Copper and the liver

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- **Copper is a essential micronutrient**

Enzyme	Function
Cytochrome-*c* oxidase	Electron transport, terminal oxidase
Superoxide dismutase	Superoxide dismutation
Catechol oxidase	Synthesis of melanin
Protein-lysine 6-oxidase	Collagen and elastin cross-linking
Ceruloplasmin	Ferroxidase
Amine oxidases	Deamination of primary amines
Dopamine-β-monooxygenase	Dopamine→norepinephrine
Peptidylglycine monooxygenase	α-Amidation of neuropeptides

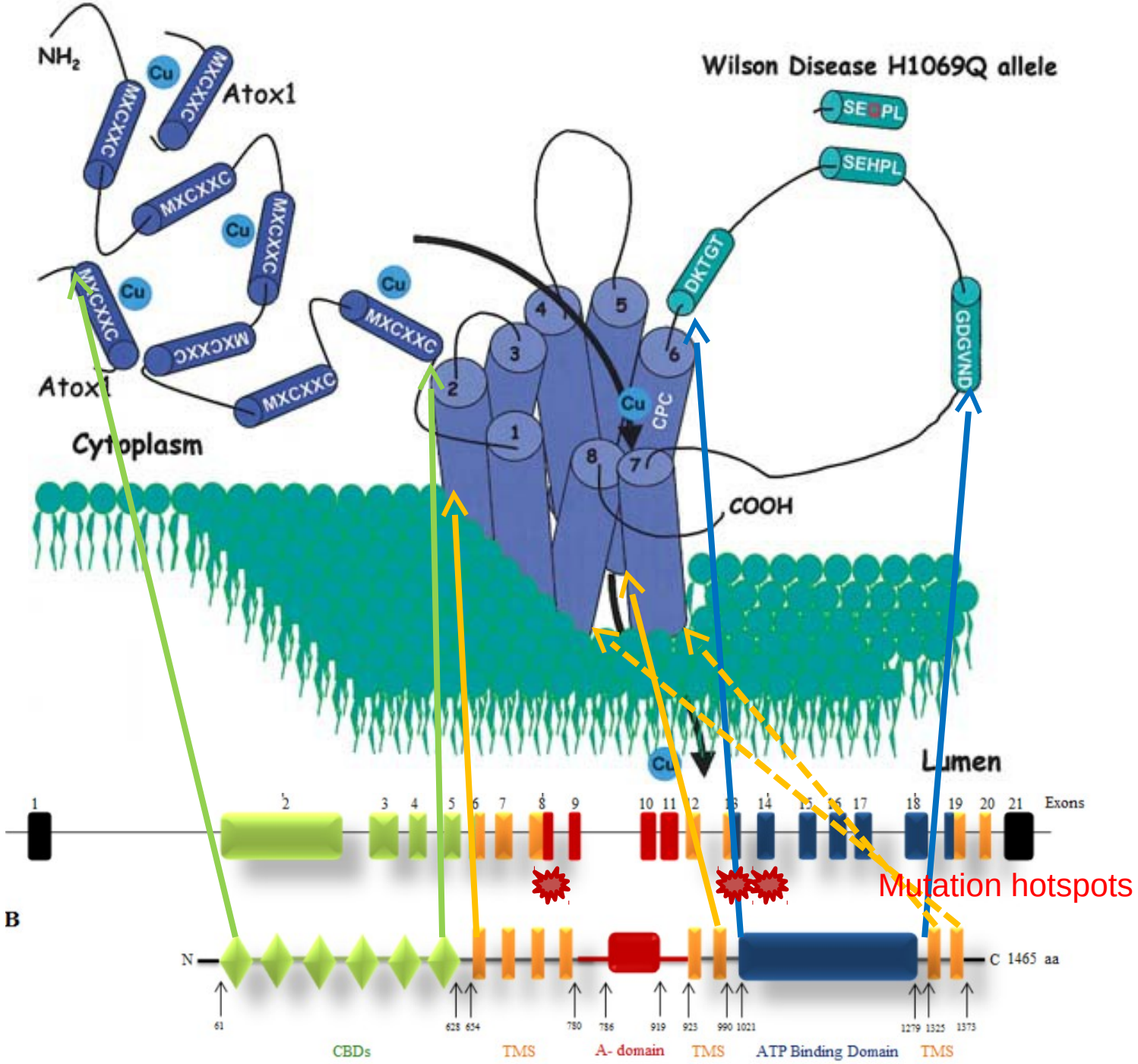
- Liver is the main storage site for copper
- Non protein bound copper is toxic

Both high and lower hepatic copper content have a pathophysiologic relevance

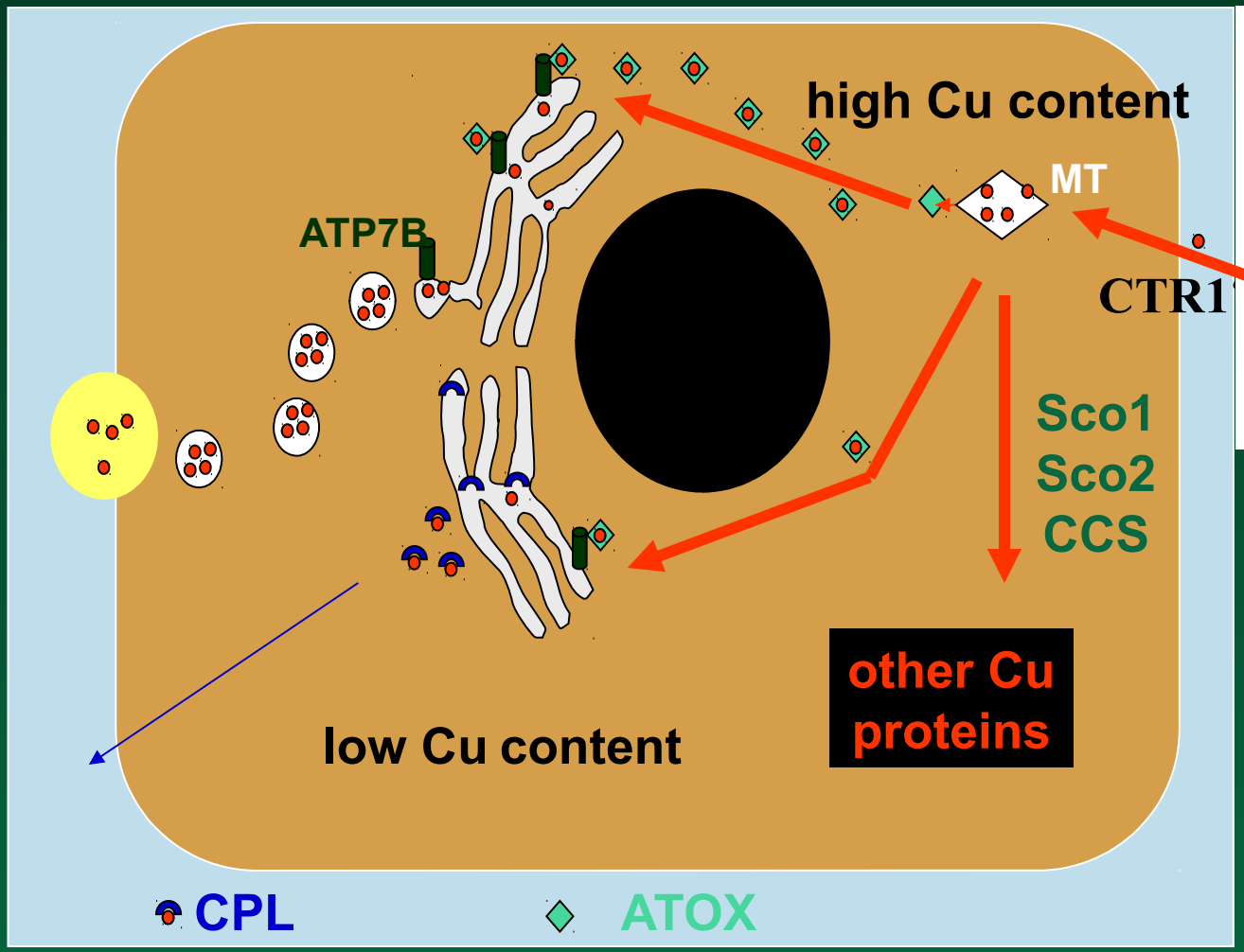
- Too much copper
  - M.Wilson
  - Cholestasis
  - Indian childhood cirrhosis
- Copper deficiency
  - NASH/NAFLD
  - Malabsorption syndromes

M.Wilson

- misconceptions in textbooks
  - Wilson disease is a very rare disease (recent data suggest a heterozygotes frequency of 1:21!)
  - Wilson disease occurs only in children and young adults
  - Wilson disease is just a neurologic disease
  - Wilson disease can be excluded if ceruloplasmin is normal


Wilson Disease H1069Q allele
NH2
Cu
MXCXXC
Atox1
Atox1
Cytoplasm
SEQPL
SEHPL
DKTGT
GDGVND
CPC
COOH
Lumen
Exons
Mutation hotspots
B
N
C 1465 aa
61
628 654
780 786
919 923
990 1021
1279 1325 1373
CBDs
TMS
A- domain
TMS
ATP Binding Domain
TMS

Copper transport in hepatocytes



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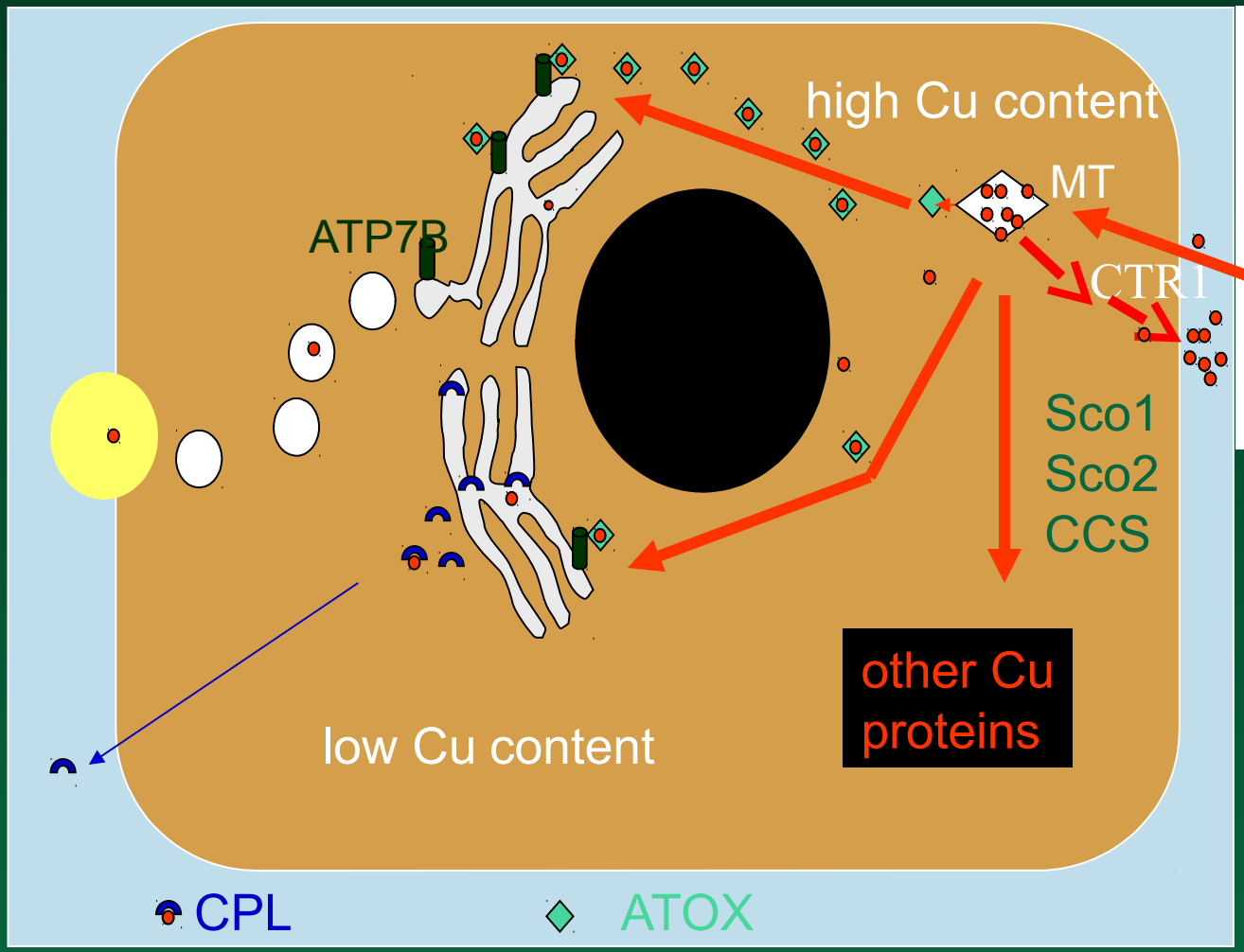
Copper transport in hepatocytes – Wilson disease



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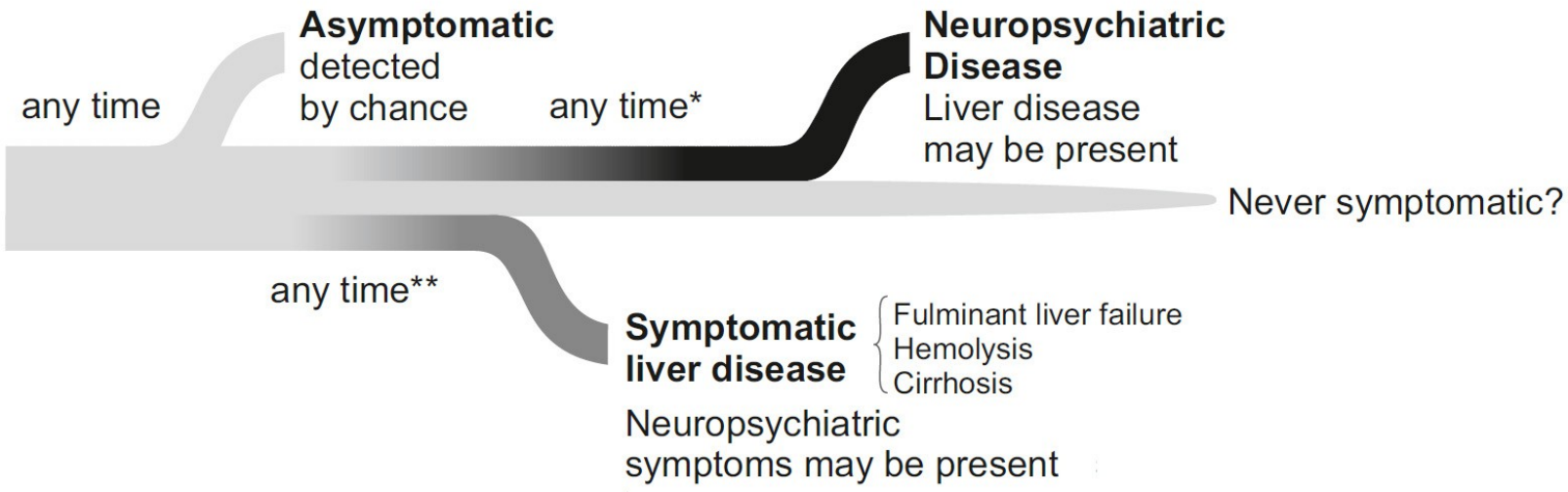
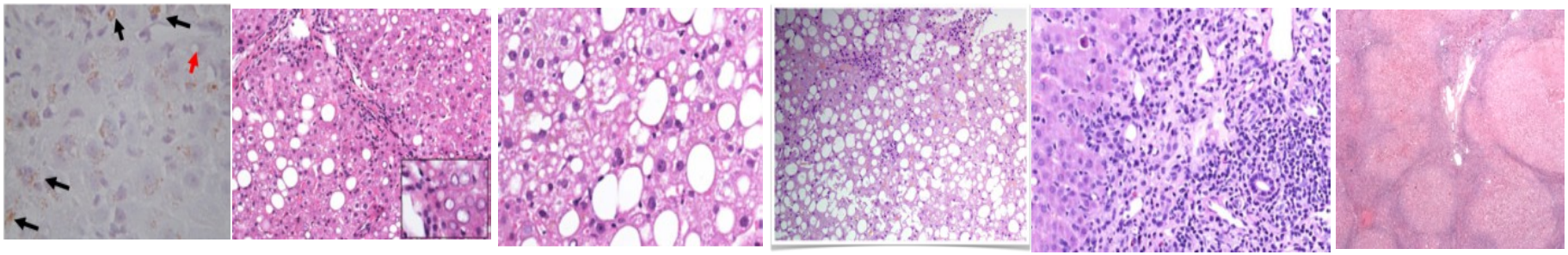


Ferenci et al, Hepatology 2019



Rhodanine pos granula (black arrow), glycogenated nuclei (red arrow)

Simple steatosis
(HE stain)

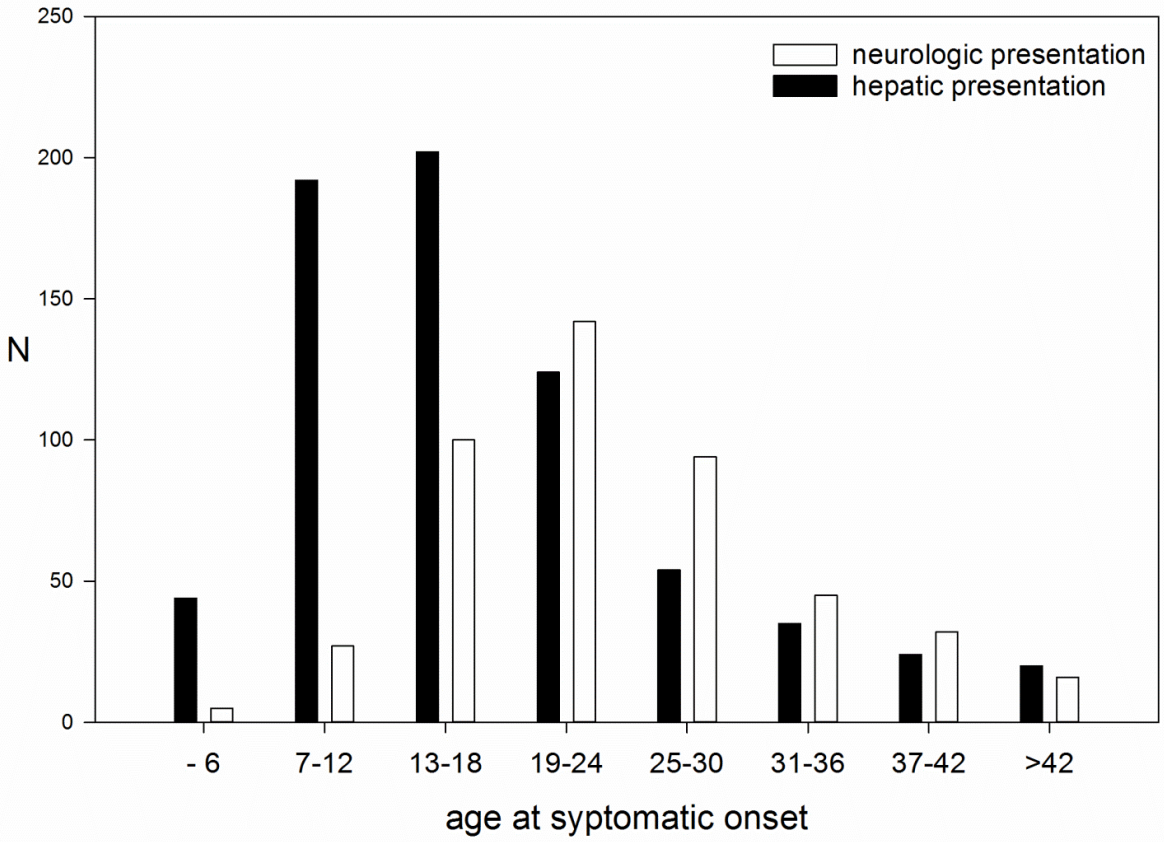
NASH like picture
(HE stain)

AIH like picture
(HE stain)

Cirrhosis
(HE stain)

Ferenci P. Ott P J. Hepatology 201

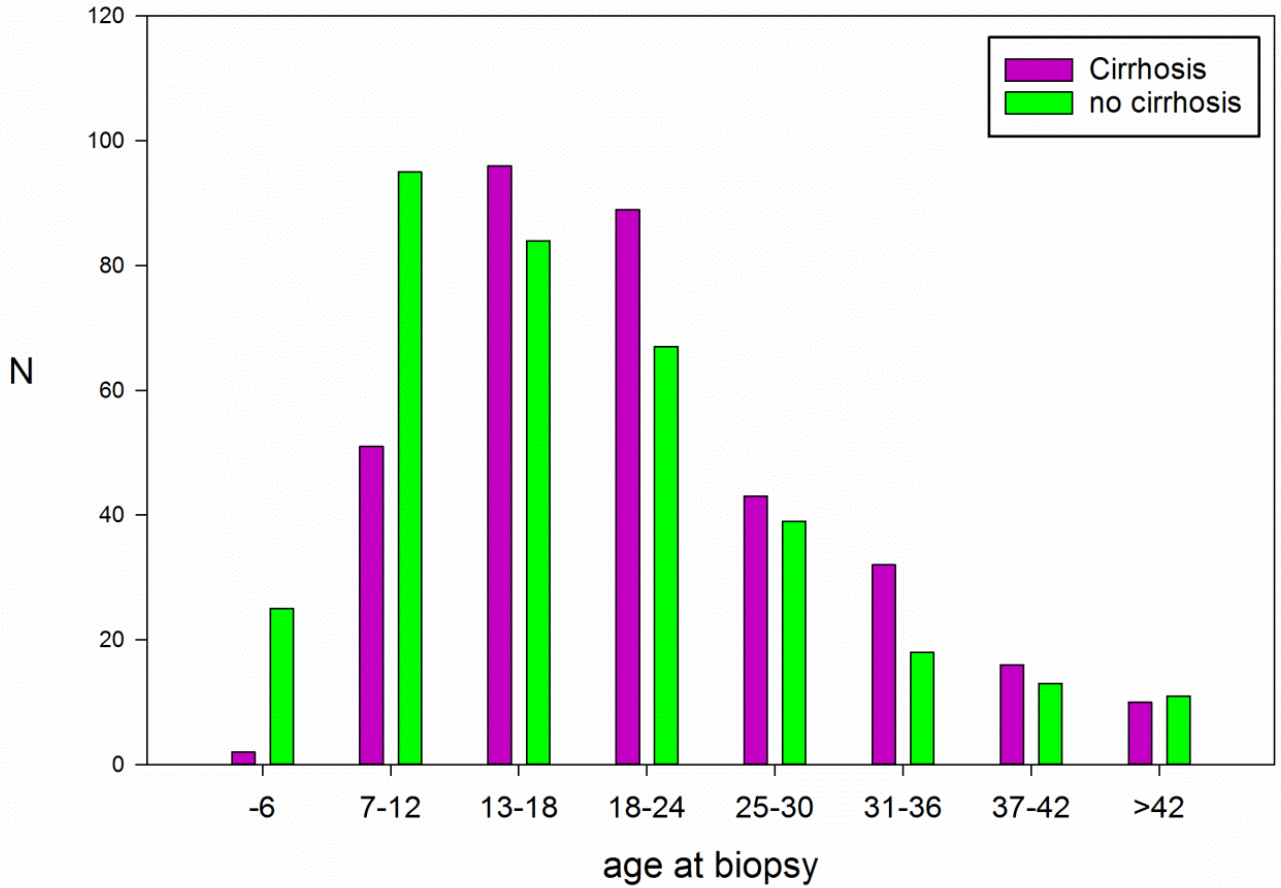
Age at symptomatic onset



may become symptomatic at any age

Ferenci et al,Hepatology 2019 (doi: 10.1002/hep.30280)

Liver biopsy findings at diagnosis

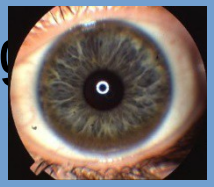


	-6	7-12	13-18	18-24	25-30	31-36	37-42	>42
N with biopsy/N total	27/65	146/269	180/356	159/296	79/167	50/87	30/59	27/41
% with cirrhosis:	7.4%	34.9%	53.3%	57.1%	52.4%	64.0%	55.1%	47.6%

only 50% develop cirrhosis

Ferenci et al,Hepatology 2019 (doi: 10.1002/hep.30280)

Diagnostic tests for Wilson disease

	abnormal	caveat
KF-Ring 	present	
Neuropsych. symptoms	present	May be nonspecific
Caeruloplasmin	< 20 mg/dl	May be normal in hepatic WD Low in malabsorption
Urinary Cu excretion	>100 mg/24h	Not useful when anuric
Hepatic copper content	> 250µg/g dry weight	May be <250 µg/g in 20%, increased in cholestasis
genetics	2 disease causing mutations*	Present only in 75%

* definition?

Ceruloplasmin for diagnosis of Wilson disease

- Low ceruloplasmin – low sensitivity and specificity (useless for screening!!)
- ceruloplasmin level increased by:
  - inflammation (e.g.. hepatitis)
  - female sex hormones (pregnancy, oral contraceptives)
- ceruloplasmin level decreased by:
  - low copper supply (celiac disease, malnutrition)
  - decoppering therapy
  - decreased hepatic production (liver failure!)
  - aceruloplasminemia

Urinary copper excretion for diagnosis of Wilson disease

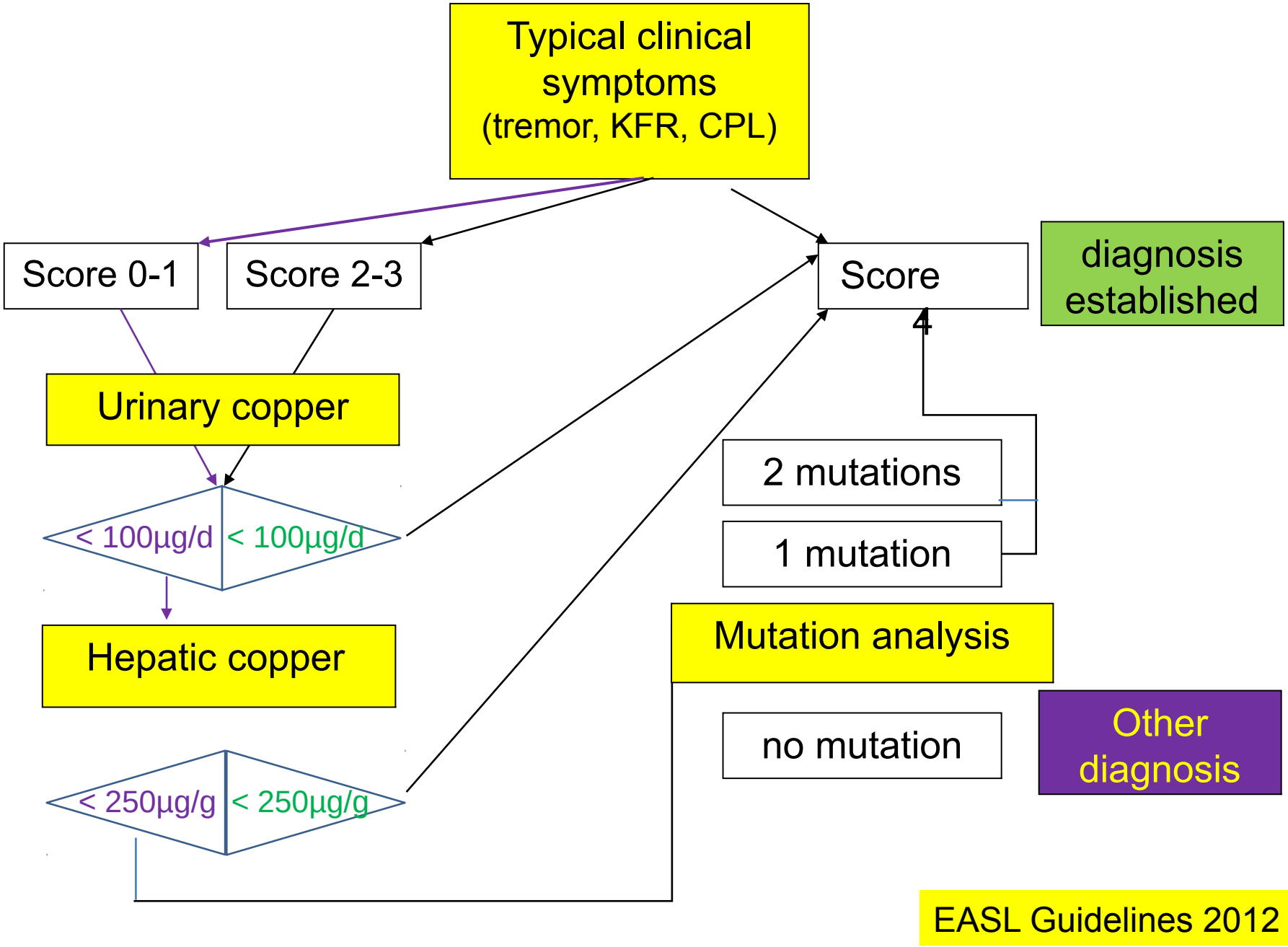
- reflects „free“ copper in blood
  - only increased when hepatic (and extrahepatic?) copper storage capacity is exhausted or
  - when copper is released from necrotic hepatocytes
- cannot be measured in anuric pts.

WD-diagnostic score (Leipzig 2001)

- KFR (2/0)
- CPL < 10 (2), 10-20 (1), > 20 (0) mg/dl
- Neuro (2/0)
- hepatic copper > 250 (2), 50-250 (1), < 50 (-1) µg/g
- urinary copper (2/0)
- Disease causing mutation 2 (4), 1 (1)

SCORE ≥4	diagnostic
SCORE 3	diagnosis possible
SCORE ≤ 2	diagnosis unlikely

EASL Guidelines 2012


Typical clinical symptoms (tremor, KFR, CPL)
Score 0-1
Score 2-3
Score
diagnosis established
Urinary copper
2 mutations
1 mutation
< 100µg/d
< 100µg/d
Hepatic copper
Mutation analysis
no mutation
Other diagnosis
< 250µg/g
< 250µg/g
EASL Guidelines 2012

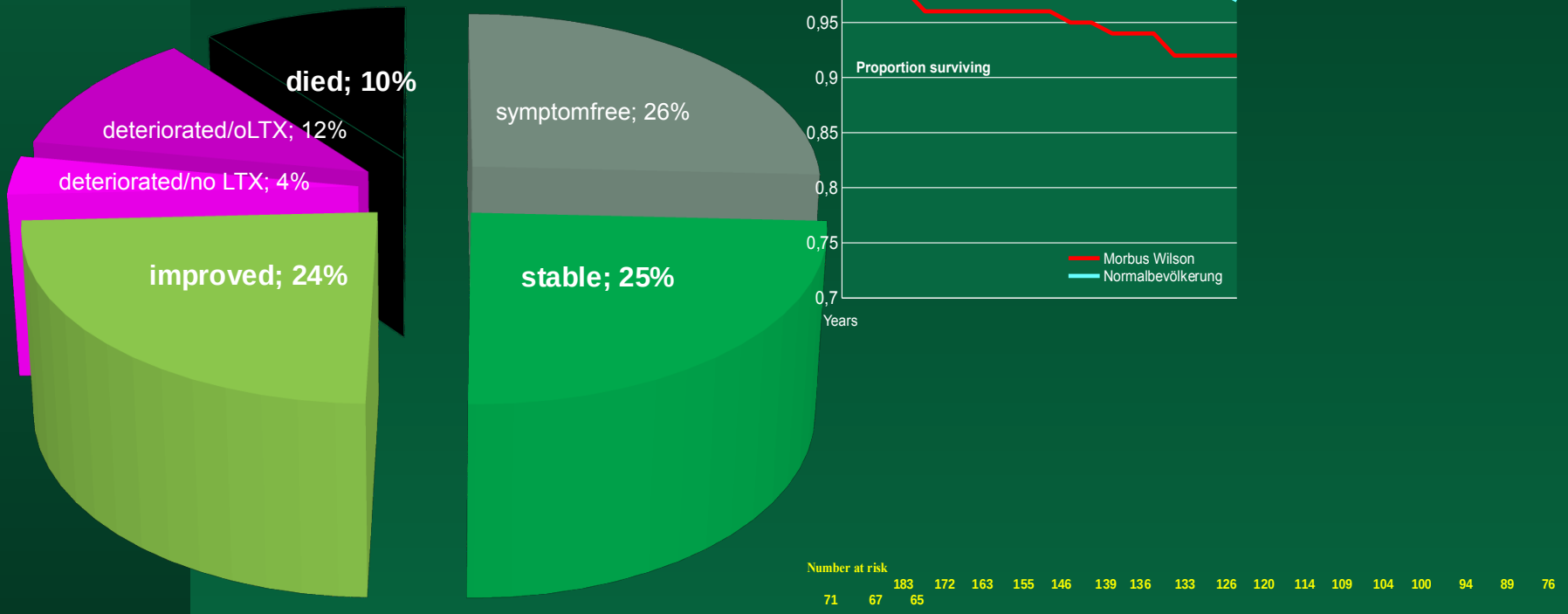
M.Wilson- treatment

- copper chelators
  - D-Penicillamine
  - Trientine
  - (BAL)
  - (tetrathiomolybdate)
- Inhibition of intestinal copper uptake
  - Zinc-acetate
- LTX

EBM? - Evidence or eminence based medicine

Longterm outcome of patients with Wilson disease

229 Austrian patients
mean observation period was 14.8 – 11.4 years (range, 0.5–52.0 years)



Beinhardt et al, CGH 2014

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Take home message Wilson disease

- Highly variable clinical presentation
- No clear cut evidence for a correlation genotype-phenotype
- Cofactors:
  - Genetic factors?
  - Environmental factors?
- Message for hepatologists: consider Wilson disease in any patient with unclear liver disease! IT IS TREATABLE!

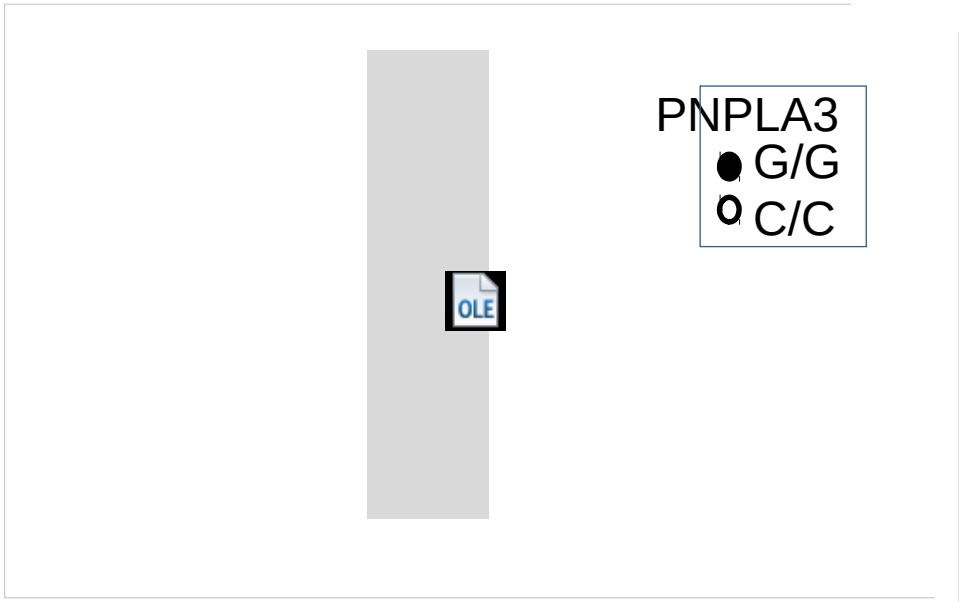
- Copper deficiency
  - NASH/NAFLD

NAFLD and hepatic copper content

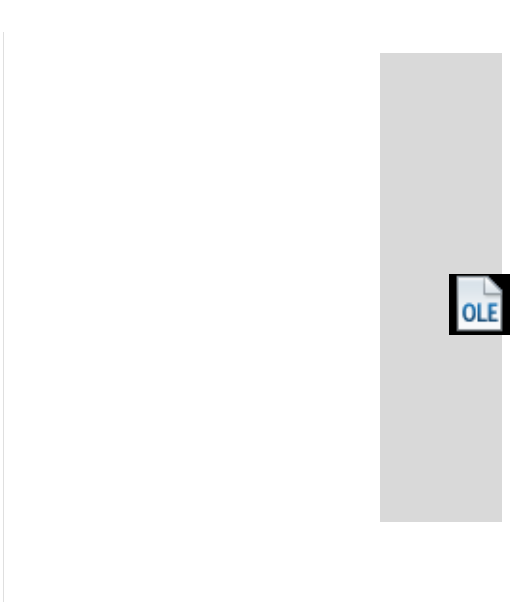
- 174 Caucasian patients with biopsy proven NAFLD/NASH (69% male, mean age 49a,mean BMI 30.2)
- Measurement of hepatic copper content (flame photometry)
- Semiquantitative assessment of hepatic steatosis
- Determination of *PNPLA3* and *TM6SF2*

Stättermayer et al, J of Trace Elements in Medicine and Biology 2017

Hepatic copper content in NAFLD



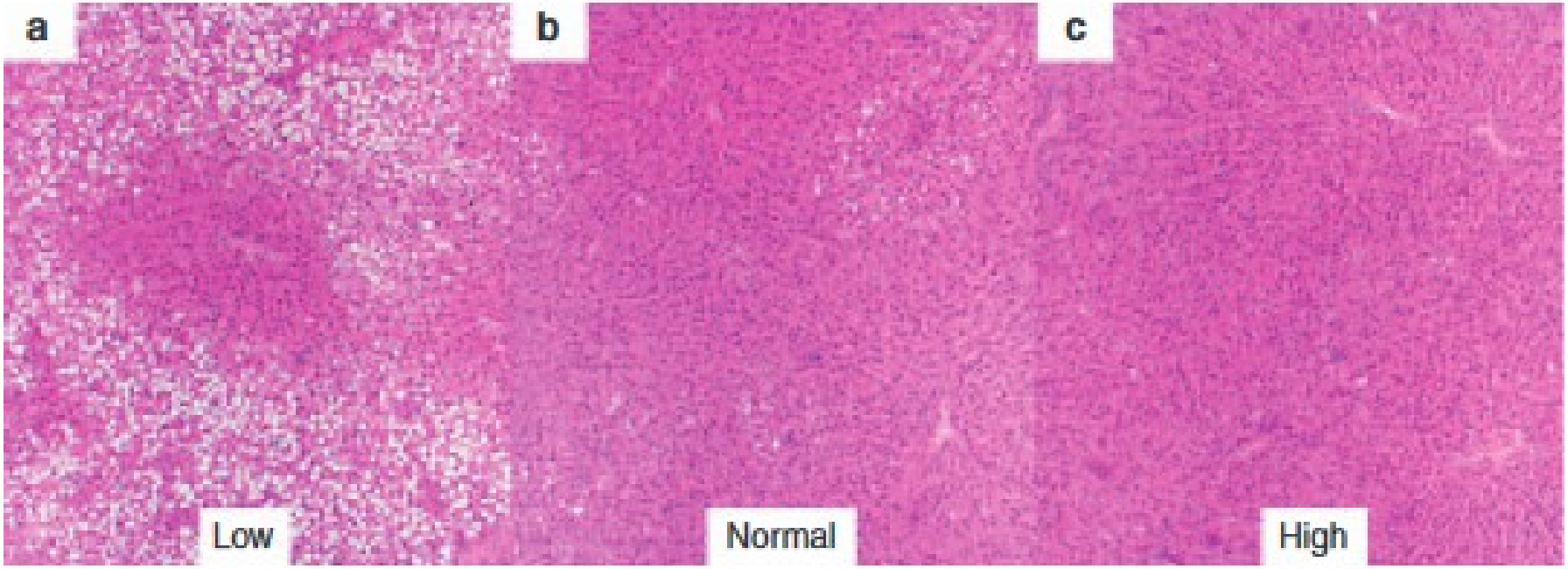
without metabolic syndrome



with metabolic syndrome

Stättermayer et al, J of Trace Elements in Medicine and Biology 2017

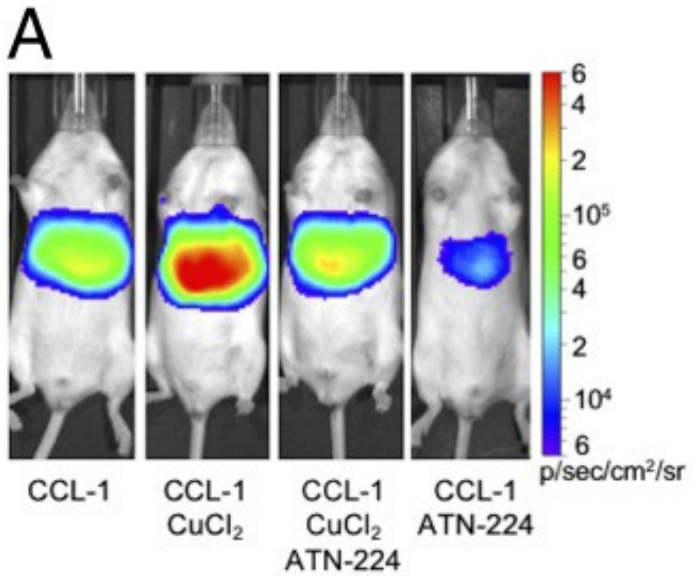
Effect of dietary copper on hepatic steatosis in rats

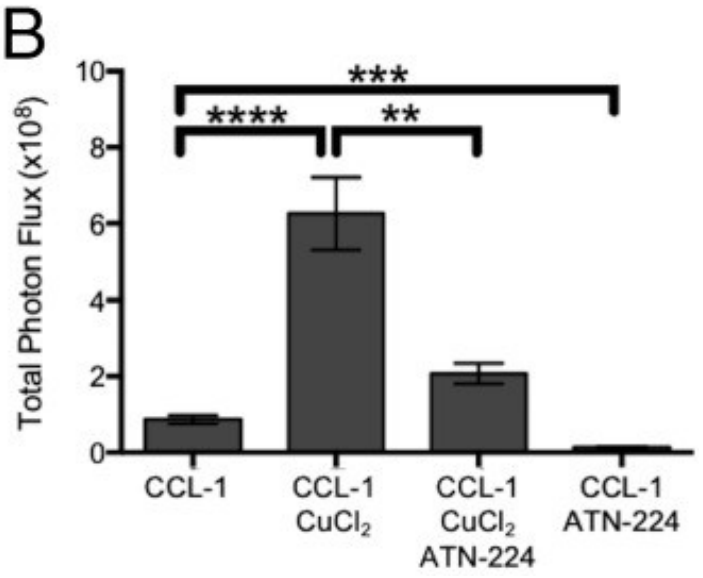


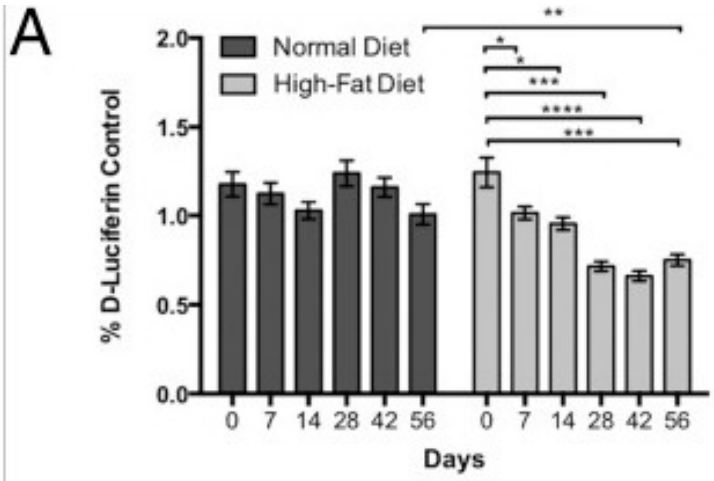
Copper in diet

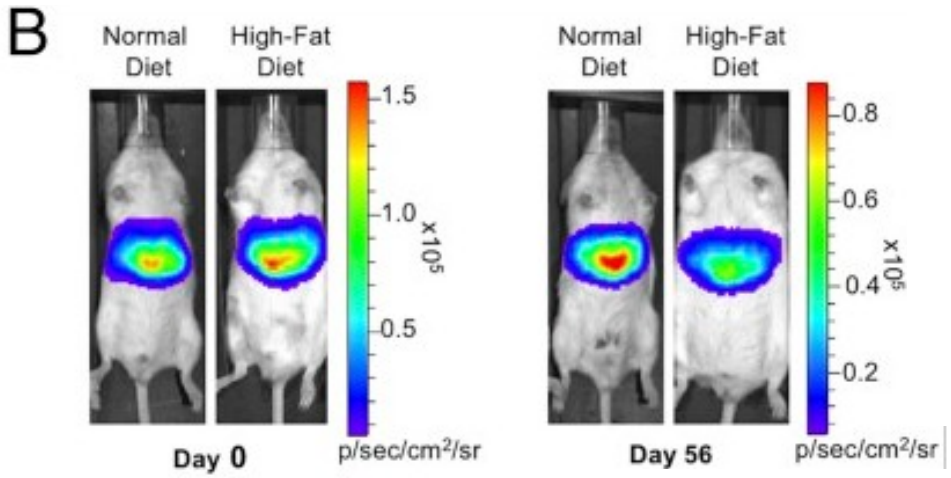
Aigner et al, Am J Gastro 2010

Effect of a high fat diet on in vivo hepatic copper content



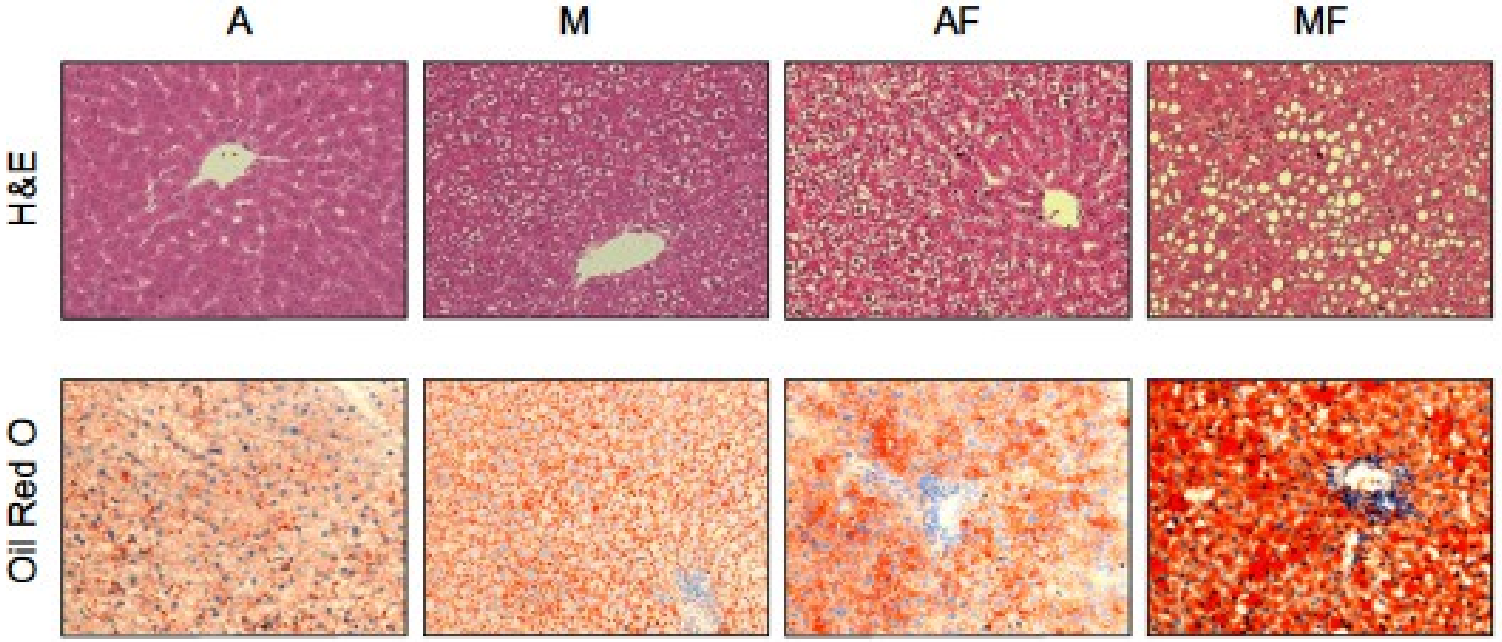






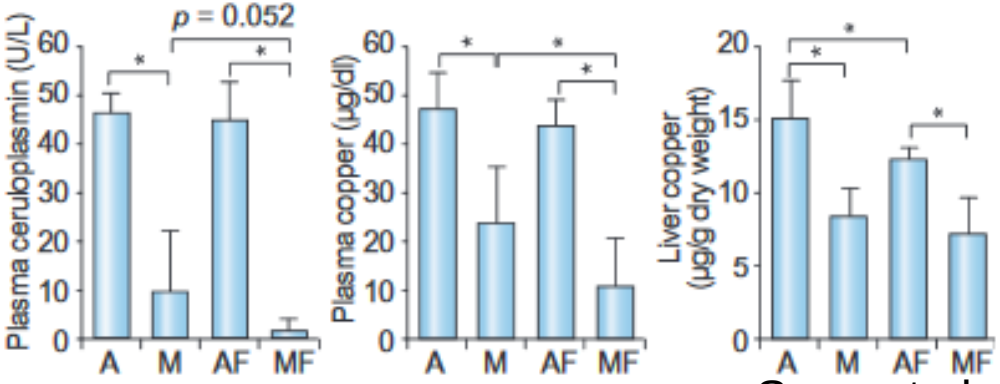
Heffern et al, PNAS 2016

Effect of high fructose feeding in rats

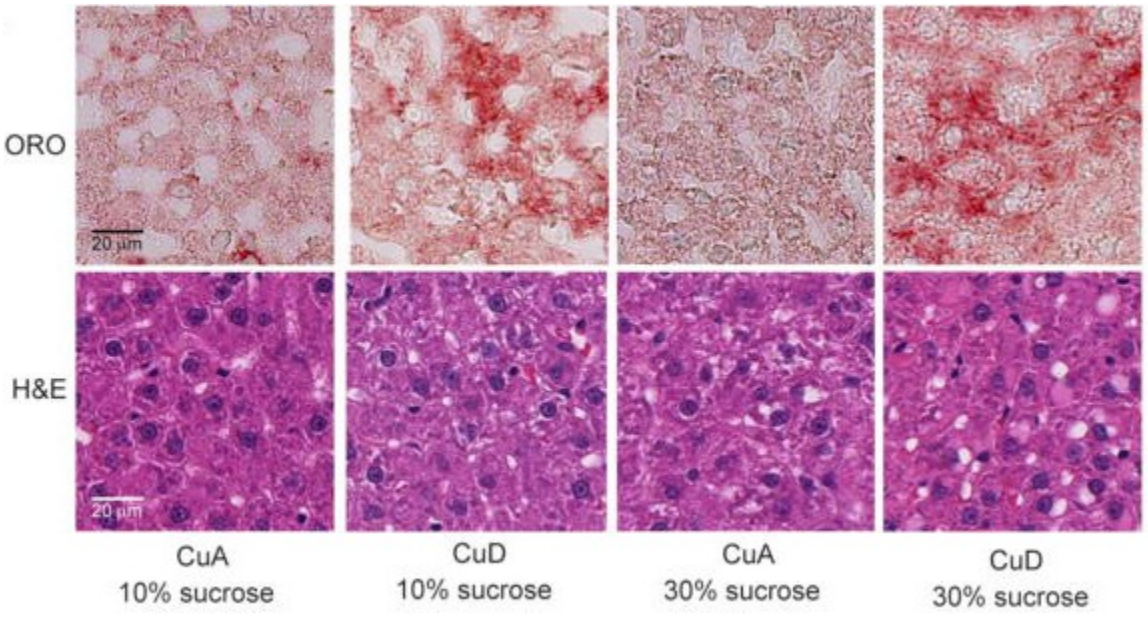


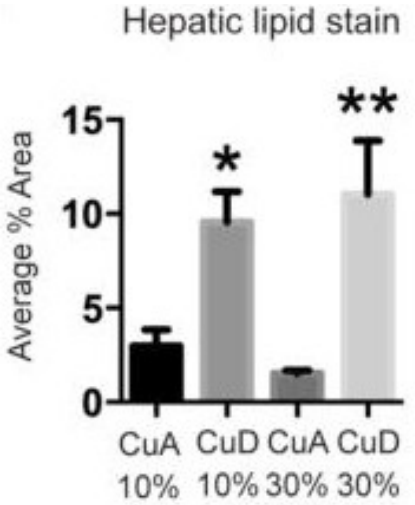
A: adaequate copper feeding
M: marginal copper feeding

AF: A+ 30% fructose
MF: M+30%fructose



Song et al, J, Hepatol 2012





- Low dietary Cu and sucrose consumption promote inflammation and fibrosis gene expression changes consistent with NAFLD
- Low dietary Cu promotes steatosis as well as gene expression changes in lipid synthe
- Cu deficiency and sucrose consumption promote NAFLD-like pathology

Tollino et al, J Nutr. Biochem 2015

NAFLD – copper connection

- Experimental:
  - Low copper diet leads to NAFLD
  - Low copper diet + high sucrose – highest degree of steatosis
  - High fat diet decreases hepatic copper content
- Patients:
  - Low hepatic copper content in (lean) NAFLD
  - Interaction with hepatic iron

Liver biopsy for diagnosis of Wilson disease

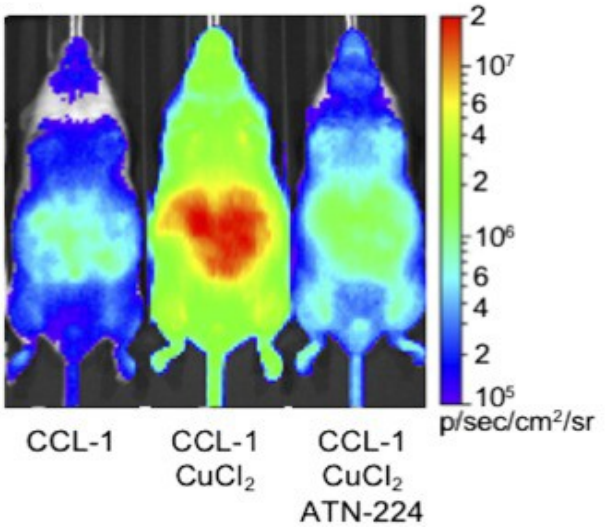
- light microscopy: steatosis and megamitochondria (DD: NASH!) should raise suspicion of pathologist
- Histochemical staining for copper of limited value (pos. only in ¼ patients)
- quantification of copper by atom absorption spectroscopy

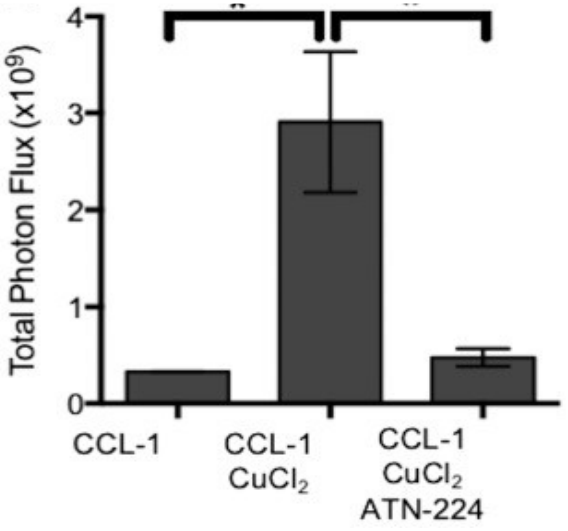
Frequency of Wilson disease

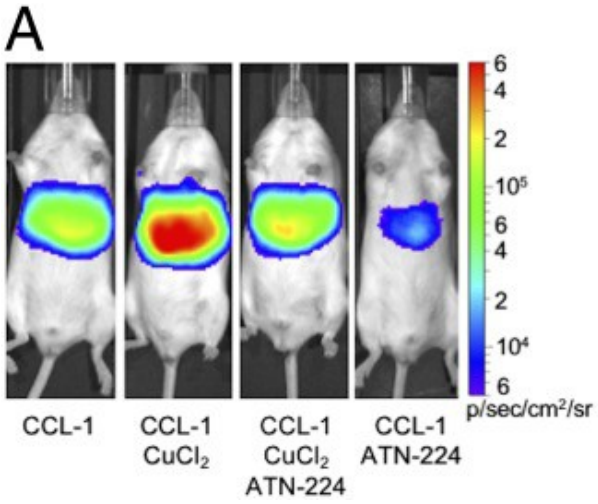
- „pre molecular genetic“ studies
  - Symptomatic 1:30,000, heterozygote frequency 1/90
- H1069Q mutation in young males at draft in Austria
  - 1/125 (accounting for 44% alleles in WD pats.) ~ 1/55
- PCR for the 6 most common mutations
  - Yang et al (S-Korea, 2017) heterozygote frequency 1/53
- Whole exon sequencing of healthy subjects
  - Coffey et al (UK,2013) heterozygote frequency 1/25
- Next generation sequencing of healthy subjects
  - Collet et al (France,2018) heterozygote frequency 1/31

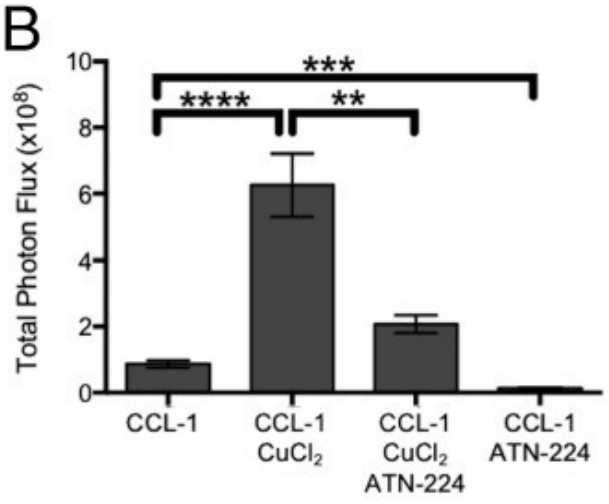
Based on these numbers symptomatic Wilson disease should be 4x as common

In vivo measurement of hepatic copper content









CCL-1 Copper-Caged Luciferin-1, ATN-224 copper chelator

Heffern et al, PNAS 2016