Wilson "India" 2020

'Webinar'

Exchangeable Copper

A New biological tool in Wilson Disease

Djebrani-Oussedik Nouzha[1,2], Poujois Aurélia[2,3], Woimant France[2,3], Poupon Joël[1,2].

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ASSISTANCE PUBLIQUE HÔPITAUX DE PARIS

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CRMR Maladie de Wilson et autres maladies rares liées au cuivre

ALIAGE CUIVRE ET AUTRES MÉTAUX

centre de référence maladies rares

Wilson "India" 2020

'Webinar'

Nouzha Djebrani-Oussedik , PharmD, PhD

Toxicology Department
French National reference center for Wilson Disease and other rare copper diseases
Hôpital Universitaire Lariboisière, Paris, France

Disclosure of Interest: Conferences for GMP-Orphan

WD Diagnosis

A combination of majors

- Clinical symptoms
- Imaging
- Biological determinations
- Genetic tests

WD Biology

A classical triad

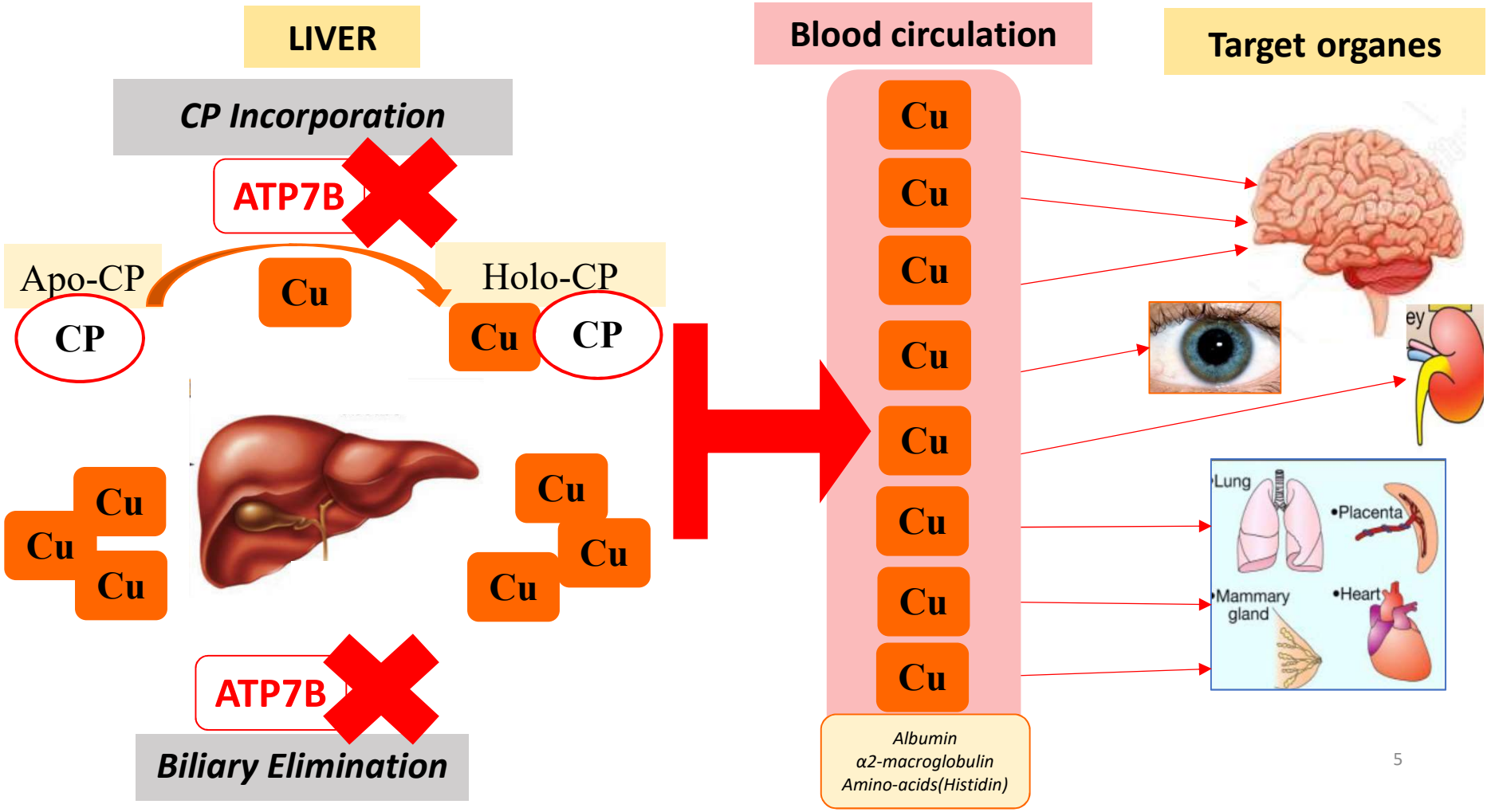
		Reference values	Wilson Disease	Often insufficient to diagnose or exclude WD
Serum ceruloplasmin (CP)	(g/L)	**0,2 - 0,5**	**Low**	*- Normal in some WD subjects* *- Low: 20% heterozygous subjects* *- Increased: inflammation* *- Low: Menkes, Aceruloplasminemia, ACD*
Serum Total Copper (CuT)	(µmol/L)	**12,7 - 22,2**	**Low**	
24 h urinary copper	(µmol/24h)	**0,3 - 0,6**	**Increased > 1,6**	*Collection errors* *Renal impairment* *Other Liver diseases*
Hepatic copper	(µmol/g)	**< 0,4**	**Increased > 4**	*Invasive sampling* *Inhomogeneous liver Cu ➔ False Neg*

Need of another biomarker

WD Physio-Pathology

«Unbound, Free, Toxic" Copper

Hepatic disease → **Multi systemic disease**



Estimating «Unbound, Free, Toxic" Copper

2 distinct ways

Calculated ➔ Non-CP-bound Copper (NCC)

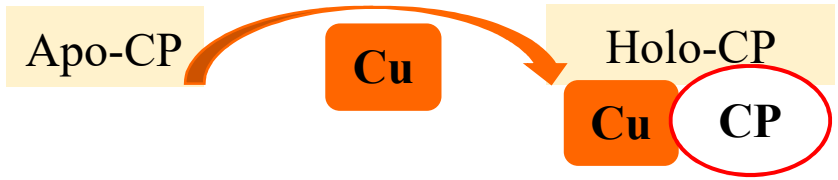
Direct measurement

NCC = Total Cu - 0,049 X CP

Different methods to determine copper fraction in literature

Adequacy of Total Cu and CP measurements

- ❑ **Routine: Immunological detection**
  - ✓ **Apo + Holo**
    - ➔ **Overestimation of Cp-Copper**
    - ➔ **NCC negative values**
  - ✓ **Low CP levels < LoD**
- ❑ **Enzymatic activity determination**
  (Not in all labs)



10% of WD patients have Negative values of NCC at diagnosis

Poujois A, Trocello JM, Djebrani-Oussedik N, et al. Exchangeable copper: a reflection of the neurological severity in Wilson's disease. Eur J Neurol 2017;24,154-60.

Direct determination of Unbound Copper

Narrow collaboration in Paris Lariboisière Hospital

CRMR Maladie de Wilson et autres maladies rares liées au cuivre

Clinical departments

Biological Toxicology Laboratory
Inorganic section

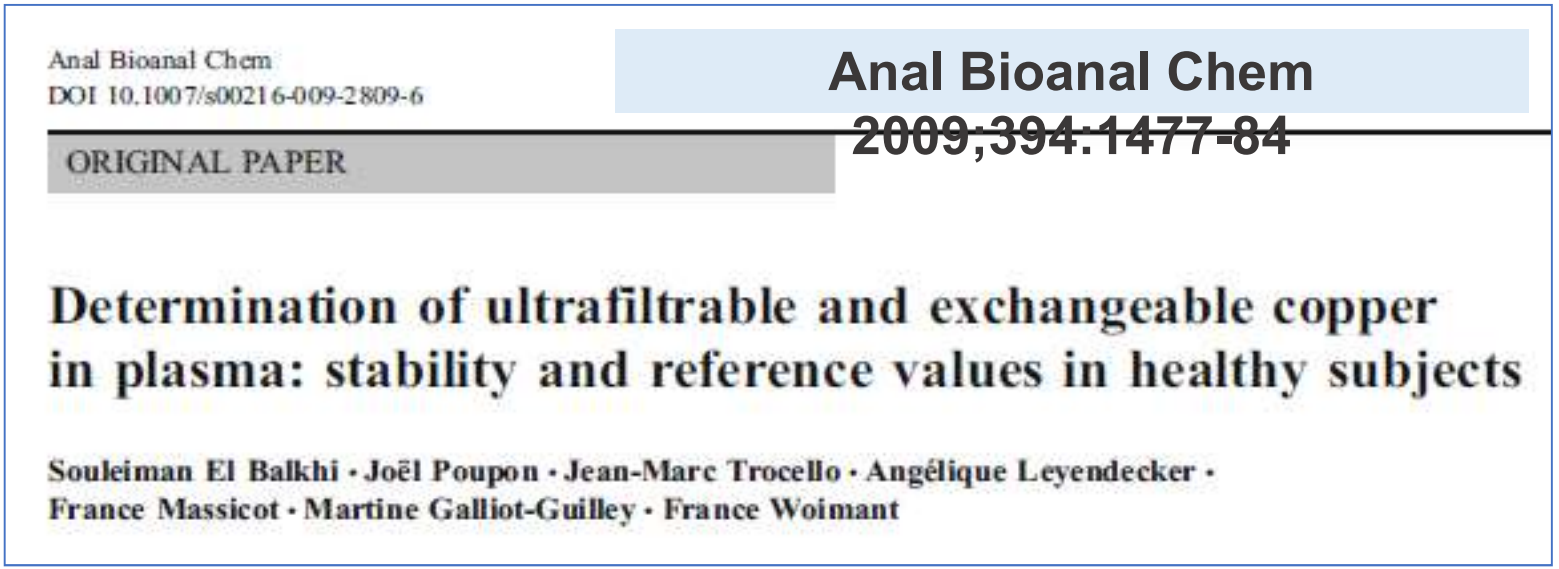
Exchangeable Copper (CuEx)

Relative Echangeable Copper (REC)
REC = Exchangeable Copper / Total Copper (%)

- ✓ **Method**
- ✓ **Contribution in current practice**

Exchangeable Copper (CuEx)

Method



Anal Bioanal Chem
DOI 10.1007/s00216-009-2809-6

Anal Bioanal Chem 2009;394:1477-84

ORIGINAL PAPER

Determination of ultrafiltrable and exchangeable copper in plasma: stability and reference values in healthy subjects

Souleiman El Balkhi · Joël Poupon · Jean-Marc Trocello · Angélique Leyendecker · France Massicot · Martine Galliot-Guilley · France Woimant

- 44 healthy adult volunteers
- 3 WD patients

3 steps

1. Incubation of serum with a chelator
2. Ultra filtration of serum dilution
3. Direct measurement of Cu in ultra filtrate

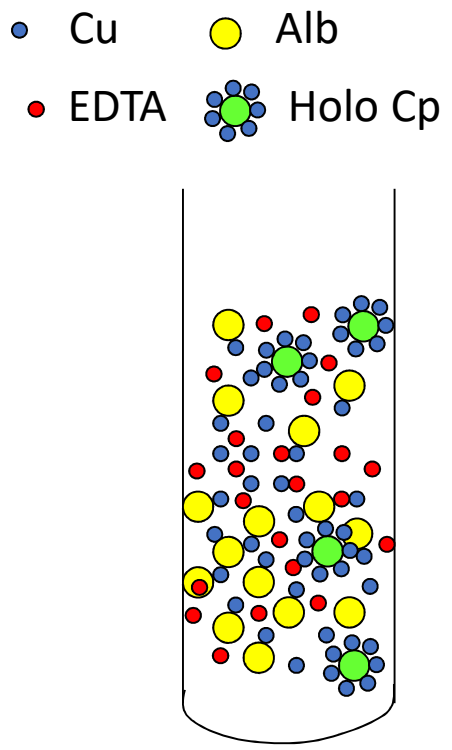
Exchangeable Copper (CuEx)

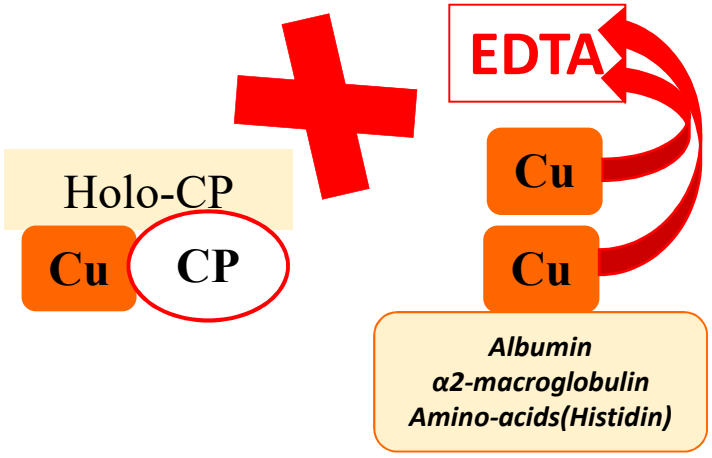
Anal Bioanal Chem
2009;394:1477-84

1. Incubation of serum with a chelator

Copper mobilization

- *Serum + EDTA 3 g/L (v/v)*
- *60 min*
- *Room temperature (20-25 °C)*





Exchangeable Copper (CuEx)

Method

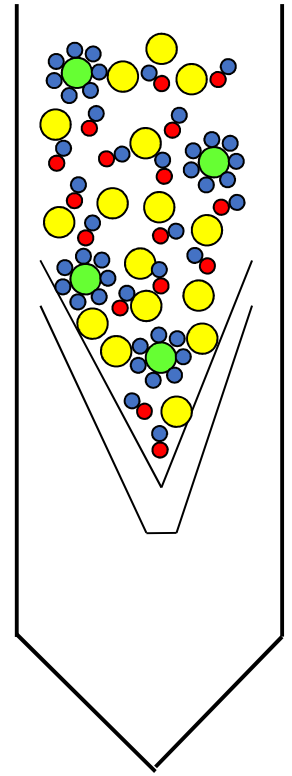
Anal Bioanal Chem 2009;394:1477-84

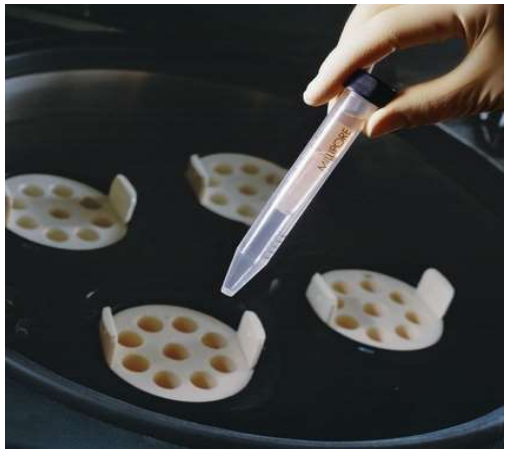
2. Ultra filtration

- Membrane filtration / *Centrifugation*
- *Cut-off* = **30 000 Da**
- **Retained** ➔ Proteins of higher molecular weight
  - ❑ **Ceruloplasmin : 132 000 Da**
  - ❑ **Albumin : 67 000 Da**

Cu Alb

EDTA Holo Cp





Amicon® Ultracel® 30K

Exchangeable Copper (CuEx)

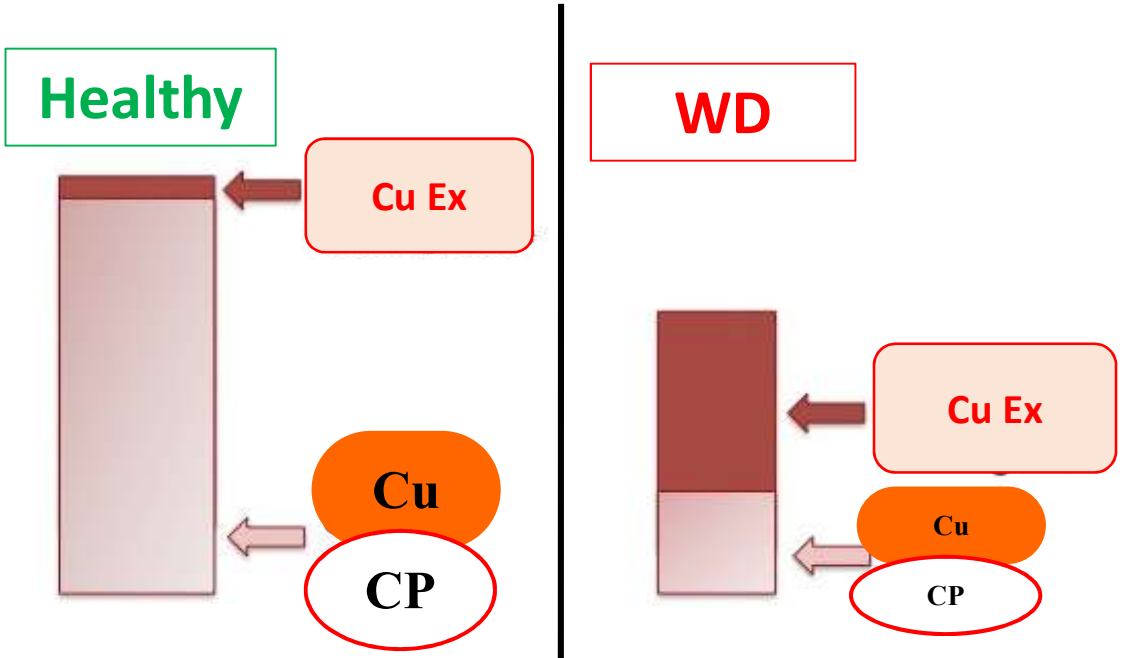
3. Direct measurement of Copper in ultra filtrate

	Total Copper (T Cu)		Exchangeable Copper (CuEx)
Analytical techniques	Healthy	WD	Healthy and WD
Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES)	+	-	-
Flame Atomic Absorption spectroscopy (AAS)	+	-	-
Electro Thermal Atomic Absorption spectroscopy (ET- AAS) *(graphite tube)*	+	+	+
Induced Plasma Mass Spectrometry (ICP-MS)	+	+	+

Relative Exchangeable Copper

REC = Exchangeable Copper / Total Copper (%)

The part of toxic copper



REC (%)	3 - 8%	> 18.5%

Slightly Lower Reference values and Cut off in children
(Lyon: unpublished Data)

CuEx and REC

Sampling, Stability, Transport

Samples Collection

EDTA Tubes ➔ Prohibited

Stability

CuEx

Room T°	**24 - 48 Hours**
– 20 °C	**Stable to 90 days**

Specimen Handling and Transport

- One day shipping ➔ **Possible transport at room T° or refrigerated**
- Longer shipping ➔ **Specific cautions**
  - ✓ *Tube centrifugation*
  - ✓ *Serum / Plasma Transfert*
  - ✓ ***Frozen***
  - ✓ ***Transport frozen***

CuEx and Relative Exchangeable Copper

Contribution in current practice

REC

WD Diagnosis

REC WD Diagnosis

Clinica Chimica Acta. 2011;412(23-24):2254-60

Clinica Chimica Acta 412 (2011) 2254–2260

Contents lists available at SciVerse ScienceDirect

Clinica Chimica Acta

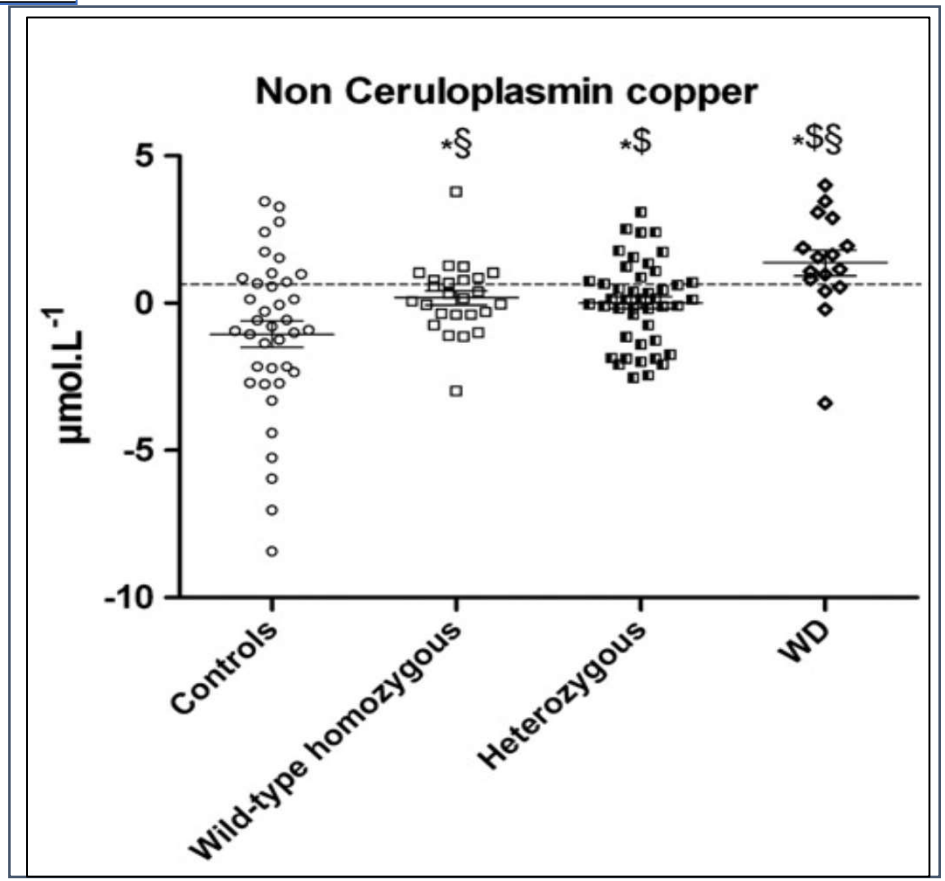
journal homepage: www.elsevier.com/locate/clinchim

Relative exchangeable copper: A new highly sensitive and highly specific biomarker for Wilson's disease diagnosis

Souleiman El Balkhi [a,d], Jean-Marc Trocello [b,d], Joël Poupon [a,*], Philippe Chappuis [c], France Massicot [e], Nadège Girardot-Tinant [d], France Woimant [b,d]

- *Controls: 62 healthy adult volunteers*
- *25 wild type homozygous*
- *45 heterozygous*
- *16 newly diagnosed WD before treatment*

REC (cut off = 18.5%)
≈ 100 % Sensitivity
≈ 100 % Specificity



- ❖ **False +/- with all other tested parameters**
- ❖ **NCC :**
  - ➢ **2 Negative values**
  - ➢ **2 false negative**
  - ➢ **27% false positive**

Very Quick results

CuEx and Relative Exchangeable Copper

Contribution in current practice

REC

WD Diagnosis

WD Family screening

REC WD Family screening

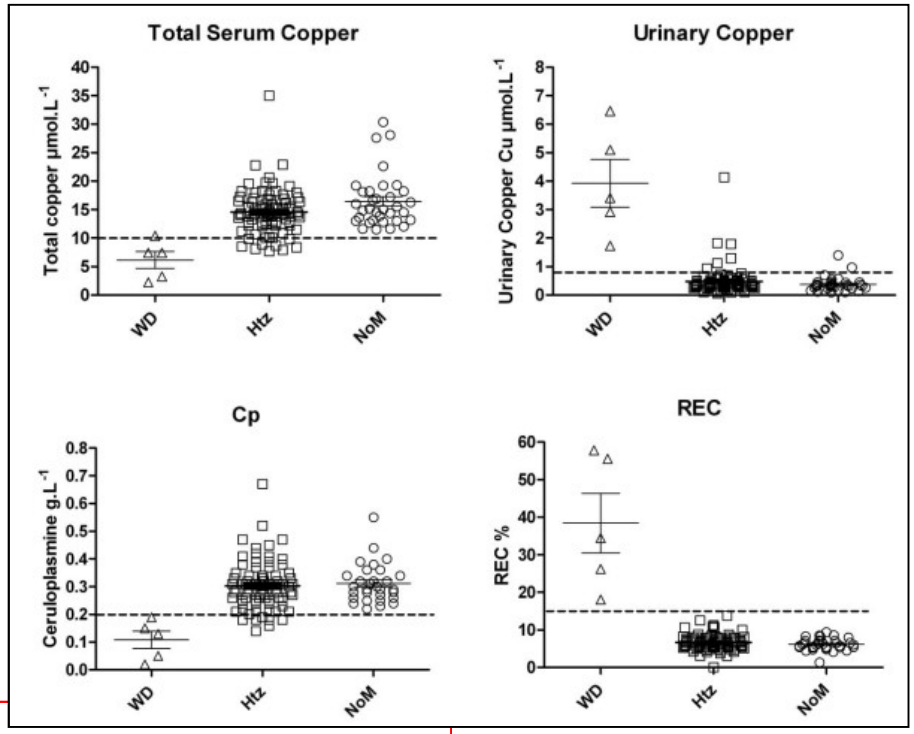
Relative Exchangeable Copper: A Promising Tool for Family Screening in Wilson Disease

Jean-Marc Trocello, MD, PhD,[1*]
Souleiman El Balkhi, PhD,[1,2] France Woimant, MD,[1]
Nadège Girardot-Tinant, PhD,[1] Philippe Chappuis, PhD,[3]
Carla Lloyd, MD,[4] and Joël Poupon, PhD[2]

Mov. Disord., 2014, 29(4) : 558-562

126 clinically asymptomatic subjects
in context of family screening for WD (3 groups):

- *WD (n=5)*
- *Htz (n=87)*
- *NoM (n=34)*



REC (Cut off = 15%)

➔ The only parameter tested able to discriminate Htz and WD (No Overlapping results between Htz / WD)

➔Practical and fast answer for parents or siblings

➔Avoid more invasive investigations

CuEx and Relative Exchangeable Copper

Contribution in current practice

REC

WD Diagnosis

WD Family screening

WD Differential Diagnosis

REC WD Differential Diagnosis

30 patients: Non-Wilsonian cirrhotic hepatopathies

➔REC : Normal for all patients

➔No false positives

➔13%: copper tests abnormalities

REC: Quickly eliminates or confirms a WD

El balkhi S., Poupon J., Marc Trocello J.-M. Woimant F., Tinant N., Ozenne V. Relative exchangeable copper in non Wilson cirrhotic patients.
63rd Annual meeting of the AASLD, Boston, November 9-13, 2012.

REC WD Differential Diagnosis

Received: 28 October 2016 | Accepted: 13 July 2017
DOI: 10.1111/liv.13520

Liver Int 2018;38:350-7

WILEY Liver INTERNATIONAL

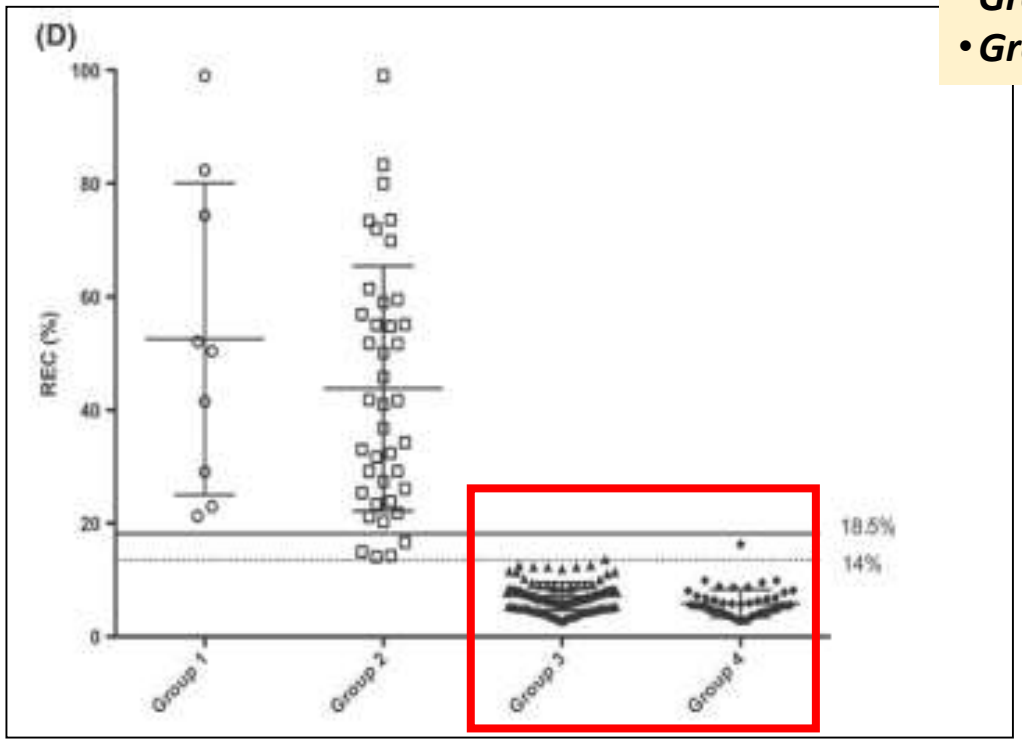
ORIGINAL ARTICLE

Relative exchangeable copper: A valuable tool for the diagnosis of Wilson disease

Olivier Guillaud[1,2] | Anne-Sophie Brunet[1,3] | Isabelle Mallet[1,4] | Jérôme Dumortier[1,2,5] | Martine Pelosse[1,3] | Sophie Heissat[1,3] | Christine Rivet[1,3] | Alain Lachaux[1,3,5] | Muriel Bost[1,4,6]

201 patients

- ***Group 1: 9 WD at diagnosis or non-compliant***
- ***Group 2: 40 WD treated***
- ***Group 3: 103 adults non-WD hepatic diseases***
- ***Group 4: 49 children non-WD hepatic diseases***



➔ **Normal REC (<18.5%) in all "Non-WD hepatic diseases"**

REC WD Differential Diagnosis

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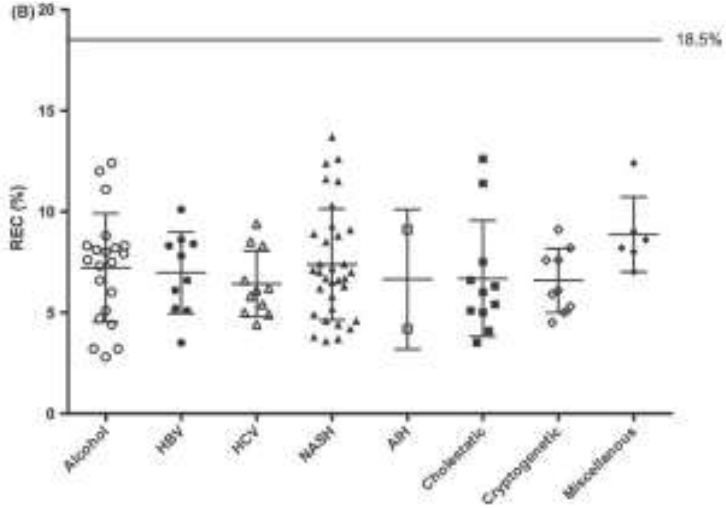
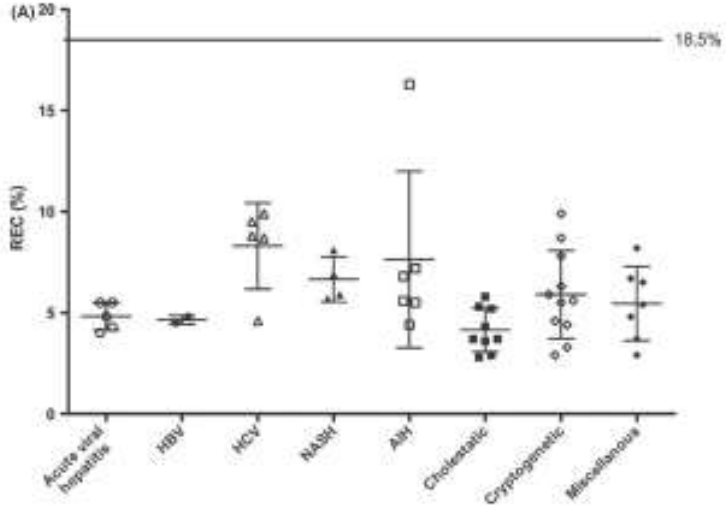
High Specificity

➔REC is normal in a variety of other chronic liver diseases of adults and children

Adults



Children



CuEx and Relative Exchangeable Copper

Contribution in current practice

REC

- **WD Diagnosis**
- **WD Family screening**
- **WD Differential Diagnosis**

CuEx

- **WD Extra hepatic severity**

CuEx WD Extra hepatic severity

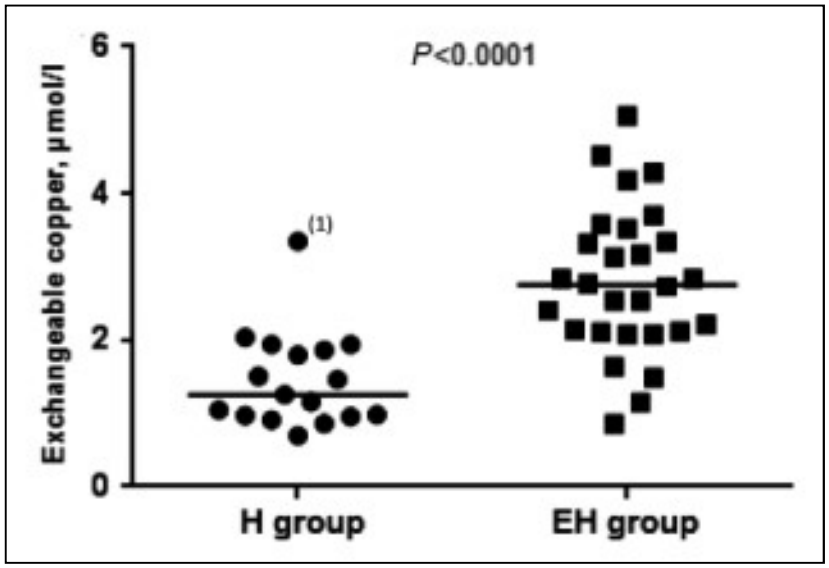
european journal of neurology
the official journal of the european academy of neurology
ean

Original Article

Exchangeable copper: a reflection of the neurological severity in Wilson's disease

A. Poujois, J.-M. Trocello, N. Djebrani-Oussedik, J. Poupon, C. Collet, N. Girardot-Tinant, R. Sobesky, D. Habès, D. Debray, C. Vanlemmens, F. Fluchère, F. Ory-Magne, J. Labreuche, C. Preda, F. Woimant

First published: 14 October 2016 | https://doi.org/10.1111/ene.13171 | Cited by: 10



48 new WD patients

- ***17 Hepatic***
- ***28 Extra Hepatic***

CuEx

➔**Higher in patients with extra-hepatic involvement**

➔**If > 2.08 µmol/L : look after neurological signs**

➔**Correlated with:**

- ✓ **Severity of neurological impairment (UWDRS)**
- ✓**Copper diffusion in the eyes and brain (Corneal deposits/MRI cerebral lesions)**

➔ **Not correlated with hepatic severity**

CuEx and Relative Exchangeable Copper

Contribution in current practice

REC

- **WD Diagnosis**
- **WD Family screening**
- **WD Differential Diagnosis**

CuEx

- **WD Extra hepatic severity**
- **Follow Up of treated patients**

CuEx Follow Up of treated patients

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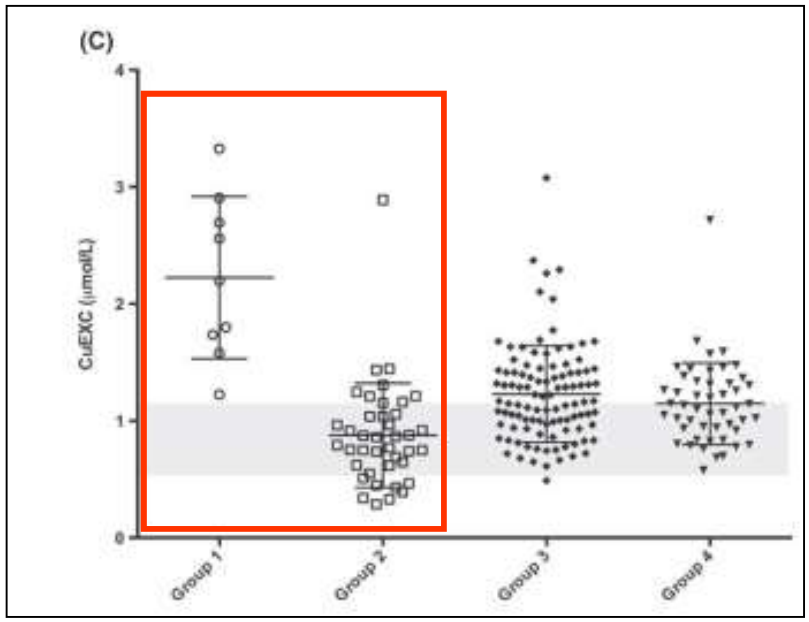
Liver Int 2018;38:350-7
WILEY Liver INTERNATIONAL

201 patients

- ***9 WD at diagnosis or non-compliant***
- ***40 WD treated***
- *103 adults non-WD hepatic diseases*
- *49 children non-WD hepatic diseases*

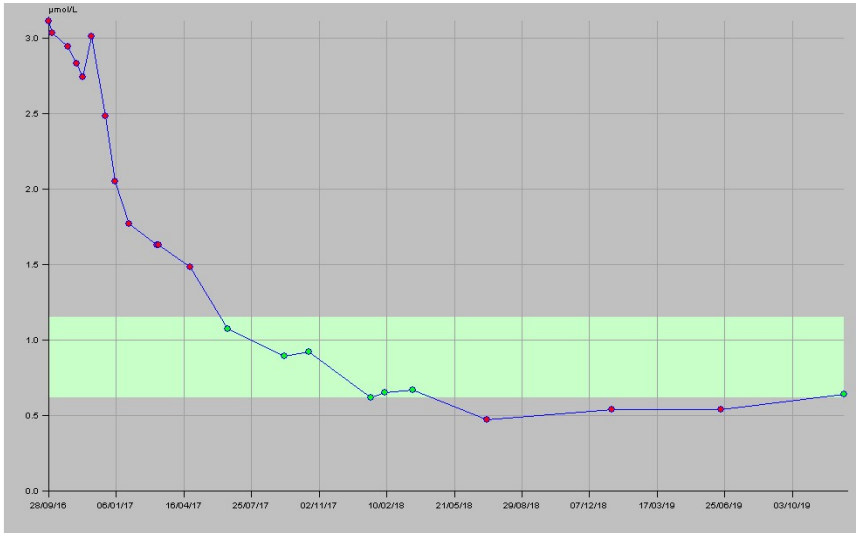


➔ **Increased CuEX In non-compliant patients**
➔ **Normal or low CuEX in compliant patients**

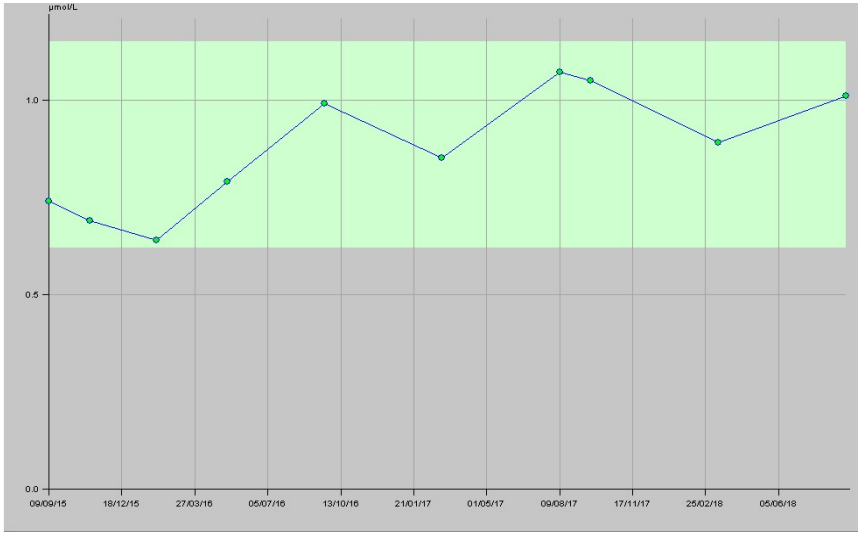
CuEx

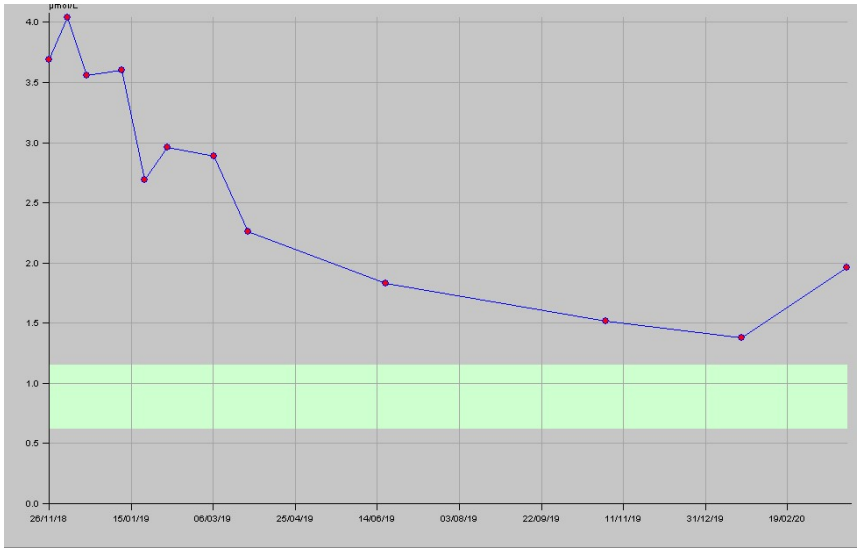
Follow Up of treated patients

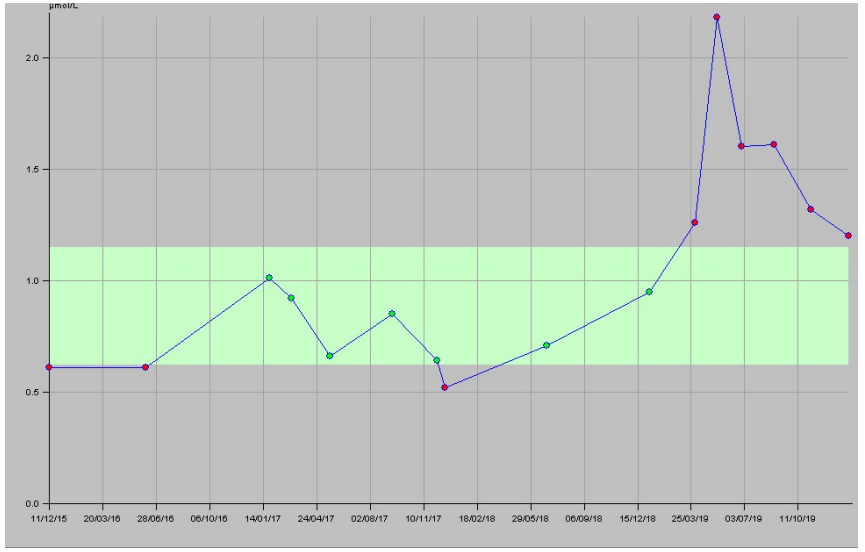
CuEx (µmol/L)



CuEx (µmol/L)







CuEx and Relative Exchangeable Copper

REC

- WD Diagnosis
- WD Family screening
- WD Differential Diagnosis

CuEx

- WD Extra hepatic severity
- Follow Up of treated patients

CuEx and REC in animal models

CuEx and Relative Exchangeable Copper

CuEx and REC ➔ Validated in animal models

Schmitt F, Podevin G, Poupon J, et al. Evolution of exchangeable copper and relative exchangeable copper through the course of Wilson's disease in the Long Evans Cinnamon rat.
PLoS One 2013;8:e82323.

- *Control : **LE rats***
- *WD model : **LEC rats***

REC :
➔ Non-invasive and reliable tool for WD diagnostic in LEC rats
➔ Not influenced by liver damage or by the copper intake regimen

Heissat S, Harel A, Um K, Brunet AS, et al. Evaluation of the accuracy of exchangeable copper and relative exchangeable copper (REC) in a mouse model of Wilson's disease. J Trace Elem Med Biol 2018;50:652-7.

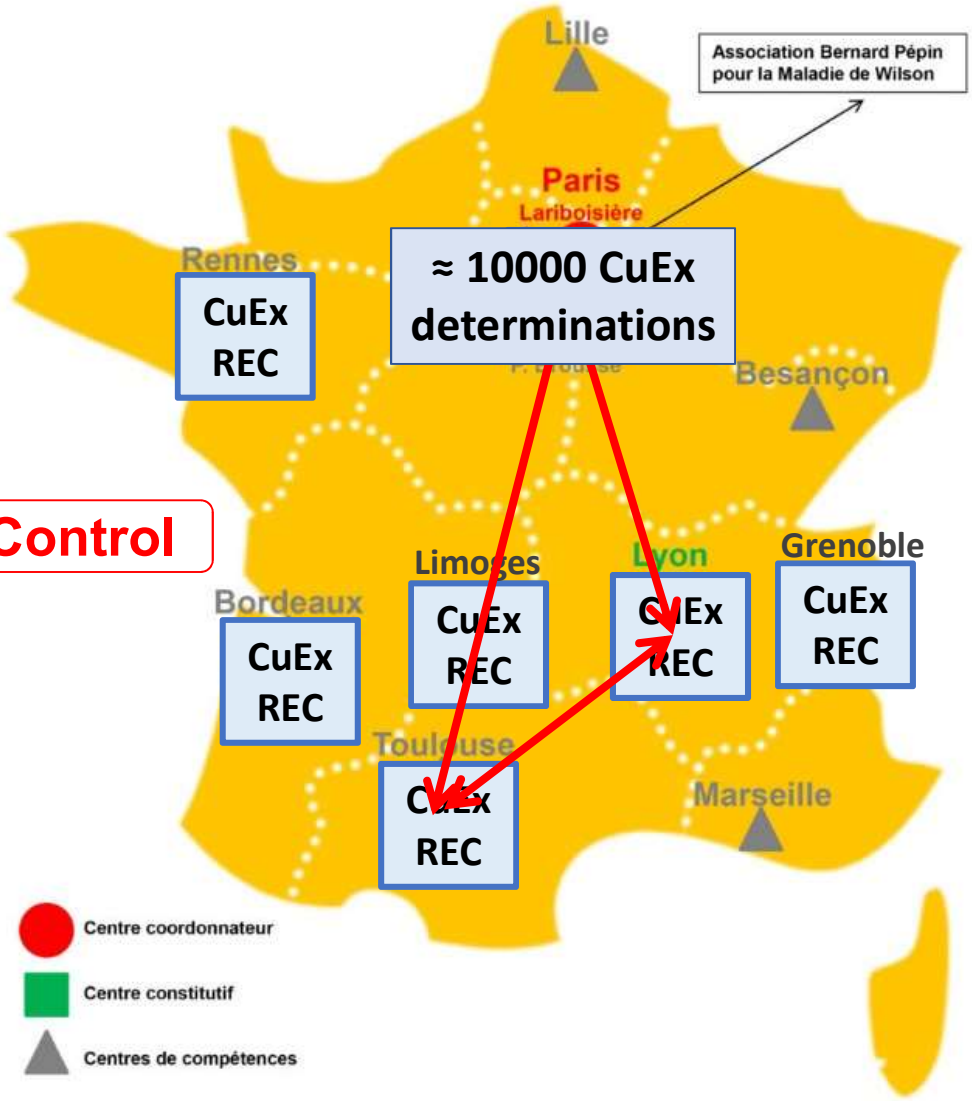
- Control : **Atp7b−/−**
- WD model : **WT (C57BL/6)**

➔ REC Significantly higher in Atp7b −/− mice
➔ CuEx Lower in treated mice

Conclusion

10 years of CuEx and REC

Inter Laboratory Control



Other countries

Conclusion

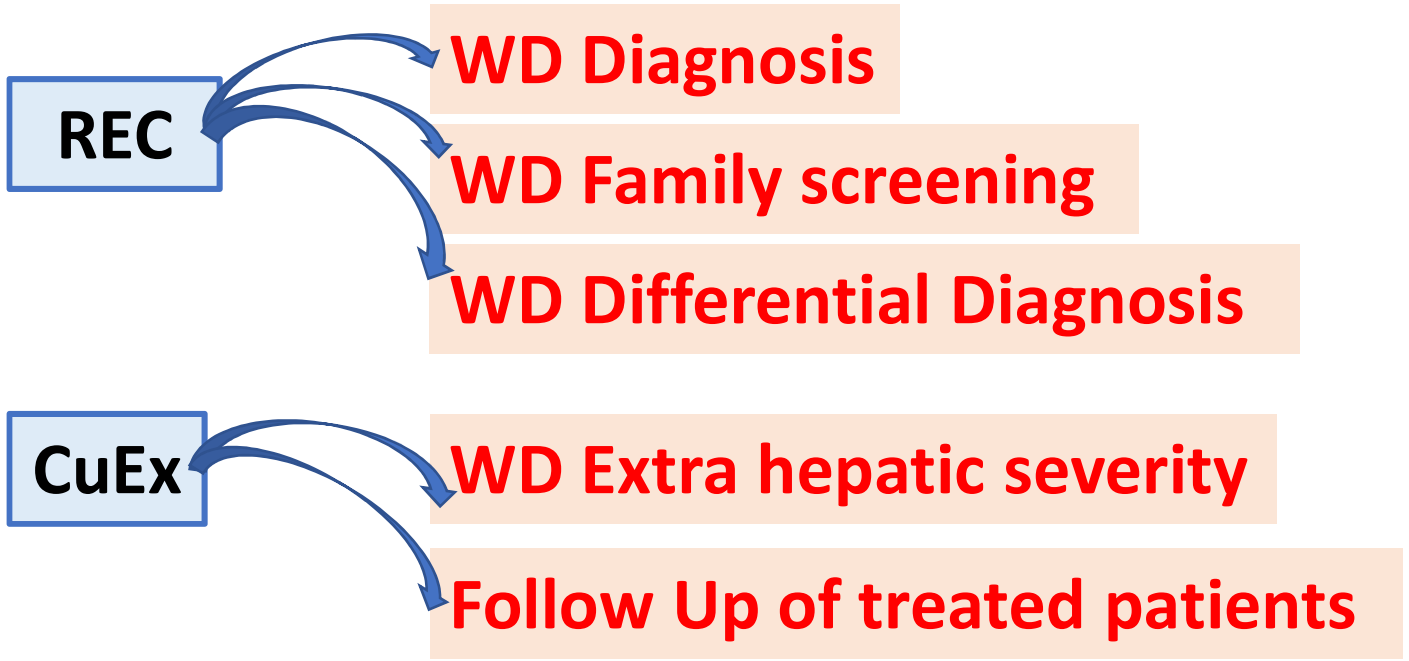
WD Biology: The classical triad + New tools

		Reference values	Wilson Disease
Serum ceruloplasmin (CP)	(g/L)	**0,2 - 0,5**	**Low**
Serum Total Copper (CuT)	(µmol/L)	**12,7 - 22,2**	**Low**
24 h urinary copper	(µmol/24h)	**0,3 - 0,6**	**Increased > 1,6**
Hepatic copper	(µmol/g)	**< 0,4**	**Increased > 4**
Exchangeable Copper (CuEX)	(µmol/L)	**0,62 - 1,15**	**Normal or increased**
Relative CuEx (REC)	(%)	**3 - 8%**	**> 18%**

✓Quick
✓Reliable
✓Useful

Conclusion

✓New tools introduced in routine frame work



✓New tools Can be used in clinical trials

Data demonstrating reliability and usefulness

Validated in animal models → **Preclinical steps**

Wilson "India" 2020

'Webinar'

Thank you