Update on Hemochromatosis and Other Genetic Liver Diseases

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Hereditary Hemochromatosis
Classification of Iron Overload Disorders

- Primary Iron Overload Disorders
- Secondary Iron Overload
- Other Iron Overload Disorders
- Iron Loading due to Liver Disease

Secondary Iron Overload

- Dietary iron overload
  - African Iron Overload
- Parenteral iron overload
- Ineffective erythropoiesis
- Long-term hemodialysis
- Liver Disease
  - End-stage liver disease
  - Alcoholic cirrhosis
  - Portocaval shunt
- Porphyria cutanea tarda
- Dysmetabolic iron overload syndrome
Primary Iron Overload

- Hereditary hemochromatosis
  - HFE
  - non-HFE
  - Ferroportin disease
- Aceruloplasminemia
- A(hypo)transferrinemia
- H-ferritin-associated iron overload
- African iron overload?
- African-American iron overload?

OMIM Classification of Hemochromatosis

- Type 1: “Classical” HFE-associated HH
- Type 2A: Juvenile HH (HJV mutation [1q21HJV])
- Type 2B: Juvenile HH (Hepcidin mutation [19q13.1])
- Type 3: TfR2 mutation-associated HH
- Type 4: Ferroportin-associated HH
Genetics of Type I HH

- Putative gene between HLA A and B
- HFE (HLA-H) identified in 1996
- HFE gene encodes a Class I protein
- Two mutations identified
  - Cys $\rightarrow$ Tyr at aa 282
  - His $\rightarrow$ Asp at aa 63

Gene Mutation Patterns in HFE-HH

- C282Y homozygotes most important mutation
  - Have the highest level of HIC
  - Uniform hepatic iron deposition
  - Majority of Caucasian patients (80-90%)
- C282Y/H63D comprise 1.5-7%
  - Have lower HIC compared to C282Y homozygotes
- S65C, H63D mutations unimportant
  - May be lead to iron overload with another risk factor
### C282Y +/- mutation in HH

<table>
<thead>
<tr>
<th>Location</th>
<th>Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>USA (Feder)</td>
<td>83%</td>
</tr>
<tr>
<td>USA (Beutler)</td>
<td>82%</td>
</tr>
<tr>
<td>Norway (Oslo)</td>
<td>81%</td>
</tr>
<tr>
<td>Germany (North)</td>
<td>95%</td>
</tr>
<tr>
<td>France (Brittany)</td>
<td>91%</td>
</tr>
<tr>
<td>Spain (Barcelona)</td>
<td>87%</td>
</tr>
<tr>
<td>Portugal (Porto)</td>
<td>84%</td>
</tr>
<tr>
<td>France (Toulouse)</td>
<td>72%</td>
</tr>
<tr>
<td>USA (Barton)</td>
<td>59%</td>
</tr>
<tr>
<td>USA (Rochester)</td>
<td>60%</td>
</tr>
<tr>
<td>Italy (North)</td>
<td>69%</td>
</tr>
<tr>
<td>Greece (Athens)</td>
<td>50%</td>
</tr>
</tbody>
</table>

### Hepatic Iron Stain

- Stainable iron in hepatocytes
- Periportal iron staining
**Predictors of Cirrhosis in HHC**

- 182 patients with HHC based on:
  - HIC>4,000 mcg/g
  - HII>1.9
  - >4 g mobilizable iron
- Liver biopsy reviewed
- Bridging Fibrosis and cirrhosis recorded
- Alcohol<20g/day
- No risk factors for NASH

Juvenile Hemochromatosis

- Distinct from classical "HFE-linked" hemochromatosis
- Early onset of disease, no gender predominance
- Greatly increased iron stores
- Clinical symptoms similar to HFE hemochromatosis
- Hypogonadism and cardiac disease common
- Premature deaths due to cardiac disease

TfR2-associated HH

- Southern Europe
  - Portugal, Italy
- Japan
- Similar to Type 1 HH in:
  - Degree of iron loading
  - Pattern of hepatic iron deposition
  - Response to phlebotomy
- Possible earlier age at onset
Ferroportin Disease

- Isolated hyperferritinemia or normal or slightly increased TS
- Progressive hepatic iron overload
- Iron accumulation in Kupffer cells
- Therapeutic phlebotomies may be poorly tolerated
- Less severe than HFE-related hemochromatosis

Diagnosis
Diagnosis of HHC

Screening tests

- TS%
- UIBC
- Ferritin

HFE test → Liver Biopsy

Prognosis HFE+
Diagnosis And Prognosis HFE-

Treatment of iron overload

- Phlebotomy therapy:
- Reduce serum ferritin to <50
- Reduce serum TS to <50
- Weekly 500 cc phlebotomy
- Reduce hematocrit to 75% of baseline
- Avoid vitamin C supplementation
**HFE Genotype and Survival After OLT**

*Kowdley et al., Gastroenterol 2005*

**Wilson Disease**
Wilson Disease

• Pathophysiology related to copper overload
• Gene localized to chromosome 13
• Encodes a p-type ATP-ase (ATP7B)
• Decreased biliary copper excretion
• Hepatic copper accumulation
• Copper deposition in extrahepatic sites
Clinical Features

- Neurologic
- Chronic active hepatitis
- Cirrhosis, portal hypertension
- Acute liver failure

Clinical Pearls

- Coombs hemolytic anemia
- Hypouricemia
- Low alkaline phosphatase
- High bilirubin:alk phos ratio
Wilson Disease

Atlas of the Liver 2nd edition

Hepatic Histology and Histochemistry in Wilson Disease

Courtesy of Dr. Michael Schilsky
Diagnosis of Wilson Disease

- **Normal**
  - Serum Copper (micgm/dl) 80-140 <80
  - Urine Copper (mcg/24 hr) <40 >100
  - Serum ceruloplasmin (mg/dl) 20-40 <20
  - Hepatic copper (micg/gm dw) 15-50 250-300

- **Wilson’s**

- **Serum Free-Copper Concentration**
  - Total Cu - Ceruloplasmin X 3.15
  - Free Cu usually < 100 μg/L
  - Wilson’s Disease: Free Cu >200 μg/L

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### Diagnosis of WD in patients with ALF

<table>
<thead>
<tr>
<th>Group</th>
<th>Screening Test</th>
<th>Sensitivity %</th>
<th>Specificity %</th>
<th>Likelihood Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute:</td>
<td>Cp&lt;sub&gt;i&lt;/sub&gt; (mg/dL)&lt;20 by oxidase</td>
<td>21</td>
<td>84</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Cp (mg/dL) &lt;20 by nephelometry</td>
<td>56</td>
<td>63</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Hemoglobin (g/dL) &lt;10</td>
<td>94</td>
<td>74</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>AP:TB&lt;sub&gt;i&lt;/sub&gt; ratio &lt;4</td>
<td>94</td>
<td>96</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>AP:TB ratio &lt;4 + AST:ALT ratio &gt;2.2</td>
<td>100</td>
<td>100</td>
<td>NA&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Cu* (μg/dL) &gt;200</td>
<td>75</td>
<td>96</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>AST:ALT ratio &gt;2.2</td>
<td>94</td>
<td>86</td>
<td>7</td>
</tr>
<tr>
<td>Chronic:</td>
<td>Cp (mg/dL) &lt;20 by oxidase</td>
<td>71</td>
<td>97</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>Cp (mg/dL) &lt;20 by nephelometry</td>
<td>71</td>
<td>79</td>
<td>3&lt;br&gt;Korman et al Hepatology 2009</td>
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Serum and Urine Copper in WD

Hepatic Copper Levels

<table>
<thead>
<tr>
<th>Disease</th>
<th>Mean Hepatic [Cu]</th>
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<tbody>
<tr>
<td>Wilson’s Disease</td>
<td>730 μg/g dry weight</td>
</tr>
<tr>
<td>Primary biliary cirrhosis</td>
<td>410 μg/g dry weight</td>
</tr>
<tr>
<td>Primary sclerosing cholangitis</td>
<td>245 μg/g dry weight</td>
</tr>
<tr>
<td>Extrahepatic biliary obstruction</td>
<td>130 μg/g dry weight</td>
</tr>
<tr>
<td>Indian childhood cirrhosis</td>
<td>1830 μg/g dry weight</td>
</tr>
<tr>
<td>Alcoholic/Cryptogenic cirrhosis</td>
<td>40 μg/g dry weight</td>
</tr>
<tr>
<td>Normal</td>
<td>30 μg/g dry weight</td>
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</table>
Wilson Disease Mutations

**ATP7B Mutations**

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Exon</th>
<th>Region</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>H1069Q</td>
<td>Exon 14</td>
<td>Central, Eastern and Northern Europe</td>
<td>30-70%</td>
</tr>
<tr>
<td>2299insC, G710S</td>
<td>Exon 8</td>
<td>Central, Eastern and Northern Europe</td>
<td>&lt; 10%</td>
</tr>
<tr>
<td>3400delC</td>
<td>Exon 15</td>
<td>Central, Eastern and Northern Europe</td>
<td>&lt; 10%</td>
</tr>
<tr>
<td>R969Q</td>
<td>Exon 13</td>
<td>Central, Eastern and Northern Europe</td>
<td>&lt; 10%</td>
</tr>
<tr>
<td>-441-427 del 5' UTR</td>
<td></td>
<td>Sardinia</td>
<td>&gt;50%</td>
</tr>
<tr>
<td>M645R</td>
<td>Exon 6</td>
<td>Spain</td>
<td>&gt;50%</td>
</tr>
<tr>
<td>R778L</td>
<td>Exon 8</td>
<td>Asia</td>
<td>14-49%</td>
</tr>
</tbody>
</table>

Affsho and Schilsky Curr Op 2010

Treatment

- **Eliminate copper rich foods**
  - Organ meats
  - Shellfish
  - Nuts
  - Chocolate
  - Mushrooms
  - Dried fruits or beans
  - Peas
Chelation Therapy

• Penicillamine
  – Reduces affinity of protein for copper
  – Increases urinary copper excretion
  – Increases hepatic ligand, metallothionein
  – Fever & Rash most common side effects
  – Nausea, vomiting and anorexia
  – Aplastic anemia
  – Proteinuria → Nephrotic Syndrome
  – Neurologic symptoms may initially worsen
  – Pyridoxine 25mg qD

Chelation Therapy

• Trientine
  – Increased urinary copper excretion
  – Decreases intestinal absorption of copper
  – Gastritis
  – Iron deficiency anemia
  – Becoming more first line
Chelation Therapy

• Zinc
  – Zinc acetate preferred over zinc sulfate
  – Induces intestinal cell metallothionein and blocks absorption of copper into circulation
  – Induces hepatic metallothionein and binds toxic copper in the liver
  – Initial therapy use in conjunction with trientine
  – Used in maintenance after urinary Cu excretion <500 μg/day

Genetic Testing for Wilson Disease

<table>
<thead>
<tr>
<th>Name of Laboratory</th>
<th>Web Site</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ambry genetics</td>
<td><a href="http://www.ambrygen.com">www.ambrygen.com</a></td>
</tr>
<tr>
<td>Mayo Medical Laboratories</td>
<td><a href="http://www.mayomedicallaboratories.com">www.mayomedicallaboratories.com</a></td>
</tr>
<tr>
<td>PreventionGenetics Molecular Diagnostics and Biobanking</td>
<td><a href="http://www.preventiongenetics.com/">http://www.preventiongenetics.com/</a></td>
</tr>
<tr>
<td>Coats Children’s Hospital Molecular Genetics Laboratory</td>
<td><a href="http://www.coatchildrens.org/healthcareprofessionals/access-services/diagnostic-services/geneticslab/">www.coatchildrens.org/healthcareprofessionals/access-services/diagnostic-services/geneticslab/</a></td>
</tr>
<tr>
<td>University of Chicago Genetic Services Laboratory</td>
<td><a href="http://www.genes.uchicago.edu/LabPDF/01WD-%20families.pdf">www.genes.uchicago.edu/LabPDF/01WD-%20families.pdf</a></td>
</tr>
<tr>
<td>University of Oklahoma Health Science Center Genetics Laboratory</td>
<td><a href="http://www.genetics.ouhsc.edu/wilson.asp">http://www.genetics.ouhsc.edu/wilson.asp</a></td>
</tr>
</tbody>
</table>
Alpha 1 Antitrypsin Deficiency

Epidemiology

- Most common genetic cause
  - of emphysema
  - of liver disease in infants and children
  - of OLT in children
- 1:1600 – 1:2000 live births
  - up to 1:5000 in Sweden
- autosomal recessive

Sveger NEJM 1976; 294:1316
Perlmutter J Clin Invest 1989; 84:1555
Pathogenesis

• Single amino acid substitution causes “Z”
  – glutamic acid 342 → lysine 342

• Abnormal folding:
  – amino acid Δ → slowing of protein folding
  – accumulation of α₁AT in ER
    • unstable and polymerizes
    • unable to further traverse secretory path

  Lomas Nature 1992; 357:605

Biochemical Aspects

• expressed mainly (95%) in hepatocytes

• a serine protease inhibitor (serpin)
  – ~90% of the α₁-globulin fraction
  – a glycoprotein
    • 394 amino acids long and 55 kDaltons

• principal physiologic function
  – inhibit destructive neutrophil proteases
    • elastase, cathepsin G, proteinase 3

  Perlmutter Sem Liver Dis 2000; 4:387
Clinical Manifestations

Late Childhood/Adulthood

– most common → asymptomatic cirrhosis
– portal HTN +/- severe liver dysfunction
– may see hepatocellular carcinoma

α₁AT should be considered in dDx of any adult who presents with chronic hepatitis, cirrhosis, portal HTN, or HCC of unknown etiology

Diagnosis of A₁AT deficiency

• Alpha₁ AT phenotype
  – PiZZ most common
  – Up to 75 allelic variants described

• Serum concentration of α₁AT
  • Generally 10-15% of normal values
  • α₁AT is an acute phase reactant
  • may ↑ 3-4 fold during inflammation
Heterozygotes

- Chronic liver disease seen in:
  - PiZZ
  - PiSZ
  - PiM\textsubscript{malton}/M\textsubscript{malton}
  - PiM\textsubscript{Z}/M\textsubscript{Z}
  - PiM\textsubscript{Z}/M\textsubscript{Z}

- self-limited hepatic dysfunction is not rare
  - 15-19% at 2 months
  - ~1% by 12 months

Reid Gastroenterology 1987;93:181
Crowley Gastroenterology 1987;93:242
Sveger Acta Paediatr Scand 1988;77:847
Sveger Hepatology 1995;22:514
Heterozygous A1AT and Liver Disease

• May lead to chronic liver disease
• 599 liver transplant recipients studied
• 8.2% had PiMZ versus 2-4%
• 27% classified as “cryptogenic”
• PiMZ may serve as a co-factor


Co-existent liver disease in A₁AT

* Prior exposure