

Elevated cortical zinc in Alzheimer disease

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Abstract—Objective: To determine whether changes in brain biometals in Alzheimer disease (AD) and in normal brain tissue are tandemly associated with amyloid β -peptide ($A\beta$) burden and dementia severity. **Methods:** The authors measured zinc, copper, iron, manganese, and aluminum and $A\beta$ levels in postmortem neocortical tissue from patients with AD ($n = 10$), normal age-matched control subjects ($n = 14$), patients with schizophrenia ($n = 26$), and patients with schizophrenia with amyloid ($n = 8$). Severity of cognitive impairment was assessed with the Clinical Dementia Rating Scale (CDR). **Results:** There was a significant, more than twofold, increase of tissue zinc in the AD-affected cortex compared with the other groups. Zinc levels increased with tissue amyloid levels. Zinc levels were significantly elevated in the most severely demented cases (CDR 4 to 5) and in cases that had an amyloid burden greater than 8 plaques/mm². Levels of other metals did not differ between groups. **Conclusions:** Brain zinc accumulation is a prominent feature of advanced Alzheimer disease (AD) and is biochemically linked to brain amyloid β -peptide accumulation and dementia severity in AD.

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Neocortical amyloid β -peptide ($A\beta$) accumulation is a cardinal neuropathologic finding in patients with Alzheimer disease (AD).¹ Abnormal metal (zinc, copper, and iron) homeostasis is implicated in the deposition of $A\beta$ in AD.² $A\beta$ is reversibly precipitated by zinc and copper^{3,4} and coordinates these (and not other) metals in plaques.^{5,6} $A\beta$ is not toxic in cell culture unless bound to copper or iron, whereupon the peptide forms a catalytic complex, generating toxic radicals ($A\beta_{1-42} > A\beta_{1-40}$).^{5,7-11}

Zinc and copper are concentrated in the neocortex, and their regulation is stringent, as these metals are potentially neurotoxic in excess or deficiency. Zinc ions are released by neurotransmission from glutamatergic neocortical fibers, achieving concentrations up to 300 μM .^{12,13} Fifteen to 30% of brain zinc is concentrated by ZnT3 into synaptic vesicles in these fibers.^{14,15} Genetic ablation of ZnT3 markedly reduces cerebral amyloid deposition and congophilic angiopathy in the Tg2576 transgenic mouse model of AD.^{15,16} Copper ions (about 15 μM)¹⁷ are released by hippocampal neurons upon *N*-methyl-D-aspartate stimulation.¹⁸

Previous brain metals surveys in AD postmortem neocortical samples have identified an elevation of

zinc,¹⁹ but whether the elevations of cortical zinc and $A\beta$ in AD are interdependent events is not known. To address this question, we measured metals in postmortem cortical tissue that was previously characterized in a report on Alzheimer neuropathology in chronic schizophrenia (compared with AD and normal subjects), which found that the cognitive deterioration of chronic schizophrenia was not associated with increased amyloid burden.²⁰

Methods. Human postmortem tissue. We obtained postmortem tissue samples from pathologically and clinically confirmed AD cases ($n = 10$), normal age-matched (to the AD cases) control subjects ($n = 14$), elderly schizophrenic patients with mild amyloid pathology ($n = 8$), and schizophrenic patients without amyloid pathology ($n = 26$).

We chose these samples because they belonged to a well-characterized bank of postmortem specimens with characteristics and neuropathology by the Consortium to Establish a Registry for Alzheimer's Disease criteria²¹ previously reported.²⁰ In brief, normal elderly comparison subjects showed no history of neuropsychiatric disease, were not demented, and were free of discernible neuropathologic lesions. Patients with schizophrenia (diagnosed according to Diagnostic and Statistical Manual for Mental Disorders [3rd rev. ed.] criteria) with and without amyloid pathology served as additional control groups for this study.

Fixed, paraffin-embedded sections were stained with modified Bielschowsky, modified thioflavine S, anti- β amyloid antibody (4G8), and anti-tau antibody (AD2) according to published proce-

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Table 1 Descriptive characteristics of the study population by subgroup

	AC, n = 14	Schizophrenia, n = 26	Schizophrenia plus amyloid, n = 8	AD, n = 10	ANOVA
Age, y	82.8 (11.2)	70.3 (13.1)	76.0 (14.6)	81.6 (11.0)	0.02*
Female/male, n	12/2	8/18	5/3	7/3	0.01†
Postmortem interval, min	479 (370)	686 (377)	735 (360)	532 (608)	0.36
CDR	0.25 (0.38)	1.85 (1.14)	2.25 (1.39)	4.10 (1.20)	<0.0001*
Metals, nmol/g wet wt					
Zinc	171.2 (28.8)	162.1 (27.3)	159.1 (27.3)	393.9 (209.1)	<0.0001*
Copper	43.8 (13.8)	44.5 (13.8)	38.9 (7.7)	35.7 (15.4)	0.33
Iron	608.8 (87.7)	584.2 (1.7)	645.6 (133.3)	675.4 (229.8)	0.23
Manganese	3.1 (0.9)	2.5 (0.4)	2.7 (0.5)	2.7 (0.9)	0.09
Aluminum	24.8 (40.7)	37.4 (92.6)	29.3 (40.7)	11.5 (3.7)	0.69
Amyloid					
Total A β , pmol/g wet wt	742.6 (47–7,094)	529 (38–5,078)	1,631.2 (0–4402)	2,210 (876–6,985)	0.01*
A β x-42, pmol/g wet wt	3.6 (3.6–3,228)	36.9 (3.6–5,980)	350.6 (116–635)	383.3 (3.6–9,010)	0.0007*
A β x-40, pmol/g wet wt	79.0 (3.6–243)	165.6 (3.6–2,089)	237.5 (43–424)	875.1 (139–3,045)	0.0004*
Plaques/mm ²	0 (0–8)	0 (0–7.2)	7.1 (1.6–10.4)	18.2 (10.4–34.4)	0.0001*

Results are presented as mean (\pm SD) except where indicated by an asterisk, which indicates median (range). ANOVA was performed with log-transformed tissue amyloid levels to obtain normality.

† Gender differed across the groups. However, this result is entirely driven by the schizophrenia group. Gender did not differ in AD patients ($p = 0.35$) and schizophrenics with AD ($p = 0.22$), when compared with normal controls (AC).

CDR = Clinical Dementia Rating Scale; A β = amyloid β -peptide.

dures.²² Amyloid plaques were defined according to neuritic plaque criteria²³ as adapted²⁴: the presence of amyloid, degenerating neuronal processes, neurofibrillary tangles (NFTs), and a minimum diameter of 50 μ m. Amyloid plaque density was averaged across five neocortical areas including middle frontal gyrus to give the plaque score number used in analyses.²⁰ Counts from the middle frontal cortex were used to assign pathologic categories: age-matched normal subjects and schizophrenia cases had ≤ 2 plaques/mm², cases of schizophrenia with mild amyloid pathology had more than 2 but less than 8 plaques/mm², and AD cases had ≥ 8 plaques/mm² (table 1). NFTs from the superior temporal gyrus were assessed semiquantitatively (none, sparse, moderate, severe).

A β levels were assessed from the dorsolateral prefrontal cortex (Brodmann area 46) and metal levels from ipsilateral superior temporal gyrus (Brodmann area 22). These areas were chosen because they are typically affected by pathology early in the course of AD. Zn levels are only markedly higher than neocortex in the amygdala and hippocampus and are expected to be roughly uniform for both zinc²⁵ and amyloid²⁶ burden between the two sampled areas.

The antemortem and postmortem procedures employed in this study were approved by the institutional review boards of Pilgrim Psychiatric Center, Mount Sinai School of Medicine, the Bronx VA Medical Center, and the Karolinska Institutet. Informed consent was obtained from the relatives of participants. Antemortem assessment and postmortem chart reviews were used²⁰ to determine the degree of dementia present in each case according to the Clinical Dementia Rating Scale (CDR).²⁷

Metal measurements. Concentrated HNO₃ (0.1 mL; Aristar, BDH) was added to each lyophilized, preweighed tissue sample and allowed to digest overnight at room temperature. Samples were further digested by heating them for 20 minutes at 90 °C. Then 0.1 mL of hydrogen peroxide (Aristar) was added immediately to each sample for 30 minutes, before heating again for a further 15 minutes at 70 °C. Samples were diluted with 1% HNO₃ in acid-washed 5-mL polypropylene tubes and measured in triplicate. As an internal control for the digestion procedure, triplicate preparations of NIST Bovine Liver SRM 1557B were also included.

Measurements were made using a Varian UltraMass ICPMS instrument under operating conditions suitable for routine multielement analysis. The instrument was calibrated using 0, 10, 50, and 100 ppb of a certified multielement ICPMS standard solution (ICP-MS- CA12-1; Accustandard). A certified internal standard

solution containing 100 ppb of yttrium (⁸⁹Y) was used as an internal control (ICP-MS- IS-MIX1-1; Accustandard). Plastic ware was acid washed, Teflon or plastic dissection instruments were used to avoid metal contamination, and all buffers were checked for metal contamination.

ELISA. Measurements of total A β , A β 40, and A β 42, after extraction from the brain, were performed using a sandwich ELISA as described previously.²⁰ In brief, 6E10 (Signet Labs) was used as the capture antibody and end-specific affinity-purified anti-A β x-40 and anti-A β x-42 polyclonal antibodies were used for detection and quantification of A β x-40 and A β x-42. Total A β was detected using the biotinylated monoclonal 4G8 antibody. Results were recorded as nanograms per gram of wet weight of cortical tissue.

Statistics. A β and metal levels were obtained by operators blinded to the neuropathologic category and clinical history data. Total metal levels were analyzed by pathologic subgroups (age-matched normal controls [AC], AD, schizophrenia, schizophrenia plus amyloid) using means \pm SD. Spearman correlation coefficients were used to describe relationships between continuous variables, and analysis of variance (ANOVA) was employed to test for mean differences between groups.

Because metal levels may change with age and AD pathology is associated with age, we additionally fitted an age-adjusted general linear-regression model. Metal values were transformed into a log scale to obtain normality. Statistical analyses were performed with STATA 8.0.

Results. Descriptive characteristics of the study population, as well as metal levels, A β levels, and amyloid plaques by subgroup are given in table 1. Schizophrenia patients were younger than control subjects and AD patients (including schizophrenia with mild AD pathology). There was no difference in postmortem delay between groups (table 1). Copper and zinc levels did not correlate to each other (Spearman $\rho = 0.10$; $p = 0.4$).

Age-related changes. We initially analyzed associations between brain extracted A β species, amyloid plaque, and metal levels as a function of age at death to determine the contribution of age as a confounding variable to potential associations between the other neurochemical factors. We differentiated neuritic plaque levels from extractable

Table 2 Spearman ρ correlations of tissue amyloid β -peptide (A β) levels with tissue metal concentrations in the entire study group

	Total A β	A β 42	A β 40	Plaques
Metals, nmol/g wet wt				
Zinc	0.2*	0.3**	0.4**	0.35***
Copper	-0.18	0.08	-0.2	-0.32**
Iron	0.04	0.1	0.03	0.11
Manganese	0.12	-0.06	-0.2*	-0.05
Aluminum	-0.05	0.2	-0.05	-0.18
Age				
Age	0.43***	-0.09	0.06	0.29**
Amyloid, pmol/g wet wt				
Total A β	—	0.18	0.25	0.60***
A β 42	—	—	0.52***	0.48***
A β 40	—	—	—	0.53***

* $p < 0.1$; ** $p < 0.05$; *** $p < 0.001$.

A β x-40/42 levels because several studies have indicated that the classic plaque count does not correlate well with severity of cognitive loss and that various modified species of A β (e.g., soluble oligomers, intracellular A β) could be more pernicious than plaques.^{28,29} A β 1-42 is more liable to be cross-linked by copper into soluble oligomers than A β 1-40,⁴ whereas plaque deposition of both forms seems to be mediated by synaptic zinc.¹⁶ Brain A β levels rise with age,³⁰ and therefore we tested whether this was true of our current samples. Pooling data from all categories, we found that total A β levels ($p < 0.001$) and plaque burden ($p < 0.05$) rose, significantly correlating, with age (table 2). There were no significant correlations between age and levels of A β x-40 or A β x-42 (table 2), possibly because the sample size was too small to achieve sufficient power.

For the analysis of age effects on metal levels, we again pooled data from all clinicopathologic categories. Of all the metals studied, only copper levels showed a decrease with age ($p < 0.0001$) after adjusting for clinicopathologic category, A β plaques/mm², and A β tissue levels (total, A β x-40, and A β x-42) (figure 1).

Brain metal levels in AD. There was a twofold increase in zinc levels in AD brain tissue as compared with tissue from age-matched control subjects (AC) and patients with schizophrenia, after adjusting for age ($p < 0.0001$) (figure 2A). There was a trend to lower copper levels in AD brain tissue ($p = 0.08$) (figure 2B). There were no significant

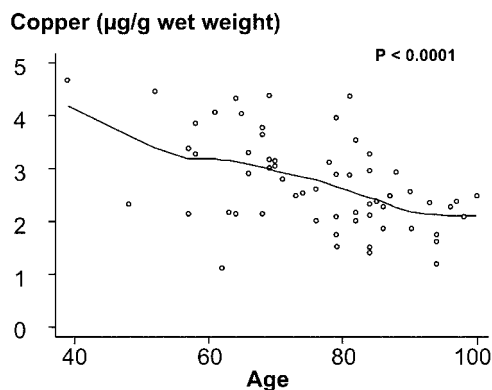


Figure 1. Predicted relationship of copper and age. The locally weighted regression line (Stata 8.0) is shown.

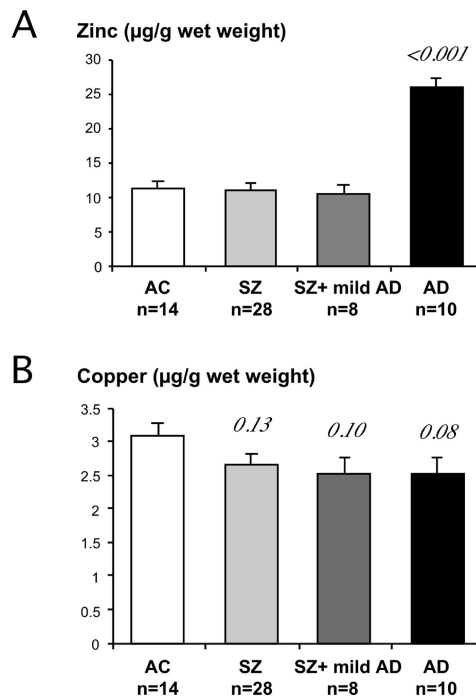


Figure 2. Levels of brain zinc and copper in each pathologic category. Data are age-adjusted mean \pm SEM levels of zinc (A) and copper (B) ($\mu\text{g/g}$ wet wt). AC = age-matched normal tissue; AD = Alzheimer disease; SZ = schizophrenia. The p value of the difference in individual means of each group compared with the normal control group is shown in italics.

differences between clinical categories in levels of iron, manganese, or aluminum (table 1).

Association of brain metals with amyloid. To test whether amyloid accumulation drove the increase in brain zinc levels observed, we performed correlations of metals with A β 42, A β 40, total A β , and histologic plaque burden across all pathologic groups (table 2; figure 3).

After adjusting for age, zinc levels were found to increase in tandem with levels of total A β ($p = 0.03$) (figure 3), A β 40 ($p = 0.02$), and A β 42 ($p = 0.02$). We then further analyzed the association of plaque pathology with zinc levels. A threshold of 8 plaques/mm² was used as a neuropathologic criterion for the diagnosis of AD in this cohort. Therefore, we compared zinc levels in tissue where plaque was absent with tissue where there was some plaque but less than the threshold for the AD diagnosis (i.e., $0 < \text{plaque count} < 8$) and with tissue where plaque count was $\geq 8/\text{mm}^2$. This analysis revealed that there was a significant and marked zinc elevation in the subjects who had the highest plaque burden (figure 4). However, tissue with only some plaques (< 8) did not have discernibly higher zinc levels than subjects with no plaques (figure 4).

This result may explain why there was no increase in zinc levels in tissue from subjects who had schizophrenia with mild AD pathology, because their average plaque count was less than 8/mm² (7.1/mm²) (table 1). This result is consistent with the elevation of zinc being associated with advanced AD pathology.

We also found an inverse correlation between copper and amyloid plaques but no association with A β 42, A β 40, or total A β (table 2). However, this correlation was not

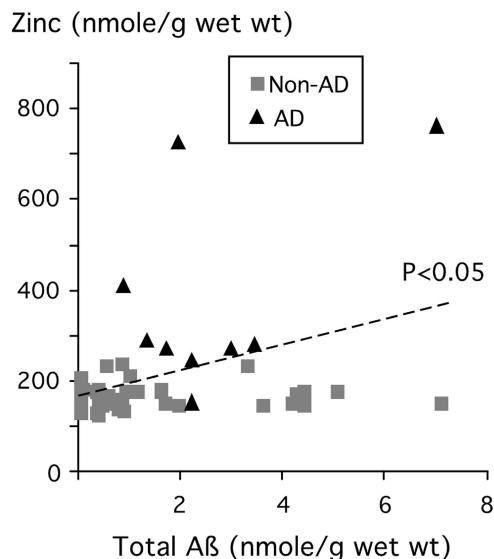


Figure 3. Predicted relationship of zinc to total amyloid β -peptide ($A\beta$) levels. The fitted line represents the predicted linear relationship of zinc to total $A\beta$ levels (age-adjusted p value = 0.034) across all clinicopathologic groups. The values for the Alzheimer disease (AD) samples are in triangles, and the remainder of the cohort (referred to as “non-AD”) are in squares.

significant after adjusting for age, probably because copper itself significantly decreased with age in this study group (figure 1). There was no correlation between $A\beta$ species, plaque load (table 2), or CDR (not shown) with levels of iron, aluminum, or manganese.

Association of brain metals with NFTs. NFTs were present only in AD tissue; hence, we excluded individuals with schizophrenia from this analysis. There were 5 individuals with severe NFTs, 4 with sparse to moderate, and 15 with no NFTs (including $n = 14$ AC tissue). Compared with no NFTs, severe NFTs were associated with higher zinc levels (age-adjusted β coefficient = 0.69; $p = 0.04$). However, this association became nonsignificant when con-

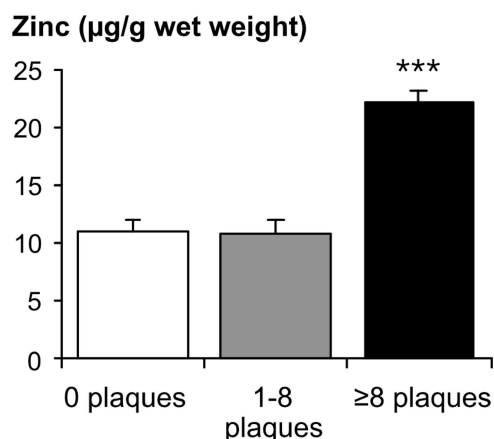


Figure 4. Relationship of brain zinc levels with plaque burden. Results represent age-adjusted mean zinc levels stratified by 0 plaques, 1 to 8 plaques, and ≥ 8 plaques (bars are SEs). Data were pooled from all clinicopathologic groups. *** $p < 0.0001$ compared with values from plaque = 0.

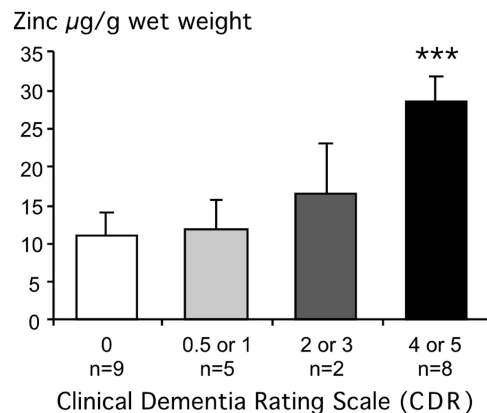


Figure 5. Relationship of Clinical Dementia Rating Scale (CDR) severity with brain zinc levels. Zn levels in combined Alzheimer disease (AD) and age-adjusted normal control (AC) subjects were analyzed by CDR score. Data are age-adjusted mean \pm SEM zinc levels ($\mu\text{g/g}$ wet wt). *** $p < 0.0001$ compared with values from CDR = 0.

trolling for amyloid plaques, suggesting a modulatory effect of amyloid on this relationship.

Association of brain metals with dementia severity. Because higher $A\beta$ load is associated with more severe dementia, we tested whether elevated zinc levels were associated with disease severity as rated by CDR among age-matched subjects and AD subjects. For this analysis, we excluded the schizophrenia cases, because the CDR might not be an appropriate test to use in schizophrenia patients and might not be comparable with that of AD patients. Zinc was significantly elevated in brains from subjects with higher scores on the CDR scale (figure 5). In an age-adjusted linear-regression analysis, CDR increased by 0.13 unit ($n = 24$; β -coefficient = 0.13; $p < 0.0001$) per increase of 1 $\mu\text{g/g}$ wet wt of zinc. An association with CDR was not detected for copper concentrations (data not shown).

Discussion. Our findings indicate that the homeostatic defect causing zinc accumulation in the neocortex in AD is linked to the severity of $A\beta$ burden, NFTs, and dementia. These findings are in concordance with previous reports of elevated zinc levels involving several brain regions in AD including hippocampus,³¹⁻³³ amygdala,³¹⁻³⁶ basal nucleus of Meynert,³⁴ the olfactory region,^{33,35} and frontal, temporal, and parietal (inferior) cortices.^{31,33} Studies that have not found zinc elevations in AD brain have either used small sample sizes³⁷ or used formalin-fixed tissue,^{38,39} which artifactually lowers zinc levels because of denatured zinc binding sites. Our findings demonstrate that the elevated zinc levels in cortex in AD are biochemically linked to $A\beta$ burden and to dementia severity (CDR).

It is notable that aluminum levels were not elevated in our cohort, nor were they correlated with $A\beta$ burden. It is likely that early work reporting aluminum enrichment in AD neuropathology was confounded by the abundance of aluminum as a buffer and glassware contaminant (a factor we have controlled for). Our current findings are consistent with

later work showing that there is no enrichment of aluminum in the plaque pathology of carefully prepared tissue samples.⁴⁰

Previously, zinc has been shown to accumulate within amyloid plaques and cerebrovascular amyloid in humans^{36,41} and in amyloid precursor protein (APP) transgenic mice.^{15,16,42} Zinc released from glutamatergic neurotransmission fosters both amyloid and total extracted A β , which are both markedly attenuated by genetic ablation of the synaptic zinc transporter, ZnT3.^{15,16} However, the elevation of zinc we observed is unlikely to be explained solely by the accumulation of zinc within amyloid. This is because whereas the levels of A β in the AD cases were 1.45 nmol/g wet wt greater than levels in age-controlled tissue (table 1), the associated elevation in Zn was about 150-fold greater (222 nmol/g wet wt) than the accompanying increase in A β . Because 1 mol of A β can bind up to (but not more than) 3 mol of Zn,⁴³ the elevated zinc in AD probably reflects accumulation within cells rather than merely reflecting zinc sequestered into amyloid. Supporting this possibility, chemically exchangeable Zn²⁺ has been found to accumulate within the bodies of neurons of AD-affected neocortex.⁴¹ One caveat with our data is that we analyzed A β and Zn from different cortical regions. Whereas both regions chosen were likely to be affected by A β pathology similarly²⁶ and have similar zinc levels,²⁵ the procedure may have reduced the strength of biochemical associations (i.e., increased the probability of a type 2 error). Further studies of identical areas may find even stronger associations for the trends that we identified (e.g., decreased Cu being associated with increased A β). Our procedures would not, however, confound the differences we observed between AD and AC groups (i.e., increase the chance of type 1 error) because these comparisons were from the same cortical areas.

Because the elevation in zinc correlates with A β levels (table 2), it is still possible that A β and Zn accumulations are causally associated through a mechanism that is yet unclear. However, we cannot yet determine whether altered zinc levels precede or follow deposition of amyloid plaques.

We previously published marked differences between APP transgenic mice and actual AD in brain metal changes associated with A β deposition. The Tg2576 mouse model for AD accumulates cerebral A β with concentrations far in excess of the human brain in AD, driven in large part by zinc release from ZnT3-dependent synaptic activity.^{15,16} However, Zn levels in Tg2576 brain tissue decline modestly with advancing age despite amyloid pathology becoming progressively more abundant.⁴⁴ This observation supports our interpretation that the accumulation of zinc in the AD-affected brain is not due to simple trapping by A β accumulation and indicates that the Tg2576 model does not reiterate the zinc neurochemical derangement of AD.

Our findings that Zn elevation correlated with dementia severity (CDR) (figure 4) raises the hypothe-

sis that Zn accumulation, rather than A β on its own, causes the neuronal dysfunction that leads to dementia. The cellular fraction that contains the elevated zinc is undetermined, but possibilities include trapping in NFTs.⁴⁵ Excess zinc is neurotoxic⁴⁶ and is normally removed by metallothionein III in neocortex,⁴⁷ which may be depleted in AD-affected neocortical tissue.⁴⁸ Excess zinc elevation inhibits mitochondrial respiration,⁴⁹ induces abnormal microtubule assembly,⁵⁰ inhibits proteasome activity,⁵¹ and may impact also upon the metabolism of zinc metalloproteinases involved in the APP/A β metabolic pathway such as insulin-degrading enzyme and neprilysin.⁵² Zinc elevation in brain has recently been shown to induce memory deficits in rats.⁵³ The pool of zinc responsible for A β precipitation has been demonstrated not to come solely from synaptic release in transgenic mice and could be in communication with the blood.¹⁵ Hence, deterioration of the blood-brain barrier, which in health excludes zinc from the brain, may contribute to elevated brain zinc in AD.

In this study, we also found a significant age-dependent decline in cortical Cu levels across clinicopathologic groups over a wide range of age groups (figure 1). In rats and mice, brain copper levels rise markedly after the neonatal period,^{54,55} but there are no data on the effects of advanced age. There remains the possibility that genetic or dietary factors, particularly in the schizophrenia group, might have contributed to our result. Although values have been adjusted for clinicopathologic groups, an ideal study design would include longitudinal data of healthy study participants. Further studies to answer this question are needed.

We found a trend toward lower levels of Cu in AD cortex compared with age-controlled tissue (figure 2B). As previous reports found significantly decreased Cu levels in AD brain,^{31,56-58} our sample size may possibly not be large enough to detect a significant decrease compared with age-matched controls. Whereas copper binds to A β in the AD brain,^{6,36,59} most evidence indicates that there is a decrease in bulk tissue levels in AD-affected neocortex.^{56,60-62} At variance with a picture of copper deficiency in AD cortex, marginally increased copper levels have been reported in the neuropil.³⁶ However, this analysis was confined to the amygdala and used micro-PIXE to probe heavily amyloid-burdened tissue. The remaining reports utilized bulk tissue analysis. Taken together, a complex picture emerges where copper abnormally redistributes in AD and collects outside the cell, leaving the tissue relatively deficient. This may explain the deficiency of copper-dependent enzymatic activities in AD such as cytochrome *c* oxidase⁶³⁻⁶⁶ and superoxide dismutase-1^{67,68} (also decreased in APP transgenic animals)⁶⁹ as well as the reported elevation of serum copper.^{70,71}

Whereas APP transgenic mice do not exhibit the elevation of brain zinc observed in AD, they do reiterate the decrease in Cu levels observed in AD brain.

In APP transgenic mice, this is due to the overexpression of APP and not dependent upon amyloid pathology.^{44,72,73} Conversely, APP knockout mice accumulate copper in cortical neurons.^{74,75} Correction of the decreased brain copper levels in APP transgenic mice, either by dietary Cu supplementation⁷² or by a mutant allele of the (Wilson disease) Cu-ATPase7B Cu transporter,⁷³ improves survival of the mice and results in decreased A β and amyloid plaque load. This may be related to decreased antioxidant Cu/Zn superoxide dismutase activity in the APP transgenics.⁷² Further studies will be needed to determine whether age-related changes in APP production may account for the decrease in copper levels that we observed accompany human aging, which possibly increases the likelihood of oxidation and amyloid deposition.

We did not observe an elevation in iron in AD-affected cortex, at variance with some reports in the literature.⁷⁶ However, our assay could not exclude accumulation of an adverse valence state of the Fe (e.g., Fe²⁺, the pro-oxidant form) or exclude microscopic accumulations of iron (e.g., within tangle-bearing neurons).⁴⁵

Our results impact upon our understanding of the therapeutic mechanism of metal-complexing agents such as clioquinol, a drug that slowed cognitive decline and decreased plasma A β 42 in a pilot Phase 2 clinical trial.⁷⁷ Despite recent problems with scale-up synthesis of clioquinol, the drug and its class are still advancing in AD clinical trials (<http://www.alzforum.org/new/detail.asp?id=1179>) and in preclinical studies for other neurologic indications where metals are implicated in protein aggregation.^{78,79} Oral clioquinol treatment on Tg2576 mice markedly decrease brain amyloid burden,⁸⁰ an effect replicated with a similar hydrophobic chelator, DP109.⁸¹ Clioquinol does not inhibit A β deposition by simply removing metals from the brain as in Tg2576 mice, clioquinol acts as an ionophore, elevating brain Cu and Zn to normal levels.⁸⁰ Based upon our current findings, to normalize brain metal levels in AD, a drug would need to increase copper levels and decrease zinc levels while preventing pooling of these metal ions in the amyloid mass. As we have recently shown that clioquinol binds metals in amyloid in Tg2576 brain,¹⁹ it appears that clioquinol acts a ionophore buffer, redistributing metals away from areas of abnormal accumulation, whether intracellular or within protein aggregates, and toward areas of metal deficiency. Indeed, in AD patients treated with clioquinol, there was a significant elevation (normalization) in plasma zinc levels compatible with movement of excess zinc out of the brain.^{15,77}

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