Cryptogenic Cirrhosis: An Approach To The Diagnosis In The Era Of Molecular Medicine

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Cryptogenic Cirrhosis

- Definition
- Incidence
  - Children
  - Adults
- Role of genomic sequencing/molecular pathology in the work up in liver disease of unknown etiology
  - Inherited metabolic disorders
  - Viral hepatitis
  - Future direction

Incidence of Cryptogenic Cirrhosis

- 1965: HBsAg
- 1969: FFAIC
- 1980: HCV
- 2004: Occult HCV
- 2009: WES

- <1970: >60%
- 1970-90: 5-30%
- 1990-2000: 5-10%
- 2000-2017: 5-10%

- Etiology of cirrhosis remains unknown despite work-up
  - Clinical history
  - Laboratory work-up
  - Pathologic analysis

Cryptogenic Cirrhosis: Definition

- Inherited genetic & metabolic causes are more common in children
- Viral hepatitis & FLD are more common in young adults

Etiology of Cirrhosis In Children and Young Adults (n=187)
A. Gorung, S. Vilarinho, P. Misra, D. Jain

- Incidence of cryptogenic cirrhosis in children (<18Y) and young adults (18-40Y) is similar (~10%)
- Incidence of cryptogenic cirrhosis and etiology of cirrhosis in young adults (18-40Y) are similar to those reported in adults
- Etiology of cirrhosis in children is different from adults
  - Inherited/genetic & metabolic causes are more common in children
  - Viral hepatitis & FLD are more common in young adults

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Dr. Dhanpat Jain declares he has no conflict of interest to disclose.
ETIOLOGY OF CIRRHOSIS

Children:

- Congenital cholestatic syndromes: 44%
- Metabolic & genetic diseases: 22%
- Autoimmune diseases: 18%
- Cryptogenic: 9%
- Fatty liver disease: 3%
- Viral hepatitis: 4%

Adults:

- Congenital cholestatic syndromes: 5%
- Metabolic & genetic diseases: 4%
- Autoimmune diseases: 7%
- Cryptogenic: 6%
- Fatty liver disease: 15%
- Viral hepatitis: 4%

- NAFLD/NASH
- Autoimmune hepatitis
- Chronic cholestatic/biliary disorders
- AAT inclusion disease
- Hepatic vascular disorders
- Occult alcoholic liver disease

Cryptogenic cirrhosis: Histologic analysis

- NAFLD/NASH
- Autoimmune hepatitis
- Chronic cholestatic/biliary disorders
- AAT inclusion disease
- Hepatic vascular disorders
- Occult alcoholic liver disease

Role of molecular diagnostics in Cryptogenic cirrhosis??
Genomic sequencing

- NGS is now readily available, reasonably cheap and is used in clinical application including the work up of liver disorders of unknown etiology
  - Whole genome analysis
  - Whole exome analysis
    - 1% of whole genome, but 85% of all disease causing variants
  - Selected gene panel

Case example

- 25 years old female found to have features suggestive of cirrhosis with esophageal varices, splenomegaly and mild thrombocytopenia
  - Labs:
    - Liver transaminases and alkaline phosphatase were mildly elevated AST 141 u/l, ALT 114 u/l, alkaline phosphatase 596 u/l, GGT 734 u/l, albumin 4.2 gm/dl and conjugated bilirubin 1.6 mg/dl
    - Viral (Hepatitis A, B and C) were negative and autoantibody screen was also negative.
    - Serum immunoglobulin levels were normal
    - Increased 24h urine copper (> 100 mcg)
    - Ceruloplasmin level was normal (38 mg/dl (ref 18–46), normal serum free copper (0.6 μg/dl (ref 0.0–10.0))
  - Liver biopsy
Diagnosis: Wilson disease with cirrhosis

Treatment:
- Started on zinc therapy, but was switched to Trientine due to GI side effects.
- After >12 months of treatment her transaminase levels did not improve and urine copper remained elevated.
- Mutations for Wilson disease (ATP7B) were negative
- Diagnosis: Cryptogenic cirrhosis

Progressive familial intrahepatic cholestasis (PFIC-3)

Another Case!
- 5 Y female child from India result of an consanguineous marriage presented with cirrhosis
- Based on clinical features and laboratory data diagnosed as Wilson disease
  - Mutations for Wilson disease (ATP7B) were negative
  - Underwent OLT at age 9
  - Explant showed Cirrhosis and HCC (2 nodules, 1.9 and 1.5cm)
  - Post-Tx developed progressive neurodegenerative symptoms and died at 10y

**ABCB4 mutations (MDR3)**

<table>
<thead>
<tr>
<th>Nucleotide change</th>
<th>Amino-acid change</th>
<th>Location</th>
<th>Zygosity</th>
<th>Reference/Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>c984T&gt;G</td>
<td>pY328</td>
<td>Exon 9</td>
<td>heterozygous</td>
<td>Nonsense mutation</td>
</tr>
<tr>
<td>c3218G&gt;A</td>
<td>pC1073Y</td>
<td>Exon 25</td>
<td>heterozygous</td>
<td>Unclassified novel variant</td>
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</table>

Presentation of Progressive Familial Intrahepatic Cholestasis Type 3 Mimicking Wilson Disease: Molecular Genetic Diagnosis and Response to Treatment

- Correct diagnosis was established based on WES
- Identified novel mutations of a known genetic inherited genetic metabolic disorder
- Helped institute appropriate therapy
WES of the proband revealed a homozygous missense mutation in MPV17 gene (c.148T>C)

Both parents were heterozygous carriers for the same mutation

Mitochondrial depletion disorder leading to neurodegenerative disease
- Neurologic symptoms (90%)
  - Developmental delay
  - Hypotonia, muscle weakness, seizures, ataxia, peripheral neuropathy
- Liver involvement (90%)
  - Liver failure or cirrhosis in early childhood

By boy seen in liver clinic with intermittently elevated transaminases
- (AST 30-131 U/L, ALT, 19-297 U/L), normal GGT levels and preserved liver synthetic functions
- Turkish decent, parents are 2nd cousins
- Full term baby with mildly delayed developmental milestones
- Transaminase elevations noted since 8 month of age
- Normal growth (weight and height between 25th-50th and 50th-75th percentile, respectively)
- At 6.5y developed some neurologic symptoms
- Liver and spleen not palpable
WES revealed homozygous mutation in \textit{ACOX2} gene (premature termination at codon 69).

- \textit{ACOX2} encodes a branched-chain Acyl-CoA oxidase, a peroxisomal enzyme expressed in the liver and kidney.

Both parents and brother found to be heterozygous, but clinically normal.

Elevated serum levels of 3α,7α-dihydroxy-5β-cholestanolic acid (DHCA) and 3α,7α,12α-dihydroxy-5β-cholestanolic acid.

Low levels of Cholic acids and its conjugates.

- ACOX2

- ACOX2 (Acyl CoA oxidase)
Subsequent follow-up

- Patient put on replacement bile acids
- Normalization of transaminases
- Long term outcome awaited

ACOX2 deficiency: A disorder of bile acid synthesis with transaminase elevation, liver fibrosis, ataxia, and cognitive impairment

- Identified novel genetic inherited disorder based on WES
- Identification of the specific defect predicted biochemical and laboratory abnormalities
- Understanding the pathophysiology helped institute appropriate therapy

How useful is genomic sequencing?

- Retrospective analysis of cryptogenic cirrhosis (age <40Y)(n=30) from FPPE liver biopsies
  - Satisfactory DNA sample (n=15)
  - Mutations identified (n=5)

<table>
<thead>
<tr>
<th>Patient</th>
<th>Abnormality detected</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>2g/M</td>
<td>ATP7B : compound/comp</td>
<td>Wilson Disease</td>
</tr>
<tr>
<td>2/M</td>
<td>MFV17 (p.R880)</td>
<td>7MDS</td>
</tr>
<tr>
<td>37/F</td>
<td>ABCB4 (p.G93X)</td>
<td>?PFIC3</td>
</tr>
<tr>
<td>39/F</td>
<td>ABCB4 (p.R102Q)</td>
<td>?PFIC3</td>
</tr>
</tbody>
</table>

What about viral infections??

- Negative HCV antibodies undetectable viral load, but evidence of HCV infection by
  - HCV-PCR from liver tissues (extra-hepatic tissues)
  - HCV-PCR from PBMC
  - In-situ hybridization
- Can occur
  - Post therapy
  - Self clearance of virus
- Incidence varies between studies
- The evidence for OCI is substantial and growing
- Natural history of OCI is not yet fully defined
Cryptogenic cirrhosis: Suspected etiologies

- Metabolic/ inherited disorders
  - Bile salt transporter defects
  - Bile salt synthetic defects
  - Mitochondrial disorders
  - Short telomere syndrome
  - Keratin 8 and 18 mutations
  - Glutathione S-transferase mutations
  - A1A1 disease
  - Wilson disease
  - Iron overload disorders (HFE or non-HFE)

Future directions

- Genomic analysis is likely to find more application is routine clinical practice in the work up of liver disorders of unknown etiology
  - Whole genome analysis
  - Whole exome analysis
  - Selected gene panel
- Many new genetic alterations are likely to be identified and help
  - Refine the existing knowledge of known disorders
  - Define new disorders
  - Identification of cryptic infections
**Cryptogenic cirrhosis: Definition**

- Etiology of the cirrhosis remains unknown despite work-up
  - Clinical history
  - Laboratory work-up
  - Pathologic analysis
  - Molecular and genetic analysis

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**CRYPTOGENIC CIRRHOSIS**

- 1965: HBsAg, PFIC
- 1969: HCV, Occult HCV
- 1989: HCV, Castle HCV
- 2004: HCV, CASTLE HCV
- 2009: WES
- 2017: Refinement of AIH criteria
- 2020: Awareness of NAFLD/NASH

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