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Commentary

An urgent need to assess safe levels of inorganic copper in nutritional supplements/parenteral nutrition for subset of Alzheimer's disease patients

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1. Introduction

Continuous progress in understanding the functioning of various biometals coupled with pinpointing the roles of metals in several diseases; it has now become clear that metal dyshomeostasis can't be merely considered as a simple "all or none phenomenon". Instead metal dyshomeostasis in neurodegeneration, a finely synchronized process that occurs in response to pathological conditions, is regulated by specific chain of events. Among various biometals, redox active copper has an important role in various physiological and pathological conditions. In scientific literature, there is lot of debate going on "cause-effect" of copper dyshomeostasis in various neurodegenerative diseases; however, one fact is certain that aberrant copper metabolism has a key role in development/progression of neurodegenerative diseases. This notion stems from the fact that excess of copper results in generation of extremely damaging free hydroxyl radicals from hydrogen peroxide through Fenton reaction and other reactive oxygen species, which ultimately leads to oxidative stress, mitochondrial dysfunction, cytotoxicity, and aging. Oxidative stress causes damage to various biomolecules like DNA cleavage, protein oxidation, and lipid peroxidation. As neuronal membranes consist mainly of polyunsaturated lipids, central nervous system is particularly susceptible to oxidative stress elicited lipid peroxidation (reviewed by Bulcke et al., 2017; Pal, 2014; Rivera-Mancia et al., 2010). Further, oxidative stress has also been implicating in inducing cognitive waning in animals as well as humans (Hajjar et al., 2018; Lima et al., 2008; Rahman et al., 2009; Revel et al., 2015).

Wilson's disease and Menke's Disease, inherited disorders of copper metabolism, corroborates the essential role of copper metabolism homeostasis for proper central nervous system functioning (Ferreira and Gahl, 2017). Notwithstanding, in last three decades research on the role of copper in other neurodegenerative diseases, especially sporadic Alzheimer's disease, has been gaining momentum thanks to several landmark studies. In this commentary, we will briefly overview the key clinical study findings wherein copper role in Alzheimer's disease pathogenesis has been demonstrated, focusing on the effect of indiscriminate use of copper in nutritional supplements/parenteral nutrition on central nervous system functioning along with discussing future prospects/challenges.

2. Conundrum over safe levels of copper

Copper, through its vital role in key biological and signaling processes (Kardos et al., 2018), have put researchers in dilemma due to difficulty in setting up precise Recommended Dietary Allowance (RDA) for it primarily because of complex interplay between copper and iron metabolism. The conundrum of precise RDA for copper is due to lack of specific and sensitive biomarker/s for marginal copper excess, and other factors (reviewed elsewhere by Pal, 2014). Currently RDA for copper is in the range of 1.5–3.0 mg copper/day (Recommended dietary allowances, 1989), which is quite a broad range, and way above the actual daily requirement of < 1.5 mg copper/day in diet (Turnlund et al., 1989).

The problem just not ends here; there are concerns over safe levels of copper in drinking water (reviewed by Brewer, 2017, 2012; Pal et al.,

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2014) and nutritional supplements/parenteral nutrition too (Brewer, 2017; Vanek et al., 2015; Brewer, 2015, 2012), which provide inorganic copper and hence augments the inorganic copper pool of body significantly. Higher levels of copper in various food stuffs further exacerbate the problem (reviewed by Pal et al., 2015).

3. Copper and Alzheimer's disease: evidence from clinical studies

Scientific literature is full of preclinical *in vitro* (Atwood et al., 1998; Huang et al., 1999; White et al., 1999a) and *in vivo* (Singh et al., 2013; Sparks et al., 2006; White et al., 1999b) evidences to support the role of copper in onset and/or progression of sporadic Alzheimer's disease. As preclinical studies linking Alzheimer's disease with copper is out of theme for current commentary, readers are referred to extensive review by Pal et al., 2015 for the same. In addition to preclinical studies, lots of clinical studies have also garnered robust evidences to link copper with sporadic Alzheimer's disease (discussed in next paragraph). The obvious clinical manifestations of gross copper toxicity are easy to diagnose but the real issue is to detect marginal copper excess which if left undiagnosed for years can become an initiative/risk factor and/or contributory factor in development of neurodegeneration, neurobehavioral impairments and cognitive deterioration (extensively reviewed by Pal et al., 2015; Siotto and Squitti, 2018).

In Wilson's disease, there is increase in serum non-ceruloplasmin bound copper (Non-Cp-Copper) above > 1.6 μmol/L, and it has also been proposed as a diagnostic test for detection of Wilson's disease (European Association for Study of Liver, 2012). Non-Cp-Copper has a very low (< 1.6 μmol/L) concentration in human physiology. Various seminal clinical studies have shown that this low molecular weight Non-Cp-Copper concentration are also increased in Alzheimer's disease i.e. Non-Cp-Copper higher than normal reference value. These intriguing clinical studies have shown that (1) in normal subjects, serum Non-Cp-Copper levels were inversely correlated with cognitive function (Salustri et al., 2010) and approximately 3% Non-Cp-Cu can cross the blood brain barrier (BBB; Squitti et al., 2006); (2) Mini-Mental state examination (MMSE) is inversely proportional to serum Non-Cp-Copper levels (Squitti et al., 2002a, 2005; Squitti et al., 2006) and Non-Cp-Copper levels were predictive of MMSE score worsening in Alzheimer's disease patients (Squitti et al., 2009; Brewer et al., 2010); (3) Non-Cp-Copper increases in mild cognitive impairment (Squitti et al., 2011), and it is also predictive of conversion from mild cognitive impairment to Alzheimer's disease (Squitti et al., 2014a); (4) SNPs in transmembrane domain of Wilson's disease gene (copper ATPase7B or ATP7B) may have a strong association with Alzheimer's disease risk (Squitti et al., 2013a); (5) Alzheimer's disease patients harboring ATP7B gene variants demonstrated increased Non-Cp-Copper levels (Squitti et al., 2013b); (6) loci of susceptibility of Alzheimer's disease is present in the ATP7B gene (Bucossi et al., 2012); (7) genetic variations in ATP7B gene might contribute to Alzheimer's disease pathogenesis in Chinese population (Liu et al., 2013); (8) serum Non-Cp-Copper and copper levels were found higher in apolipoprotein E ε4 carriers (Squitti et al., 2007); (9) meta-analysis of Alzheimer's disease patients serum sample showed higher levels of serum copper than healthy controls (Squitti et al., 2014b).

James et al., 2012 have also shown elevated levels of exchangeable cupric (Cu^{2+}), which were correlated with tissue oxidative damage. In fact, there is one case report which reported the presence of Kayser-Fleisher (KF) rings in an Alzheimer's disease patient. It is noteworthy here that KF ring is pathological hallmark for neurological Wilson's disease and is caused by deposition of excess copper on the inner surface of the cornea in the Descemet membrane (Amtage et al., 2014). Various meta-analysis also supports the notion of copper imbalance in Alzheimer's disease i.e. decrease in brain copper level (Schrag et al., 2011) and small increase in blood copper (Wang et al., 2015). In recent years, this notion is also supported by many other lab group studies: five studies showed increased serum/plasma copper level whereas three studies reported decreased brain copper level in Alzheimer's disease patients (reviewed by Siotto and Squitti, 2018).

To the contrary, Rembach et al. (2013) study reported no change of Non-Cp-Copper levels in mild cognitive impairment and Alzheimer's disease patients. However, a year later meta-analysis published by these authors agreed in Non-Cp-Copper abnormalities in Alzheimer's disease (Squitti et al., 2014b). These observations can be explained on the basis of expanded blood Non-Cp-Copper pool (Squitti et al., 2014a, 2014b) (reviewed by Siotto and Squitti, 2018; Sensi et al., 2018). Taken together, the evidence presented here points towards higher serum Non-Cp-Copper and lower brain copper levels in subset of Alzheimer's disease patients. Therefore, aforementioned evidences robustly support "copper subtype of Alzheimer's disease" hypothesis (reviewed by Pal et al., 2015). This kind of stratification (sub-grouping) for Alzheimer's disease is necessary considering its chronic (Alzheimer's disease starts insidiously many decades before full dementia becomes apparent) multifactorial pathoetiology and incompatibility of amyloid cascade theory with clinical observations of sporadic Alzheimer's disease (Sporadic Alzheimer's disease accounts for ~90% of total Alzheimer's disease cases; Sensi et al., 2018).

To further ascertain the role of copper in Alzheimer's disease, two clinical trials using copper supplementation (Kessler et al., 2008) and copper chelation (Squitti et al., 2002b) have been also reported. Kessler et al., 2008 studied the effect of copper intake as Copper-II-orotate-dihydrate having 8 mg copper for 12 months in 29 patients (average age of 69 years) in mild Alzheimer's disease patients, and reported that over the course of the clinical trial there was no effect of increased copper intake on cognitive endpoints. 2002b) using D-penicillamine in a pilot study demonstrated that chelation therapy was able to mitigate the hydrogen peroxide induced oxidative stress along with increasing the urinary excretion of copper in Alzheimer's disease patients (n = 34). There was no reported apparent benefit on cognitive outcomes after D-penicillamine therapy in these nine Alzheimer's disease patients; however, the study was stopped before schedule finish due to high patient dropout and only nine Alzheimer's disease patients completed the six month clinical trial. These observations can be explained in part on the basis of the fact that D-penicillamine therapy leads to slower improvement of symptoms (may be observed even after three years) in patients with neurologic Wilson's disease (Brewer et al., 1987).

The intriguing question is that why the inorganic copper (in the form of copper sulphate) of nutritional supplements/drinking water is so much more hazardous to neural and cognition functioning than the organic copper found in diet? The answer lies in the way the cells utilize copper. Human cells first reduce cupric (Cu^{2+}) to cuprous (Cu^{+}) state with the help of specific reductases proteins e.g. Dcytb1 and Steap 1/2, before its transportation via copper transporters located on plasma membrane. The large influx of dietary copper along with inorganic copper from nutritional supplements/drinking water/parenteral nutrition may exceed the reductases potential and/or copper handling machinery of body/cell to reduce it, and hence results in increase of Non-Cp-Copper in blood which can cross BBB (Squitti et al., 2006). Mammals including humans have developed sophisticated cellular copper handling machinery (Fig. 1) in such a way that it can handle Cu^{+} safely, but not Cu^{2+}.

The intestinal copper transport system consist of Ctr1 (a cell membrane copper tran;1;sporter) which channels Cu^{+} (after copper reduction by reductases outside the cell) to the liver. Subsequently, copper handling machinery of cell incorporates it in safe intracellular channels in reduced form. The Ctr1 transports Cu^{+} and not Cu^{2+}, and probably excess of copper beyond the reduction capacity of intestinal reductases finds its way into the circulation and causes increase in Cu^{2+} pool of the blood. A percentage of Alzheimer's disease patients may have, at least in part, Cu^{2+} excess as a initiating and/or aggravating factor in the Alzheimer's disease pathogenesis [the basis of copper subtype of Alzheimer's disease hypothesis has been extensively reviewed elsewhere (Pal et al., 2015)].

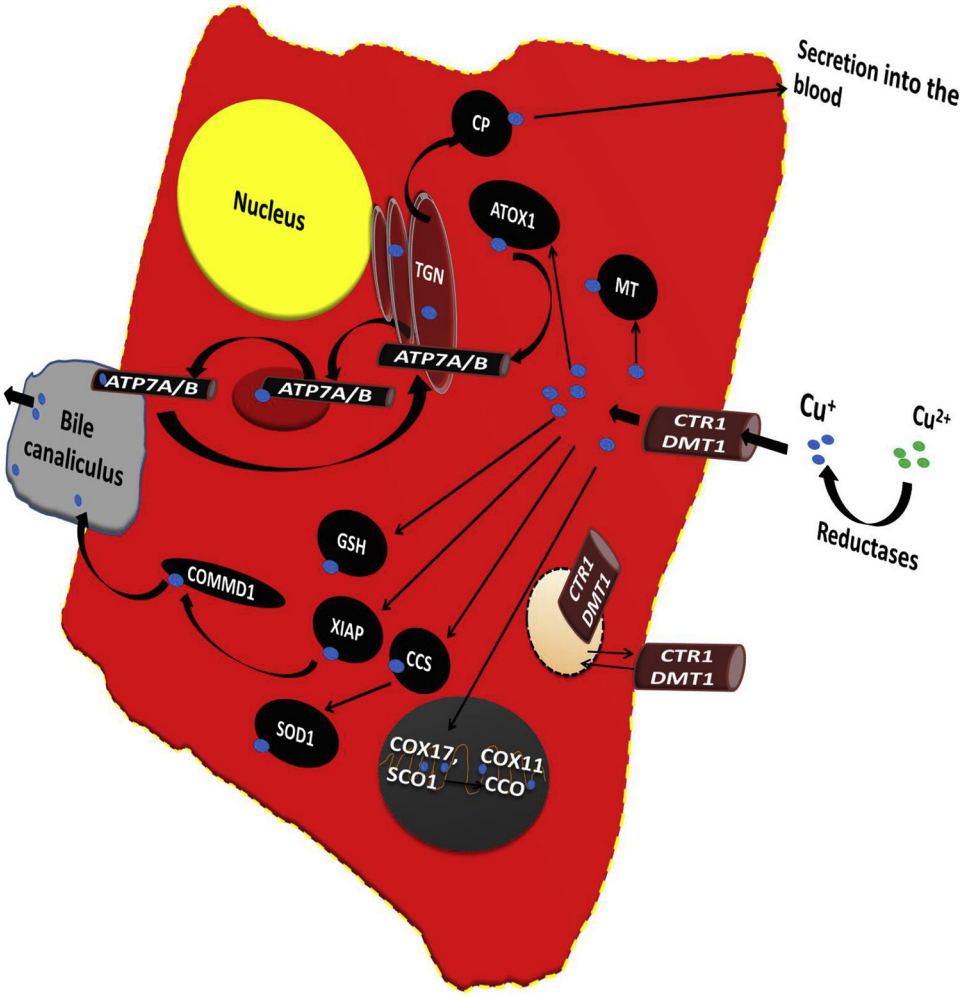

Fig. 1. Generalized copper handling machinery in a mammalian cell. In extracellular fluids, specific copper carriers and cuproenzymes are bound with copper^{2+} (green circles). CTR1 or DMT1 (copper transporters) are situated at the plasma membrane through which copper^{+} (blue circles) enters the cell after reduction by reductases. Copper chaperones (CCS, ATOX1, Cox17, Cox11 and Sco1) function is to transport copper to specific proteins/organelles. Copper sequestration and storage function is performed by MT (different tissues have different isoforms of MT I/II/III). The ATP7A/B (different tissues have different isoform of copper ATPase performing the same function) is required to transfer Copper into the lumen of the TGN for maturation of secreted cuproenzymes like dopamine-β-hydroxylase and ceruloplasmin. Under increased intracellular copper condition, ATP7A/B translocate to plasma membrane from the TGN, and aids in excretion of excess intracellular Copper. Abbreviations: ATP7A, copper-transporting ATPase A; ATP7B, copper-transporting ATPase B; CCS, copper chaperone for SOD1; CCO, cytochrome c oxidase; COMMD1, Copper metabolism (Murr1) domain containing protein1; COX11, cytochrome c oxidase assembly protein Cox11; Cox17, cytochrome c oxidase copper chaperone; Cp, ceruloplasmin; CTR1, copper transporter1; DMT1, divalent metal transporter 1; GSH, Glutathione; MT, metallothionein; Sco1, protein SCO1 homolog; SOD1, superoxide dismutase 1; TGN, trans-Golgi network; XIAP, X-linked inhibitor of apoptosis (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).
Reference: Pal (2014)

Concerns over use of copper containing nutritional supplement for safe parenteral nutrition/fortified foods have been raised in past too along with different guidelines for preterm neonates, children, adult/aged population and in different diseases (Asensio-Sanchez, 2014; Fessler, 2013; Finch, 2015; Jin et al., 2017; Krishnadev et al., 2010; McBurney et al., 2017; Vanek et al., 2015; Wong, 2012). Importantly, due to immaturity of biliary excretion system in very young children, the copper levels in fortified food should be strictly regulated.

Whereas, it's obvious to avoid copper containing supplements for patients suffering from Wilson's disease, Indian childhood cirrhosis, Idiopathic copper toxicosis and cholestasis; notwithstanding, various studies have also shown that it's better to reduce copper intake in other diseases/conditions especially individuals/Alzheimer's disease patients harboring ATP7B heterozygous condition (reviewed by Pal et al., 2015). This problem is further worsened by indiscriminate use of inorganic copper in nutritional supplements/parenteral nutrition and high copper levels in drinking water.

Due to wider acceptance for the role of excess of Non-Cp-Copper for neurological manifestation in Wilson's disease and sporadic Alzheimer's disease, there has been considerable scrutiny over the levels of inorganic copper in nutritional supplements/parenteral nutrition, which according to authors is quite obvious. As nutritional supplements/parenteral nutrition contains very high levels of inorganic copper, mostly in the form of inorganic copper sulphate, indiscriminate use of mineral supplements (mostly containing 2 mg inorganic copper/500 mg tablet) can cause hepatic/neurological/cognitive deterioration manifestations in long run especially in ATP7B heterozygous subjects.

As discussed previously, increase of inorganic copper pool in the circulation can have deleterious effect on central nervous system as it has been shown that around 3% Non-Cp-Copper can cross the BBB in humans (Squitti et al., 2006). Given the very high frequency (1:90) of ATP7B carriers in population and unknown single nucleotide polymorphisms (SNP)/novel mutations in ATP7B (Kumari et al., 2018), ATP7B modifier genes/factors (reviewed by Lutsenko, 2014) along with genes acting downstream of ATP7B gene, this increase of serum Non-Cp-Copper in genetically susceptible individuals can cause/initiate neuropathological conditions.

Due to redox active property of copper, free radicals (like hydroxyl radical) are produced when hydrogen peroxide reacts with Cu^{+} by Fenton reaction. Therefore, increase of Non-Cp-Cu can cause oxidative stress which can lead to reactive astrogliosis, microgliosis, neuroinflammation and alteration in zinc to copper ratio differentially in different regions of brain. These pathological changes along with oxidative stress eventually lead to aging and cellular degeneration. Reactive astrogliosis (observation of activated astrocytes at the site of Aβ deposits) is a common factor in Alzheimer's disease. In addition, extents of reactive astrogliosis as well as the amount of accrued Aβ in astrocytes have been shown to correlate with the severity of Alzheimer's disease associated tissue damage. These observations strengthen the view that astrocytes play an important role in Alzheimer's disease pathology (for comprehensive review, refer to Allaman et al., 2011). Also, astrocytes serve as copper depots of brain (reviewed by Pal and Prasad, 2014) and also thought to play a key role in learning and memory consolidation (reviewed by Gibbs et al., 2008).

As reactive astrogliosis is the common denominator in copper dyshomeostasis associated neurological disorders, it will be interesting to

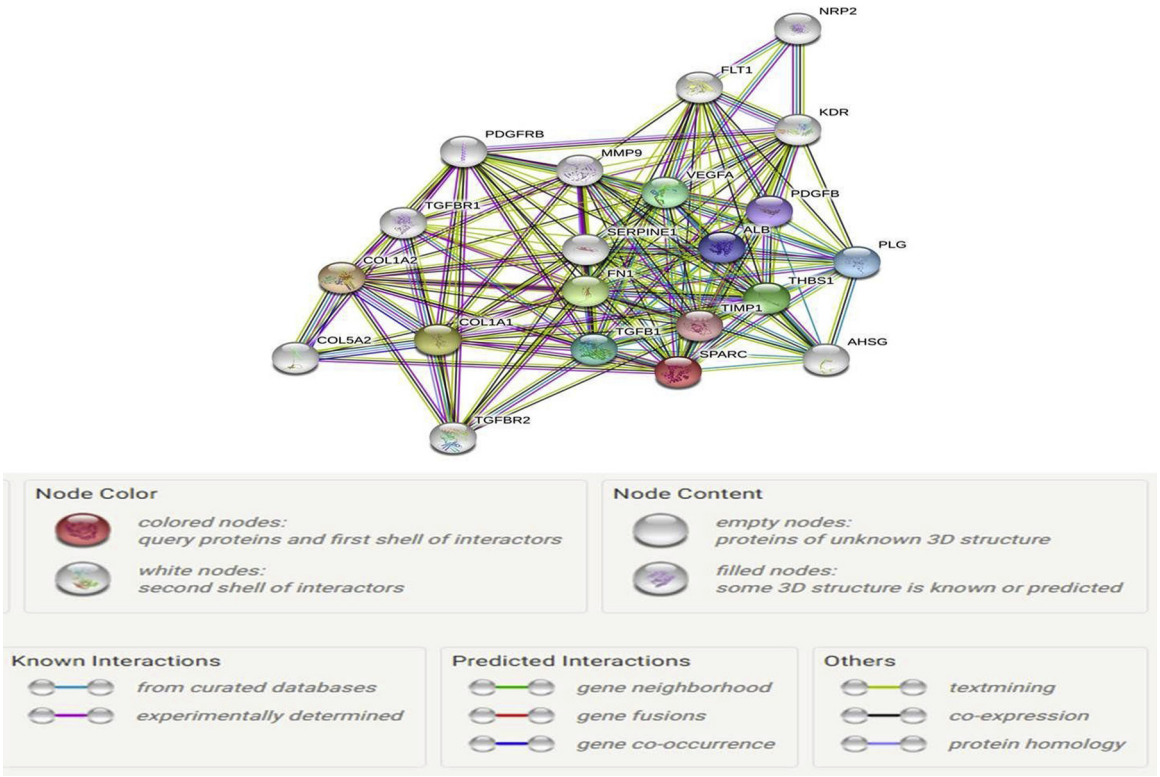

Fig. 2. Protein–protein interaction network of *Homo sapiens* SPARC protein with other proteins (Source: STRING database; http://string.embl.de/). Box below with colored bars specify the basis of protein–protein interaction as shown by the colored lines in the SPARC protein-protein interaction network above. Proteins are represented by the node (circle) and biological interactions between the proteins are represented by edge (line). Abbreviations: ALB, Serum albumin; COL1A2, Collagen alpha-2(I) chain; COL1A1, Collagen alpha-1(I) chain; FN1, Fibronectin type III domain containing; PLG, Plasminogen; PDGFB, Platelet-derived growth factor subunit B; SPARC, secreted protein acidic and rich in cysteine; THBS1, Thrombospondin-1;TGFB1, Transforming growth factor beta-1; TIMP1, Metalloproteinase inhibitor 1; VEGFA, Vascular endothelial growth factor A.

evaluate specific subtype of astrocytes which serve as copper depots of brain and which are also pathologically involved in Alzheimer's disease. It will be interesting to explore whether this phenomenon is associated with particular subpopulations of astrocytes (refer to review of Matyash and Kettenmann, 2010 for heterogeneity of astrocytes), while the surrounding astrocytes remain either unaffected or become reactive? Besides, future studies examining whether there is an intercellular communication between neurons and astrocytes for the copper export from astrocytes under physiological/neuropathological settings will be highly worthy (Pal and Prasad, 2014).

The alterations in zinc to copper ratio in brain are deleterious for neuronal health as zinc serves as a second messenger for intracellular signaling at lower concentration. However, at higher concentration zinc becomes neurotoxic. The neurobiological activity of zinc is complex. Zinc and copper homeostasis are closely regulated. Zinc levels are altered in Alzheimer's disease, and zinc also affects both amyloid-dependent and -independent mechanisms critical for the Alzheimer's disease progression. The increase of copper can displace zinc bound to metallothionein, which can have neuropathological consequences (for role of zinc in Alzheimer's disease refer to Sensi et al., 2018).

4. Future perspectives and challenges

There are still unanswered questions which remain to be answered to establish a definitive "cause-effect" relation of copper with Alzheimer's disease. Given the complex heterogeneous nature of Alzheimer's disease, and the most likely change in biometals distribution (particularly copper, iron and zinc) in different regions of brain, blood and liver that changes dynamically depending on increasing age, course of disease and treatment, then arguably the most promising approach is to study effect of two or more variables (for example two or more biometals). However, this approach is confounded by complex interlinked interplay among different essential/toxic metals, and the ever expanding data on the metal-protein, protein-protein and metal-metal interactions that subserve various critical pathways of central nervous system functioning under physiological and pathological conditions. As biometals dyshomeostasis is a common factor in Alzheimer's disease, there is need to study effect of various biometals along with their inter-and intra-protein-protein interactions in Alzheimer's disease. This approach can substantially increase the limited "success" as well as reduce the "failures" in clinical trials targeting "Biometal and Alzheimer's disease" pathway by employing either chelation, modulation or supplementation of a particular metal (Adlard and Bush, 2018).

System biology can play a pivotal role in this regard as it help in analyzing large set of data in a systemic way. Network biology studies are a novel way to see multivariate effect of more than two factors to study old research problem. We used Cytoscape software to identify novel copper and iron metabolism proteins among all the reported copper and iron metabolism proteins of *Homo Sapiens* in Alzheimer's disease, and *in silico* data demonstrated the plausible association of secreted protein acidic and rich in cysteine (SPARC/osteonectin) protein with Alzheimer's disease (Kumar et al., 2019). It is emphasized here that copper and iron binding domain of SPARC mediates cell survival via interaction with Integrin 1 and activation of Integrin-linked Kinase Weaver et al., 2008). Interestingly hevin (SPARC like protein 1), a secreted protein with high structural similarity to SPARC/osteonectin, is reported to be involved in Alzheimer's disease (Medway et al., 2010; Seddighi et al., 2018; Vafadar-Isfahani et al., 2012).

We used SPARC as a template and constructed a protein-protein interaction network (Fig. 2) and gene co-expression data (Fig. 3) for SPARC with the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database, limiting the search only to information

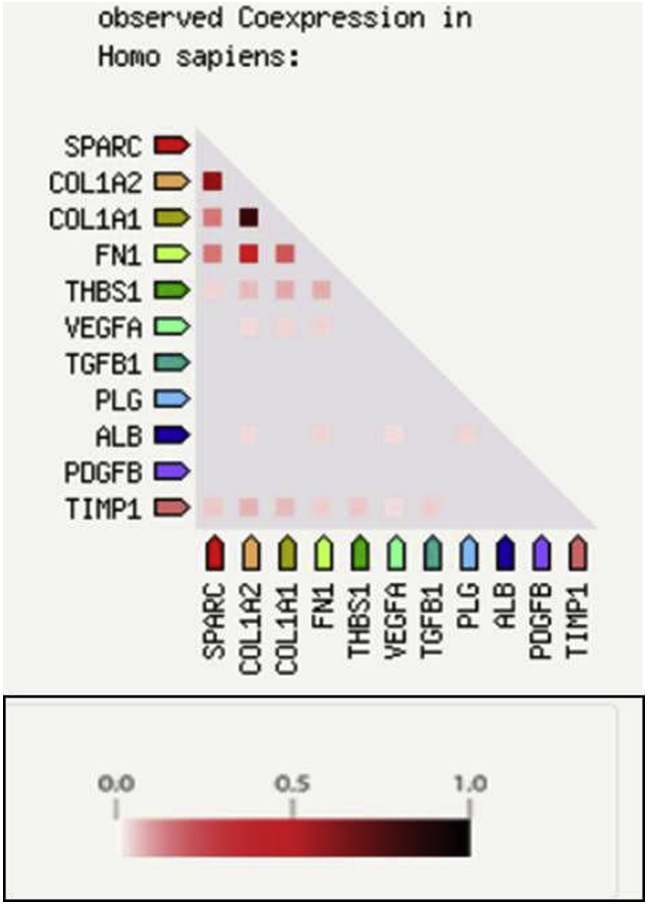

Fig. 3. Gene co-expression data of SPARC protein of *Homo Sapiens* (Source: STRING database; http://string.embl.de/). Coexpression predicts functional association. Coexpression scores were based on RNA expression patterns and protein co-regulation. In the triangle-matrices, the intensity of colour indicates the level of confidence that two proteins are functionally associated, given the overall expression data in the organism. Abbreviations: ALB, Serum albumin; COL1A2, Collagen alpha-2(I) chain; COL1A1, Collagen alpha-1(I) chain; FN1, Fibronectin type III domain containing; PLG, Plasminogen; PDGFB, Platelet-derived growth factor subunit B; SPARC, secreted protein acidic and rich in cysteine; THBS1, Thrombospondin-1;TGFB1, Transforming growth factor beta-1; TIMP1, Metalloproteinase inhibitor 1; VEGFA, Vascular endothelial growth factor A.

available in *Homo sapiens* (http://string.embl.de/). Fig. 2 shows 21 nodes and 150 edges for SPARC protein. Node refers to proteins, and edge depicts biological interactions between the proteins. Fig. 3 demonstrates the co-expression scores of SPARC gene based on RNA expression patterns and protein co-regulation. SPARC protein appears to regulate cell growth through interactions with the extracellular matrix and cytokines. In addition, it binds calcium and copper, several types of collagen, albumin, thrombospondin, and cell membranes. Given the magnitude of its node and edge interactions, there lays a huge scope to investigate SPARC protein role in neurodegeneration. Different region of brain expresses SPARC differentially with microglia of the cerebellum and cerebellar Bergmann glia having the maximum expression. It is also expressed in astrocytes of cerebellum and hippocampus. However, there are only a few reports on role of SPARC protein in neurodegeneration; nonetheless, emerging evidences are quite robust to support its involvement in neurodegeneration (reviewed by Jayakumar et al., 2017).

During data mining, we also found that there are reports linking F5 gene [encodes coagulation factor V which contains a copper ion (Mann et al., 1984), and is part of non-exchangable copper pool of plasma (reviewed by Siotto and Squitti, 2018)], SNP with Alzheimer's disease (Melville et al., 2012). In addition, F5 SNP rs2213865 was demonstrated to be significantly associated with rate of cognitive decline among 331 Alzheimer's disease patients (Sherva and Farrer, 2011). There are also reports linking other coagulation factor (XIIIa) with Alzheimer's disease. It has been shown that coagulation factor XIIIa crosslinks amyloid β into dimers and oligomers and to blood proteins (Hur et al., 2019). Further, coagulation factor XIIIa colocalizes with deposited β-amyloid in cerebral amyloid angiopathy and forms unique complexes with β-amyloid (de Jager et al., 2016). Given the rapid progress in system biology derived evidences together with basic, experimental and clinical studies, we can hope to address the unanswered questions related to role of biometals (especially copper) in Alzheimer's disease.

The take home message is that nutritional supplements (especially containing copper) should be taken only after physician prescription only, as indiscriminate and self-medication for long term can have a deleterious effect in certain ATP7B carriers and Wilson's disease patients. In addition, there should be a closer scrutiny over the use and levels of inorganic copper added in supplements/parenteral nutrition/fortified foods for different age groups/disease conditions. Given the harmful effects of higher levels of the inorganic copper in nutritional supplements/parenteral nutrition which can lead to sporadic Alzheimer's disease in certain (genetically) susceptible individuals, attempts should be made to make universally acceptable guidelines for safe levels of inorganic copper in such supplements. Even a set of preliminary guidelines (Dietary and lifestyle guidelines) to help individuals who wish to reduce their risk of Alzheimer's disease was published in a seminal article (Barnard et al., 2014) and copper reduction was among one of the seven guidelines proposed. Moreover, more studies are warranted to search for sensitive and specific biomarkers for marginal copper excess which will eventually help in accurately assessing the copper store of the body (reviewed by Pal. 2014) and will help in setting up precise guidelines for safe levels of copper in mineral supplements/parenteral nutrition.

Conflict of interest statement

The authors declare no competing financial interest.

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References

Adlard, P.A., Bush, A.I., 2018. Metals and Alzheimer's disease: how far have we come in the clinic? J. Alzheimers Dis. 62, 1369–1379.
Allaman, I., Belanger, M., Magistretti, P.J., 2011. Astrocyte-neuron metabolic relationships: for better and for worse. Trends Neurosci. 34, 76–87.
Amtage, F., Birnbaum, D., Reinhard, T., Niesen, W.D., Weiller, C., Mader, I., et al., 2014. Estrogen intake and copper depositions: implications for Alzheimer's disease? Case Rep. Neurol. 6, 181–187.
Asensio-Sanchez, V.M., 2014. Copper in multivitamin supplements. Arch. Soc. Esp. Oftalmol. 89, 342–343.
Atwood, C.S., Moir, R.D., Huang, X., Scarpa, R.C., Bacarra, N.M., Romano, D.M., Hartshorn, M.A., Tanzi, R.E., 1998. Bush AI Dramatic aggregation of Alzheimer abeta by Cu(II) is induced by conditions representing physiological acidosis. J. Biol. Chem. 273, 12817–12826.
Barnard, N.D., Bush, A.I., Ceccarelli, A., Cooper, J., de Jager, C.A., Erickson, K.I., et al., 2014. Dietary and lifestyle guidelines for the prevention of Alzheimer's disease. Neurobiol. Aging 35 (September (Suppl 2)), S74–8.
Brewer, G.J., 2012. Copper toxicity in Alzheimer's disease: cognitive loss from ingestion of inorganic copper. J. Trace Elem. Med. Biol. 26, 89–92.
Brewer, G.J., 2015. Copper-2 ingestion, plus increased meat eating leading to increased copper absorption, are major factors behind the current epidemic of Alzheimer's disease. Nutrients 7, 10053–10064.
Brewer, G.J., 2017. Copper-2 hypothesis for causation of the current Alzheimer's disease epidemic together with dietary changes that enhance the epidemic. Chem. Res. Toxicol. 30, 763–768.
Brewer, G.J., Terry, C.A., Aisen, A.M., Hill, G.M., 1987. Worsening of neurologic

syndrome in patients with Wilson's disease with initial penicillamine therapy. Arch. Neurol. 44, 490–493.
Brewer, G.J., Kanzer, S.H., Zimmerman, E.A., Celmins, D.F., Heckman, S.M., Dick, R., 2010. Copper and ceruloplasmin abnormalities in Alzheimer's disease. Am. J. Alzheimer's Dis. Dement. 25, 490–497.
Bucossi, S., Polimanti, R., Mariani, S., Ventriglia, M., Bonvicini, C., Migliore, S., et al., 2012. Association of K832R and R952K SNPs of Wilson's disease gene with Alzheimer's disease. J. Alzheimers Dis. 29, 913–919.
Bulcke, F., Dringen, R., Scheiber, I.F., 2017. Neurotoxicity of copper. Adv. Neurobiol. 18, 313–343.
de Jager, M., Boot, M.V., Bol, J.G., Breve, J.J., Jongenelen, C.A., Drukarch, B., et al., 2016. The blood clotting Factor XIIIa forms unique complexes with amyloid-beta (Abeta) and colocalizes with deposited Abeta in cerebral amyloid angiopathy. Neuropathol. Appl. Neurobiol. 42, 255–272.
European Association for Study of Liver, 2012. EASL clinical practice guidelines: wilson's disease. J. Hepatol. 56, 671–685.
Ferreira, C.R., Gahl, W.A., 2017. Disorders of metal metabolism. Transl. Sci. Rare Dis. 2, 101–139.
Fessler, T.A., 2013. Trace elements in parenteral nutrition: a practical guide for dosage and monitoring for adult patients. Nutr. Clin. Pract. 28, 722–729.
Finch, C.W., 2015. Review of trace mineral requirements for preterm infants: what are the current recommendations for clinical practice? Nutr. Clin. Pract. 30, 44–58.
Gibbs, M.E., Hutchinson, D., Hertz, L., 2008. Astrocytic involvement in learning and memory consolidation. Neurosci. Biobehav. Rev. 32, 927–944.
Hajjar, I., Hayek, S.S., Goldstein, F.C., Martin, G., Jones, D.P., Quyyumi, A., 2018. Oxidative stress predicts cognitive decline with aging in healthy adults: an observational study. J. Neuroinflammation 15 (1), 17.
Huang, X., Cuajungco, M.P., Atwood, C.S., Hartshorn, M.A., Tyndall, J.D., Hanson, G.R., Stokes, K.C., Leopold, M., Multhaup, G., Goldstein, L.E., Scarpa, R.C., Saunders, A.J., Lim, J., Moir, R.D., Glabe, C., Bowden, E.F., Masters, C.L., Fairlie, D.P., Tanzi, R.E., Bush, A.I., 1999. Cu(II) potentiation of alzheimer abeta neurotoxicity. Correlation with cell-free hydrogen peroxide production and metal reduction. J. Biol. Chem. 274, 37111–37116.
Hur, W.S., Mazinani, N., Lu, X.J.D., Yefet, L.S., Byrnes, J.R., Ho, L., et al., 2019. Coagulation factor XIIIa crosslinks amyloid b into dimers and oligomers and to blood proteins. J. Biol. Chem. 294 (2), 390–396. https://doi.org/10.1074/jbc.RA118.005352.
James, S.A., Volitakis, I., Adlard, P.A., Duce, J.A., Masters, C.L., Cherny, R.A., et al., 2012. Elevated labile Cu is associated with oxidative pathology in Alzheimer disease. Free Radic. Biol. Med. 52, 298–302.
Jayakumar, A.R., Apeksha, A., Norenberg, M.D., 2017. Role of matricellular proteins in disorders of the central nervous system. Neurochem. Res. 42, 858–875.
Jin, J., Mulesa, L., Carrilero Rouillet, M., 2017. Trace elements in parenteral nutrition: considerations for the prescribing clinician. Nutrients 28, 9. https://doi.org/10.3390/nu9050440. pii: E440.
Kardos, J., Heja, L., Simon, A., Jablonkai, I., Kovacs, R., Jemnitz, K., 2018. Copper signalling: causes and consequences. Cell Commun. Signal 16, 71.
Kessler, H., Bayer, T.A., Bach, D., Schneider-Axmann, T., Supprian, T., Herrmann, W., et al., 2008. Intake of copper has no effect on cognition in patients with mild Alzheimer's disease: a pilot phase 2 clinical trial. J. Neural Transm. Vienna (Vienna) 115, 1181–1187.
Krishnadev, N., Meleth, A.D., Chew, E.Y., 2010. Nutritional supplements for age-related macular degeneration. Curr. Opin. Ophthalmol. 21, 184–189.
Kumar, A., Gupta, S., Sharma, P., Prasad, R., Pal, A., 2019. *In silico* method for identification of novel copper and iron metabolism proteins in various neurodegenerative disorders. Neurotoxicology 73, 50–57. https://doi.org/10.1016/j.neuro.2019.02.020. [Epub ahead of print].
Kumari, N., Kumar, A., Thapa, B.R., Modi, M., Pal, A., Prasad, R., 2018. Characterization of mutation spectrum and identification of novel mutations in ATP7B gene from a cohort of Wilson disease patients: functional and therapeutic implications. Hum. Mutat. 39, 1926–1941. https://doi.org/10.1002/humu.23614.
Lima, F.D., Souza, M.A., Furian, A.F., Rambo, L.M., Ribeiro, L.R., Martignoni, F.V., et al., 2008. Na+,K+ ATPase activity impairment after experimental traumatic brain injury: relationship to spatial learning deficits and oxidative stress. Behav. Brain Res. 193, 306–310.
Liu, H.P., Lin, W.Y., Wang, W.F., Tsai, C.H., Wu, W.C., Chiou, M.T., et al., 2013. Genetic variability in copper-transporting P-type adenosine triphosphatase (ATP7B) is associated with Alzheimer's disease in a Chinese population. J. Biol. Regul. Homeost. Agents 27, 319–327.
Lutsenko, S., 2014. Modifying factors and phenotypic diversity in Wilson's disease. Ann. N. Y. Acad. Sci. 1315, 56–63.
Mann, K.G., Lawler, C.M., Vehar, G.A., Church, W.R., 1984. Coagulation factor V contains copper ion. J. Biol. Chem. 259, 12949–12951.
Matyash, V., Kettenmann, H., 2010. Heterogeneity in astrocyte morphology and physiology. Brain Res. Rev. 63, 2–10.
McBurney, M.I., Hartunian-Sowa, S., Matusheski, N.V., 2017. Implications of US nutrition facts label changes on micronutrient density of fortified foods and supplements. J. Nutr. 147, 1025–1030.
Medway, C., Shi, H., Bullock, J., Black, H., Brown, K., Vafadar-Isfahani, B., et al., 2010. Using in silico LD clumping and meta-analysis of genome-wide datasets as a complementary tool to investigate and validate new candidate biomarkers in Alzheimer's disease. Int. J. Mol. Epidemiol. Genet. 1, 134–144.
Melville, S.A., Buros, J., Parrado, A.R., Vardarajan, B., Logue, M.W., Shen, L., et al., 2012. Multiple loci influencing hippocampal degeneration identified by genome scan. Ann. Neurol. 72, 65–75.
Pal, A., 2014. Copper toxicity induced hepatocerebral and neurodegenerative diseases: an urgent need for prognostic biomarkers. Neurotoxicology 40, 97–101.
Pal, A., Prasad, R., 2014. Recent discoveries on the functions of astrocytes in the copper homeostasis of the brain: a brief update. Neurotox. Res. 26, 78–84.
Pal, A., Jayamani, J., Prasad, R., 2014. An urgent need to reassess the safe levels of copper in the drinking water: lessons from studies on healthy animals harboring no genetic deficits. Neurotoxicology 44C, 58–60.
Pal, A., Siotto, M., Prasad, R., Squitti, R., 2015. Towards a unified vision of copper involvement in Alzheimer's disease: a review connecting basic, experimental, and clinical research. J. Alzheimers Dis. 44 (2), 343–354.
Rahman, M.F., Wang, J., Patterson, T.A., Saini, U.T., Robinson, B.L., Newport, G.D., et al., 2009. Expression of genes related to oxidative stress in the mouse brain after exposure to silver-25 nanoparticles. Toxicol. Lett. 187, 15–21.
Recommended Dietary Allowances, 1989. Recommended Dietary Allowances, 10th edition. The National Academies Press.
Rembach, A., Doecke, J.D., Roberts, B.R., Watt, A.D., Faux, N.G., Volitakis, I., et al., 2013. Longitudinal analysis of serum copper and ceruloplasmin in Alzheimer's disease. J. Alzheimers Dis. 34, 171–182.
Revel, F., Gilbert, T., Roche, S., Drai, J., Blond, E., Ecochard, R., Bonnefoy, M., 2015. Influence of oxidative stress biomarkers on cognitive decline. J. Alzheimers Dis. 45 (2), 553–560.
Rivera-Mancia, S., Perez-Neri, I., Rios, C., Tristan-Lopez, L., Rivera-Espinosa, L., Montes, S., 2010. The transition metals copper and iron in neurodegenerative diseases. Chem. Biol. Interact. 186, 184–199.
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. Clin. Neurophysiol. 121 (4), 502–507.
Schrag, M., Mueller, C., Oyoyo, U., Smith, M.A., Kirsch, W.M., 2011. Iron, zinc and copper in the Alzheimer's disease brain: a quantitative meta-analysis. Some insight on the influence of citation bias on scientific opinion. Prog. Neurobiol. 94, 296–306.
Seddighi, S., Varma, V.R., An, Y., Varma, S., Beason-Held, L.L., Tanaka, T., et al., 2018. SPARCL1 accelerates symptom onset in alzheimer's disease and influences brain structure and function during aging. J. Alzheimers Dis. 61, 401–414.
Sensi, S.L., Granzotto, A., Siotto, M., Squitti, R., 2018. Copper and zinc dysregulation in Alzheimer's disease. Trends Pharmacol. Sci. 39, 1049–1063.
Sherva, R., Farrer, L.A., 2011. Power and pitfalls of the genome-wide association study approach to identify genes for Alzheimer's disease. Curr. Psychiatry Rep. 13, 138–146.
Singh, I., Sagare, A.P., Coma, M., Perlmutter, D., Gelein, R., Bell, R.D., Deane, R.J., Zhong, E., Parisi, M., Ciszewski, J., Kasper, R.T., Deane, R., 2013. Low levels of copper disrupt brain amyloidbeta homeostasis by altering its production and clearance. Proc. Natl. Acad. Sci. U. S. A. 110, 14771–14776.
Siotto, M., Squitti, R., 2018. Copper imbalance in Alzheimer's disease: overview of the exchangeable copper component in plasma and the intriguing role albumin plays. Coord. Chem. Rev. 371, 86–95.
Sparks, D.L., Friedland, R., Petanceska, S., Schreurs, B.G., Shi, J., Perry, G., Smith, M.A., Sharma, A., Derosa, S., Ziolkowski, C., Stankovic, G., 2006. Trace copper levels in the drinking water, but not zinc or aluminum influence CNS Alzheimerlike pathology. J. Nutr. Health Aging 10, 247–254.
Squitti, R., Lupoi, D., Pasqualetti, P., Dal Forno, G., Vernieri, F., Chiovenda, P., Rossi, L., Cortesi, M., Cassetta, E., Rossini, P.M., 2002a. Elevation of serum copper levels in Alzheimer's disease. Neurology 59, 1153–1161.
Squitti, R., Rossini, P.M., Cassetta, E., Moffa, F., Pasqualetti, P., Cortesi, M., Colloca, A., Rossi, L., Finazzi-Agro, A., 2002b. Dpenicillamine reduces serum oxidative stress in Alzheimer's disease patients. Eur. J. Clin. Invest. 32, 51–59.
Squitti, R., Pasqualetti, P., Dal Forno, G., Moffa, F., Cassetta, E., Lupoi, D., Vernieri, F., Rossi, L., Baldassini, M., Rossini, P.M., 2005. Excess of serum copper not related to ceruloplasmin in Alzheimer disease. Neurology. 64, 1040–1046.
Squitti, R., Barbati, G., Rossi, L., Ventriglia, M., Dal Forno, G., Cesaretti, S., et al., 2006. Excess of nonceruloplasmin serum copper in AD correlates with MMSE, CSF [beta]-amyloid, and h-tau. Neurology 67, 76–82.
Squitti, R., Ventriglia, M., Barbati, G., Cassetta, E., Ferreri, F., Dal Forno, G., et al., 2007. 'Free' copper in serum of Alzheimer's disease patients correlates with markers of liver function. J. Neural Transm. 114 (12), 1589–1594.
Squitti, R., Bressi, F., Pasqualetti, P., Bonomini, C., Ghidoni, R., Binetti, G., Cassetta, E., Moffa, F., Ventriglia, M., Vernieri, F., Rossini, P.M., 2009. Longitudinal prognostic value of serum "free" copper in patients with Alzheimer disease. Neurology 72, 50–55.
Squitti, R., Ghidoni, R., Scrascia, F., Benussi, L., Panetta, V., Pasqualetti, P., Moffa, F., Bernardini, S., Ventriglia, M., Binetti, G., Rossini, P.M., 2011. Free copper distinguishes mild cognitive impairment subjects from healthy elderly individuals. J. Alzheimers Dis. 23, 239–248.
Squitti, R., Polimanti, R., Bucossi, S., Ventriglia, M., Mariani, S., Manfellotto, D., et al., 2013a. Linkage disequilibrium and haplotype analysis of the ATP7B gene in Alzheimer's disease. Rejuvenation Res. 16, 3–10.
Squitti, R., Polimanti, R., Siotto, M., Bucossi, S., Ventriglia, M., Mariani, S., et al., 2013b. ATP7B variants as modulators of copper dyshomeostasis in Alzheimer's disease. Neuromol. Med. 15, 515–522.
Squitti, R., Ghidoni, R., Siotto, M., Ventriglia, M., Benussi, L., Paterlini, A., Magri, M., Binetti, G., Cassetta, E., Caprara, D., Vernieri, F., Rossini, P.M., Pasqualetti, P., 2014a. Value of serum nonceruloplasmin copper for prediction of mild cognitive impairment conversion to Alzheimer disease. Ann. Neurol. 75, 574–580.
Squitti, R., Simonelli, I., Ventriglia, M., Siotto, M., Pasqualetti, P., Rembach, A., et al., 2014b. Meta-analysis of serum non-ceruloplasmin copper in Alzheimer's disease. J. Alzheimers Dis. 38, 809–822.
Turnlund, J.R., Keyes, W.R., Anderson, H.L., Acord, L.L., 1989. Copper absorption and retention in young men at three levels of dietary copper by use of the stable isotope

65Cu. Am. J. Clin. Nutr. 49, 870–878.
Vafadar-Isfahani, B., Ball, G., Coveney, C., Lemetre, C., Boocock, D., Minthon, L., et al., 2012. Identification of SPARClike 1 protein as part of a biomarker panel for Alzheimer's disease in cerebrospinal fluid. J. Alzheimers Dis. 28, 625–636.
Vanek, V.W., Borum, P., Buchman, A., Fessler, T.A., Howard, L., Shenkin, A., et al., 2015. A call to action to bring safer parenteral micronutrient products to the U.S. market. Nutr. Clin. Pract. 30, 559–569.
Weaver, M.S., Workman, G., Sage, E.H., 2008. The copper binding domain of SPARC mediates cell survival in vitro via interaction with integrin beta1 and activation of integrin-linked kinase. J. Biol. Chem. 283, 22826–22837.
Wang, Z.X., Tan, L., Wang, H.F., Ma, J., Liu, J., Tan, M.S., et al., 2015. Serum Iron, zinc, and copper levels in patients with Alzheimer's disease: a replication study and meta-analyses. J. Alzheimers Dis. 47, 565–581.
White, A.R., Multhaup, G., Maher, F., Bellingham, S., Camakaris, J., Zheng, H., Bush, A.I., Beyreuther, K., Masters, C.L., Cappai, R., 1999a. The Alzheimer's disease amyloid precursor protein modulates copper-induced toxicity and oxidative stress in primary neuronal cultures. J. Neurosci. 19, 9170–9179.
White, A.R., Reyes, R., Mercer, J.F., Camakaris, J., Zheng, H., Bush, A.I., Multhaup, G., Beyreuther, K., Masters, C.L., Cappai, R., 1999b. Copper levels are increased in the cerebral cortex and liver of APP and APLP2 knockout mice. Brain Res. 842, 439–444.
Wong, T., 2012. Parenteral trace elements in children: clinical aspects and dosage recommendations. Curr. Opin. Clin. Nutr. Metab. Care 15, 649–656.