Nutrition and Metabolic Insights

ORIGINAL RESEARCH

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

Anthony J. Russo, Andrea P. Bazin, Richard Bigega, Robert S. Carlson III, Martin G. Cole, Dilenia C. Contreras, Matthew B. Galvin, Sayde S. Gaydorus, Sierra D. Holik, Gavin P. Jenkins, Brandon M. Jones, Penelope A. Languell, Padraic J. Lyman, Kareem P. March, Katie A. Meuer, Serena R. Peterson, Matthew T. Piedmonte, Michael G. Quinn, Nicole C. Smaranda, Patrick L. Steves, Heather P. Taylor, Teagan E. Waddingham and Janine S. Warren

Hartwick College, Oneonta, NY 13820. Corresponding author email: russoa2@hartwick.edu

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

Nutrition and Metabolic Insights 2012:5 41–47

doi: 10.4137/NMI.S8761

This article is available from http://www.la-press.com.

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 signaling[22] 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.[59,60]

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 syndrome[37] malnutrition[38] and Malabsorption.[39] Elevated copper levels are associated with infections,[40] inflammation,[41] trauma,[42] Wilson's disease,[43] excessive dietary intake[44] 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

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 (4C) 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

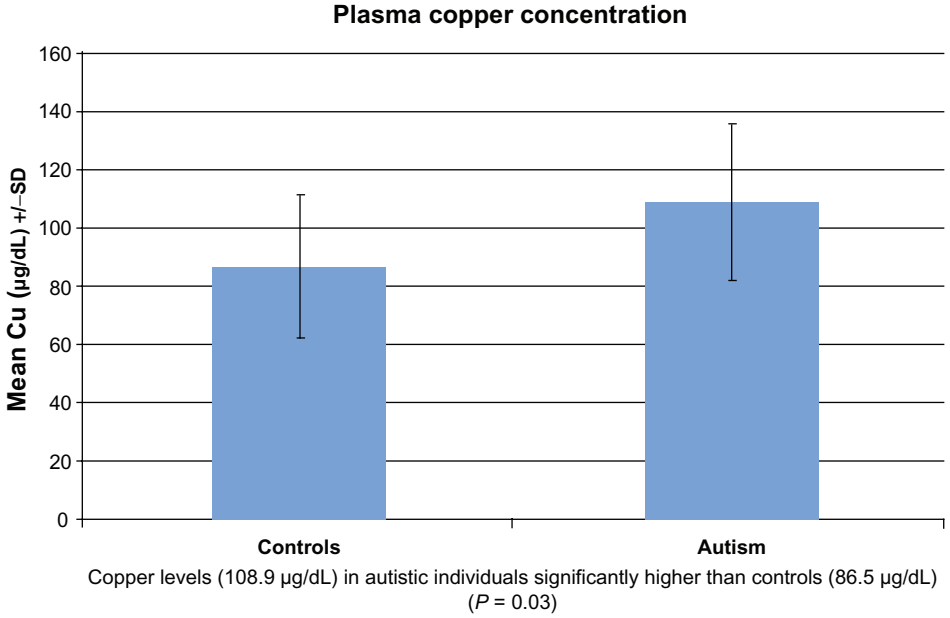

Figure 1. Plasma copper concentration was significantly higher in autistic individuals compared to controls.
Note: $P = 0.003$.

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

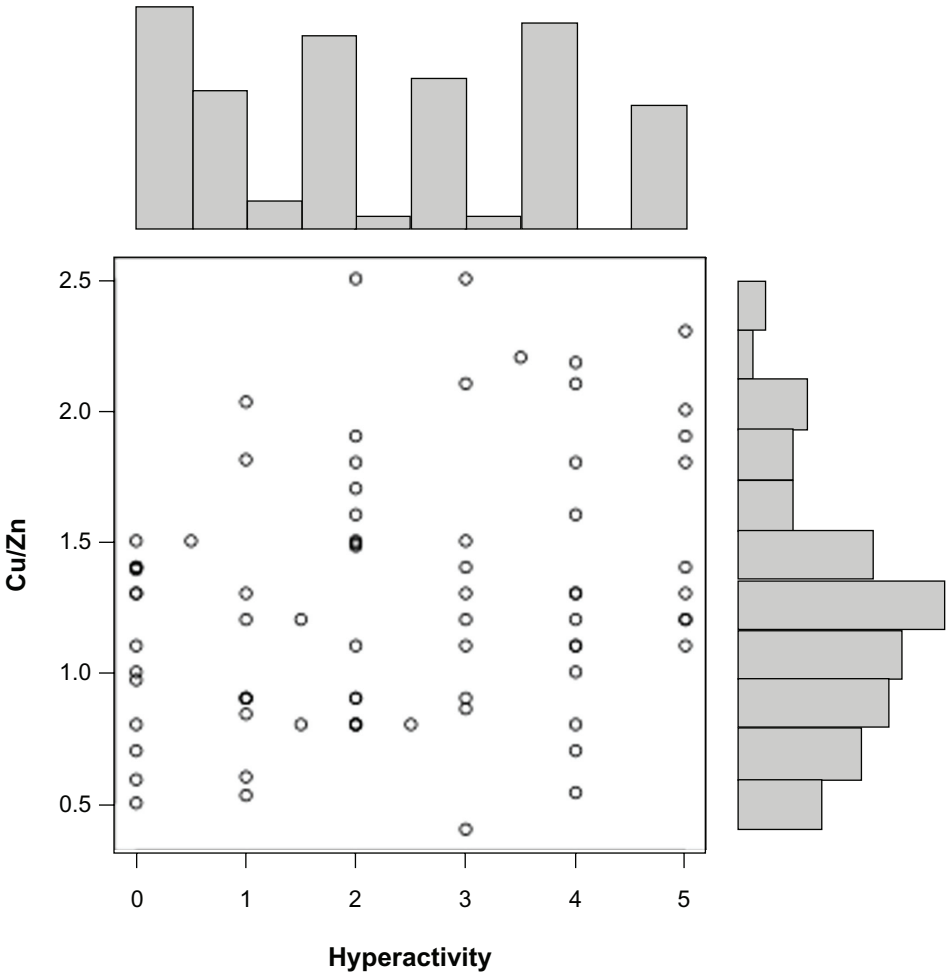

Figure 2. Severity of hyperactivity correlated significantly with Cu/Zn [r = 0.3, n = 79, *P* = 0.01] in autistic individuals.

the GABAergic system, which is responsible for synaptic inhibition in the adult brain, may be involved in autism.[47–49]

Zinc has been found to be associated with GABA and glutamate regulation, particularly through anxiolytic activity, modulating GABAergic inhibition and seizure susceptibility.[50–52] Zinc deficiency has also been found to be associated with GABAergic impairment.[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.[54] Data strongly suggest that Cu and Zn might interact with each other with $GABA_A$ receptor complex and participate in modulation of synaptic transmission.[55]

Dopamine-β-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.[57,58] Increased norepinephrine levels have been found in autistic individuals,[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,[61,62] and that in ADHD, lower Cu/Zn SOD is associated with increased copper.[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

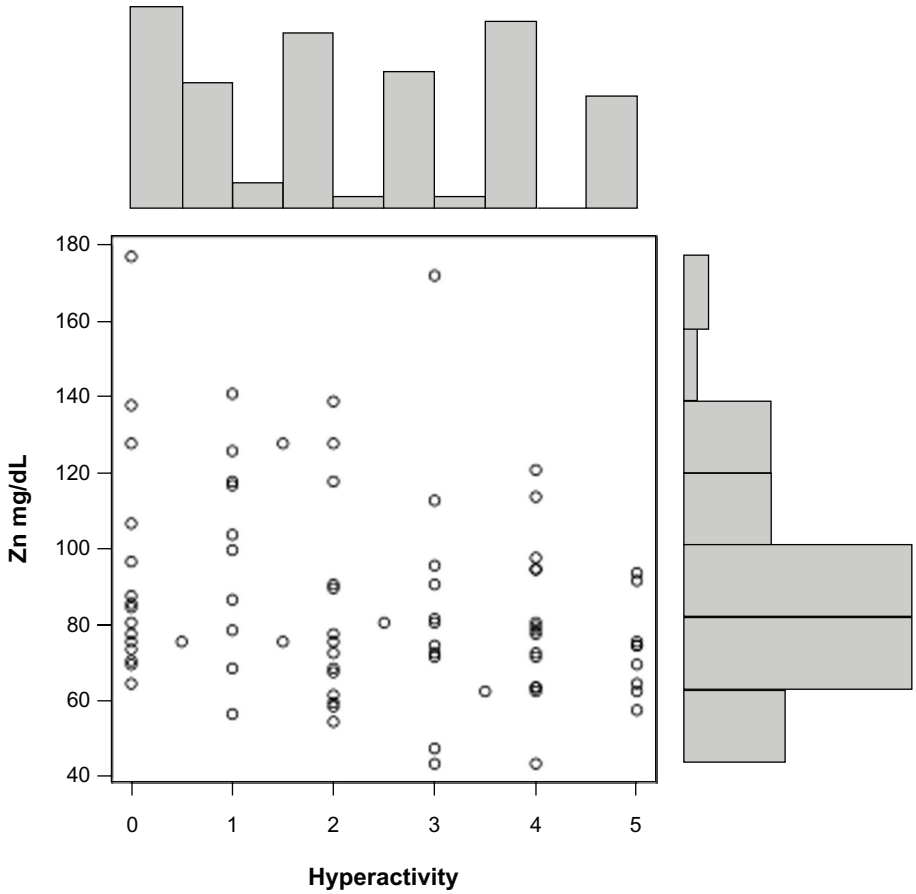

Figure 3. Severity of hyperactivity correlated significantly with decreased Zn [r = –0.3, n = 79, *P* = 0.02] in autistic individuals.

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.

Funding

This research was funded by the Autism Research Institute.

Disclosures and Ethics

As a requirement of publication author(s) have provided to the publisher signed confirmation of compliance with legal and ethical obligations including but not limited to the following: authorship and contributorship, conflicts of interest, privacy and confidentiality and (where applicable) protection of human and animal research subjects. The authors have read and confirmed their agreement with the ICMJE authorship and conflict of interest criteria. The authors have also confirmed that this article is unique and not under consideration or published in any other publication, and that they have permission from rights holders to reproduce any copyrighted material. Any disclosures are made in this section. The external blind peer reviewers report no conflicts of interest.

References

1. Muhle R, Trentacoste SV, Rapin I. The Genetics of Autism. *Pediatrics.* 2004;113:e472–86.
2. Yeargin-Allsopp M, Rice C, Karapurkar T, Doernberg N, Boyle C, Murphy C. Prevalence of autism in a US metropolitan area. *J Am Med Assoc.* 2003;289:49–55.
3. Schanen CN. Epigenetics of autism spectrum disorders. *Human Molecular Genetics*. 2006;15:138–50.
4. Fombonne E. Epidemiological surveys of autism and other pervasive developmental disorders: an update. *J Autism Dev Disord.* 2003;33:365–82.
5. Barrett S, Beck JC, Bernier R, et al. An autosomal genomic screen for autism. Collaborative linkage study of autism. *Am J Med Genet.* 1999;88:609–15.
6. International Molecular Genetics Study of Autism Consortium. Further characterization of the autism susceptibility locus AUTS1 on chromosome 7q. *Hum Mol Genet.* 2001;10:973–82.
7. Yonan AL, Alarcon M, Cheng R, et al. A genomewide screen of 345 families for autism-susceptibility loci. *Am J Hum Genet.* 2003;73:886–97.
8. Hutcheson HB, Olson LM, Bradford Y, et al. Examination of NRCAM, LRRN3, KIAA0716, and LAMB1 as autism candidate genes. *BMC Med Genet.* 2004;5:12.
9. Valicenti-McDermott M, McVicar K, Rapin I, Wershil BK, Cohen H, Shinnar S. Frequency of gastrointestinal symptoms in children with autistic spectrum disorders and association with family history of autoimmune disease. *J Dev Behav Pediatr.* 2006;27:S128–36.
10. Jyonouchi H, Geng L, Ruby A, Zimmerman-Bier B. Dysregulated innate immune responses in young children with autism spectrum disorders: their relationship to gastrointestinal symptoms and dietary intervention. *Neuropsychobiology.* 2005;51:77–85.
11. White JF. Intestinal Pathophysiology in *Autism Exp Biol Med* (Maywood). 2003;228:639–49.
12. Russo AJ, Krigsman A, Jepson B, Wakefield A. Low serum alpha-1 antitrypsin associated with Anti-PR3 ANCA in autistic children with GI disease. *Genomics Insights.* 2009;2:1–9.
13. Russo AJ, Krigsman A, Jepson B, Wakefield A. Anti-PR3 and anti-MPO IgG ANCA in autistic children with chronic GI disease. *Immunology and Immunogenetics Insights.* 2009;2:21–8.
14. Russo AJ. Anti-Metallothionein IgG and levels of metallothionein in autistic families. *Swiss Med Weekly.* 2008;138(5–6):70–7.
15. Russo AJ. Anti-metallothionein IgG and levels of metallothionein in autistic children with GI disease. *Drug, Healthcare and Patient Safety.* 2009;1:1–8.
16. Raulin J. Etudes clinique sur la vegetation. *Annales des Scienceas Naturelle: Botanique*. 1869;11:93–299.
17. Maze P. Influences respectives des elements de la solution gmineral du mais. *Annales de l'Institut Pasteur (Paris)*. 1914;28:21–69.
18. Todd WR, Elvehjem CA, Hart EB. Zinc in the nutrition of the rat. *American Journal of Physiology.* 1934;107:146–56.
19. Vallee BL, Auld DS. Zinc coordination, function, and structure of zinc enzymes and other proteins. *Biochemistry.* 1990;29:5647–59.
20. Miller WJ, Blackmon DM, Gentry RP, Pitts WJ, Powell GW. Absorption, excretion, and retention of orally administered zinc-65 in various tissues of zinc-deficient and normal goats and calves. *Journal of Nutrition.* 1967;92:71–8.
21. Brown RS, Sander C, Argos P. The primary structure of transcription factor IIIA has 12 consecutive repeats. *FEBS Letters.* 1985;186:271–4.
22. McNulty TJ, Taylor CW. Extracellular heavy- metal ions stimulate Ca^{2+} mobilization in hepatocytes. *Biochemical Journal.* 1999;339 (Pt 3):555–61.
23. Zalewski PD, Forbes IJ, Betts WH. Correlation of apoptosis with change in intracellular labile Zn(II) using zinquin [(2-methyl-8-p-toluenesulphonamido-6 quinolyloxy)acetic acid], a new specific fluorescent probe for Zn(II). *Biochemical Journal.* 1993;296:403–8.
24. Prasad AS. Clinical manifestations of zinc deficiency. *Annual Review of Nutrition.* 1985;5:341–63.
25. Cunnane SC. Role of zinc in lipid and fatty acid metabolism and in membranes. *Progress in Food and Nutrition Science.* 1988;12:151–88.
26. Endre L, Katona Z, Gyurkovits K. Zinc deficiency and cellular immune deficiency in acrodermatitis enteropathica. *Lancet.* 1975;2:119–6.
27. Aggett PJ. Acrodermatitis enteropathica. *Journal of Inherited Metabolic Disease.* 1983;1:39–43.
28. Halsted JA, Ronaghy HA, Abadi P. Zinc deficiency in man. *American Journal of Medicine.* 1972;53:277–84.
29. Prasad AS. Role of zinc in human health. *Boletin de la Asociacion Medica de Puerto Rico*. 1991;83:558–60.
30. Vallee BL, Falchuk KH. The biochemical basis of zinc physiology. *Physiological Reviews.* 1993;73:79–118.
31. Hambidge M. Human zinc deficiency. *J Nutr*. 2000;130(5S Suppl):1344S–9.
32. Ho E, Ames B. Low intracellular zinc induces oxidative DNA damage, disrupts p53, NFκB, and AP1 DNA binding, and affects DNA repair in a rat glioma cell line. *Proc Natl Acad Sci U S A.* 2002;99(26):16770–5.
33. Song Y, et al. Zinc deficiency affects DNA damage, oxidative stress, antioxidant defenses, and DNA repair in rats. *Journal of Nutrition.* 2009;139:1626–31.
34. Powell S. The antioxidant properties of zinc. *Journal of Nutrition.* 2000;130:1447S–54.
35. Vassiliev V, Harris ZL, Zatta P. Ceruloplasmin in neurodegenerative diseases. *Brain Res Rev*. 2005;49:633–40.
36. Arnaud P, Gianazza E, Miribel L. Ceruloplasmin. *Methods Enzymol*. 1988;163:441–52.
37. Horn N, Tonnesen T, Tumer Z. Menkes disease: an X-linked neurological disorder of the copper metabolism. *Brain Pathol*. 1992;2:351–62.
38. Wendland BE, Greenwood CE, Weinberg I, Young KW. Malnutrition in institutionalized seniors: the iatrogenic component. *J Am Geriatr Soc*. 2003;51:85–90.
39. Kumar N. Copper deficiency myelopathy (human swayback). *Mayo Clin Proc.* 2006;81:1371–84.
40. Kassu A, Yabutani T, Mahmud ZH, et al. Alterations in serum levels of trace elements in tuberculosis and HIV infections. *Eur J Clin Nutr*. 2006;60:580–6.
41. Ko WS, Guo CH, Yeh MS, et al. Lin Blood micronutrient, oxidative stress and viral load in patients with chronic hepatitis C. *World J Gastroenterol*. 2005;11:4697–702.
42. Molteni A, Ward W.F, Kim Y.T, et al. Lomont Serum copper concentration as an index of clinical lung injury. *Adv Exp Med Biol*. 1989;258:273–85.
43. Das SK, Ray K. Wilson's disease: an update. *Nat Clin Pract. Neurol*. 2006;2:482–93.
44. Wapnir RA. Copper absorption and bioavailability. *Am J Clin Nutr*. 1998;67:141–3.
45. Yilmaz A, Sari RA, Gundogdu M, Kose N, Dag E. Trace elements and some extracellular antioxidant proteins levels in serum of patients with systemic lupus erythematosus. *Clin Rheumatol*. 2005;24:331–5.
46. Chauhan A, et al. Increased copper-mediated oxidation of membrane phosphatidylethanolamine in autism. *American Journal of Biochemistry and Biotechnology.* 2008;4 (2):95–100.
47. Collins A, et al. Investigation of autism and GABA receptor subunit genes in multiple ethnic groups *Neurogenetics.* 2006;7(3):167–74.
48. Ma DQ, et al. Identification of significant association and gene-gene interaction of GABA receptor subunit genes in autism. *Am J Hum Genet.* 2005;77(3):377–88.
49. Ashley-Koch AE, et al. An analysis paradigm for investigating multi-locus effects in complex disease: examination of three GABA receptor subunit genes on 15q11-q13 as risk factors for autistic disorder. *Ann Hum Genet.* May 2006;70(Pt 3):281–92.
50. Xie X, Smart T. Properties of GABA-mediated synaptic potentials induced by zinc in adult rat hippocampal pyramidal neurones. *J Physiol*. 1993;460:503–23.
51. Ben-Ari Y, Cherubini E. Zinc and GABA in developing brain. *Nature.* 1991;353:220.
52. Takeda A, Hirate M, Tamano H, Oku N. Release of glutamate and GABA in the hippocampus under zinc deficiency. *J Neurosci Res*. 2003;72(4):537–42.

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 Cu^{2+} sensitivity of αβ3γ2L $GABA_A$ 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:e38. 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.