide aggregation and neurotoxicity. *Brain Res* 1991;563:311-314. [PubMed: 1786545]
158. Pike CJ, Burdick D, Walencewicz AJ, Glabe CG, Cotman CW. Neurodegeneration induced by beta-amyloid peptides in vitro: the role of peptide assembly state. *J Neurosci* 1993;13:1676-1687. [PubMed: 8463843]
159. Prasad AS, Miale A Jr, Farid Z, Sandstead HH, Schulert AR. Zinc metabolism in patients with the syndrome of iron deficiency anemia, hepatosplenomegaly, dwarfism, and hypogonadism. *J Lab Clin Med* 1963;61:537-549. [PubMed: 13985937]
160. Price D, Joshi JG. Ferritin: a zinc detoxicant and a zinc ion donor. *Proc Natl Acad Sci U S A* 1982;79:3116-3119. [PubMed: 6212927]
161. Ritchie CW, Bush AI, Mackinnon A, Macfarlane S, Mastwyk M, MacGregor L, Kiers L, Cherny R, Li QX, Tammer A, Carrington D, Mavros C, Volitakis I, Xilinas M, Ames D, Davis S, Beyreuther K, Tanzi RE, Masters CL. Metal-protein attenuation with iodocholehydroxyquin (clioquinol) targeting Abeta amyloid deposition and toxicity in Alzheimer disease: a pilot phase 2 clinical trial. *Arch Neurol* 2003;60:1685-1691. [PubMed: 14676042]
162. Rivlin AA, Chan YL, Wool IG. The contribution of a zinc finger motif to the function of yeast ribosomal protein YL37a. *J Mol Biol* 1999;294:909-919. [PubMed: 10588896]
163. Rogaeva E, Meng Y, Lee JH, Gu Y, Kawarai T, Zou F, Katayama T, Baldwin CT, Cheng R, Hasegawa H, Chen F, Shibata N, Lunetta KL, Pardossi-Piquard R, Bohm C, Wakutani Y, Cupples LA, Cuenco KT, Green RC, Pinessi L, Rainero I, Sorbi S, Bruni A, Duara R, Friedland RP, Inzelberg R, Hampe W, Bujo H, Song YQ, Andersen OM, Willnow TE, Graff-Radford N, Petersen RC, Dickson D, Der SD, Fraser PE, Schmitt-Ulms G, Younkin S, Mayeux R, Farrer LA, St George-Hyslop P. The neuronal sortilin-related receptor SORL1 is genetically associated with Alzheimer disease. *Nat Genet* 2007;39:168-177. [PubMed: 17220890]
164. Rubin H, Koide T. Stimulation of DNA synthesis and 2-deoxy-D-glucose transport in chick embryo cultures by excessive metal concentrations and by a carcinogenic hydrocarbon. *J Cell Physiol* 1973;81:387-396. [PubMed: 4197395]
165. Rulon LL, Robertson JD, Lovell MA, Deibel MA, Ehmann WD, Markesbery WR. Serum zinc levels and Alzheimer's disease. *Biol Trace Elem Res* 2000;75:79-85. [PubMed: 11051598]
166. Samudralwar DL, Diprete CC, Ni BF, Ehmann WD, Markesbery WR. Elemental imbalances in the olfactory pathway in Alzheimer's disease. *J Neurol Sci* 1995;130:139-145. [PubMed: 8586977]
167. Segal D, Ohana E, Besser L, Hershinkel M, Moran A, Sekler I. A role for ZnT-1 in regulating cellular cation influx. *Biochemical and Biophysical Research Communications* 2004;323:1145-1150. [PubMed: 15451416]
168. Selkoe DJ. Translating cell biology into therapeutic advances in Alzheimer's disease. *Nature* 1999;399:A23-31. [PubMed: 10392577]
169. Seshadri S, Beiser A, Selhub J, Jacques PF, Rosenberg IH, D'Agostino RB, Wilson PW, Wolf PA. Plasma homocysteine as a risk factor for dementia and Alzheimer's disease. *N Engl J Med* 2002;346:476-483. [PubMed: 11844848]
170. Shore D, Henkin RI, Nelson NR, Agarwal RP, Wyatt RJ. Hair and serum copper, zinc, calcium, and magnesium concentrations in Alzheimer-type dementia. *J Am Geriatr Soc* 1984;32:892-895. [PubMed: 6512128]
171. Sinha S, Anderson JP, Barbour R, Basi GS, Caccavello R, Davis D, Doan M, Dovey HF, 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, Suomensaaari SM, Wang S, Walker D, Zhao J, McConlogue L, John V. Purification and cloning of amyloid precursor protein beta-secretase from human brain. *Nature* 1999;402:537-540. [PubMed: 10591214]
172. Sisodia SS, St George-Hyslop PH. gamma-Secretase, Notch, Abeta and Alzheimer's disease: where do the presenilins fit in? *Nat Rev Neurosci* 2002;3:281-290. [PubMed: 11967558]
173. Skhulachev VP, Christyakov VV, Jasaitis AA, Smirnova EG. Inhibition of the respiratory chain by zinc ions. *Biochem Biophys Res Commun* 1967;26:1-6. [PubMed: 4291553]

174. Skoog I, Lernfelt B, Landahl S, Palmertz B, Andreasson LA, Nilsson L, Persson G, Oden A, Svanborg A. 15-year longitudinal study of blood pressure and dementia. *Lancet* 1996;347:1141–1145. [PubMed: 8609748]
175. Smith JL, Xiong S, Markesbery WR, Lovell MA. Altered expression of zinc transporters-4 and -6 in mild cognitive impairment, early and late Alzheimer's disease brain. *Neuroscience* 2006;140:879–888. [PubMed: 16580781]
176. Snowden DA, Kemper SJ, Mortimer JA, Greiner LH, Wekstein DR, Markesbery WR. Linguistic ability in early life and cognitive function and Alzheimer's disease in late life. Findings from the Nun Study. *Jama* 1996;275:528–532. [PubMed: 8606473]
177. Snowden DA, Greiner LH, Markesbery WR. Linguistic ability in early life and the neuropathology of Alzheimer's disease and cerebrovascular disease. Findings from the Nun Study. *Ann N Y Acad Sci* 2000;903:34–38. [PubMed: 10818486]
178. Sommer B. Alzheimer's disease and the amyloid cascade hypothesis: ten years on. *Curr Opin Pharmacol* 2002;2:87–92. [PubMed: 11786314]
179. St George-Hyslop PH, Tanzi RE, Polinsky RJ, Haines JL, Nee L, Watkins PC, Myers RH, Feldman RG, Pollen D, Drachman D, et al. The genetic defect causing familial Alzheimer's disease maps on chromosome 21. *Science* 1987;235:885–890. [PubMed: 2880399]
180. St George-Hyslop, PH. The molecular genetics of Alzheimer's disease. In: Terry, RD.; Katzman, R.; Bick, LL., editors. *Alzheimer's disease*. Raven Press; New York: 1994. p. 345-352.
181. Stengaard-Pedersen K, Fredens K, Larsson LI. Comparative localization of enkephalin and cholecystokinin immunoreactivities and heavy metals in the hippocampus. *Brain Res* 1983;273:81–96. [PubMed: 6351958]
182. Stoltenberg M, Bruhn M, Sondergaard C, Doering P, West MJ, Larsen A, Troncoso JC, Danscher G. Immersion autometallographic tracing of zinc ions in Alzheimer beta-amyloid plaques. *Histochem Cell Biol* 2005;123:605–611. [PubMed: 15981003]
183. Strozzyk D, Launer LJ, Adlard PA, Cherny RA, Tsatsanis A, Volitakis I, Blennow K, Petrovitch H, White LR, Bush AI. Zinc and copper modulate Alzheimer Abeta levels in human cerebrospinal fluid. *Neurobiol Aging*. 2007 Epub ahead of print. 10.1016/j.neurobiolaging.2007.10.012
184. Takeda A. Movement of zinc and its functional significance in the brain. *Brain Res Brain Res Rev* 2000;34:137–148. [PubMed: 11113504]
185. Tonder N, Johansen FF, Frederickson CJ, Zimmer J, Diemer NH. Possible role of zinc in the selective degeneration of dentate hilar neurons after cerebral ischemia in the adult rat. *Neurosci Lett* 1990;109:247–252. [PubMed: 2330128]
186. Vallee BL, Falchuk KH. The biochemical basis of zinc physiology. *Physiol Rev* 1993;73:79–118. [PubMed: 8419966]
187. Vallee BL. The function of metallothionein. *Neurochem Int* 1995;27:23–33. [PubMed: 7655345]
188. Vandenberg RJ, Mitrovic AD, Johnston GA. Molecular basis for differential inhibition of glutamate transporter subtypes by zinc ions. *Mol Pharmacol* 1998;54:189–196. [PubMed: 9658205]
189. Vassar R, Bennett BD, Babu-Khan S, Kahn S, Mendiaz EA, Denis P, Teplow DB, Ross S, Amarante P, Loeloff R, Luo Y, Fisher S, Fuller J, Edenson S, Lile J, Jarosinski MA, Biere AL, Curran E, Burgess T, Louis JC, Collins F, Treanor J, Rogers G, Citron M. Beta-secretase cleavage of Alzheimer's amyloid precursor protein by the transmembrane aspartic protease BACE. *Science* 1999;286:735–741. [PubMed: 10531052]
190. Wallace DC. Mitochondrial genetics: a paradigm for aging and degenerative diseases? *Science* 1992;256:628–632. [PubMed: 1533953]
191. Walsh DM, Lomakin A, Benedek GB, Condron MM, Teplow DB. Amyloid beta-protein fibrillogenesis. Detection of a protofibrillar intermediate. *J Biol Chem* 1997;272:22364–22372. [PubMed: 9268388]
192. Walsh DM, Klyubin I, Fadeeva JV, Cullen WK, Anwyl R, Wolfe MS, Rowan MJ, Selkoe DJ. Naturally secreted oligomers of amyloid beta protein potently inhibit hippocampal long-term potentiation in vivo. *Nature* 2002;416:535–539. [PubMed: 11932745]
193. Wang HW, Pasternak JF, Kuo H, Ristic H, Lambert MP, Chromy B, Viola KL, Klein WL, Stine WB, Krafft GA, Trommer BL. Soluble oligomers of beta amyloid (1-42) inhibit long-term

- potentiation but not long-term depression in rat dentate gyrus. *Brain Res* 2002;924:133–140. [PubMed: 11750898]
194. Wenstrup D, Ehmann WD, Markesbery WR. Trace element imbalances in isolated subcellular fractions of Alzheimer's disease brains. *Brain Res* 1990;533:125–131. [PubMed: 2085723]
 195. Westerman MA, Cooper-Blacketer D, Mariash A, Kotilinek L, Kawarabayashi T, Younkin LH, Carlson GA, Younkin SG, Ashe KH. The relationship between Abeta and memory in the Tg2576 mouse model of Alzheimer's disease. *J Neurosci* 2002;22:1858–1867. [PubMed: 11880515]
 196. Williams, RJP. An introduction to the biochemistry of zinc. In: Mills, CF., editor. *Zinc in Human Biology*. Springer-Verlag; London: 1989. p. 15-31.
 197. Williams TI, Lynn BC, Markesbery WR, Lovell MA. Increased levels of 4-hydroxynonenal and acrolein, neurotoxic markers of lipid peroxidation, in the brain in Mild Cognitive Impairment and early Alzheimer's disease. *Neurobiol Aging*. 2005
 198. Wilquet V, De Strooper B. Amyloid-beta precursor protein processing in neurodegeneration. *Curr Opin Neurobiol* 2004;14:582–588. [PubMed: 15464891]
 199. Yamaguchi M, Kura M, Okada S. Role of zinc as an activator of mitochondrial function in rat liver. *Biochem Pharmacol* 1982;31:1289–1293. [PubMed: 6212062]
 200. Yang JP, Merin JP, Nakano T, Kato T, Kitade Y, Okamoto T. Inhibition of the DNA-binding activity of NF-kappa B by gold compounds in vitro. *FEBS Lett* 1995;361:89–96. [PubMed: 7890047]
 201. Yatin SM, Varadarajan S, Link CD, Butterfield DA. In vitro and in vivo oxidative stress associated with Alzheimer's amyloid beta-peptide (1-42). *Neurobiol Aging* 1999;20:325–330. discussion 339–342. [PubMed: 10588580]
 202. Yin HZ, Weiss JH. Zn(2+) permeates Ca(2+) permeable AMPA/kainate channels and triggers selective neural injury. *Neuroreport* 1995;6:2553–2556. [PubMed: 8741761]
 203. Yokoyama M, Koh J, Choi DW. Brief exposure to zinc is toxic to cortical neurons. *Neurosci Lett* 1986;71:351–355. [PubMed: 3796893]
 204. Zabel U, Schreck R, Baeuerle PA. DNA binding of purified transcription factor NF-kappa B. Affinity, specificity, Zn²⁺ dependence, and differential half-site recognition. *J Biol Chem* 1991;266:252–260. [PubMed: 1985897]
 205. Zalewski PD, Forbes IJ, Seamark RF, Borlinghaus R, Betts WH, Lincoln SF, Ward AD. Flux of intracellular labile zinc during apoptosis (gene-directed cell death) revealed by a specific chemical probe, Zinquin. *Chem Biol* 1994;1:153–161. [PubMed: 9383385]
 206. Zeng J, Heuchel R, Schaffner W, Kagi JH. Thionein (apometallothionein) can modulate DNA binding and transcription activation by zinc finger containing factor Sp1. *FEBS Lett* 1991;279:310–312. [PubMed: 2001744]