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Copper dysfunction in Alzheimer's disease: From meta-analysis of biochemical studies to new insight into genetics

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ABSTRACT

The involvement of body copper metabolism in the development of Alzheimer's disease (AD) – the most common form of dementia – is a deeply investigated issue in recent years. Copper is essential for life, but in excess it can be toxic. Recently, it has been hypothesized that copper toxicity may be a contributory factor in the etiology of the neurodegenerative disease AD. Studies on copper evaluation in AD vs. healthy controls collected in the latest 30 years and merged in a meta-analysis demonstrate that serum copper is slightly increased in AD. A specific form of copper, the copper non-bound to ceruloplasmin, or 'free' copper, seems to best characterize this increase in copper in AD patients. Clinical studies from us and other groups have demonstrated that free copper is associated with the typical deficits of AD, incipient AD and mild cognitive impairment, and specific cerebrospinal markers. Moreover, very recent data addressing molecular processes underlying copper dysfunction in AD have indicated that genetic variations of K832R and R952K Single Nucleotide Polymorphisms (SNPs) of the Wilson's disease gene ATP7B are associated also with sporadic AD. Specifically, ATP7B encodes for the protein ATPase 7B which controls free copper status in the body, and both R allele in K832R and K allele in R952K ATP7B SNPs are associated with an increased risk of having AD. Even though copper dysfunction cannot be assumed as a determinant of the disease, its causative, rather than associated, role in AD pathology as risk factor can be claimed.

Introduction

Researchers and physicians agree on the increase in frequency in Alzheimer's disease (AD). This is the most common form of dementia, and it is classified in two categories of the basis of the age of onset. Early-onset AD has a Mendelian inheritance with an autosomal dominant trait, in which mutations in Amyloid Precursor Protein (b-APP), Presenilin 1, and Presenilin 2 genes can cause the disease [1]. The late-onset form is a multifactorial sporadic complex disease in which oligo or polygenes as well as epigenetic and non-genetic factors contribute to the onset of the disease [1]. The two forms share the same neuropathology consisting of extracellular amyloid beta (Aβ) plaques and intracellular neurofibrillary tangles. The causes of AD has been closely related to Aβ aggregation in plaques, and the bulk of the evidence gathered in the last 20 years strongly supports a role of metal disarrangement as a potential risk for AD onset, affecting the rate of Aβ oligomers formation [2,3] and toxicity [4–6].

The most salient and basic studies [7,8] supporting a 'metal causative role' in AD demonstrate that b-APP binds and reduces copper from Cu(II) to Cu(I), which modulates copper-induced toxicity and oxidative stress through the production of H_2O_2 [9]; that Aβ and metals are packed together in plaques, disturbing brain physiology; and that the use of chelating agents sequesters metals within Aβ, dissolving the plaques [7].

Copper dysfunction in AD: our contribution for a systemic view

In a series of clinical studies, we demonstrated that patients with AD suffer from an altered copper metabolism, consisting primarily of increases in a specific fraction of serum copper, i.e., copper non-bound to ceruloplasmin (also called 'free' copper). Normally, 85–95% of the copper in serum is tightly bound to ceruloplasmin [10]. The remaining copper, that is free copper, is loosely bound and exchanged among albumin, alpha 2 macroglobulin, and low-molecular-weight compounds such as peptides and amino acids. The two pools differ in the fact that the free copper crosses the Blood–Brain–Barrier (BBB) [11,12], as exemplified in Wilson's disease, the paradigmatic disease of free copper toxicity or accumulation [13,14].

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Table 1
Studies on serum copper levels in AD patients vs. healthy controls.

Study	Alzheimer's disease				Healthy controls				*p* value	SMD	95% CI
	N°	Sex (% women)	Mean age (years)	MMSE	N°	Sex (% women)	Means age (years)	MMSE			
Jeandel et al. (1989)	55	73	81.7 ± 5.7	Lower than 25	24	79	76.4 ± 6.1	–	NS	0.14	[−0.34; 0.62]
Kapaki et al. (1989)	5	20	54	–	28	36	46	–	NS	−0.81	[1.79; 0.16]
Molina et al. (1998)	26	46	73.1 ± 8.2	13.2 ± 5.7	28	43	70.8 ± 7.3		NS	0.16	[−0.37; 0.70]
Gonzàlez et al. (1999)	51	35	70.3 ± 4	–	40	90	74.5 ± 2.3	–	0.048	0.39	[−0.02; 0.81]
Ozcankaya et al. (2002)	27	30	72.3 ± 6.5	16.8 ± 1.3	25	36	64.4 ± 7.2	28.2 ± 2.4	NS	−0.10	[−0.64; 0.45]
Squitti et al. (2002)	79	68	74.5 ± 7.4	17.3 ± 4.9	76	56	70.1 ± 10.8	27.7 ± 2.2	<0.001	1.34	[0.99; 1.69]
Smorgon et al. (2004)	8	–	79 ± 5	–	11	–	78 ± 9	–	<0.001	2.23	[1.02; 3.44]
Bocca et al. (2005)	60	67	74.6 ± 6.39	–	44	25	Older than 45	–	NS	0.25	[−0.14; 0.64]
Sedighi et al. (2006)	50	48	76.4	14.3 ± 4.6	50	50	67.8	25.8 ± 1.5	NS	0.08	[−0.32; 0.47]
Sevym e al. (2007)	98	66	72.1 ± 6.7	–	76	59	70.3 ± 5.7	–	0.001	0.50	[0.20; 0.81]
Argawal et al. (2008)	50	38	59.96 ± 11.57	14.07 ± 7.59	50	34	55.32 ± 10.88	–	0.002	0.70	[0.30; 1.11]
Baum et al. (2009)	44	66	74.3 ± 8.7	–	41	49	79.1 ± 6	–	NS	0.28	[−0.14; 0.71]

Free copper associates with some typical clinical [15–18], neurophysiological deficits [19,20] and cerebrospinal fluid (CSF) markers of the disease [15], findings which recently have received confirmation [21,22]. Free copper is also increased in CSF [23] and brain parenchyma of AD patients [24].

Copper dysfunction in AD: meta-analysis of biochemical studies

To corroborate our earlier data on the involvement of copper dysfunction in AD and put them into context with previously published data from other researchers, we completed a meta-analysis of all the data published between 1983 and 2010 specifically addressing the issue of the comparison of copper concentrations in serum, plasma, and CSF between patients with AD and healthy controls [25] Table 1.

Considering either studies on serum or plasma, data from 966 patients with AD and 831 healthy controls were merged together; the analysis revealed that AD patients had slightly but significantly increased levels of circulating copper. This result, specifically that concerning copper increase in serum, previously published [25], was maintained when we repeated the meta-analysis after excluding 4 of the 5 studies from our laboratory [15,18,20,26], to avoid the possibility that studies carried out in the same laboratory could bias the result ($p = 0.024$), as shown in Fig. 1.

Copper dysfunction in AD: new insight into genetics

Our original hypothesis was that a mild defect in copper incorporation into ceruloplasmin could be at the basis of the slight but significant increase of free copper found in AD, and that when reaching the brain it could interact with Aβ, triggering Aβ toxicity [27]. A chain of events would have revealed the underlying biological processes expected by this hypothesis: (1) increased levels of apoceruloplasmin in serum, derived by the failure of copper bonding into ceruloplasmin during its biosynthesis in the liver [28]; (2) signs of liver dysfunction due to free copper disturbance of hepatocytes [26]; (3) increases of free copper tightly related to the clinical picture, which is the same as saying that it could have effects on brain functions, as for example cognitive state or electrophysiological changing [16,17,20], implying increased levels of free copper in the brain; (4) evidence of the move of free copper from the serum to the CSF, crossing the BBB [23], and of free copper interaction with CSF markers of AD [15]. However, all the evidence that we collected lies on a correlative level, precluding any conclusion about the causative rather than accompanying role of copper dysfunction in AD pathology.

To answer this question, we improved our original hypothesis, shifting our interest to the ATP7B gene, which codes for ATPase 7B. ATPase 7B is a copper chaperone that regulates body copper levels in normal conditions by controlling either the copper loading into ceruloplasmin or the rate of copper excretion through the bile. Its failure generates free copper increases in serum, which characterize Wilson's disease [13]. From the above, we have found the direct role of ATP7B in the risk of developing AD. In other words, we have developed a new hypothesis considering the possibility that the ATP7B gene is a potential harbor of multiple rare variants associated with an increased risk of AD, suggesting that they may account for the 'missing' heritability of AD [5]. To verify this idea, we developed a hypothesis-driven gene-candidate project, investigating ATP7B gene variants in AD.

In this new line of research, we explored the hypothesis that ATP7B sequence changes in exons 2, 5, 8, 10, 14 and 16 – where most of the Mediterranean mutations causing Wilson's disease lie – have a higher frequency in a group of patients affected by mild or