Diagnosis of Inborn Errors of Liver Metabolism

with particular reference to disorders of bile acid synthesis

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Funded by Great Ormond Street Hospital Children's Charity

When to suspect an inborn error of bile acid synthesis - 1

- Typical phenotype of a peroxisomal disorder:
  - Dysmorphic features
  - Hypotonia
  - Seizures
  - Retinopathy with reduced ERG
  - Sensorineural deafness
  - Failure to thrive
  - Liver dysfunction

When to suspect an inborn error of bile acid synthesis - 2

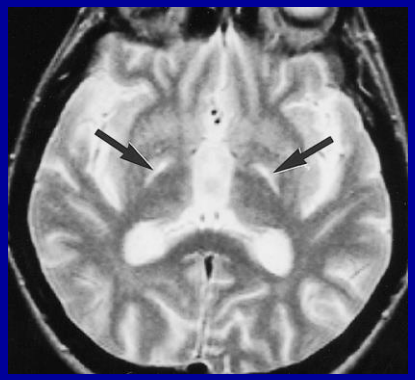
- Conjugated hyperbilirubinaemia of early onset (typically neonatal)
  - Raised transaminases but normal γ-GT
  - Evidence of fat-soluble vitamin malabsorption
    - Especially rickets and low plasma vitamin E
  - Low cholesterol
  - Low total plasma bile acids by 3α-hydroxysteroid dehydrogenase assay
    - Exceptions
      - amidation defects,
      - peroxisomal disorders

Inborn Errors of Bile Acid Synthesis presenting with Jaundice

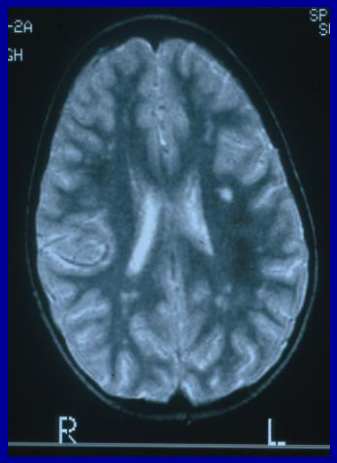
Deficient Enzyme	Gene	Location
3β-Hydroxy-Δ5-C27steroid dehydrogenase	***HSD3B7***	16p11.2-12
Δ4-3-Oxosteroid-5β-reductase	***SRD5B1***	7q31
Sterol 27-hydroxylase (CTX)	***CYP27A1***	2q33-qter
Oxysterol 7α-hydroxylase	***CYP7B1***	8q21.3
α-Methyl-acyl-CoA racemase	***AMACR***	5p13.2 - q11.1
Bile acid CoA: amino acid N-acyl transferase	***BAAT***	9q22.3
Bile acid CoA ligase – resynthesis and ?jaundice	***SLC27A5***	19q13,43
Peroxisome biogenesis	Several	Several
Transporter for THCA-CoA into peroxisome	***ABCD3***	1p21.3

When to suspect an inborn error of bile acid synthesis - 3

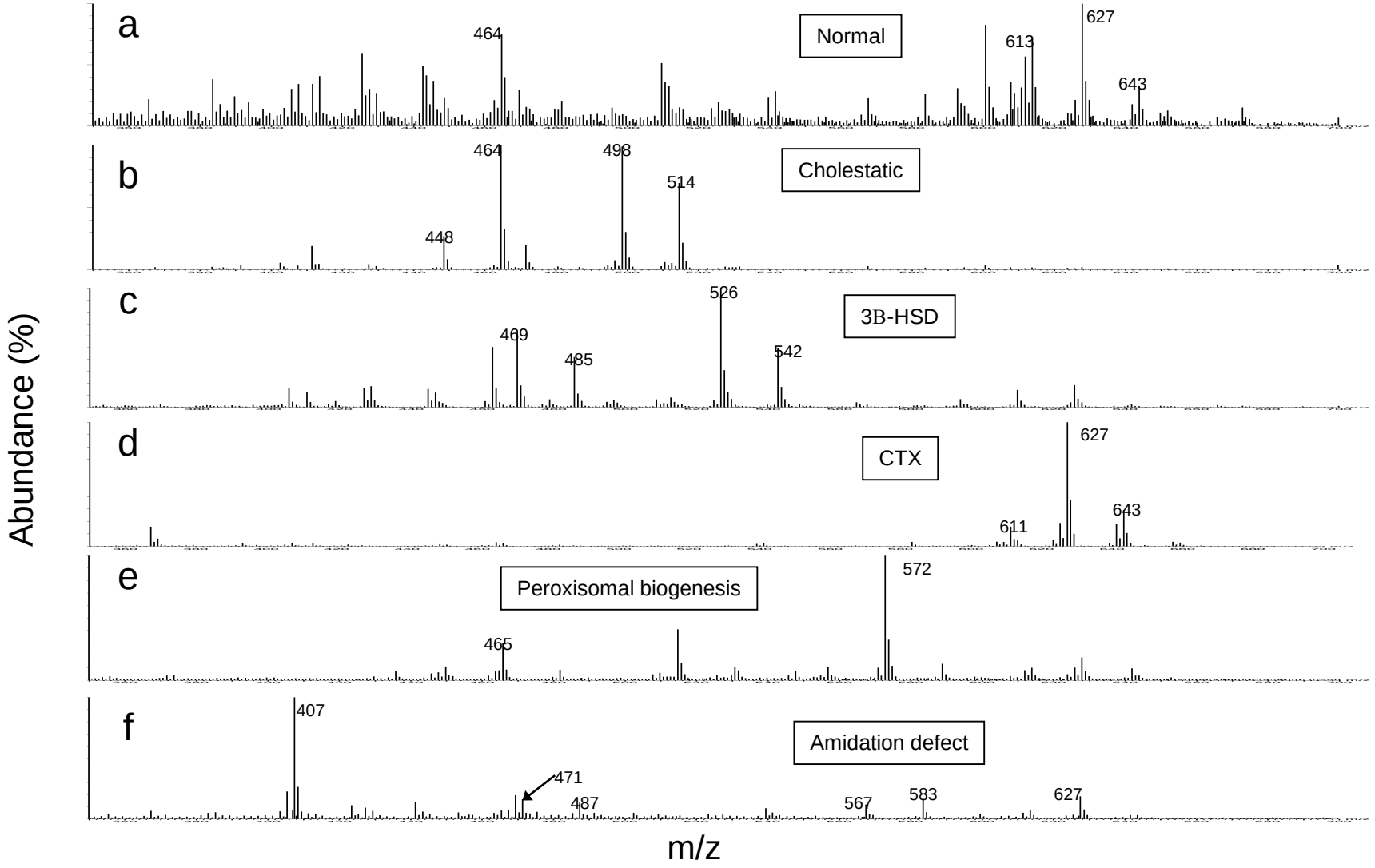
- Progressive neurological disease in an older child / adult with white matter changes on MRI
  - Cerebrotendinous xanthomatosis
  - Hereditary spastic paraplegia
  - Leukodystrophy in patients with peroxisomal disorders



CTX

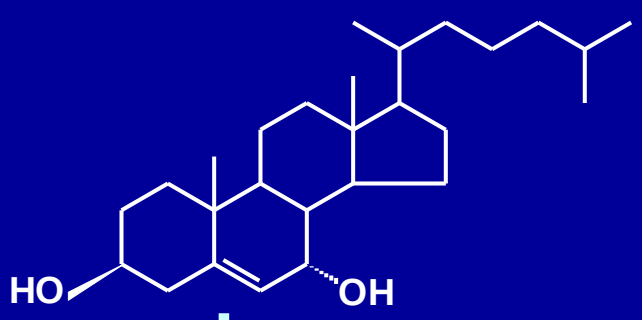


Peroxisomal biogenesis defect

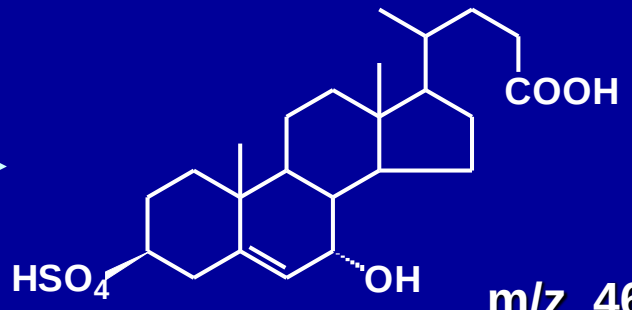


ESI-MS Profiles in Inborn Errors affecting Bile Acid Synthesis

3β-Hydroxy-Δ^5-C$_{27}$-steroid dehydrogenase deficiency



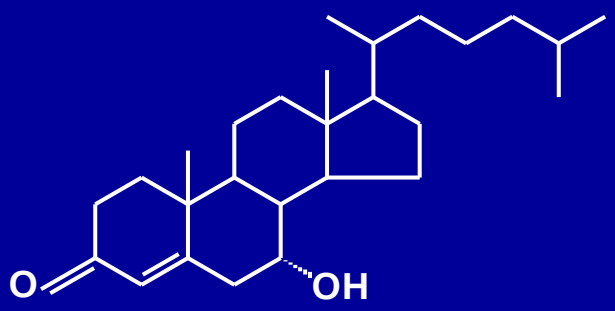
Side chain oxidation + sulphation



m/z 469

Gly conj = m/z 526

SCO + 12α OH + sulphation

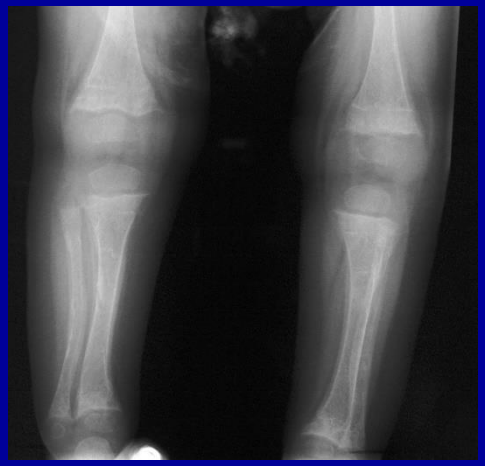


m/z 485

Gly conj = m/z 542

3β-Dehydrogenase Deficiency

- Presenting features;
  - neonatal conjugated hyperbilirubinaemia (11/18)
  - rickets (8/18)
    - including one with hypocalcaemic tetany and seizures
  - hepatomegaly (7/18)
  - pruritus (3/18)
  - steatorrhoea and failure to thrive (3/18)
- Biochemistry
  - low 25-OH vitamin D in 10/18
    - 8 also had low vitamin E levels
    - 6 also had low vitamin A level
    - 1 also had a prolonged prothrombin time responsive to vitamin K
- Liver biopsy
  - giant cell change and hepatocyte disarray in all cases
  - added features of cholestasis in the majority
  - many had bridging fibrosis.

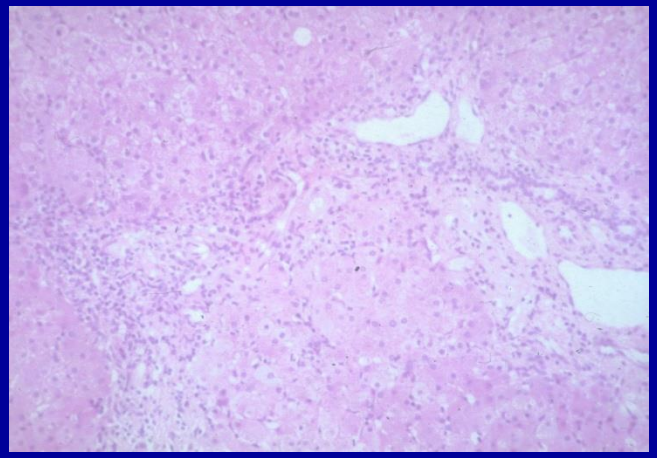


Subramaniam et al. 2010. J Ped Gastro Nutr 50:1-7

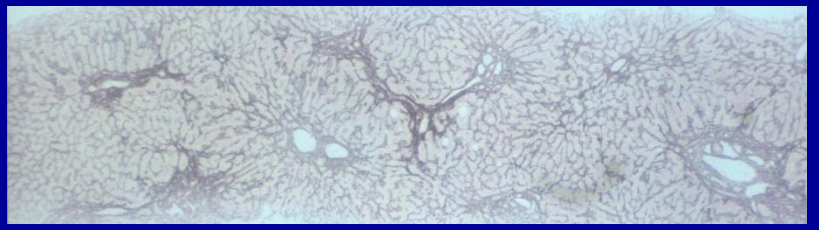
Treatment of 3β-Dehydrogenase Deficiency

- Normalisation of liver function tests
- Improvement in liver biopsy
- Correction of fat soluble vitamin malabsorption
- Improved growth and weight gain
- Achieved with
  - Chenodoexycholic acid alone
  - Chenodeoxycholic acid + cholic acid
    - Subramaniam et al. 2010
  - Cholic acid alone
    - Gonzalez et al. 2009
- Maintained long term (>15y)
  - successful pregnancies

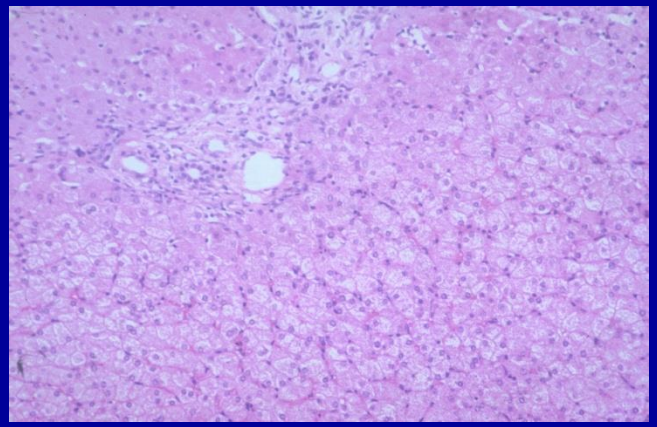
Effect of Cheno on Liver Biopsy

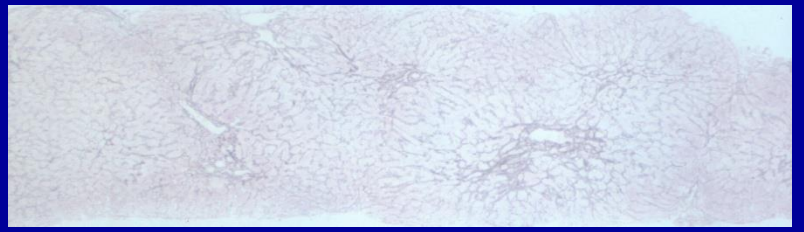


Reduced inflammatory infiltrate



Reduced reticulin (fibrosis)



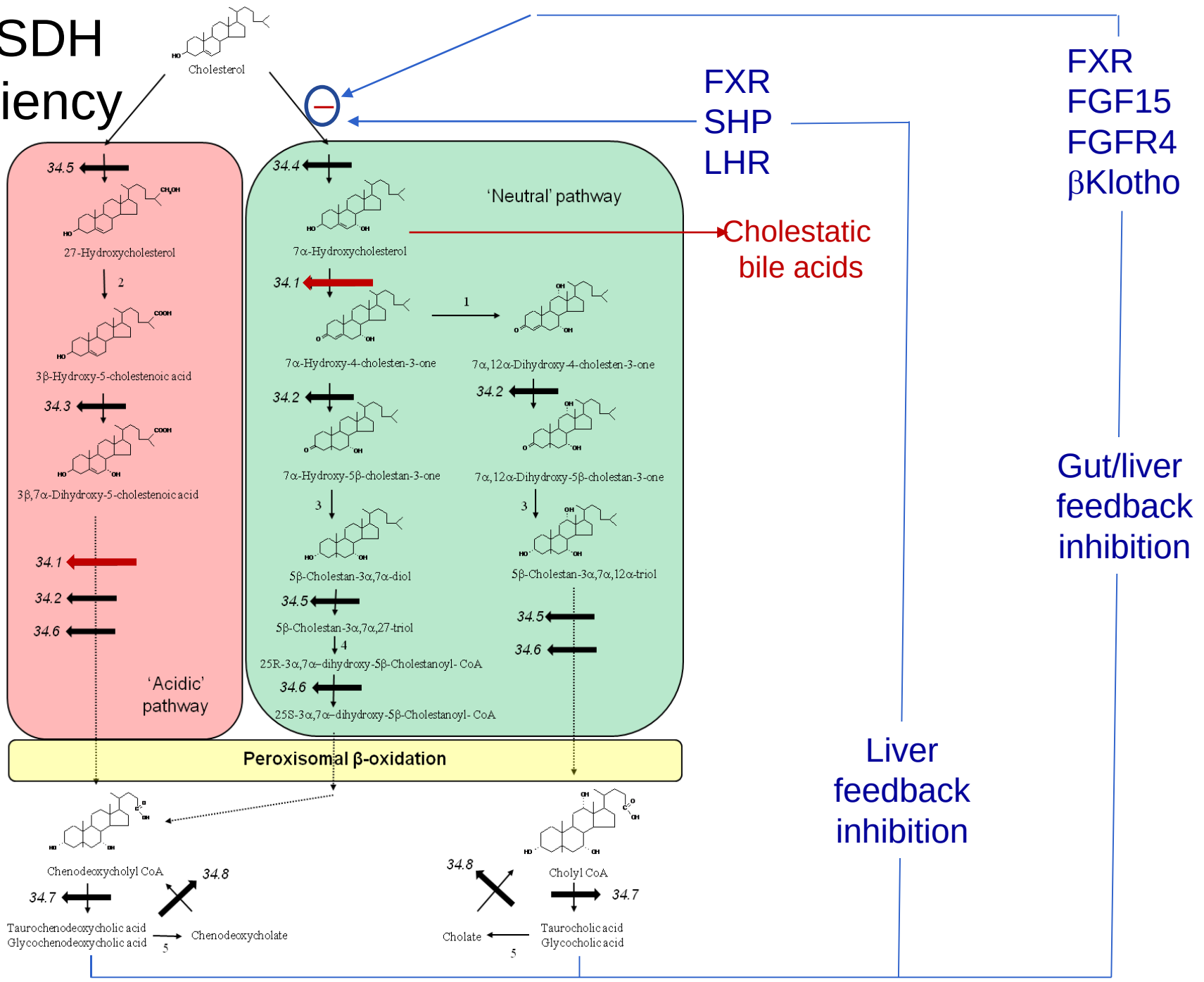


Mechanism of Cholestasis and Effect of Cheno and Cholic

- Patient synthesises little or no conjugates of cheno and cholic
  - No fuel for bile-acid dependent bile secretion
- The abnormal 3β-hydroxy-Δ5 bile acids

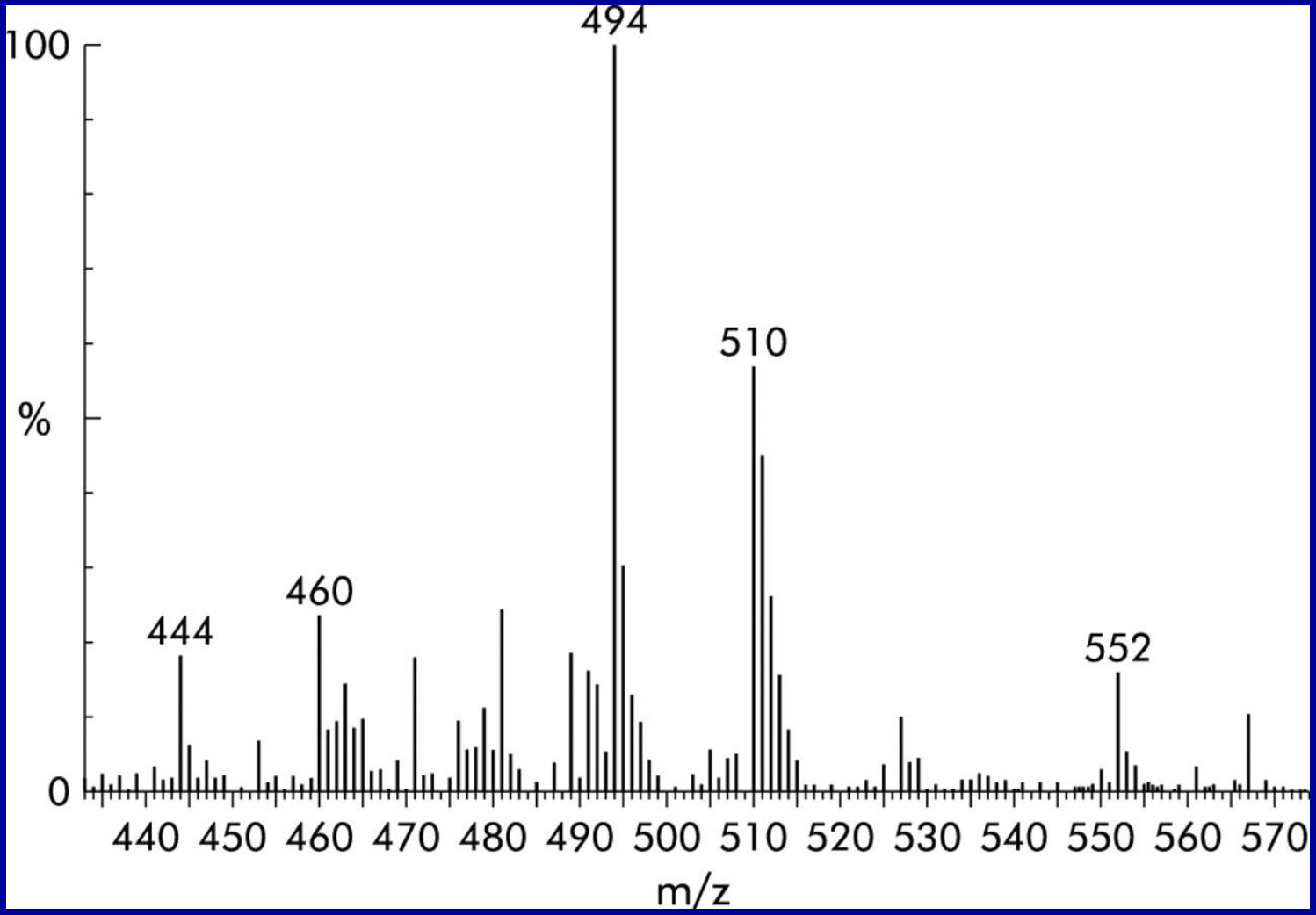
  actually inhibit BA-dependent bile secretion
- Cheno/cholic given as treatment has at least two effects
  - Fuels BA-dependent bile secretion
  - Inhibits the synthesis of abnormal bile acids
    - CDCA and CA are ligands for FXR receptor, down-regulate cholesterol 7α-hydroxylase

3-βHSDH Deficiency



5β-Reductase Deficiency

Negative ion liquid secondary ionisation mass spectrometry (LSIMS) analysis of a urine sample.

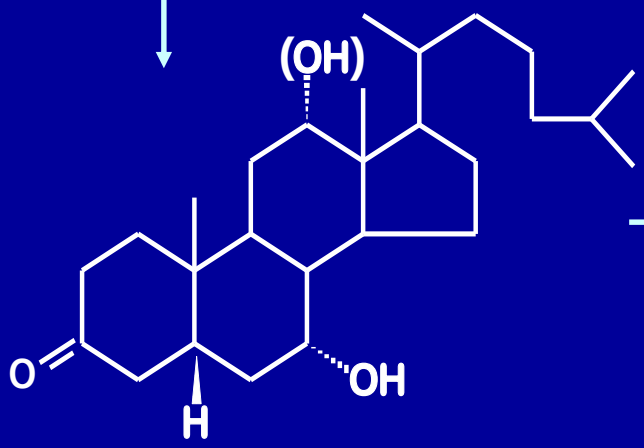


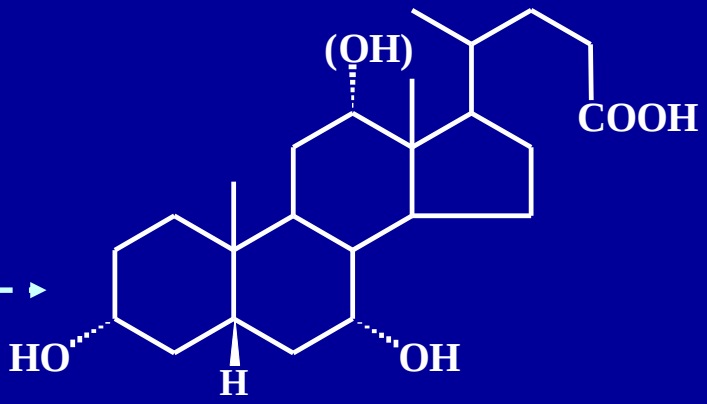
Lemonde H A et al. Gut 2003;52:1494-1499

Δ^4-3-Oxosteroid 5β-reductase deficiency

Glycine conjugates m/z 444, 460
Taurine conjugates m/z 494, 510

5β-reductase





Glycine conjugates m/z 448, 464
Taurine conjugates m/z 498, 514

Response to Treatment in 5β-Reductase Deficiency

Mutations	Start of treatment		Treatment	Outcome	Ref
	Age	INR (post vit K)			
P198L / P198L	8 mo	1.0	Chenodeoxycholic acid + cholic acid	Alive and well	Lemonde et al. 2003
P133R / R261C	8 mo	?	Cholic acid	Alive and well	Gonzalez et al. 2004
c.511delT/ c.511delT	2 mo	1.4	Chenodeoxycholic acid + cholic acid	Transplanted	Lemonde et al. 2003
L106F/ L106F	3 mo	2.0	Chenodeoxycholic acid	Died	Lemonde et al. 2003
R261C / R261C	6 mo	2.5	Cholic acid	Died	Unpublished

Secondary 5β-Reductase Deficiency

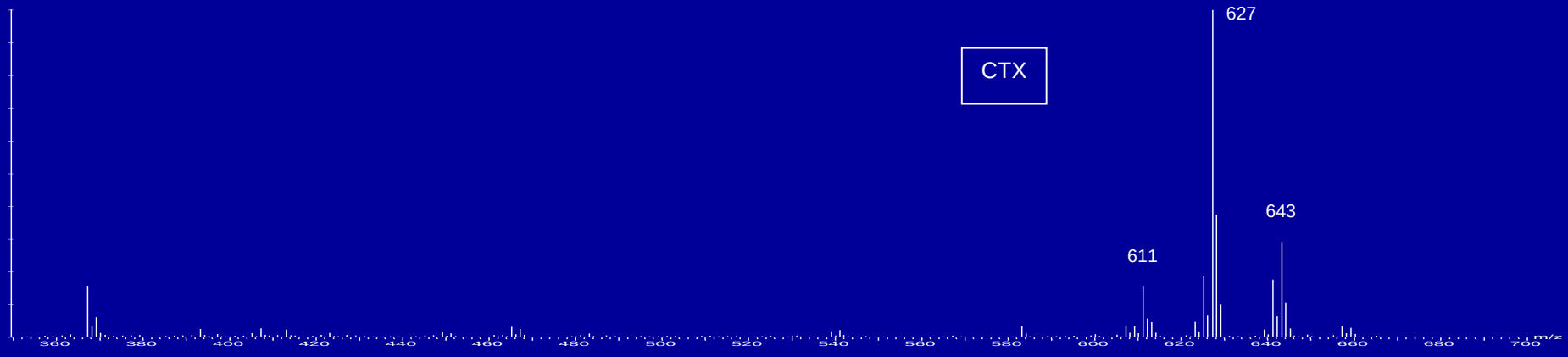
- 3-Oxo-Δ4 bile acids >80% total urine BA
- But: Diagnosis found that explains liver disease
- Improvement in UBA profile on treatment of primary disorder (sometimes demonstrable)
  - Hepatitis B
  - Tyrosinaemia
  - Neonatal haemochromatosis
  - Cystic fibrosis
  - Biliary atresia
- Excretion of 3-oxo-Δ4 bile acids usually associated with severe derangement of liver function (INR > 2.0 after vitamin K)

Cerebrotendinous xanthomatosis (CTX) Sterol 27-hydroxylase deficiency

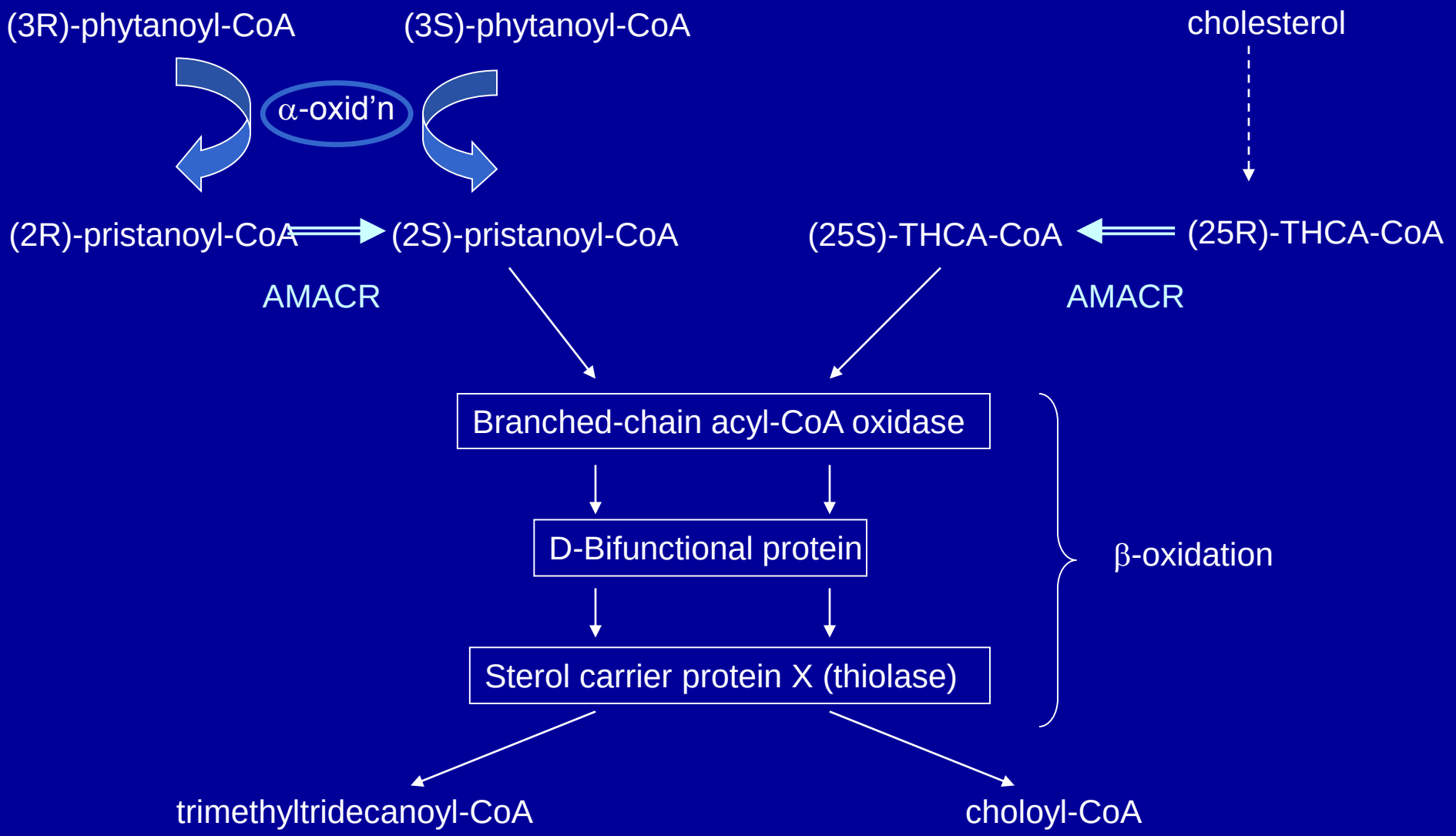
- Wide range of clinical presentations
- Early infancy
  - Cholestatic liver disease
    - Fatal
      - Von Bahr et al. 2005
    - Spontaneous resolution with subsequent presentation with neurological disease
    - Resolution with bile acid therapy
      - Clayton et al. 1995, 2002

Cerebrotendinous xanthomatosis (CTX)

- Raised plasma cholestanol, bile acid precursors and C25-tetrol
- Increased urine bile alcohol glucuronide excretion
- Liver disease improves with cheno + cholic
- Neurological disease of adults improves with cheno



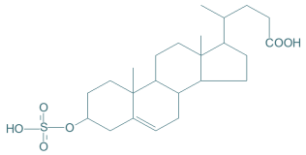
Peroxisomal β-oxidation of branch chain substrates



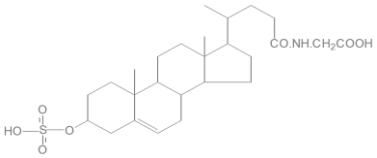
α-Methyl-Acyl-CoA Racemase Deficiency

- Neonatal presentation
  - Vitamin K deficient coagulopathy
  - Mild cholestatic jaundice with
    - raised AST
    - raised γ-GT (contrast other BA synth defects)
    - urine bile acid profile with m/z 572 (tauro-tetrahydroxy-C27 bile acids - like other peroxisomal disorders)
    - inreased C27 bile acids in plasma (25R-THCA)
    - increased plasma pristantate if cow's milk in diet and urine (older children / adults)

Oxysterol 7α-Hydroxylase Deficiency

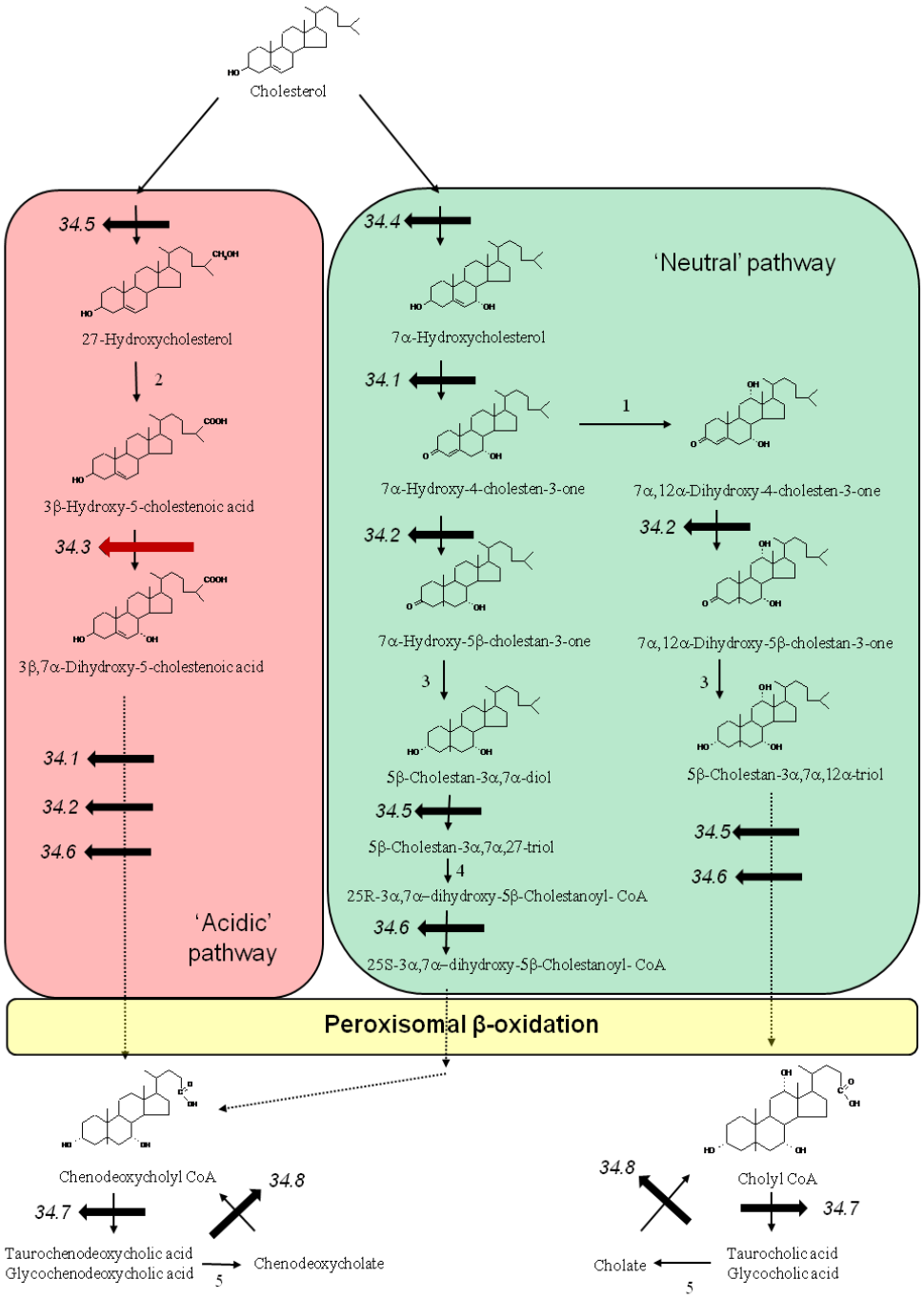


3β-Hydroxy-5-cholenoic acid 3-sulphate (m/z 453)



Glycine conjugate (m/z 510

Cholestatic bile acids



Oxysterol 7α-Hydroxylase Deficiency – 1st cases

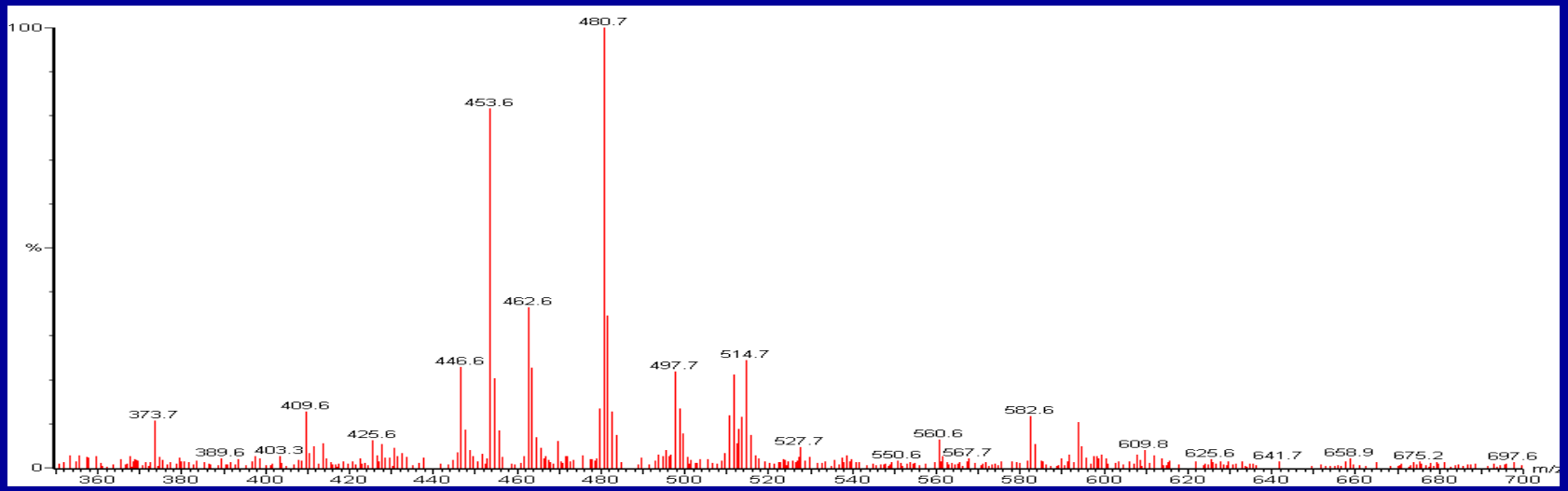
- Setchell et al.1998 and Ueki et al. 2008
  - Cholestatic jaundice in first 6 mo of life
  - Hepato(spleno)megaly
  - Elevated ALT, alk phos, normal GGT
  - Liver biopsy: Cirrhosis
  - No improvement with ursodeoxycholic acid
  - No clinical improvement with cholic acid (Setchell et al.)
- Urine MS
  - m/z 453 and 510
  - 3β-hydroxy-5-cholenoic acid 3-sulphate (453) + glycine conjugate (510)
- Plasma bile acids
  - ↑ 3β-hydroxy-5-cholenoic and -5-cholestenoic acids
- Plasma oxysterols
  - ↑ 27-hydroxycholesterol
- DNA
  - Nonsense mutations in *CYP7B1*

Oxysterol 7α-Hydroxylase Deficiency First UK Case

- Pakistani parents
- 3 weeks
  - Jaundice
    - mostly unconjugated bilirubin
- 3 - 4mo
  - irritability
  - prolonged PT
  - low albumin
  - episodes of hypoglycaemia
- Investigations
  - Bili 130 μM
  - ALT 60-70 u/L
  - GGT normal
- Response to treatment
  - Ursodeoxycholic acid
    - condition slowly worsening
  - Chenodeoxycholic acid
    - improved within 2 – 3 days
  - Previous patient failed to respond to cholic
    - Setchell et al 1998

Oxysterol 7α-Hydroxylase Deficiency

– Urine ESI –MS/MS



373 453* 480 510*

Consistent with 3β-hydroxy-5-cholenoic acid (free, sulphated, taurine conjugated, glycine conjugated and sulphated

* Major peaks in Setchell et al case

Effect of treatment with cheno on oxysterols and 3β-hydroxy-Δ5 bile acids:

Dramatic reduction (plasma and urine)

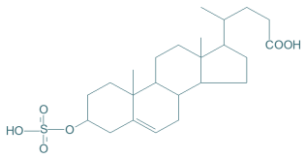
Analysis of *CYP7B1* and Outcome

- Homozygous p.Arg417Cys
  - Known *CYP7B1* mutation
  - Cause of hereditary spastic paraparesis!
    - Onset of spastic paraparesis at 10y and 12y in 2 sibs
    - Pes cavus when examined at >50y
- Follow-up (6y old)
  - On cheno (11 mg/kg/d)
  - Height and weight 50th – 75th centile
  - Normal liver examination and LFT's
  - "Trips a lot when he runs"
  - Examination:
    - Bilateral pes cavus
    - No evidence of spastic paraparesis

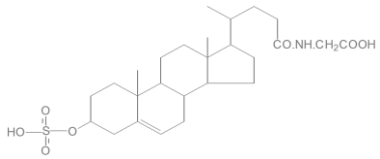
CDCA Treatment

Cheno inhibits but undesirable!

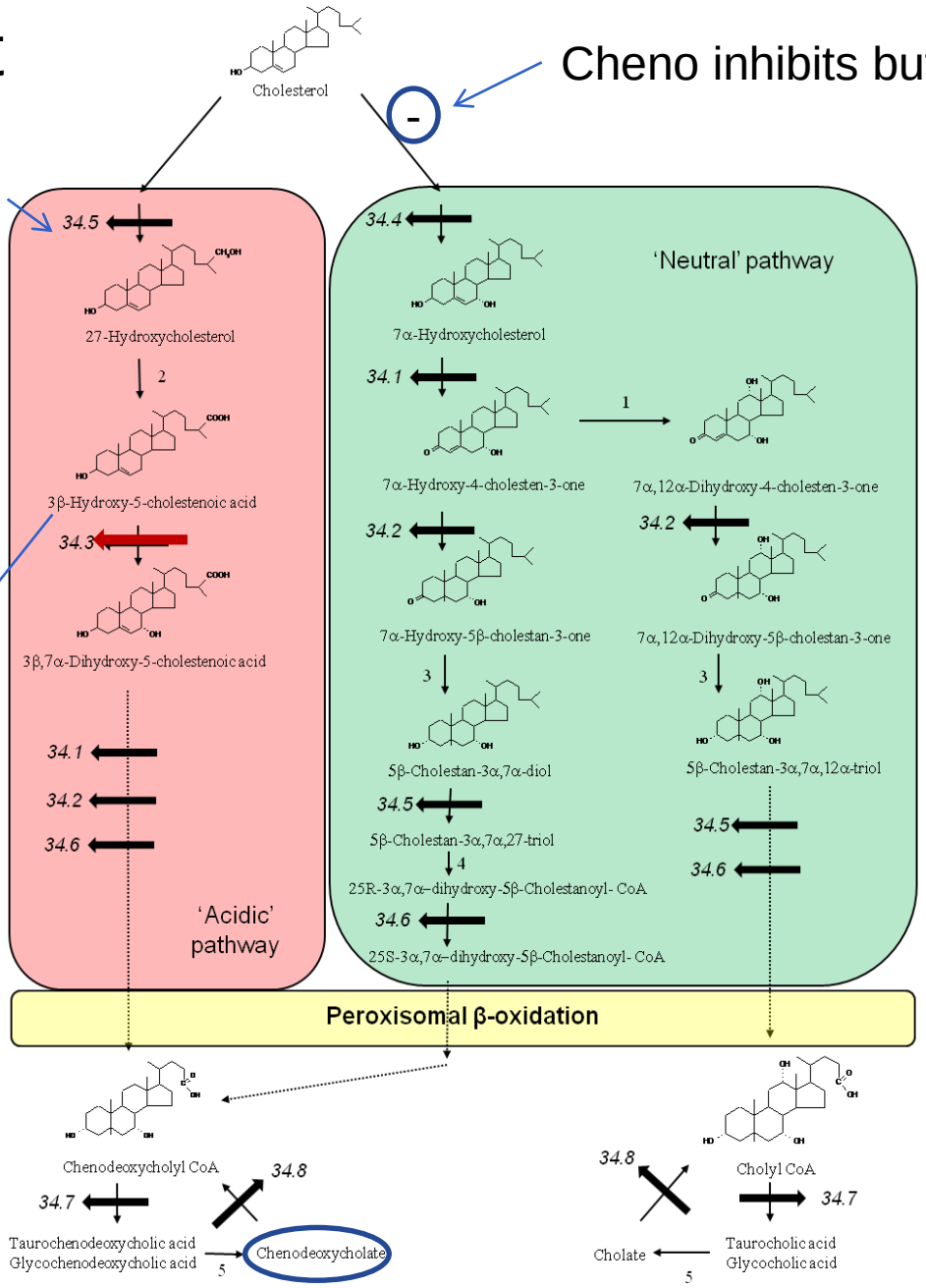
Can cheno inhibit the first step of the acidic pathway?



3β-Hydroxy-5-cholenoic acid 3-sulphate (m/z 453)



Glycine conjugate (m/z 510



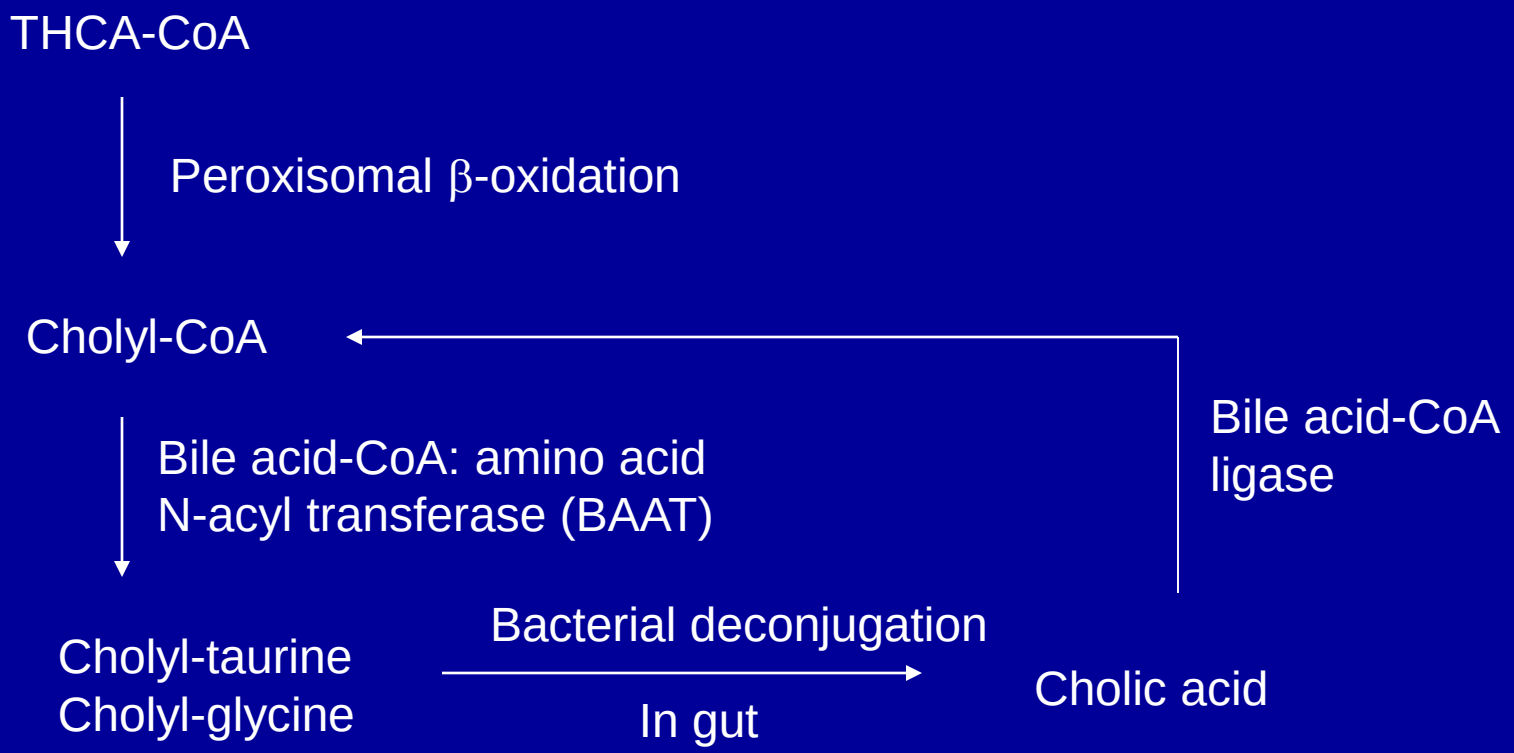
Cholestatic bile acids

Fuels bile flow

Inhibition of Cholesterol 27-Hydroxylase by Bile Acids

- Majority of studies:
  - bile acids, particularly hydrophobic bile acids such as chenodeoxycholic acid can reduce cholesterol 27-hydroxylase activity
- Species differences
- Human subjects given chenodeoxycholic acid or cholestyramine
  - modest effect on cholesterol 27-hydroxylase
- Effect of correction of marked cheno deficiency may be greater

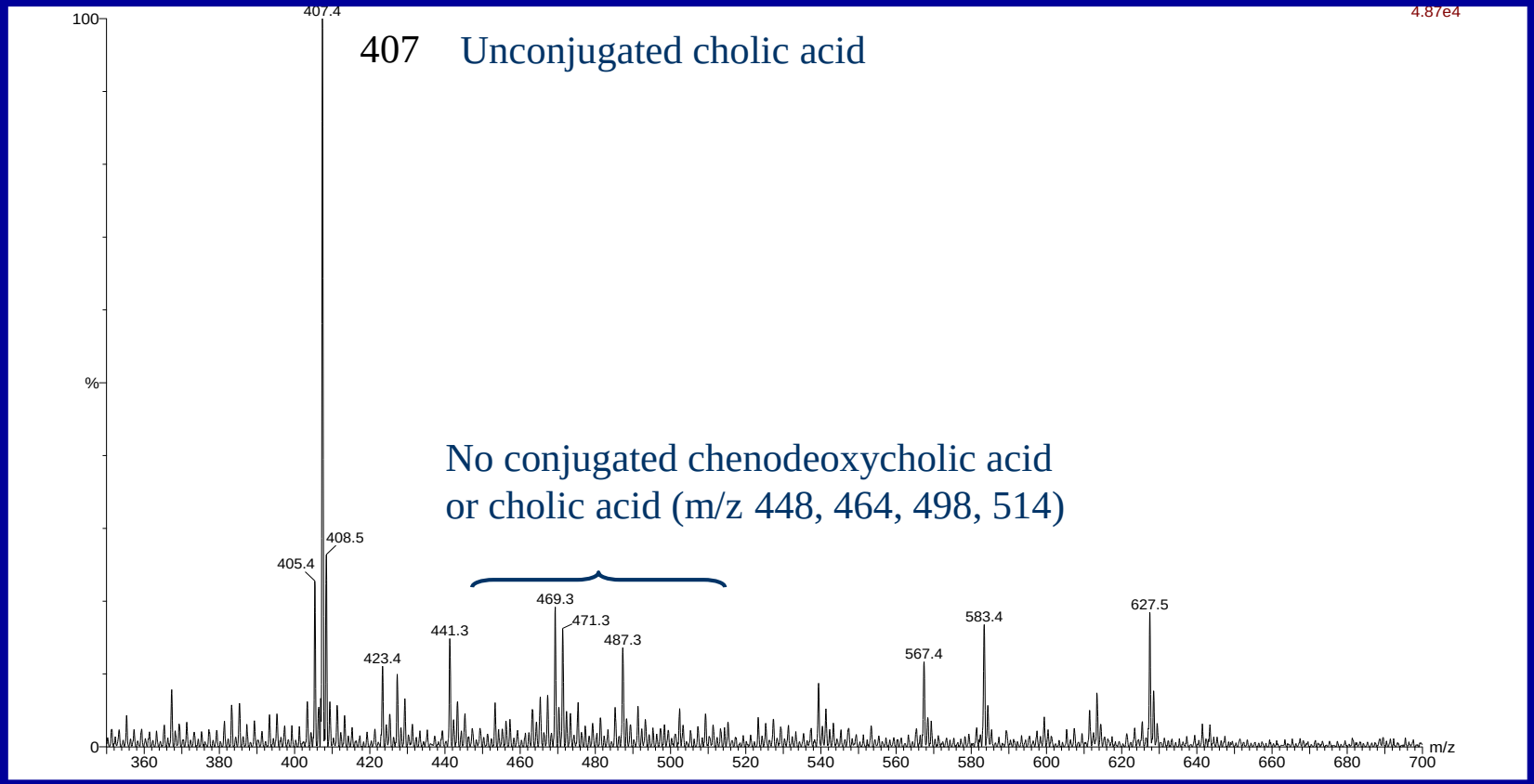
Amidation Defects



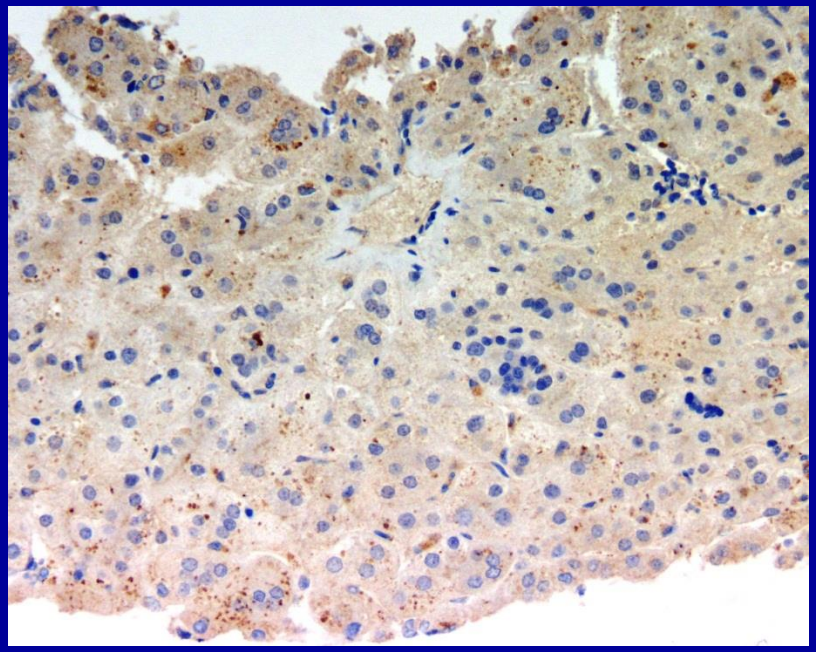
BAAT Deficiency

- First child of first cousin parents
- Mild jaundice noted at 5 weeks
- At 3 months:
  - Poor weight gain
  - Pale bulky stools
  - Rickets
- Investigation
  - Bili 80 μM (conj 40 μM)
  - ALT 90 u/L
  - Low vitamin D, E levels
  - Prolonged PT responsive to vit K
  - Urinary cholanoid profile consistent with amidation defect
  - Homozygous for mutations in BAAT gene

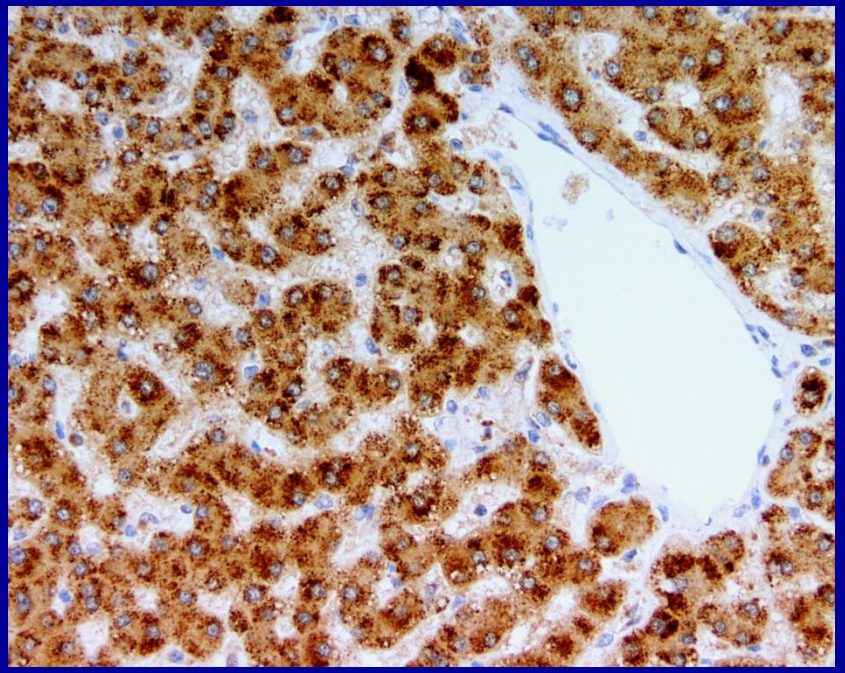
BAAT Deficiency – ESI-MS of Urinary Cholanoids



BAAT Deficiency - Immunostaining



BAAT Deficiency



Control

Bile Acid-CoA Ligase Deficiency

- First child of parents of Pakistani origin
- Born at 27 weeks gestation
- Required a prolonged period of parenteral nutrition
- Developed conjugated hyperbilirubinaemia which persisted until the age of 12 months
- Liver biopsy showed portal to portal bridging fibrosis
- Between 12 months and 18 months liver function tests returned to normal
- Now well aged 4y
- Urine bile acid profile indicating an amidation defect
- No mutations in *BAAT* gene

Bile Acid-CoA Ligase Deficiency

- H338Y/ H338Y in *SLC27A5* in two sisters
  - Both have urine bile acid profile indicating an amidation defect (and no mutations in *BAAT*)
  - First born at 27/40
    - required prolonged TPN
    - developed cholestatic liver disease with extensive fibrosis on biopsy
    - homozygous for mutations in bile salt export pump (N591S) as well as in SLC27A5
    - liver disease has not progressed
  - Second born at term
    - asymptomatic with normal LFT's and fat soluble vitamins

Disorders causing neonatal conjugated hyperbilirubinaemia / cholestasis

- Bile acid synthesis
  - including peroxisomal disorders
- Transporters of biliary constituents
- Cholesterol synthesis
- Cholesterol transport +
  - Niemann-Pick C
- Carbohydrate metabolism
  - Galactosaemia (Gal-1-PUT and UDPGal-4-epimerase)
  - Fructose intolerance
  - Transaldolase deficiency
  - Glucose-6-phosphate dehydrogenase deficiency
  - Pyruvate kinase deficiency

Disorders causing neonatal conjugated hyperbilirubinaemia / cholestasis

- Mitochondrial respiratory chain
  - mtDNA disorders
  - Nuclear encoded
    - Disorders of mt DNA replication
    - Disorders of mitochondrial mRNA translation
    - Disorders of respiratory chain complexes / assembly factors
- Disorder of lipid remodelling leading to respiratory chain dysfunction
  - MEGDHEL syndrome
- Glycosylation / glycoprotein secretion
  - congenital disorders of glycosylation
  - α1-anititrypsin deficiency
- Fatty acid oxidation

Disorders causing neonatal conjugated hyperbilirubinaemia / cholestasis

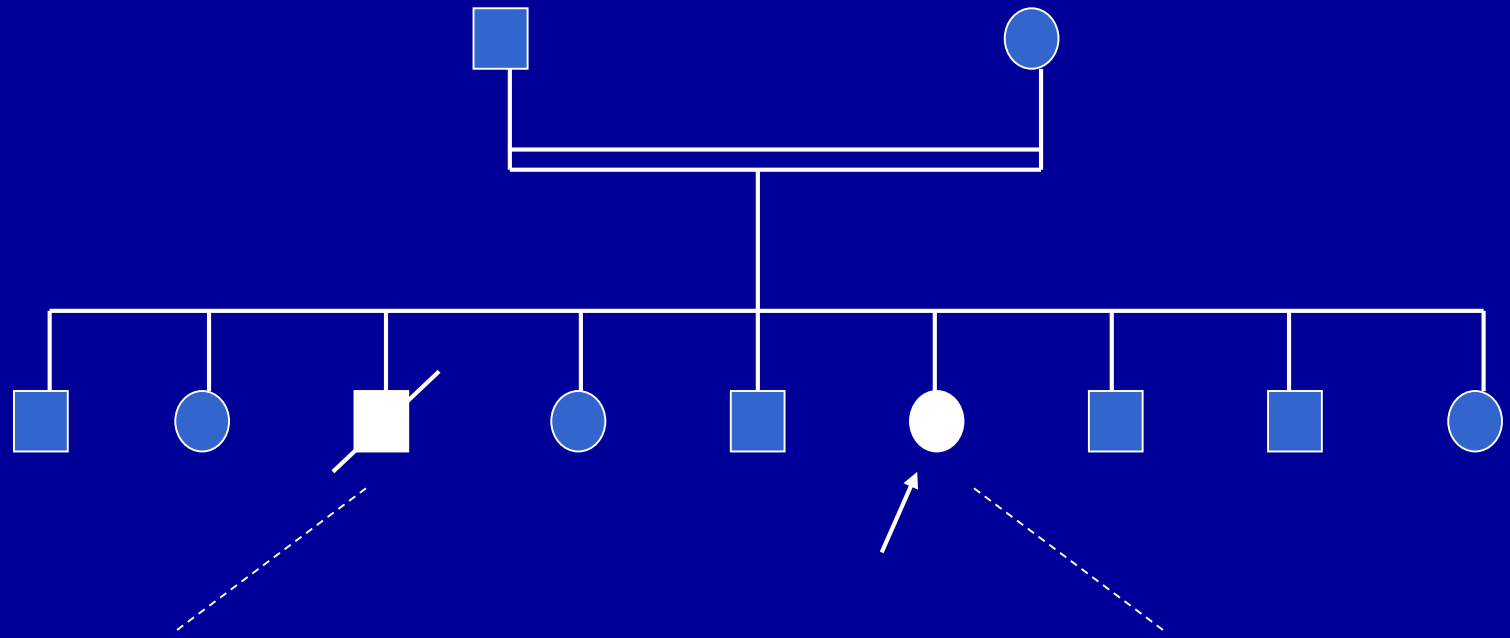
- Mitochondrial transporters
  - Citrullinaemia type II
- Amino acid catabolism
  - Tyrosinaemia
  - Disorders affecting methionine metabolism
    - Adenosine kinase deficiency
    - S-Adenosyl-homocysteine hydrolase deficiency
  - Urea cycle
    - Occasional cholestatic presentation e.g. arginase
- Lysososomal storage
- Metals
  - Neonatal haemochromatosis
    - Many are not inborn errors of metabolism
    - Some are e.g. Mitochondrial respiratory chain disorders

Investigation of Metabolic Causes of Cholestatic Liver Disease / Liver Failure in Infancy

- Specific biochemical tests based on clinical suspicion
- Biochemical screen
- DNA panel / exome / genome

- Two principal aims:
  - Detection of disorders for which specific treatment is effective
  - Detection of candidates for transplantation

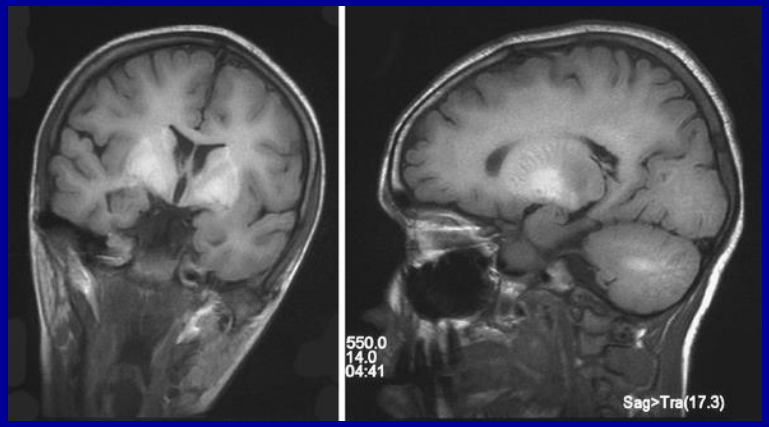
GOSH case of familial cirrhosis with dystonia

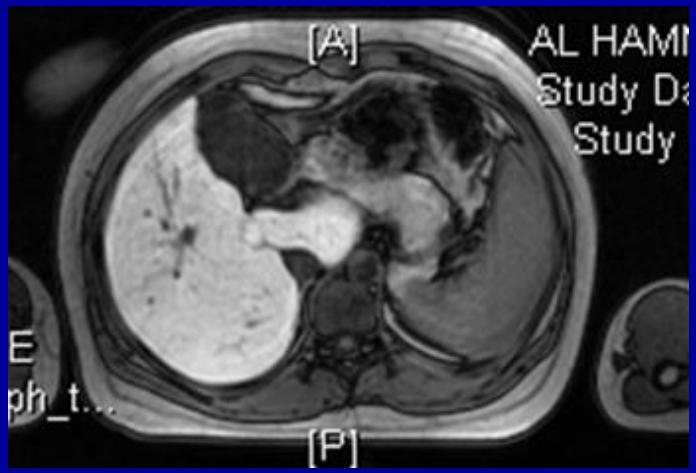


3y: Difficulty walking
18y Died of complications of cirrhosis

11y: Difficulty with walking
Examination - four limb dystonia with abnormal gait

Failure of Biliary Excretion of Mn^{2+} - *SLC30A10* mutations



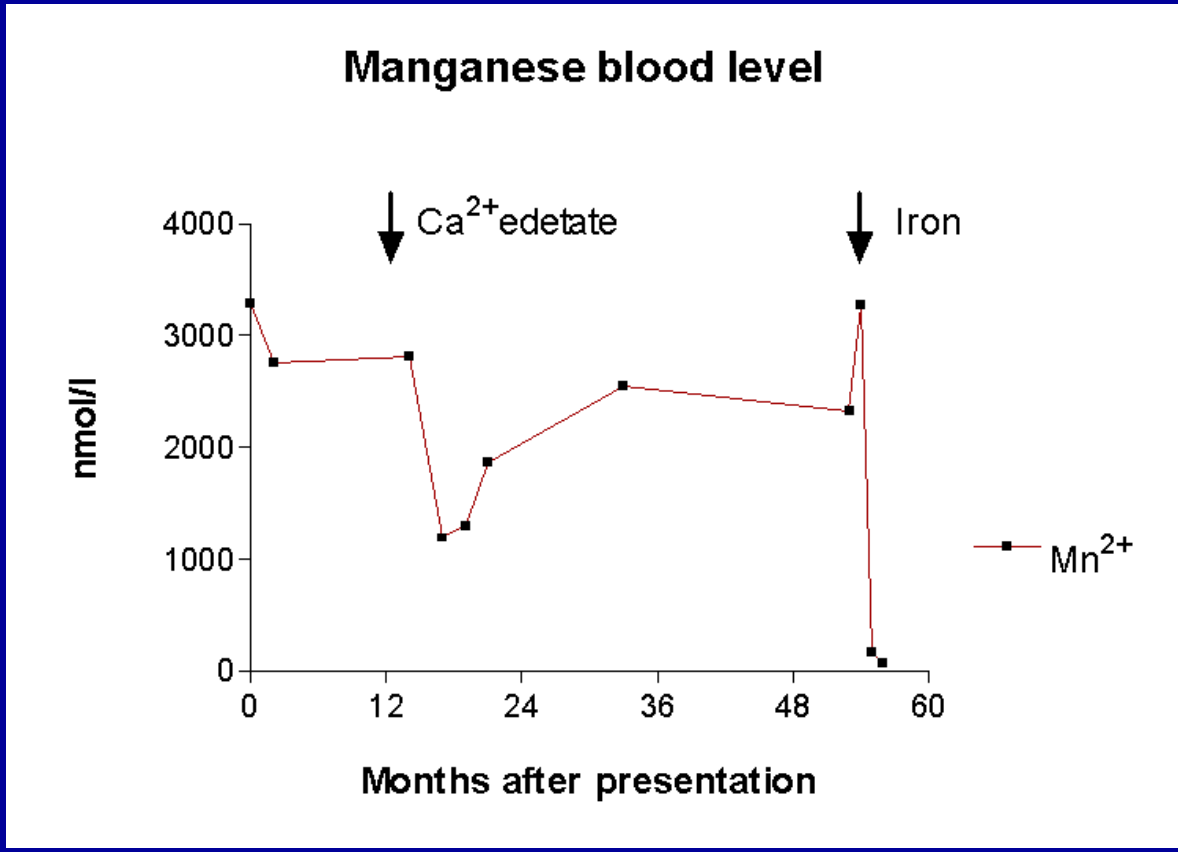


- Chronic liver disease
  - deaths from complications
- Parkinsonism / dystonia
- Polycythaemia
- High blood Mn^{2+}

- T1-weighted MRI
- High signal from:
  - Several areas of brain
    - esp. basal ganglia
  - Liver

Response to Treatment

- After 4y treatment:
  - handwriting, tremor and stiffness much better
  - liver manganese normal (1µg/g)
  - liver histology: less inflammation and fibrosis
  - no polycythaemia



Metabolic Liver Disease

- Clinical signs can assist diagnosis
- Some biochemical tests have the ability to detect treatable disorders quickly
  - BA synthesis defects
- In an infant with liver failure there is a need to diagnose the treatable and the transplantable
  - Gene panel / exome / genome may be the quickest way to cover the whole range of disorders
    - Good for mitochondrial hepatopathy
- In older children with liver disease, “new” treatable disorders can still be found