Copper and the liver

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Copper is a essential micronutrient

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

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
Copper transport in hepatocytes

- ATP7B
- CTR1
- Sco1
- Sco2
- CCS
- MT
- low Cu content
- high Cu content
- other Cu proteins
- CPL
- ATOX

Ferenci
Copper transport in hepatocytes – Wilson disease

- CPL
- ATP7B
- MT
- Sco1
- Sco2
- CCS
- other Cu proteins

low Cu content → high Cu content

Other Cu proteins

Ferenci
Rhodanine positive granula (black arrow), glycogenated nuclei (red arrow)

Asymptomatic detected by chance

any time

Neuropsychiatric Disease
Liver disease may be present

Never symptomatic?

any time**

Symptomatic liver disease

Fulminant liver failure
Hemolysis
Cirrhosis

Neuropsychiatric symptoms may be present

asymptomatic or absent liver disease
ALT normal or increased

symptomatic liver disease

neurologic symptoms

In this study:
* 23.6 (3-74) years of age
** 17.6 (2-63) years of age

Ferenczi et al, Hepatology 2019

Simple steatosis (HE stain)

NASH like picture (HE stain)

AIH like picture (HE stain)

Cirrhosis (HE stain)

Ferenczi P. Ott P J. Hepatology 2019
Age at symptomatic onset

may become symptomatic at any age

Liver biopsy findings at diagnosis

only 50% develop cirrhosis

### Diagnostic tests for Wilson disease

<table>
<thead>
<tr>
<th>Test</th>
<th>Abnormal</th>
<th>Caveat</th>
</tr>
</thead>
<tbody>
<tr>
<td>KF-Ring</td>
<td>Present</td>
<td></td>
</tr>
<tr>
<td>Neuropsych. symptoms</td>
<td>Present</td>
<td>May be nonspecific</td>
</tr>
<tr>
<td>Caeruloplasmin in</td>
<td>&lt; 20 mg/dl</td>
<td>May be normal in hepatic WD</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Low in malabsorption</td>
</tr>
<tr>
<td>Urinary Cu excretion</td>
<td>&gt; 100 mg/24h</td>
<td>Not useful when anuric</td>
</tr>
<tr>
<td>Hepatic copper content</td>
<td>&gt; 250 µg/g dry weight</td>
<td>May be &lt;250 µg/g in 20%, increased in cholestasis</td>
</tr>
<tr>
<td>Genetics</td>
<td>2 disease causing mutations*</td>
<td>Present only in 75%</td>
</tr>
</tbody>
</table>

*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
- **3** diagnosis possible
- **≤2** diagnosis unlikely

EASL Guidelines 2012
Typical clinical symptoms (tremor, KFR, CPL)

Score 0-1
- Urinary copper: < 100µg/d

Score 2-3
- Hepatic copper: < 250µg/g

Score
- 2 mutations
- 1 mutation
- Mutations
- no mutation

EASL Guidelines 2012

diagnosis established

Other diagnosis
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)

- symptomfree; 26%
- stable; 25%
- improved; 24%
- deteriorated/o LTX; 12%
- deteriorated/no LTX; 4%
- died; 10%

Beinhardt et al, CGH 2014

Graph showing survival rates over years with Morbus Wilson and Normalbevölkerung.
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

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: adequate 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 synthesis
• Cu deficiency and sucrose consumption promote NAFLD-like pathology

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 $\frac{1}{4}$ 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