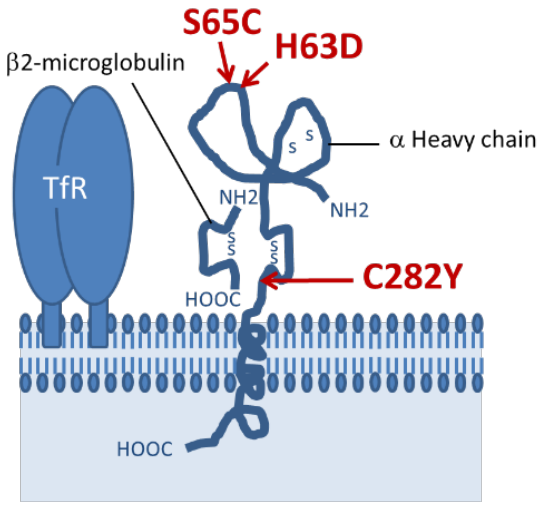
Chapter 8. Genetic liver diseases

8.1 Hereditary Hemochromatosis (HH)

Epidemiology & Pathophysiology

- **Causes of Iron Overload**
  - **Hereditary Hemochromatosis (HFE-related)**
    - C282Y/C282Y homozygotes (>90%)
    - C282Y/H63D or C282Y/S65C compound heterozygotes (5%)
  - **Non-HFE related**
    - Acquired iron overload
    - Iron-loading anemias
    - Transfusion and parenteral iron overload
    - Other liver diseases (ETOH, NAFLD, HCV, etc.)
    - Miscellaneous
- **Genetics of HH**
  - Genetic disorder of iron (Fe) metabolism
  - 1 in 240 (0.4%) of population (most common in Northern Europeans)
  - Autosomal recessive (AR) inheritance with HFE gene identified in 1996
  - MHC I-like molecule → binds to transferrin receptor & modulates iron absorption
  - C282Y and H63D mutations are seen in approximately 10% of Caucasian population

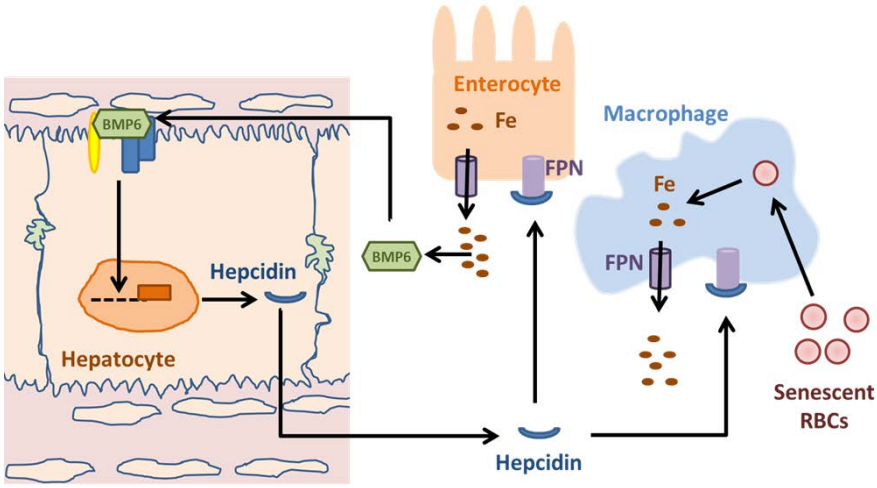
    NOTE: H63D homozygotes do NOT get iron overload (need one C282Y mutation)



Adapted from Bacon B, et al. Hepatology 2011; 54(1): 328-345.

- **Pathogenesis**
  - **Crypt Villous Hypothesis**
    - HFE gene mutations decrease the affinity of transferrin receptor (TfR) for transferrin
    - This leads to a false signal that body iron stores are low
    - Enterocytes in crypt synthesize more divalent metal transporters (DMT)
    - Enterocytes with more DMT migrate up from crypt to villi where more iron is absorbed
    - This leads to eventual iron overload in tissues
  - **Hepcidin Hypothesis**
    - Hepcidin is the principle iron-regulatory hormone and is produced in hepatocytes
    - Hepcidin binds ferroportin on macrophages (plays a role in anemia of chronic disease) and basolateral surface of enterocytes
    - Hepcidin – ferroportin is internalized and degraded, inhibiting iron export by these cells

- BMP6 pathway regulates this pathway and the HFE/TfR influences hepcidin expression



Adapted from Bacon B, et al. Hepatology 2011; 54(1): 328-345.

Diagnosis & Natural History

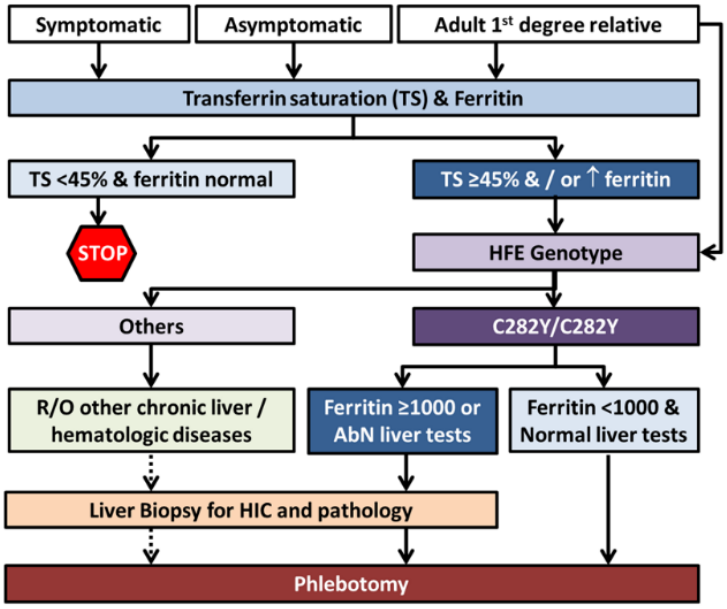
- Typically patients have mild to moderate elevation of ALT or AST

  NOTE: HH does not cause severe elevation of ALT or AST, and if ferritin is elevated with a severe elevation of ALT (>1000) it is usually due to an acute phase reaction and not HH

- Screening tests include transferrin saturation (TS) and ferritin (see below)
  - TS % = Fe (iron) / TIBC (total iron binding concentration) is more specific
  - Ferritin is more sensitive, but less specific
    - DDx for elevated ferritin includes 1) HH, 2) other chronic liver disease, 3) other inflammatory conditions (e.g. inflammatory bowel disease, rheumatoid arthritis)
    - If TS% >45% then order HFE genetic testing
    - Ferritin > 1000 ng/mL with TS% <45% should not necessary prompt HFE genetic testing (*Choosing Wisely Canada™ Recommendation*)
  - Tests are not perfect

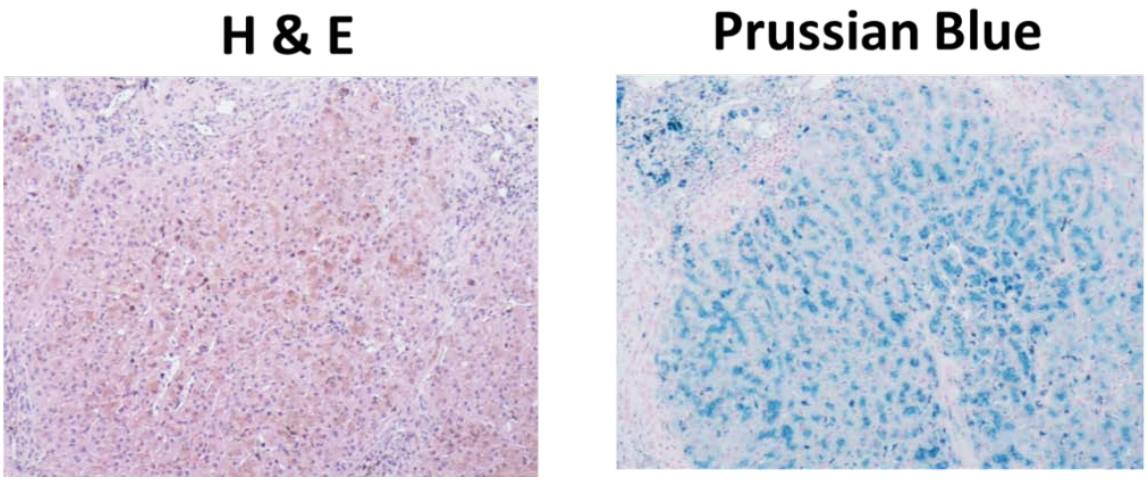
- 1/3 HH may have TS ≤ 45%
- 50% ETOH, HCV, NASH have abnormal ferritin (but negative HFE gene studies)
- 30% C282Y homozygotes have normal ferritin

o **Suggested Testing and Treatment for HH (AASLD)**



Adapted from Bacon B, et al. Hepatology 2011; 54(1): 328-345.

- **Liver biopsy** can be done to qualitatively estimate iron overload (0→4) which is seen as a brown pigment on H&E stain but should be confirmed to be iron using **Prussian Blue stain**
  - o **Dry weight iron** can be performed to quantitate the amount of iron
  - o **HIC = Hepatic Iron Content** should be > 1800 µg/g dry weight (>32 µmol/g) if HH
  - o **HII = Hepatic Iron Index** = HIC (mmol/g) ÷ patients age (years) and should be > 1.9 if HH, but this is not that useful now that we have genetic testing



- **Clinical Features** related to iron deposition in organs
  - **Liver** = hepatitis, cirrhosis, hepatocellular carcinoma (HCC)
  - **Joints** = arthritis with classic finding of arthritis is 2nd & 3rd MCP joints (knuckles)
  - **Heart** = congestive heart failure, arrhythmias
  - **Skin** = bronze pigmentation
  - **Pancreas & endocrine** = diabetes, impotence (testicular atrophy), amenorrhea
- **Natural History**
  - Can progress to cirrhosis and associated with high risk of HCC
  - **Phlebotomy** can change natural history[1]
    - ↓ of tissue iron stores to normal
    - ↑ **survival if phlebotomy started before cirrhosis and diabetes**
    - ↑ sense of well-being and energy
    - ↑ cardiac function
    - ↑ control of diabetes
    - ↓ in abdominal pain

- ↓ in skin pigmentation
- Normalization of liver enzymes
- Reversal of hepatic fibrosis (~30% of cases)
- No reversal of established cirrhosis
- Elimination of risk of HCC if iron removal before cirrhosis
- ↓ in portal hypertension in patients with cirrhosis
- No (or minimal) improvement in arthritis
- No reversal of testicular atrophy

Treatment

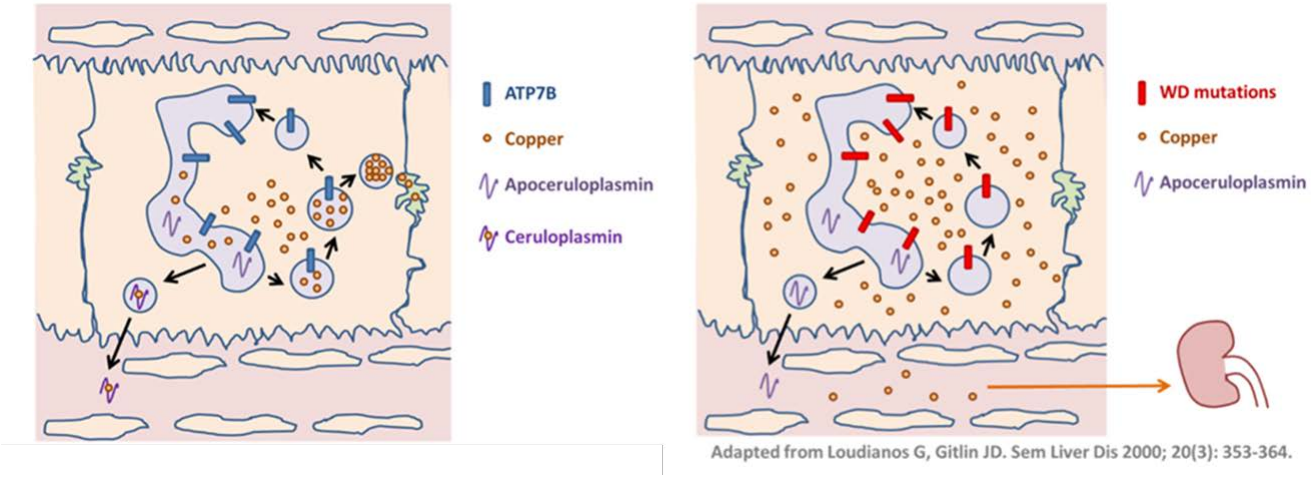
- **Phlebotomy** (removal of 500 mL blood)
  - Removes 250 mg of iron per phlebotomy
  - Some will have 20-30 grams of excessive iron at the time of diagnosis
  - Initially do weekly phlebotomy
  - Check hemoglobin prior to each phlebotomy
  - Allow hemoglobin to fall by no more than 20%
  - Check ferritin level every 10-12 phlebotomies
  - Stop weekly phlebotomies when ferritin 50 g/L
  - Then continue phlebotomy every 2-4 months to keep ferritin between 50-100 g/L
- Avoid vitamin C supplements (increase dietary absorption of iron) and iron supplements but no need to avoid red meat or other iron rich foods

8.2 Wilson's Disease (WD)

Epidemiology & Pathophysiology

- **Copper (Cu)** is an essential trace metal important for cellular respiration, iron homeostasis, pigment formation, neurotransmitter production, connective tissue synthesis, and antioxidant defense (immune system function)
- It is absorbed from stomach & duodenum (4-6 mg absorbed daily in diet) and normal copper stores (100 mg Cu) are maintained by balance of GI absorption + bile excretion
- Wilson's disease was described in 1912 in a family who had neurologic disease with associated cirrhosis and the role of copper was recognized in 1948
- In 1953 it was found to be an autosomal recessive condition and the gene defect (ATP7B copper transporter mutations) was found in 1993
- It occurs in all ethnic groups in approximately 1 in 30,000 births (carrier rate 1 in 90)
- **Pathophysiology**
  - Within hepatocytes, high intracellular copper normally leads to increased biliary excretion of excess copper (preferred way to eliminate excess copper)
    - This requires vesicles (produced from the trans Golgi network) to carry copper to bile canaliculus for excretion into bile
    - **ATP7B** is a copper transporter on the trans Golgi network
    - In the Golgi, free copper is bound to **apoceruloplasmin** and is released as **ceruloplasmin** into the circulation for transport of copper to other organs
  - **ATP7B mutations of Wilson's Disease** leads to abnormal uptake of copper into the Golgi
    - This results in a lack of biliary excretion of copper

- Copper is also not bound to apoceruloplasmin
- Apoceruloplasmin has a shorter half-life (compared to copper bound ceruloplasmin) and is removed from circulation more quickly
- When the lab measures "ceruloplasmin" it is measuring both apoceruloplasmin + ceruloplasmin and the levels are therefore lower in WD
- The **screening test** for WD is therefore a **low ceruloplasmin** level
- The free copper the spills from the liver goes to the kidney for excretion and results in the **high urinary copper levels** seen in WD



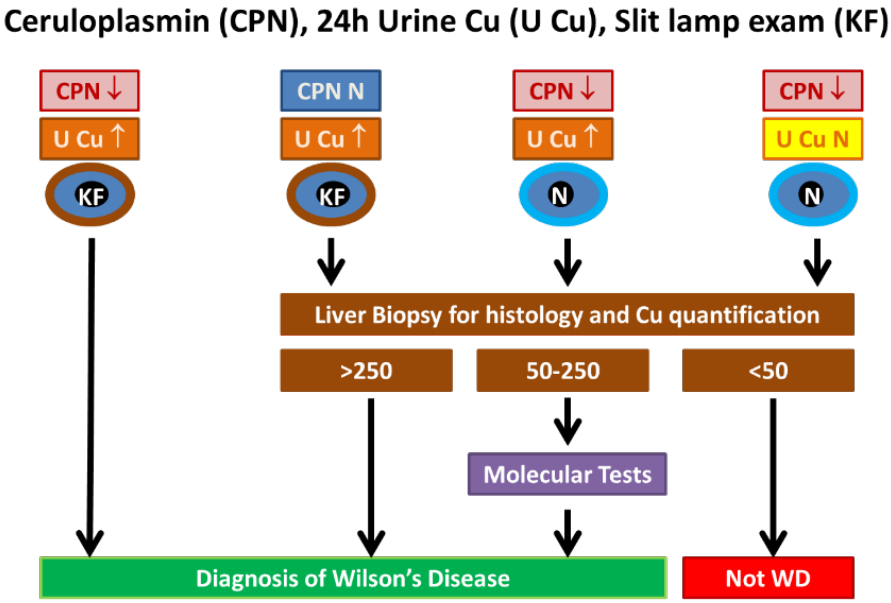
Adapted from Loudianos G, Gitlin JD. Sem Liver Dis 2000; 20(3): 353-364.

Diagnosis & Natural History

- **Clinical Presentations**
  - **Liver disease**
    - Suspect in young people with elevated liver tests, cirrhosis or acute liver failure
    - Hepatic dysfunction usually occurs 10 years before neurologic symptoms
    - May be asymptomatic with abnormal liver tests (mild, moderate or severe ↑ ALT)
    - They may have chronic hepatitis (with liver biopsy findings that can mimic NAFLD, AIH, viral hepatitis) and it can progress to cirrhosis

    - Acute Liver Failure (ALF) presents with jaundice, severely elevated ALT, elevated bilirubin (which may be partly unconjugated as copper is toxic to red blood cells and leads to hemolytic anemia) with disproportionately low ALP
  - **Neurologic disease**
    - Initial presentation in 60% (3rd or 4th decade)
    - Parkinsonian features (Cu in basal ganglia), with dystonia (rigidity and contractures), tremors, dysarthria and dysphonia, gait disturbance and choreiform movements
    - Psychiatric symptoms include abnormal behavior, personality changes, depression, cognitive loss (poor school performance), psychoses
- Other features
  - **Kayser-Fleischer (KF) rings** (copper deposits in limbus) seen as brown ring around iris, which is best seen on slit lamp examination
  - Renal disease includes aminoaciduria (Fanconi's) and nephrocalcinosis
- **Diagnosis** (see below, N = normal)
  - First-line
    - Low serum ceruloplasmin (CPN), but this is normal in 5%
    - KF rings on slit lamp exam
    - Raised 24h urine copper (U Cu), which should be >40 μg/day
  - Second-line
    - Liver biopsy for copper concentration (>250 μg/g dry weight)

- Third-line
  - Genetic studies which are difficult as there are multiple mutations of the ATP7B gene (unlike the few mutations that cause HH)



Adapted from Roberts EA, Schilsky ML. Hepatology 2008; 47(6): 2089-2111.

- **Natural History**
  - Can progress to cirrhosis and its complications *[see Chapter 14]*
  - Acute liver failure always requires liver transplantation *[see Chapter 10]*

Treatment

- Copper chelation to increase urinary copper excretion
  - **Penicillamine**
    - Side-effects include drug induced systemic lupus erythematosus (SLE), proteinuria, bone marrow suppression → therefore must monitor urinalysis and CBC

- **Trientine**
  - Better tolerated but very expensive and can cause gastritis and sideroblastic anemia

- Decrease copper absorption
  - **Zinc**
    - Very well tolerated but can cause gastrointestinal upset
    - Used for maintenance after the patient has been de-coppered with chelation

- Monitor response with yearly 24h urine copper measurement
  - On chelation the 24 hour urine Cu should be 3-8 μmol/day
  - On zinc alone the 24 hour urine Cu should be < 1.2 μmol/day

8.3 Alpha 1 Antitrypsin Deficiency (A1AT def)

Epidemiology & Pathophysiology

- A1AT is a glycoprotein made by the liver and is a major protease inhibitor in blood
  - Secretion from liver requires glycosylation
  - Amino acid substitutes determine the type of carbohydrate (CHO) side chains attached to the A1AT protein
  - Each person inherits 2 alleles (Pi phenotype)
    - Named for how fast they travel on a protein electrophoresis gel (M $\rightarrow$ Z)
    - Most common normal variant being M
    - Most common deficiency variant is Z

- Those homozygous for Z allele do not properly excrete A1AT into plasma
- This leads to globules being retained in liver (disease of excess) leading to abnormal autophagy with subsequent fibrosis and cirrhosis
- The lack of A1AT in the circulation and the lungs (disease of deficiency) leading to an inability to inhibit neutrophil elastase and results in **emphysema**

- Allele frequencies in population
  - PiM = 0.95
  - PiS = 0.03
  - PiZ = 0.01
  - Therefore PiSZ in 1/800 and PiZZ in 1/3500 births
- Consequences
  - MM = normal level
  - MS = 80% of normal level
  - MZ = 60% of normal level
  - SZ = 40% of normal level
  - ZZ = 10% of normal level
- USA population estimates
  - 2% of the population is heterozygote for Z allele
  - 0.05% (1 in 2000 births) are homozygous ZZ

Diagnosis & Natural History

- Childhood

- Most common genetic cause of liver disease in children where it can lead to **neonatal cholestasis**
- However, only in 20% of PiZZ infants will be symptomatic
- Neonatal hepatitis syndrome has variable outcomes
  - Cholestatic jaundice, pruritus, failure to thrive, hepatosplenomegaly
  - Liver tests can resolve with little residual damage (80% free of clinically apparent liver disease by age 18)
  - Can progress to cirrhosis as toddler or teenager
  - Cirrhosis may not be apparent until adulthood

- Adults
  - Only 10% PiZZ adults have overt liver disease (poor genetic penetrance)
  - Mechanism of liver disease is unclear
  - Typically only mild ALT elevation (like ETOH should not cause ALT > 300)
  - Heterozygotes (~3% Caucasians) may have an increased risk of cirrhosis or liver cancer if they have another liver disease (e.g. ETOH, HCV, or NAFLD)

- **Diagnosis**
  - Screen with A1AT level and if low order phenotyping (M, S, Z)
  - Liver biopsy will show globules which are Periodic Acid Schiff (PAS) positive but Diastase resistant on **PAS-D stain**

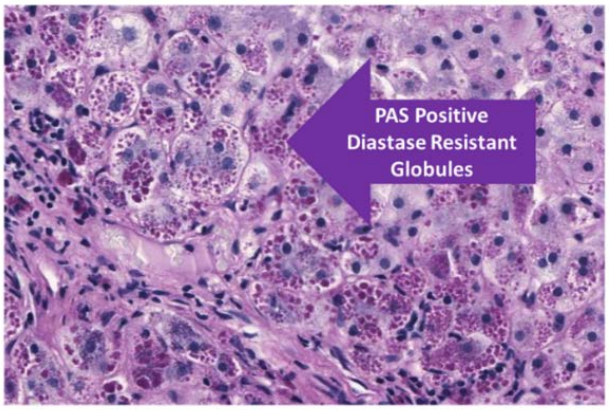


Image Source: http://en.wikipedia.org/wiki/Alpha_1-antitrypsin_deficiency

Treatment

- There is no effective therapy for A1AT deficiency
- Some patients will require liver transplantation (rare indication)

Abbreviations

AR – autosomal recessive

CBC – complete blood count

CHO – carbohydrates

CPN – ceruloplasmin

Cu – copper

DDx – differential diagnosis

DMT – divalent metal transporters

HIC – hepatic iron content

HII – hepatic iron index

KF rings – Kayser-Fleischer rings

MCP joint – metacarpophalangeal joint

SLE – systemic lupus erythematosus

TfR – transferrin receptor

U Cu – urine copper

Figure Citations

HFE gene product, Hepcidin hypothesis, HH Diagnosis and Management. Adapted from Bacon BR, Adams PC, Kowdley KV, Powell LW, Tavill AS, American Association for the Study of Liver Diseases. Diagnosis and management of hemochromatosis: 2011 practice guideline by the American Association for the Study of Liver Diseases. *Hepatology* 2011; 54(1):328-343.

Hemochromatosis Pathology (H&E and Prussian Blue). Slides courtesy of Dr. Stefan Urbanski.

Wilson's Disease Pathogenesis. Adapted from Loudianos G, Gitlin JD. Wilson's Disease. *Sem Liver Dis* 2000; 20(3):353-364.

Wilson's Disease Diagnosis. Adapted from Roberts EA, Schilsky ML, American Association for the Study of Liver Diseases. Diagnosis and treatment of Wilson disease: an update. *Hepatology* 2008; 47(6):2089-2111.

A1AT Deficiency Pathology. Alpha 1-antitrypsin deficiency. Retrieved July 20, 2017 from Wikipedia. https://en.wikipedia.org/wiki/Alpha_1-antitrypsin_deficiency

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3. European Association for the Study of the Liver. EASL Clinical Practice Guidelines: Wilson's Disease. *J Hepatol* 2012; 56(3):671-685.
4. Fairbanks KD, Tavill AS. Liver Disease in Alpha 1-Antitrypsin Deficiency: A Review. *Am J Gastroenterol* 2008; 103(8):2136-2141.