Wilson Disease

Sihoun Hahn, MD, PhD

Professor, Department of Pediatrics
University of Washington, Seattle, WA
Children’s Hospital and Regional Medical Center
Wilson Disease

- Autosomal Recessive
- Incidence:
  - 1/30,000
  - 1/15,000~25,000 in Japan
  - 1/3,000 in Sardinia
  - 6/90 birth
    - Wilson disease: high prevalence in a mountaineous area of Crete (Ann Hum Genet 2005)
- Carrier frequency: ~1/87
- Characteristics
  - Defective biliary excretion of copper
  - Impairment in the incorporation of copper into ceruloplasmin
# Copper Transport Defect

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Wilson disease</th>
<th>Menkes disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intestinal absorption</td>
<td>2 mg</td>
<td>2 mg</td>
<td>0.1-0.2 mg</td>
</tr>
<tr>
<td>Biliary excretion</td>
<td>2 mg</td>
<td>0.2-0.4 mg</td>
<td>Not known</td>
</tr>
<tr>
<td>Urinary excretion</td>
<td>0.04 mg</td>
<td>1 mg</td>
<td>Increase</td>
</tr>
<tr>
<td>Net balance</td>
<td>0</td>
<td>positive</td>
<td>negative</td>
</tr>
</tbody>
</table>
Oral intake (1.5-4 mg/d) → Intestinal absorption → Plasma albumin (rapid clearance) → Menkes disease

Intestinal absorption

Other tissues, proteins; brain, eye, kidney, enzymes

Liver

apoceruloplasmin

Cu(I)

Menkes disease

urine

Other proteins

Wilson disease

Biliary excretion (1-4 mg)

ceruloplasmin

ceruloplasminemia

Ferritin, Fe(II)

Transferrin, Fe(III)

Cox DW, J Gastroenterol Hepatol, 1997
Copper binding domain

NH2

ATP binding domain

Transduction

Phosphorylated D residue

Conserved CPC motif

COOH
# Mutation Spectrum

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Caucasian</th>
<th>Asian</th>
</tr>
</thead>
<tbody>
<tr>
<td>H1069Q</td>
<td>&gt;50%</td>
<td>0%</td>
</tr>
<tr>
<td>2301insC</td>
<td>~10%</td>
<td>0%</td>
</tr>
<tr>
<td>2347delC</td>
<td>~10%</td>
<td>0%</td>
</tr>
<tr>
<td>R778L</td>
<td>0%</td>
<td>~30%</td>
</tr>
<tr>
<td>A874V</td>
<td>&lt;2%</td>
<td>~10%</td>
</tr>
<tr>
<td>N1270S</td>
<td>&lt;2%</td>
<td>~10%</td>
</tr>
<tr>
<td>2304delC</td>
<td>&lt;1%</td>
<td>&lt;2%</td>
</tr>
<tr>
<td>L1083F</td>
<td>&lt;1%</td>
<td>&lt;2%</td>
</tr>
</tbody>
</table>
# Clinical Characteristics


<table>
<thead>
<tr>
<th>Hepatic</th>
<th>Asymptomatic hepatomegaly</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Isolated splenomegaly</td>
</tr>
<tr>
<td></td>
<td>Persistently elevated serum aminotransferase activity (AST, ALT)</td>
</tr>
<tr>
<td></td>
<td>Fatty liver</td>
</tr>
<tr>
<td></td>
<td>Acute hepatitis</td>
</tr>
<tr>
<td></td>
<td>Resemblance to autoimmune hepatitis</td>
</tr>
<tr>
<td></td>
<td>Cirrhosis</td>
</tr>
<tr>
<td></td>
<td>Fulminant hepatic failure</td>
</tr>
<tr>
<td>Neurologic</td>
<td>Movement disorders (tremor, involuntary movements)</td>
</tr>
<tr>
<td></td>
<td>Drooling, dysarthria</td>
</tr>
<tr>
<td></td>
<td>Rigid dystonia</td>
</tr>
<tr>
<td></td>
<td>Pseudobulbar palsy</td>
</tr>
<tr>
<td></td>
<td>Seizures</td>
</tr>
<tr>
<td></td>
<td>Migraine headaches</td>
</tr>
<tr>
<td></td>
<td>Insomnia</td>
</tr>
<tr>
<td>Psychiatric</td>
<td>Depression</td>
</tr>
<tr>
<td></td>
<td>Neuroses</td>
</tr>
<tr>
<td></td>
<td>Personality changes</td>
</tr>
<tr>
<td></td>
<td>Psychosis</td>
</tr>
<tr>
<td>Other symptoms</td>
<td>Renal abnormalities: aminoaciduria and nephrolithiasis</td>
</tr>
<tr>
<td></td>
<td>Skeletal abnormalities: premature osteoporosis and arthritis</td>
</tr>
<tr>
<td></td>
<td>Cardiomyopathy, dysrhythmias</td>
</tr>
<tr>
<td></td>
<td>Pancreatitis</td>
</tr>
<tr>
<td></td>
<td>Hypoparathyroidism</td>
</tr>
<tr>
<td></td>
<td>Menstrual irregularities: infertility, repeated miscarriages</td>
</tr>
</tbody>
</table>


Diagnosis

- Serum ceruloplasmin: < 20 mg/dl
- Slit lamp examination
- Serum copper: < 0.75 ug/ml
- Serum transaminases: elevation
- Liver copper: > 250 ug/g dry tissue
- Urinary copper excretion: > 100 ug/day
- DNA test
  - Direct sequencing of whole exons
Liver Copper

- Patients: > 250 ug/g dry tissue (<35)
- Carriers: up to 125 ug/g dry tissue

- Other condition with >250 ug/g tissue
  - Chronic cholestatic conditions
    - Primary biliary cirrhosis
    - Biliary atresia
    - Alagille syndrome (arteriohepatic dysplasia)
  - Extremely rare with other conditions
Comparison of copper between fresh and block liver

Copper μg/g dry wt

Fresh liver

Block liver
Diagnostic Value of Quantitative Hepatic Copper Determination in Patients With Wilson’s Disease

PETER FERENCI,* PETRA STEINDL-MUNDA,* WOLFGANG VOGEL,† WOLFGANG JESSNER,* MICHAEL GSCHWANTLER,§ RUDOLF STAUBER,‖ CHRISTIAN DATZ,¶ FRANZ HACKL,# FRITZ WRBA,** PETER BAUER,*** and OSKAR LORENZ‡‡

- non-cholestatic liver disease (n=219)
  - 1.4% : >250 ug /g

- Wilson disease (n=114)
  - 83.3 % : >250 ug/g
  - 13.2 % : between 50 and 250 g/g
  - 3.5 % : <50 ug/g
• 17% (19/114) had copper less than 250 ug/g in liver tissue

• 63% of those less than 250 ug had two mutations... DNA test helpful in determining the diagnosis

• 17% (19/114) had copper less than 250 ug/g in liver tissue

• 63% of those less than 250 ug had two mutations... DNA test helpful in eliminating the diagnosis in 37%
DNA test

• Diagnostic dilemmas
  liver copper over or under 200 ug/g and urine copper over 100 ug/24 hours and low/normal ceruloplasmin

• Variable methods
  • Partial sequencing or scanning
  • Direct sequencing of entire gene
# Negative

<table>
<thead>
<tr>
<th>Case</th>
<th>Liver Cu (10-35ug/g)</th>
<th>Urine Cu (&lt;60ug/day)</th>
<th>Serum Cu (0.75-1.45 ug/ml)</th>
<th>CP (&gt;20 mg/dl)</th>
<th>DNA results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>386</td>
<td>18</td>
<td>0.55</td>
<td>17.8</td>
<td>negative</td>
</tr>
<tr>
<td>2</td>
<td>36</td>
<td>1019</td>
<td>NA</td>
<td>16</td>
<td>negative</td>
</tr>
<tr>
<td>3</td>
<td>860</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>negative</td>
</tr>
<tr>
<td>4</td>
<td>1414</td>
<td>NA</td>
<td>NA</td>
<td>31.4</td>
<td>negative</td>
</tr>
</tbody>
</table>
Case #1

• 23 year old male presenting with
  • Flu-like episodes, persistent abdominal pain & nausea
  • Dizziness, trouble focusing
  • Persistent elevated total bilirubin
    • Total Bilirubin: 1.4~2.9, Direct:0.26~0.3
  • Abnormal liver function tests
    • AST: 36~48, ALT: 92~141, GGT: normal
  • Elevated liver copper
    • 386 ug/g of dried tissue (<35)
  • Slightly low serum ceruloplasmin and copper
    • 17.8 mg/dl (22.9-43.0), 0.55 ug/dl (0.75-1.45)
• Urine copper in the normal range
→ Treatment started with zinc
Case #1

- **Wilson disease**
  - DNA sequencing performed; NO mutations identified
  - Doing well with no symptoms under no medication
  - Wilson disease (affected) AND carrier state have been ruled out

- **Gilbert syndrome**
  - Homozygous for the A(TA)$_7$TAA allele of UGT1A1 (2q37)
    - Specific polymorphism in promoter region
    - Homozygous polymorphism identified in 80-100% of Caucasian individuals with Gilbert syndrome
## Carrier

<table>
<thead>
<tr>
<th>Case</th>
<th>Liver Cu (10-35ug/g)</th>
<th>Urine Cu (&lt;60ug/day)</th>
<th>Serum Cu (0.75-1.45 ug/ml)</th>
<th>CP (&gt;20 mg/dl)</th>
<th>DNA results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>385</td>
<td>30</td>
<td>0.26</td>
<td>7</td>
<td>R1319X/wild</td>
</tr>
<tr>
<td>2</td>
<td>243</td>
<td>94</td>
<td>0.48</td>
<td>10.5</td>
<td>4091_4092delGT/Wild type</td>
</tr>
<tr>
<td>3</td>
<td>NA</td>
<td>NA</td>
<td>0.77</td>
<td>19.2</td>
<td>P1379S/Wild</td>
</tr>
<tr>
<td>4</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>G1281D/Wild</td>
</tr>
</tbody>
</table>
Case #2

• **MW, age 15 years**

• **Reason for referral:**
  • To evaluate possibility of WD and/or heterozygote status

• **Present Illness:**
  • Healthy adolescent with no evidence of clinical symptoms
  • Mother diagnosed with WD at age of 19 years
Case #2

- At age of 3 years, liver biopsy due to very low ceruloplasmin (7 mg/dl)
  - Copper 246 ug/g dried wt (<35)
  - Urine copper 14 ug/24 h (15-60)
- At least three separate urine copper tests at Michigan – either normal or heterozygote
- 8.2004 Liver biopsy
  - Copper 386 ug/g dried wt
  - Urine copper normal, liver function normal
  - Treatment with zinc acetate started
## Patients

<table>
<thead>
<tr>
<th>Case</th>
<th>Liver Cu (10-35ug/g)</th>
<th>Urine Cu (&lt;60ug/day)</th>
<th>Serum Cu (0.75-1.45 ug/ml)</th>
<th>CP (&gt;20 mg/dl)</th>
<th>DNA results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1176*</td>
<td>98</td>
<td>0.97</td>
<td>22</td>
<td>H1069Q/H1069Q</td>
</tr>
<tr>
<td>2</td>
<td>1061*</td>
<td>refused</td>
<td>0.51</td>
<td>8.1</td>
<td>H1069Q/T762S</td>
</tr>
<tr>
<td>3</td>
<td>1446*</td>
<td>NA</td>
<td>NA</td>
<td>17</td>
<td>H1069Q/H1069Q</td>
</tr>
<tr>
<td>4</td>
<td>1021</td>
<td>125</td>
<td>0.88</td>
<td>23</td>
<td>H1069Q/E1064A</td>
</tr>
</tbody>
</table>
The Genetic Test Underlying the Medical Decision; A time to change the Diagnostic Algorithm for Wilson Disease
At least half of patients with WD are never diagnosed and die of untreated disease

- Dr. George Brewer, 1999

Recognition, screening, and differential diagnosis of WD still challenge physicians today

Natural Course

- Asymptomatic
- Hemolysis
- Hepatic necrosis
- Neurologic Damage
- Liver cirrhosis
Prevention
Ceruloplasmin

a. Oxidase activity
b. Immunoturbidimetric assay
c. Radial immunodiffusion

NOT APPROPRIATE FOR MASS SCREENING!
A case from pilot study

- 32 month old *HEALTHY (?)* boy
- K-F ring (-)
- Lab:
  - Ceruloplasmin = 2.3 mg/dL (20-60)
  - Serum Copper = 0.27 ug/ml (0.70-1.55)
  - Urine 24 hour Copper = 654.1 ug/day (15-50)
  - SGOT/SGPT/GGT = 314/207/83 U/L
WD Pilot Study at Mayo Clinic

Eligibility Criteria

- Males & Females ages 3 months - 18 years
- No acute infection
- No oral contraceptive use
- No pregnancy
Has anybody measured ceruloplasmin in dried blood spot from newborns with Wilson disease?
Retrospective Determination of CP in Newborn Screening Blood Spots of Patients with WD

Table 1
Ceruloplasmin measurement in original newborn screening dried blood spots retrieved from 2 WD patients and controls

<table>
<thead>
<tr>
<th></th>
<th>CP (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1 (18 years old)</td>
<td>2.8</td>
</tr>
<tr>
<td>Age-matched controls (n = 3)</td>
<td>12.6, 15.8, 18.4</td>
</tr>
<tr>
<td>Patient 2 (11 years old)</td>
<td>2.6</td>
</tr>
<tr>
<td>Age-matched controls (n = 3)</td>
<td>8.1, 30.2, 54.6</td>
</tr>
</tbody>
</table>

### Prospective Study of Newborn Screening for Wilson Disease in Minnesota

<table>
<thead>
<tr>
<th>Category</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Period</td>
<td>2005-2006</td>
</tr>
<tr>
<td>Volume</td>
<td>7,382</td>
</tr>
<tr>
<td>Age of subjects</td>
<td>1-7 days</td>
</tr>
<tr>
<td>Cutoff value</td>
<td>5.0 mg/dL (~0.5%ile)</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>n/a</td>
</tr>
<tr>
<td>Specificity</td>
<td>99.3%</td>
</tr>
<tr>
<td>Detection rate</td>
<td>n/a</td>
</tr>
<tr>
<td>False positive rate</td>
<td>0.7%</td>
</tr>
<tr>
<td>Positive predictive value</td>
<td>n/a</td>
</tr>
</tbody>
</table>
WHO Criteria for Population Screening

- Latent stage
- Suitable test
- Test acceptable to population
- Agreement on whom to treat
- Natural history understood
- Cost effective
Novel Human Ceruloplasmin Peptide Quantification Method By LC/MS/MS for the Newborn Screening of Wilson Disease
Acute Liver Failure in the US: Results of a multi-center study
-Wilson Disease-
## Spontaneous Survival

<table>
<thead>
<tr>
<th>Condition</th>
<th>Survival Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. ACM</td>
<td>68%</td>
</tr>
<tr>
<td>2. Hepatitis A</td>
<td>64%</td>
</tr>
<tr>
<td>3. Shock</td>
<td>59%</td>
</tr>
<tr>
<td>4. Pregnancy</td>
<td>50%</td>
</tr>
<tr>
<td>5. Drug</td>
<td>25%</td>
</tr>
<tr>
<td>6. Hepatitis B</td>
<td>23%</td>
</tr>
<tr>
<td>7. Budd-Chiari</td>
<td>20%</td>
</tr>
<tr>
<td>8. Other</td>
<td>17%</td>
</tr>
<tr>
<td>9. Indeterminate</td>
<td>17%</td>
</tr>
<tr>
<td>10. Autoimmune</td>
<td>15%</td>
</tr>
<tr>
<td>11. Wilson</td>
<td>0%</td>
</tr>
<tr>
<td>12. Malignancy</td>
<td>0%</td>
</tr>
</tbody>
</table>
Transplantation

1. Wilson 75%
2. Hepatitis B 64%
3. Budd-Chiari 60%
4. Drug 53%
5. Indeterminate 51%
6. Autoimmune 46%
7. Other 33%
8. Hepatitis A 21%
9. ACM 6%
10. Shock 0%
11. Pregnancy 0%
12. Malignancy 0%
Wilson Disease in Septuagenarian Siblings: Raising the Bar for Diagnosis

Aftab Ala,^1^ Jimo Borjigin,^2^ Arnold Rochwarger,^3^ and Michael Schilsky^3,4^

Wilson Disease (WD) usually presents in the first decades of life, although rare patients have a later presentation. We report the clinical features, diagnostic evaluation, and outcome with treatment of two septuagenarian siblings evaluated as part of a research trial for treatment of neurological WD. The index case was a 72-year-old woman who suffered progressive neurological disability, then developed sub-fulminant liver failure. Her sibling was a 70-year-old man with minimal neurological symptoms and a mild depressive disorder. His liver biopsy revealed only steatosis and minimal fibrosis and an elevated hepatic copper content (671 µg/g dry weight liver). Molecular studies demonstrated compound heterozygosity for disease specific \( ATP7B \) mutations E1064A and H1069Q in both patients. Both individuals were...
To the Editor:

We read with interest the article by Ala et al.\textsuperscript{1} regarding the diagnosis of Wilson’s Disease in siblings, both of whom were in their eighth decade of life. We recently diagnosed Wilson’s Disease in a 60-year-old woman, underscoring the conclusions reached by Ala et al.

This finding underscores the conclusion reached by Ála et al. that the diagnosis of Wilson’s Disease should be entertained in patients of all ages who present with evidence of liver disease, neurological disease, or psychiatric symptoms.

Roman E. Perri, M.D.\textsuperscript{1}
Si Houen Hahn, M.D., Ph.D.\textsuperscript{2}
Matthew J. Ferber, Ph.D.\textsuperscript{2}
Patrick S. Kamath, M.D.\textsuperscript{1}

\textsuperscript{1}Division of Gastroenterology and Hepatology
\textsuperscript{2}Department of Laboratory and Medical Pathology
Mayo Clinic College of Medicine
Rochester, MN
Summary

• Diagnostic algorithm:
  • Liver block specimen could be contaminated with copper leading to false positive
  • DNA test should be considered prior to biopsy

• Newborn screening for WD is possible
  • Appropriate method to be determined
  • MSMS or sandwich ELISA

• WD should be considered at any age
Acknowledgements

Mayo Clinic
  Chuck Kroll
  Jennifer Brown
  Jesse Schafer
  Saba Zafari
  Marie Pogatschnik
  Sara Minnich
  Shannon Hodel-Hanson
  Kate Hibbs
  Stacy Hartman
  Dr. Robert Jacobson
  CPAM Staffs
  Nursing Staffs
  Tammy Schmit
  Dr. Brian Dawson
  Dr. Matt Ferber
  Loretta Christiansen
  Kara Mensink
  Sharon Wiesner
  Todd Juen
  Richard Hurt, Jr

Wilson Disease Association
  Mary Graper
  Kimberly Symmonds

CA DOH
  Dr. Fred Lorey
  Dr. John Sherwin
  Dr. George Cunningham

University of Washington
Children’s Hospital
  Katerina Sadilkova
  Martin Sadilek
  Valeria Vasta
  Amy DeWilde

UW Medicine
School of Medicine