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Review Article

Copper phenotype in Alzheimer's disease: dissecting the pathway

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Abstract: Alzheimer's disease (AD) is the most common form of dementia. Several hypotheses have been put forward to explain the basis of disease onset and progression. Unfortunately, none of these seems to clarify the complexity of the pathogenesis. In fact, diverse and independent pathogenetic pathways can be disrupted at the same time, and each contributes to disease etiology. In recent years, researchers have begun studying biometals more deeply. A number of studies have shown that metal dyshomeostasis may enhance AD onset and progression. Specifically, different authors have hypothesized that alterations in metal metabolism are associated with an increased in metal-related oxidative stress and beta-amyloid oligomer formation and precipitation. Studies conducted *in vivo*, *in vitro*, *in living patients* and *in silico* studies have demonstrated that local and systemic defects in copper metabolism are characteristic signs of AD. This strongly supports the hypothesis that copper pathways may be disrupted by the disease. More specifically, a copper phenotype can be proposed for AD, based on defects found in genes involved in copper metabolism. In this review, we describe copper dyshomeostasis in AD patients and attempt to explain the basis of the AD copper phenotype. Dissecting copper pathways, we highlight mechanisms which may be at the basis of the disease. We also discuss various associated translation outcomes.

Keywords: Alzheimer's disease, copper, metals, neurodegeneration, etiology, pathogenesis, systems biology, diagnosis, drugs, Wilson's disease

Introduction

Alzheimer's disease (AD) is the most frequent cause of dementia [1]. The late onset form of the disease is sporadic and has a complex disease etiology, with disease family history and age being the most widely accepted risk factors [2]. The cause of the disease appears to be closely related to the aggregation within the brain of the beta-amyloid (Aβ) peptide and tau proteins, which form Aβ plaques and neurofibrillary tangles, respectively [1]. Moreover, the epsilon4 allele of the apolipoprotein E (*APOE*) gene has been proven to increase AD risk and decrease the average age of the disease onset [3]. The 'amyloid cascade', which has been touted as the most popular Alzheimer hypothesis, has now taken many forms as new details about the disease emerge [4]. In fact, diverse pathogenetic pathways have been postulated to contribute to AD onset and progression. For example, in addition to Aβ oligomers and toxic tau aggregates, oxidative stress, aberrant inflammation, and impaired energy metabolism have been identified among the pathogenic pathways involved in the disease cascade. There is abundant evidence that oxidative stress, mainly via Fenton and Haber Weiss chemistry, inflicts damage upon the AD brain. Various *in vitro* and animal studies have uncovered the central role played by copper in these processes [5, 6]. Specifically, it has been proposed that the hypermetallation of the Aβ peptide could be at the basis of redox cycles of oxidative stress and H_2O_2 production, Aβ oligomer formation and precipitation. A systemic derangement of metal homeostasis may feed into this noxious circle within the brain generating pleiotropic effects on the AD cascade. The possibility that abnormalities in body copper balance are a risk factor for AD is further sustained by solid clinical, epidemiological, and

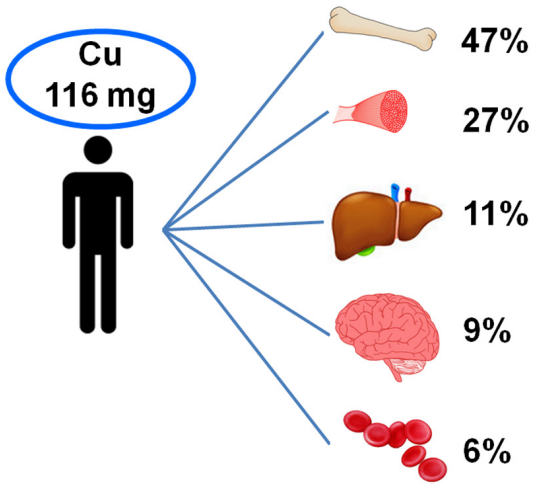

Figure 1. Copper distribution in human tissues.

meta-analysis data [7]. The bulk of the evidence achieved on this matter has now reached such a critical mass that a copper-related phenotype in AD may be hypothesized. Important translational outcomes may be developed from this hypothesis both in terms of diagnostic and prognostic tools, and in terms of preventive and therapeutic approaches. Recent knowledges gained about copper dyshomeostasis in AD is reported herein, along with our insights into copper-related AD pathogenesis, and our future prospective about anti-copper treatments for AD patients.

Copper metabolism

Copper is essential for life. Indeed, it is a cofactor of a number of proteins and enzymes, involved in many crucial molecular pathways, including energy generation, oxygen transportation, hematopoiesis, cellular metabolism, and signal transduction [8]. Copper is absorbed mainly from food [9]. An average of 0.6 - 1.6 mg of copper per day is ingested and no more than 110 mg are present in a 70 kg-healthy man [10]. Tipically, copper is distributed in human tissues thusly: 47% in bones, 27% in skeletal muscles, 11% in the liver, 9% in the brain, and 6% in the blood [11] (**Figure 1**). The mechanisms of copper absorption, distribution, and storage are finely regulated in mammals and also in low eukaryotes. This indicates a remarkable evolutionary conservation of these regulating mechanisms, which reflects the high chemical reactivity of this element [8]. This is in line with the double facet of copper: when copper is kept under control, bound to special proteins as a co-factor, it yields key-properties, which are essential for life processes; when it deranges and spirals out of control, it is exchanged among small compounds (it is loosely bound to them), and its redox activity makes it dangerous for cell viability [12].

The maintenance of copper homeostasis mainly requires the actions of two kinds of proteins: membrane copper transporters and copper chaperones. Three transmembrane proteins have been classified as membrane copper transporters: ATP7A, ATP7B, and CTR1. ATP7A protein is widely distributed throught human tissues; it is localized to the trans-Golgi network, where it supplies copper to copper-dependent enzymes in the secretory pathway [13]. ATP7B protein is mainly expressed in the liver; it exports copper out of the hepatic cell into the bile, excreting the copper exceeding cell requirements [14]. Moreover, ATP7B supplies copper to nascent ceruloplasmin in the trans-Golgi network, which - in its holo-form - is released from the hepatocyte into the blood stream. CTR1 protein is a homotrimer transporting dietary copper within the cell [15]. Regarding copper chaperones, at least three delivery systems have been described: the Cox17 system in mitochondria [16], the copper chaperone for the Superoxide Dismutase (CCS) system [17], and the ATOX1 system in trans-Golgi network [18].

Dyshomeostasis in copper metabolism is associated with several life-threatening conditions (**Table 1**). The most studied copper-related disorders are Wilson's (WD) and Menkes (MD) diseases. Both WD and MD are monogenetic diseases caused by point mutation in the genes encoding for ATP7B and ATP7A proteins, respectively [13, 19]. WD (MIM# 277900) is an autosomal recessive disease, in which ATP7B dysfunction causes copper accumulation in the liver, brain, and other tissues, leading to the manifestation of hepatic, neurological, psychiatric, and ophthalmological symptoms [20]. WD manifestations are caused by a reduction of cuproenzyme biosynthesis, and ceruloplasmin and by an increase of copper-related oxidative damage [21]. MD (MIM# 309400) is a multisystemic disorder with an X-linked recessive inheritance, and its main features are progressive neurodegeneration, marked connective tissue abnormalities, and – tipically - sparse and

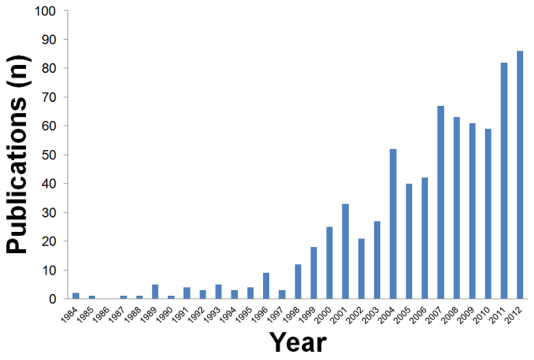

Figure 2. Temporal trend of scientific publications in PubMed about copper and Alzheimer's disease.

steely hair [22]. The clinical signs of MD are principally attributed to the malfunction and reduced catalytic activity of different cuproenzymes. Animal models with a defect in the *ATP7A* gene have already confirmed the mechanisms at the basis of MD phenotype expression [23]. In addition to WD and MD, other pathologic conditions seem to be related to copper dysfunction, affecting heart development, brain and liver function, lipid metabolism, inflammation, and resistance to chemotherapeutic drugs [24]. Specifically, relevant findings have been collected regarding the role that copper homeostasis plays in brain function. Molecular studies have indicated that the human brain requires copper for its normal development and function (e.g. as cofactor of many enzymes), and its distribution is not uniform in the central nervous system (CNS), suggesting the key role of copper homeostasis in healthy brain [25]. In line with this evidence, both WD and MD showed neurologic symptoms [26]. Furthermore, research has indicated that increased copper levels can be observed in patients with different forms of dementia and, in particular, with AD-like dementia [27-29].

Copper dyshomeostasis in AD

A large body of literature is currently available regarding the role that copper dyshomeostasis plays in AD. To date, over 700 articles are present in PubMed under the query "Copper AND Alzheimer's disease" (**Figure 2**). In these articles, different approaches (*in vivo*, *in vitro*, *in living patients,* and *in silico*) have been used to better define disrupted copper pathways linked to AD onset or progression. *In vitro* investigations have revealed a strict interaction between cellular copper levels and the toxic properties of amyloid precursor proteins (APPs) and Aβ [30, 31]. Furthermore, copper toxicity is not limited to its interactions with APPs. Rather, it also induces neurodegeneration via oxidative stress [32]. Experiments with animal models have confirmed that increased copper levels are significantly related to cognitive impairments and to the exacerbation of AD pathology [33, 34]. Clinical studies on living patients have been performed in order to evaluate whether AD effects the mean value of copper measured in diverse biological districts (i.e. serum, plasma, and cerebral spinal fluid [CSF]) [35-37]. Recent meta-analyses have demonstrated that copper levels in CSF are not significantly different between AD patients and healthy elderly controls [38, 39]. Conversely, plasma/serum copper levels are significantly higher in AD patients than in controls [38, 39].

Recently, a meta-analysis evaluating studies measuring copper concentrations within the brain [40] demonstrated decreased levels of total copper in the more affected areas of the AD brain vs. the healthy control brain, even though reports were not univocal [41]. This result has been replicated by some authors [42] in a recent study, which demonstrated that, even though a lower concentration of copper in the Brodman (BA46) and in the temporal lobe (BA22) areas can be detected, the quote of labile copper - which resembles non-ceruloplasmin bound copper (Non-Cp) copper in serum - was increased of about 25% in these areas.

Diverse studies conducted on copper metabolism have confirmed that the Non-Cp copper, rather than absolute serum copper levels, is a pivotal concept in interpreting copper findings even in living AD patients [43]. Specifically, increased levels of Non-Cp copper, which is also known as 'free' copper, have been observed in many studies [9, 44-47], although they have not been observed in all studies conducted on the subject [48]. The study of Rembach and colleagues [48] has caused some concerns; its details are available at http://www.j-alz.com/letterseditor/index.html#March2013. Generalizing, the increase in Non-Cp copper seems to account for the increase in total copper found in the serum of AD patients [39].

The strict relationship between increased Non-Cp copper in serum and increased Non-Cp copper in the more severely affected areas of the AD brain strongly sustains a systemic disruption of copper metabolism with dramatic effects upon local, sensible brain areas.This could account for the cognitive worsening that has been extensively documented in living AD patients [49].

About 85-95% of the copper found in healthy individuals is bound to ceruloplasmin, whereas Non-Cp copper is loosely bound and exchanged among albumin, transcuprein, alpha 2 macroglobulin, and low-molecular-weight compounds. These are capable of reaching tissues, including the brain, crossing the Blood–Brain–Barrier (BBB) [7]. This copper is potentially toxic, and it is available for oxidative stress reactions via Haber Weiss and Fenton's chemistry also interacting with APPs or Aβ itself. Additional evidence can be gleaned from studies that evaluated CSF, which also confirms the increased of Non-Cp copper in AD [35, 50]. Comparing the accumulated biochemical evidence of the last 5 years (regarding AD and WD) the paradigmatic disease of Non-Cp copper toxicosis or accumulation suggests that the diseases are quite similar [6, 51, 52]. For example, molecular studies conducted on WD patients indicate that neurologic symptoms are caused by Non-Cp copper damage. This proving that the alteration enhances neurodegenerative processes [53, 54]. Several authors have described anatomical, clinical, and molecular homologies between AD and WD patients. Brain investigations of WD patients have also revealed similar neurodegenerative processes at work in WD and AD patients [55, 56]. Moreover, neurologic and neuropsychological evaluations of WD patients have depicted a number of features (i.e. dysarthric, dystonic, tremor, pseudosclerotic, parkinsonian, chorea, athetosis, myoclonus, seizures, ataxia, pyramidal signs, drooling, eye movement abnormalities, impulsivity, promiscuity, impaired social judgment, apathy, decreased attention, executive dysfunction with poor planning and decision making, emotional lability, slowness of thinking, memory loss, and cortical signs of aphasia, apraxia, or agnosia) that may resemble Alzheimer's-like dementia [57]. Additional molecular homologies, besides altered levels of Non-Cp copper, have been documented by genetic studies. *ATP7B* mutations cause WD, but some loci in the *ATP7B* gene appear to also be associated with an increased risk of having AD [58]. In addition to these similarities, important differences distinguish AD from WD. For example, the severity of the copper dysfunction and the related hepatic presentation, which typify WD, may vary. However, several neurodegenerative diseases have shown diverse phenotypes with distinct clinical manifestations, and these are associated with the age of onset [59-61]. MD and occipital horn syndrome, for instance, share the same genetics (i.d. mutations in the ATP7A even though in diverse domains of the protein), but they are two distinct clinical entities [62]. Understanding the traits shared by WD and AD, has been the objective of our latest studies, which have focused on dissecting the pathway of the AD copper phenotype. For example, considering the age-of-onset of WD, two clinical phenotypes can be observed: a juvenile WD with hepatic symptoms (mainly), and an adult WD with neurologic (mainly) traits [57]. This difference in age-of-onset-related WD phenotypes is probably explained by the complexity of WD genetics. Indeed, *ATP7B* mutations cause WD, but other genetic and non-genetic factors may interact with the disease and its phenotype expression. This phenomenon has been also reported regarding other monogenetic disorders [63].

Although it is incorrect to describe AD as the "senile" form of WD, the previously described findings regarding copper dyshomeostasis in AD patients suggest that shared pathogenetic mechanisms can be activated in both of these diseases. Specifically, a copper phenotype may be hidden in the complexity of the AD forms, and copper dysfunction can account for a percentage of the AD risk. Defining this risk can assist in discriminating the AD phenotype and in orientating clinical interventions.

Pathogenetic pathway of AD copper phenotype

Although relevant information has been collected about AD, its etiology is not yet completely understood. To date, two forms of AD are recognized: early-onset and late-onset AD. Early-onset AD is an autosomal dominant trait caused by genetic mutations in four genes, which encode proteins related to deposition of Aβ (i.e.

Table 1. Metabolic and neurodegenerative disorders associated with copper dyshomeostasis. The causative/associated copper-related genes are also reported

Gene	Metal-metabolic disorder	Neurodegenerative disorders	References
SOD1	-	Amyloid Lateral Sclerosis	[80]
COX17	Cox deficiency	-	[81]
ATP7A	Menke's disease	Occipital horn syndrome	[13]
ATP7B	Wilson's disease	Alzheimer's disease	[51]

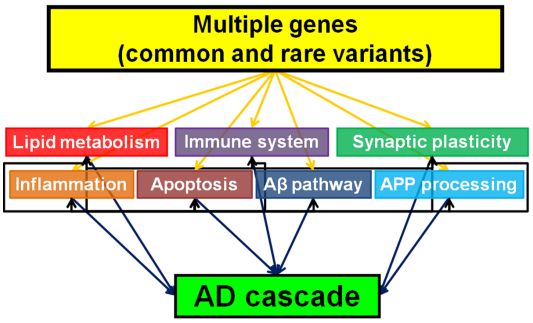

Figure 3. Genetic complexity of Alzheimer's disease pathogenesis.

APP, *PSEN1*, *PSEN2*, and *SOLR1*). It affects 5% of AD patients [1]. Late-onset AD showed a complex pathogenesis, in which multiple independent factors contribute to disease onset [1]. Among the AD risk factors, genetics seems to account about 70% of overall AD risk [1]. However, gene-candidate studies and genome-wide association studies (GWASs) uncovered only a small fraction of genetic predisposition of late-onset AD [64]. Specifically, to date the most relevant gene associated with AD is *APOE*: individuals with one ε4 allele have a three-fold increase in AD risk, and those with two ε4 alleles have a 15-fold increase in AD risk, when compared with individuals with the *APOE* ε3ε3 genotype [64]. Although the *APOE* genotype is associated with an high AD risk, not all ε4 carriers get sick. Therefore, *APOE* is not sufficient to explain the genetics of AD. GWASs have revealed that other genetic polymorphisms are also significantly associated with AD, but the relative risk of these genetic variants is very small and does not explain the probability of having AD [65]. However, these genetic variants may help us to understand the pathways involved in AD pathogenesis. Using the Alzgene database (available at http://www.alzgene.org/), we can consider: *APOE* related to lipid transport and metabolism, Aβ pathway, synaptic plasticity, and neuroinflammation; *CLU* related to Aβ pathway, lipid metabolism, immune system, inflammation, and apoptosis; *CR1* related to immune system, and Aβ pathway; *PICALM* related to synaptic cell functioning, Aβ toxic effects, and APP processing; *BIN1* related to synaptic cell functioning, and caspase-independent apoptosis; *EPHA1* related to immune system; ABCA7 related to cholesterol metabolism, immune system, and APP processing; *MS4A4A/MS4A6E* related to immune system, and cell surface signalling; *CD33* related to immune system, and synaptic cell functioning; *CD2AP* related to synaptic cell functioning, and actin cytoskeleton. Nevertheless, these data are not currently sufficient to develop new strategies to manage or to treat the disease. Different hypotheses may explain the forgetfulness of this picture in terms the causes of late-onset AD. Recent gains in knowledge have led researchers to propose a new scenario regarding the genetics of complex diseases: the genetic predisposition of complex diseases can be explained by multiple rare variants rather than common disease variants [66]. This hypothesis accounts for the limitations experienced by GWASs investigations in AD. Indeed, the GWAS approach is not suitable to analyze rare variants [67]. Furthermore, the genetic background of AD is likely not explained by a single gene but by different variants in diverse genes that are involved in the same pathogenetic mechanism that contributes to AD onset and sustains its progression [64] (**Figure 3**). Finally, multiple pathogenetic pathways may activate the disease cascade with independent mechanisms. For example, *APOE*, lipid metabolism, inflammation, and copper dysfunction can be investigated in the AD cohort or in the single patient, allowing stratification and definition of the phenotype to become more prominent. Screening for these disease risks could allow us to overcome the difficulties in clinical and molecular studies on AD and the heterogeneity observed in their outcomes. In other words, the

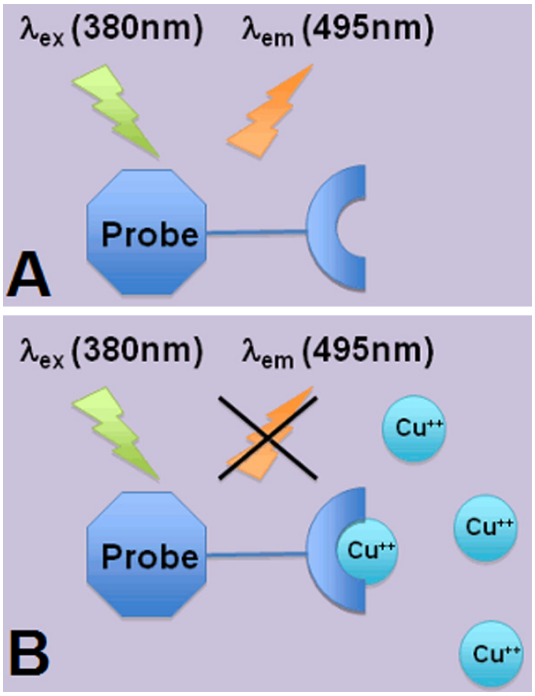

Figure 4. Operating mechanism of the direct fluorescent assay to measure serum Non-Cp copper. A. Free copper does not bind the probe, which emit fluorescence after 380nm-irradiation. B. Free copper binds the probe, which does not emits the 495nm-fluorescence after 380nm irradiation.

analysis of late-onset AD, which aids in distinguishing AD patients on the basis of their potential pathogenetic mechanisms, may improve our understanding of the disease and our capacity to prevent, manage, and treat at least some traits that can delay the natural history of the disease. Considering this point of view, *APOE* may underline a pathogenetic mechanism that could explain a percentage of AD patients, and other genetic and non-genetic factors may interact with *APOE* in the activation of this pathogenetic pathway.

Evidence collected about copper dysfunction appears, for the most part, to support the hypothesis that abnormalities in systemic copper metabolism are one of the pathogenetic pathways involved in the AD cascade [5, 17]. Specifically, our hypothesis is that systemic deficits in copper trafficking and supply are related to an increased AD risk. Even though copper dysfunction is less severe than that of WD, Non-Cp copper, entering the Aβ peptide pathogenic pathways, or others, as for example oxidative stress, can eventually accelerate AD progression. Our data suggest that in 60% of Italian AD patients the copper dysfunction risk is evident [68]. Although a number of studies have demonstrated the association of copper with the clinical and molecular features of AD, few research groups have investigated the causes. More precisely, it has been recently postulated that the ingestion of inorganic copper in drinking-water, which enters the water through copper pipe-lines may contribute to an increase in the probability of having AD [6]. We believed that in specific individuals with a predisposition to a copper dysfunction, these environmental pollutants may have some effects. Our hypothesis is in accordance with the current knowledge available about the contribution of the environment to the worsening of the condition of individuals affected by copper-related disorders, such as WD [69]. Concerning the interplay of copper genetics with AD, our studies focused on *ATP7B*, demonstrating that *ATP7B* Loss-of-Function variants in transmembrane domains are strongly associated with disease risk [58, 70, 71]. Furthermore, we found that the complex structure and presence of multiple rare variants in the ATP7B gene may have concealed this gene from those with significant outcomes in genome-wide association studies [51]. Finally, we observed that copper dysfunction in patients with AD is significantly modulated by ATP7B variants [68]. Although our findings strongly suggested the presence of a AD copper phenotype, in which genetics plays a key role, further studies are needed to confirm and validate this insight. To do this, we are currently performing an analysis of a large study population, in which biochemical, genetic, and epidemiological approaches are being used. Biochemical analyses are performed to characterize copper dysfunction in AD patients and to identify individuals, in which a copper phenotype is present. Specifically, we have used innovative technology to measure the Non-Cp copper. Until today, studies have valuated Non-Cp copper levels as an indirect calculation from ceruloplasmin and copper using the Walshe's formula [72]. We have developed a direct fluorescent assay to measure serum Non-Cp copper [73] (**Figure 4**), confirming our previous data with a large correlation. We are planning to perform a genetic analysis using next-generation sequencing technologies, and we are considering not only the *ATP7B* gene but also other genes related to copper metabolism

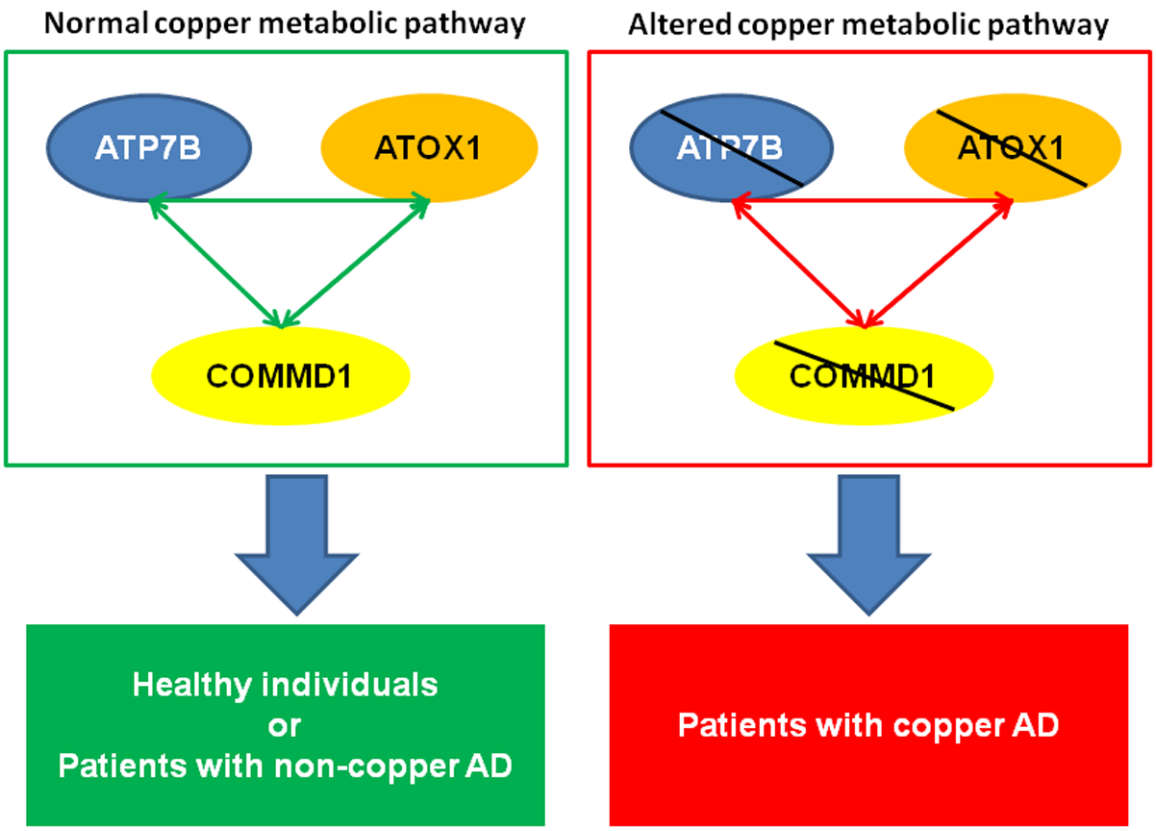

Figure 5. Schematic representation of the pathogenetic mechanism of AD copper phenotype. Individuals with normal copper metabolism does not develop AD copper phenotype (left). Individuals with significant alteration in copper metabolism develop AD copper phenotype (right).

(i.e. *ATOX1*, and *COMMD1*). In **Figure 5**, the schematic representation of the expected pathogenetic mechanism is reported. Moreover, genetic data will be correlated with epidemiological investigations carried out to evaluate the contribution of environment to copper dyshomeostasis in AD patients and elderly healthy controls. This multi-faceted study may uncover the complex and interactive mechanism at the basis of copper dysfunction in AD.

By confirming the copper hypothesis, strategies to prevent, manage, and treat patients with copper AD could become immediately available. Indeed, knowledge about WD has widely demonstrated that copper dyshomeostasis is a condition which can be modified by life-style changes or treated by drugs [74].

Translational prospective of copper-related knowledges in Alzheimer's disease

AD is one of the main problems for the national health systems, because of its social and economic costs. Huge gains in the knowledge of AD have been made in the last decade. However, important enhancements in the patients' management are still needed, especially, in diagnosis and therapy. Currently, only symptomatic treatments have been approved for the cure of cognitive symptoms [75]. The increasing tendency of AD prevalence makes it absolutely imperative to develop useful tools for early and reliable diagnosis and effectiveness therapies.

Our copper hypothesis in AD pathogenesis may rapidly open new scenarios that could aid in the developing of new means to manage the AD risk, in terms of prevention and treatment.

An understanding of the genetic predisposition to the AD copper phenotype may furnish the basis for a genetic test for the early detection of individuals with high AD risk related to dysfunction copper. Specifically, this test could be based on the investigation on multiple genes

related to copper trafficking. Furthermore, uncovering the role of environment copper may help to delineate environmental prevention campaigns, both for populations exposed to high amounts of inorganic copper and for individuals with genetic defects in copper metabolic system.

In addition to the improvement of the prevention and diagnosis of AD, our copper hypothesis may greatly impact the treatment of affected individuals. Indeed, the work with WD patients has demonstrated that copper in AD is a modifiable risk factor that could be manipulated in order to return to normal copper metabolism [74]. WD patients are treated with metal chelating agents in order to release copper accumulations and to increase the urinary excretion of copper [76]. Generally, D-penicillamine is the first-line drug used in WD patients, to which ammonium tetrathiomolybdate and zinc therapy may be added or may be used as substitutes. In most of the cases, these therapies increased the survival rates of the affected individuals and have coincided with a diminution or a disappearance of copper accumulation symptoms [54]. Regarding AD, different clinical trials with metal modulators are currently in progress, in order to assess the effectiveness of these therapies [77]. These studies have pointed out that the progression of AD was slowed in some cases [78]. In accordance with these outcomes, AD animal models have shown that chelating agents can be associated with a complete reversion of cognitive symptoms [34, 79]. The effectiveness of copper anticopper agents in delay AD progression AD appears underestimated. In fact, a copper dysfunction criterion should be fulfilled by the patient before entering a clinical trial with anticopper treatment, that would allow a more accurate evaluation of these treatments.

Concluding remarks

This review tried to collect and organize the most recent knowledge regarding copper dysfunction in AD, suggesting the existence of a specific copper-related AD phenotype with high Non-Cp copper levels. Although a number of findings corroborate the pivotal role of copper disarrangement in AD pathogenesis, the mechanism of this altered pathogenetic pathway has not yet been clarified. Our hypothesis is that some people prone to a copper dysfunction on a genetic basis are at risk to develop AD. Those AD patients with a copper dysfunction, i.e. with a Non-Cp copper of a higher than normal value, can be said to have an AD copper phenotype. More precisely, multiple rare variants located in genes encoding for protein associated with copper trafficking may enlighten its genetics, explaining a portion of the hereditability of AD missed by GWASs. This genetic background, coupled with environmental exposure to inorganic copper, may worsen the AD risk. According to this scenario, individuals with genetic defects in proteins handling copper when exposed to higher quantities of inorganic copper or other metals, may be more likely to become sick. In order to confirm this hypothesis, a large multi-approach investigation is needed (in progress). The validation of our suggestion can open new routes to prevent, manage, and treat AD patients, with improvements in quality of life observed in a percentage of patients.

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References

[1] Ballard C, Gauthier S, Corbett A, Brayne C, Aarsland D and Jones E. Alzheimer's disease. Lancet 2011; 377: 1019-1031.

[2] Querfurth HW and LaFerla FM. Alzheimer's disease. N Engl J Med 2010; 362: 329-344.

[3] Verghese PB, Castellano JM, Garai K, Wang Y, Jiang H, Shah A, Bu G, Frieden C and Holtzman DM. ApoE influences amyloid-beta (Abeta) clearance despite minimal apoE/Abeta association in physiological conditions. Proc Natl Acad Sci U S A 2013; 110: E1807-16.

[4] Reitz C. Alzheimer's disease and the amyloid cascade hypothesis: a critical review. Int J Alzheimers Dis 2012; 2012: 369808.
[5] Squitti R. Metals in Alzheimer's disease: a systemic perspective. Front Biosci 2012; 17: 451-472.
[6] Brewer GJ. Copper toxicity in Alzheimer's disease: cognitive loss from ingestion of inorganic copper. J Trace Elem Med Biol 2012; 26: 89-92.
[7] Squitti R. Copper dysfunction in Alzheimer's disease: from meta-analysis of biochemical studies to new insight into genetics. J Trace Elem Med Biol 2012; 26: 93-96.
[8] Gaggelli E, Kozlowski H, Valensin D and Valensin G. Copper homeostasis and neurodegenerative disorders (Alzheimer's, prion, and Parkinson's diseases and amyotrophic lateral sclerosis). Chem Rev 2006; 106: 1995-2044.
[9] Brewer GJ. Toxicity of copper in drinking water. J Toxicol Environ Health B Crit Rev 2010; 13: 449-452; author reply 453-449.
[10] Linder MC and Hazegh-Azam M. Copper biochemistry and molecular biology. Am J Clin Nutr 1996; 63: 797S-811S.
[11] Linder MC. Biochemistry of Copper. New York: Plenum Press, 1991.
[12] Squitti R, Siotto M, Salustri C and Polimanti R. Metal dysfunction in Alzheimer's disease. New York: Springer, 2013.
[13] Kaler SG. ATP7A-related copper transport diseases-emerging concepts and future trends. Nat Rev Neurol 2011; 7: 15-29.
[14] Lutsenko S, Barnes NL, Bartee MY and Dmitriev OY. Function and regulation of human copper-transporting ATPases. Physiol Rev 2007; 87: 1011-1046.
[15] Wee NK, Weinstein DC, Fraser ST and Assinder SJ. The mammalian copper transporters CTR1 and CTR2 and their roles in development and disease. Int J Biochem Cell Biol 2013; 45: 960-963.
[16] Amaravadi R, Glerum DM and Tzagoloff A. Isolation of a cDNA encoding the human homolog of COX17, a yeast gene essential for mitochondrial copper recruitment. Hum Genet 1997; 99: 329-333.
[17] Culotta VC, Klomp LW, Strain J, Casareno RL, Krems B and Gitlin JD. The copper chaperone for superoxide dismutase. J Biol Chem 1997; 272: 23469-23472.
[18] Klomp LW, Lin SJ, Yuan DS, Klausner RD, Culotta VC and Gitlin JD. Identification and functional expression of HAH1, a novel human gene involved in copper homeostasis. J Biol Chem 1997; 272: 9221-9226.
[19] Kumar SS, Kurian G, Eapen CE and Roberts EA. Genetics of Wilson's disease: a clinical perspective. Indian J Gastroenterol 2012; 31: 285-293.
[20] Behari M and Pardasani V. Genetics of Wilsons disease. Parkinsonism Relat Disord 2010; 16: 639-644.
[21] Mufti AR, Burstein E, Csomos RA, Graf PC, Wilkinson JC, Dick RD, Challa M, Son JK, Bratton SB, Su GL, Brewer GJ, Jakob U and Duckett CS. XIAP Is a copper binding protein deregulated in Wilson's disease and other copper toxicosis disorders. Mol Cell 2006; 21: 775-785.
[22] Tumer Z. An overview and update of ATP7A mutations leading to menkes disease and occipital horn syndrome. Hum Mutat 2013; 34: 417-429.
[23] Qin Z, Itoh S, Jeney V, Ushio-Fukai M and Fukai T. Essential role for the Menkes ATPase in activation of extracellular superoxide dismutase: implication for vascular oxidative stress. FASEB J 2006; 20: 334-336.
[24] Lutsenko S. Human copper homeostasis: a network of interconnected pathways. Curr Opin Chem Biol 2010; 14: 211-217.
[25] Lutsenko S, Bhattacharjee A and Hubbard AL. Copper handling machinery of the brain. Metallomics 2010; 2: 596-608.
[26] Kodama H, Fujisawa C and Bhadhprasit W. Pathology, clinical features and treatments of congenital copper metabolic disorders–focus on neurologic aspects. Brain Dev 2011; 33: 243-251.
[27] Eskici G and Axelsen PH. Copper and oxidative stress in the pathogenesis of Alzheimer's disease. Biochemistry 2012; 51: 6289-6311.
[28] Brewer GJ. Risks of copper and iron toxicity during aging in humans. Chem Res Toxicol 2010; 23: 319-326.
[29] Squitti R, Pasqualetti P, Polimanti R, Salustri C, Moffa F, Cassetta E, Lupoi D, Ventriglia M, Cortesi M, Siotto M, Vernieri F and Rossini PM. Metal-score as a potential non-invasive diagnostic test for Alzheimer's disease. Curr Alzheimer Res 2013; 10: 191-198.
[30] White AR, Multhaup G, Maher F, Bellingham S, Camakaris J, Zheng H, Bush AI, Beyreuther K, Masters CL and Cappai R. The Alzheimer's disease amyloid precursor protein modulates copper-induced toxicity and oxidative stress in primary neuronal cultures. J Neurosci 1999; 19: 9170-9179.
[31] Wang H, Wang M, Wang B, Li M, Chen H, Yu X, Zhao Y, Feng W and Chai Z. The distribution profile and oxidation states of biometals in APP transgenic mouse brain: dyshomeostasis with age and as a function of the development of Alzheimer's disease. Metallomics 2012; 4: 289-296.
[32] Roberts BR, Ryan TM, Bush AI, Masters CL and Duce JA. The role of metallobiology and amyloid-beta peptides in Alzheimer's disease. J Neurochem 2012; 120 Suppl 1: 149-166.

[33] Mao X, Ye J, Zhou S, Pi R, Dou J, Zang L, Chen X, Chao X, Li W, Liu M and Liu P. The effects of chronic copper exposure on the amyloid protein metabolisim associated genes' expression in chronic cerebral hypoperfused rats. Neurosci Lett 2012; 518: 14-18.

[34] Ceccom J, Cosledan F, Halley H, Frances B, Lassalle JM and Meunier B. Copper chelator induced efficient episodic memory recovery in a non-transgenic Alzheimer's mouse model. PLoS One 2012; 7: e43105.

[35] Capo CR, Arciello M, Squitti R, Cassetta E, Rossini PM, Calabrese L and Rossi L. Features of ceruloplasmin in the cerebrospinal fluid of Alzheimer's disease patients. Biometals 2008; 21: 367-372.

[36] Squitti R, Ventriglia M, Barbati G, Cassetta E, Ferreri F, Dal Forno G, Ramires S, Zappasodi F and Rossini PM. 'Free' copper in serum of Alzheimer's disease patients correlates with markers of liver function. J Neural Transm 2007; 114: 1589-1594.

[37] Squitti R, Barbati G, Rossi L, Ventriglia M, Dal Forno G, Cesaretti S, Moffa F, Caridi I, Cassetta E, Pasqualetti P, Calabrese L, Lupoi D and Rossini PM. Excess of nonceruloplasmin serum copper in AD correlates with MMSE, CSF [beta]-amyloid, and h-tau. Neurology 2006; 67: 76-82.

[38] Bucossi S, Ventriglia M, Panetta V, Salustri C, Pasqualetti P, Mariani S, Siotto M, Rossini PM and Squitti R. Copper in Alzheimer's disease: a meta-analysis of serum,plasma, and cerebrospinal fluid studies. J Alzheimers Dis 2011; 24: 175-185.

[39] Ventriglia M, Bucossi S, Panetta V and Squitti R. Copper in Alzheimer's disease: a meta-analysis of serum, plasma, and cerebrospinal fluid studies. J Alzheimers Dis 2012; 30: 981-984.

[40] Schrag M, Mueller C, Oyoyo U, Smith MA and Kirsch WM. 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 2011; 94: 296-306.

[41] Andrasi E, Farkas E, Scheibler H, Reffy A and Bezur L. Al, Zn, Cu, Mn and Fe levels in brain in Alzheimer's disease. Arch Gerontol Geriatr 1995; 21: 89-97.

[42] James SA, Volitakis I, Adlard PA, Duce JA, Masters CL, Cherny RA and Bush AI. Elevated labile Cu is associated with oxidative pathology in Alzheimer disease. Free Radic Biol Med 2012; 52: 298-302.

[43] Squitti R, Pasqualetti P, Dal Forno G, Moffa F, Cassetta E, Lupoi D, Vernieri F, Rossi L, Baldassini M and Rossini PM. Excess of serum copper not related to ceruloplasmin in Alzheimer disease. Neurology 2005; 64: 1040-1046.

[44] Squitti R, Quattrocchi CC, Salustri C and Rossini PM. Ceruloplasmin fragmentation is implicated in 'free' copper deregulation of Alzheimer's disease. Prion 2008; 2: 23-27.

[45] Squitti R, Ghidoni R, Scrascia F, Benussi L, Panetta V, Pasqualetti P, Moffa F, Bernardini S, Ventriglia M, Binetti G and Rossini PM. Free copper distinguishes mild cognitive impairment subjects from healthy elderly individuals. J Alzheimers Dis 2011; 23: 239-248.

[46] Arnal N, Cristalli DO, de Alaniz MJ and Marra CA. Clinical utility of copper, ceruloplasmin, and metallothionein plasma determinations in human neurodegenerative patients and their first-degree relatives. Brain Res 2010; 1319: 118-130.

[47] Lopez N, Tormo C, De Blas I, Llinares I and Alom J. Oxidative stress in Alzheimer's disease and mild cognitive impairment with high sensitivity and specificity. J Alzheimers Dis 2013; 33: 823-829.

[48] Rembach A, Doecke JD, Roberts BR, Watt AD, Faux NG, Volitakis I, Pertile KK, Rumble RL, Trounson BO, Fowler CJ, Wilson W, Ellis KA, Martins RN, Rowe CC, Villemagne VL, Ames D, Masters CL, AIBL research group, Bush AI. Longitudinal analysis of serum copper and ceruloplasmin in Alzheimer's disease. J Alzheimers Dis 2013; 34: 171-182.

[49] Howells C, Saar K, Eaton E, Ray S, Palumaa P, Shabala L, Adlard PA, Bennett W, West AK, Guillemin GJ and Chung RS. Redox-active Cu(II)-Abeta causes substantial changes in axonal integrity in cultured cortical neurons in an oxidative-stress dependent manner. Exp Neurol 2012; 237: 499-506.

[50] Boll MC, Alcaraz-Zubeldia M, Montes S and Rios C. Free copper, ferroxidase and SOD1 activities, lipid peroxidation and NO(x) content in the CSF. A different marker profile in four neurodegenerative diseases. Neurochem Res 2008; 33: 1717-1723.

[51] Squitti R and Polimanti R. Copper hypothesis in the missing hereditability of sporadic Alzheimer's disease: ATP7B gene as potential harbor of rare variants. J Alzheimers Dis 2012; 29: 493-501.

[52] Hoogenraad TU. Paradigm shift in treatment of Alzheimer's disease: zinc therapy now a conscientious choice for care of individual patients. Int J Alzheimers Dis 2011; 2011: 492686.

[53] Burkhead JL, Gray LW and Lutsenko S. Systems biology approach to Wilson's disease. Biometals 2011; 24: 455-466.

[54] Walshe JM. Serum 'free' copper in Wilson disease. QJM 2012; 105: 419-423.

[55] Miyakawa T and Murayama E. An autopsy case of the "demyelinating type" of Wilson's disease. Acta Neuropathol 1976; 35: 235-241.

[56] Shimoji A, Miyakawa T, Watanabe K, Yamashita K, Katsuragi S and Kabashima K. Wilson's disease with extensive degeneration of cerebral white matter and cortex. Jpn J Psychiatry Neurol 1987; 41: 709-717.
[57] Lorincz MT. Neurologic Wilson's disease. Ann N Y Acad Sci 2010; 1184: 173-187.
[58] Squitti R, Polimanti R, Bucossi S, Ventriglia M, Mariani S, Manfellotto D, Vernieri F, Cassetta E, Ursini F and Rossini PM. Linkage disequilibrium and haplotype analysis of the ATP7B gene in Alzheimer's disease. Rejuvenation Res 2013; 16: 3-10.
[59] Koedam EL, Lauffer V, van der Vlies AE, van der Flier WM, Scheltens P and Pijnenburg YA. Early-versus late-onset Alzheimer's disease: more than age alone. J Alzheimers Dis 2010; 19: 1401-1408.
[60] Halliday GM and McCann H. The progression of pathology in Parkinson's disease. Ann N Y Acad Sci 2010; 1184: 188-195.
[61] Scalfari A, Neuhaus A, Daumer M, Ebers GC and Muraro PA. Age and disability accumulation in multiple sclerosis. Neurology 2011; 77: 1246-1252.
[62] Moller LB, Tumer Z, Lund C, Petersen C, Cole T, Hanusch R, Seidel J, Jensen LR and Horn N. Similar splice-site mutations of the ATP7A gene lead to different phenotypes: classical Menkes disease or occipital horn syndrome. Am J Hum Genet 2000; 66: 1211-1220.
[63] Accurso FJ and Sontag MK. Gene modifiers in cystic fibrosis. J Clin Invest 2008; 118: 839-841.
[64] Bettens K, Sleegers K and Van Broeckhoven C. Genetic insights in Alzheimer's disease. Lancet Neurol 2013; 12: 92-104.
[65] Pedersen NL. Reaching the limits of genome-wide significance in Alzheimer disease: back to the environment. JAMA 2010; 303: 1864-1865.
[66] Luo L, Boerwinkle E and Xiong M. Association studies for next-generation sequencing. Genome Res 2011; 21: 1099-1108.
[67] Navon O, Sul JH, Han B, Conde L, Bracci P, Riby J, Skibola CF, Eskin E and Halperin E. Rare Variant Association Testing Under Low-Coverage Sequencing. Genetics 2013; Epub ahead of print.
[68] Squitti R, Polimanti R, Siotto M, Bucossi S, Ventriglia M, Mariani C, Vernieri F, Scrascia F, Trotta L and Rossini PM. ATP7B variants as modulators of copper dyshomeostasis in Alzheimer's disease. Neuromolecular Med 2013; Accepted.
[69] Siaj R, Sauer V, Stoppeler S, Spiegel HU, Kohler G, Zibert A and Schmidt HH. Dietary copper triggers onset of fulminant hepatitis in the Long-Evans cinnamon rat model. World J Gastroenterol 2012; 18: 5542-5550.
[70] Bucossi S, Mariani S, Ventriglia M, Polimanti R, Gennarelli M, Bonvicini C, Pasqualetti P, Scrascia F, Migliore S, Vernieri F, Rossini PM and Squitti R. Association between the c. 2495 A>G ATP7B Polymorphism and Sporadic Alzheimer's Disease. Int J Alzheimers Dis 2011; 2011: 973692.
[71] Bucossi S, Polimanti R, Mariani S, Ventriglia M, Bonvicini C, Migliore S, Manfellotto D, Salustri C, Vernieri F, Rossini PM and Squitti R. Association of K832R and R952K SNPs of Wilson's disease gene with Alzheimer's disease. J Alzheimers Dis 2012; 29: 913-919.
[72] Walshe JM and Clinical Investigations Standing Committee of the Association of Clinical B iochemists. Wilson's disease: the importance of measuring serum caeruloplasmin non-immunologically. Ann Clin Biochem 2003; 40: 115-121.
[73] Colabufo N, Squitti R. (2012) Patent: Method and kit for determination of free copper in serum. European Patent Office (EPO) (RO/EP), Patent number: PCT-SAFE Version 3.51.056.232 MT/FOP 20121001/0.20.5.19.
[74] Rodriguez B, Burguera J and Berenguer M. Response to different therapeutic approaches in Wilson disease. A long-term follow up study. Ann Hepatol 2012; 11: 907-914.
[75] Funke SA and Willbold D. Peptides for therapy and diagnosis of Alzheimer's disease. Curr Pharm Des 2012; 18: 755-767.
[76] Delangle P and Mintz E. Chelation therapy in Wilson's disease: from D-penicillamine to the design of selective bioinspired intracellular Cu(I) chelators. Dalton Trans 2012; 41: 6359-6370.
[77] Crouch PJ and Barnham KJ. Therapeutic redistribution of metal ions to treat Alzheimer's disease. Acc Chem Res 2012; 45: 1604-1611.
[78] Bonda DJ, Liu G, Men P, Perry G, Smith MA and Zhu X. Nanoparticle delivery of transition-metal chelators to the brain: Oxidative stress will never see it coming! CNS Neurol Disord Drug Targets 2012; 11: 81-85.
[79] Quinn JF, Harris CJ, Cobb KE, Domes C, Ralle M, Brewer G and Wadsworth TL. A copper-lowering strategy attenuates amyloid pathology in a transgenic mouse model of Alzheimer's disease. J Alzheimers Dis 2010; 21: 903-914.
[80] Carri MT and Cozzolino M. SOD1 and mitochondria in ALS: a dangerous liaison. J Bioenerg Biomembr 2011; 43: 593-599.
[81] Darin N, Moslemi AR, Lebon S, Rustin P, Holme E, Oldfors A and Tulinius M. Genotypes and clinical phenotypes in children with cytochrome-c oxidase deficiency. Neuropediatrics 2003; 34: 311-317.