Inborn Errors of Bile Acid Metabolism- Amidation Defects

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

Cincinnati Children's
changing the outcome together

Financial Disclosures

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

Cincinnati Children's
changing the outcome together

Outline

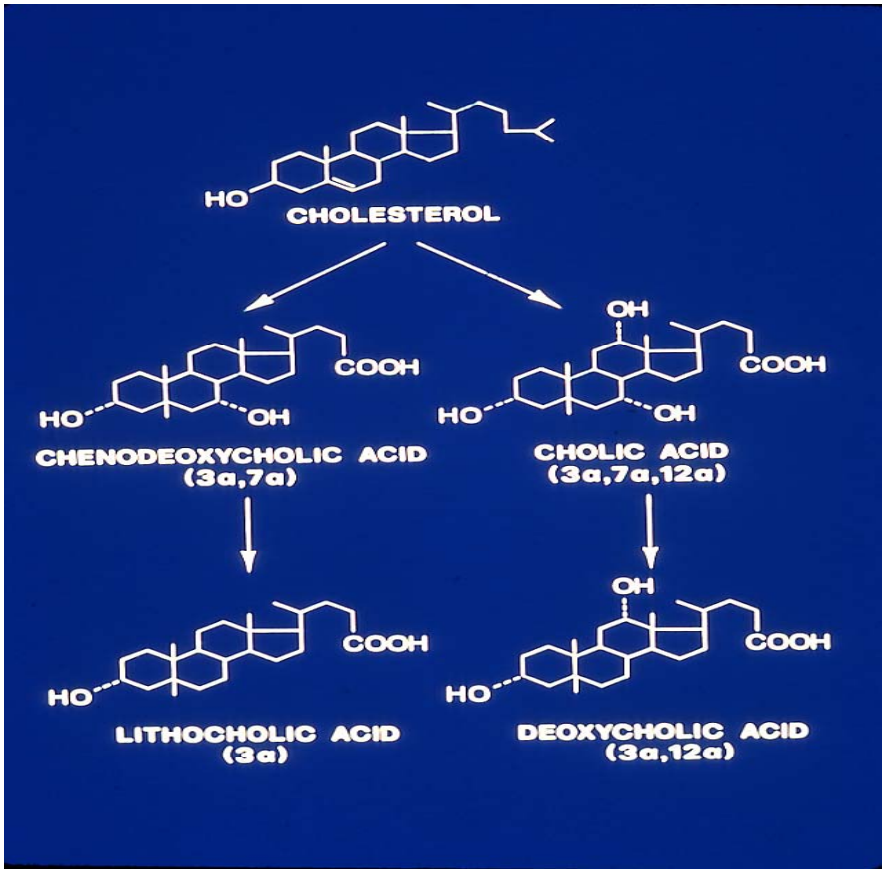
- Function of bile acids
- Enterohepatic circulation and metabolism of bile acids
- Clinical and Pathological manifestations of amidation defects
- Treatment

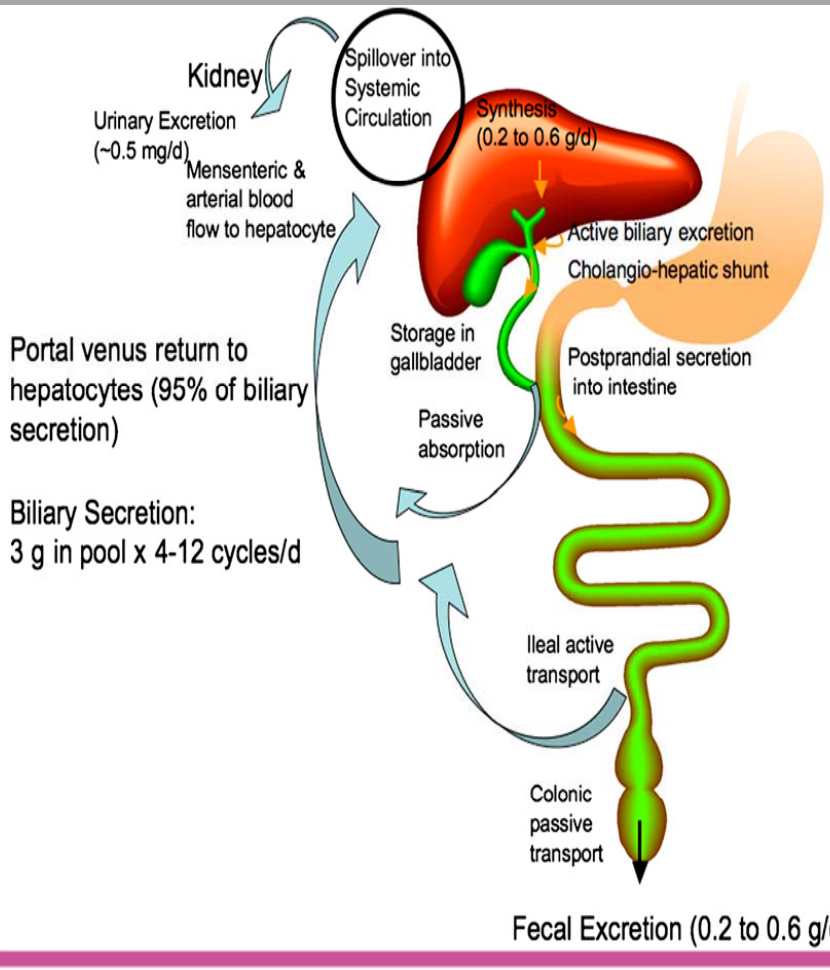
Cincinnati Children's
changing the outcome together

Role of Bile Acids

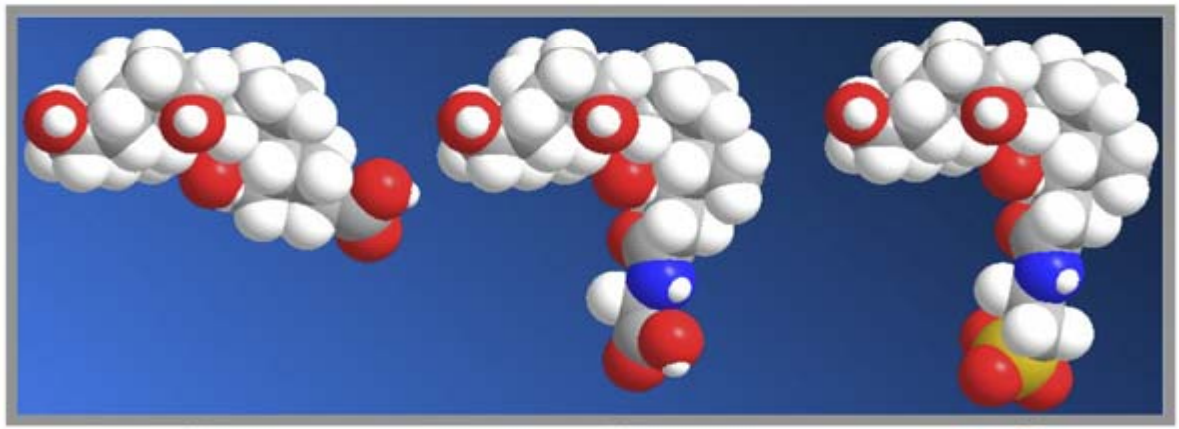
- Major metabolic pathway for elimination of cholesterol
- Promote formation/ secretion of bile
- Fat and fat soluble vitamin absorption
- Cathartic action-induce water and electrolyte secretion
- Bacteriostatic properties
- Role in signaling pathways

EHC and BA Metabolism 101



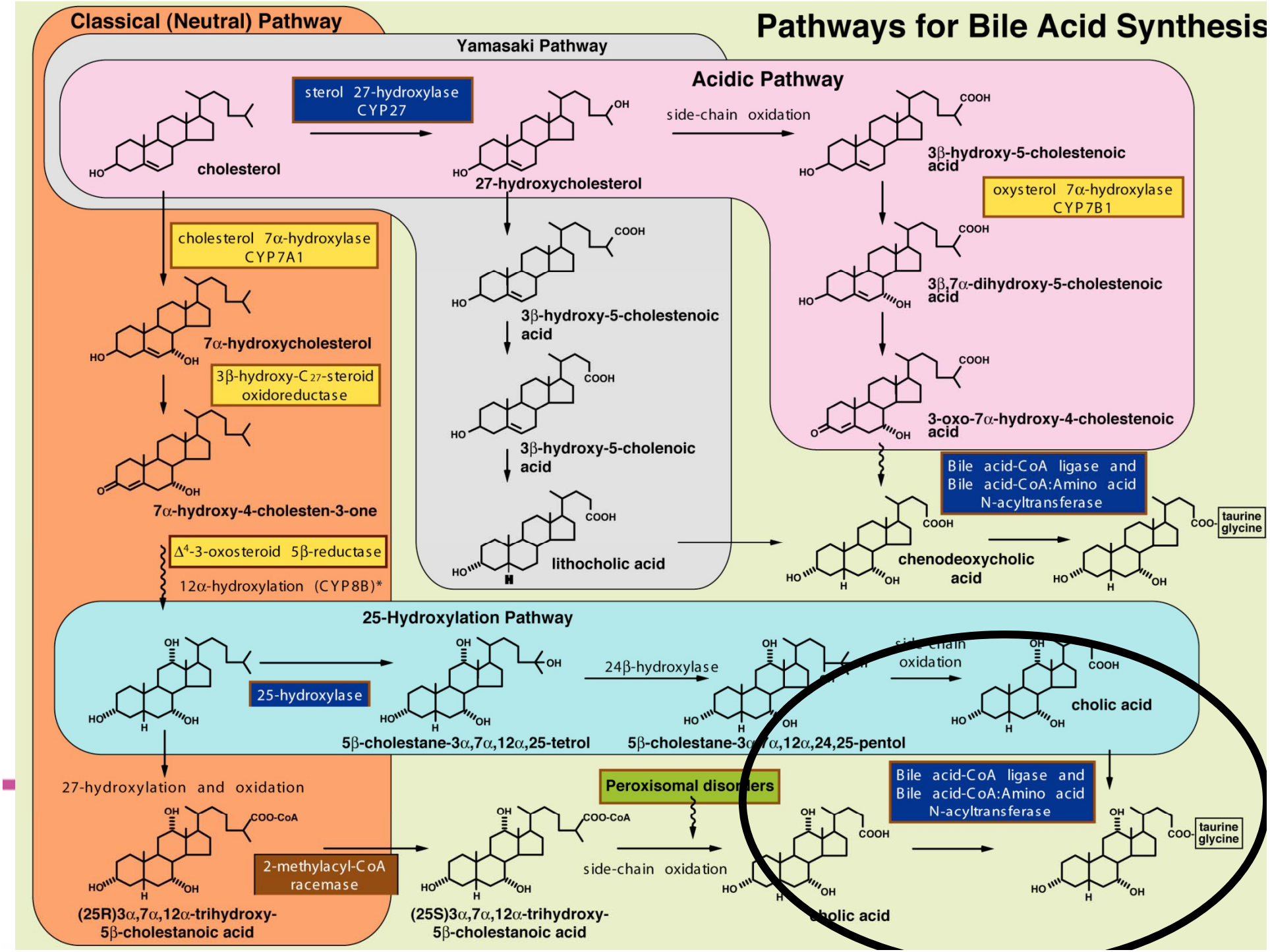


Physico-chemical characteristics of cholic acid and conjugates

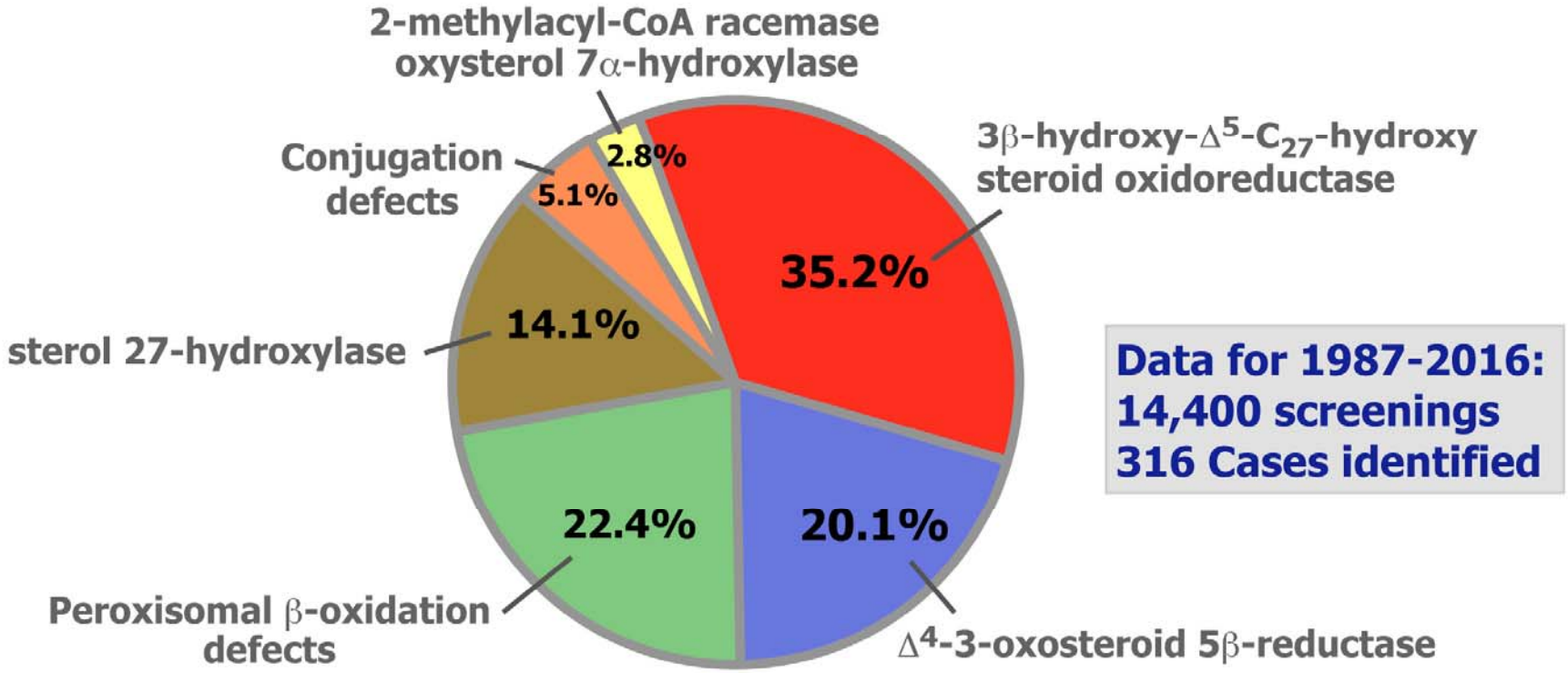


Cholic	Glycocholic	Taurocholic
■ pK_a 5.0, non-ionized	■ pK_a 3.9, ionized	■ pK_a <2.0, ionized
■ insoluble	■ soluble	■ soluble
■ absorbed by passive diffusion in small and large intestine	■ absorbed solely by active transport in terminal ileum	■ absorbed solely by active transport in terminal ileum
■ CMC = 11 mM	■ CMC = 10 mM	■ CMC = 6 mM

Cincinnati Children's
changing the outcome together


Pathways for Bile Acid Synthesis
Classical (Neutral) Pathway
Yamasaki Pathway
Acidic Pathway
sterol 27-hydroxylase CYP27
cholesterol
27-hydroxycholesterol
side-chain oxidation
3β-hydroxy-5-cholestenoic acid
oxysterol 7α-hydroxylase CYP7B1
3β,7α-dihydroxy-5-cholestenoic acid
3-oxo-7α-hydroxy-4-cholestenoic acid
cholesterol 7α-hydroxylase CYP7A1
7α-hydroxycholesterol
3β-hydroxy-C27-steroid oxidoreductase
7α-hydroxy-4-cholesten-3-one
Δ4-3-oxosteroid 5β-reductase
12α-hydroxylation (CYP8B)*
3β-hydroxy-5-cholestenoic acid
3β-hydroxy-5-cholenoic acid
lithocholic acid
Bile acid-CoA ligase and Bile acid-CoA:Amino acid N-acyltransferase
chenodeoxycholic acid
taurine glycine
25-Hydroxylation Pathway
25-hydroxylase
5β-cholestane-3α,7α,12α,25-tetrol
24β-hydroxylase
5β-cholestane-3α,7α,12α,24,25-pentol
side-chain oxidation
cholic acid
27-hydroxylation and oxidation
(25R)3α,7α,12α-trihydroxy-5β-cholestanoic acid
2-methylacyl-CoA racemase
(25S)3α,7α,12α-trihydroxy-5β-cholestanoic acid
Peroxisomal disorders
side-chain oxidation
Bile acid-CoA ligase and Bile acid-CoA:Amino acid N-acyltransferase
taurine glycine

Defects in Bile Acid Synthesis: 'The Cincinnati Experience'

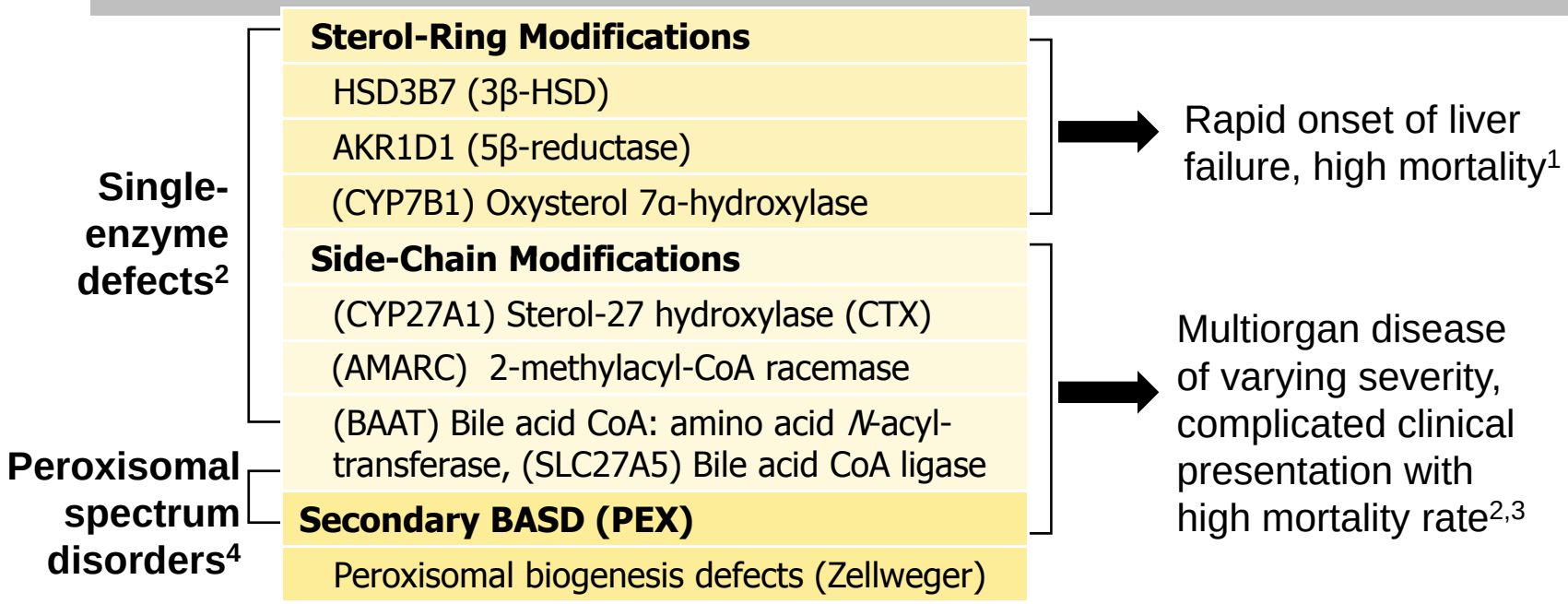


Data for 1987-2016:
14,400 screenings
316 Cases identified

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

1. Heubi JE, et al. Semin Liver Dis. 2007;27(3):282-294.
2. Bove KE, et al. Pediatr Dev Pathol. 2000;3(1):1-16.
3. Setchell KD, Heubi JE. J Pediatr Gastroenterol Nutr. 2006;43(suppl 1):S17-S22

Clinical Sequelae of BASD: SED vs PD



Clinical phenotype is highly variable — high index of suspicion based on physical examination and laboratory evaluation

Cincinnati Children's
changing the outcome together

The Lancet, August 6, 1988

Hypothesis

Defective Bile Acid Amidation: Predicted Features of a New Inborn Error of Metabolism

Alan F. Hofmann[1] Birgitta Strandvik[2]
Department of Medicine, University of California at San Diego, La Jolla, California 92093, USA,[1] and Department of Pediatrics, Huddinge Hospital, Sweden[2]

Summary Biochemical and clinical features are predicted for an as yet unreported inborn error of metabolism, in which bile acids cannot be conjugated with glycine or taurine. **Unconjugated cholic acid will be secreted into bile, be absorbed from the intestine, and become the predominant bile acid in bile and plasma.** Other bile acids will be esterified with glucuronate and secreted into bile, but undergo little enterohepatic circulation. **Cholestasis will not be present;** the bile acid pool will be diminished and lipid absorption, especially that of **fat-soluble vitamins, will be impaired....**

Conjugation Defects

- Two recognized defects
  - Bile Acid; amino acid N acyltransferase (BAAT)
    - Recent report of 10 patients with bile acid: amino acid -N acyltransferase deficiency (Setchell, Heubi et al Gastroent 2013; 2013; 144:945-955)
  - Bile acid acyl-CoA ligase (BACL)
    - 27 week gestation Pakastani infant (Chong CPK et al, J Inherited Metab Dis 2012; 35:521-530)
  - Diagnosis
    - Urine FAB-MS: absent amidated bile acids
    - Cholestasis gene sequencing

BAAT Defect Characteristics

Patient no.	Sex	Age at diagnosis	Consanguinity	Origin/ethnicity	Liver	Serum AST/ALT	Serum direct bilirubin	Serum fat-soluble vitamin levels
1	M	14 y	Not known	Laotian	Hepatomegaly	Normal	Elevated	Low
2	M	4 y	Yes	Saudi Arabia/Asian	Hepatomegaly - portoenterostomy	Elevated	Elevated	Low
3	F	8 y	Yes	Saudi Arabia/Asian	—	Normal	—	Low
4	F	1 y	No	United States/Hispanic	Normal	Elevated	Normal	Low
5	M	3 mo	Yes	United States/Hispanic	Liver failure/orthotopic liver transplantation	Elevated	Elevated	—
6	F	11 y	Yes	United States/Hispanic	Normal	Normal	Normal	Low
7	F	10 y	Yes	United States/Hispanic	Normal	Normal	Normal	Low
8	F	3 mo	Not known	United States/Amish	Normal	Normal	Normal	Low
9	M	6 mo	No	United States/Hispanic	Hepatomegaly	Elevated	Elevated	Low
10	F	6.5 y	Not known	United States/white	—	—	—	

M, male; F, female; AST, aspartate aminotransferase.
[a]Body weight at birth and 3½ months of age.

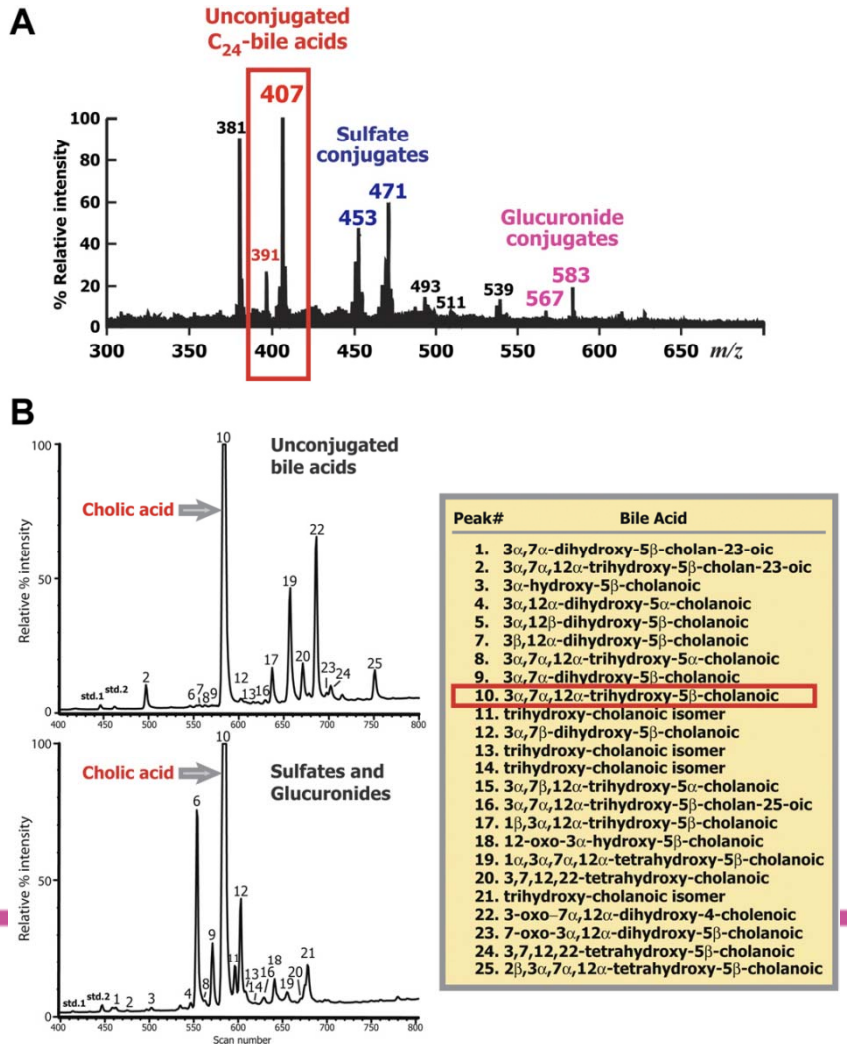
Cincinnati Children's
changing the outcome together

Patient Characteristics

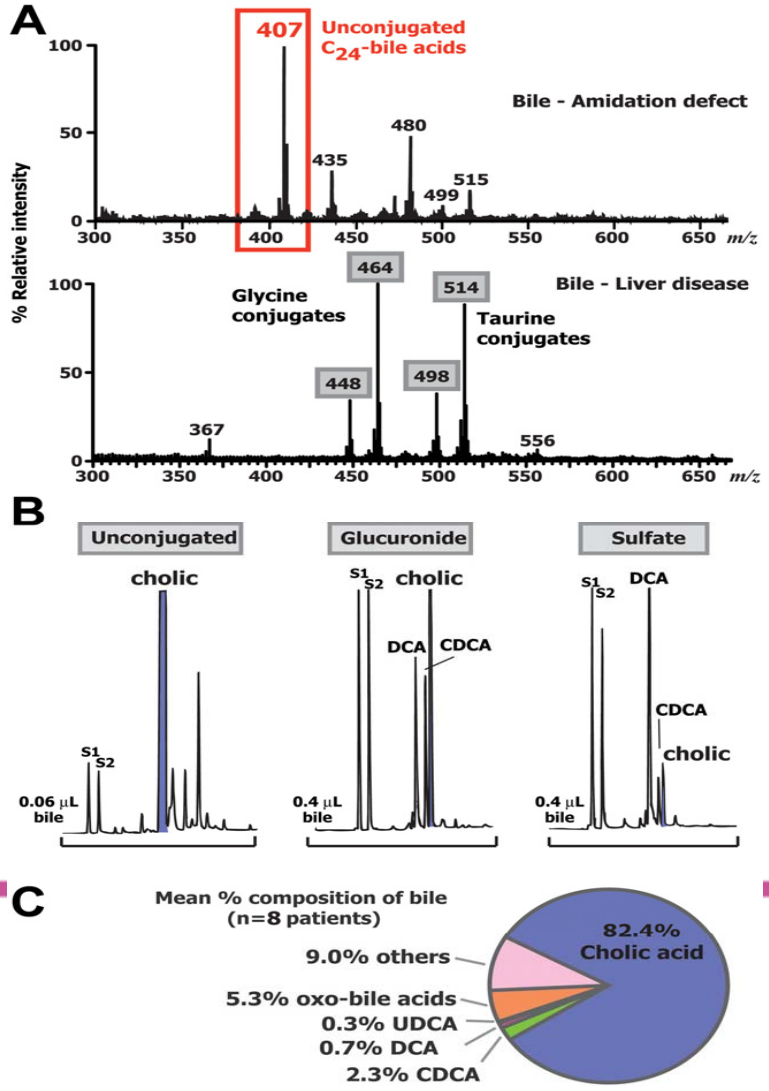
Body wt (percentile)	Bone	Urine FAB-MS analysis: amidated bile acids (present/absent)	Total urinary bile acid concentration ($\mu mol/L$)	Unconjugated bile acids in urine (%)	Cholic acid in urine (%)	Total duodenal bile acid concentration ($\mu mol/L$)	Unconjugated biliary acids (%)	Cholic acid in bile (%)
<5th	Rickets	Absent	217	80.8	65.5	5528	99.7	95.1
Normal	Rickets with bone fracture	Absent	1111	64.9	59.2	35,806	99.9	58.7
<50th	Rickets with fractures	Absent	—	—	—	—	—	—
<3rd	Rickets	Absent	173	80.6	56.5	76	92.0	76.9
75th[a]	—	Absent	153	78.8	50.7	472	82.6	85.8
50th	—	Absent	135	72.4	49.6	3023	97.1	92.0
10th	—	Absent	—	—	—	24,083	98.2	93.3
25th	—	Absent	82.5	95.7	79.0	23,509	99.4	94.0
<3rd	Rickets	Absent	—	—	—	—	—	—
<5th	—	Absent	1156	82.7	23.7	3997	96.8	63.3

Cincinnati Children's
changing the outcome together

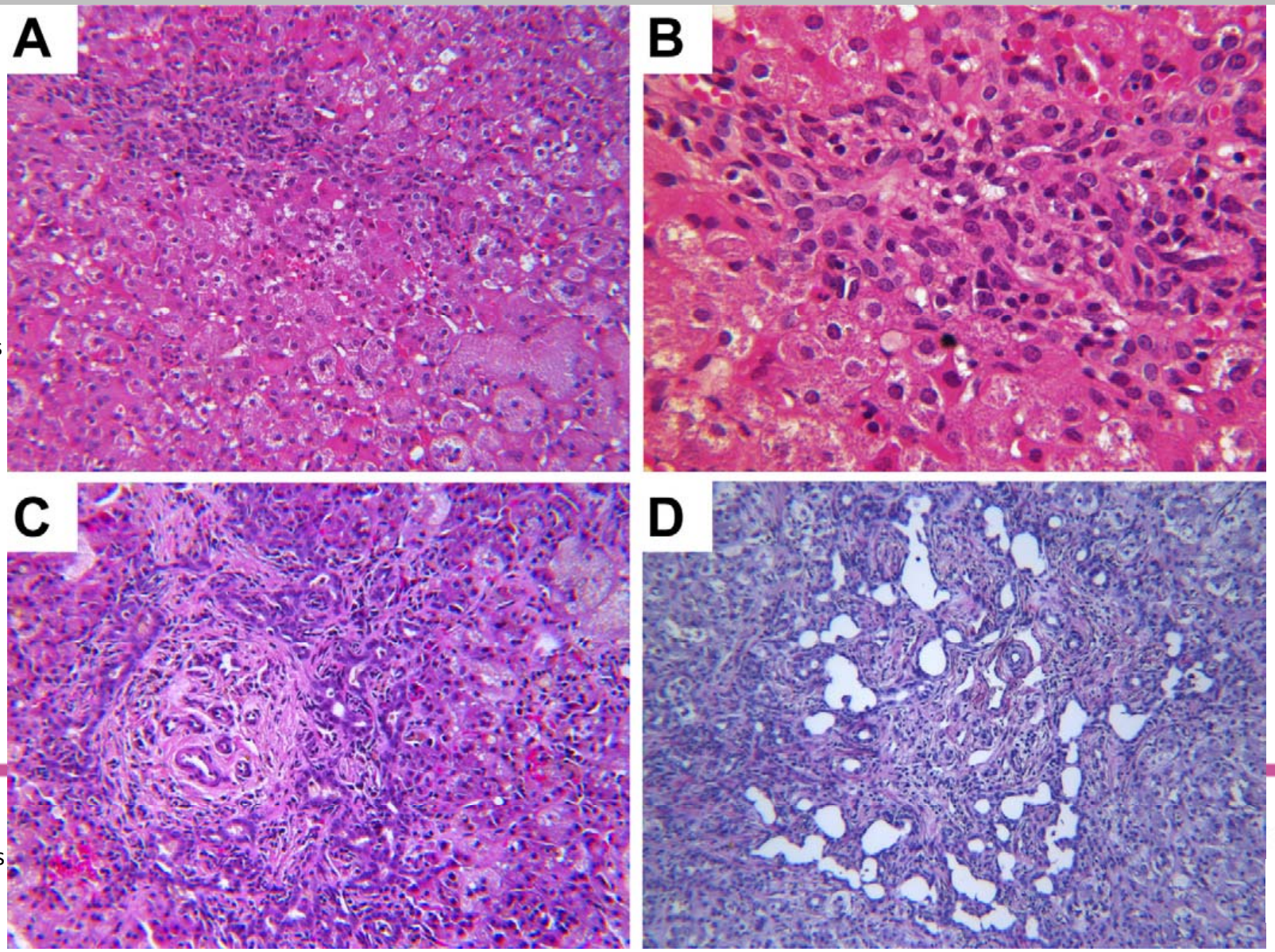
BAAT Defects: Bile and Urine BA



Peak#	Bile Acid
1.	3α,7α-dihydroxy-5β-cholan-23-oic
2.	3α,7α,12α-trihydroxy-5β-cholan-23-oic
3.	3α-hydroxy-5β-cholanoic
4.	3α,12α-dihydroxy-5α-cholanoic
5.	3α,12β-dihydroxy-5β-cholanoic
7.	3β,12α-dihydroxy-5β-cholanoic
8.	3α,7α,12α-trihydroxy-5α-cholanoic
9.	3α,7α-dihydroxy-5β-cholanoic
10.	3α,7α,12α-trihydroxy-5β-cholanoic
11.	trihydroxy-cholanoic isomer
12.	3α,7β-dihydroxy-5β-cholanoic
13.	trihydroxy-cholanoic isomer
14.	trihydroxy-cholanoic isomer
15.	3α,7β,12α-trihydroxy-5α-cholanoic
16.	3α,7α,12α-trihydroxy-5β-cholan-25-oic
17.	1β,3α,12α-trihydroxy-5β-cholanoic
18.	12-oxo-3α-hydroxy-5β-cholanoic
19.	1α,3α,7α,12α-tetrahydroxy-5β-cholanoic
20.	3,7,12,22-tetrahydroxy-cholanoic
21.	trihydroxy-cholanoic isomer
22.	3-oxo–7α,12α-dihydroxy-4-cholenoic
23.	7-oxo-3α,12α-dihydroxy-5β-cholanoic
24.	3,7,12,22-tetrahydroxy-5β-cholanoic
25.	2β,3α,7α,12α-tetrahydroxy-5β-cholanoic



BAAT Defects: Histopathology

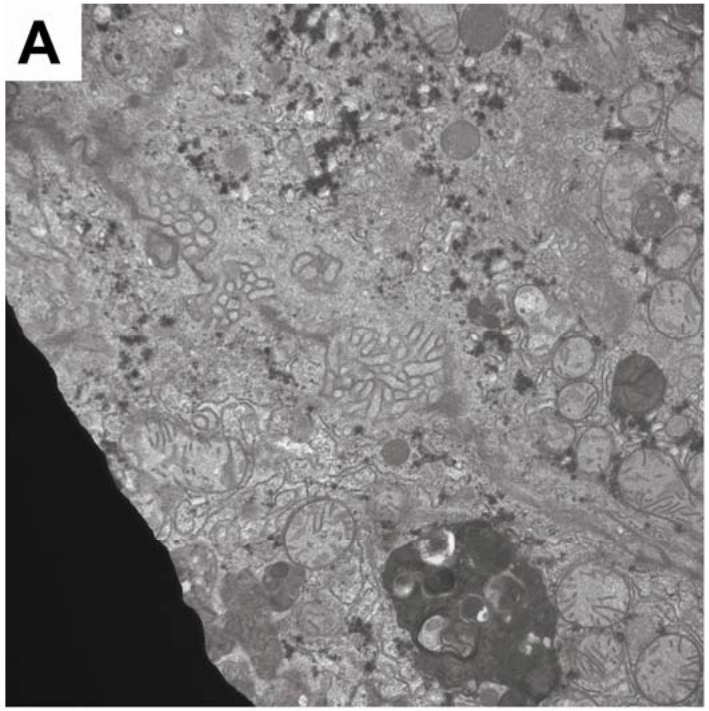


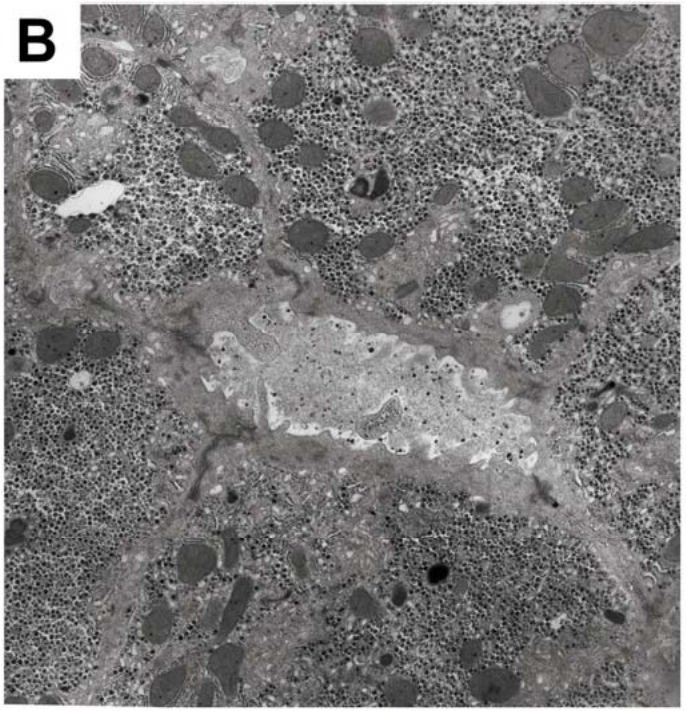
Pt 2,
5 weeks

Pt 5,
10 weeks

Pt 5,
6 mos

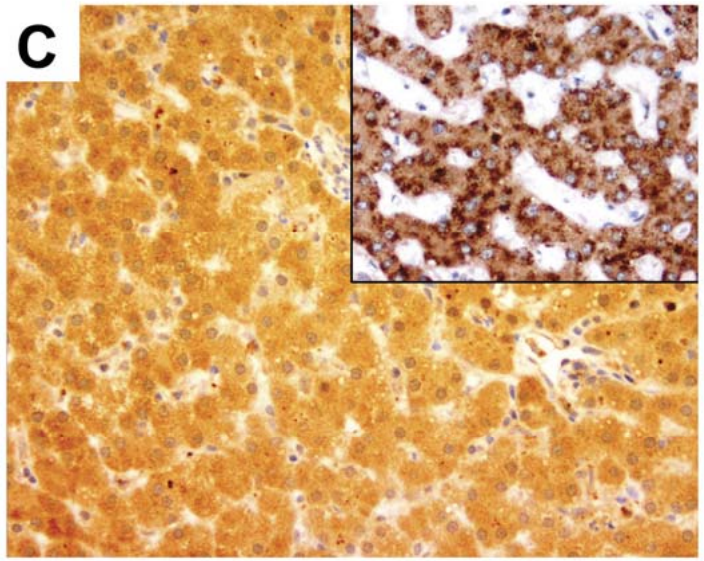
Pt 5,
10 weeks

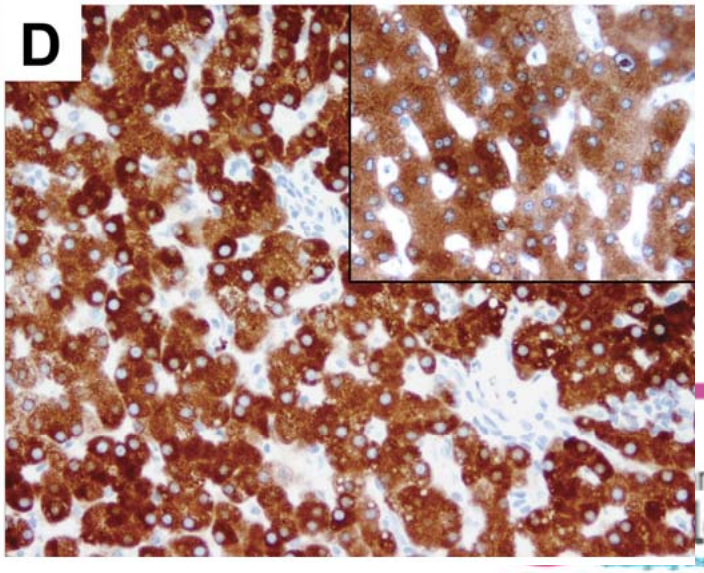




Pt 2,
4.5 years

Pts 2, 3, 5
Anti-BAAT
stain

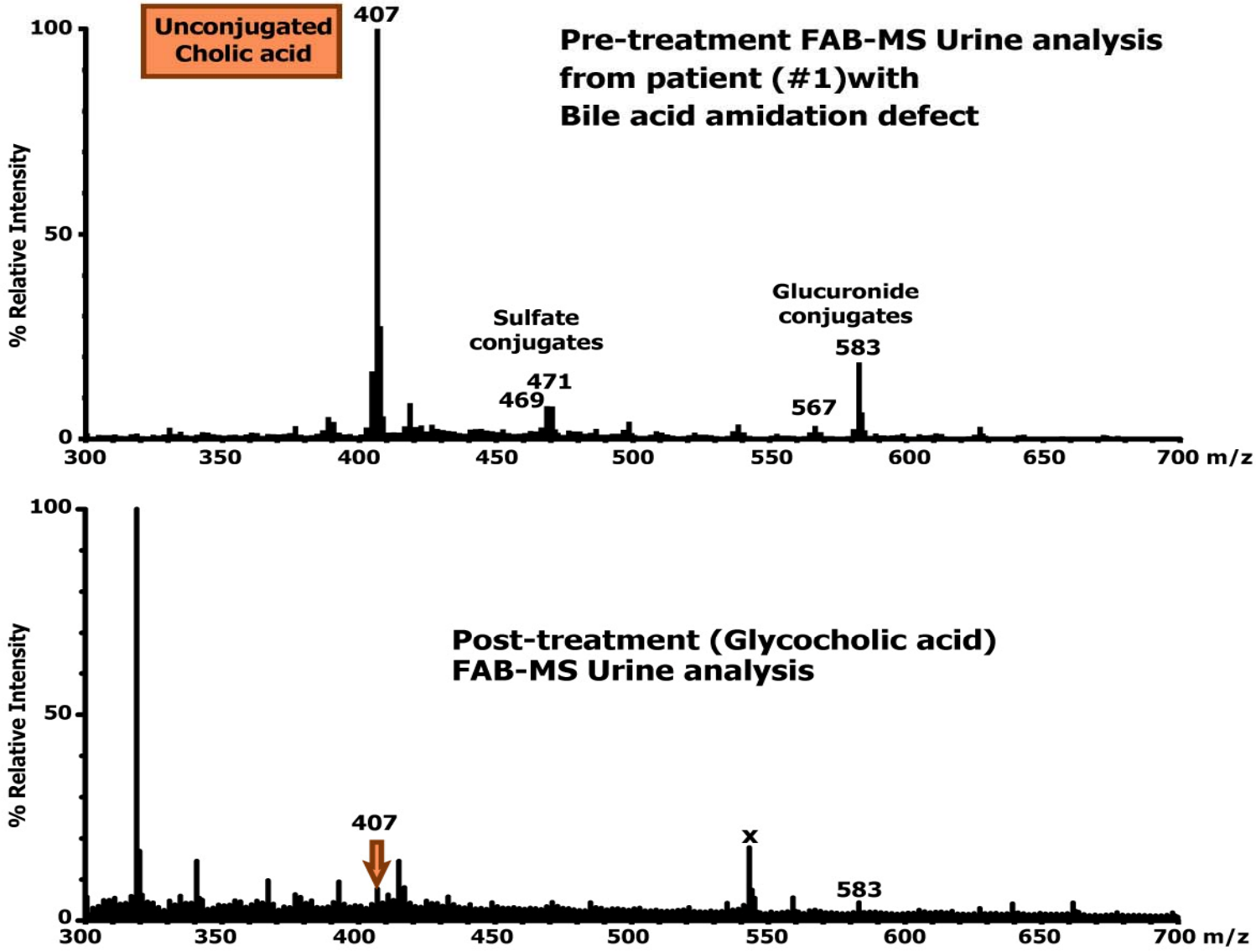




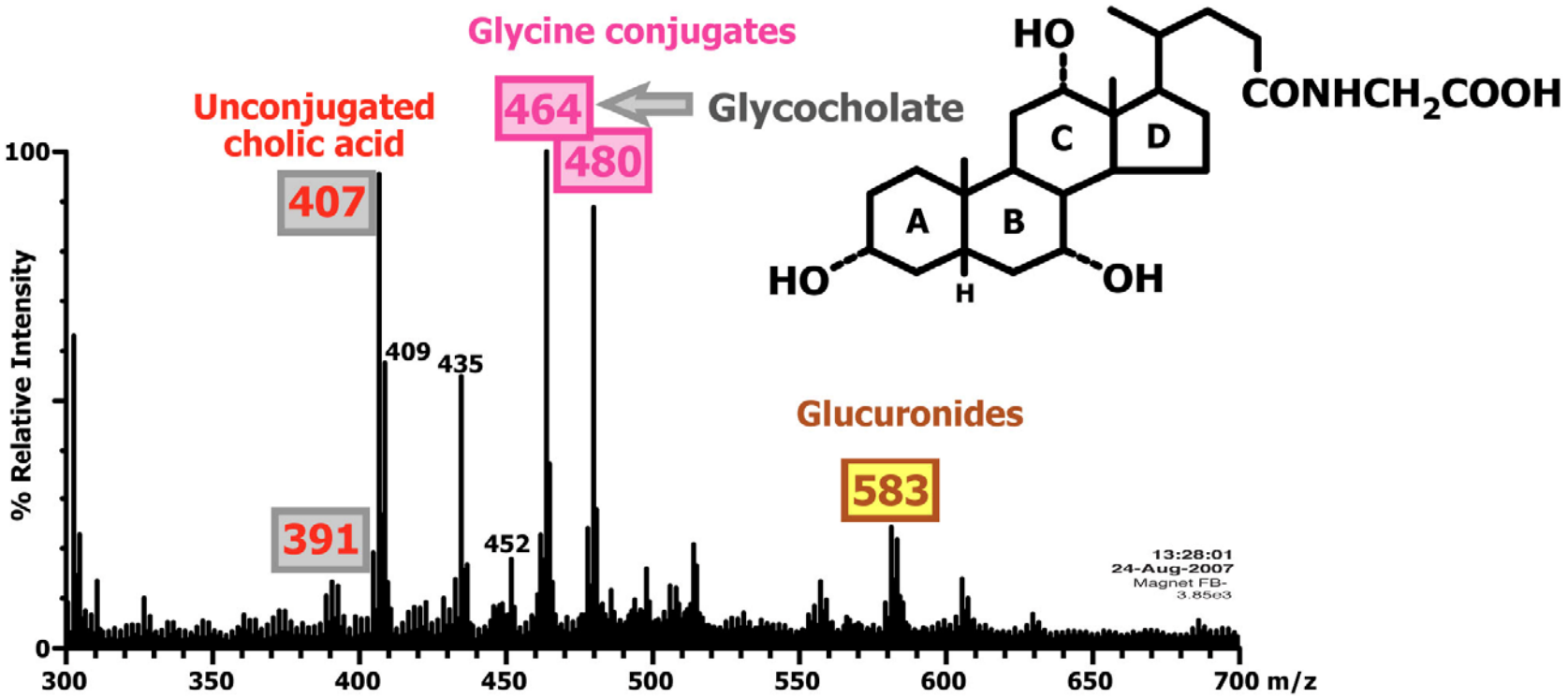
Pts 2,3,5
Anti-BACL
Stain

BAAT Defects-Genetics

Family	Patient No.	Nucleotide Δ	Nature of mutation	Homozygous
1	1	-	--	--
2	2	c.1156G→A	Missense	Yes
	3	c.1156G→A	Missense	Yes
3	4	c.206A→T	Missense	Yes
4	5	c. 58C→T	Premature stop	Yes
	6	c. 58C→T	Premature stop	Yes
	7	c. 58C→T	Premature stop	Yes
5	8	c250C→A	Missense	Yes
6	9	No mutation	--	--
7	10	-	--	--

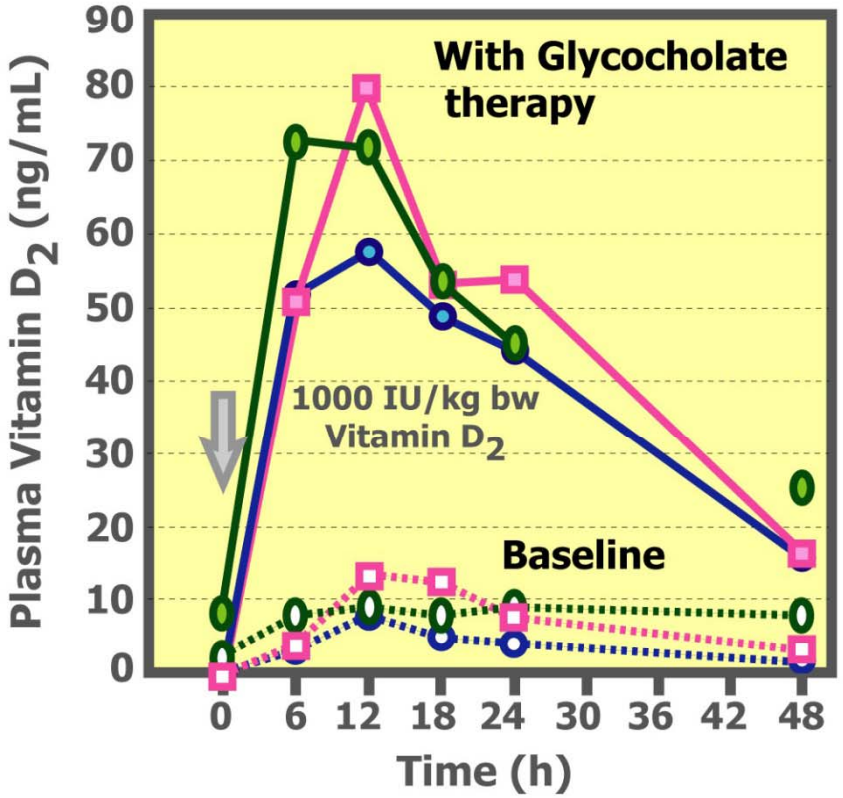

Unconjugated
Cholic acid
407
Pre-treatment FAB-MS Urine analysis
from patient (#1)with
Bile acid amidation defect
% Relative Intensity
100
50
0
Sulfate
conjugates
469
471
Glucuronide
conjugates
567
583
300
350
400
450
500
550
600
650
700 m/z
Post-treatment (Glycocholic acid)
FAB-MS Urine analysis
407
X
583

FAB-MS Analysis of Bile After Glycocholic Acid Therapy



1. Glycocholic acid administered orally is absorbed transported to the liver and secreted in bile
2. Concomitant with biliary enrichment with glycocholic acid, a correction of the fat-soluble vitamin malaborption occurs

Improvement in Vitamin D2 Absorption in Patients with a Bile Acid Conjugation Defect Treated with GCA



Vitamin D_2 tolerance Test:

Performed at baseline and 6-12 months after oral glycocholic acid therapy (10-15 mg/kg/day)

HEPATOLOGY
Official Journal of the American Association for the Study of Liver Diseases
AASLD

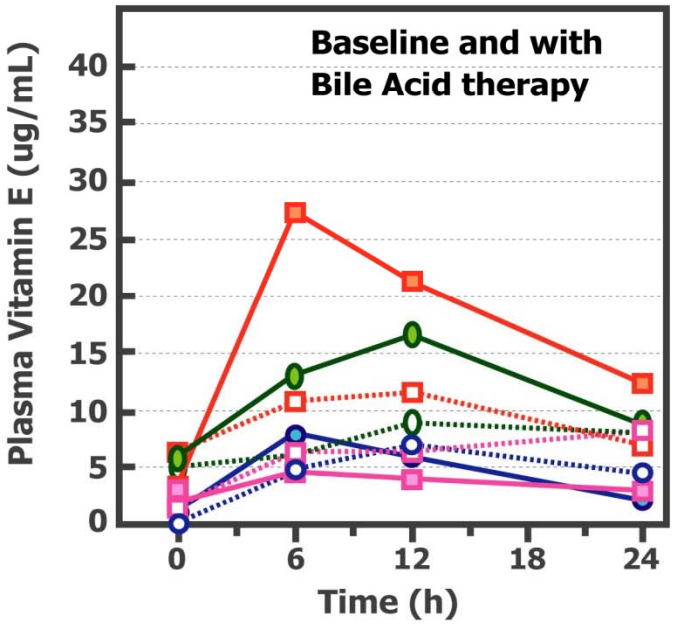
Treatment of Bile Acid Amidation Defects With Glycocholic Acid

James E. Heubi,[1,2] Kenneth D.R. Setchell,[3] Pinky Jha,[3] Donna Buckley,[1,2] Wujuan Zhang,[3] Philip Rosenthal,[4] Carol Potter,[5] Simon Horslen,[6] and David Suskind[6]

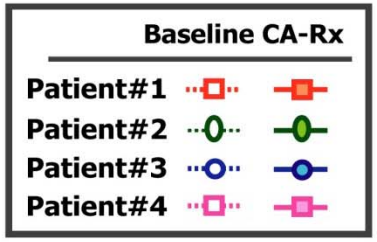
Hepatology 2015;61:264-278

- **Cholic acid therapy improved Vitamin D2 absorption in patients with bile acid conjugation defects**

Effect of Glycocholic Acid Therapy on Vitamin E Absorption



Vitamin E tolerance test performed at baseline and 6-12 months after GCA therapy 10-15 mg/kg/day



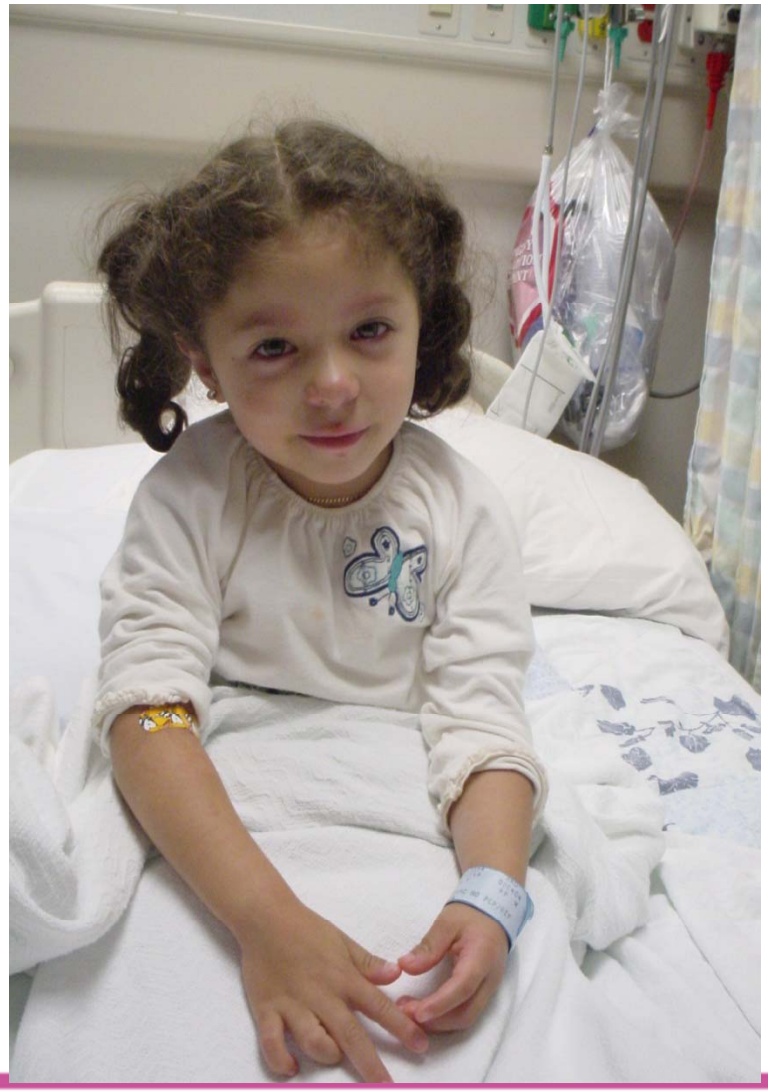
Cincinnati Children's
changing the outcome together

Conjugation Defects:Anthropometrics

	Age(mos)	Weight %ile	Height %ile
Patient 1 Baseline	11	3	75
Patient 1 Follow up	72	75	50
Patient 2 Baseline	33	50*	10
Patient 2 Follow up	78	25	25
Patient 3 Baseline	9	10	10
Patient 3 Follow up	40	90	97

Cincinnati Children's
changing the outcome together

*5 cans Peptamen Jr/Day



Cincinnati Children's
changing the outcome together

BAAT Defects: Longitudinal Treatment

- Treated with Glycocholic acid for 40+ patient years
- 3 independently identified patients (OH, WA, OR)
- 2 siblings of affected infant with FHF (CA)
- Age 11 months to 15yrs
- Phenotype: Minimal liver disease, ± growth failure, fat soluble vitamin deficiency → coagulopathy with bruising/bleeding with immunization
- Add duration of treatment and growth outcomes

Cincinnati Children's
changing the outcome together

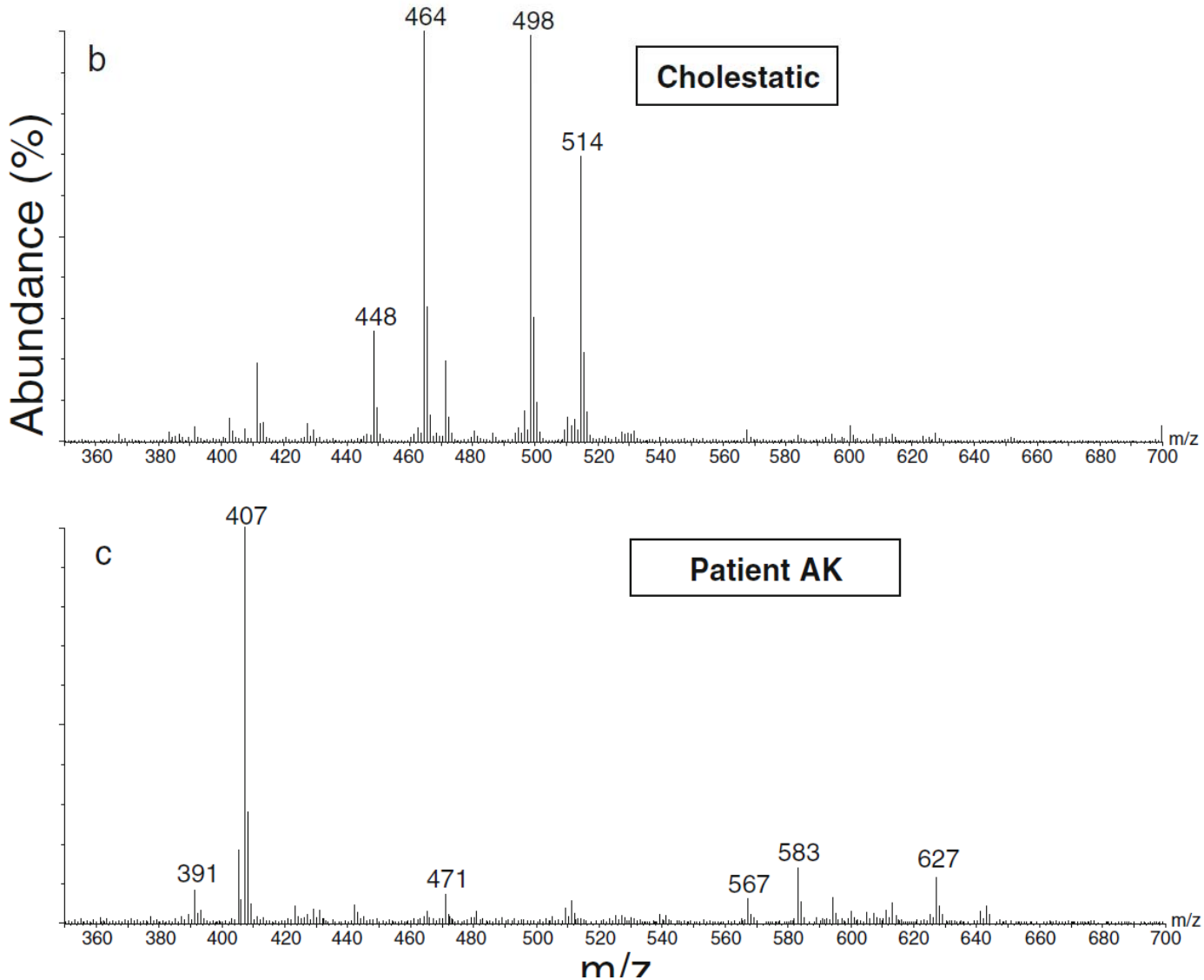
Bile Acid CoA Ligase Deficiency (BACL)

- Limited experience
  - Pakistani born at 27 weeks, parents first cousins
  - On TPN x 35 days: ↑ conjugated bili, AST,ALT, nl GGT
  - Serum ↓vitamin A and E
  - Evaluated at 13 weeks and tx with UDCA and FSV
  - Resolution of biochemical abnormalities
  - At age 8 months, normal biochemistries

Chong CPK et al. J Inherit Metab Dis 2012; 35:521-530

Cincinnati Children's
changing the outcome together

Serum MS analysis



Cincinnati Children's
changing the outcome together

Plasma BA characteristics

Table 4 Results of analysis of plasma bile acids from patient AK by GC-MS

Bile acid	Total bile acid concentration[a] (μM) [normal range]	Unconjugated bile acid concentration[b] (μM)	Unconjugated (%)	Unconjugated in controls (%)
Chenodeoxycholic acid	20.9 [0.22–12.4]	18.4	88%	<25%
Cholic acid	3.25 [0.05–4.55]	2.95	91%	<25%
Ursodeoxycholic acid	4.07 [0–2.09]	3.75	92%	<25%

Cincinnati Children's
changing the outcome together

Genetic Analysis

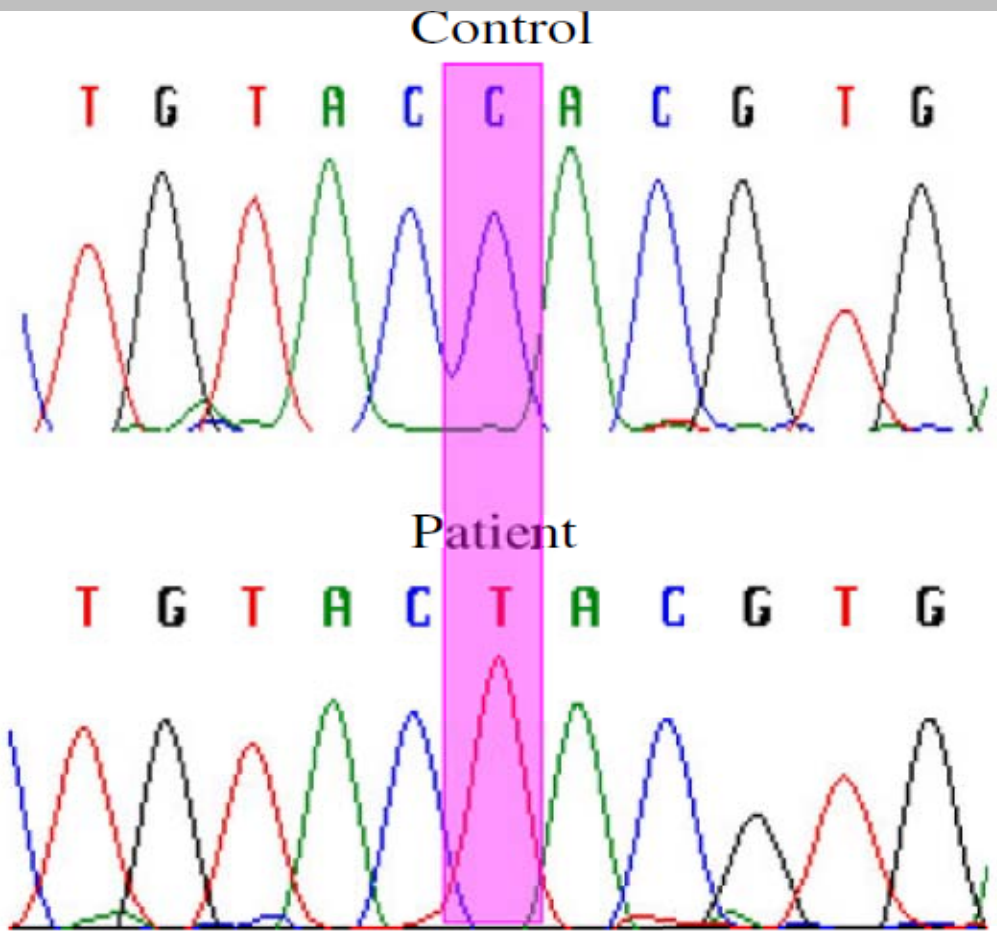
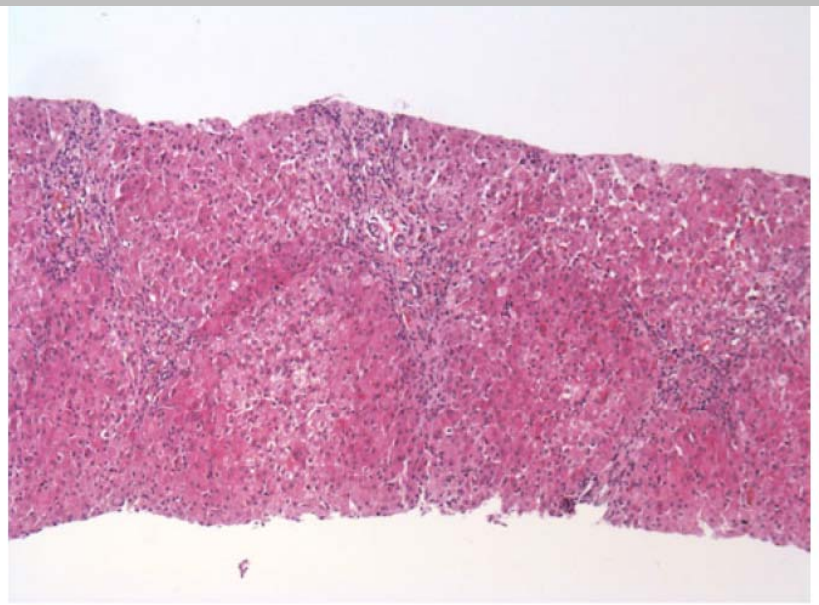
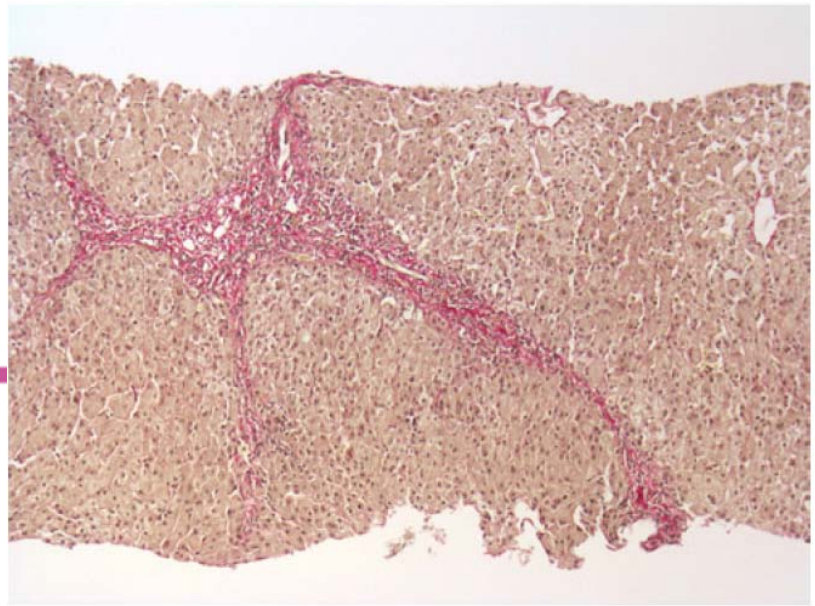


Fig. 3 Electropherograms showing the homozygous mutation (c.1012C> T; H338Y) in the *SLC27A5* gene of patient AK

Cincinnati Children's
changing the outcome together

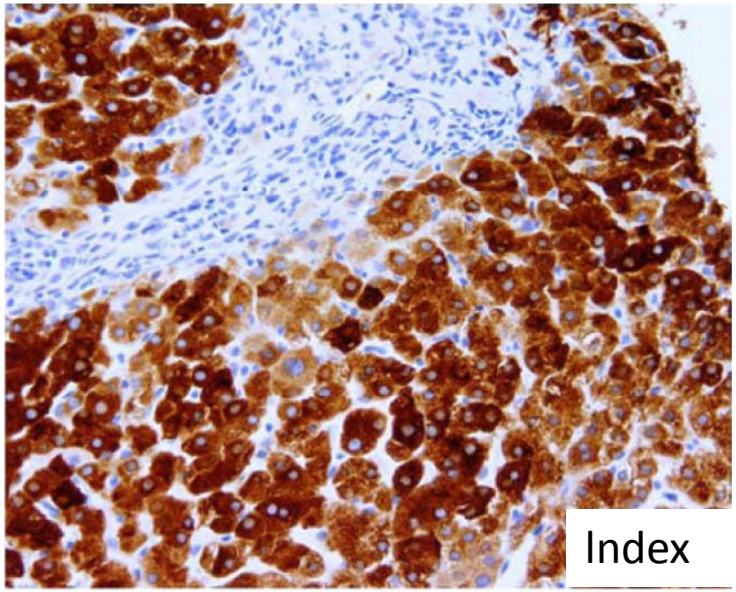
Histopathology



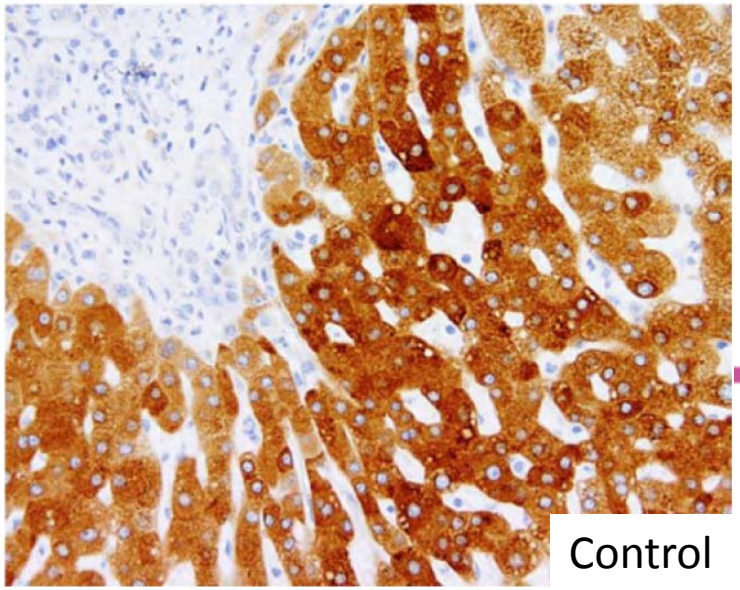


Cincinnati Children's
changing the outcome together

BACL Immunostaining



Index



Control

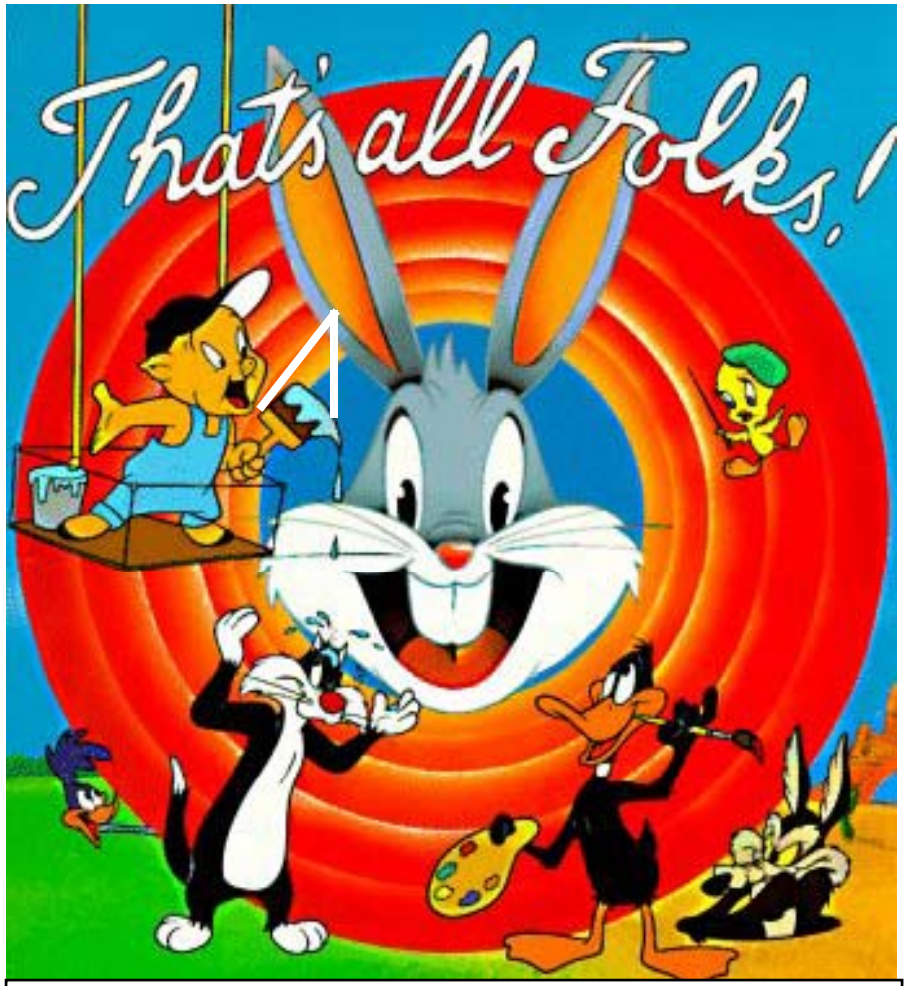
Immunostain for BASL in affected and control

Cincinnati Children's
changing the outcome together

BACL outcomes

- Limited data
- Cholestasis resolved without intervention
- Well at age 5 years with need for fat soluble vitamin supplementation with normalization
- Normal growth Wt and Ht 25-50%ile

Cincinnati Children's
changing the outcome together



Gracias

Cincinnati Children's
changing the outcome together