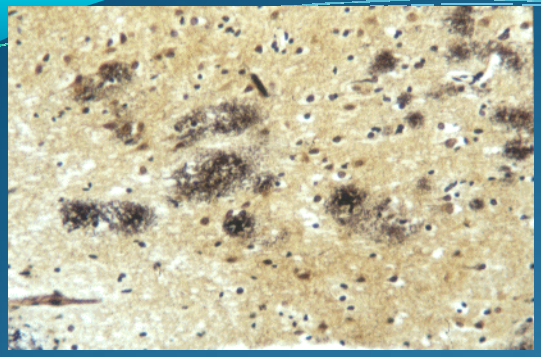
Copper Dysfunction in Alzheimer's Disease

FONDAZIONE FATEBENEFRATELLI DIVISIONE RICERCA

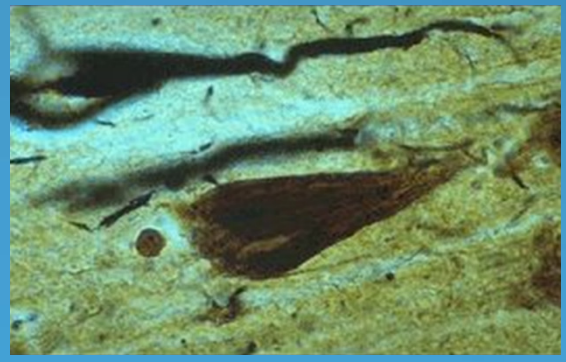


Rosanna Squitti,
Department of Neuroscience,
Fatebenefratelli Foundation,
AFaR Division
Rome, Italy



Signs of Alzheimer's disease

- In a schematic representation, five primary functional and anatomical features characterize the AD brain:
- (a) loss of neurons and synapses in the cerebral cortex
- (b) density and distribution of extracellular amyloid plaques
- (c) presence of intracellular neurofibrillary tangles containing hyper-phosphorylated Tau
- (d) increased oxidative damage of lipids, proteins and nucleic acids
- (e) loss of biometal homeostasis



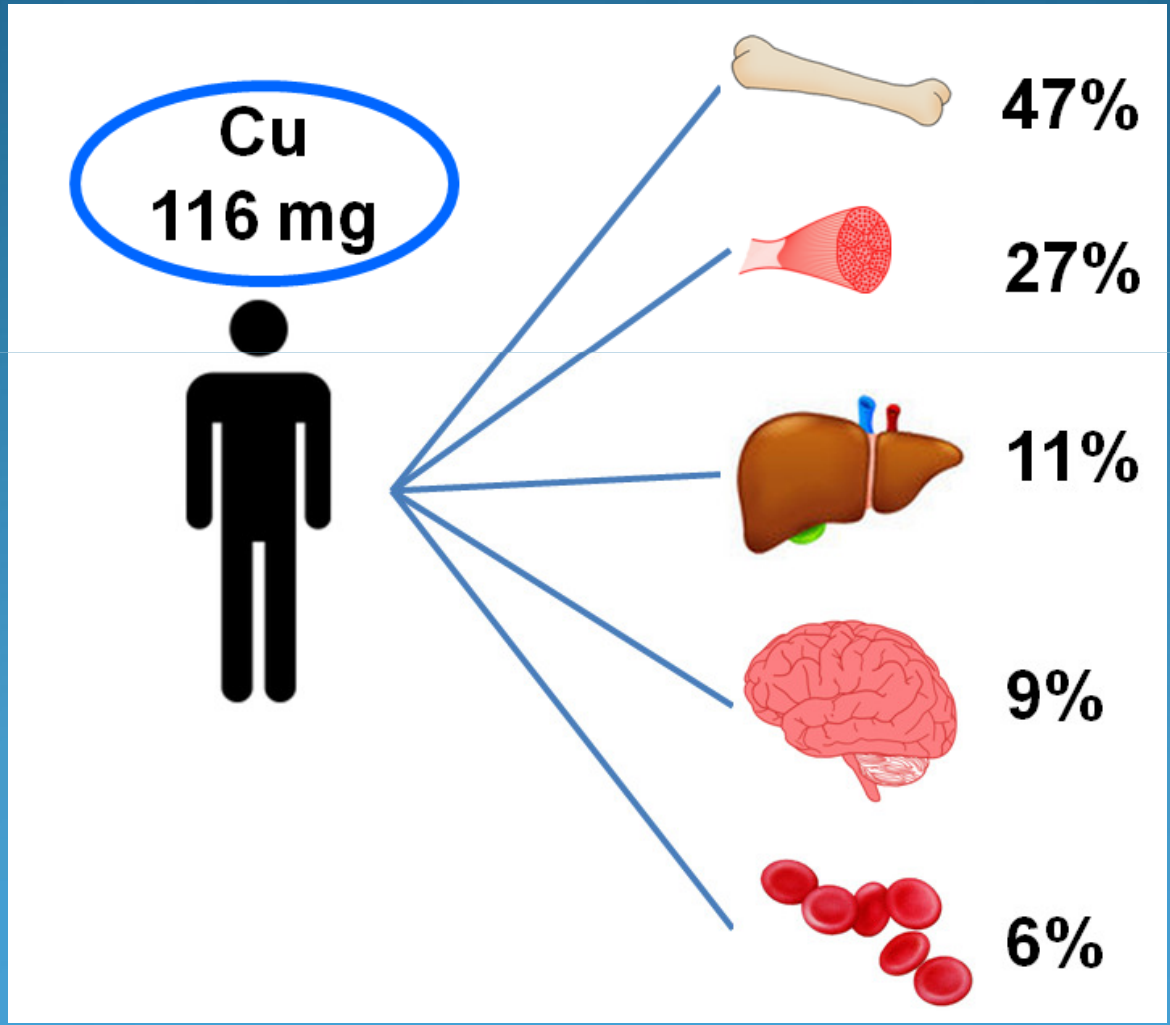
Copper and AD (> 1394)

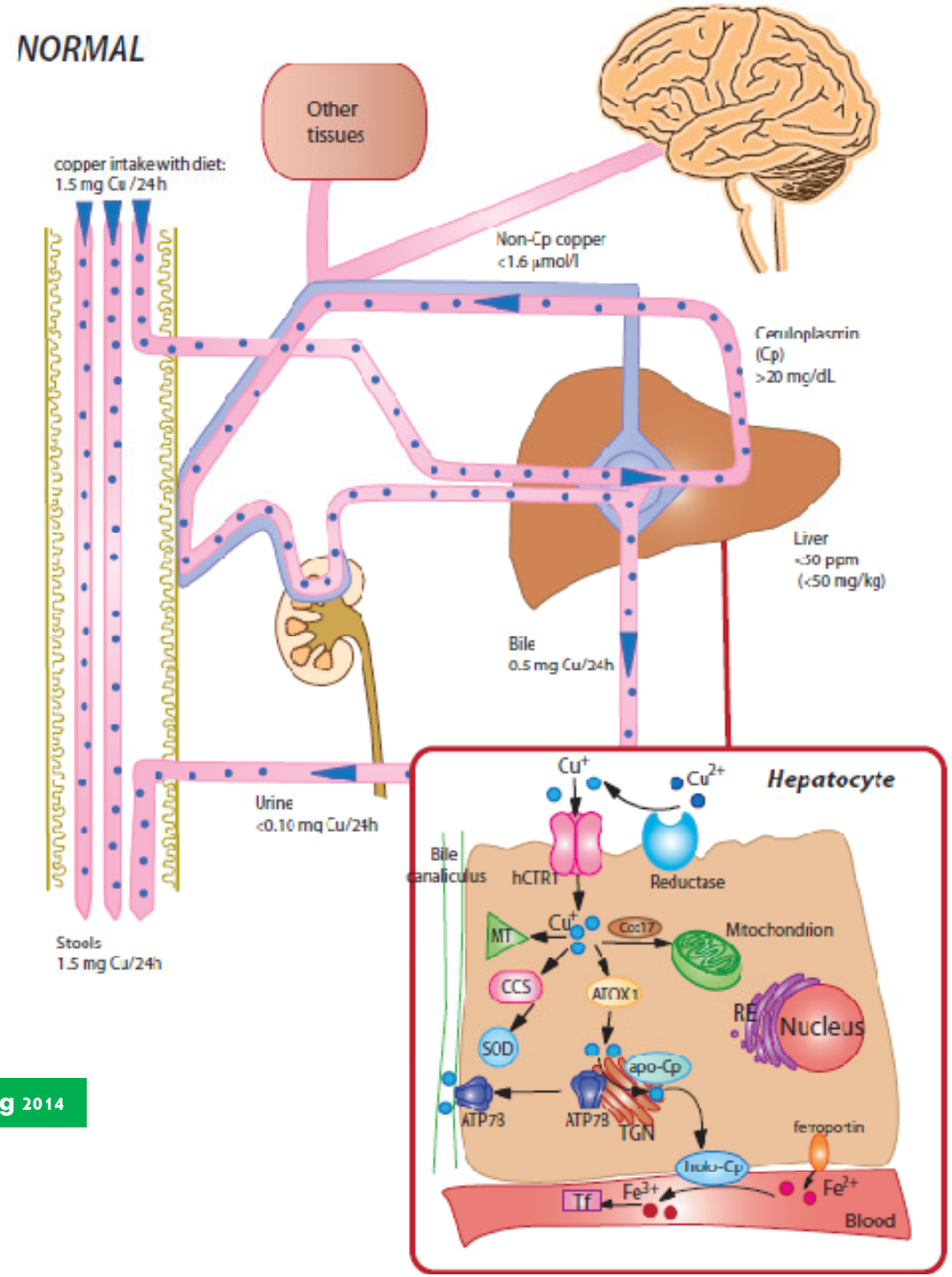


Evidence sustaining the role of copper dysfunction in Alzheimer's disease

- Biochemical in vitro
- Clinical
- Epidemiological
- Meta-analyses
- Genetics

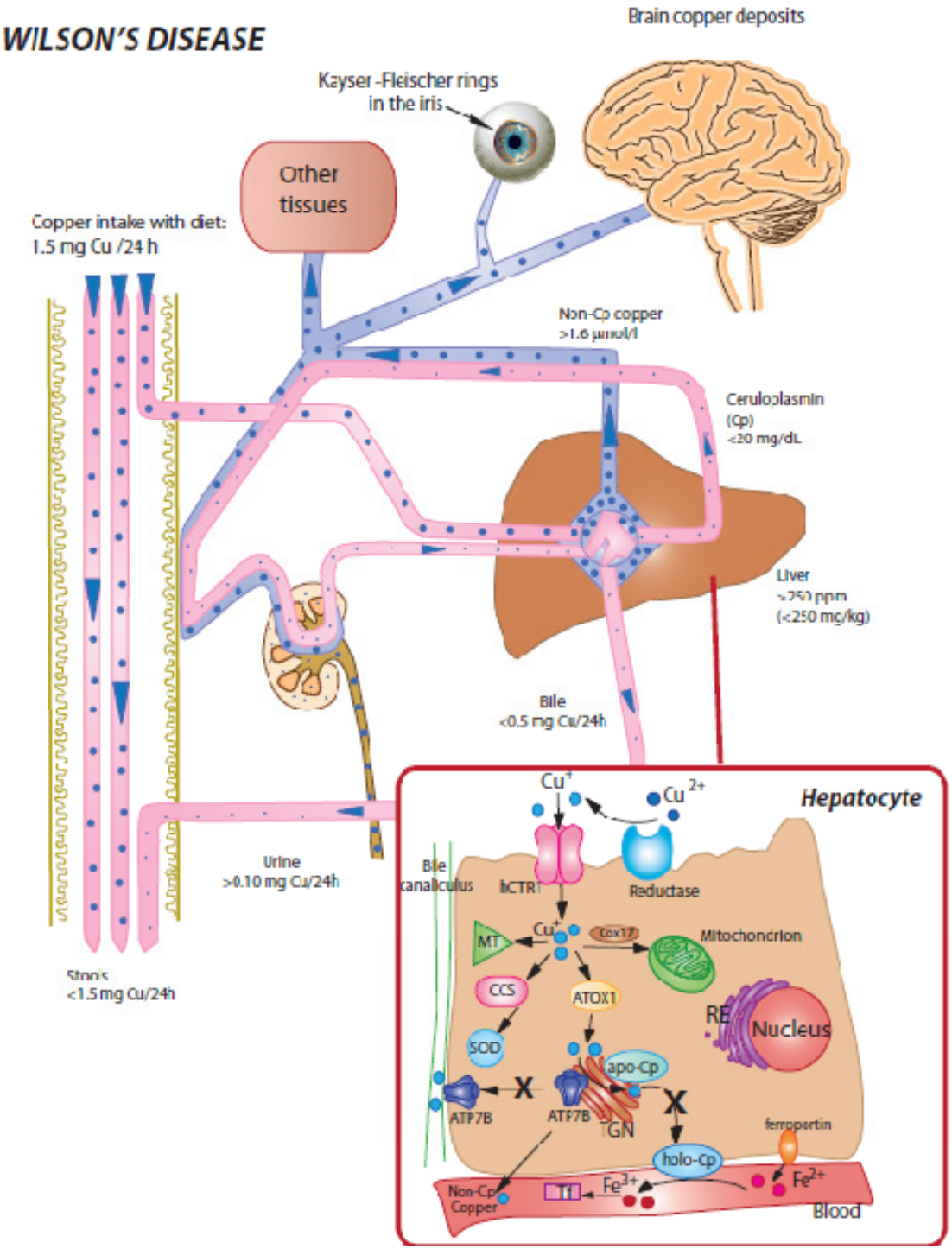
Copper in physiology: body distribution





Squitti et al., Neurobiol Aging 2014

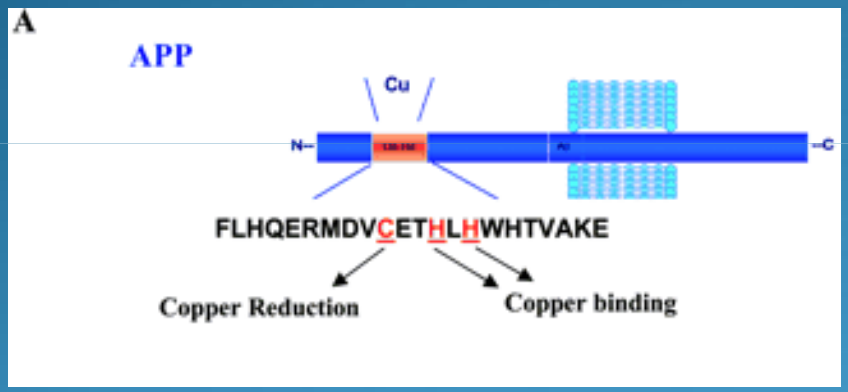
WILSON'S DISEASE



Copper dysfunction in Alzheimer's disease:

Biochemical evidence *in vitro*

The CuBD sequence



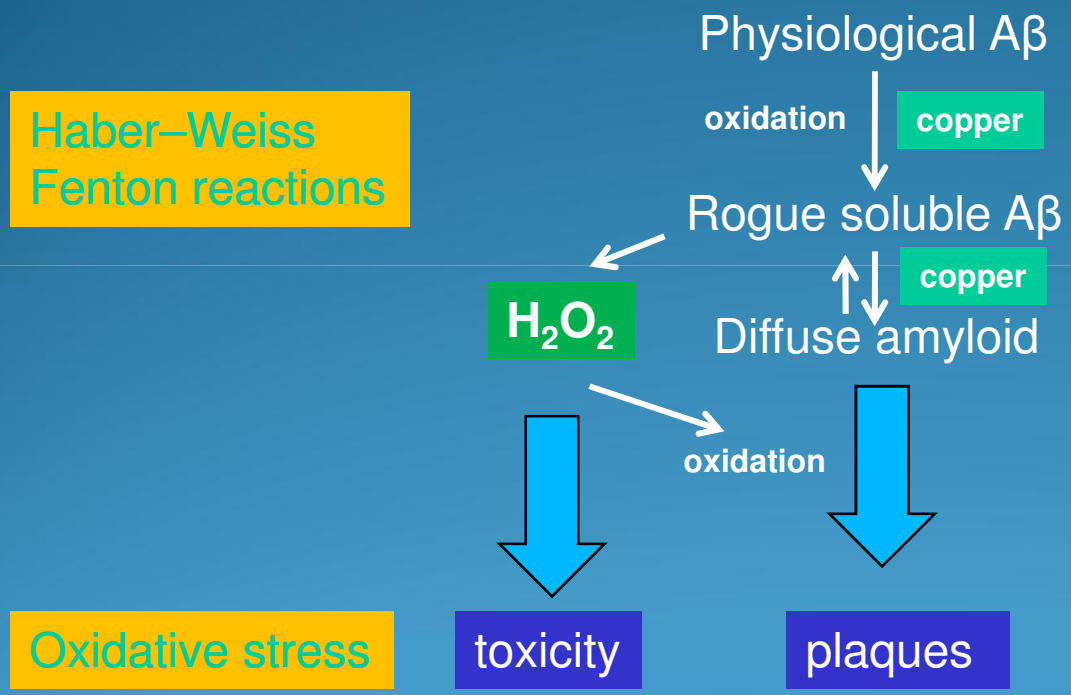
APP reduces Cu (II) a Cu(I)
(Multhaup G et al., Science 1996)

H_2O_2 production by AβCu2
(Opazo et al., J Biol Chem. 2002)

Aβ deposits dissolution by biometal depletion
(Cherny et al., J Biol Chem 1999)

APP/Aβ metalloprotein controlling [Cu]in
(White AR et al., Brain Res 1999)
(Barnham et al. J Biol Chem 2003)

Model of copper toxicity



(modified by Bush AI Trends Neurosci 2003)

What happens in living patients?
Does a copper dysfunction occur in AD patients?

Copper dysfunction in Alzheimer's disease:

Clinical evidence

AD copper subtype: 12 years of evidence

2002 **Squitti et al.,** Elevation of serum copper levels in Alzheimer's disease - ***Neurology***

2003 **Squitti et al.,** Elevation of serum copper levels discriminates Alzheimer's disease from vascular dementia - ***Neurology***

2005 **Squitti et al.,** Excess of serum copper not related to ceruloplasmin in Alzheimer disease - ***Neurology***

2006 **Squitti et al.,** Excess of nonceruloplasmin serum copper in AD correlates with MMSE, CSF [beta]-amyloid, and h-tau - ***Neurology***

2007 **Babiloni et al.,** Free copper and resting temporal EEG rhythms correlate across healthy, mild cognitive impairment, and Alzheimer's disease subjects - ***Clin Neurophysiol***

2007 **Squitti et al.,** 'Free' copper in serum of Alzheimer's disease patients correlates with markers of liver function - ***J Neural Transm***

2008 **Squitti et al.,** Ceruloplasmin **fragmentation** is implicated in 'free' copper deregulation of Alzheimer's disease - ***Prion***

2009 Squitti et al., Longitudinal prognostic value of serum "free" copper in patients with Alzheimer disease - ***Neurology***

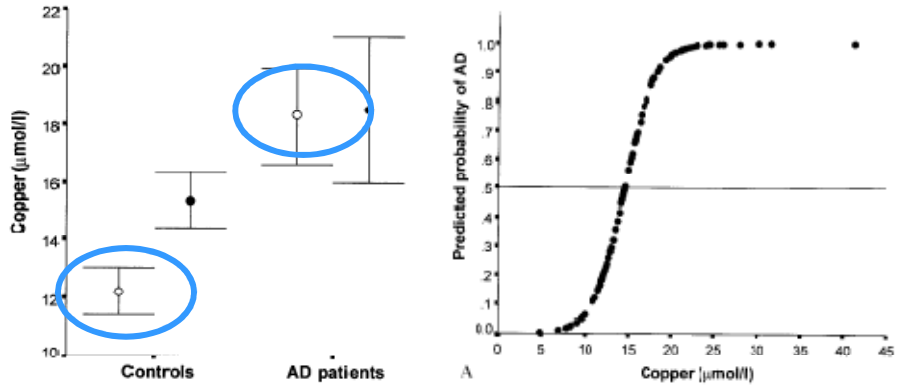
2014 Squitti R et al, Value of serum non-ceruloplasmin copper for prediction of MCI conversion to AD. ***Ann Neurol***

Elevation of serum copper levels in Alzheimer's disease

R. Squitti, PhD; D. Lupoi, MD; P. Pasqualetti, PhD; G. Dal Forno, MD; F. Vernieri, MD; P. Chiovenda, PhD; L. Rossi, PhD; M. Cortesi, MD; E. Cassetta, MD; and P.M. Rossini, MD

AD:79
Ctrl:76

NEUROLOGY 2002;59:1159–1161



Excess of serum copper not related to ceruloplasmin in Alzheimer

R. Squitti, PhD; P. Pasqualetti, PhD; G. Dal Forno, MD, PhD; F. Moffa, D. Lupoi, MD; F. Vernieri, MD; L. Rossi, PhD; M. Baldassini, MD; an

NEUROLOGY 2005;64:1040–1046

AD:47
VAD: 24
Ctrl:44

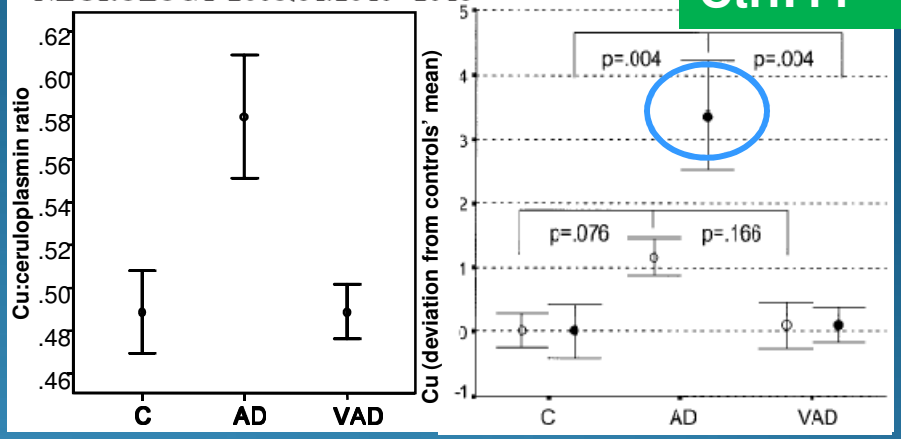


Table 3 Correlation between outcomes of neuropsychological examination and peroxides, copper, and TRAP level

Neuropsychological test	Peroxides, n = 54	Copper, n = 54	TRAP, n = 54
AVLT Immediate Recall	$r = -0.231, p = 0.093$	$r = -0.413, p = 0.002$	$r = 0.285, p = 0.039$
AVLT Delayed Recall	$r = -0.303, p = 0.026$	$r = -0.391, p = 0.003$	$r = 0.304, p = 0.027$
Immediate Visual Memory	$r = -0.158, p = 0.254$	$r = -0.246, p = 0.073$	$r = 0.303, p = 0.028$
Copy Drawing	$r = -0.242, p = 0.078$	$r = -0.376, p = 0.005$	$r = 0.242, p = 0.081$
Copy Drawing with Landmarks	$r = -0.257, p = 0.061$	$r = -0.427, p = 0.001$	$r = 0.315, p = 0.022$
Raven's Progressive Matrices	$r = -0.289, p = 0.036$	$r = -0.439, p = 0.001$	$r = 0.354, p = 0.01$
Sentences Construction	$r = -0.031, p = 0.825$	$r = -0.204, p = 0.139$	$r = 0.129, p = 0.357$
Verbal Fluency	$r = -0.106, p = 0.446$	$r = -0.354, p = 0.009$	$r = 0.357, p = 0.009$
Digit span	$r = -0.262, p = 0.056$	$r = -0.285, p = 0.037$	$r = 0.233, p = 0.094$
Corsi Test	$r = -0.173, p = 0.21$	$r = -0.288, p = 0.035$	$r = 0.255, p = 0.065$
MMSE	$r = -0.227, p = 0.089$	$r = -0.331, p = 0.012$	$r = 0.252, p = 0.061$

Excess of nonceruloplasmin serum copper in AD correlates with MMSE, CSF β-amyloid, and h-tau

R. Squitti, PhD; G. Barbati, PhD; L. Rossi, PhD; M. Ventriglia, PhD; G. Dal Forno, MD, PhD; S. Cesaretti, PhD; F. Moffa, PhD; I. Caridi, MD; E. Cassetta, MD; P. Pasqualetti, PhD; L. Calabrese, PhD; D. Lupoi, MD; and P.M. Rossini, MD

NEUROLOGY 2006;67:76–82

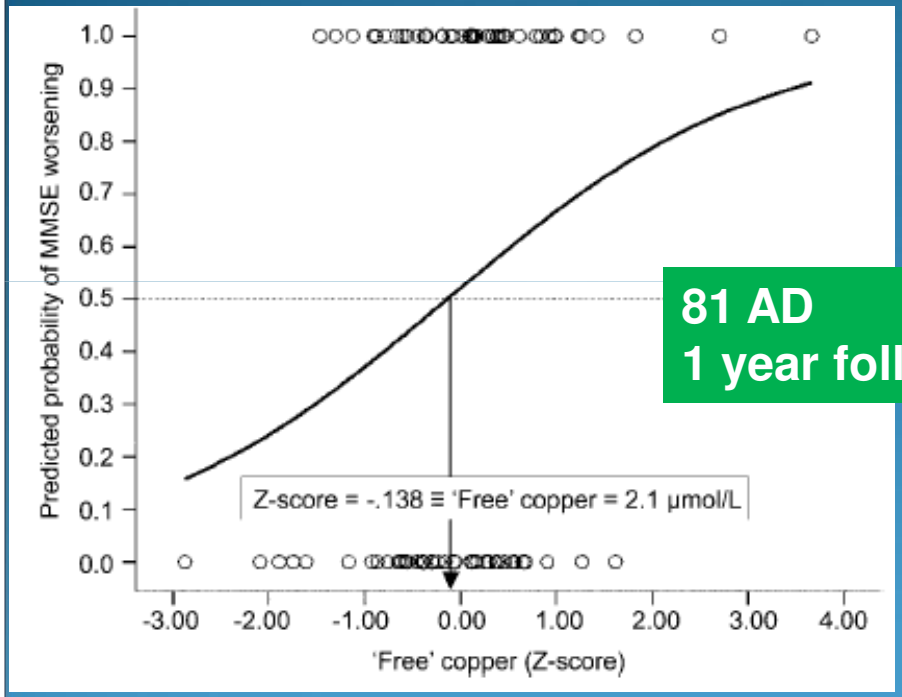
Table 3 Correlations between copper explained and unexplained by ceruloplasmin in the serum and CSF markers of neurodegeneration

	CSF		
Serum	β-Amyloid (ng/mL) (n = 42)	h-tau (ng/mL) (n = 43)	P-tau (ng/mL) (n = 43)
Copper explained (μmol/L)			
r	−0.1	−0.21	−0.12
p	0.52	0.18	0.43
Copper unexplained (μmol/L)			
r	−0.46	0.49	−0.21
p	0.002	0.001	0.17

Pearson correlation coefficient (*r*) significant at the 0.05 level.

	Factor 1 Visuo-spatial	Factor 2 Verb Mem	Factor 3 Languag e	MMSE
β-amyloid (ng/ml)	r= .03 p= .92 (n= 17)	**r=0.43 p= .03 (n= 25)**	r= .19 p= .37 (n= 24)	**r= .46 p= .002 (n= 43)**
h-tau (ng/ml)	r= -.28 p= .27 (n= 17)	r= -.33 p= .11 (n= 25)	r= -.26 p= .22 (n= 24)	r= -.29 p= .06 (n= 44)
P-tau (ng/ml)	r= .20 p= .45 (n= 17)	r= .23 p= .26 (n= 25)	r= .26 p= .22 (n= 24)	r= .16 p= .29 (n= 43)
CSF copper (μmol/l)	**r= -.524 p= .05 (n= 14)**	r= -.039 p= .869 (n= 20)	r= -.32 p= .2 (n= 19)	r= -.001 p=.99 (n= 42)
Bound Copper (μmol/l)	r= .26 p= .35 (n= 14)	r= -.08 p= .72 (n= 21)	r= .10 p= .68 (n= 20)	r= -.27 p= .07 (n= 43)
'Free' Copper (μmol/l)	r= -.37 p= .20 (n= 14)	**r= -.45 p= .04 (n= 21)**	r= -.41 p= .07 (n= 20)	**r= -.45 p= .003 (n= 43)**

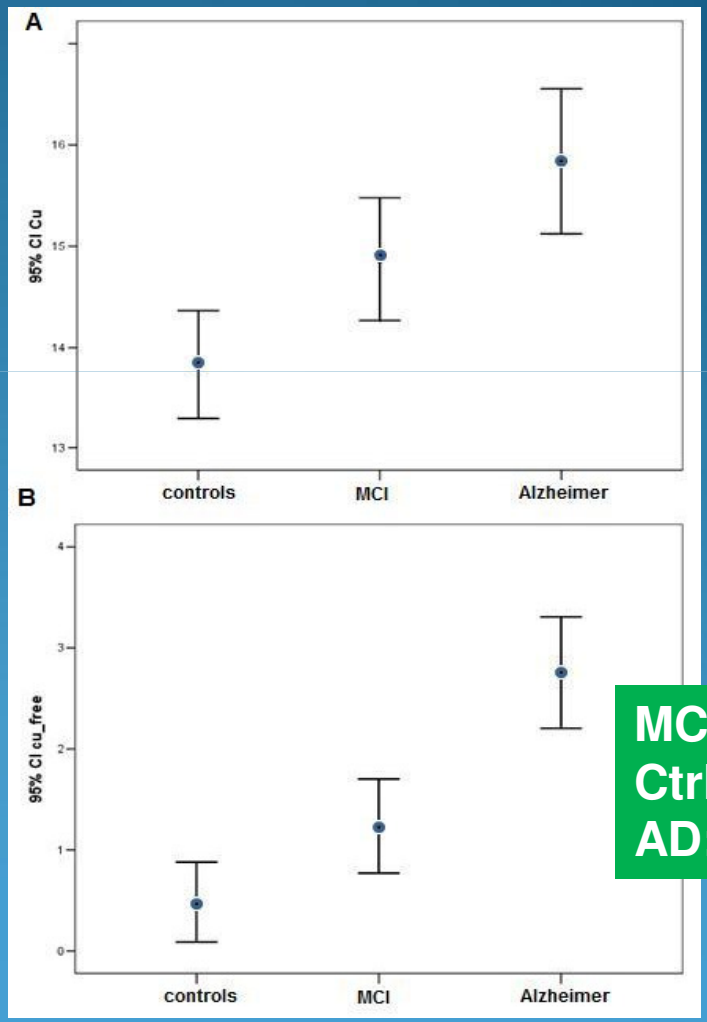
Non-Cp Cu AD Prognosis



81 AD
1 year follow-up

(Squitti et al., Neurology 2009)

Non-Cp Cu in MCI



MCI: 83
Ctrl:100
AD:105

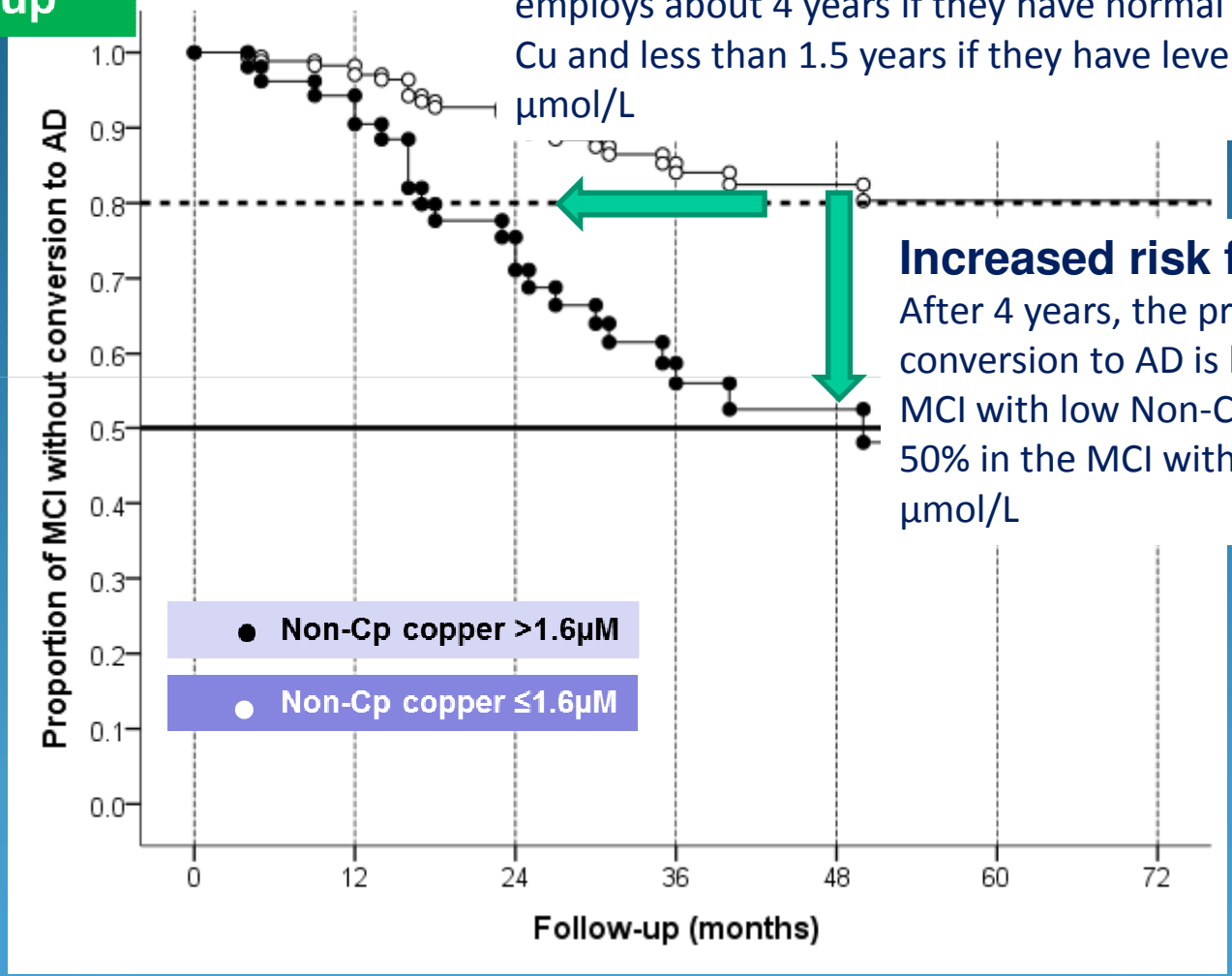
(Squitti et al., J Alzh Dis 2010)

Non-Cp Cu MCI conversion

42 MCI converters
99 MCI stable
6 year follow-up

Faster rate of conversion to AD:

Among the MCI, the 20% more rapid to convert to AD employs about 4 years if they have normal levels of Non-Cp-Cu and less than 1.5 years if they have levels higher than 1.6 µmol/L



Increased risk for AD:

After 4 years, the probability of conversion to AD is less than 20% in MCI with low Non-Cp-Cu and almost 50% in the MCI with higher than 1.6 µmol/L

(Squitti et al., Annals of Neurology 2014)

Copper dysfunction in Alzheimer's disease:

epidemiologic data

ORIGINAL CONTRIBUTION

Dietary Copper and High Saturated and *trans* Fat Intakes Associated With Cognitive Decline

Martha Clare Morris, ScD; Denis A. Evans, MD; Christine C. Tangney, PhD; Julia L. Bienias, ScD; Julie A. Schneider, MD; Robert S. Wilson, PhD; Paul A. Scherr, ScD, PhD

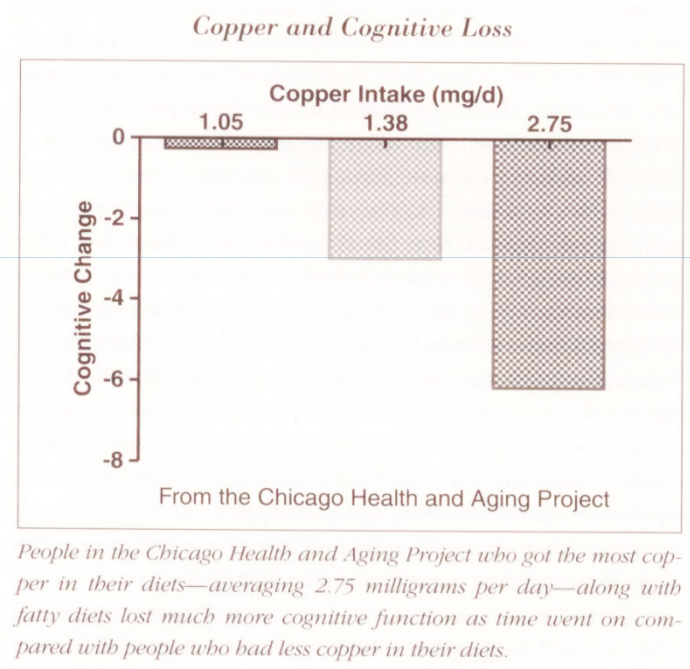
Table 2. Age- and Multiple-Adjusted Differences in the Estimated Annual Rate of Change in Cognitive Score During 6 Years by Intake Level of Copper, Zinc, and Iron Among 3718 Participants in the Chicago Health and Aging Project, 1993-2002*

Dietary Supplements and Mortality Rate in Older Women (The Iowa Women's Health Study) the use of vitamin and mineral supplements in relation to total mortality in 38 772 older women: Copper was associated with a 18% increased of mortality

(Mursu et al, Arch Intern Med.)

Relationship between Mortality from Alzheimer's Disease and Soil Metal Concentration in Mainland China.

Shen Positive J Alzheimers Dis. 2014



People in the Chicago Health and Aging Project who got the most copper in their diets—averaging 2.75 milligrams per day—along with fatty diets lost much more cognitive function as time went on compared with people who had less copper in their diets.

(Morris et al Arch Neurol 2006)

Copper dysfunction in Alzheimer's disease:

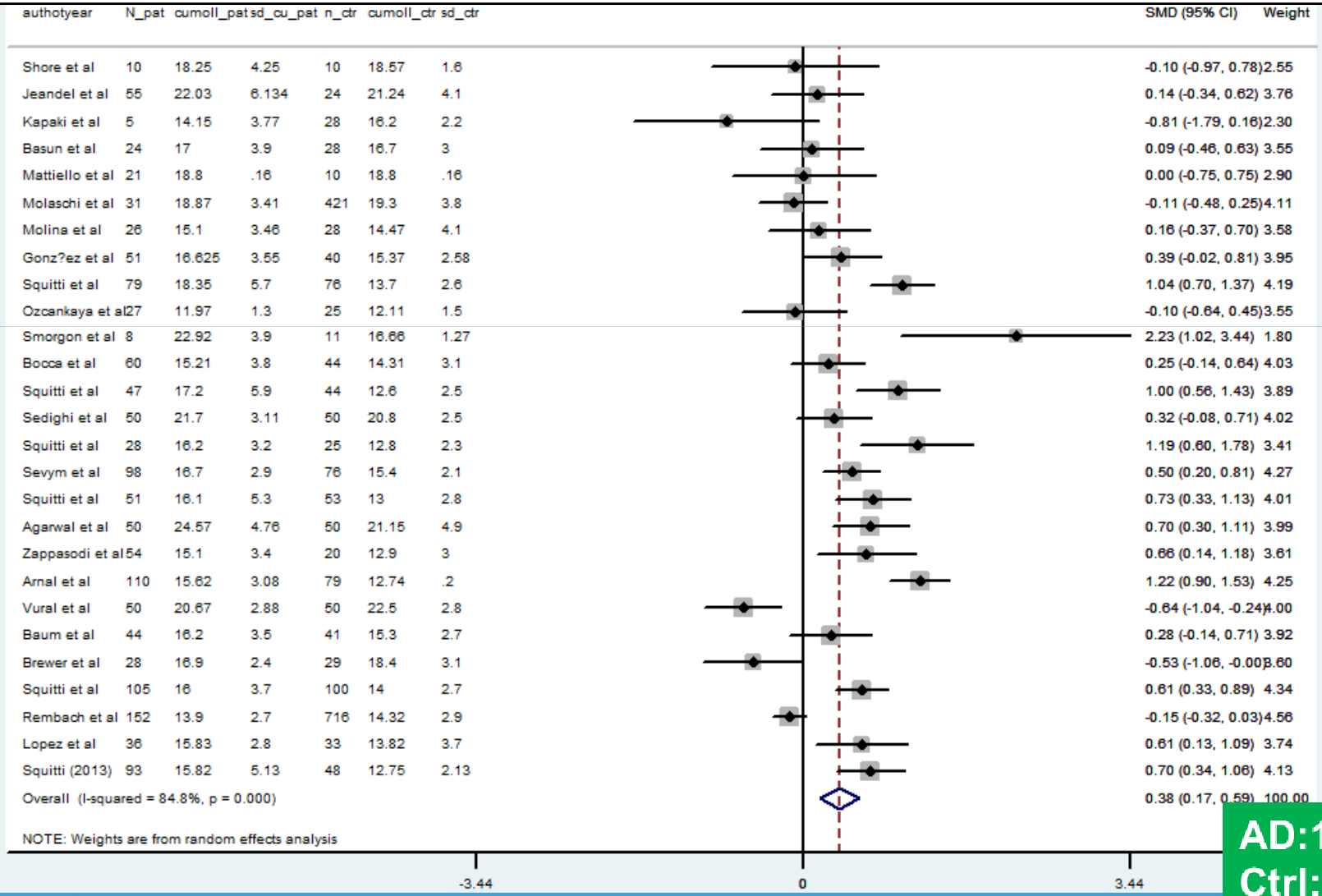
Meta-analytic data

Journal of Alzheimer's Disease xx (20xx) x–xx
DOI 10.3233/JAD-131247
IOS Press

Meta-Analysis of Serum Non-Ceruloplasmin Copper in Alzheimer's Disease

Rosanna Squitti[a,b,*], Ilaria Simonelli[c,d], Mariacarla Ventriglia[a], Mariacristina Siotto[e], Patrizio Pasqualetti[c,d], Alan Rembach[f], James Doecke [g,h] and Ashley I. Bush[f]

Proof



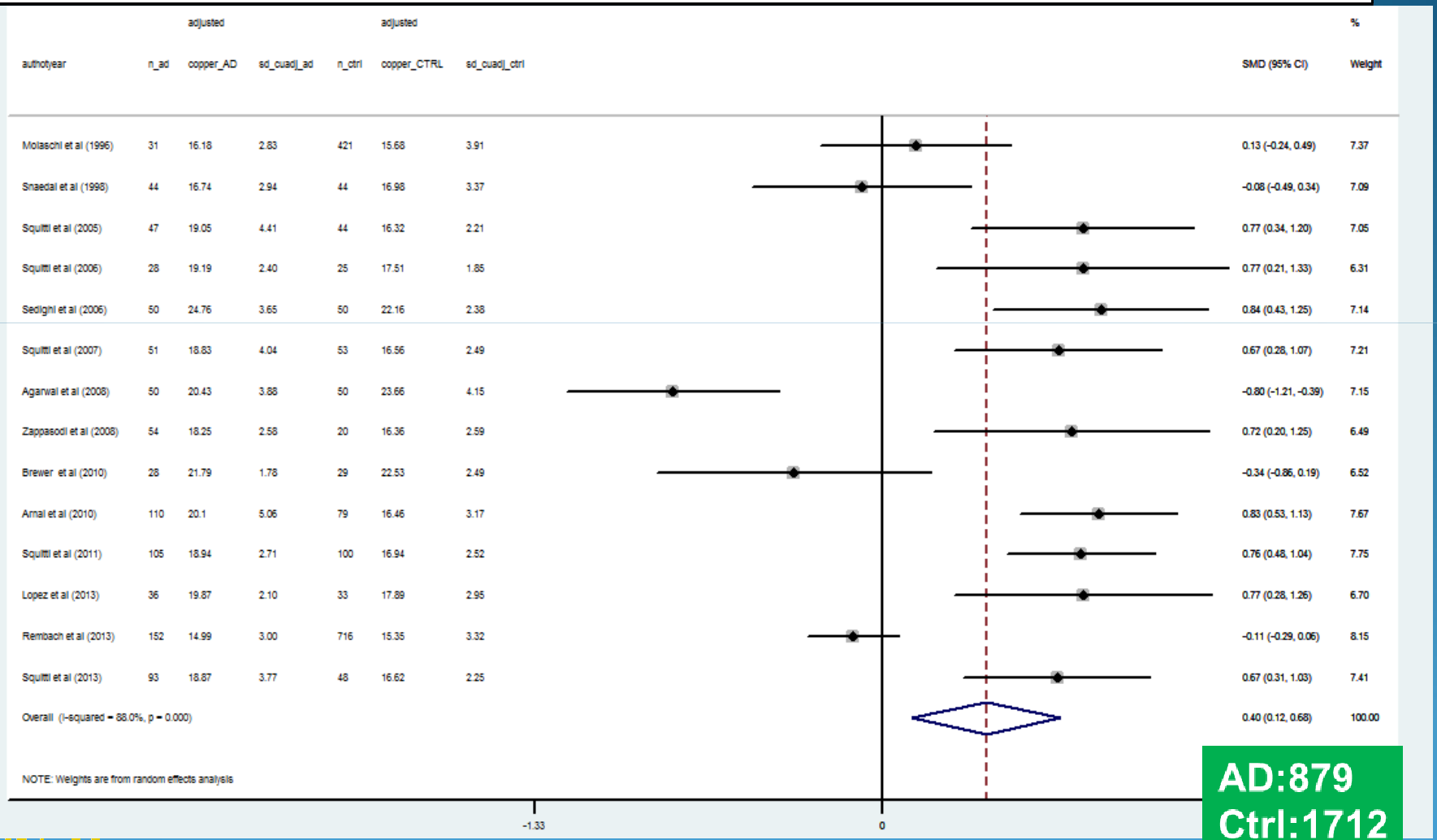
AD:1393
Ctrl:2159

Journal of Alzheimer's Disease xx (20xx) x–xx
DOI 10.3233/JAD-131247
IOS Press

Meta-Analysis of Serum Non-Ceruloplasmin Copper in Alzheimer's Disease

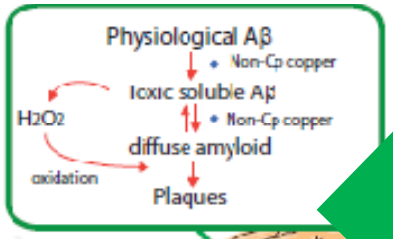
Rosanna Squitti[a,b,*], Ilaria Simonelli[c,d], Mariacarla Ventriglia[a], Mariacristina Siotto[e], Patrizio Pasqualetti[c,d], Alan Rembach[f], James Doecke [g,h] and Ashley I. Bush[f]

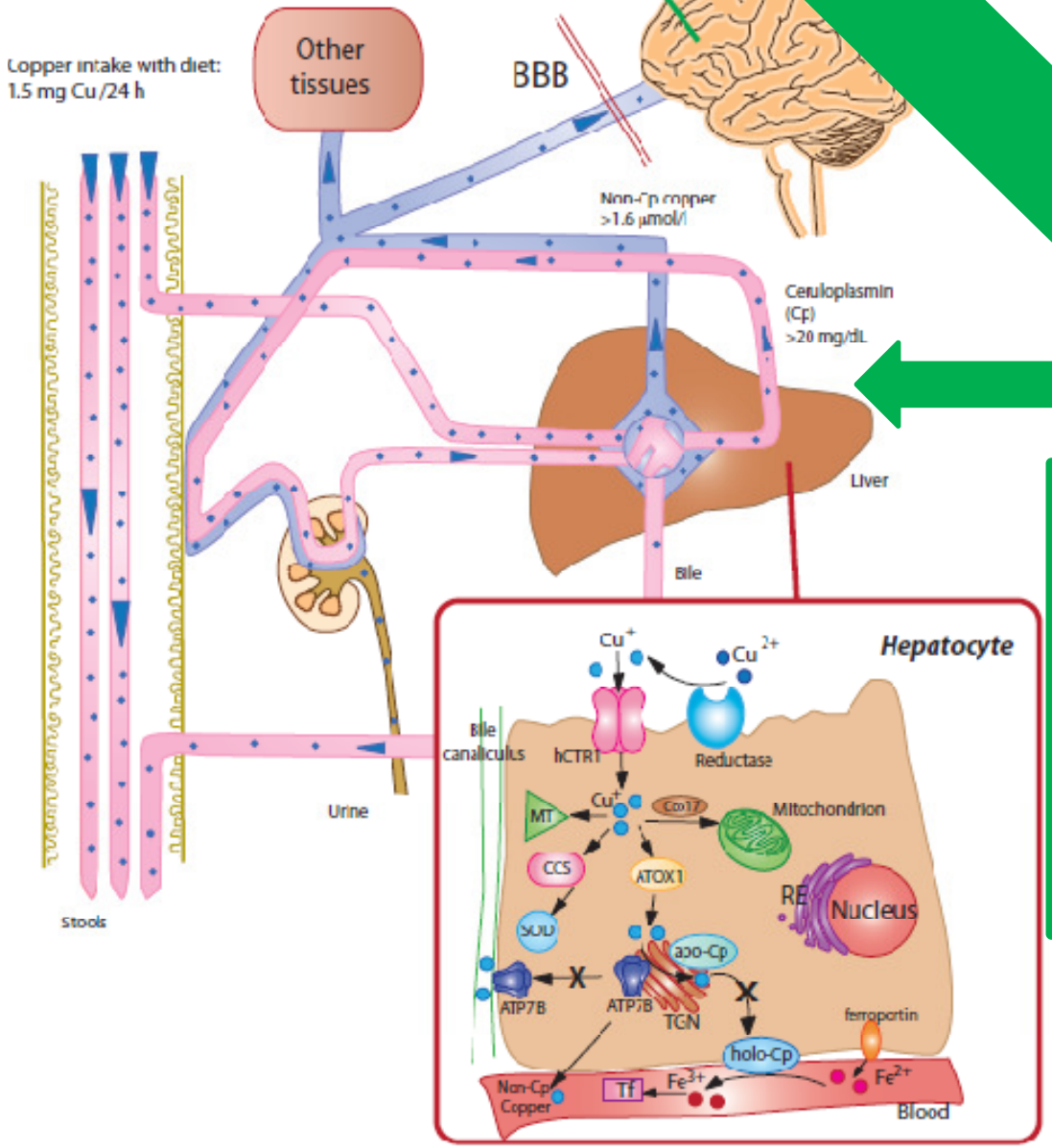
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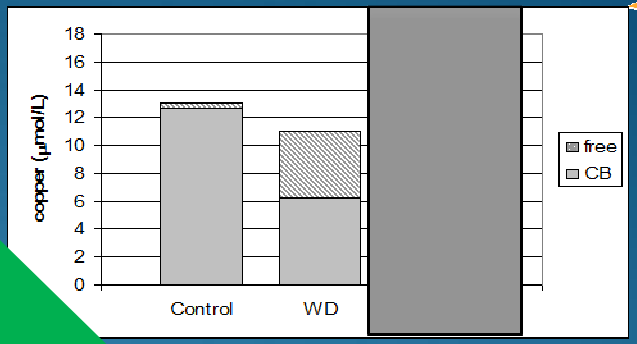
AD:879
Ctrl:1712

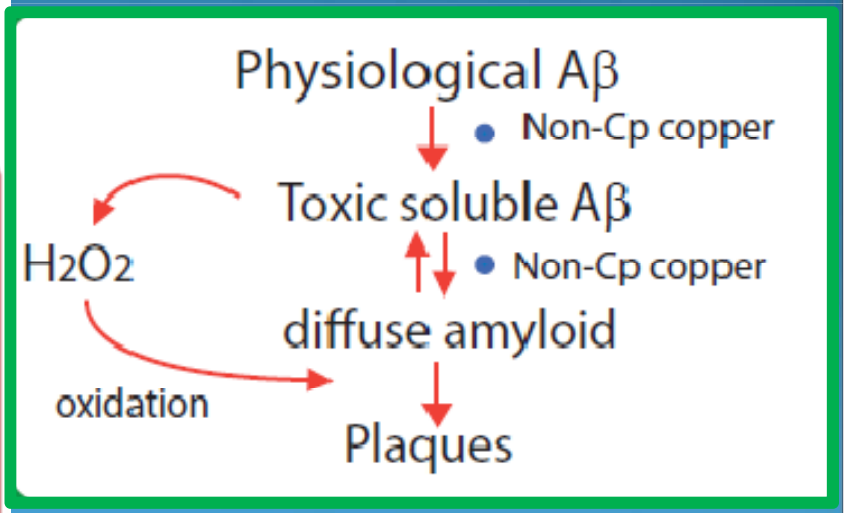
25% Labile copper increased in AD in Brodman area
(James et al Free Radic Biol Med 2012)





Non-Cp-Cu in the AD cascade



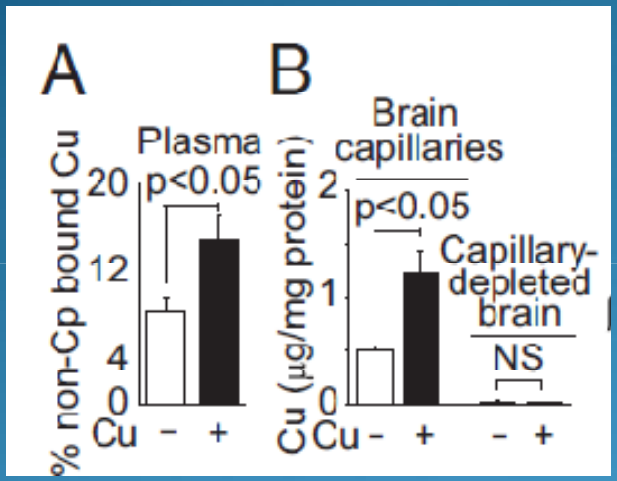


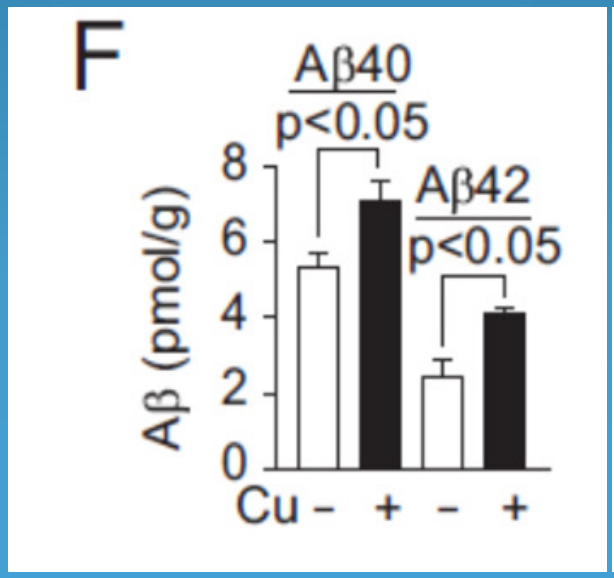
Low levels of copper disrupt brain amyloid-β homeostasis by altering its production and clearance

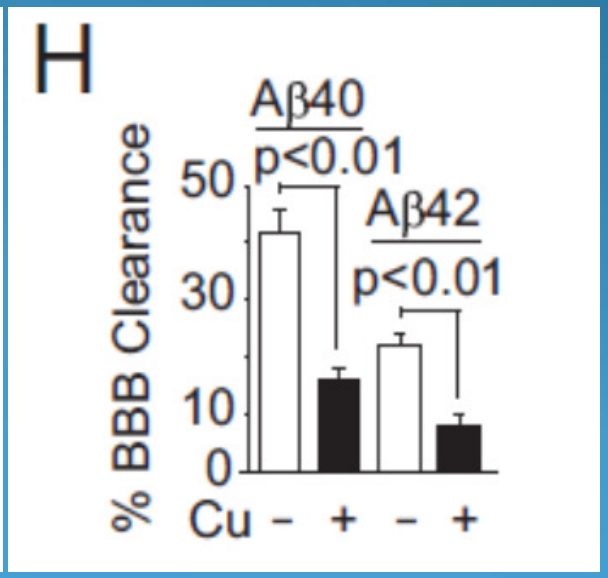
Itender Singh[a,b,c], Abhay P. Sagare[a,b], Mireia Coma[a,b], David Perlmutter[a,b], Robert Gelein[d], Robert D. Bell[a,b], Richard J. Deane[a,b], Elaine Zhong[a,b], Margaret Parisi[a,b], Joseph Ciszewski[a,e], R. Tristan Kasper[a,b,e], and Rashid Deane[a,b,e,1]

Departments of [a]Neurosurgery, [c]Pediatrics, and [d]Environmental Medicine, and Centers for [b]Neurodegenerative and Vascular Brain Disorders and [e]Translational Neuromedicine, University of Rochester Medical Center, Rochester NY 14642

Edited by Thomas C. Südhof, Stanford University School of Medicine, Stanford, CA, and approved July 18, 2013 (received for review February 2, 2013)







Copper dysfunction in Alzheimer's disease:

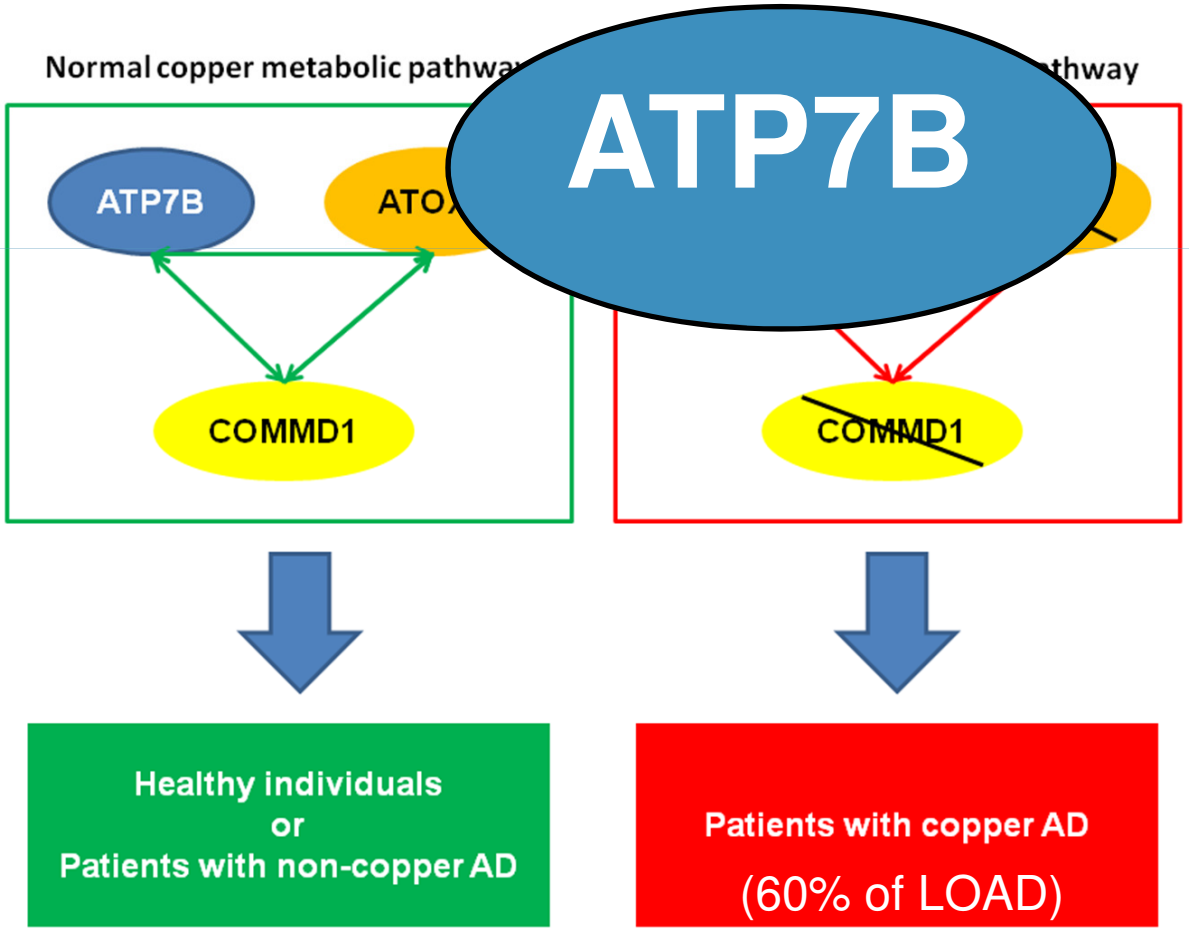
genetics

Am J Neurodegener Dis 2013;2(2):XXX-XXX
www.AJND.us /ISSN:2165-591X/AJND1305004

Review Article

Copper phenotype in Alzheimer's disease: dissecting the pathway

Rosanna Squitti[1,2], Renato Polimanti[3]



The copper gene ATP7B associates with AD

2013 Squitti R, et al., ATP7B Variants as Modulators of Copper Dyshomeostasis in Alzheimer's Disease - Neuromolecular Med.

2013 Bucossi et al., Intronic rs2147363 variant in ATP7B transcription factor-binding site associated with Alzheimer's disease - J Alzheimers Dis.

2013 Squitti R et al., Linkage disequilibrium and haplotype analysis of the ATP7B gene in Alzheimer's disease - Rejuvenation Res.

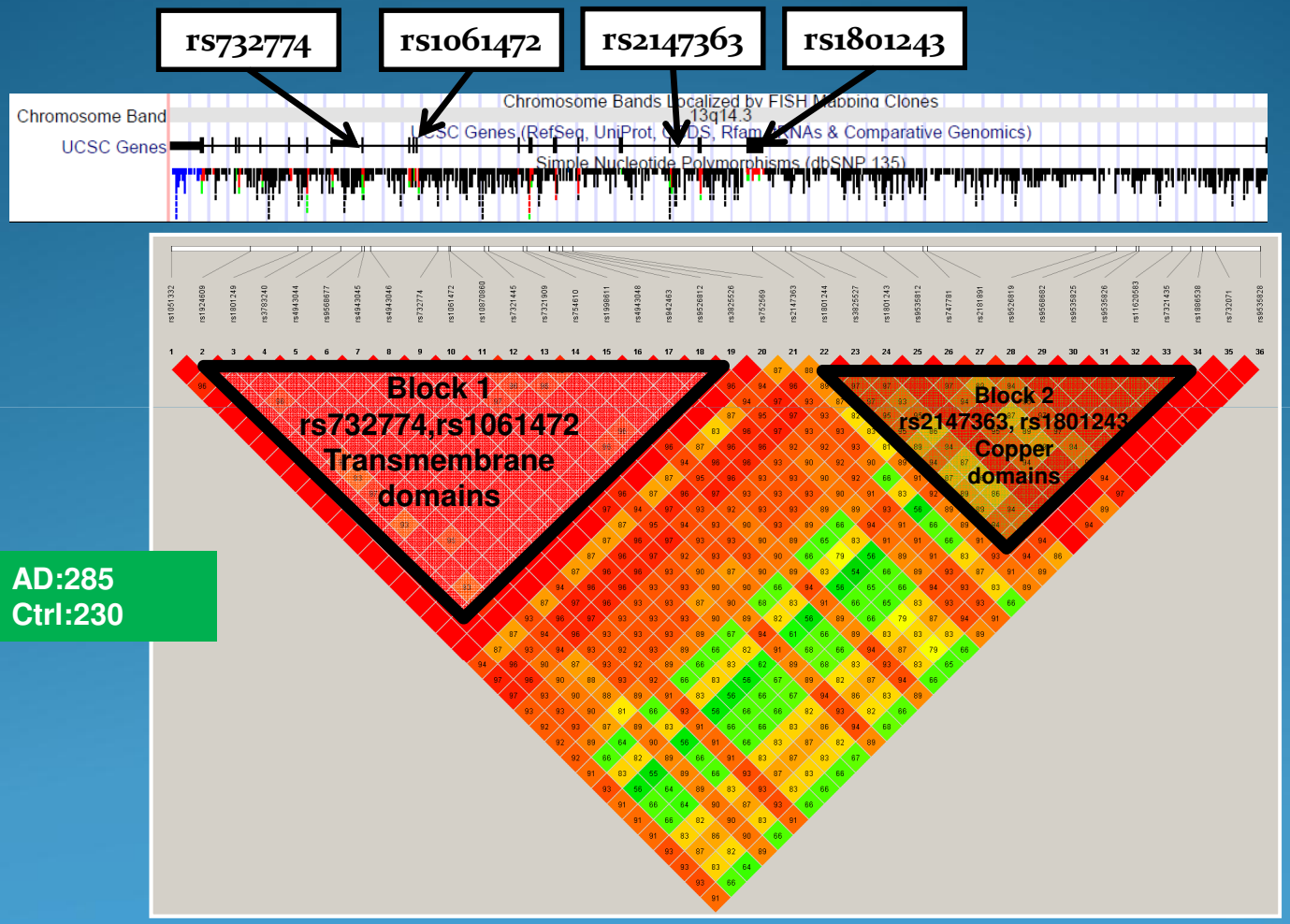
2012 Squitti R. Copper dysfunction in Alzheimer's disease: from meta-analysis of biochemical studies to new insight into genetics - J Trace Elem Med Biol

2012 Bucossi et al., Association of K832R and R952K SNPs of Wilson's disease gene with Alzheimer's disease - J Alzheimers Dis.

2012 Squitti R, Polimanti R. Copper hypothesis in the missing hereditability of sporadic Alzheimer's disease: ATP7B gene as potential harbor of rare variants - J Alzheimers Dis

2011 Bucossi et al., Association between the c. 2495 A>G ATP7B Polymorphism and Sporadic Alzheimer's Disease - Int J Alzheimers Dis.

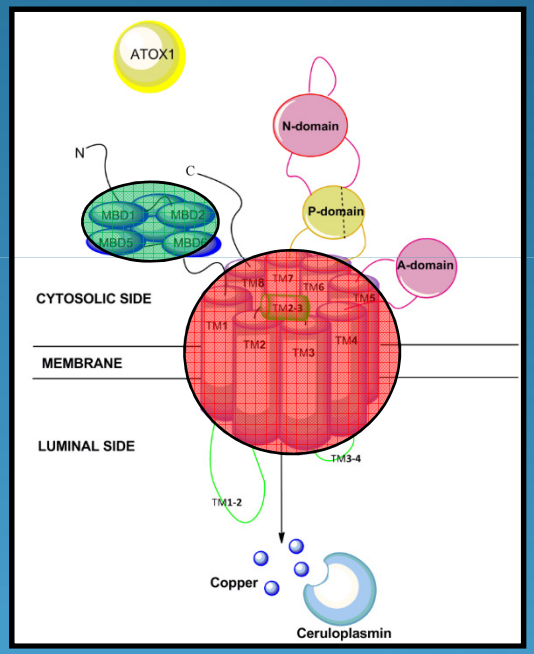
ATP7B informative SNPs in AD risk



(Squitti et al., 2013 Rejuvenation Res. 16:3-10)

ATP7B loci in transmembrane domains associate with an increased risk for AD

Block	SNP ID	Best genetic model	OR (95%CI)	adjusted P value
1	rs732774	recessive	2.31 (1.41-3.77)	< 0.001
	rs1061472	Log-additive	1.73 (1.23-2.43)	0.002
2	rs2147363	Log-additive	1.58 (1.11-2.25)	0.010
	rs1801243	Log-additive	1.52 (1.10-2.09)	0.010



(Squitti et al., 2013 Rejuvenation Res. 16:3-10)

ATP7B SNPs modulate Non-Cp copper in AD patients

AD:399
Ctrl:303

		Copper			Ceruloplasmin			Free Copper		
		WH	AD	CT	WH	AD	CT	WH	AD	CT
rs1801243	Codominant									
	Dominant									
	Recessive									
rs2147363	Codominant									
	Dominant									
	Recessive									
rs1061472	Codominant									
	Dominant									
	Recessive									
rs732774	Codominant									
	Dominant									
	Recessive									
GCGA haplotype										

[Non-Cp copper] in AD and rs732774

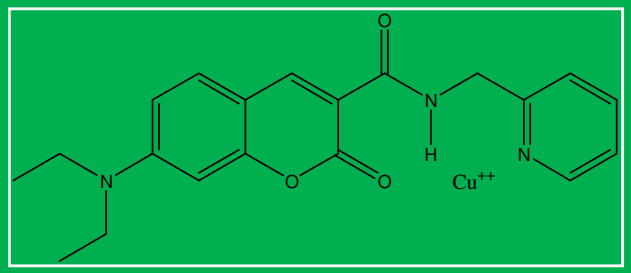
AD carriers of GG genotype have higher levels of Non-Cp copper than carriers of AA+AG genotypes

(p value = 0.005)

(Squitti et al., Neuromolecular Med. 2013)

Copper dysfunction in Alzheimer's disease:

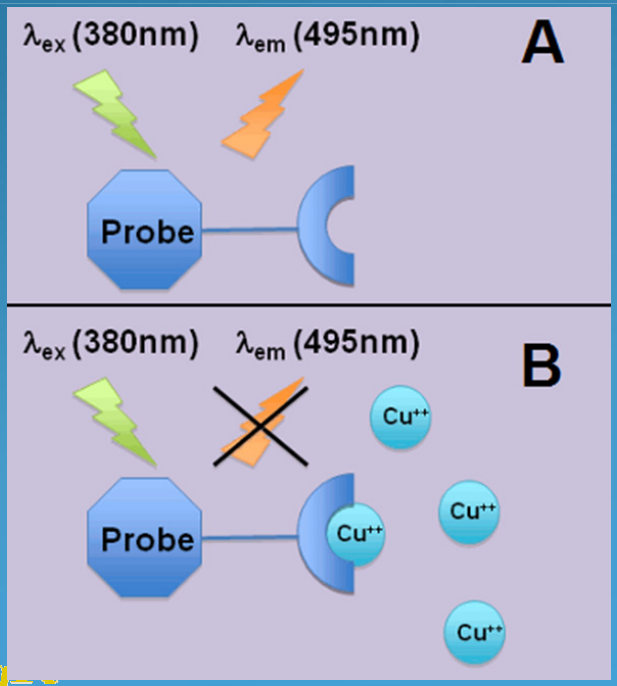
1 patent,
1 CE-certified
blood test for
Non-Cp-Cu
(C4D)

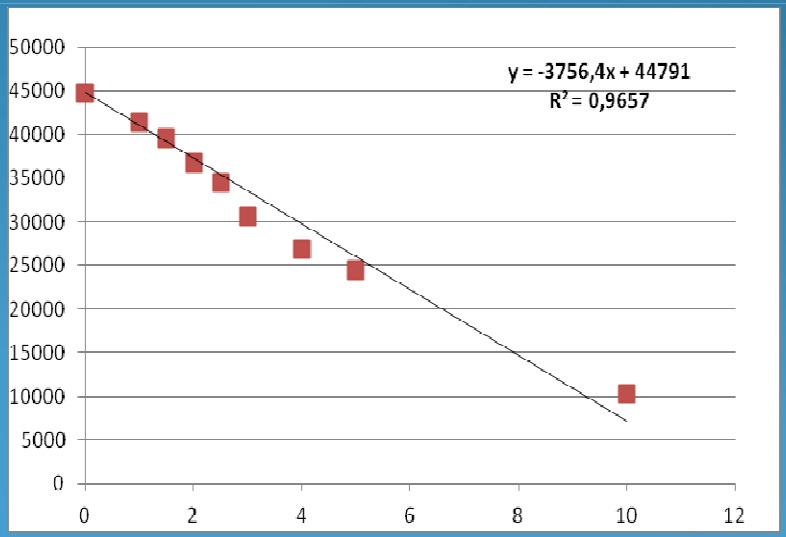


kit to measure Non-Cp copper

Ref Patent:
PCT/EP2012/072063

switch-off coumarin fluorescent probe for detecting Non-Cp copper

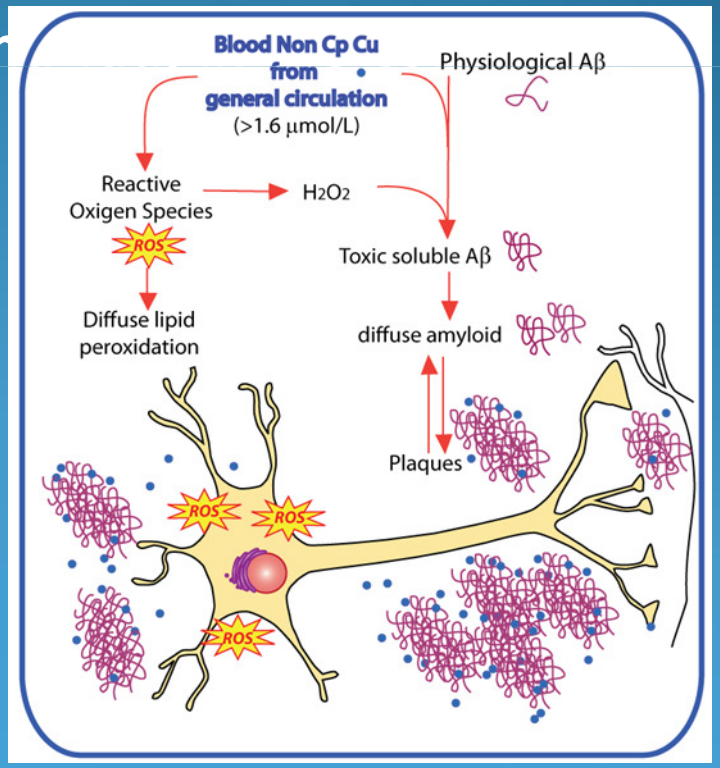




Conclusions

Serum Non-Cp-Cu increases HIGHER than normal values are present in about 50% of amnestic MCI subjects and 60% of AD patients. Non-Cp-Cu correlates with:

- **the major deficits of AD**
- **markers of AD (beta-amyloid ar**
- **Copper in CSF**
- **Clinical worsening**
- **MCI state**
- **MCI conversion to AD**
- **Copper dysfunction has a genetic basis on ATP7B gene variants**



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