Improving clinical practice through research:

Where are we going?

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Cincinnati Children's Hospital Medical Center

Conflict of interests

- Molecular Genetics Laboratory, CCHMC
  - Research funding
- Gilead
  - Principal Investigator: Anti-HBV Clinical Trial
  - Sub-Investigator: Anti-HCV Clinical Trials 1 & 2

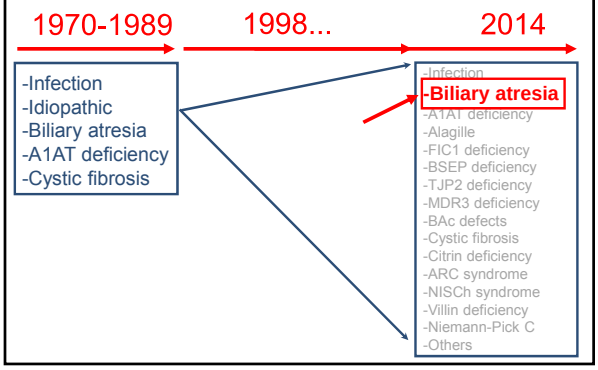
Pediatric hepatology research

- 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

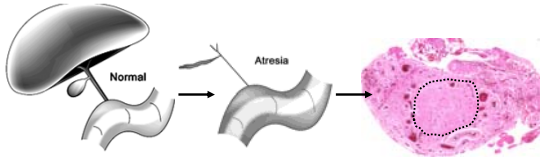
- Battling an epidemic: NAFLD research
- De-personalizing care: DAAV for HCV
- Personalizing care: Cholestasis syndromes
  - Recent advances
  - Tools for diagnostics and therapeutics
  - Research aims for 2020 – 20/20 clarity?
- Beyond 2020: Livers in a dish

Causes of neonatal cholestasis



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



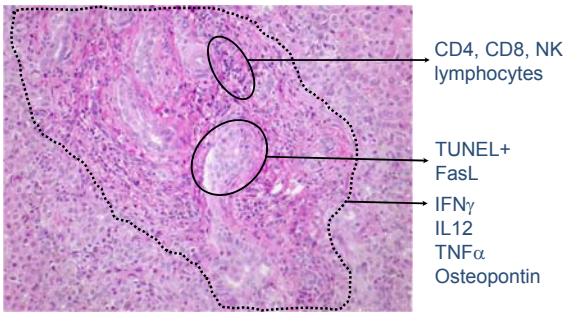
Biliary atresia

Recent advances

- *Pathogenesis*
- *Treatment*

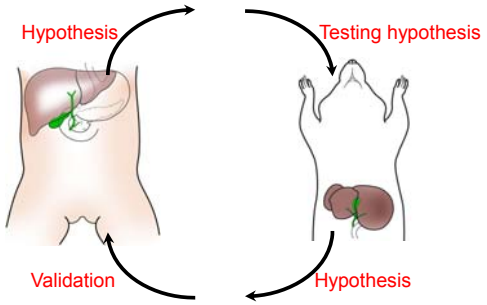
Mechanisms of duct injury

Pro-inflammatory signature

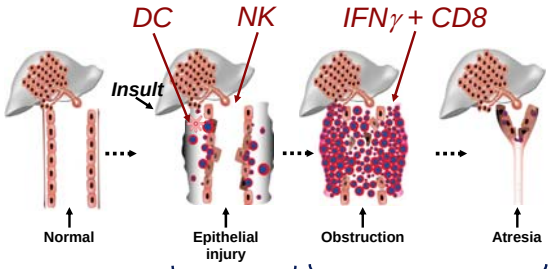


Non-specific response or mechanism of injury?

Pathogenesis of disease



Guilty: Cellular effectors of biliary injury and duct obstrution



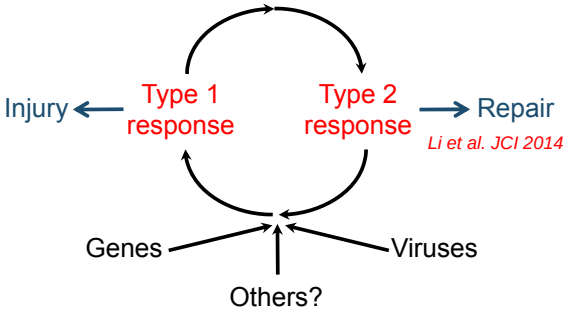
Shivakumar et al. JCI 2004
Shivakumar et al. Gastroenterology 2007
Shivakumar et al. JCI 2009
Saxena et al. Sci Transl Med 2011
Li et al. JCI 2011

Onset of injury

Progression of disease

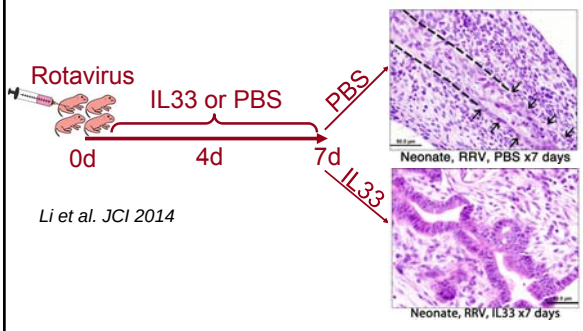
Biliary atresia

Inflammatory response targeting ducts



Li et al. JCI 2014

Redemption: Recovery of biliary epithelium after an injury



Li et al. JCI 2014

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
  - ~50% in US (Lao Am J Surg 2010)
  - >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

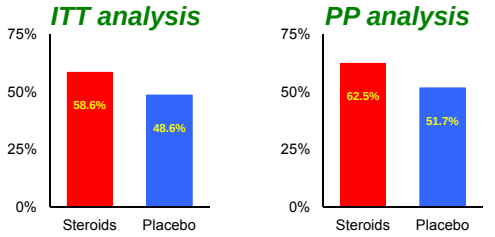
BA + HPE

Steroids	Placebo
Week 1-2: 4 mg/kg/d	Same volume
Week 3-4: 2 mg/kg/d	Same volume
Week 5-6: 1 mg/kg/d	Same volume
Week 7-13: Wean to off	Same volume

- 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

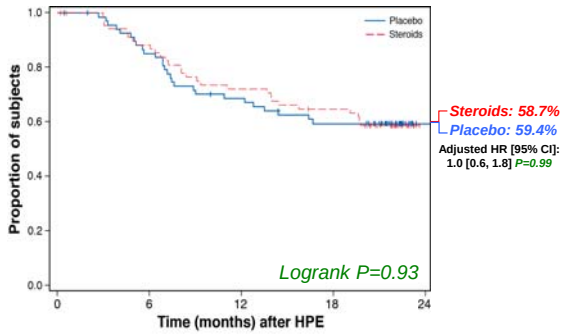


Adjusted RR [95% CI]: 1.14 [0.83, 1.57] *P=0.43*

Adjusted RR [95% CI]: 1.14 [0.81, 1.61] *P=0.44*

Secondary end point

Survival with native liver at 2 yr



Biliary atresia

Research goals for 2020

20/20 Clarity: Personalized trial

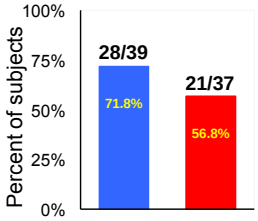
- *Age at diagnosis*
- *Biological stages*

Age at diagnosis: <70 days

Total bilirubin < 1.5 mg/dL at 6 months

Open-label trials

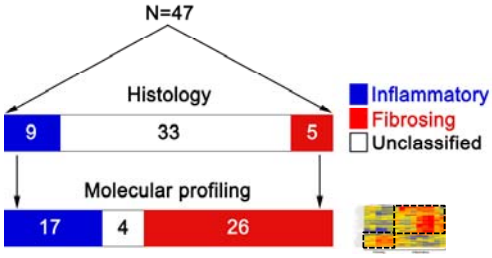
- Davenport et al. Hepatology 2007
- Davenport et al. J Hepatol 2013
- Lower bilirubin early after HPE in children receiving steroids
- Patient <70 days of age



Adjusted RR [95% CI]: 1.23 [0.79, 1.89] *P=0.36*

Biological stages at diagnosis

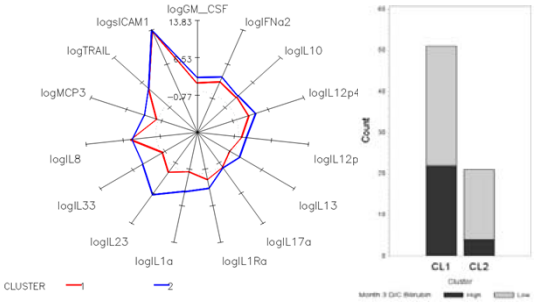
Moyer et al Genome Med 2010;2:33



Validity of subtypes
- Biological plausibility
- Clinical relevance

Groups of patients by serum markers

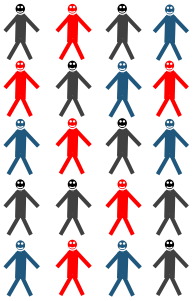
Serum cytokines, chemokines



Squires J et al. AASLD 2013, abstract

Individualized care

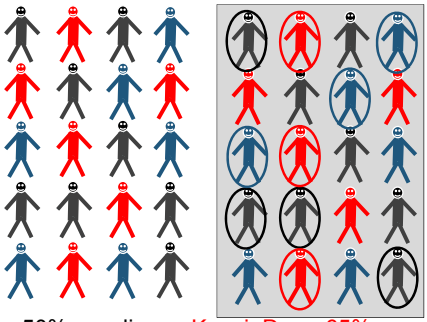
Goal: To decrease inflammation in liver



Kasai: 50% own liver

Individualized care

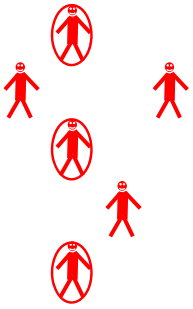
Goal: To decrease inflammation in liver



Kasai: 50% own liver Kasai+Drug: 65% own liver

Individualized care

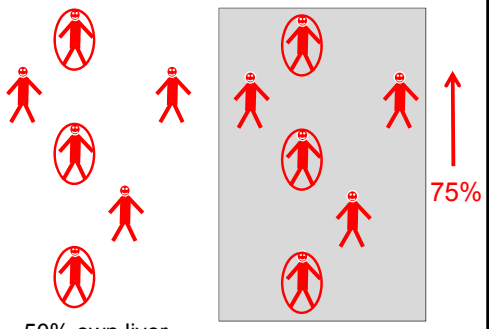
Goal: To decrease inflammation in liver



Kasai: 50% own liver

Individualized care

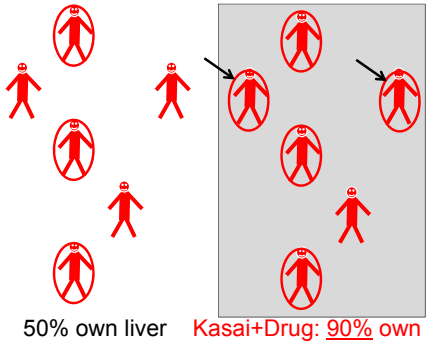
Goal: To decrease inflammation in liver



Kasai: 50% own liver

Individualized care

Goal: To decrease inflammation in liver



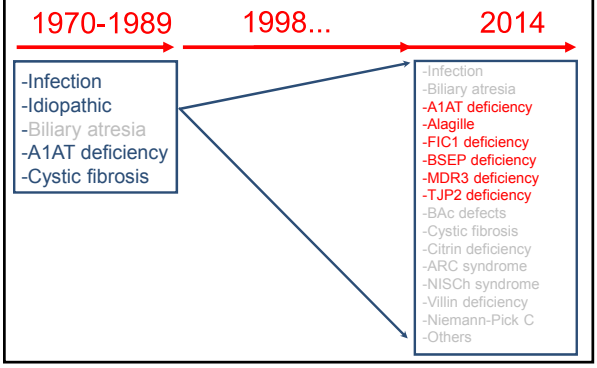
Kasai: 50% own liver Kasai+Drug: 90% own liver

Intrahepatic cholestasis

Recent advances

- *Genetics*
- *Old and new syndromes*

Causes of neonatal cholestasis



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

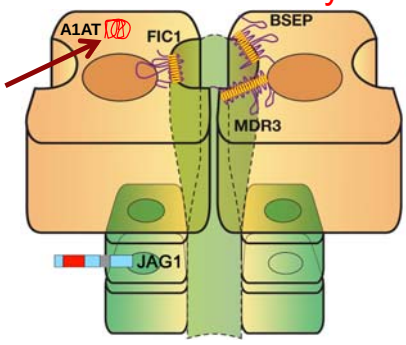
Discoveries

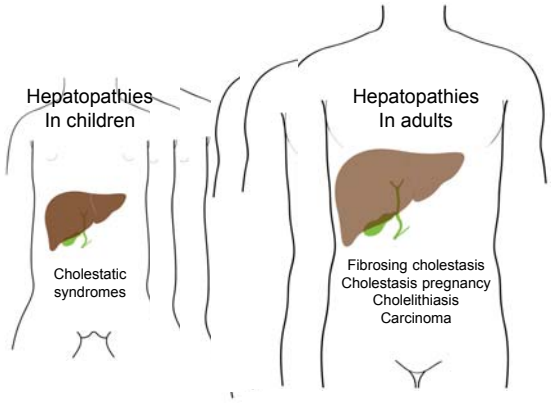
Genetic basis of liver disease

Year, investigators	Disorder	Gene
1969 - Sharp H et al.	A1AT deficiency	*SERPINA1*
1998 - Oda T et al. Spinner NB et al.	Alagille syndrome	*JAG1*
1998 - Bull LN et al.	PFIC-1, BRIC-1	*ATP8B1*
1998 - Strautnieks S et al.	PFIC-2	*ABCB11*
1996 - Deleuze JF et al. 1998 - de Vree JM et al.	PFIC-3	*ABCB4*
1998 - Setchell K et al.	BASD, Reductase	*AKR1D1*

Molecular basis of cholestasis

A1AT deficiency





Spectrum of syndrome

FIC1 deficiency

Year, investigators	Disorder	Gene
1998 - Bull LN et al.	PFIC-1 BRIC-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. 1998 - de Vree JM et al.	PFIC3	*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

ABCB4 Heterozygous Gene Mutations Associated With Fibrosing Cholestatic Liver Disease in Adults

MARIANNE ZIOL,*,‡ VÉRONIQUE BARBU,§,‖ OLIVIER ROSMORDUC,¶,‖ ANNONCIADE FRASSATI-BIAGGI,* NATHALIE BARGET,*,# BRIGITTE HERMELIN,§,‖ GEORGES L. SCHEFFER,** SELMA BENNOUNA,# JEAN-CLAUDE TRINCHET,#,‡‡ MICHEL BEAUGRAND,#,‡‡ and NATHALIE GANNE-CARRIÉ#,‡‡

GASTROENTEROLOGY 2008;135:131–141

- 32 adults with chronic cholestasis
- Unknown etiology
- Histology
  - Portal fibrosis with ductular reaction
  - Portal tract with macrophage infiltration
- Heterozygous mutations in *ABCB4* 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

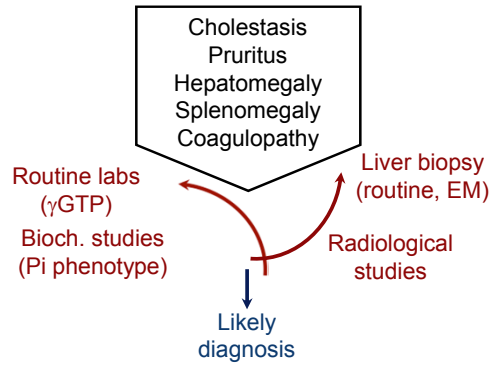
Intrahepatic cholestasis

Research goals for 2020

20/20 Clarity: Personalized care

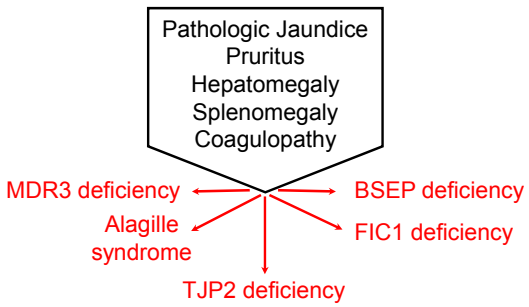
- *Diagnostics*
- *Therapeutics*

Diagnostics

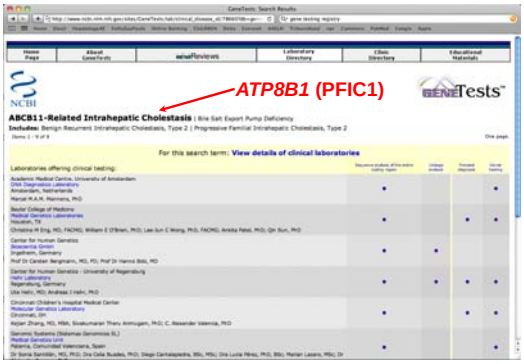


Diagnostics

Mutation screening

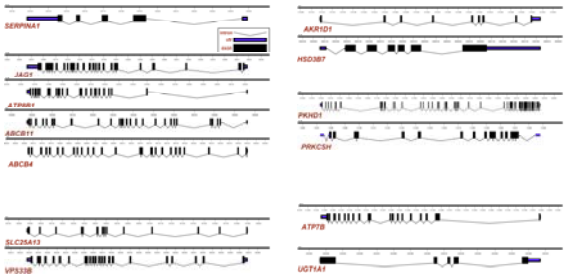


Diagnostics: Laboratories

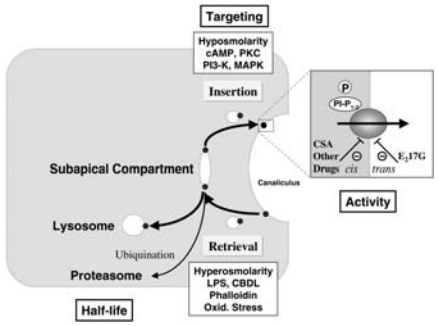


Customization of platforms

- One stop-shop for liver genes?



Posttranscriptional regulation of canalicular transport systems.



Trauner M , and Boyer J L Physiol Rev 2003;83:633-671

Physiological Reviews

Personalized therapeutics

Successful mutation-specific chaperone therapy with 4-phenylbutyrate in a child with progressive familial intrahepatic cholestasis type 2

Emmanuel Gonzales[1,2], Brigitte Grosse[2], Doris Cassio[2], Anne Davit-Spraul[3], Monique Fabre[4], Emmanuel Jacquemin[1,2,*]

- 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 ➔ 237 μmol/L
  - ALT 125 ➔ 19 Total bili 12 ➔ <1

J Hepatol 2012; 57: 695–698

Personalized therapeutics

Improved Liver Function and Relieved Pruritus after 4-Phenylbutyrate Therapy in a Patient with Progressive Familial Intrahepatic Cholestasis Type 2

Sotaro Naoi, MS[1,*], Hisamitsu Hayashi, PhD[1,*], Takeshi Inoue, MD[2], Ken Tanikawa, MD[3], Koji Igarashi, PhD[4], Hironori Nagasaka, MD, PhD[5], Masayoshi Kage, MD, PhD[3], Hajime Takikawa, MD, PhD[6], Yuichi Sugiyama, PhD[7], Ayano Inui, MD, PhD[8], Toshiro Nagai, MD, PhD[2], and Hiroyuki Kusuhara, PhD[1]

- 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

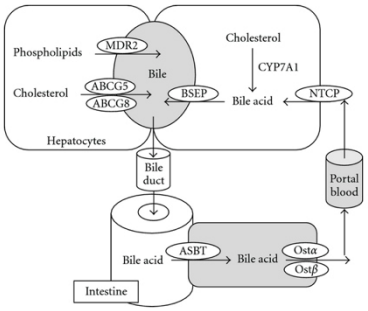
J Pediatr 2014;154L1219-27

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)
  - Heteroz (N=2) or homozygous (N=1) for *ATP8B1*
- 4-PB: Escalating doses 150-500 mg/kg/d

	Pruritus	ATX	AST, ALT	Bilirubin
Patient 1	4 ➔ 2	No Δ	No Δ	No Δ
Patient 2	4 ➔ 2	No Δ	No Δ	No Δ
Patient 3	4 ➔ 2	No Δ	No Δ	No Δ



Safety and efficacy of LUM001 in children with PFIC: NCT02057718

FIC1 deficiency and OLT

L-Hayastino A et al. Liv Transpl 2009;15:610

- 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

BSEP deficiency and OLT

- *Keitel V et al. Hepatology 2009;50:510*
- *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

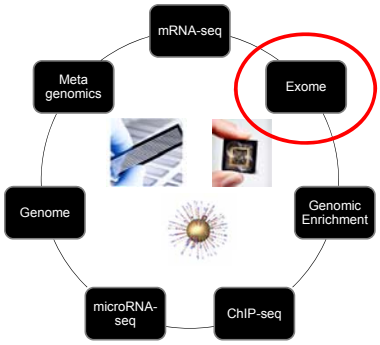
Hepatology of tomorrow

Beyond 2020

- *Genomic pediatrics*
- *Liver in a dish*

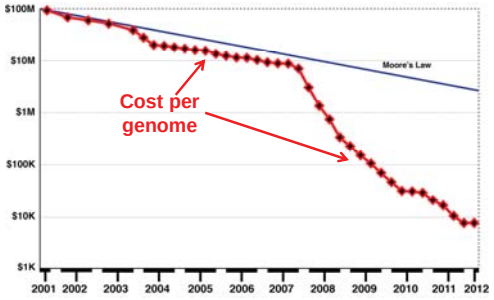
The current frontier

Next-Gen(eration) sequencing



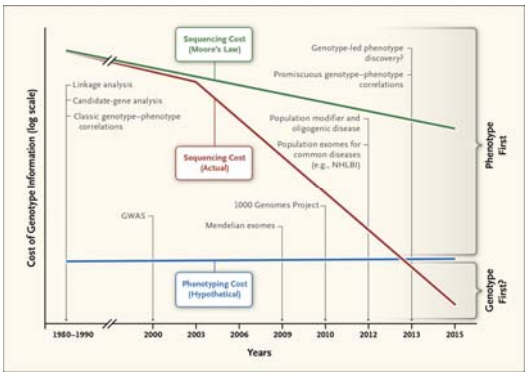
The $1000 Genome

Rapid decrease in cost of sequencing



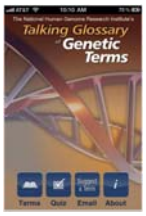
Source: National Human Genome Research Institute
www. genome.gov/sequencingcosts

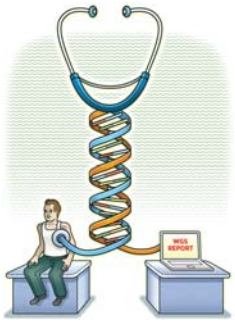
The decreasing cost of genotype information



Lu JT et al. N Engl J Med 2014;371:593-596.

Hepatologist



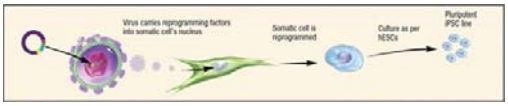


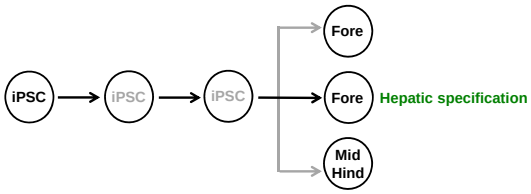
Genetic Counselor

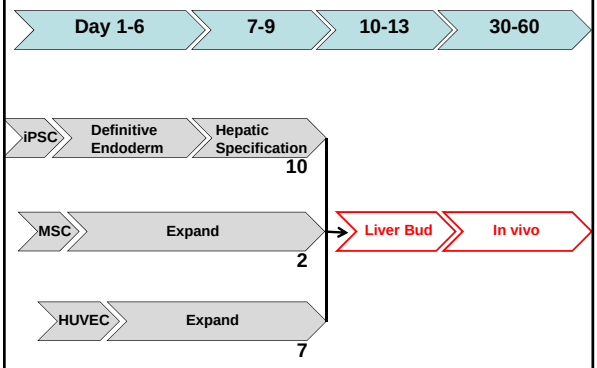
D W Nebert, G Zhang Science 2012;337:910-910

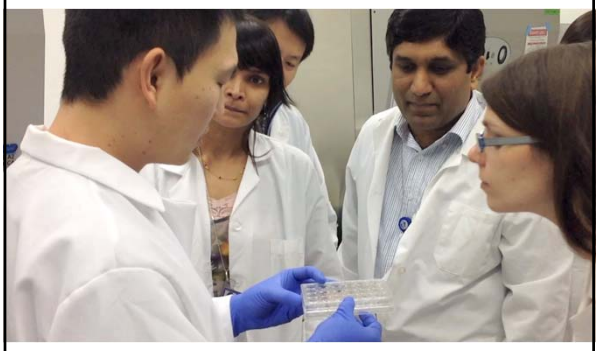
From stem cells ➔ Liver bud

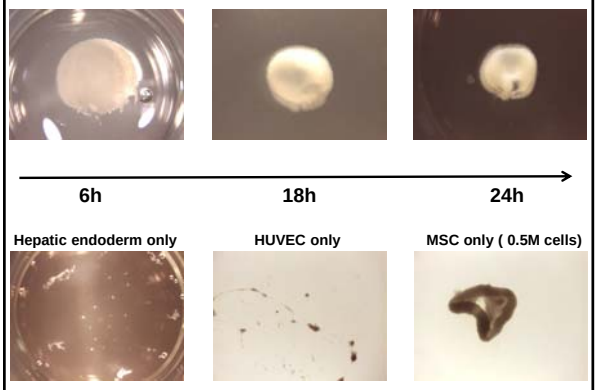
- Inducible Pluripotent Stem Cells (iPSC)





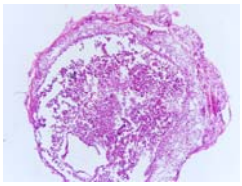






Liver “bud”

Liver “bud”



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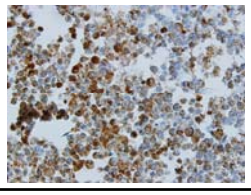
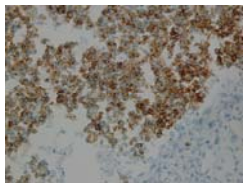


CD31



Engineering of a human liver

LETTER

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

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