Clinical Case Conference

HPI

- 17 year old female presents with a 6 day history of increasing fatigue and diffuse abdominal pain
- Dark colored urine, icteric sclera and recent onsent of pruritis
- Denies fever, emesis, diarrhea and chills.

- PMHx
  - Epilepsy (GTC) since 8 years old
- Medications: Zonisamide, Trileptal
- Allergies: Tegretol (rash)
- Family Hx
  - Non-contributory
- Social Hx
  - Increasingly poor school performance
  - Denies sexual activity, alcohol and drugs

Laboratory

- Total bilirubin 25.2, conjugated 13
- Total protein 5.1, albumin 2.8
- Amylase 53, Lipase 45
- Ammonia 68
- AST 86, ALT 42, ALP 82
- Hemoglobin 11.7
- PT 24.1, PTT 43, INR 2.26
- Abdominal Ultrasound
  - Gallbladder wall thickening
  - Normal CBD
  - Unremarkable liver and pancreas

Physical Exam

- General: tired, easily aroused
- HEENT: PERRLA, NCAT, icteric sclera
- CV: I/VI SEM at LSB, no radiation
- Resp: CTA bilaterally
- Abdomen: soft, hepatomegaly 4 cm below rcm, no palpable spleen, NABS, no mass
- Derm: jaundice
- Neuro: answering questions appropriately

Hospital Course

- Viral, bacterial and stool cultures sent
- Started Vitamin K and Ursodiol
- Repeat abdominal ultrasound
  - Hepatosplenomegaly
  - Normal doppler
- Became more lethargic, developed peripheral edema, tachycardia, tachypnea and hypotension

DIFFERENTIAL DIAGNOSIS

Acute Liver Failure: Infant

Infections	Drug/Toxin	Cardiovascular	Metabolic
Herpes simplex	Tylenol	ECMO	Galactosemia
Echovirus		Hypo. left heart	Tyrosinemia
Adenovirus		Shock	Iron storage
EBV		Asphyxia	Mitochondrial
Hepatitis B		Myocarditis	HFI
Parvovirus			Fatty Acid Ox.
CMV			Niemann-Pick

No clear etiology in at least 63% of cases

Acute Liver Failure: Child

Infections

Hep A,B,C,D
EBV
CMV
Herpes
Leptospirosis

Drug/Toxin

Valproic acid
Isoniazid
Halothane
Tylenol
Mushroom
Phosphorus
ASA

Cardiovascular

Myocarditis
Heart surgery
Cardiomyopathy
Budd-Chiari

Metabolic

FA oxidation
Reye Syndrome
Leukemia
Autoimmune
α1-Antitrypsin

Acute Liver Failure: The Adult

Infections

Hep A,B,C,D,E
Yellow fever
Dengue Fever
Lassa Fever

Drug/Toxin

Tylenol
Tetracycline
Halothane
Valproic Acid
MAO Inhibitors
Isoniazid
Bacillus cereus

Cardiovascular

Budd-Chiari
Acute failure
Heat stroke
Shock

Metabolic

Fatty Liver
Wilson's
Autoimmune

Hospital Course

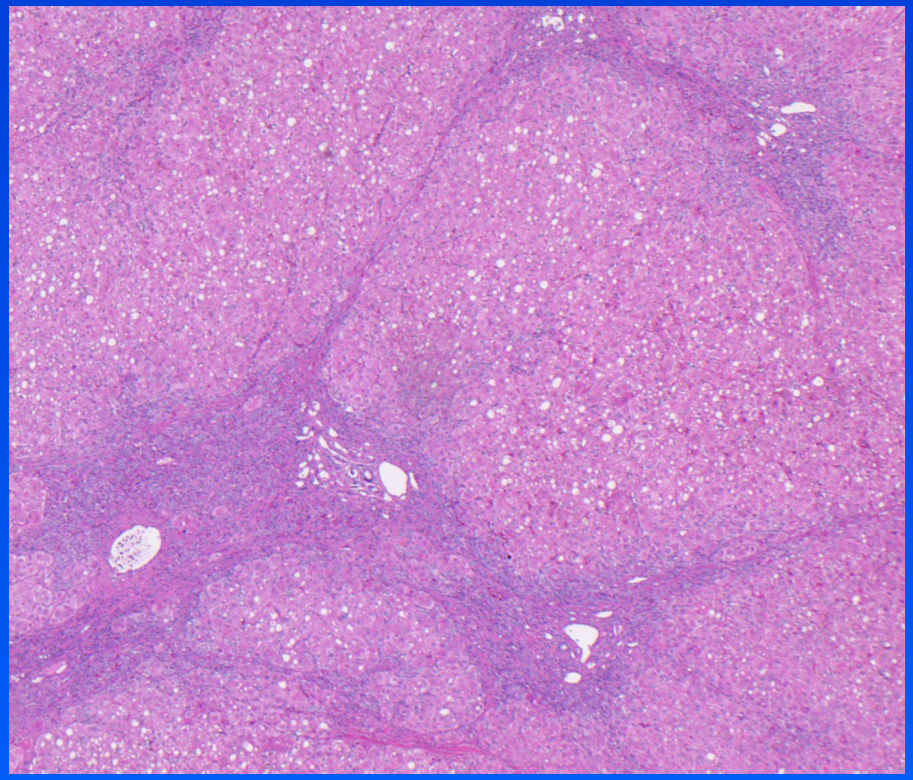
- Hemoglobin steadily falling 11.7 to 8.8
- Reticulocyte count 6.6%
- Increasing total bilirubin, peak 43.7
- GGT peaked at 325

What would you do?

Further Analysis

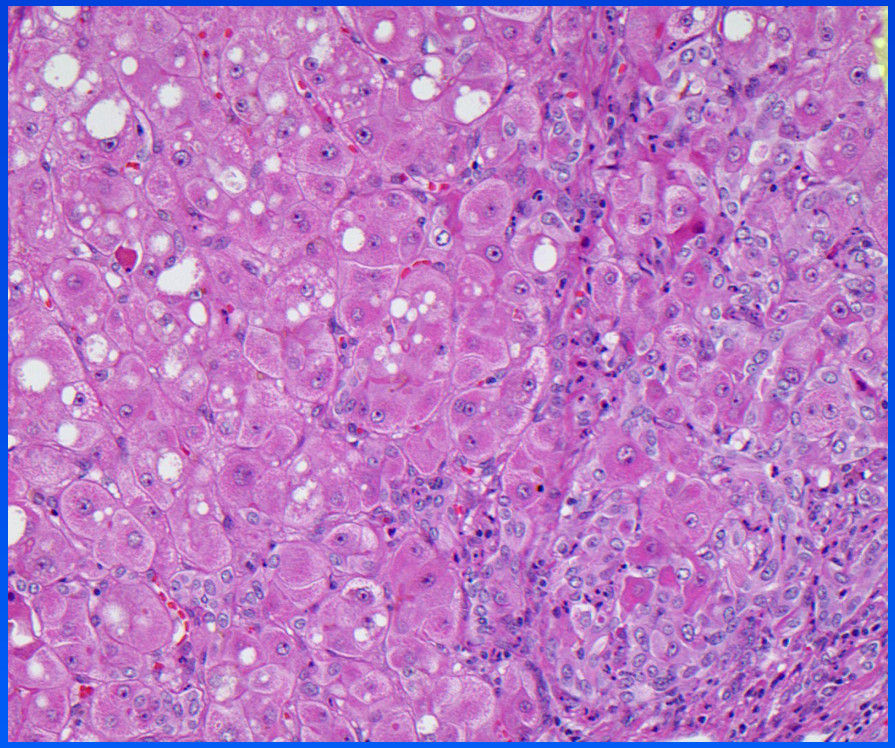
- CMV, EBV negative
- Hepatitis A,B,C negative
- Autoimmune markers negative
- Copper 120 (nl)
- Ceruloplasmin 18 (low)
- Low factor levels

Pathology – H&E



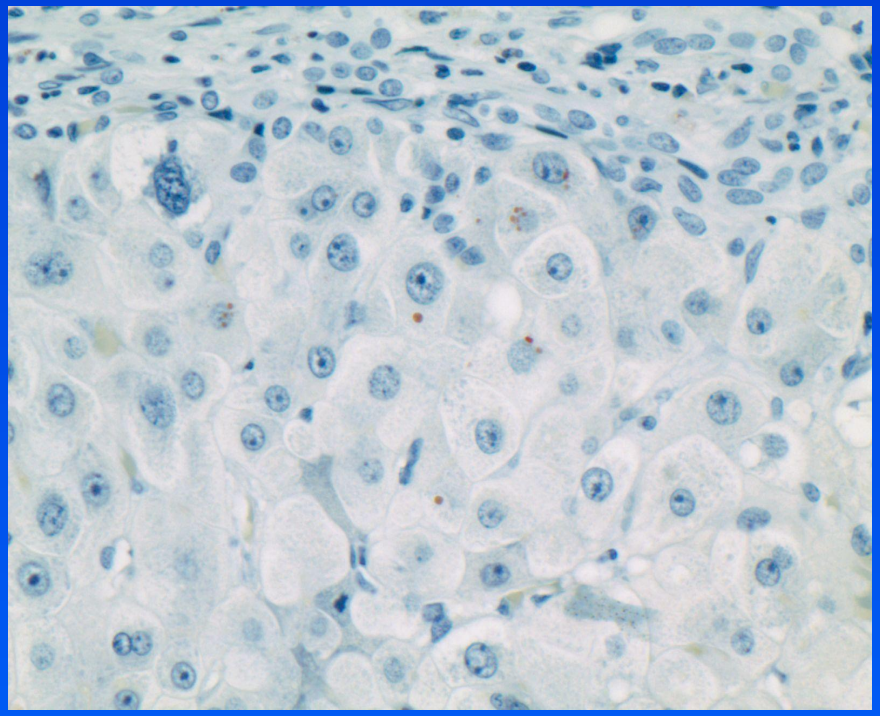
- Peri-portal fibrosis
- Peri-portal glycogenated hepatic nuclei
- Kupffer cell hyperplasia
- Hepatocyte enlargement

Pathology – H&E



- Hepatic steatosis
  - Microvesicular then macrovesicular
- Cholestasis
- False positive
  - alcoholic steatohepatitis

Copper Stain



- False positive
  - Cholestatic liver disorders
  - Indian Childhood Cirrhosis
- False negative
  - Copper not present in hepatocyte
  - Released secondary to cell injury
  - Cytosolic copper more difficult to appreciate than granular lysosomal copper

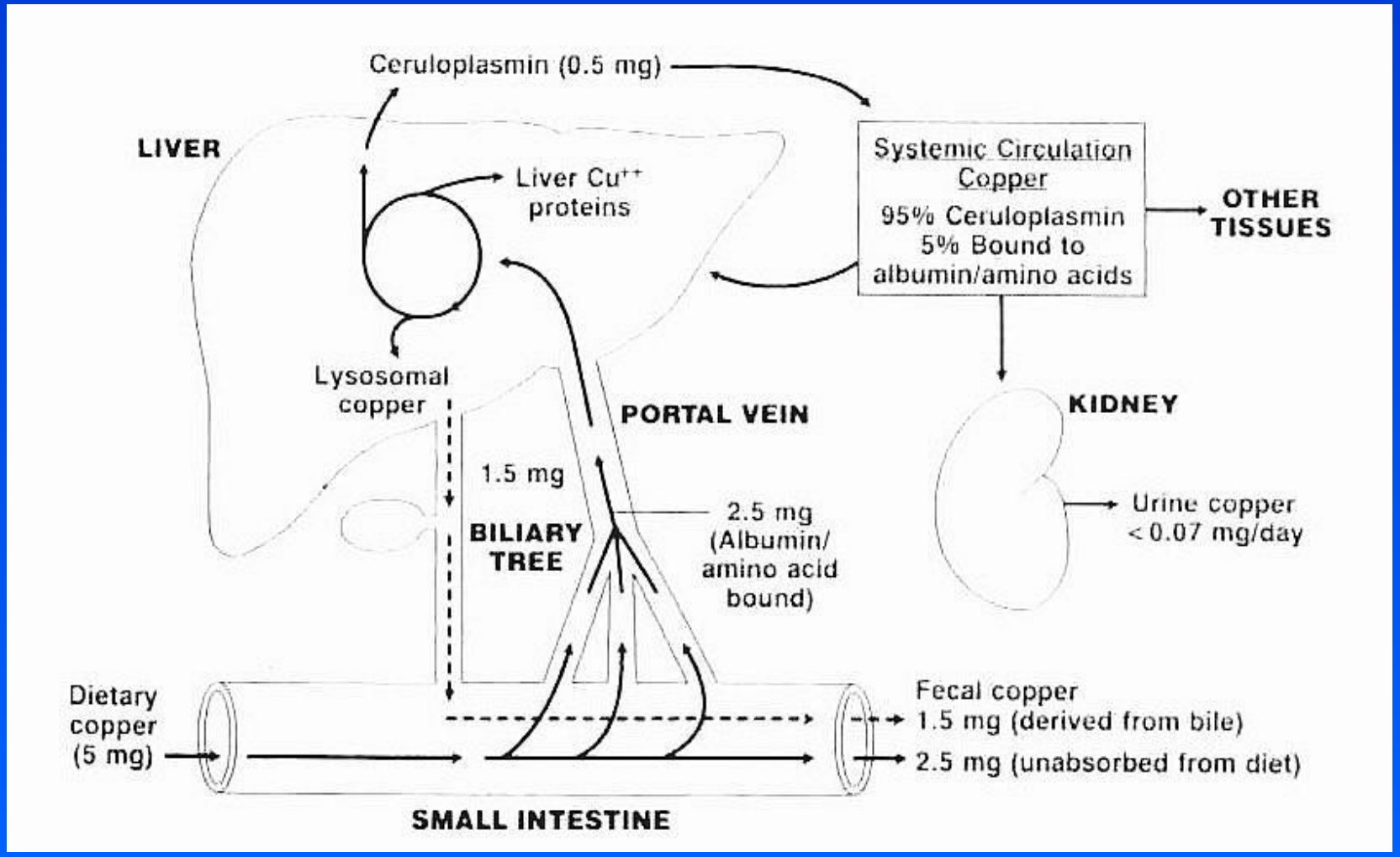
Copper = 1153 µg/g dry weight

WILSON'S DISEASE

Copper Physiology

- Most abundant in unprocessed wheat, dried beans, peas, shellfish, chocolate, liver, kidney
  - Impair copper absorption
    - Zinc, cadmium, ascorbic acid
    - Vegetarian diet
  - Aid copper absorption
    - Gastrointestinal secretions
- Absorbed copper is bound to the protein metallothionein or complexed to amino acids and transported into portal system

Copper Metabolism



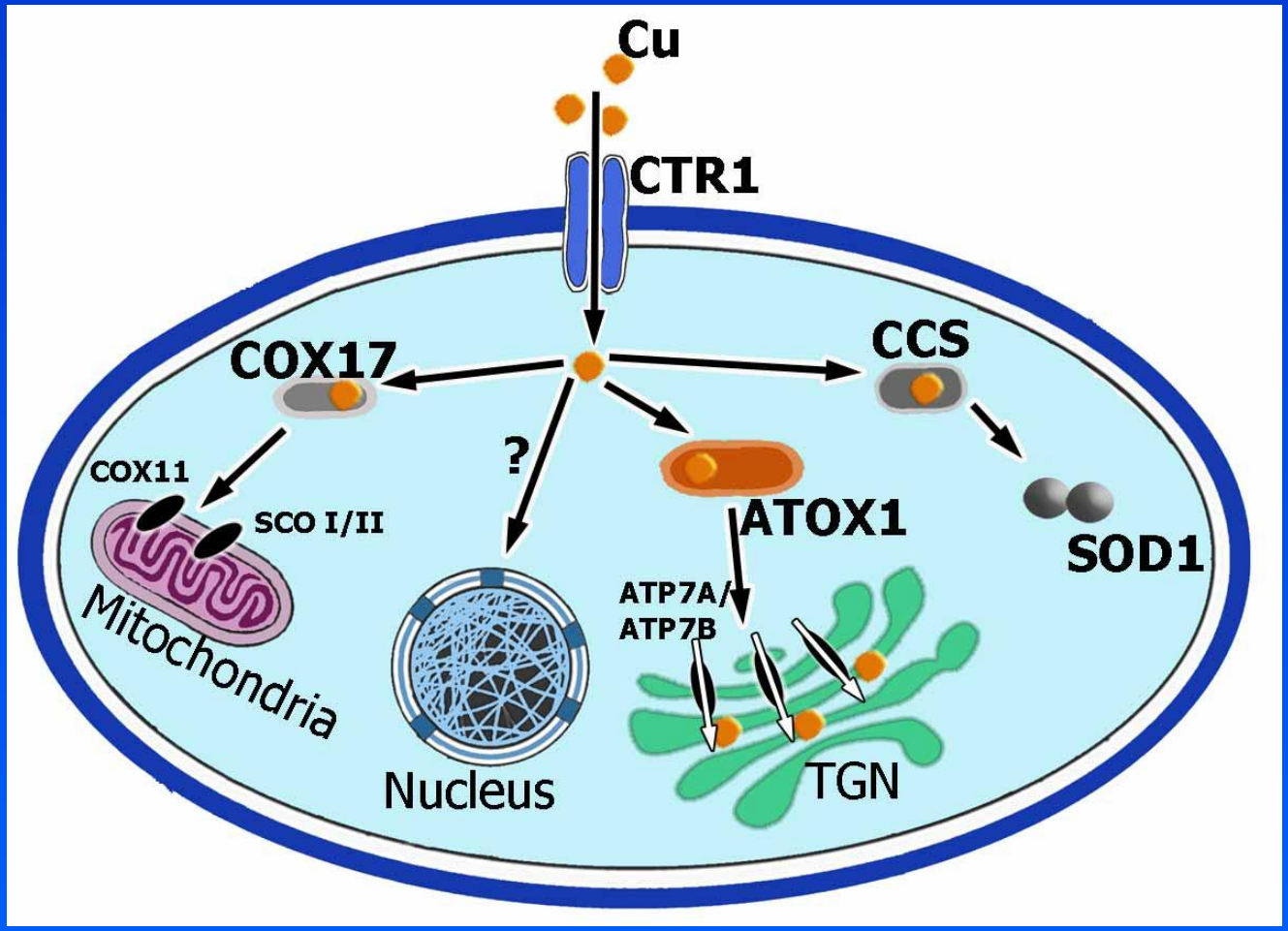
Copper Metabolism

- Copper is transported into hepatocytes by the human copper transporter (hCTR)

- In hepatocyte, copper interacts with the ligands metallothionein, glutathione and HAH1
  - Bind and detoxify excess copper
  - Transfer or store copper
  - Provide copper to chaperones

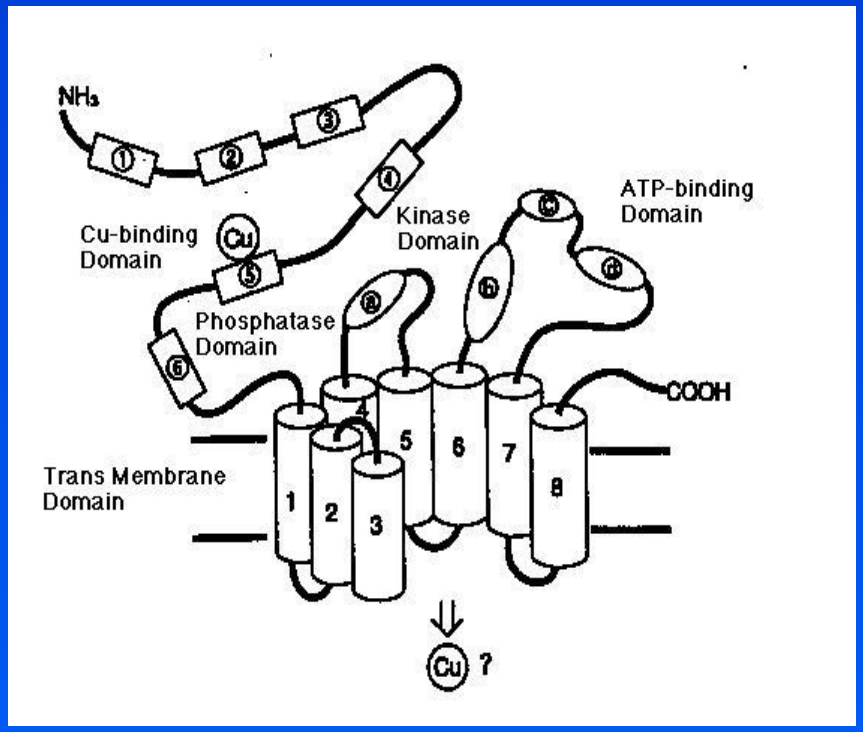
- Chaperones incorporate copper into essential proteins or assist in copper excretion into bile
  - CCS
  - COX17
  - ATOX/HAH1

Copper Metabolism



ATP7B sorts copper and incorporates into secretory vesicles and ceruloplasmin

ATP7B



Copper transporting P-type ATPase

- Copper transporting P-type ATPase
- 13q14-13q21
- 21 exons
- 6 cysteine-rich copper binding sites
- 8 transmembrane domains
- CPC (cysteine-proline-cysteine) common to other metal transporters

ATP7B

- >200 mutations identified
- Most small deletions or missense mutations
  - Missense: neurologic and later presentation
  - Deletions: hepatic and earlier presentation
- Highly expressed in liver and kidney

ATP7B

- Makes copper available for ceruloplasmin synthesis and transport of copper into vesicles

- Long-Evans Cinnamon (LEC) rat
  - ATP7B defects involve transport of copper into the vesicular pathway from Golgi apparatus to the canaliculus

Clinical Presentation

Age	Hepatic symptoms	Neuropsychiatric symptoms
< 10 years	83%	17%
10-18 years	52%	48%
> 18 years	24%	74%

Combined date from Walshe and Scheinberg & Sternleib

Hepatic

- Acute hepatitis
  - 25%
- Fulminant hepatic failure
  - Liver transplant
  - May also present after discontinuing copper chelation
- Chronic active hepatitis
  - 10-30%
  - Absence of other symptoms in Wilson's patients should prompt biochemical screening in those <40 years
- Cirrhosis
  - Absence of other symptoms in Wilson's patients should prompt evaluation >4 years

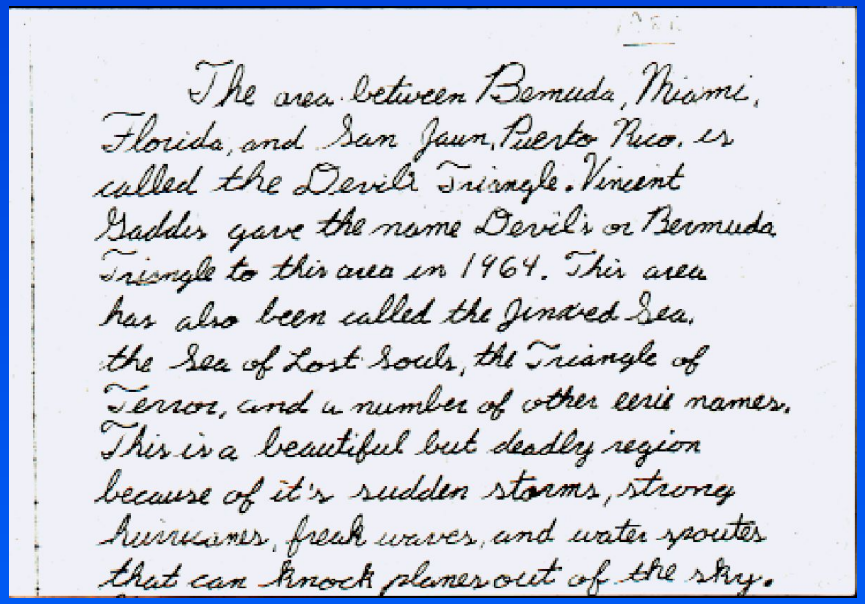
Laboratory

- Mildly elevated serum aminotransferase levels
- Low alkaline phosphatase
- Serum alkaline phosphatase to total bilirubin ratio ratio < 2

Neuropsychiatric

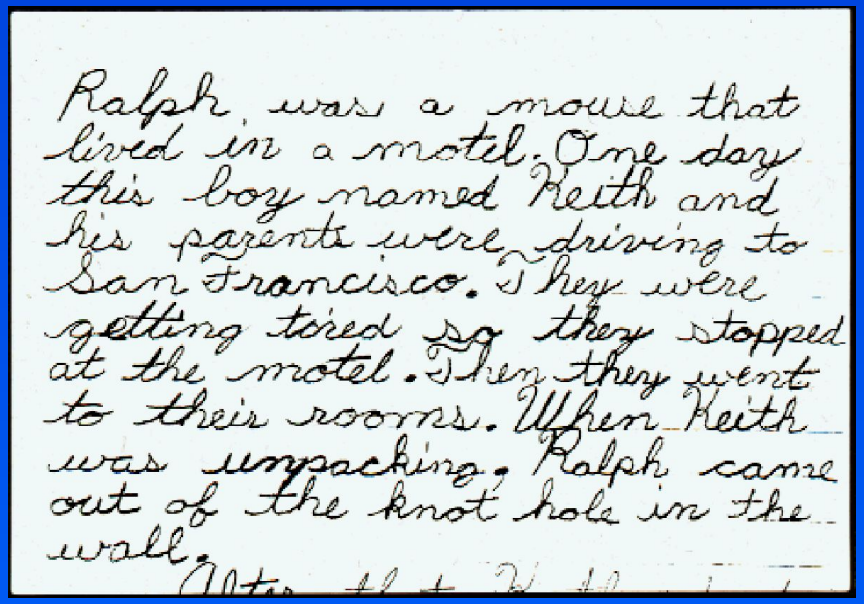
- 40-45% as presentation
- Most common 2nd to 3rd decades of life
- Extrapyramidal and cerebrellar dysfunction
- Migraine headaches
- Seizures
  - Denning et al found 13 of 200 patients with Wilson's disease had seizures
  - Prevalence rate 6.2%, exceeding epilepsy frequency by tenfold
- Gait disturbances secondary to tremor and dystonia

13 year old boy presents to a neurologist for evaluation of a deterioration in his handwriting.



The area between Bemuda, Miami, Florida, and San Jaun, Puerto Rico, is called the Devil's Triangle. Vincent Gaddis gave the name Devil's or Bermuda Triangle to this area in 1964. This area has also been called the Jinxed Sea, the Sea of Lost Souls, the Triangle of Terror, and a number of other eerie names. This is a beautiful but deadly region because of it's sudden storms, strong hurricanes, freak waves, and water spouts that can knock planes out of the sky.

Grade 5



Ralph was a mouse that lived in a motel. One day this boy named Keith and his parents were driving to San Francisco. They were getting tired so they stopped at the motel. Then they went to their rooms. When Keith was unpacking. Ralph came out of the knot hole in the wall.

Grade 6

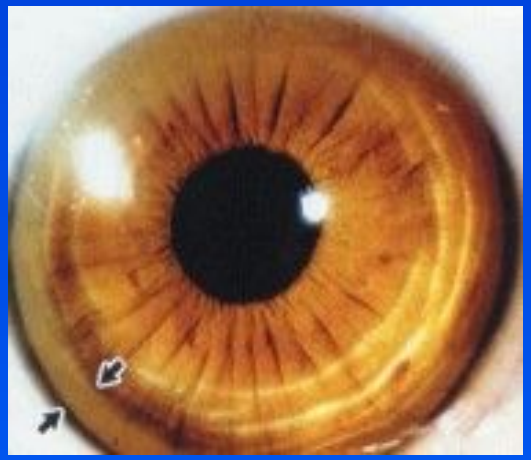
Imaging

- CT abnormalities
  - 73% ventricular dilation
  - 63% cortical atrophy
  - 55% brainstem atrophy
  - 45% hypodensity in basal ganglia
  - 10% posterior fossa atrophy

Williams and Walshe

Kayser-Fleischer Ring

- Superior poles of cornea, then inferior involvement
- Copper chelators result in resolution over 3-5 years
- Occurs after hepatic saturation of copper
  - Virtually always present when neurological or psychiatric symptoms develop
  - Frequently absent in children without neurolgic involvement but with hepatic symptoms
- False positive: hepatitis, cholestasis, primary biliary cirrhosis, TPN



Sunflower Cataract

- Copper deposition in anterior and posterior lens capsule
- False positive with foreign body lodged intraocularly (chalcosis)

Other

- Renal
  - Proximal renal tubular dysfunction (Fanconi's syndrome)
  - Renal insufficiency
  - Nephrocalcinosis
- Hematologic
  - Coombs negative hemolytic anemia
- Cardiac
  - Autonomic dysfunction
  - Cardiomyopathy
- Skeletal
  - Bone demineralization
- Dermatologic
  - Acanthosis nigrans

Diagnostic Studies

Diagnostic test	Diagnostic value	False positive	False negative
Serum ceruloplasmin	<20 mg/dl	Protein losing state Hepatic failure Aceruloplasminemia	Acute hepatitis Pregnancy Malignancy
Hepatic copper	>250 µg/g dry weight (<50 µg/g)	Primary biliary cirrhosis ICC PSC Liver tumors	Copper chelation therapy
24-hour urine copper	>100 µg (<40 µg)	PSC Cholestasis	Copper chelation therapy

Genetic Analysis

- Genetic studies are becoming more available but limited
- Kumar et al characterized ATP7B mutations by restriction fragment length polymorphism (RFLP)
- 3 mutations, Q1256R, A1003T and I1102T were characterized associated with restriction sites for AccII, Bsh1236I and EcoRI
- Mutation analysis in combination with RFLP is useful for positive diagnosis of asymptomatic Wilson's disease and elucidation of carrier status

Treatment Goals

- Reduce copper accumulation by
  - Enhancing urinary excretion
  - Decreasing intestinal absorption

Therapy

<u>Oral Chelating Agents</u> D-Penicillamine Trientine Ammonium tetrathiomolybdate	Many side effects Fewer side effects Experimental
Zinc acetate	Inhibits Cu absorption Stimulates hepatic metallothionein
Vitamin E	Antioxidant Interferes in Vitamin K-dependent clotting factors
Additional Recommendations	Low Cu diet Antioxidants

Liver Transplant

- Life-saving
  - Acute fulminant hepatic failure
  - Decompensated cirrhosis with progressive end stage liver disease

Long-Evans Cinnamon Rats

- A deletion of 900 base pairs at the 3' end of ATP7B gene, eliminating normal gene product
- Previous adenoviral gene transfer resulted in successful but transient gene expression
- In vivo administration of HIV-1-derived lentiviral vectors (LV) expressing the ATP7B gene
- Transplantation of lentivirally modified autologous hepatocytes

Results

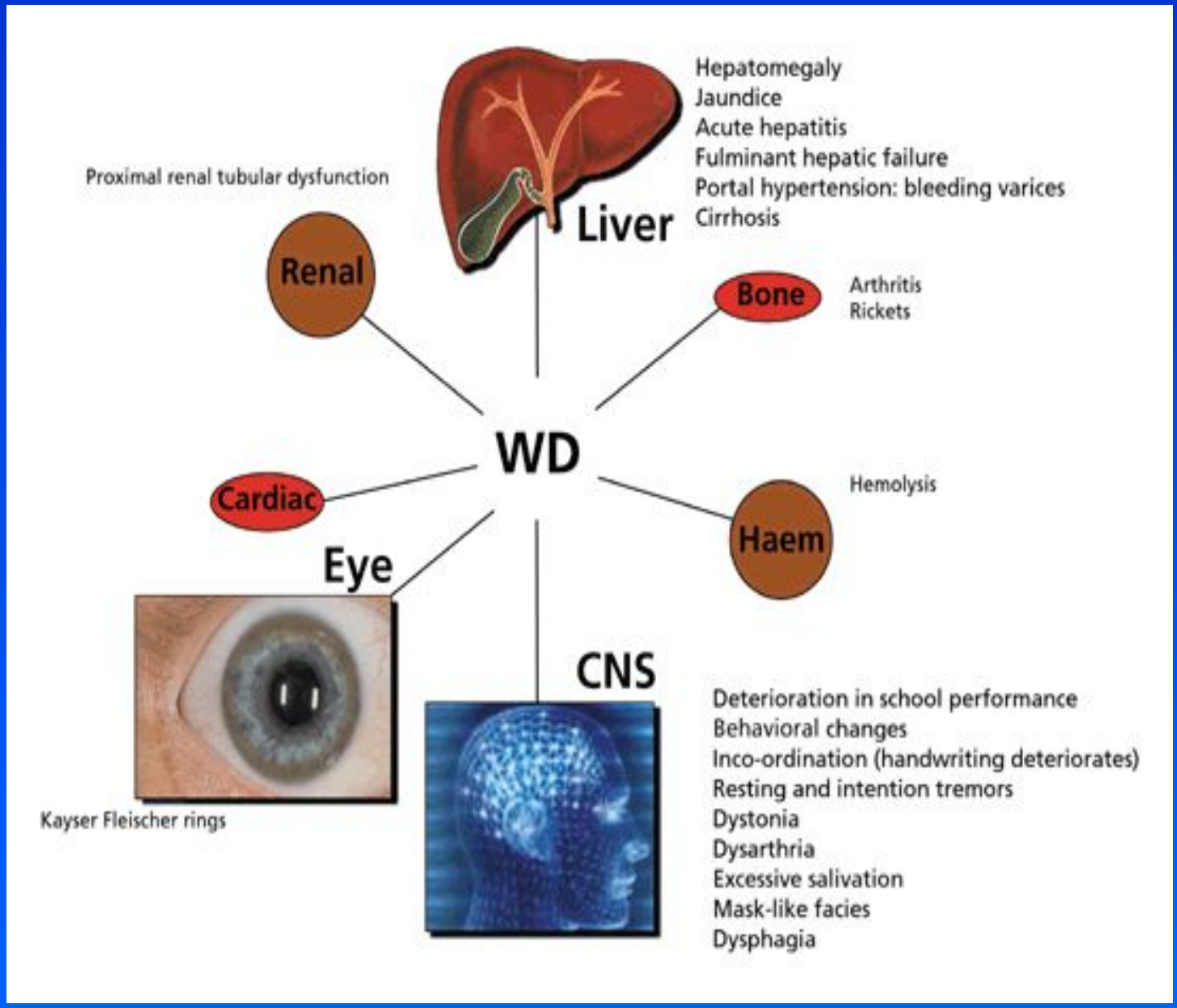
	ATP mRNA (RT-PCR)		Hepatic copper content (average) (µg/g)		Serum ceruloplasmin oxidase activity (U/l)		Histology (fibrosis)
Weeks	2	24	2	24	2	24	
LV	+	+	430	1438	7.3	1.97	Fibrous septa
Hepatocyte Tx	+	+/-	938	1152	6.1	0.9	10.7-19.8%
Untreated Rats	-	-	500	2787	0.05	0.05	48.3-57.9%

Conclusions

- Liver copper levels were lowered in all treatment groups compared to untreated LEC rats
- Histological analysis showed only fibrous septa after LV-ATP7B treatment but still with round-cell infiltration
- Lentiviral ATP7B gene transfer is feasible in Wilson's disease

Wilson's Disease

- Wilson disease is a disorder of copper transport resulting in copper deposition in multiple organs
- Clinical manifestations may be severe, but the disease is treatable if diagnosed early


Hepatomegaly
Jaundice
Acute hepatitis
Fulminant hepatic failure
Portal hypertension: bleeding varices
Cirrhosis
Liver
Proximal renal tubular dysfunction
Renal
Bone
Arthritis
Rickets
WD
Cardiac
Hemolysis
Haem
Eye
Kayser Fleischer rings
CNS
Deterioration in school performance
Behavioral changes
Inco-ordination (handwriting deteriorates)
Resting and intention tremors
Dystonia
Dysarthria
Excessive salivation
Mask-like facies
Dysphagia

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