UC Irvine

UC Irvine Previously Published Works

Title

Low concentrations of copper in drinking water increase AP-1 binding in the brain.

Permalink

<https://escholarship.org/uc/item/83j7n9wq>

Journal

Toxicology and industrial health, 31(12)

ISSN

0748-2337

Authors

Lung, Shyang
Li, Huihui
Bondy, Stephen C
et al.

Publication Date

2015-12-01

DOI

10.1177/0748233713491805

Peer reviewed

Low concentrations of copper in drinking water increase AP-1 binding in the brain

Shyang Lung, Huihui Li, Stephen C. Bondy and Arezoo Campbell

Toxicol Ind Health published online 29 May 2013

DOI: 10.1177/0748233713491805

The online version of this article can be found at:

<http://tih.sagepub.com/content/early/2013/05/29/0748233713491805>

Published by:

<http://www.sagepublications.com>

Additional services and information for *Toxicology and Industrial Health* can be found at:

Email Alerts: <http://tih.sagepub.com/cgi/alerts>

Subscriptions: <http://tih.sagepub.com/subscriptions>

Reprints: <http://www.sagepub.com/journalsReprints.nav>

Permissions: <http://www.sagepub.com/journalsPermissions.nav>

>> [OnlineFirst Version of Record](#) - May 29, 2013

[What is This?](#)

Low concentrations of copper in drinking water increase AP-1 binding in the brain

Shyang Lung¹, Huihui Li², Stephen C. Bondy² and Arezoo Campbell¹

Toxicology and Industrial Health
1–7

© The Author(s) 2012

Reprints and permissions:

sagepub.co.uk/journalsPermissions.nav

DOI: 10.1177/0748233713491805

tih.sagepub.com

Abstract

Copper (Cu) in trace amounts is essential for biological organisms. However, dysregulation of the redox-active metal has been implicated in different neurological disorders such as Wilson's, Menkes', Alzheimer's, and Parkinson's diseases. Since many households use Cu tubing in the plumbing system, and corrosion causes the metal to leach into the drinking water, there may be adverse effects on the central nervous system connected with low-level chronic exposure. The present study demonstrates that treatment with a biologically relevant concentration of Cu for 3 months significantly increases activation of the redox-modulated transcription factor AP-1 in mouse brains. This was independent of an upstream kinase indicated in AP-1 activation. Another redox-active transcription factor, NF- κ B, was not significantly modified by the Cu exposure. These results indicate that the effect of Cu on AP-1 is unique and may involve direct modulation of DNA binding.

Keywords

AP-1, copper, NF- κ B, neurodegenerative diseases, oxidative stress

Introduction

Copper (Cu) is a cofactor necessary for the function of many enzymes required in living organisms. The importance of Cu homeostasis in brain function is demonstrated by two hereditary disorders caused by mutations in genes encoding P-type cation-transporting adenosine triphosphatases. These genetic abnormalities lead to disturbances in the absorption and distribution of the metal. These disorders include Menkes' disease, which results in neurological degeneration, and Wilson's disease, which leads to liver toxicity and neurological impairments (Kitzberger et al. 2005; Strecker et al., 2006; Vulpe et al., 1993). Cu levels are also altered in age-related neurodegenerative disorders such as Alzheimer's disease (AD) and Parkinson's disease (PD). The non-ceruloplasmin-bound component of the metal is increased in the serum of patients with AD (Squitti et al., 2005) and the cerebrospinal fluid of patients with PD (Hozumi et al., 2011). One of the characteristic hallmarks of AD is senile plaques composed of amyloid fibrils derived from the amyloid precursor protein (APP). It has been demonstrated that interaction of Cu with the Cu regulatory element motif in the promoter region regulates

gene expression of APP (Bellingham et al., 2004). Therefore, an increase in Cu concentration may contribute to APP overexpression and amyloid deposition. Furthermore, prolonged occupational exposure to Cu is linked to an increased risk of PD (Gorell et al., 1999).

Many households use Cu tubing in the plumbing system and there is concern regarding the potential adverse effects on the central nervous system (CNS) connected with chronic low-level Cu exposure (Marx, 2003). The present Environmental Protection Agency's maximum recommended standard for Cu in drinking water is 1.3 ppm (Buchanan et al., 1999). However, the levels can be as high as 7.8 mg/L in tap water that has been stagnant overnight in corrosive Cu pipes (Spitalny

¹ Department of Pharmaceutical Sciences, College of Pharmacy, Western University of Health Sciences, Pomona, CA, USA

² Center for Occupational and Environmental Health, University of California, Irvine, Irvine, CA, USA

Corresponding author:

Arezoo Campbell, Department of Pharmaceutical Sciences, College of Pharmacy, Western University of Health Sciences, 309 E Second Street, Pomona, CA 91766-1854, USA.

Email: acampbell@westernu.edu

et al., 1984) and as high as 5 mg/L in running tap water (Pizarro et al., 2001). In the present study, the effect of a 3-month exposure to Cu (0.5 ppm) in the drinking water upon the activator protein-1 (AP-1) and nuclear factor- κ B (NF- κ B) activation was investigated. Activation of both these transcription factors has been reported to underlie apoptotic cell death in striatal neurons (Qin et al., 2001).

Methods

Animal treatment

Two-month-old male B/6C3F1 mice (a hybrid between C57BL/6 and C3H) were obtained from Charles River Laboratories (Wilmington, Massachusetts, USA). They were housed four per cage and maintained on a 12-h light/dark cycle in a temperature-controlled ($20 \pm 1^\circ\text{C}$) room. Food and water were provided *ad libitum*. The drinking water was supplemented with 8 μM of copper sulfate. This is equivalent to 0.5 ppm of Cu. Control mice received double distilled water. The amount of water consumed was recorded twice a week, and the weight of the animals was determined each week. The animals were exposed for 3 months. For brain tissue collection, animals were anesthetized with pentobarbital (65 mg/kg, intraperitoneally). Depth of anesthesia was ascertained by the toe pinch reflex. When animals were deeply sedated, the chest cavity was opened and mice were perfused transcardially with phosphate-buffered saline to remove blood from all organs. The brain was removed and quickly frozen.

Preparation of samples

Cytoplasmic and nuclear fractions were prepared using a previously published method (Lahiri and Ge, 2000). The brain tissue from each animal was weighed and homogenized in 2 mL of an ice-cold buffer (10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), pH 7.9, 10 mM potassium chloride (KCl), 0.1 mM ethylenediaminetetraacetic acid (EDTA), 0.1 mM ethylene glycol tetraacetic acid (EGTA), 1 mM dithiothreitol (DTT), 0.5 mM phenylmethylsulfonyl fluoride (PMSF), and 0.5% NP-40). The samples were incubated for 10 min and centrifuged (1500g) at 4°C for 1 min. The supernatant containing the cytoplasmic constituents was collected and a protease inhibitor cocktail was added. The samples were aliquoted and stored at -80°C . The nuclear pellet was resuspended in 200 μL of a buffer composed of 20 mM HEPES, pH 7.9, 400 mM sodium chloride (NaCl),

1 mM DTT, 1 mM EDTA, 1 mM EGTA, and 1 mM PMSF. The samples were then centrifuged at 11,000g for 5 min at 4°C . The supernatant (nuclear extract) was aliquoted and stored at -80°C .

Electrophoretic mobility shift assay

The extent of AP-1 and NF- κ B activation was determined in the nuclear fraction of brain tissue using a protocol developed by Promega Corporation (Madison, Wisconsin, USA). The amount of protein in 5 μL of the nuclear extract was determined by the bicinchoninic acid (BCA) protein assay method (Pierce, Rockford, Illinois, USA) and 50 μg of each sample, incubated with ^{32}P -labeled oligonucleotides containing either the AP-1 or NF- κ B consensus sequence, was loaded onto a gel. A negative control containing no cell extract, as well as competitor reactions were included. The specific competitor contained unlabeled AP-1 or NF- κ B consensus nucleotide, while the nonspecific competitor contained unlabelled SP-1 consensus oligonucleotide. The competitor reactions also contained 50 μg of nuclear fraction derived from Cu-treated mouse brains. X-Ray films were manually developed, and the intensity of each band was measured and quantitated using an image analyzer.

Western blots

The levels of interleukin-1 receptor-associated kinase 1 (IRAK-1), NF- κ B-inducing kinase (NIK), c-Jun N-terminal kinase (JNK), and phospho-JNK (p-JNK) were determined using Western blotting. Protein content was determined using the BCA protein assay kit. Each sample of 50 μg was resolved on a sodium dodecylsulfate–10% polyacrylamide gel electrophoresis and transferred onto a nitrocellulose membrane (BioRad, Hercules, California, USA). After blocking overnight in Tris-buffered saline and Tween 20 (TBST; 20 mM Tris-HCl, 150 mM NaCl, and 0.1% Tween 20)-containing 5% nonfat milk, membranes were washed four times and incubated for 1 h with primary antibodies (goat polyclonal antibody against p-JNK (1:500), rabbit polyclonal antibody against JNK (1:500), mouse monoclonal antibody against IRAK-1 (1:250), and rabbit polyclonal antibody against NIK (1:500); all purchased from Santa Cruz Biotechnology, Inc., Santa Cruz, California, USA). After washing the membranes four times with TBST, they were incubated with appropriate secondary horseradish peroxidase-conjugated antibodies (1:10,000). Bands were detected with enhanced-chemiluminescence reagents (Amersham

Pharmacia Biotech, Piscataway, New Jersey, USA) following the manufacturer's protocol. An antibody against actin (mouse monoclonal, 1:1500 dilution) purchased from Chemicon International (Temecula, California; USA) was used to insure equal loading of protein for each sample. The intensity of specific bands was measured and quantitated using an image analyzer.

Statistical analysis

The differences among control and Cu exposure were assessed by the Student's *t* test using Prism software (GraphPad Software, San Diego, California, USA). Results were considered statistically significant at $p < 0.05$ using a two-tailed distribution.

Results

A 3-month exposure to copper sulfate (8 μ M), in the drinking water, did not cause any significant changes in the weight or water consumption of treated animals when compared with the controls (data not shown). This implied that the overall health of animals was not affected by treatment. There was a significant increase in the levels of AP-1 DNA binding in Cu-exposed mouse brains compared with the controls as judged by gel shift mobility assay (Figure 1). Since the phosphorylation of JNK is an upstream event, which precedes the activation of AP-1, the levels of the active form of JNK, that is p-JNK, and JNK were assessed. These were unchanged after exposure (Figure 2). Therefore, Cu exposure may directly affect DNA binding without activating upstream signaling events. The activation of another transcription factor, NF- κ B, was also assessed (Figure 3(a)). Integrated density measurement of the main shifted band revealed that Cu exposure did not significantly alter the levels of NF- κ B DNA binding in the nuclear fraction of mouse brain (Figure 3(b)). The levels of two kinases related to NF- κ B activation were evaluated. The brain levels of the NIK were not changed. A reduction in IRAK-1 was evident but not statistically significant (Figure 4). Prolonged exposure to low levels of Cu in the drinking water modulates AP-1 DNA binding but upstream signaling pathways do not appear to be responsible for this effect.

Discussion

In the present study, the levels of AP-1 DNA binding were increased in the CNS of mice chronically exposed to biologically relevant concentrations of

Figure 1. AP-1 activation. (a) Typical gel shift mobility assay showing three separate samples per group (B: blank; C: control; Cu: copper; SC: specific competitor containing sample with unlabelled AP-1 consensus oligonucleotide; NSC: nonspecific competitor containing sample with unlabelled SP-1 consensus oligonucleotide). (b) Integrated density of the main shifted band. Bars represent mean of six individual animal determinations $\pm$ SE. *Value is significantly different ($p \leq 0.05$) from the control.

Cu. This is in agreement with a previous study showing that a 4- or 24-h exposure to high concentrations of Cu (400 μ M) increased AP-1 binding in COS-7 cells (Mattie et al., 2008). Since this transcription factor is modulated by the redox status of cells (Mattson et al., 2004), such activation may be in response to an increase in prooxidant events. Indeed, chronic exposure to Cu significantly increased the lipid peroxidation marker, malondialdehyde, and neuronal nitric oxide synthase levels in the brain (Becaria et al., 2006).

The enhancement of AP-1 DNA binding after a 3-month exposure to Cu was not associated with a concurrent increase in the phosphorylation of JNK, which activates the signaling pathway for this transcription pathway. It is possible that during an acute response, the levels of this mitogen-activated protein kinase increased but that the longer duration of exposure allows homeostasis to be reached. This is in agreement with the *in vitro* findings of Mattie et al. (2008), where a 4-h exposure caused a significant increase in JNK phosphorylation that was attenuated by a 24-h exposure. The effect of Cu on transcription factor activation may also be mediated by direct

Figure 2. Levels of p-JNK and JNK in brain cytoplasmic fractions. (a) p-JNK and JNK levels were determined in cytoplasmic fraction of mouse brains by immunoblot analysis: control (lanes 1 and 3) and Cu-treated (lanes 2 and 4). An antibody against actin was used to adjust for possible variation in sample loading. (b) Integrated density of blots (p-JNK/actin or JNK/actin). Bars represent mean of six individual determinations $\pm$ SE. JNK: c-Jun N-terminal kinase; p-JNK: phosphor-c-Jun N-terminal kinase.

modulation of the affinity of active AP-1 for its DNA-binding sites.

The activation of both AP-1 and NF- κ B has been shown to enhance apoptosis in striatal neurons (Nakai et al., 2000). Intra-striatal injection of dopamine, which can also act as an oxidant, induced apoptotic changes that were dependent on AP-1 and NF- κ B activation (Luo et al., 1999). Therefore, it may be that Cu increased the DNA binding of AP-1 by way of its prooxidant properties. This could be a mechanism by which the metal acts as a neurotoxicant and contributes to neurological impairments.

In recent years, the potential role of Cu in the pathology of AD has received much attention. Concentrations of the metal are increased in amyloid plaques, where it strongly colocalizes with the β -sheet conformation of aggregated amyloid proteins (Miller et al., 2006). There are specific Cu-binding sites on both the APP (Barnham et al., 2003) and amyloid beta (A β) peptide (Dong et al., 2003), and they increase the toxicity of the metal by reducing Cu(II) to Cu(I). This allows redox cycling, with consequent formation of

Figure 3. NF- κ B activation. (a) Typical gel shift mobility assay showing three separate samples per group (B: blank; C: control; Cu: copper; SC: specific competitor containing sample with unlabelled NF- κ B consensus oligonucleotide; NSC: non-specific competitor containing sample with unlabelled SP-1 consensus oligonucleotide). (b) Integrated density of the shifted band. Bars represent mean of six individual animal determinations $\pm$ SE. NF- κ B: nuclear factor- κ B.

reactive oxygen species within CNS tissue (Dikalov et al., 2004; Feaga et al., 2011; Guilloureau et al., 2007). In the present study, we show that Cu increases AP-1 DNA binding. Since AP-1 is modulated by the redox status of cells, the mechanism of action of Cu is more likely to initially relate to the promotion of oxidative processes (Becaria et al., 2006). This is in agreement with the work of Mattie et al. (2008), which showed that increasing oxidative stress caused enhanced AP-1 binding in COS-7 cells.

Trace amounts of Cu (0.12 ppm), present in the drinking water, increase the number of A β reactive neurons and senile plaque-like structures in a rabbit model of AD (Sparks and Schreurs, 2003). The promoter region of the β -site APP-cleaving enzyme contains multiple transcription factor binding sites, including ones for AP-1 and cyclic adenosine monophosphate response element-binding protein (Lahiri et al., 2006). Cu-induced increase in the activation of AP-1 may thus contribute to accelerated formation of A β . This may be a potential mechanism by which the metal enhances the number of A β reactive neurons (Sparks et al., 2006). This effect of metals on potentiation of AD pathology may not be unique to Cu. In monkeys, exposure to lead (Pb), during brain development,

Figure 4. Levels of IRAK-1 and NIK in brain cytoplasmic fractions. (a) IRAK-1 (M_w : 80 kDa) levels were determined using a mouse monoclonal antibody and NIK (M_w : 130 kDa) levels were examined using a rabbit polyclonal antibody in the brain cytoplasmic fraction of control (lanes 1 and 3) and Cu-treated (lanes 2 and 4) animals. An antibody against actin was used to adjust for possible variation in sample loading. (b) Integrated density of blots (IRAK/actin or NIK/actin). Bars represent mean of six individual determinations $\pm$ SE. M_w : molecular weight; IRAK-1: interleukin-1 receptor-associated kinase 1; NIK: NF- κ B-inducing kinase.

led to an increase in APP expression and amyloid deposition (Wu et al., 2008). Under SP-1 transcription regulation, fetal exposure to Pb caused latent promotion of APP expression later in life (Basha et al., 2005). The “Latent Early-life Associated Regulation” model explains how exposure to environmental agents (such as Cu) may cause changes in oxidation status of promoters, which modify latent expression of disease-associated genes (Lahiri et al., 2009).

Early gene imprinting by environmental factors such as metal exposure, and subsequent altered responses of AD-associated genes, may be an important etiological pathway for neurodegenerative disorders (Lahiri and Maloney, 2010).

Free Cu cation has been correlated with cognitive decline (Salustri et al., 2010) and this may affect the initiation of the prodrome of AD (Brewer, 2012; Campbell, 2006). It has been suggested that failure of Cu sequestration by microglia clustered around amyloid plaques may precipitate inflammatory conditions and thus exacerbate AD (Zheng et al., 2010).

Further studies are needed to determine the exact mechanism by which Cu enhances DNA binding of AP-1 and determine whether exposure to this metal at environmentally relevant levels may accelerate the progression of neurodegenerative diseases such as AD.

Funding

This study was supported by grants from Western University of Health Sciences (12085-1501) and the National Institutes of Health (ES 7992).

References

- Barnham KJ, McKinstry WJ, Multhaup G, Galatis D, Morton CJ, Curtain CC, et al. (2003) Structure of the Alzheimer's disease amyloid precursor protein copper binding domain. *Journal of Biological Chemistry* 278: 17401–17407.
- Basha MR, Wei W, Bakheet SA, Benitez N, Siddiqi HK, Ge YW, et al. (2005) The fetal basis of amyloidogenesis: exposure to lead and latent overexpression of amyloid precursor protein and beta-amyloid in the aging brain. *Journal of Neuroscience* 25: 823–829.
- Becaria A, Lahiri DK, Bondy SC, Chen D, Hamadeh A, Li H, et al. (2006) Aluminum and copper in drinking water enhance inflammatory or oxidative events specifically in the brain. *Journal of Neuroimmunology* 176: 16–23.
- Bellingham SA, Lahiri DK, Maloney B, La Fontaine S, Multhaup G, and Camakaris J (2004) Copper depletion down-regulates expression of the Alzheimer's disease amyloid-beta precursor protein gene. *Journal of Biological Chemistry* 279: 20378–20386.
- Brewer GJ (2012) Copper toxicity in Alzheimer's disease: cognitive loss from ingestion of inorganic copper. *Journal of Trace Elements in Medicine and Biology* 26: 89–92.
- Buchanan SD, Discker RA, Sinks T, Olson DR, Daniel J, and Flodman T (1999) Copper in drinking water, Nebraska, 1994. *International Journal of Occupational and Environmental Health* 5: 256–261.
- Campbell A (2006) The role of aluminum and copper on neuroinflammation and Alzheimer's disease. *Journal of Alzheimer's Disease* 10: 165–172.
- Dikalov SI, Vitek MP, and Mason RP (2004) Cupric-amyloid beta peptide complex stimulates oxidation of ascorbate and generation of hydroxyl radical. *Free Radicals in Medicine and Biology* 36: 340–347.
- Dong J, Atwood CS, Anderson VE, Siedlak SL, Smith MA, Perry G, et al. (2003) Metal binding and oxidation of amyloid-beta within isolated senile plaque cores: Raman microscopic evidence. *Biochemistry* 42: 2768–2773.

- Feaga HA, Maduka RC, Foster MN, and Szalai VA (2011) Affinity of Cu^+ for the copper-binding domain of the amyloid- β peptide of Alzheimer's disease. *Inorganic Chemistry* 50: 1614–1618.
- Gorell JM, Johnson CC, Rybicki BA, Peterson EL, Kortsha GX, Brown GG, et al. (1999) Occupational exposure to manganese, copper, lead, iron, mercury and zinc and the risk of Parkinson's disease. *Neurotoxicology* 20: 239–247.
- Guilloureau L, Combalbert S, Sournia-Saquet A, Mazarguil H, and Faller P (2007) Redox chemistry of copper-amyloid-beta: the generation of hydroxyl radical in the presence of ascorbate is linked to redox-potentials and aggregation state. *ChemBiochem* 8: 1317–1325.
- Hozumi I, Hasegawa T, Honda A, Ozawa K, Hayashi Y, Hashimoto K, et al. (2011) Patterns of levels of biological metals in CSF differ among neurodegenerative diseases. *Journal of Neurological Science* 303: 95–99.
- Kitzberger R, Madl C, and Ferenci P (2005) Wilson disease. *Metabolic Brain Disease* 20: 295–302.
- Lahiri DK, Ge Y (2000) Electrophoretic mobility shift assay for the detection of specific DNA-protein complex in nuclear extracts from the cultured cells and frozen autopsy human brain tissue. *Brain Research* 5: 257–265.
- Lahiri DK, Ge YW, Rogers JT, Sambamurti K, Greig NH, and Maloney B (2006) Taking down the unindicted co-conspirators of amyloid beta-peptide-mediated neuronal death: shared gene regulation of BACE1 and APP genes interacting with CREB, Fe65 and YY1 transcription factors. *Current Alzheimer's Research* 3: 475–483.
- Lahiri DK, Maloney B (2010) The "LEARn" (Latent Early-life Associated Regulation) model integrates environmental risk factors and the developmental basis of Alzheimer's disease, and proposes remedial steps. *Experimental Gerontology* 45: 291–296.
- Lahiri DK, Maloney B, and Zawia NH (2009) The LEARN model: an epigenetic explanation for idiopathic neurobiological diseases. *Molecular Psychiatry* 14: 992–1003.
- Luo Y, Hattori A, Munoz J, Qin ZH, and Roth GS (1999) Intrastriatal dopamine injection induces apoptosis through oxidation-involved activation of transcription factors AP-1 and NF-kappaB in rats. *Molecular Pharmacology* 56: 254–264.
- Marx J (2003) Neuroscience. Possible role for environmental copper in Alzheimer's disease. *Science* 301: 905.
- Mattie MD, McElwee MK, and Freedman JH (2008) Mechanism of copper-activated transcription: activation of AP-1, and the JNK/SAPK and p38 signal transduction pathways. *Journal of Molecular Biology* 383: 1008–1018.
- Mattson D, Bradbury CM, Bisht KS, Curry HA, Spitz DR, and Gius D (2004) Heat shock and the activation of AP-1 and inhibition of NF-kappa B DNA-binding activity: possible role of intracellular redox status. *International Journal of Hyperthermia* 20: 224–233.
- Miller LM, Wang Q, Telivala TP, Smith RJ, Lanzirrotti A, and Miklossy J (2006) Synchrotron-based infrared and X-ray imaging shows focalized accumulation of Cu and Zn co-localized with beta-amyloid deposits in Alzheimer's disease. *Journal of Structural Biology* 155: 30–37.
- Nakai IM, Qin ZH, Chen JF, Wang Y, and Chase TN (2000) Kainic acid-induced apoptosis in rat striatum is associated with nuclear factor-kappaB activation. *Journal of Neurochemistry* 74: 647–658.
- Pizarro F, Olivares M, Araya M, Gidi V, and Uauy R (2001) Gastrointestinal effects associated with soluble and insoluble copper in drinking water. *Environmental Health Perspectives* 109: 949–952.
- Qin ZH, Wang Y, Chen RW, Wang X, Ren M, Chuang DM, et al. (2001) Prostaglandin A(1) protects striatal neurons against excitotoxic injury in rat striatum. *Journal of Pharmacology and Experimental Therapeutics* 297: 78–87.
- Salustri C, Barbati G, Ghidoni R, Quintiliani L, Ciappina S, Binetti G, et al. (2010) Is cognitive function linked to serum free copper levels? A cohort study in a normal population. *Clinical Neurophysiology* 121: 502–507.
- Sparks DL, Friedland R, Petanceska S, Schreurs BG, Shi J, Perry G, et al. (2006) Trace copper levels in the drinking water, but not zinc or aluminum influence CNS Alzheimer-like pathology. *Journal of Nutrition Health and Aging* 10: 247–254.
- Sparks DL, Schreurs B (2003) Trace amounts of copper in water induce beta-amyloid plaques and learning deficits in a rabbit model of Alzheimer's disease. *Proceedings of the National Academy of Sciences United States of America* 100: 11065–11069.
- Spitalny KC, Brondum J, Vogt RL, Sargent HE, and Kappel S (1984) Drinking-water-induced copper intoxication in a Vermont family. *Pediatrics* 74: 1103–1106.
- Squitti R, Pasqualetti P, Dal Forno G, Moffa F, Cassetta E, Lupoi D, et al. (2005) Excess of serum copper not related to ceruloplasmin in Alzheimer disease. *Neurology* 64: 1040–1046.
- Strecker K, Schneider JP, Barthel H, Hermann W, Wegner F, Wagner A, et al. (2006) Profound midbrain atrophy in patients with Wilson's disease and neurological symptoms? *Journal of Neurology* 253: 1024–1029.
- Vulpe C, Levinson B, Whitney S, Packman S, and Gitschier J (1993) Isolation of a candidate gene for Menkes

disease and evidence that it encodes a copper-transporting ATPase. *Nature Genetics* 3: 7–13.

Wu J, Basha MR, Brock B, Cox DP, Cardozo-Pelaez F, McPherson CA, et al. (2008) Alzheimer's disease (AD)-like pathology in aged monkeys after infantile exposure to environmental metal lead (Pb): evidence for a

developmental origin and environmental link for AD. *Journal of Neuroscience* 28: 3–9.

Zheng Z, White C, Lee J, Peterson TS, Bush AI, Sun GY, et al. (2010) Altered microglial copper homeostasis in a mouse model of Alzheimer's disease. *Journal of Neurochemistry* 114: 1630–1638.