

# Wilson Disease

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# 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 mountainous 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

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	Normal	Wilson disease	Menkes disease
Intestinal absorption	2 mg	2 mg	0.1-0.2 mg
Biliary excretion	2 mg	0.2-0.4 mg	Not known
Urinary excretion	0.04 mg	1 mg	Increase
Net balance	0	positive	negative

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Oral intake  
(1.5-4 mg/d)

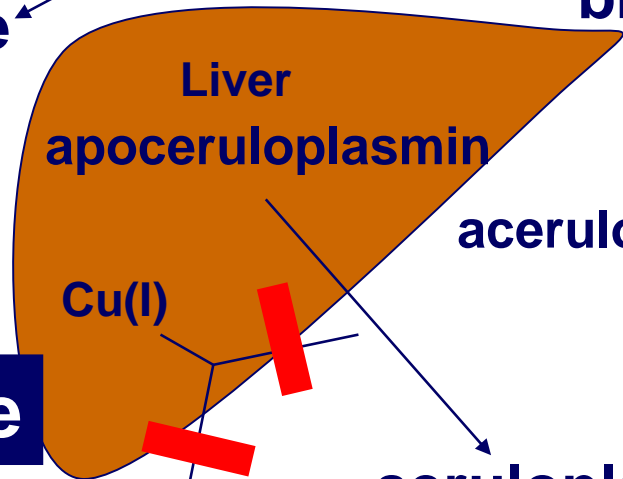
Intestinal absorption

Plasma albumin  
(rapid clearance)

**Menkes disease**

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

urine



**Wilson disease**

ceruloplasmin

Ferritin, Fe(II)

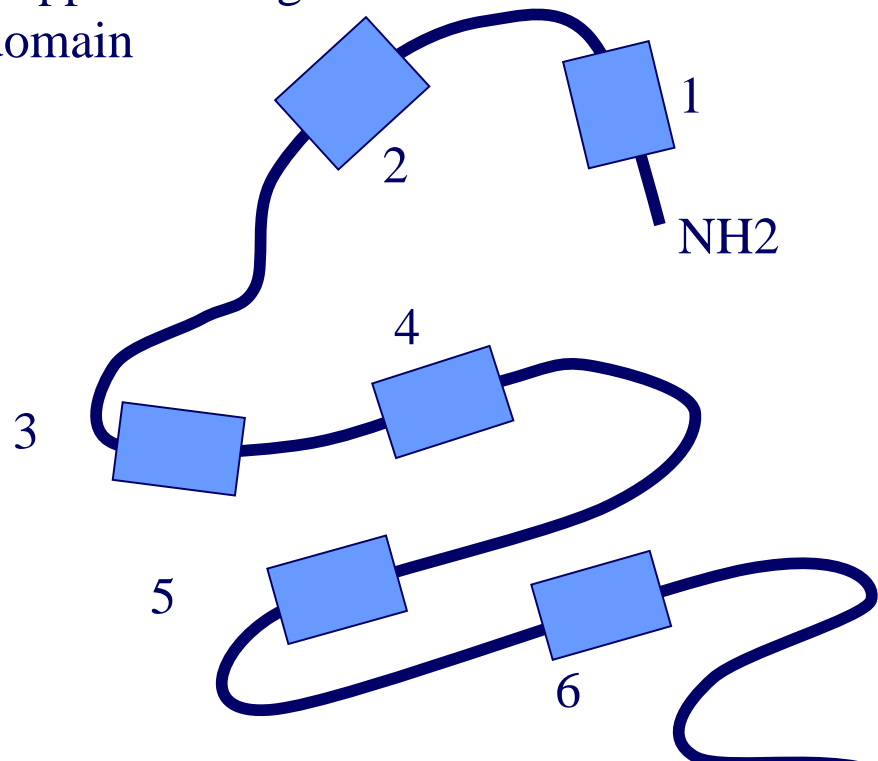
Other proteins

Transferrin, Fe(III)

Biliary excretion  
(1-4mg)

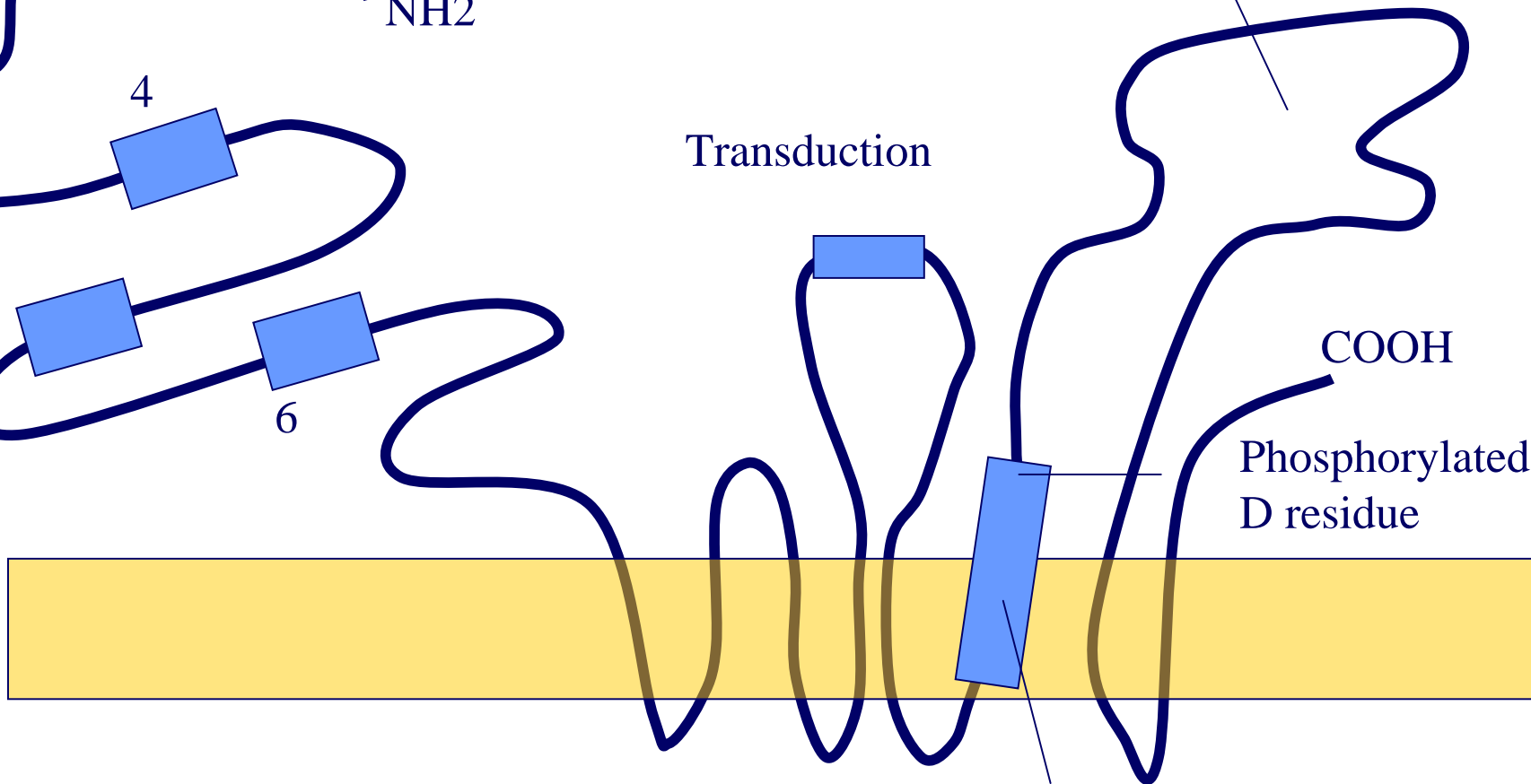
*Cox DW, J Gastroenterol Hepatol, 1997*

Copper binding domain



ATP binding domain

Transduction



COOH

Phosphorylated D residue

Conserved CPC motif

# Mutation Spectrum

Mutation	Caucasian	Asian
H1069Q	>50%	0%
2301insC	~10%	0%
2347delC	~10%	0%
R778L	0%	~30%
A874V	<2%	~10%
N1270S	<2%	~10%
2304delC	<1%	<2%
L1083F	<1%	<2%

# Clinical Characteristics

Roberts EA, Schilsky ML: A practice guideline on Wilson disease. Hepatology 2003

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

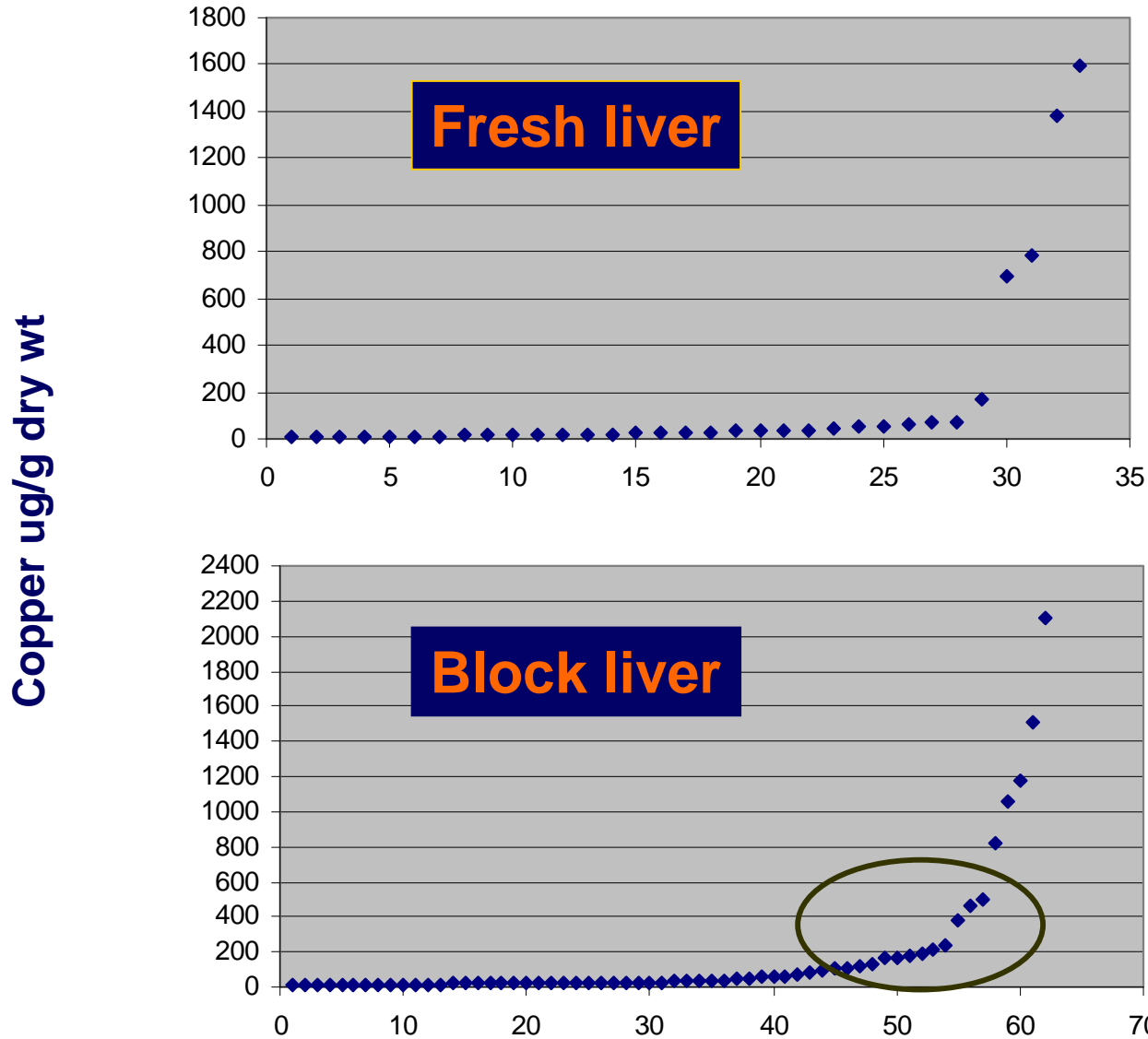
# Diagnosis

- **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



# Diagnostic Value of Quantitative Hepatic Copper Determination in Patients With Wilson's Disease

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MICHAEL GSCHWANTLER,<sup>§</sup> RUDOLF STAUBER,<sup>||</sup> CHRISTIAN DATZ,<sup>¶</sup> FRANZ HACKL,<sup>#</sup> FRITZ WRBA,\*\*  
PETER BAUER,<sup>††</sup> and OSKAR LORENZ<sup>§§</sup>

- 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**
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- **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

Case	Liver Cu (10-35ug/g)	Urine Cu (<60ug/day)	Serum Cu (0.75-1.45 ug/ml)	CP (>20 mg/dl)	DNA results
1	386	18	0.55	17.8	negative
2	36	1019	NA	16	negative
3	860	NA	NA	NA	negative
4	1414	NA	NA	31.4	negative

# 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)<sub>7</sub>TAA allele of UGT1A1 (2q37)
    - Specific polymorphism in promoter region
    - Homozygous polymorphism identified in 80-100% of Caucasian individuals with Gilbert syndrome

# Carrier

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

# Case #2

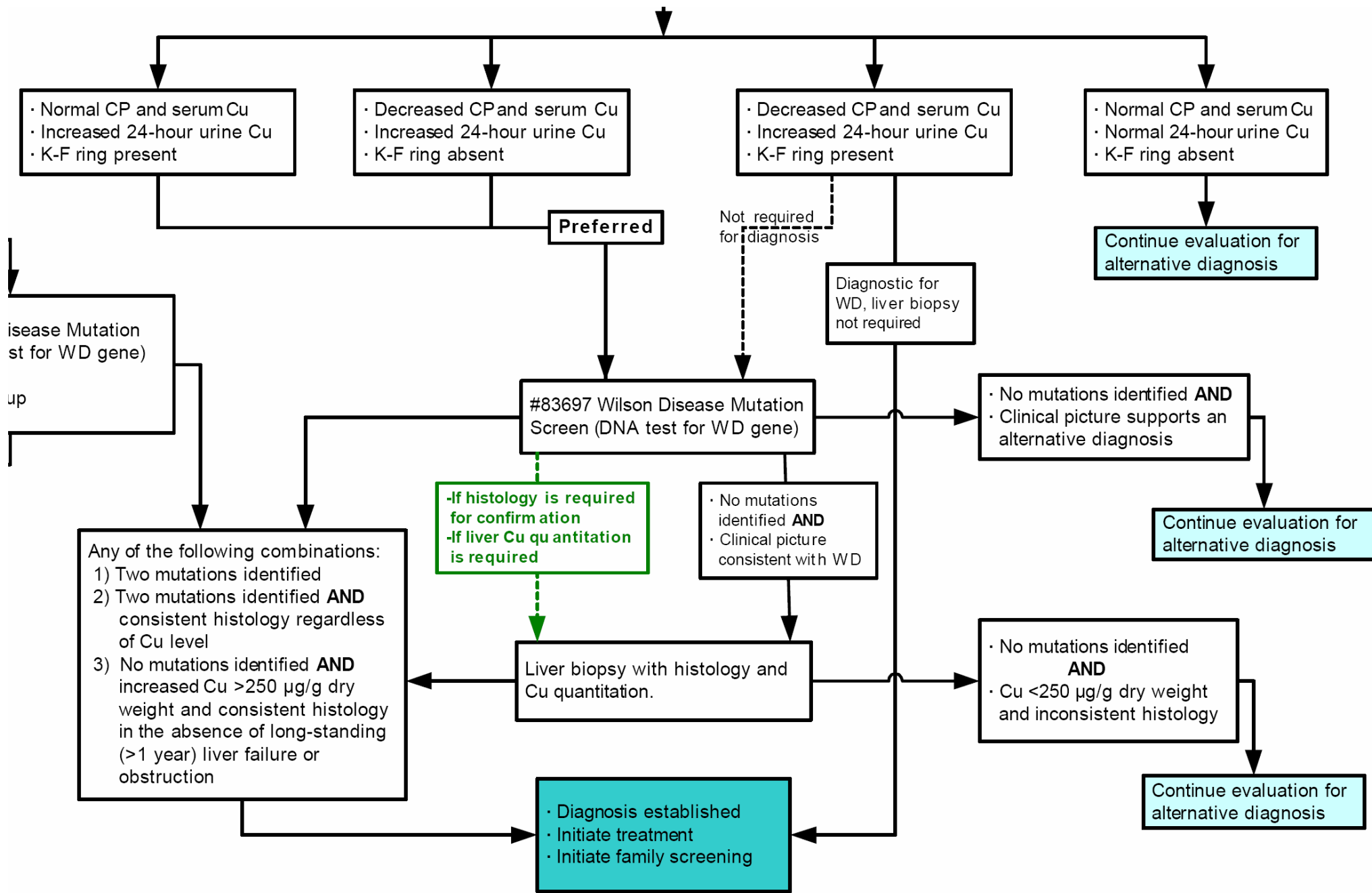
- **MW, age 15 years**
- **Reason for referral:**
  - **To evaluate possibility of WD and/or heterozygote status**
- **Present Illness:**
  - **Healthy adolescent with no evidence of clinical symptoms**
  - **Mother diagnosed with WD at age of 19 years**

# 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

Case	Liver Cu (10-35ug/g)	Urine Cu (<60ug/day)	Serum Cu (0.75-1.45 ug/ml)	CP (>20 mg/dl)	DNA results
1	1176*	98	0.97	22	H1069Q/H1069Q
2	1061*	refused	0.51	8.1	H1069Q/T762S
3	1446*	NA	NA	17	H1069Q/H1069Q
4	1021	125	0.88	23	H1069Q/E1064A



***The Genetic Test Underlying the Medical Decision;  
A time to change the Diagnostic Algorithm  
for Wilson Disease***

# Natural Course

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- At least half of patients with WD are never diagnosed and die of untreated disease
  - *Dr. George Brewer, 1999*



asymptomatic

Hemolysis  
Hepatic necrosis

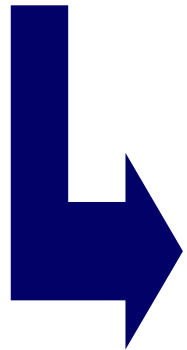
Neurologic Damage  
Liver cirrhosis

- Recognition, screening, and differential diagnosis of WD still challenge physician today

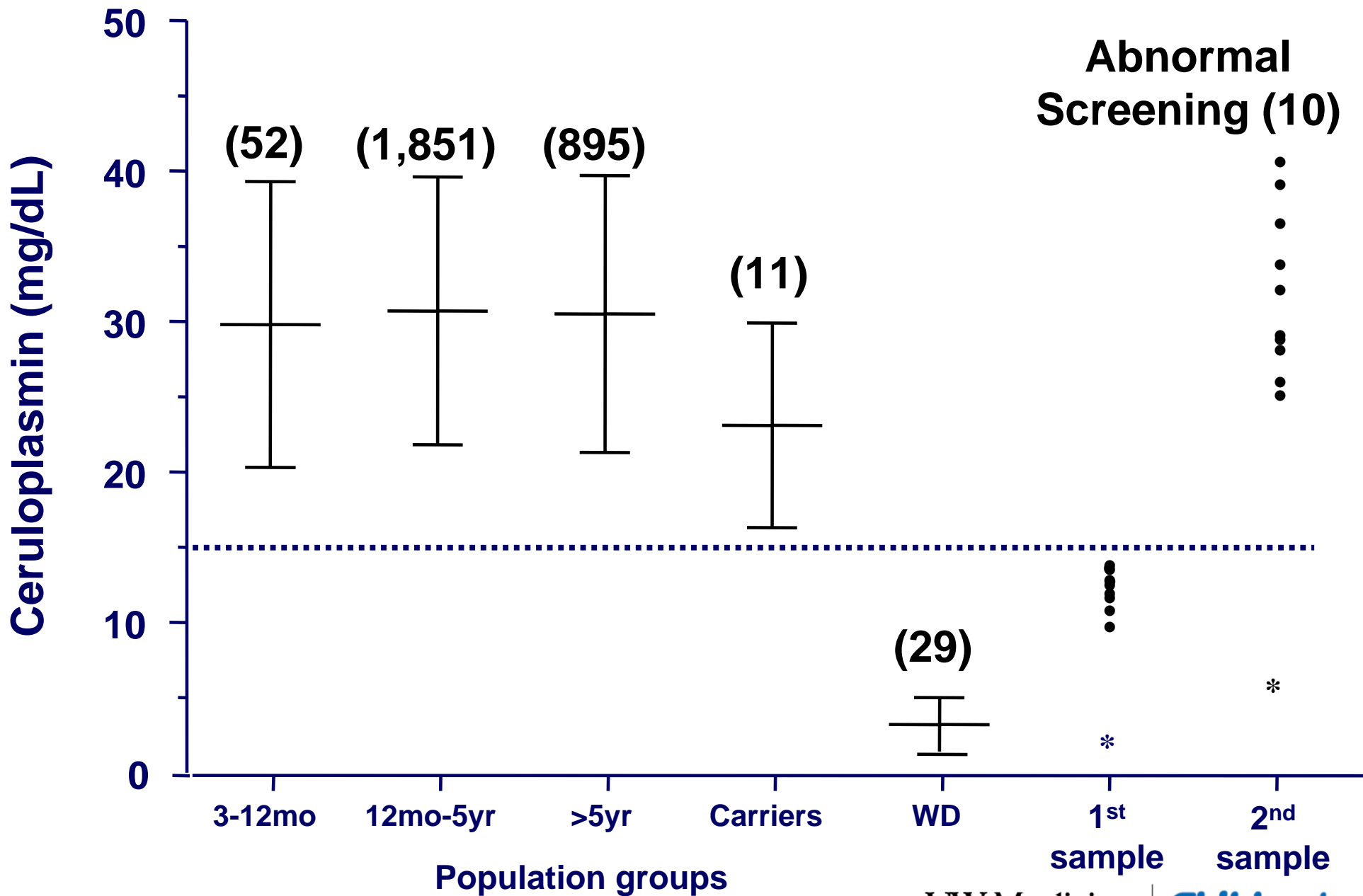
# Prevention

# Ceruloplasmin

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



**NOT APPROPRIATE FOR  
MASS SCREENING !**




# A case from pilot study

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- 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


 MAYO CLINIC

## VOLUNTEERS NEEDED


*Mayo Clinic Pediatric Screening for Wilson Disease Pilot Study*


Children and young adults between the ages of 3 months and 18 years

- Wilson disease may be the most frequent and most preventable cause of chronic liver disease in children.
- Wilson disease is treatable and serious symptoms can be avoided if a diagnosis is made early.
- Mayo Clinic is investigating the effectiveness and feasibility of screening pediatric patients.




If interested, please call our study hotline anytime at (507) 284-2993.



 MAYO CLINIC

## Pediatric Screening for Wilson Disease



Mayo Clinic  
Rochester, MN

## 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

	CP (mg/dL)
Patient 1 (18 years old)	2.8
Age-matched controls ( $n = 3$ )	12.6, 15.8, 18.4
Patient 2 (11 years old)	2.6
Age-matched controls ( $n = 3$ )	8.1, 30.2, 54.6

***Kroll et al. Mol Genet Metab 89:134-138, 2006***

# Prospective Study of Newborn Screening for Wilson Disease in Minnesota

<b>Period</b>	<b>2005-2006</b>
<b>Volume</b>	<b>7,382</b>
<b>Age of subjects</b>	<b>1-7 days</b>
<b>Cutoff value</b>	<b>5.0 mg/dL (~0.5%ile)</b>
<b>Sensitivity</b>	<b>n/a</b>
<b>Specificity</b>	<b>99.3%</b>
<b>Detection rate</b>	<b>n/a</b>
<b>False positive rate</b>	<b>0.7%</b>
<b>Positive predictive value</b>	<b>n/a</b>

# 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-**

**Schioldt et al. Etiology and outcome for  
295 patients with acute liver failure in  
the United States. Liver Transpl Surg.  
5:29-34, 1999**

# Spontaneous Survival

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1. ACM	68%
2. Hepatitis A	64%
3. Shock	59%
4. Pregnancy	50%
5. Drug	25%
6. Hepatitis B	23%
7. Budd-Chiari	20%
8. Other	17%
9. Indeterminate	17%
10. Autoimmune	15%
11. <b>Wilson</b>	<b>0%</b>
12. Malignancy	0%

# Transplantation

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<b>1. Wilson</b>	<b>75%</b>
<b>2. Hepatitis B</b>	<b>64%</b>
<b>3. Budd-Chiari</b>	<b>60%</b>
<b>4. Drug</b>	<b>53%</b>
<b>5. Inderminate</b>	<b>51%</b>
<b>6. Autoimmune</b>	<b>46%</b>
<b>7. Other</b>	<b>33%</b>
<b>8. Hepatitis A</b>	<b>21%</b>
<b>9. ACM</b>	<b>6%</b>
<b>10.Shock</b>	<b>0%</b>
<b>11.Pregnancy</b>	<b>0%</b>
<b>12.Malignancy</b>	<b>0%</b>

# Wilson Disease in Septuagenarian Siblings: Raising the Bar for Diagnosis

Aftab Ala,<sup>1</sup> Jimo Borjigin,<sup>2</sup> Arnold Rochwarger,<sup>3</sup> and Michael Schilsky<sup>3,4</sup>

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  $\mu\text{g/g}$  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.<sup>1</sup> 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 Ala 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.

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# Summary

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- **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**

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