Plasma Copper and Zinc Concentration in Individuals with Autism Correlate with Selected Symptom Severity

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Abstract

Aim: To assess plasma zinc and copper concentration in individuals with autism and correlate these levels with symptom severity.

Subjects and methods: Plasma from 102 autistic individuals, and 18 neurotypical controls, were tested for plasma zinc and copper using inductively-coupled plasma-mass spectrometry. Copper and zinc levels and Cu/Zn were analyzed for possible correlation with severity of 19 symptoms.

Results: Autistic individuals had elevated plasma levels of copper and Cu/Zn and lower, but not significantly lower, plasma Zn compared to neurotypical controls. There was a correlation between Cu/Zn and expressive language, receptive language, focus attention, hyperactivity, fine motor skills, gross motor skills and Tip Toeing. There was a negative correlation between plasma zinc concentration and hyperactivity, and fine motor skills severity.

Discussion: These results suggest an association between plasma Cu/Zn and severity of symptoms associated with autism.

Keywords: autism, zinc, copper
Introduction

Autism is a complex, behaviorally defined neurodevelopmental disorder characterized by social deficits, language impairments, and repetitive behaviors. There has been a dramatic increase in the diagnosis of autism over the past decade.1,2

The etiology of this complex disease is highly heritable, but likely involves environmental factors.3 Twin studies demonstrate concordance rates of 82%–92% in monozygotic twins and 1%–10% concordance rate in dizygotic twins.1 Sibling recurrence risk (6%–8%) is 35 times the population prevalence.1,4

Genetic analysis suggests that as many as 15 genes might be involved in autism spectrum disorders (ASD), including variants on chromosomes 2q, 7q, 15q, and 17q.5–8

Children with ASD frequently have accompanying gastrointestinal, immunological, or nonspecific neurological symptoms.9–15

Zinc has a unique and extensive role in biological processes. Since the discovery of this element as an essential nutrient for living organisms,16–18 many diverse biochemical roles for it have been identified. These include roles in enzyme function,19 nucleic acid metabolism,20,21 cell signaling22 and apoptosis.23 Zinc is essential for physiological processes including growth and development,24 lipid metabolism,25 brain and immune function.24,26

Dietary factors that reduce the availability of zinc are the most common cause of zinc deficiency. However, inherited defects can also result in reduced zinc. Both nutritional and inherited zinc deficiency produce similar symptoms, such as dermatitis, diarrhea, alopecia and loss of appetite.27 With more prolonged deficiency causing growth impairment and neuropsychological changes such as emotional instability, irritability and depression.28–31

Deficiency of zinc in man has now been recognized to occur not only as a result of nutritional factors, but also in various disease states, including malabsorption syndromes, acrodermatitis enteropathica, Crohn’s disease, alcoholism and cirrhosis of the liver.32,33

Low intracellular zinc has been found to be associated with DNA damage, oxidative stress, antioxidant defenses, and DNA repair,32,33 and zinc may serve as an important anti-oxidant.34

Copper (Cu), a trace metal, is also an essential element for living cells. It plays an important role in redox reactions because of its easy conversion from Cu+ to Cu++. Copper is transported mainly by ceruloplasmin, a copper-binding antioxidant protein that is synthesized in several tissues, including brain.35,36

Copper levels are low in Menke’s kinky hair syndrome37 malnutrition38 and Malabsorption.39 Elevated copper levels are associated with infections,40 inflammation,41 trauma,42 Wilson’s disease,43 excessive dietary intake44 systemic lupus erythematosus,45 as well as autism.46

Because of the potential association between Zn and Cu levels and autism, we tested patients with autism for plasma concentration of these elements and then compared those levels with severity of disease symptoms.

Materials and Methods

Subjects

Experimental and Control

Plasma from consecutive individuals with diagnosed autism and neurotypical controls was obtained from patients presenting consecutively at the Health Research Institute/Pfeiffer Treatment Center. These individuals meet the DSM-IV criteria and many were diagnosed using The Autism Diagnostic Interview-Revised—ADI-R before presenting for treatment at the Pfeiffer Treatment Center, Warrenville, Il.*

Patient consent was obtained from all patients involved in this study and this study was approved by the IRB of the Health Research Institute/Pfeiffer Treatment Center.

Severity of disease

An autism questionnaire was used to evaluate symptoms. The questionnaire (Pfeiffer Questionnaire) asked parents or caregivers to assess the severity of the following symptoms: Awareness, Expressive Language, Receptive Language, (Conversational) Pragmatic Language, Focus, Attention, Hyperactivity, Impulsivity, Perseveration, Fine Motor Skills, Gross Motor Skills, Hypotonia (low muscle tone), Tip Toeing, Rocking/Pacing, Stimming, Obsessions/Fixations, Eye Contact, Sound Sensitivity, Light Sensitivity, Tactile Sensitivity, Pica/eats dirt, metal, Tics and Seizures. The symptoms were rated on a
Copper and zinc levels correlate with symptom severity in autism

scale of 0–5 (5 being the highest severity) for each of these behaviors.

**Serum/Plasma**
All experimental and control plasmas were treated in an identical fashion—refrigerated (4°C) immediately after collection and cell/serum separation, then used within 4 hours for inductively-coupled plasma-mass spectrometry.

**Statistics**
Inferential statistics were derived from t-test with 95% confidence intervals and correlation data was obtained using Pearson Product Moment Correlation.*

**Results**
Autistic individuals had elevated plasma levels of copper (108.9 µg/dL) compared to controls (86.5 µg/dL) \( (P = 0.003) \) (Fig. 1) and elevated Cu/Zn (1.41) compared to controls (1.19) \( (P = 0.06) \), but not significantly lower plasma Zn (80.5 µg/dL) compared to neurotypical controls (84.7 µg/dL) \( (P = 0.4) \).

In 452 random individuals presenting to the Health Research Institute, we found no significant differences between the copper \( (P = 0.24) \) or zinc \( (P = 0.52) \) levels in 6 different age groups (0–19; 20–29; 30–39; 40–49; 50–59; and above 60 years old), and in this same group, we found no difference in copper \( (P = 0.78) \) and zinc \( (P = 0.63) \) associated with gender.

We found a correlation between Cu/Zn and expressive language \( [r = 0.3, n = 45, P = 0.05] \), receptive language \( [r = 0.4, n = 43, P = 0.01] \), focus attention \( [r = 0.23, n = 84, P = 0.03] \), hyperactivity \( [r = 0.3, n = 79, P = 0.01] \), fine motor skills \( [r = 0.32, n = 74, P = 0.004] \), gross motor skills \( [r = 0.41, n = 68, P = 0.0004] \) and Tip Toeing \( [r = 0.3, n = 71, P = 0.03] \). There was a negative correlation between plasma zinc concentration and hyperactivity \( [r = -0.3, n = 79, P = 0.02] \), and fine motor skills severity, \( [r = -0.3, n = 74, P = 0.005] \). Figures 2 and 3 show correlation between Hyperactivity and Cu/Zn and plasma zinc concentration, respectively.

**Discussion**
There is much support for the role of GABA in the etiology of autism. Alterations in levels of GABA and GABA receptors in autistic patients indicate that

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*The Pfeiffer Treatment Center is a comprehensive treatment and research center, specializing in the care of with neurological disorders, including autism.*

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**Figure 1.** Plasma copper concentration was significantly higher in autistic individuals compared to controls.  
Note: \( P = 0.003 \).
the GABAergic system, which is responsible for synaptic inhibition in the adult brain, may be involved in autism.\textsuperscript{47-49} 

Zinc has been found to be associated with GABA and glutamate regulation, particularly through anxiolytic activity, modulating GABAergic inhibition and seizure susceptibility.\textsuperscript{50-52} Zinc deficiency has also been found to be associated with GABAergic impairment.\textsuperscript{53} 

Copper, on the other hand, has been found to be a potent inhibitor of GABA-evoked responses, particularly in Purkinje cells. Copper toxicity, notably in Wilson’s disease, could result, to some extent, from chronic GABAA receptor blockade.\textsuperscript{54} Data strongly suggest that Cu and Zn might interact with each other with GABA\textsubscript{\lambda} receptor complex and participate in modulation of synaptic transmission.\textsuperscript{55} 

Dopamine-\textbeta-hydroxylase (DBH) is a neurotransmitter, synthesizing enzyme, which catalyzes the formation of norepinephrine from dopamine. Copper is a co-factor required for this enzyme’s activity.\textsuperscript{57,58} Increased norepinephrine levels have been found in autistic individuals,\textsuperscript{56} which, at least in part, could be explained by excess copper. 

Our lab has also found that Cu/Zn SOD is decreased in autistic children, as well as in individuals with ADHD,\textsuperscript{61,62} and that in ADHD, lower Cu/Zn SOD is associated with increased copper.\textsuperscript{62} This suggests that increased copper in autism may also be associated with increased oxidative stress.

Our study shows that autistic individuals have lower levels of zinc and significantly higher levels of copper when compared to neurotypical controls, and copper/zinc correlates with selected symptom severity in autistic children.

It is tempting to suggest that plasma copper concentration and/or copper/zinc could be used as a
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biomarker for diagnosis of autism, but copper and zinc levels are altered in many other disease states and nutritional deficiencies.

We suggest that low zinc and high copper may modulate GABA receptors, ultimately changing transmitter concentration. High copper may also be associated with high norepinephrine found in autistic children, and high epinephrine may, in turn, manifest as excitability and hyperactivity associated autistic symptoms. To evaluate this relationship, future studies will assess more patients with autism and evaluate GABA and norepinephrine levels, as they are associated with Cu and Zn levels.

Author Contributions
All authors shared responsibility for data collection/entry/analysis and assistance with manuscript preparation. AJR was responsible for the study design and preparation of the manuscript. All authors read and approved the final manuscript.

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53. Takeda A, Itoh H, Imano S, Oku N. Impairment of GABAergic neurotransmitter system in the amygdala of young rats after 4-week zinc deprivation. *Neurochem Int.* 2006;49(8):746–50.

54. Sharonova IN, Vorobjev VS, Haas HL. High-affinity copper block of GABA(A) receptor-mediated currents in acutely isolated cerebellar Purkinje cells of the rat. *Eur J Neurosci.* 1998;10(2):522–8.

55. Kim H, Macdonald RL. An N-terminal histidine Is the primary determinant of α subunit-dependent Cu²⁺ sensitivity of αβγ2L GABA₅ receptors. *Molecular Pharmacology.* 2003;64:1145–52.

56. Lake CR, Ziegler MG, Murphy DL. Increased norepinephrine levels and decreased dopamine-beta-hydroxylase activity in primary autism. *Arch Gen Psychiatry.* May 1977;34(5):553–6.

57. Rahman K, et al. Dopamine-β-hydroxylase (DBH), Its cofactors and other biochemical parameters in the serum of neurological patients in Bangladesh. *Int J Biomed Sci.* 2009;5(4):395–401.

58. Deinum J, et al. *DBH* gene variants that cause low plasma dopamine β hydroxylase with or without a severe orthostatic syndrome. *J Med Genet.* 2004;41:138. doi:10.

59. Prasad AS. The role of zinc in gastrointestinal and liver disease. *Clin Gastroenterol.* Sep 1983;12(3):713–41.

60. Prasad AS. Clinical manifestations of zinc deficiency. *Annu Rev Nutr.* 1985;5:341–63.

61. Russo AJ. Decreased serum Cu/Zn SOD in children with autism. *Nutrition and Metabolic Insights.* 2009;2:27–35.

62. Russo AJ. Decreased serum Cu/Zn SOD associated with high copper in children with attention deficit hyperactivity disorder (ADHD). *Journal of Central Nervous System Disease.* 2010;2:1–6.

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