Inborn Errors of Bile Acid Metabolism

James E. Heubi, M.D.
Professor, Department of Pediatrics
Associate Dean for Clinical and Translational Research
Director of Center for Clinical and Translational Science and Training
University of Cincinnati College of Medicine
Financial Disclosures

• Equity interest in Asklepion Pharma, LLC.
• Funding: NCATS, NIDDK, NICHD, and CFF
• Consultant to Nordmark, Retrophin, Alnylam
Outline

• Causes and evaluation for neonatal cholestasis
• Role of bile acids in cholestasis and fat/fat soluble vitamin absorption
• Pathophysiology of inborn errors of BA metabolism
• Diagnosis of inborn errors of BA metabolism
• Commonly identified defects
• Treatment
Differential - “Neonatal Cholestasis” 1973

- "Neonatal Hepatitis" (25%)
- Biliary Atresia (10%)
- "Viral" (TORCH) (3%)
- A-1-AT deficiency (7%)
- Miscellaneous* (55%)
Differential - “Neonatal Cholestasis” 2016

- "Neonatal Hepatitis" 25%
- "PFIC" & Alagille 25%
- Metabolic Disease 15%
- Biliary Atresia 10%
- Viral 5%

"A-1-AT deficiency"
Role of Bile Acids

• Major metabolic pathway for elimination of cholesterol
• Promote formation/secretion of bile
• Fat and fat soluble vitamin absorption
• Cathartic action-induce water and electrolyte secretion
• Bacteriostatic properties
• Role in signaling pathways
EHC and BA Metabolism 101

**Cholesterol Metabolism**

Cholesterol can be converted into various bile acids:
- **Chenodeoxycholic Acid (3a,7a)**
- **Cholic Acid (3a,7a,12a)**
- **Lithocholic Acid (3a)**
- **Deoxycholic Acid (3a,12a)**

**Biliary Secretion**

- Portal venous return to hepatocytes (95% of biliary secretion)
- Biliary Secretion: 3 g in pool x 4-12 cycles/d

**Excretion**

- Urinary Excretion (~0.5 mg/d)
- Storage in gallbladder
- Passive absorption
- Postprandial secretion into intestine
- Ileal active transport
- Colonic passive transport

**Fecal Excretion** (0.2 to 0.6 g/d)

**Kidney**

- Spillover into systemic circulation

**Cholangio-hepatic shunt**
Biochemical Anomalies/ Hepatotoxicity of Bile Acid Synthesis Disorders\textsuperscript{1-3}

- Lack of, or markedly diminished synthesis of primary bile acids, CA and CDCA→ poor bile flow

- Concomitant production and accumulation of precursors in the pathway proximal to enzyme defect→ metabolites may be directly toxic

Clinical Sequelae of BASD: SED vs PD

<table>
<thead>
<tr>
<th>Sterol-Ring Modifications</th>
<th>Side-Chain Modifications</th>
<th>Secondary BASD (PEX)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSD3B7 (3β-HSD)</td>
<td>(CYP27A1) Sterol-27 hydroxylase (CTX)</td>
<td>Peroxisomal biogenesis defects (Zellweger)</td>
</tr>
<tr>
<td>AKR1D1 (5β-reductase)</td>
<td>(AMARC) 2-methylacyl-CoA racemase</td>
<td></td>
</tr>
<tr>
<td>(CYP7B1) Oxysterol 7α-hydroxylase</td>
<td>(BAAT) Bile acid CoA: amino acid N-acyl-transferase, (SLC27A5) Bile acid CoA ligase</td>
<td></td>
</tr>
</tbody>
</table>

Rapid onset of liver failure, high mortality\(^1\)

Multiorgan disease of varying severity, complicated clinical presentation with high mortality rate\(^2,3\)

Clinical phenotype is highly variable — high index of suspicion based on physical examination and laboratory evaluation
Cumulative Numbers of Patients Diagnosed with Inborn Errors in Bile Acid Synthesis

Total number of samples screened at CCHMC = 14,400

Frequency of Bile Acid disorders = 2.2%

Screening program commenced at CCHMC
Defects in Bile Acid Synthesis: ‘The Cincinnati Experience’

- 2-methylacyl-CoA racemase oxysterol 7α-hydroxylase (2.8%)
- Conjugation defects (5.1%)
- 3β-hydroxy-Δ5-C27-hydroxy steroid oxidoreductase (35.2%)
- Sterol 27-hydroxylase (14.1%)
- Peroxisomal β-oxidation defects (20.1%)
- Δ4-3-oxosteroid 5β-reductase (22.4%)

Data for 1987-2016: 14,400 screenings 316 Cases identified

- Age at diagnosis and clinical presentation is highly variable ranging from early infancy to adulthood - Can be a cause of late-onset chronic cholestasis

Typical FAB-MS (-ve) spectra comparing urine from a cholestatic newborn with healthy infant.

**Cholestatic Newborn**
- Taurine conjugates
- Glycine conjugates
- Glyco-sulfate conjugates

**Normal Infant**
- Urine - 0.05 mL
- Total bile acid concentration <20 nmol/mL

Setchell 07-005
## Comparing Intrahepatic Cholestasis in Bile Acid Synthesis and Transporter Disorders

<table>
<thead>
<tr>
<th>Features</th>
<th>Bile acid defects</th>
<th>PFIC-1, 2, 4 and 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at presentation:</td>
<td>variable, late onset</td>
<td>&lt; 1 year</td>
</tr>
<tr>
<td>Liver:</td>
<td>hepatosplenomegaly</td>
<td>hepatosplenomegaly</td>
</tr>
<tr>
<td>Pruritus:</td>
<td>absent</td>
<td>severe</td>
</tr>
<tr>
<td>Growth failure:</td>
<td>mild/absent</td>
<td>severe</td>
</tr>
<tr>
<td>Jaundice:</td>
<td>+/-</td>
<td>mild/severe</td>
</tr>
<tr>
<td>Serum transaminases:</td>
<td>elevated</td>
<td>slight to ↑ elevations</td>
</tr>
<tr>
<td>Serum GGT:</td>
<td>generally normal</td>
<td>normal</td>
</tr>
<tr>
<td>Serum cholesterol:</td>
<td>low</td>
<td>low</td>
</tr>
<tr>
<td>Fat-soluble vitamins:</td>
<td>malabsorption</td>
<td>malabsorption</td>
</tr>
<tr>
<td>Bile acids:</td>
<td>no primary bile acids</td>
<td>↑ primary bile acids</td>
</tr>
<tr>
<td>Treatment:</td>
<td>Bile acid therapy</td>
<td>Transplantation</td>
</tr>
<tr>
<td>Prognosis:</td>
<td>excellent</td>
<td>Fatal 1st-2nd decades</td>
</tr>
</tbody>
</table>
Diagnosis of Inborn Errors of Bile Acid Metabolism

• Diagnosis confirmed in Commercial lab
  • www.genetests.org

• CCHMC (formerly jaundice chip)
  • www.cincinnatichildrens.org/service/h/hereditary-liver/tests/

• Emory
  • http://geneticslab.emory.edu/tests/MM340

• Supported by Retrophin
  • http://testcholestasis.com
3β-OH steroid dehydrogenase/isomerase deficiency

- Presents with cholestasis in infancy
- Fat soluble vitamin deficiency (rickets, bleeding)
- Older siblings of affected infants/children
- Low serum gamma GT concentration
- Indolent course → cirrhosis
- May have rapidly progressive course → transplantation
- May present in later childhood/ adulthood
Pedigree of Arab-Iranian Family with 3β-hydroxy-Δ5-C27-steroid oxidoreductase deficiency

Asymptomatic at 32y age

Cirrhosis of unknown etiology jaundice, elevated LFT’s, splenomegaly portal hypertension, varices, ascites

Homozygous for 2 basepair deletion in exon 1 of HSD3B7 (c.45_46del AG) FAB-MS of serum confirmed bile acid synthetic defect

Molho-Pessach, V. et al Hepatology 2012;55:1139-1145
Biochemical defect in 3β-Hydroxysteroid dehydrogenase/isomerase deficiency

Metabolism by side chain oxidation and 12 α-hydroxylation

3β, 7α-dihydroxy-5-chenoic acid

urinary excretion as the glycine and sulphate conjugates

3β, 7α, 12α-trihydroxy-5-chenoic acid
Biochemical Characteristics of Patients with HSD3B7 Deficiency

- Range of baseline values for 19 patients with a biochemically confirmed 3β-hydroxy-Δ5-C27-steroid oxidoreductase (HSD3B7) deficiency
Metabolic Basis for Cholic Acid Therapy

Transcription Factor
SHP-1 downregulation

Cholesterol

CYP7A1

7α-hydroxycholesterol

Atypical bile acids

LRH1 RXR FXR

Cholic acid

restore feedback inhibition

Bile acids

Orally administered Cholic acid
Clinical Manifestation of Inborn Errors in Bile Acid Synthesis and Effect of Primary Bile Acid Therapy

Therapeutic goals:

1. Inhibit endogenous bile acid synthesis - reduce accumulation of atypical bile acids

2. Provide stimulus for bile secretion - generate choleresis

3. Increase intraluminal bile salts - facilitate absorption of fats and fat-soluble vitamins
Ursodeoxycholic acid may improve ALT/AST but not suppress synthesis and prevent liver injury
Suppression of Atypical Bile Acids with Cholic Acid Therapy - Urinary FAB-MS profiles

Pre-treatment

After Cholic acid therapy
10-15 mg/kg bw/day
3β-Hydroxy-Δ^5-C_{27}-steroid oxidoreductase deficiency
- Effect of Cholic Acid Therapy on Atypical Bile Acids

Oral cholic acid therapy 10-15 mg/kg body weight/day
Summary of long-term therapy in 19 patients
Effect of Oral Cholic Acid Therapy on LFTs in Patients with $3\beta$-Hydroxy-Δ$^5$-C$_{27}$-steroid oxidoreductase Deficiency

Cholic acid therapy: 10-15 mg/Kg bw/day (19 Patients)

Time after initiating oral cholic acid therapy
Pathology in 3-HSD
Long-term Effect of Cholic Acid on Growth

3β-Hydroxy-Δ^5-C_{27}-steroid oxidoreductase deficiency (n=19)

Cholic acid therapy
Oral dose 10-15 mg/kg bw/day

- Long term Cholic acid therapy leads to significant improvement in growth and body weight in most patients
- Cholic acid was well tolerated with no significant side-effects reported in >20 yrs of therapy
Δ⁴-3-oxosteroid-5β-reductase deficiency

• Initial description of monochorionic twins presenting with neonatal cholestasis
• Presented with jaundice and varying severity of liver dysfunction
• Rapidly progressive disease leading to cirrhosis in infancy: Previously presumed affected sibling died in infancy
Δ⁴-3-Oxosteroid 5β-reductase Deficiency - Biochemistry

- Cholesterol
  - cholesterol 7α-hydroxylase (CYP7A1)
  - 3β-hydroxy-Δ⁵-C₂₇-steroid oxidoreductase (HSD3B7)

- 7α-hydroxy-4-cholen-3-one (sterol-C4)
  - Δ⁴-3-oxosteroid 5β-reductase (AKR1D1)
  - primary bile acids [Not Detected]

- (+/-)12α-hydroxylation
  - 27-hydroxylation (CYP27A1)
  - and side-chain oxidation

- Conjugation with taurine and glycine

- cholestasis, liver injury

3-oxo-7α-hydroxy-4-cholenoic acid
3-oxo-7α,12α-dihydroxy-4-cholenoic acid
Effect of Therapy on Bile Acid Excretion
Biochemical Response to Therapy

- day 0: 70mg CA + 130mg UDCA
- day 86: 130mg CA + 130mg UDCA

Serum enzyme concentration (IU/L)

- AST
- ALT
- GGT

Day on bile acid therapy
Effect of cholic acid therapy on liver histology: Patient with a bile acid synthetic defect

$\Delta^4$-3-Oxosteroid $5\beta$-reductase deficiency
Cholic acid: 15 mg/kg/day

Liver: Age 3 months (Before treatment)  Liver after 16 months of cholic acid therapy
EM of liver of a patient (SG) with \( \Delta^4 \)-3-oxosteroid 5\( \beta \)-reductase deficiency

Bile ducts poorly developed, lacking normal microvillus structure and filled with electron-dense material

Before treatment

After 6 months of bile acid therapy
Sterol 27-hydroxylase Deficiency (CTX) - Metabolic Defect

27-hydroxylation (CYP27) and side-chain $\beta$-oxidation

$5\beta$-cholestane-3$\alpha$,7$\alpha$,12$\alpha$-triol

bile alcohols (22-,23-,24-,25-hydroxylated)

Urine/Bile/Feces

Cholic acid

developmental pathway

microsomal 24$\beta$-hydroxylation

Cholic acid

[Chenodeoxycholic]
Clinical History-Index Patient

- Asian male (parents 1st cousins), full term pregnancy, BW 2.6 kg
- At 8 weeks, prolonged jaundice, pale stools
  - Serum bilirubin 4.9 mg/dl, albumin 3.9 gm/dl, AST 275 u/L, ALP 1281 U/L
  - Cholic acid started
- Age 3 months, poor growth, weight 3%ile, hepatosplenomegaly
  - Serum AST 260 U/L, ALT 212 U/L, GGT 95 U/L, ALP 2555 U/L
• Impression: Neonatal hepatitis
• Age 4 months: jaundice ↓, pruritus
• Age 5 months: resolved jaundice, pruritus improved, BW at 50th%ile
• Age 8 months: LFT’s normal
• Age 13 months: seizures
Liver Function Tests in an Infant with Sterol 27-hydroxylase Deficiency

Patient: 12-week old infant diagnosed with CTX

Graph showing:
- Serum liver enzymes (IU/L) vs. Age (weeks)
- Serum total bilirubin (μmol/L) vs. Age (weeks)

Legend:
- AST
- ALT
- GGT

Cholic acid therapy indicated by shaded area.
Changes in Urinary Bile Alcohol Excretion with Cholic Acid Therapy

Patient: MM - Sterol 27-hydroxylase deficiency
Oral cholic acid 150 mg/day

![Graph showing changes in urinary bile alcohol excretion with treatment.]

- **3,7,12,24,25-pentol**
- **3,7,12,23,25-pentol**
- **3,7,12,25-pentol**

**Total Bile Alcohols**
- **Cholic acid 150 mg/day**

Days on Treatment:
- Pre-
- 12
- 27
- 55

Urinary Bile alcohol excretion (μmol/L)
Cholestanol/Cholesterol Changes in Response to Cholic Acid Therapy

Patient: MM - Sterol 27-hydroxylase deficiency
Oral cholic acid 150 mg/day

Graph showing changes in serum concentration of cholestanol (in μmol/L) and cholesterol (in mmol/L) with age (weeks) and days of treatment (Pre, 12, 27, 55) after starting cholic acid therapy.
Disorders of Peroxisomal Function

- Zellweger syndrome, Neonatal Adrenoleukodystrophy, Refsum syndrome
- Profound muscular weakness, liver disease, fatal early in life
- Characterized by absent peroxisomes or defects of enzymes in peroxisomes
- BA and defects of FA metabolism:
  - Accumulation of VLCFA, Pipecolic Acid, Phytanic, Pristanic Acids
  - Di- and Trihydroxycholestanoic acid
Effect of peroxisomal disorders on BA synthetic pathway

Metabolism via 1β-, 2β-, 6α- and 24-hydroxylation to form tetrahydroxy-cholestanoic acids

urinary excretion as the taurine conjugates

3α,7α-dihydroxy-5α-cholestanolic (DHCA) 3α,7α,12α-trihydroxy-cholestanolic (THCA)

chenodeoxycholic acid  cholic acid  C_{29}-dicarboxylic acid
Effect of CA in Peroxismal Disorders
Effect of Oral Primary Bile Acid Treatment on LFT's and Growth in a Patient with Zellwegers

Index Case:

- Cholic and chenodeoxycholic acid (15 mg/kg bw/day)

Response to Cholic Acid

Safety/Efficacy of Cholic Acid

- Bile acid therapy singled out as curative for BA defects by IOM*
- No drug-related adverse events in > 20 year use
- Impressive clinical response
  - Normalization of liver chemistries
  - Resolution of histologic abnormalities
  - Improved growth
- Treatment failures only in advanced ESLD
- Exceptions (Cholic acid does not work!)
  - Oxysterol 7α-hydroxylase deficiency
  - Conjugation defects (treated with glycocholic acid)

*IOM Report on Rare Diseases and Orphan Products 2010*
Acknowledgements

• William K Schubert, M.D.
• Bill Balistreri, M.D.
• Donna Buckley
• Nancy O’Connell
• Linda Nechemias
• Pinky Jha
• Wujuan Zhang
• Stephanie Galandi
• Kevin Bove, M.D.
• Laura Woollett, Ph.D.
• David Russell, Ph.D.

• Collaborating MDs
  • Ron Sokol
  • Carol Potter
  • Ben Shneider
  • Rob Squires
  • Saul Karpen
  • Simon Horslen
  • David Suskind
  • Phil Rosenthal

• CRC Staff
  • Nurses, dietitians
  • Andrea Smith

• Patients/families
• Sponsors
  • NIH (NCRR, NIDDK), FDA (OPG), CCHMC, Asklepion Pharm
Gracias