Sheffield Children's NHS
NHS Foundation Trust

Disorders of Bile Acid Metabolism and their Diagnosis

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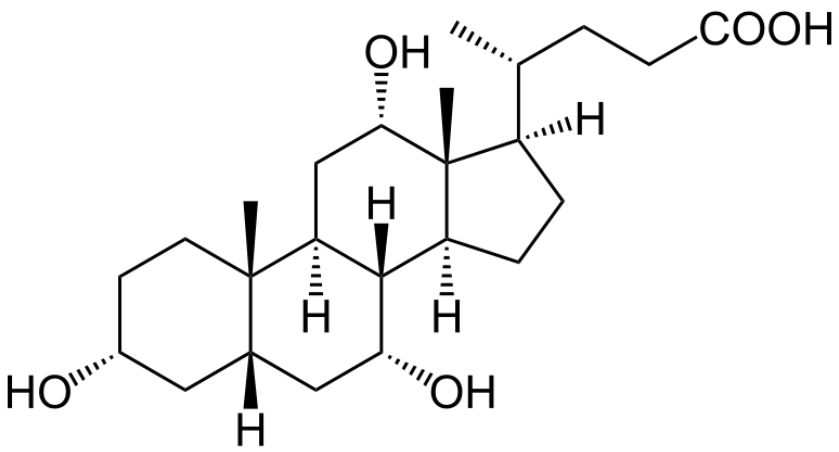
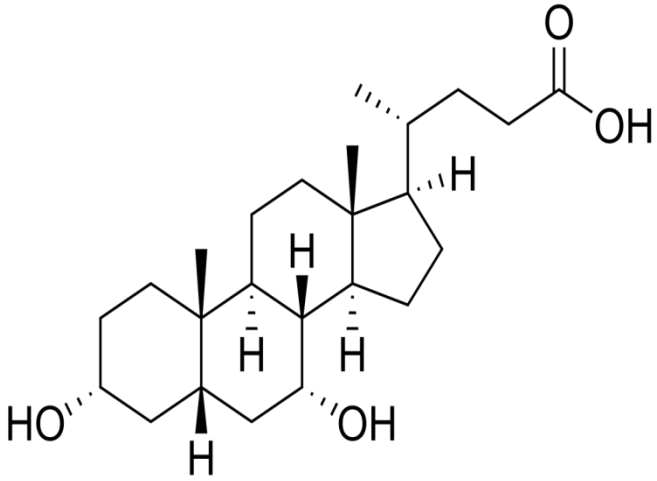
What is a Bile Acid?

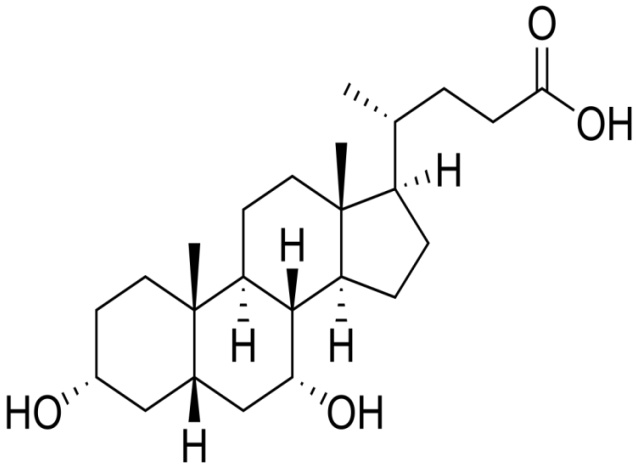


Cholic Acid
(3α, 7α, 12α trihydroxycholanoate)

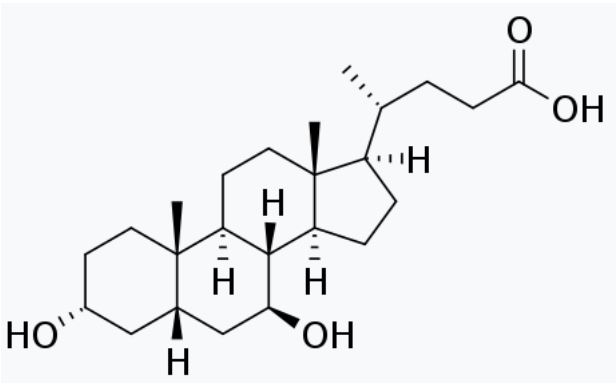


Chenodeoxycholic Acid
(3α, 7α dihydroxycholanoate)

What is Ursodeoxycholic acid?



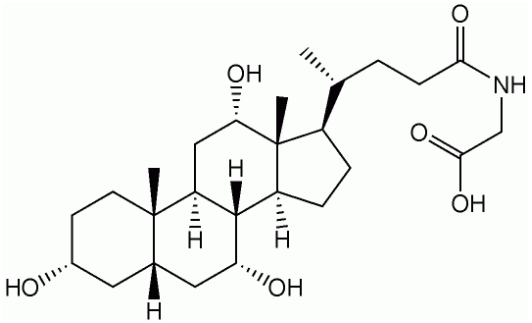
Chenodeoxycholic Acid
(3α, 7α -dihydroxycholanoate)



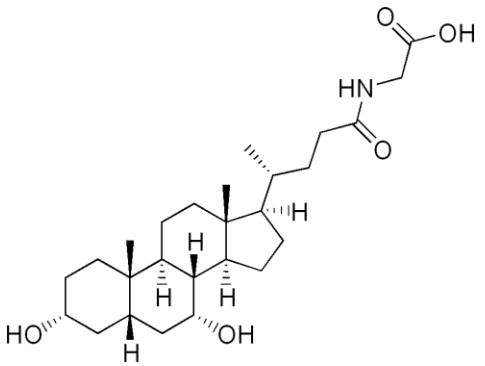
Ursodeoxycholic Acid
(3α, 7β -dihydroxycholanoate)

What is a Bile Salt?

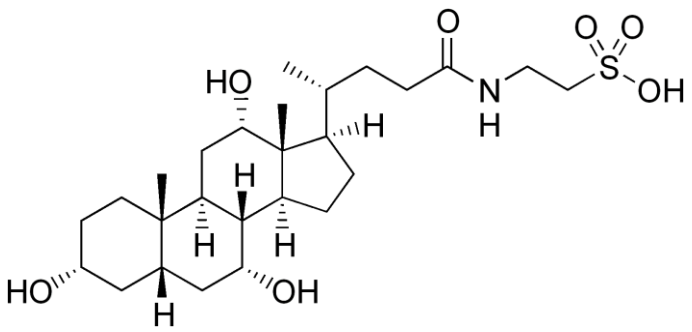
Glyco or Tauro conjugate of cholic or chenodeoxycholic acid



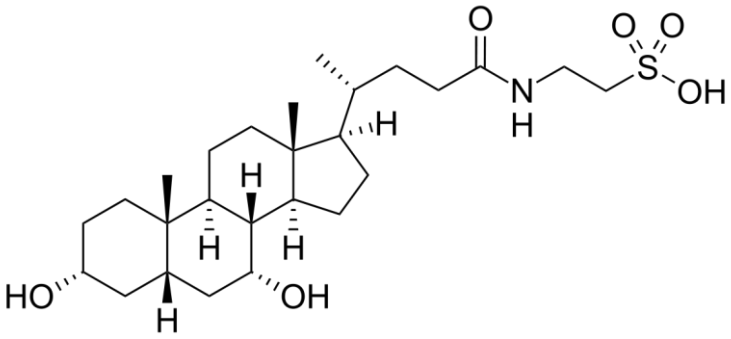
Glycocholic acid



Glycochenodeoxycholic acid

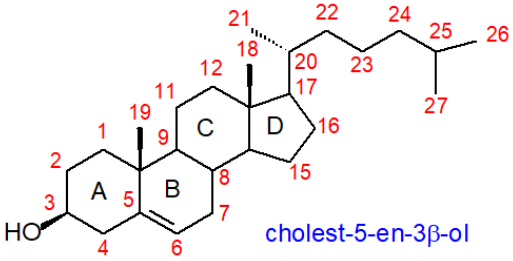


Taurocholic acid



Taurochenodeoxycholic acid

How do we make Bile Acids / Salts?



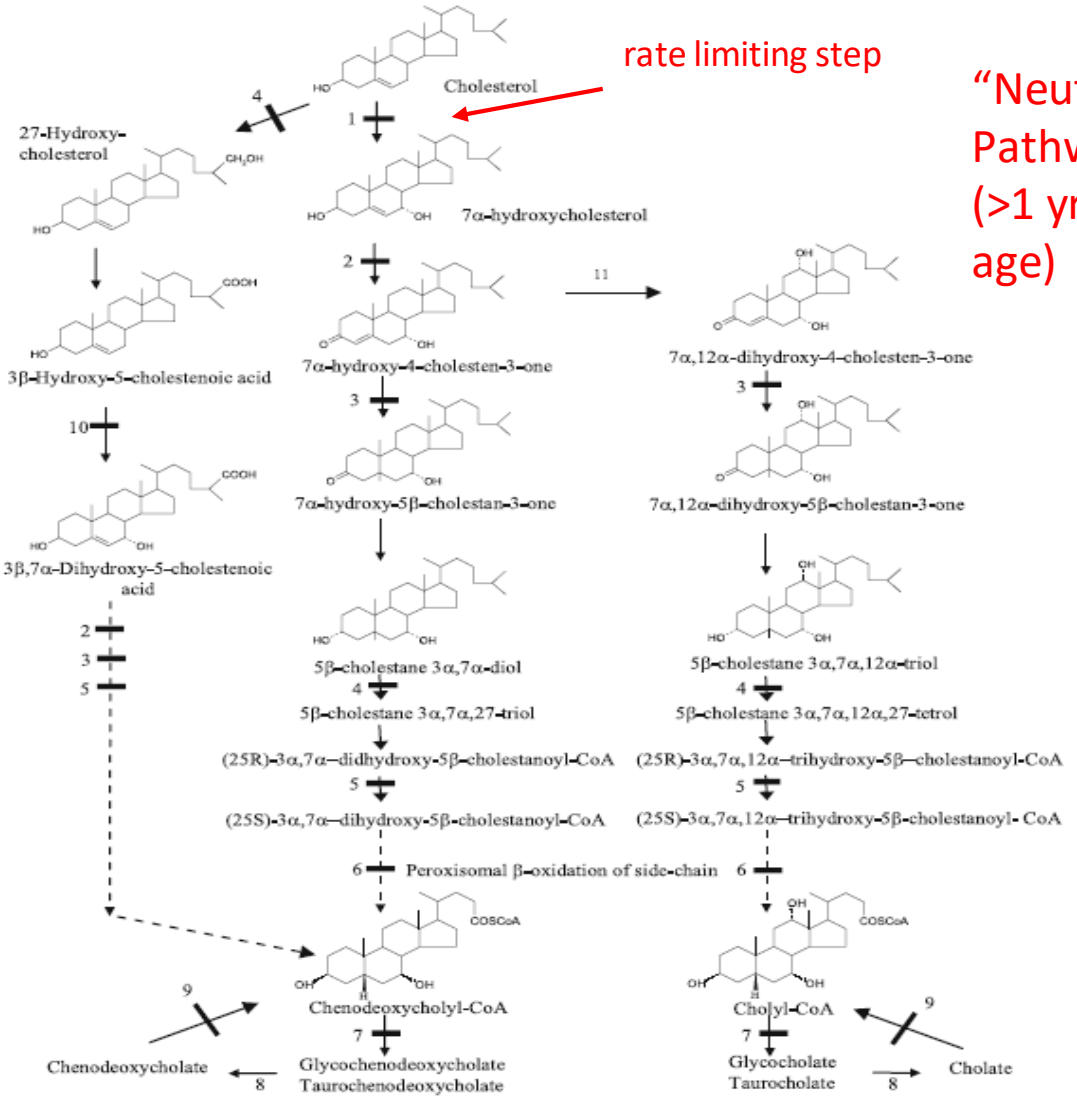
Cholesterol

Bile Acids are made predominantly in the liver using cholesterol as the precursor.
Bile Salts provide negative feedback inhibiting the pathway at cholesterol 7alpha hydroxylase (1)

"Acidic Pathway" (<1 yr of age)

rate limiting step

"Neutral Pathway" (>1 yr of age)



What are the disorders of Bile Acid /Salt Metabolism?

- Bile Acid synthesis disorders – single gene defects in the synthesis of cholic and chenodeoxycholic acid from cholesterol

  - Oxysterol 7 alpha hydroxylase deficiency (CYP7B1)
  - 3-beta-hydroxy-delta^5-C27-steroid oxidoreductase deficiency (HSD3B7)
  - Delta^4-3-oxosteroid 5 beta reductase deficiency (AKR1D1)

- Typically present with prolonged neonatal jaundice, steatorrhea, treatment resistant diarrhoea, fat soluble vitamin deficiencies (rickets, haemorrhage), pruritus, - progressing to cirrhosis / liver failure. Of note patients have a normal gamma GT

- Oxysterol 7 alpha hydroxylase deficiency may also present with hereditary spastic paraplegia (onset from childhood to adulthood)

What are the disorders of Bile Acid / Salt Metabolism?

- Cerebrotendinous Xanthomatosis / Sterol 27-hydroxylase deficiency (CYP27A1)
- Technically a bile acid synthesis disorder but usually put in category of own
- Presents with; self-limiting neonatal hepatitis (occasionally persists and progresses to cholestatic liver disease), treatment resistant diarrhoea, cataract, -and from 2nd decade onwards-xanthomas, atherosclerosis, osteoporosis, progressive ataxia and eventually dementia

What are the disorders of Bile Acid /Salt Metabolism?

- Bile Acid Amidation (conjugation) defects –failure to conjugate bile acids to taurine or glycine to form the active Bile Salts
  - Bile Acid-CoA:aminoacid N-acyltransferase deficiency (BAAT)
  - Bile Acid-CoA ligase deficiency (SLC27A5)

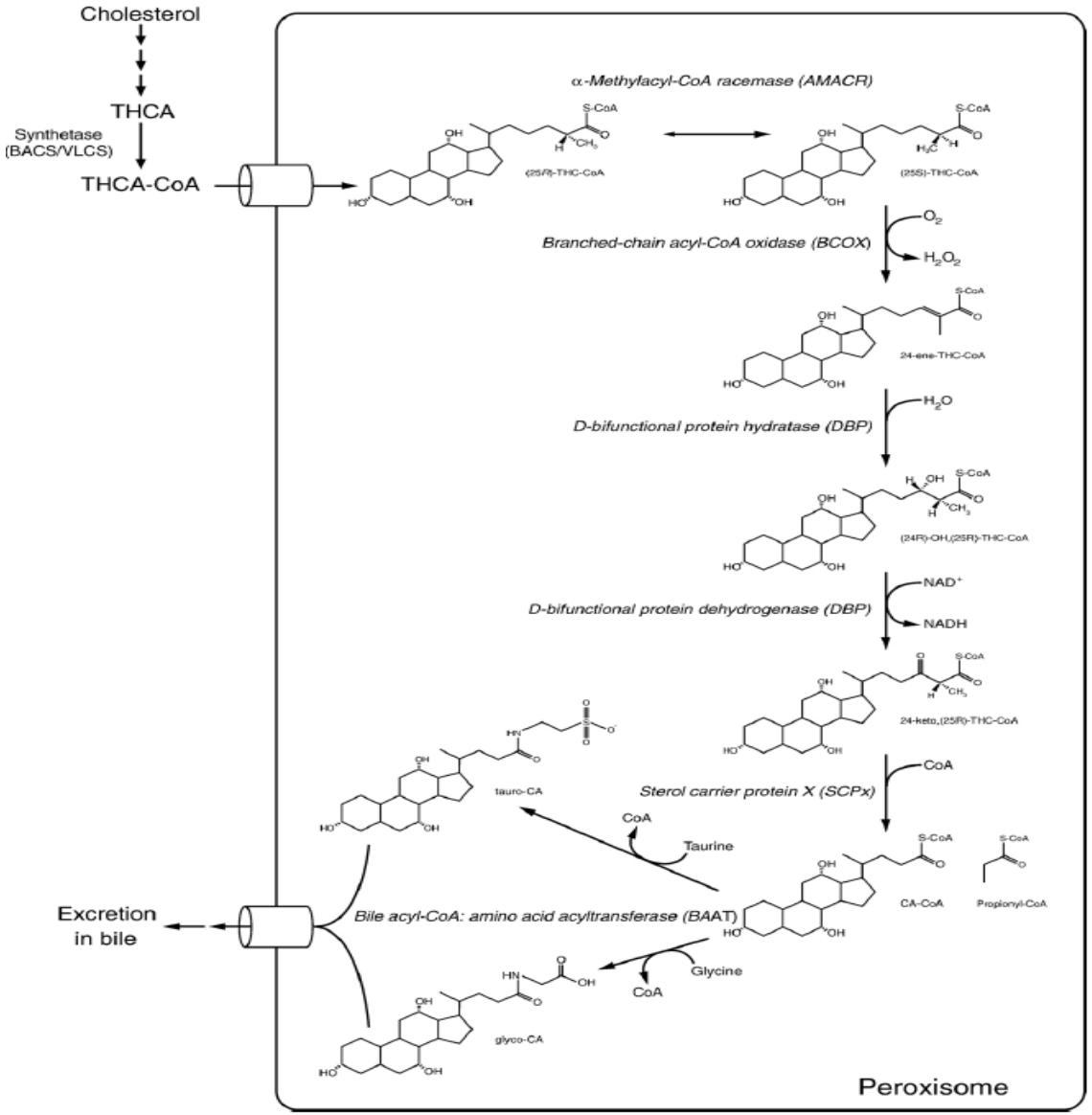
- N-acyltransferase deficiency typically presents with failure to thrive, cholestatic liver disease, fat soluble vitamin deficiency
- Ligase deficiency is of uncertain clinical significance

What are the disorders of Bile Acid / Salt Metabolism?

- Peroxisomal Disorders – several stages of bile acid synthesis occur in the peroxisome so peroxisomal biogenesis disorders and some single enzyme defects impact bile acid synthesis
  - Peroxisomal Biogenesis disorders (PEX genes)
  - D-bifunctional protein deficiency (HSD17B4)
  - 2-Methylacyl-CoA racemase deficiency (AMACR)
  - Sterol carrier protein-2 (SCP2)

  - All have a specific pattern of metabolite(s), although the pattern may not be observable in "milder" patients (good specificity, less good sensitivity)

Peroxisomal Bile Acid Metabolism



Differential Diagnosis of Peroxisomal Disorders Using Bile Acids

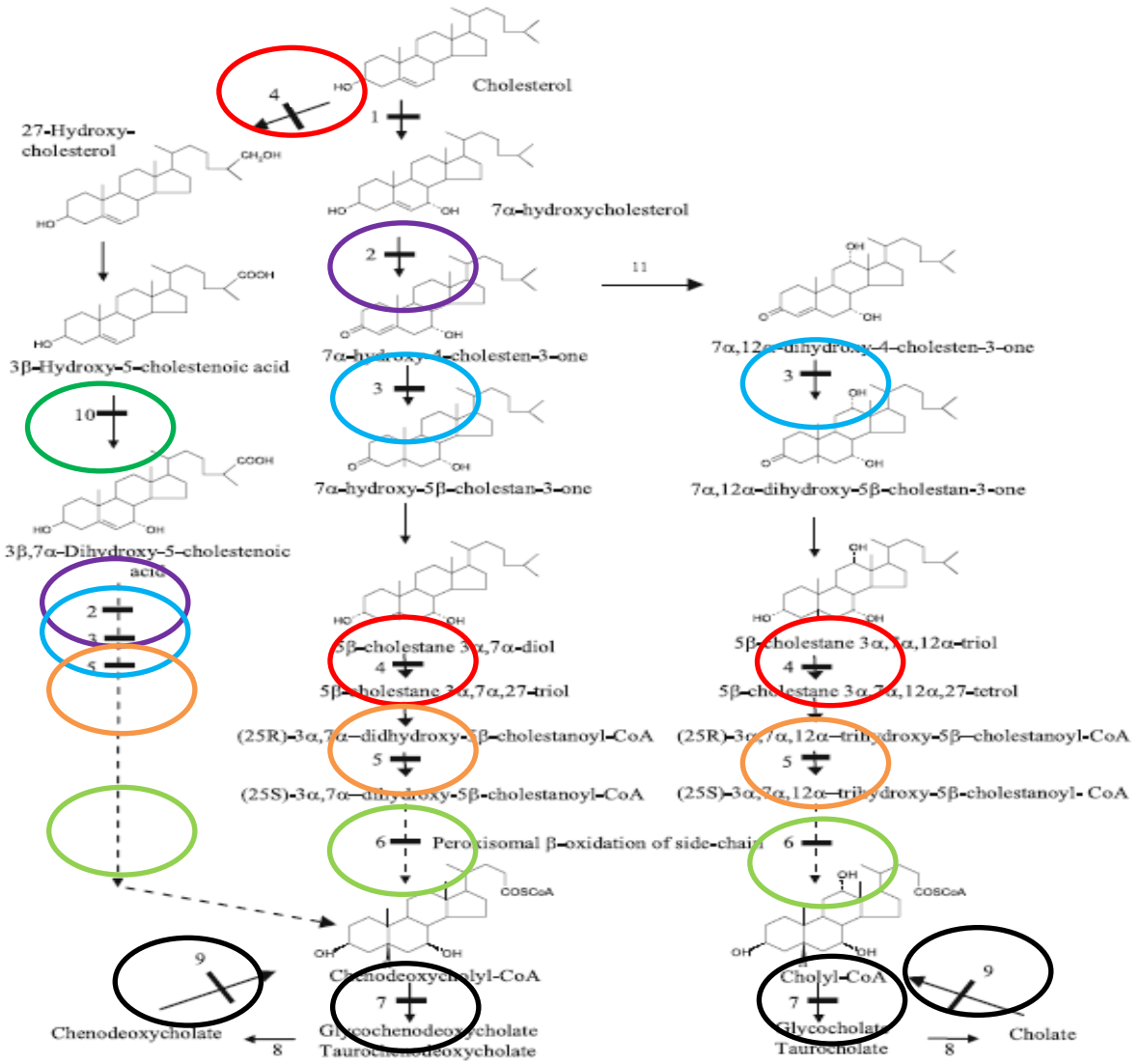
Disorder	VLCFA	Phytanate	Pristanate	Bile Acid metabolites
Peroxisomal Biogenesis Disorders	↑↑	↑*	↑*	Taurotrihydroxycholestanoate (m/z 556) + Taurotetrahydroxycholestanoate (m/z 572)
D-bifunctional protein deficiency	↑(↑)	↑*	↑*	Taurotrihydroxycholestenoate (m/z 554) + Taurotetrahydroxycholestenoate (m/z 570)
Methylacyl-CoA racemase def	N	N	↑	Taurotrihydroxycholestanoate (m/z 556)
Sterol Carrier protein X	N or sl ↑	N	↑	Variety of urine bile alcohol glucuronides (some overlap with CTX)

* Dependent on dietary intake, often not elevated in neonates

What are not disorders of Bile Acid /Salt Metabolism?

- Progressive Familial Intrahepatic Cholestasis (PFIC) disorders
  - Several different disorders of bile salt TRANSPORT
  - Present with jaundice, pruritus, hepatosplenomegaly, cirrhosis
  - Present with generalised increase in the 4 primary bile salts (cholestasis) but no distinguishing metabolites present
  - Therefore cannot differentiate between the PFIC disorders and cholestasis of other cause

How do we make Bile Salts?



CTX

Oxysterol 7alpha hydroxylase deficiency

3beta-hydroxy-delta5-C27-oxidoreductase deficiency

Delta4-3-oxosteroid 5 beta- reductase deficiency

Alpha-methyl-acyl-CoA racemase deficiency

Peroxisomal Biogenesis disorders and D-bifunctional protein deficiency

Bile Amidation defects

Analysis of Bile Acids /Salts

- ESI-MS/MS in negative ion mode ($M\text{-}H^-$ ions)
- Direct injection (no column)
- Scan across range 300-700 m/z
- 3 x “Parents of” scans
  - Parents of 74 = Glyco conjugates
  - Parents of 80 = Tauro conjugates
  - Parents of 97 = Sulpho conjugates
- Use D4-Taurotrihydroxycholanoate as internal standard for all species
- Calibration curves using glyco- and tauro- di and trihydroxycholanoate (4 primary bile salts)

Sample Preparation (Urine and Plasma)

- 50µl sample + 50µl of D4- Taurocholic acid internal standard (in acetonitrile) + 250µl acetonitrile
- Vortex mixed, centrifuged and supernatant transferred to multiwell plate
- Running buffer 1:1 acetonitrile / water

Report Format

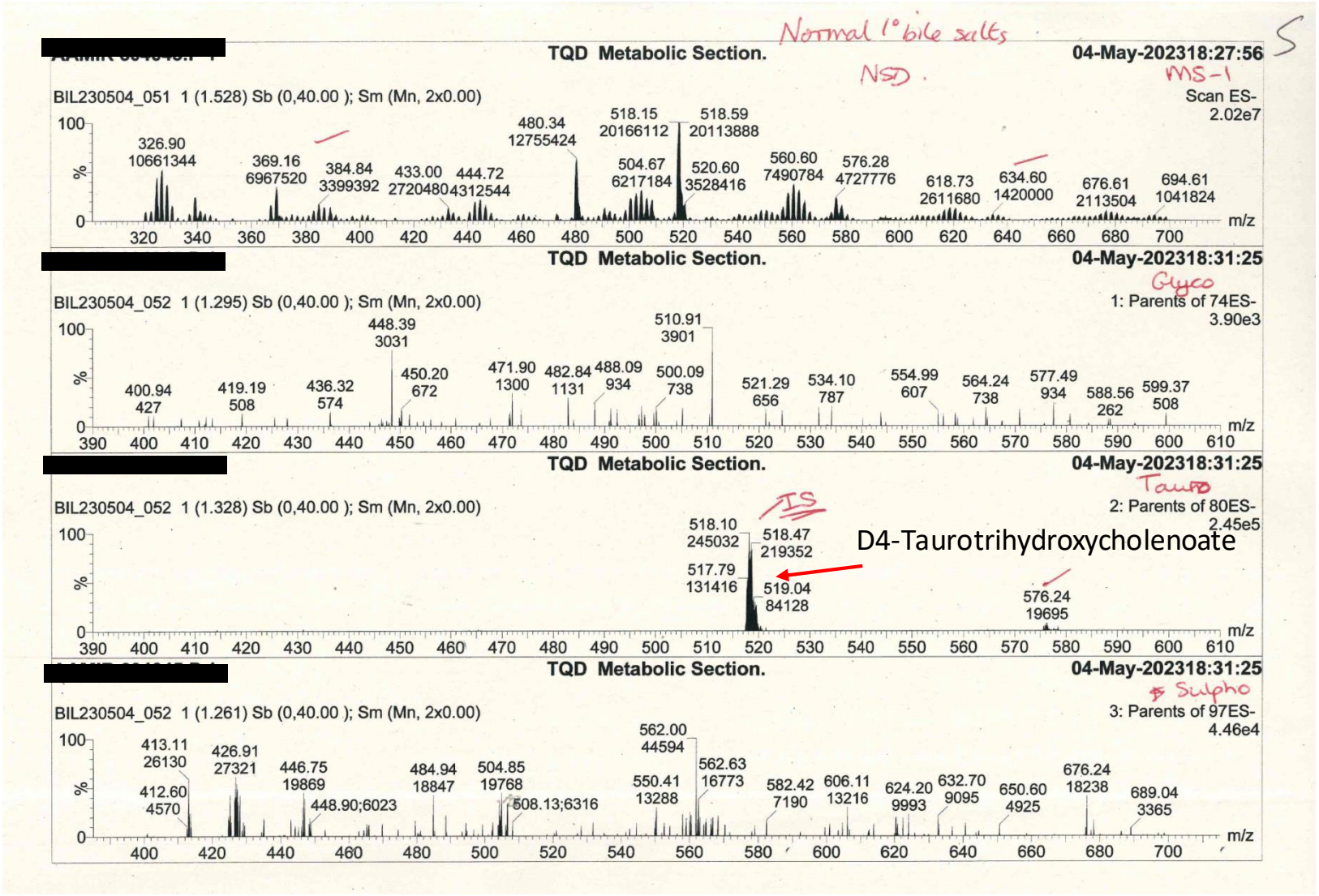
- Report Quantitative Results for 4 Primary bile salts (with reference ranges) + qualitative report on the presence or absence of any abnormal bile acid metabolites

Bile Acid Analysis by Electrospray - Mass Spectrometry

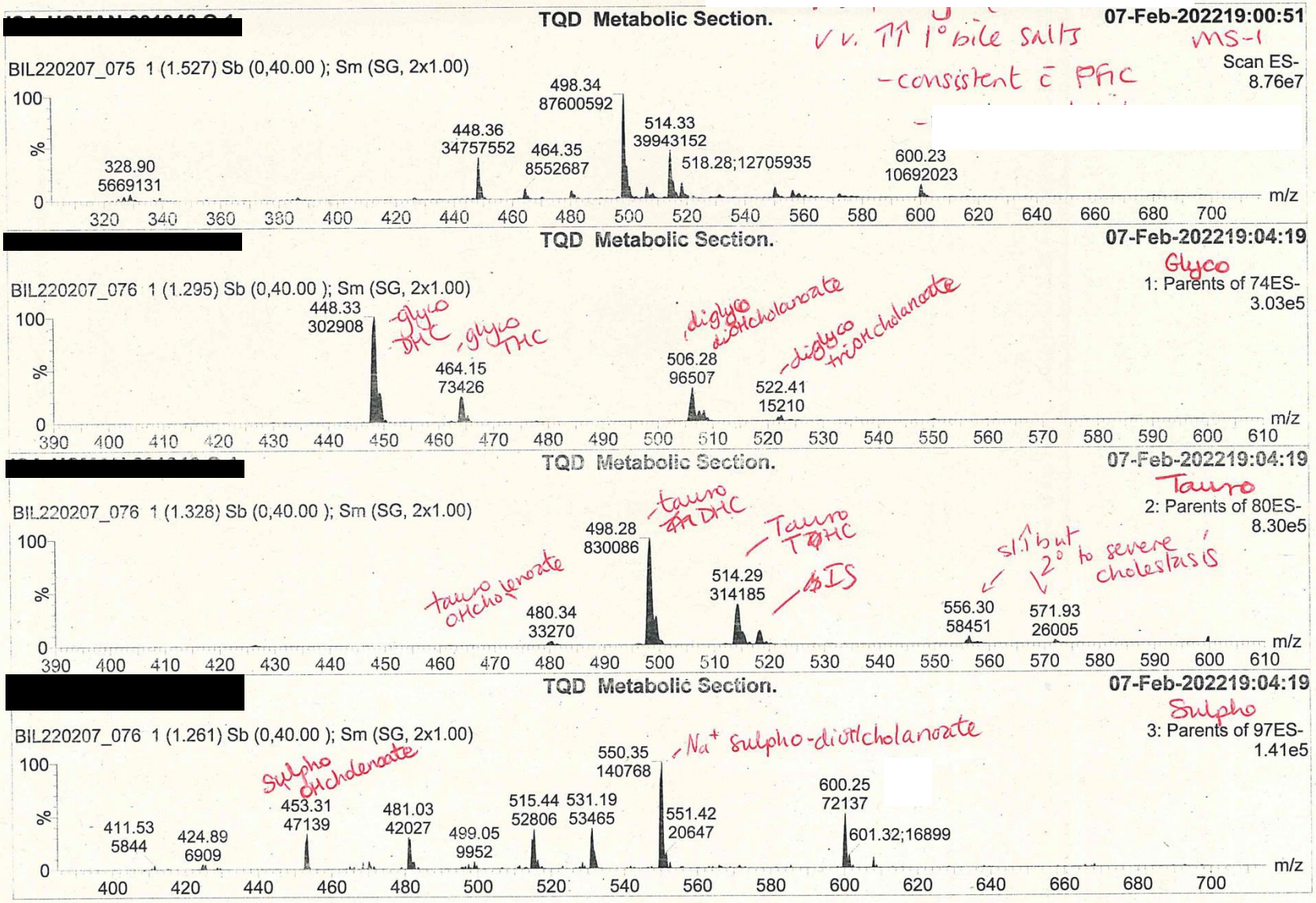
Glycodihydroxycholanoate	2	µmol/L	<6
Glycotrihydroxycholanoate	1	µmol/L	<2
Taurodihydroxycholanoate	1	µmol/L	<2
Taurotrihydroxycholanoate	<1	µmol/L	<2

Normal concentration of primary bile salts.
No abnormal conjugates of bile acid intermediates were detected, which tends to exclude disorders of bile acid biosynthesis.

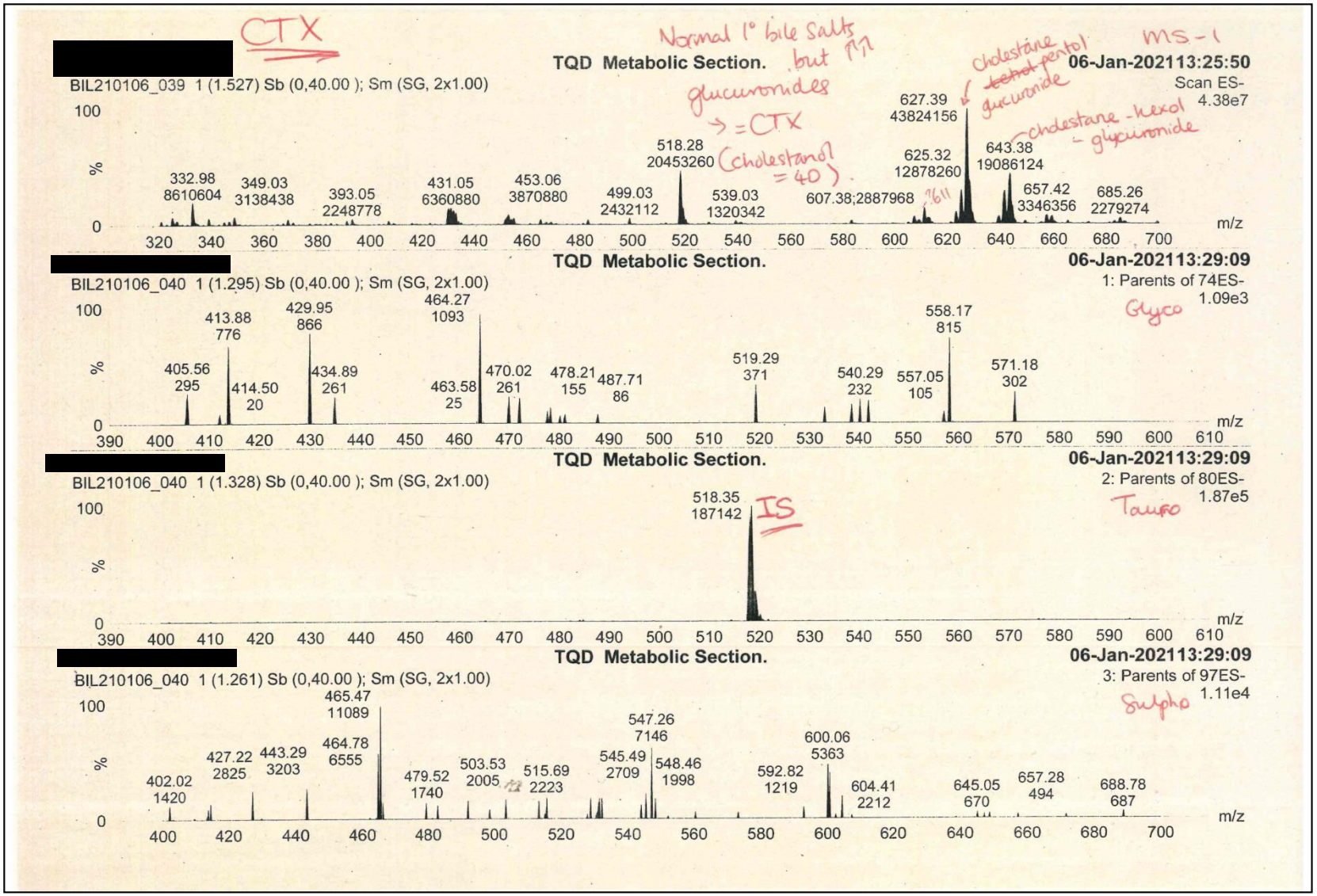
Normal Plasma Profile



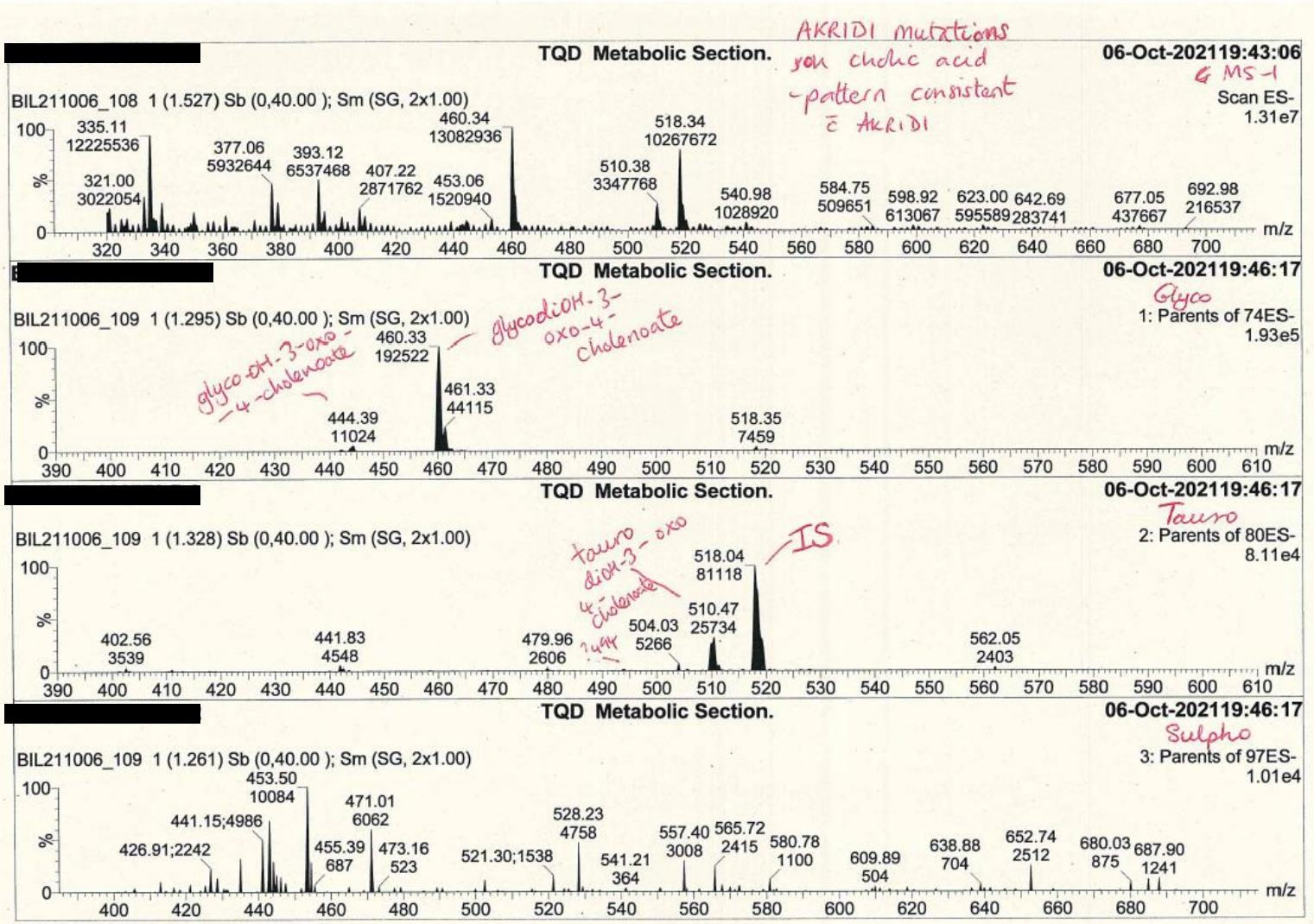
Cholestasis (PFIC 2 patient)



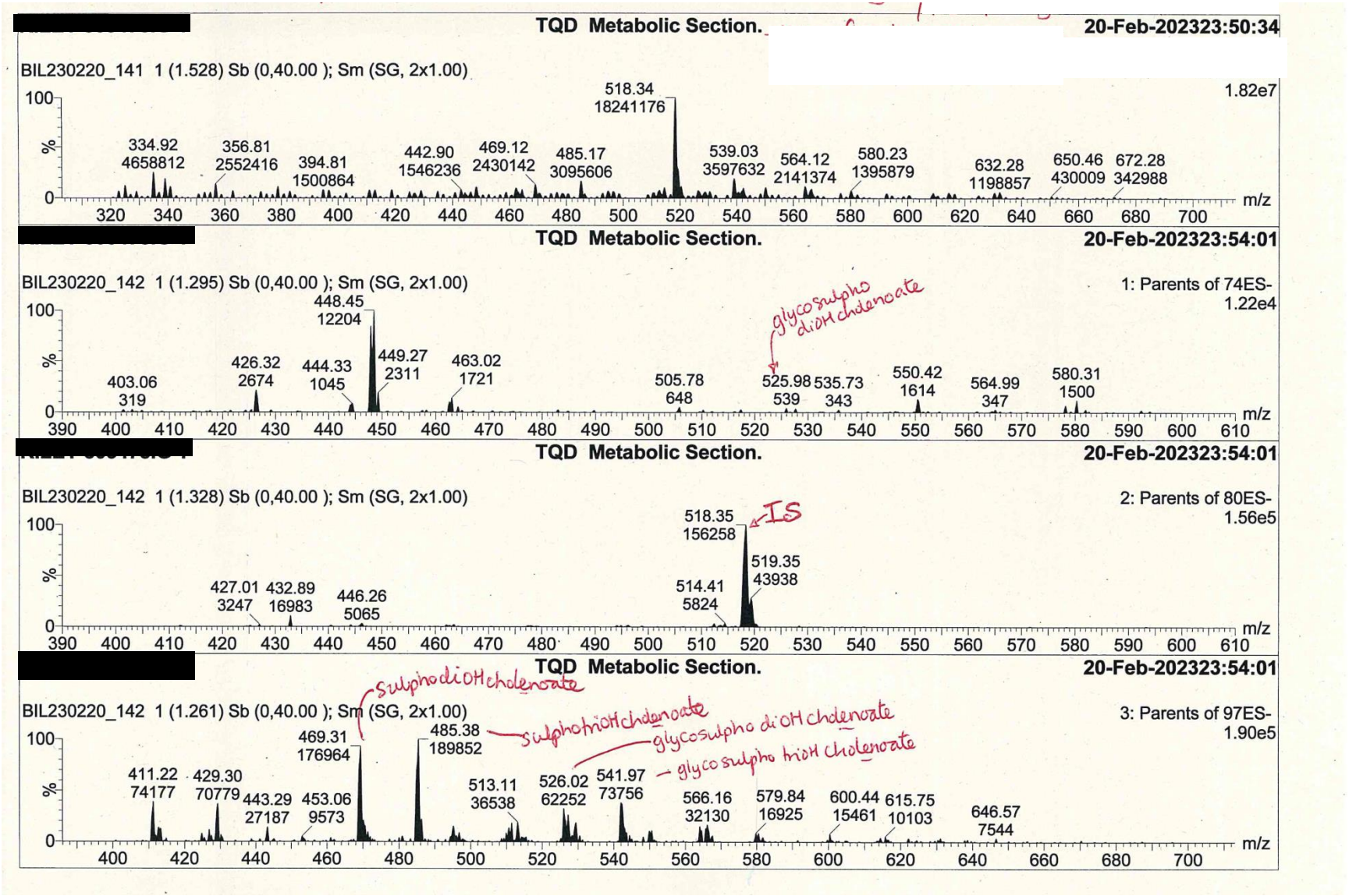
Cerebrotendinous Xanthomatosis



Delta^4-3-oxosteroid 5 beta reductase deficiency (AKR1D1)



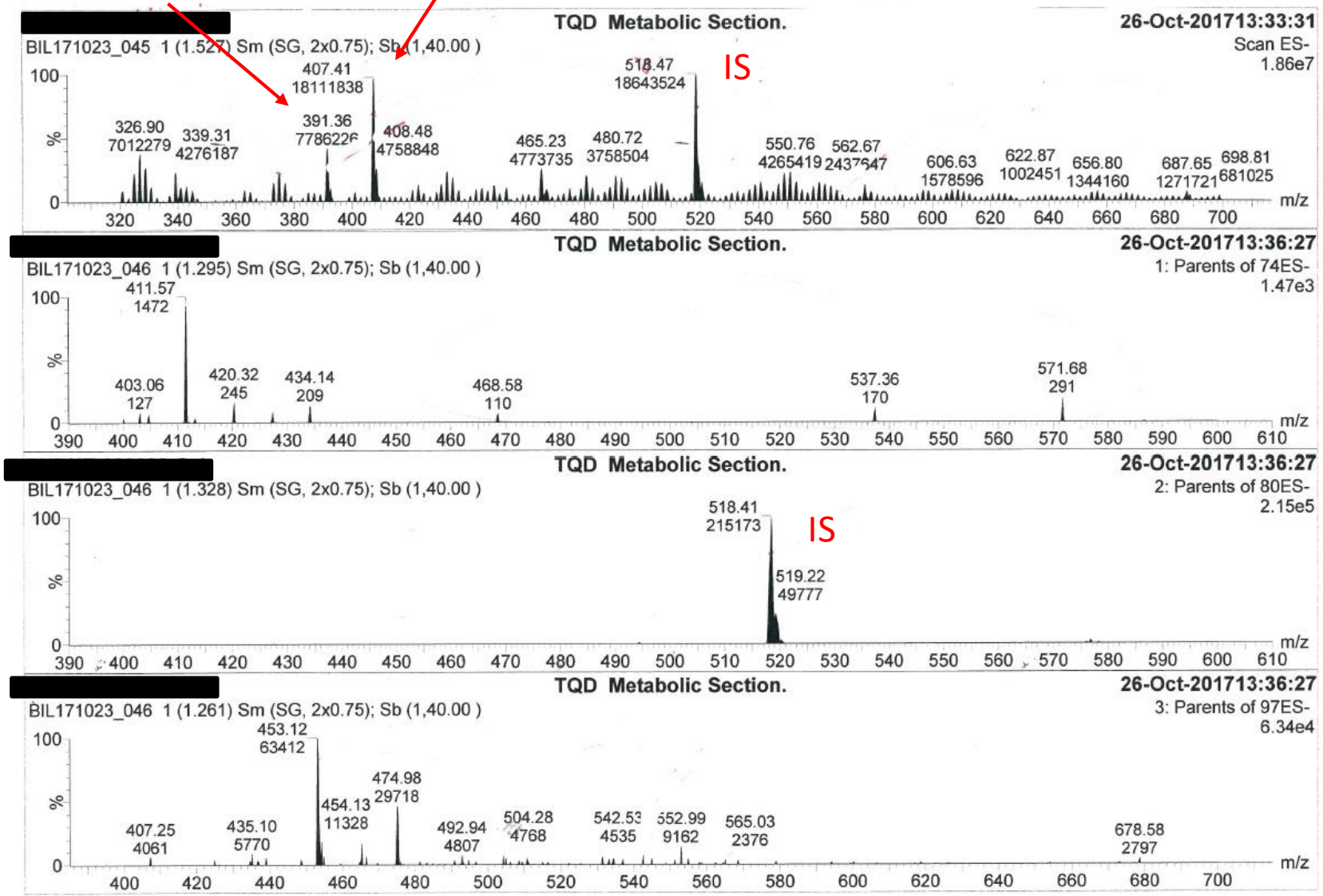
3-beta-hydroxy-delta^5-C27-steroid oxidoreductase deficiency (HSD3B7)



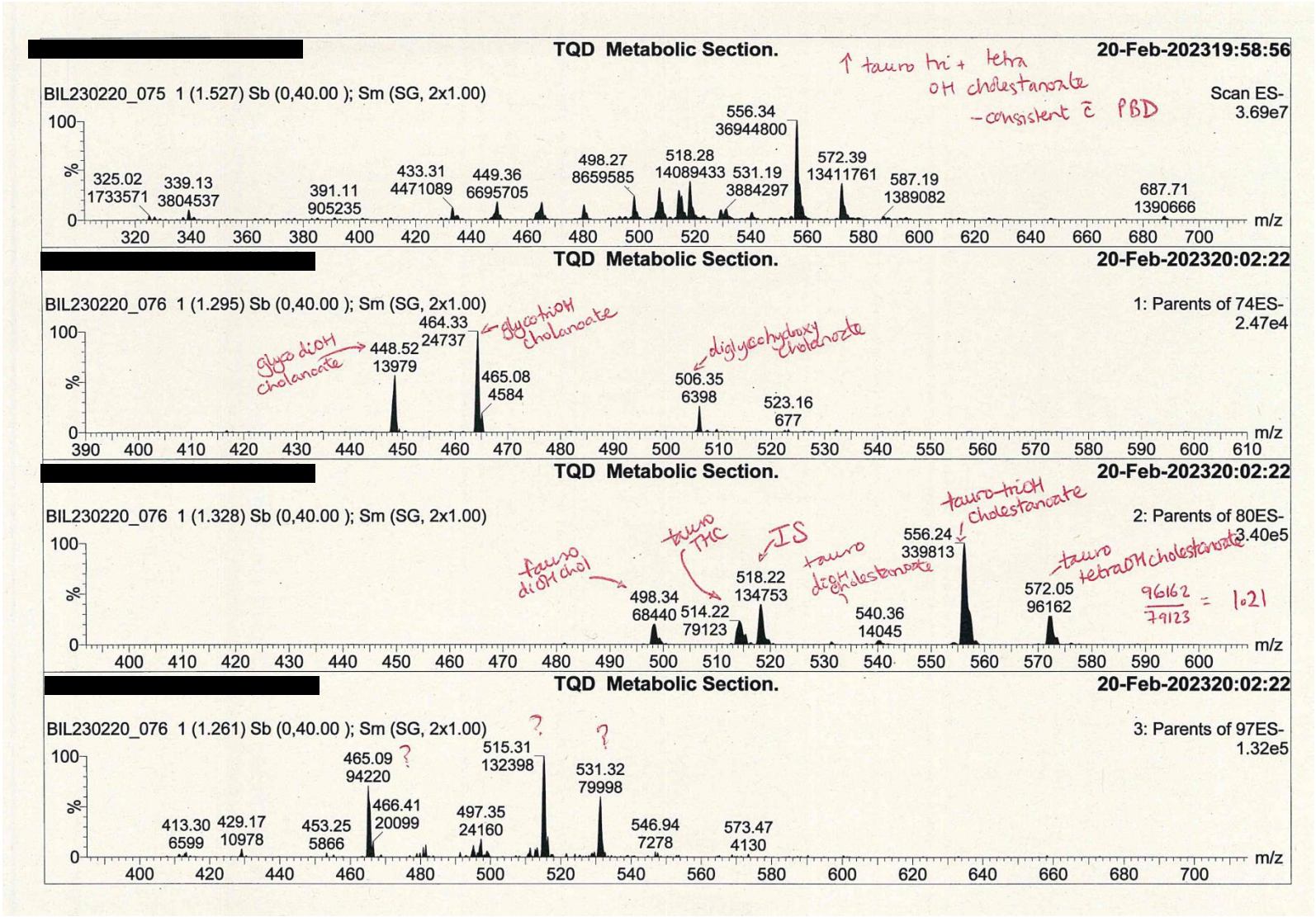
Bile Amidation / Conjugation Defect

Chenodeoxycholic acid

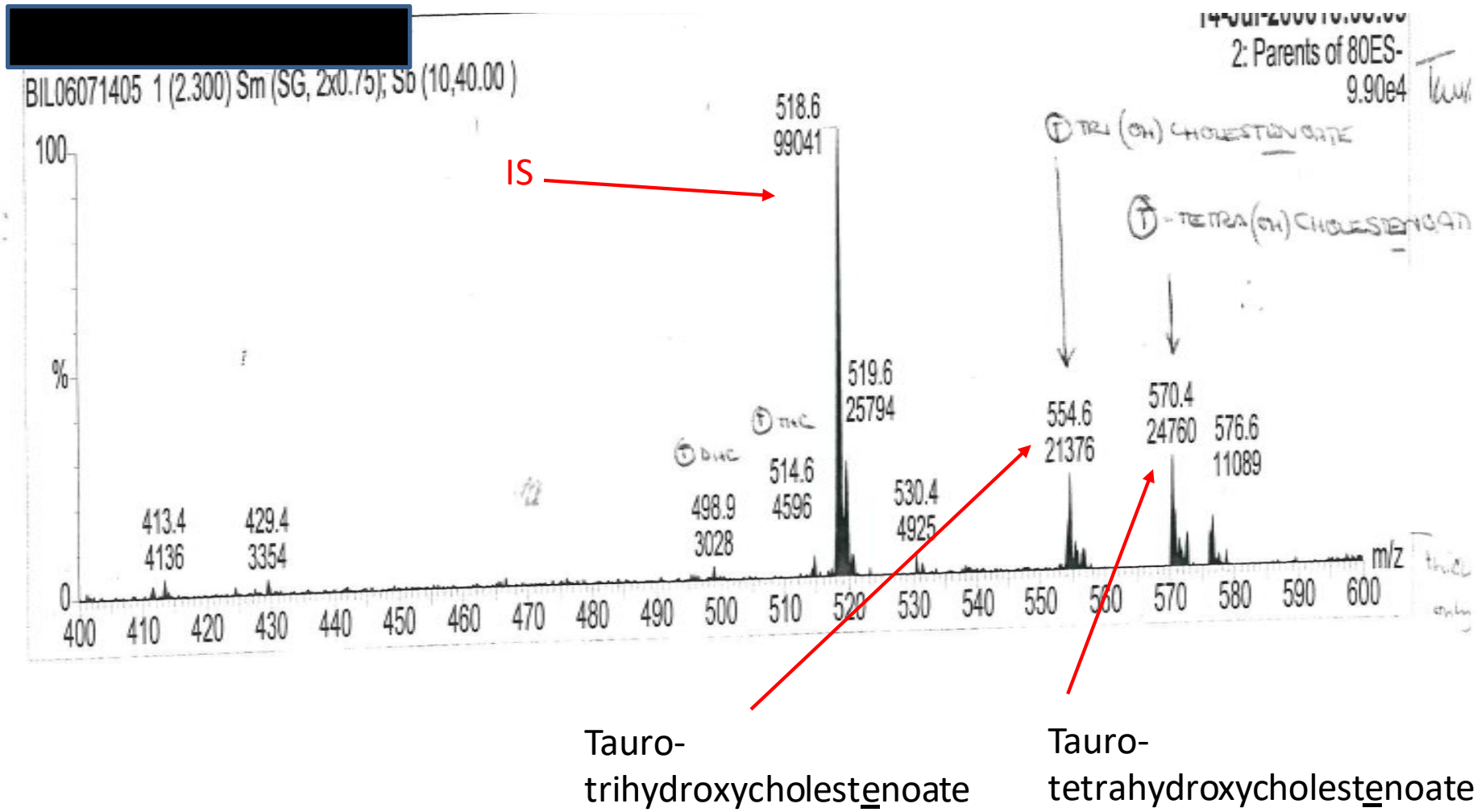
Cholic acid



Peroxisomal Biogenesis Disorder



D-Bifunctional Protein Deficiency



Urine or Plasma or Both?

- Urine definitely more sensitive for CTX and Sterol carrier protein X (glucuronides are very water soluble)
- Plasma seems to be better for Peroxisomal disorders generally
- For other disorders it seems to be variable depending on patient but often it is the same difference
- Overall the answer is “it depends” – generally it is a good idea to send both if at all possible

Diagnostic Problem 1:

Patients already treated with Ursodeoxycholic acid

- Ursodeoxycholic acid and Chenodeoxycholic acid are isobaric stereoisomers (both dihydroxycholanoate) - cannot distinguish between them by this method
- Therefore if patient treated with Urso may falsely elevate glyco and / or tauro-dihydroxycholanoate
- Patients with bile acid synthesis disorders should typically have low / low normal bile acids – but if they have been treated you will produce a cholestatic picture that can confuse things

Diagnostic Problem 2: Secondary increase in abnormal metabolites due to underlying liver disease / hepatic viruses

- Not uncommon to see secondary increases in sulpho and glyco-hydroxycholenoate (Oxysterol 7-alpha hydroxylase deficiency metabolites) – usually seen in cholestatic patients (all causes), immaturity probably also a factor
- Sometimes see secondary increases in glyco and tauro di and trihydroxy-3-oxo-4-cholenoate compounds (5beta reductase, AKR1D1 metabolites) – associated with hepatic viruses and haemochromatosis
- In theory primary patients should be clear as primary bile acids will be low or low normal, whereas in secondary patients they will be high - however if a primary patient has been given ursodeoxycholic acid already you can get a very confused picture
- Have to go by relative peak heights (are abnormal metabolites substantially bigger than primary bile acid peaks)

Diagnostic Problem 3:
Very rare disorders!

- Other than CTX and PBD –these disorders are VERY rare

- Makes it difficult to build up experience of diagnosing them

Treatment

- CTX and other primary bile acid synthesis disorders require treatment with cholic and / or chenodeoxycholic acid
- These can be converted to their tauro and glyco conjugates in liver (with exception of amidation defects) and will down regulate cholesterol-7-alpha hydroxylase and avoid build up of toxic intermediates
- Ursodeoxycholic acid doesn’t work (doesn’t down regulate)
- Liver transplant may be necessary
- Glycocholic acid used to treat amidation defects

Useful review papers

ELSEVIER

Available online at www.sciencedirect.com
ScienceDirect
Biochimica et Biophysica Acta 1763 (2006) 1427–1440

BIOCHIMICA ET BIOPHYSICA ACTA
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Review

Peroxisomes and bile acid biosynthesis

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Disorders of bile acid synthesis

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