Improving clinical practice through research: Where are we going?

Jorge A. Bezerra, M.D.

Molecular Genetics Laboratory, CCHMC
- Research funding

Gilead
- Principal Investigator: Anti-HBV Clinical Trial
- Sub-Investigator: Anti-HCV Clinical Trials 1 & 2

Conflict of interests

Pediatric hepatology research
- Battling an epidemic: NAFLD research
- De-personalizing care: DAAV for HCV
- Personalizing care: Cholestasis syndromes
  - Recent advances
  - Tools for diagnostics and therapeutics
  - Research aims for 2020 – 20/20 clarity?
- Beyond 2020: Livers in a dish

Pediatric hepatology research
- Battling an epidemic: NAFLD research
- De-personalizing care: DAAV for HCV
- Personalizing care: Cholestasis syndromes
  - Recent advances
  - Tools for diagnostics and therapeutics
  - Research aims for 2020 – 20/20 clarity?
- Beyond 2020: Livers in a dish

Causes of neonatal cholestasis

1970-1989
- Infection
- Idiopathic
- Biliary atresia
- A1AT deficiency
- Cystic fibrosis

1998...
- Biliary atresia
- A1AT deficiency
- Magtate
- FIC1 deficiency
- BSEP deficiency
- TJP2 deficiency
- MRD3 deficiency
- Bnc defects
- Cystic fibrosis
- Citrin deficiency
- ARMC syndrome
- Niemann-Pick C
- Others

2014

Biliary atresia
- Most common cause end-cirrhosis in children
- Onset within first 3 months of life
- Pathology: Inflammation and fibrosis
- Targets: Biliary epithelium, lumenal obliteration
Biliary atresia

Recent advances

- Pathogenesis
- Treatment

Pathogenesis of disease

Hypothesis
Testing hypothesis
Validation

Guilty: Cellular effectors of biliary injury and duct obstruction

DC
NK
IFN$\gamma$ + CD8

Insult

Onset of injury
Progression of disease

Redemption: Recovery of biliary epithelium after an injury

Rotavirus
IL33 or PBS
PBS

0d
4d
7d

Li et al. JCI 2014

Shivakumar et al. JCI 2004
Shivakumar et al. Gastroenterology 2007
Shivakumar et al. JCI 2009
Saxena et al. Sci Transl Med 2011
Li et al. JCI 2011

Mechanisms of duct injury

Pro-inflammatory signature

CD4, CD8, NK lymphocytes
TUNEL+
FasL
IFN$\gamma$
IL12
TNF$\alpha$
Osteopontin

Non-specific response or mechanism of injury?
Corticosteroids

- Suggested as adjuvant therapy based on its anti-inflammatory effects (uncontrolled trials)
- Meta-analysis (Sarkhy et al. Can J Gastroenterol 2011)
  - 17 publications: Unable to determine efficacy
- Surveys of use of steroids after surgery
  - >90% in Japan (Muraji J Pediatr Surg 2004)

The START Trial

Lancet 2014;311:1750-9

- **START** – **Steroids in Biliary Atresia Randomized Trial**
- Double blind, placebo-controlled (NCT00294684)
- To determine whether treatment with high-dose steroids after HPE is superior to HPE
- 14 liver centers in the U.S. – ChiLDREN

### BA + HPE

<table>
<thead>
<tr>
<th>Steroids</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week 1-2: 4 mg/kg/d</td>
<td>Same volume</td>
</tr>
<tr>
<td>Week 3-4: 2 mg/kg/d</td>
<td>Same volume</td>
</tr>
<tr>
<td>Week 5-6: 1 mg/kg/d</td>
<td>Same volume</td>
</tr>
<tr>
<td>Week 7-13: Wean to off</td>
<td>Same volume</td>
</tr>
</tbody>
</table>

- 1st dose steroids or placebo: within 72 hours of HPE
- Steroids: Methylprednisolone ➔ prednisolone
- Clinical care based on guidelines for the trial
  - UDCA, TMP-SMZ, MCT-formula, Fat-soluble vitamins

### Primary end point

Percentage of subjects with TB <1.5 mg/dL with his/her native liver 6 m after HPE

<table>
<thead>
<tr>
<th>Steroids</th>
<th>Placebo</th>
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<tr>
<td>ITT analysis</td>
<td></td>
</tr>
<tr>
<td>Adjusted RR [95% CI]: 1.14 [0.83, 1.57]</td>
<td>P=0.43</td>
</tr>
<tr>
<td>PP analysis</td>
<td></td>
</tr>
<tr>
<td>Adjusted RR [95% CI]: 1.14 [0.81, 1.61]</td>
<td>P=0.44</td>
</tr>
</tbody>
</table>

### Secondary end point

**Survival with native liver at 2 yr**

Logrank P=0.93

**Biliary atresia**

**Research goals for 2020**

20/20 Clarity: Personalized trial

- Age at diagnosis
- Biological stages
**Age at diagnosis: <70 days**

*Total bilirubin < 1.5 mg/dL at 6 months*

**Open-label trials**
- Davenport et al. Hepatology 2007
- Davenport et al. J Hepatol 2013
- Lower bilirubin early after HPE in children receiving steroids
- Patient <70 days of age

**Biological stages at diagnosis**

Moyer et al Genome Med 2010;2:33

N=47

- Histology
  - Inflammatory
  - Fibrosing
  - Unclassified

- Molecular profiling
  - Molecular profiling

Validity of subtypes
- Biological plausibility
- Clinical relevance

**Groups of patients by serum markers**

*Serum cytokines, chemokines*

Squires J et al. AASLD 2013, abstract

**Individualized care**

*Goal: To decrease inflammation in liver*

Kasai: 50% own liver

**Individualized care**

*Goal: To decrease inflammation in liver*

Kasai: 50% own liver

Kasai+Drug: 65% own liver
Intrahepatic cholestasis

Recent advances

- Genetics
- Old and new syndromes

Clinical Case

- Male infant, 11 months old
- History of transient neonatal jaundice
- Exam:
  - No dysmorphic features
  - Normal cardiac auscultation
- Laboratory studies
  - AST: 111 IU/L  ALT: 108 IU/L
  - Albumin: 3.7 g/dL  DBi: 2.8 mg/dL
  - Alk Phos: 201  γGTP: 387 IU/L

Discoveries

<table>
<thead>
<tr>
<th>Year, Investigators</th>
<th>Disorder</th>
<th>Gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>1969 - Sharp H et al.</td>
<td>A1AT deficiency</td>
<td>SERPINA1</td>
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<td>1998 - Bull LN et al.</td>
<td>Alagille syndrome</td>
<td>JAG1</td>
</tr>
<tr>
<td>1998 - Strautnieks S et al.</td>
<td>PFIC-2</td>
<td>ABCB11</td>
</tr>
<tr>
<td>1996 - Deleuze JF et al.</td>
<td>PFIC-3</td>
<td>ABCB4</td>
</tr>
<tr>
<td>1998 - de Vree JM et al.</td>
<td>BASD, Reductase</td>
<td>AKR1D1</td>
</tr>
</tbody>
</table>
Molecular basis of cholestasis

A1AT deficiency

Cholestatic syndromes
Hepatopathies
In children

Fibrosing cholestasis
Cholestasis pregnancy
Cholelithiasis
Carcinoma

Hepatopathies
In adults

Spectrum of syndrome
FIC1 deficiency

Year, investigators | Disorder          | Gene   |
--- | --- | --- |
1998 - Bull LN et al. | PFIC-1          | ATP8B1 |
1999 - Tygstrup N et al. | RFIC Faeroe Is. | ATP8B1 |
2000 - Klomp LW et al. | Greenland familial cholestasis | ATP8B1 |

FIC1 deficiency
- PFIC-1
- BRIC-1
- RFC/Faeroe Is
- Greenland FC

Spectrum of syndrome
BSEP deficiency

Year, investigators | Disorder          | Gene   |
--- | --- | --- |
1998 - Strautnieks S et al. | PFIC-2          | ABCB11 |
2004 - van Mill ST et al. | BRIC-2          | ABCB11 |
2006 - Knisely AS et al. | HCC             | ABCB11 |
2007 - Scheimann AO et al. | Cholangiocarcinoma | ABCB11 |

BSEP
- PFIC-2
- BRIC-2
- HCC
- Cholangiocarcinoma

Spectrum of syndrome
MDR3 deficiency

Year, investigators | Disorder          | Gene   |
--- | --- | --- |
1996 - Deleuze JF et al. | PFIC3          | ABCB4 |
1998 - de Vree JM et al. | ICP            | ABCB4 |
1999 - Jacquemin E et al. | ICP            | ABCB4 |
2001 - Rosmorduc O et al. | LPAC           | ABCB4 |
2003 - Lucena JF et al. | LPAC, ICP, cirrhosis | ABCB4 |

MDR3
- PFIC-3
- ICP
- LPAC
- Fibrosis, cirrhosis

MDR3 heterozygosity

ABC4 Heterozygous Gene Mutations Associated With Fibrosing Cholestatic Liver Disease in Adults

- 32 adults with chronic cholestasis
- Unknown etiology
- Histology
  - Portal fibrosis with ductular reaction
  - Portal tract with macrophage infiltration
- Heterozygous mutations in ABC4 in 11 patients (34%)
Identification of new PFIC gene
Sambrotta M et al. Nat Genet Mar 9, 2014
• Patient population
  – 29 families, 33 affected individuals
  – Chronic cholestasis
  – Low GGT relative to cholestasis
• No mutation in ATP8B1 or ABCB11
• Mutation survey
  – Target screening: Sequencing of 21 genes
  – Whole exome sequencing
  – Confirmation by Sanger sequencing

Identification of new PFIC gene
Sambrotta M et al. Nat Genet Mar 9, 2014
• Mutations in TJP2
  – 8 families, 12 individuals (36%)
  – Deletions, splice site mutations
  – Predicted to abolish protein translation
• Phenotype of liver disease
  – Age at presentation: <3 months
  – GGT: 15-109
  – OLT: 9 of 12 patients (1.5-10 yr)
  – 2 patients with stable PHTN (4 and 7 yr)
  – Unexplained hematoma; lung disease (?)

TJP2
Biology and disease phenotypes
• Biology
  – Cytosolic component of several classes of cell-cell junctions
  – Influences localization of junction components
  – Patients: No TJP2 or CLDN1
• Mutations in patients with hypercholanemia
  – Pruritus, fat malabsorption, no progressive liver disease
  – Homozygous missense mutations
• Broader phenotype: Progressive cholestasis

Intrahepatic cholestasis
Research goals for 2020
20/20 Clarity: Personalized care
• Diagnostics
• Therapeutics

Diagnostics
Pathologic Jaundice
Pruritus
Hepatomegaly
Splenomegaly
Coagulopathy
MDR3 deficiency
BSEP deficiency
FIC1 deficiency
TJP2 deficiency
Diagnostics: Laboratories

- ATP8B1 (PFIC1)

Customization of platforms

- One stop-shop for liver genes?

Posttranscriptional regulation of canalicular transport systems.

- Targeting
- Insertion

Subapical Compartment
- Lysosome
- Uncoating
- Proteosome
- Trafficking

Activity

Personalized therapeutics

- Girl: 10 yr, p.T1210P BSEP, listed for OLT
- p.T1210P BSEP expressed in MDCK cells
- Retention in ER
- 4-PB corrected canalicular localization
- 4-PB treatment x 1 year
  - Serum bile acids 493 $\rightarrow$ 237 $\mu$mol/L
  - ALT 125 $\rightarrow$ 19
  - Total bili 12 $\rightarrow$ 1

Personalized therapeutics

- Girl with neonatal cholestasis
- 2m, p.R1231Q BSEP
- Jaundice, intractable pruritus
- Effect when dose reached 500 mg/kg/day
  - Improved AST, ALT, and bilirubin
  - No improvement in serum bile acid

4-PB in PFIC

Hasegawa Y et al. OJRD 2014;9:89

- Intractable pruritus; low-GGT PFIC
- Diagnosis
  - Low mRNA and protein for FIC1 (ATP8B1)
  - Heterozyg (N=2) or homozygous (N=1) for ATP8B1
- 4-PB: Escalating doses 150-500 mg/kg/d

<table>
<thead>
<tr>
<th>Pruritus</th>
<th>ATX</th>
<th>AST</th>
<th>ALT</th>
<th>Bilirubin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1</td>
<td>4</td>
<td>2</td>
<td>No Δ</td>
<td>No Δ</td>
</tr>
<tr>
<td>Patient 2</td>
<td>4</td>
<td>2</td>
<td>No Δ</td>
<td>No Δ</td>
</tr>
<tr>
<td>Patient 3</td>
<td>4</td>
<td>2</td>
<td>No Δ</td>
<td>No Δ</td>
</tr>
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Safety and efficacy of LUM001 in children with PFIC: NCT02057718

- Liver disease after liver transplantation
  - N=11, ages: 1-18 yr
  - Macrovesicular steatosis
    - 8 of 11
    - 7 progressed to steatohepatitis
    - 6 developed bridging fibrosis; 2 with cirrhosis
  - Refractory diarrhea: In all 8 with steatosis
  - Steatosis and diarrhea improved with bile adsorptive therapy

FIC1 deficiency and OLT
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BSEP deficiency and OLT
- Jara P et al. NEJM 2010;361:14
- Lin CH et al. Liv Transpl 2014;19:1403
- Alloimmune hepatitis after liver transplantation
  - Low GTP-cholestasis at 5m-12yr after OLT
- Patient IgG recognizes BSEP
  - Autoantibodies in serum: Recognized BSEP
- Treatment
  - Increased immunosuppression
  - IV IG, anti-CD20 antibodies, plasmapheresis

Hepatology of tomorrow

Beyond 2020
- Genomic pediatrics
  - Liver in a dish

The current frontier

Next-Gen(eration) sequencing

The $1000 Genome
Rapid decrease in cost of sequencing

Source: National Human Genome Research Institute
www.genome.gov/sequencingcosts
From stem cells ➔ Liver bud

- Inducible Pluripotent Stem Cells (iPSC)

\[ \text{iPSC} \rightarrow \text{iPSC} \rightarrow \text{iPSC} \rightarrow \text{Hepatic specification} \]

- iPSC: Inducible Pluripotent Stem Cells
- MSC: Mesenchymal Stem Cells
- HUVEC: Human Umbilical Vein Endothelial Cells

Day 1-6 ➔ 7-9 ➔ 10-13 ➔ 30-60

**Liver Bud**

- Hepatic specification

- Liver Bud In vivo

- Hepatic endoderm only
- HUVEC only
- MSC only (0.5M cells)
Engineering of a human liver

**LETTER**

Vascularized and functional human liver from an iPSC-derived organ bud transplant

- Liver in a dish: Strategy to model human liver disease?
- Liver in a bucket: Organoids to fulfill a functional deficit?