# Disorders of Bile Acid Synthesis and Biliary Transport

# 34

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## Contents



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## 34.1 Introduction

The primary bile acids produced in man are the taurine and glycine conjugates of cholic and chenodeoxycholic acid. They are synthesised in the liver from cholesterol via numerous modifications of the sterol nucleus and side chain. A number of physiological roles have traditionally been assigned to bile acids. These include the end products of cholesterol catabolism that account for approximately 90 % of cholesterol excretion, the facilitation of bile flow by activating bile solute pumps (via nuclear receptors such as the farnesoid X receptor) and driving the osmotic excretion of water into the bile canaliculi and as biological detergents within the gut lumen enabling the absorption of fat-soluble compounds. More recently a wider role of bile acids is becoming apparent as hormone regulators of metabolism, with postulated effects on diverse processes such as carbohydrate and fat metabolism and the regulation of energy expenditure by thyroid hormone (Hylemon et al. 2009).

The conversion of cholesterol to the primary bile acids can occur via different pathways, which are summarised in Fig. 34.1. The two commonly described pathways are the 'neutral' pathway, starting with 7α-hydroxylation and subsequent nuclear modification prior to side-chain modification, and the 'acidic' pathway that starts with side-chain modification. The majority of the enzymes involved in bile acid synthesis are shared between these pathways, with the notable exception being those involved in 7α-hydroxylation (cholesterol 7α-hydroxylase [neutral pathway] and the oxysterol 7α-hydroxylase ['acidic pathway']) and 12α-hydroxylation (Russell 2003). The neutral pathway is considered the most significant in human adult life, whereas the acidic pathway seems to play a more prominent role in early life. Alternative pathways that involve initial 24/25 hydroxylation are described and are postulated to play an important role in cholesterol metabolism in brain and lung. The common pathway for side-chain modification is completed via peroxisomal β-oxidation.

Inborn errors of bile acid metabolism can present in a variety of ways. Most of the errors that effect transformation

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of the steroid nucleus (disorders **34.1**–**34.4**) produce abnormal metabolites that are not substrates for active transport into bile and generally present with failure of bile flow (cholestasis) and malabsorption of fat and fat-soluble vitamins. Patients may present acutely with the effects of vitamin deficiency such as haemorrhage or hypocalcaemic seizures or more insidiously with prolonged neonatal jaundice, steatorrhoea and rickets. Transaminases are significantly raised with a conjugated hyperbilirubinaemia, but gamma-glutamyl transpeptidase (γGT) is characteristically normal. Notable exceptions include cholesterol 7α-hydroxylase deficiency, which presents with statin-resistant hyperlipidaemia and gallstones in later life. This presumably reflects the ability of the 'acidic' pathway to compensate for the deficit in the 'neutral' pathway. Also, oxysterol 7α-hydroxylase deficiency has been described in patients with neonatal cholestasis, but also in patients presenting with hereditary spastic paraplegia (HSP). The acidic pathway has been implicated in cholesterol catabolism in the central nervous system, and significantly elevated levels of 27-hydroxycholesterol have been found in the CSF of patients with HSP.

Inborn errors that effect the modification of the cholesterol side chain (disorders **34.5**, **34.6** and the peroxisomal disorders) produce cholanoids (bile acids and alcohols) that, to some extent, can drive bile flow. Symptoms appear to be caused mainly by accumulation of intermediates proximal to the site of the block and conversion of these intermediates to a product which is deposited in various tissues of the body (Verrips et al. 2000). The deposition of cholestanol and cholesterol in CTX can lead to the formation of cataracts, mental retardation in the first decade and neurological regression with dementia and motor dysfunction in later life. The lipid deposition also produces tendon xanthomata and premature atherosclerosis. CTX can, however, also cause cholestasis in infancy. In several of the peroxisomal disorders, there is impaired bile acid synthesis and some impairment of liver function, although other pathways are often impaired and neurological disease usually predominates. These disorders are considered elsewhere. α-Methylacyl-CoA racemase deficiency (**34.6**) can present with neonatal cholestasis (Setchell et al. 2003) and is considered in this chapter, but is also mentioned in Chap. 24 on peroxisomal diseases.

In the defects of bile acid amidation (**34.7**, **34.8**), the primary bile acids are synthesised but the final step of conjugation with glycine or taurine is defective. Bile acids synthesised de novo are produced as cholyl-CoA esters and require only the peroxisomal enzyme bile acid-CoA/amino acid N-acyltransferase (BAAT) to produce conjugated products (Pellicoro et al. 2007). Bile acids that are deconjugated by intestinal flora and returned to the liver via enterohepatic circulation require bile acid-CoA ligase to form the bile acid-CoA esters prior to reconjugation by BAAT. Deficiency of either enzyme leads to the production of unconjugated bile acids, which are substrates for active transport into bile thus driving bile flow, but are inefficient biological detergents. Thus, patients with BAAT deficiency can present with steatorrhoea and fat-soluble vitamin deficiency with mild or absent jaundice.

The clinical presentation of bile acid-CoA ligase deficiency is unclear as the only symptomatic child described had a possible concurrent diagnosis of TPN-related cholestasis and mutations in the bile salt export pump gene (Chong et al. 2012).

The simplest way to screen a symptomatic individual for inborn errors of bile acid synthesis is to analyse urine by a soft ionisation (usually electrospray) mass spectrometry technique (ESI-MS). Other methods are available for some of the individual disorders. Prompt and accurate diagnosis of inborn errors of bile acid metabolism is paramount, as many of these disorders are amenable to simple oral therapy if instituted before the onset of significant hepatic damage – treatment is further discussed in Sect. 34.10.

Defects in bile transporters are commonly identified in patients with inherited forms of cholestasis (e.g. conjugated hyperbilirubinaemia). The more severe protein defects manifest in early life, whilst milder abnormalities may become apparent only when the transport processes are under stress such as in pregnancy or after specific drug ingestion. Defects of at least six proteins that facilitate transport of different bile constituents are known (Fig. 34.2). Bile constituents that include bile acids, bilirubin, cholesterol, phospholipids and other products of metabolism are secreted into biliary canaliculi in an energy-dependent manner. Transmembrane transporter proteins mediate the secretory function of hepatocytes and biliary epithelial cells.

Progressive familial intrahepatic cholestasis (PFIC) is characterised by persistent conjugated hyperbilirubinaemia and in PFIC1 and PFIC2, low or normal serum γGT values and progressive liver damage that requires liver transplantation in childhood. Patients with PFIC1/2 have reduced concentrations of primary bile acids in bile. Mutations in *ATP8B1* (PFIC1) and *ABCB11* (PFIC2) were found to be the cause of disease in the majority of patients, although there is still a proportion of patients without mutations in either of the genes. There are some clinical differences in the presentation of ATP8B1 and ABCB11 disease; most notably patients with ATP8B1 have a range of extrahepatic manifestations such as diarrhoea and episodes of pancreatitis. Patients with *ABCB11* mutations are at increased risk of hepatobiliary malignancy. In addition to the classical PFIC, some mutations in both *ATP8B1* and *ABCB11* cause the so-called benign recurrent intrahepatic cholestasis (BRIC), when cholestasis can completely resolve between relapses. It is now clear that a spectrum of severity between PFIC and

BRIC exists. ABCB4 (PFIC3) deficiency results in impaired excretion of phosphatidylcholine (PC) into bile and can result in a spectrum of cholestatic disorders including neonatal hepatitis and biliary cirrhosis with patients typically having high serum γGT values.

Mutations in *ABCC2* result in Dubin-Johnson syndrome, a condition characterised by recurrent episodes of cholestatic jaundice without other clinical/biochemical indications of hepatobiliary injury. Liver biopsy shows intrahepatocyte deposits of dark pigment but no other abnormalities. Rotor syndrome is phenotypically very similar to Dubin-Johnson syndrome and manifests with mild cholestatic jaundice that can be detected in the neonatal period or in childhood. It differs from Dubin-Johnson syndrome in that no intrahepatocyte pigment deposits can be found in Rotor syndrome patients and there is a delayed plasma clearance of unconjugated bromsulphthalein. Mutations in *SLCO1B1* and *SLCO1B3* genes, encoding organic anion-transporting polypeptides OATP1B1 and OATP1B3, have to be present simultaneously to cause Rotor syndrome.

## 34.2 Nomenclature

Disorders of peroxisome biogenesis and defects of peroxisomal β-oxidation (such as D-bifunctional protein deficiency) affect bile acid synthesis but are considered elsewhere. α-Methylacyl-CoA racemase is located both in peroxisomes and mitochondria; it is considered here because in common with other disorders of bile acid synthesis, it can present with neonatal cholestatic jaundice without neurological abnormalities. An identical argument can be made for BAAT deficiency. Both disorders can also be found in Chap. 24 on peroxisomal disease.

| No. | Disorder | Alternative name | Abbreviation | Gene symbol | Chromosomal localisation | Affected protein | OMIM no. | Subtype |
|---|---|---|---|---|---|---|---|---|
| **34.1** | 3β-Hydroxy-Δ5-C27-steroid dehydrogenase/isomerase deficiency | 3β-Dehydrogenase deficiency | C27-3β-HSD | *HSD3B7* | 16p11.2 | 3β-Hydroxy-Δ5-C27-steroid dehydrogenase/isomerase | 607764 | All forms |
| **34.2** | Δ4-3-Oxosteroid-5β-reductase deficiency | 5β-Reductase deficiency | SRD5B1 | *AKR1D1* | 7q32-q33 | Delta(4)-3-oxosteroid-5β-reductase | 604741 | All forms |
| **34.3** | Oxysterol 7α-hydroxylase deficiency | Spastic Paraplegia 5A | CYP7B1 | *CYP7B1* | 8q12.3 | Oxysterol 7α-hydroxylase | 603711 | All forms |
| **34.4** | Cholesterol 7α-hydroxylase deficiency | | CYP7A1 | *CYP7A1* | 8q12.1 | Cholesterol 7α-hydroxylase | 118455 | All forms |
| **34.5** | Sterol 27-hydroxylase deficiency | Cerebrotendinous Xanthomatosis | CTX | *CYP27A1* | 2q35 | Sterol 27-hydroxylase | 213700 | All forms |
| **34.6** | α-Methylacyl-CoA racemase deficiency | AMACR deficiency | AMACR | *AMACR* | 5q13.2 | α-Methylacyl-CoA racemase | 604489 | All forms |
| **34.7** | Bile acid-CoA: amino acid N-acyltransferase deficiency | Bile acid amidation defect | BAAT | *BAAT* | 9q31.1 | Bile acid-CoA: amino acid N-acyltransferase | 602938 | All forms |
| **34.8** | Bile acid-CoA ligase deficiency | | BA CoA LD | *SLC27A5* | 19q13.43 | Bile acid-CoA ligase | 603314 | All forms |
| **34.9** | ATP8B1 deficiency | Progressive familial intrahepatic cholestasis type 1 | PFIC1 | *ATP8B1* | 18q21.31 | ATP8B1 (type 4 P-type ATPase) | 211600 | All forms |
| **34.10** | ABCB11 deficiency | Progressive familial intrahepatic cholestasis type 2 | PFIC 2 | *ABCB11* | 2q31.1 | ABCB11 (bile salt export pump [BSEP]) | 603201 | All forms |
| **34.11** | ABCB4 deficiency | Progressive familial intrahepatic cholestasis type 3 | ABCB4 | *ABCB4* | 7q21.12 | ABCB4 (MDR3) | 602347 | All forms |
| **34.12** | OATP1B1 and OATP1B3 disease | Rotor syndrome | OATP1B1 and OATP1B3 | *SLCO1B1 and SLCO1B3* | 12p12.2-p12.1 and 12p12.2 | OATP1B1 and OATP1B3 | 237450, 604843, 605495 | All forms |
| **34.13** | ABCC2 deficiency | Dubin-Johnson syndrome | ABCC2 or DJS | *ABCC2* | 10q24.2 | ABCC2 (cMOAT) | 237500, 601107 | All forms |

## 34.3 Metabolic Pathways

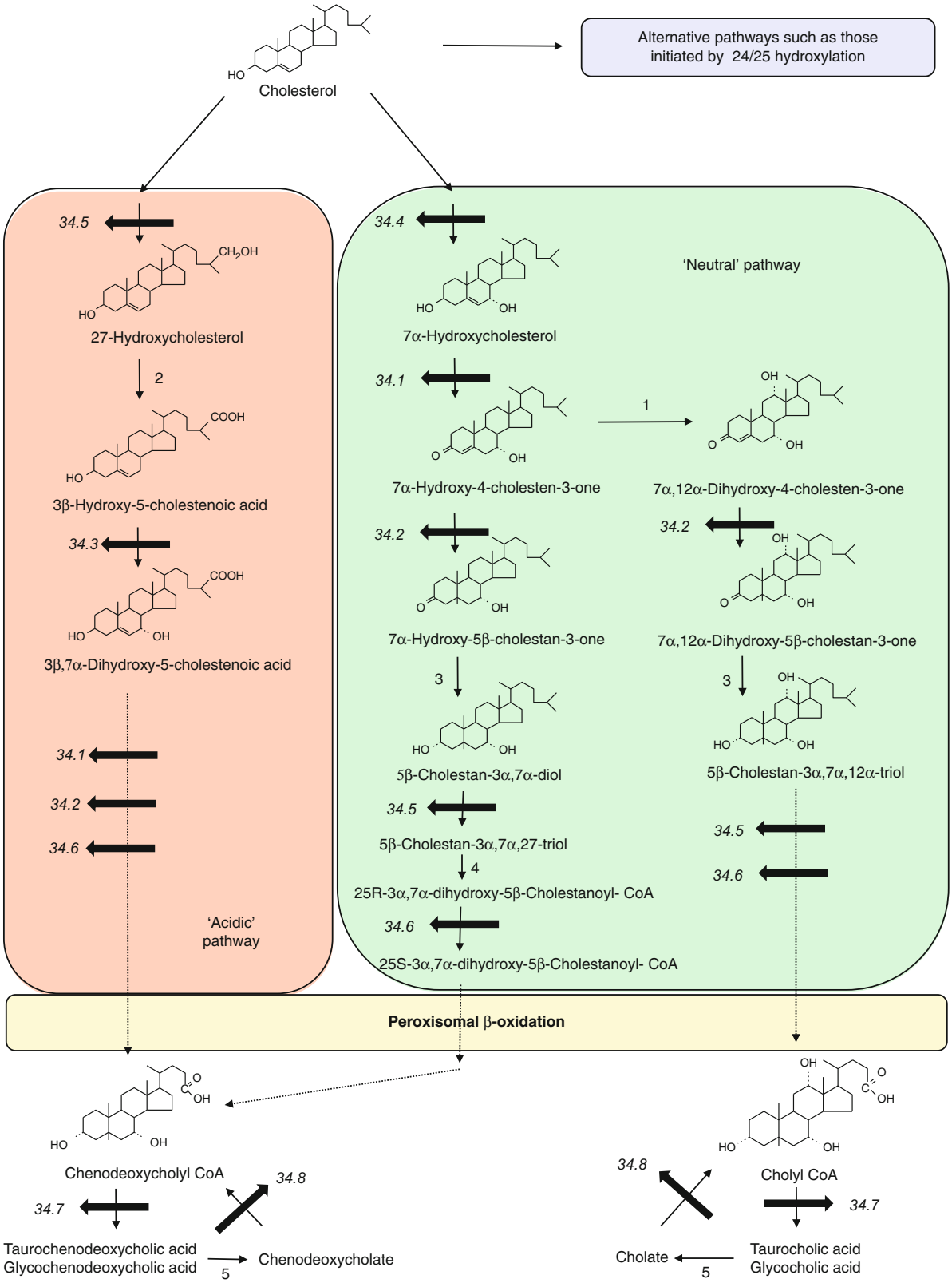


**Fig. 34.1** Simplified scheme of the known pathways for the synthesis of bile acids from cholesterol, including enterohepatic recycling. The 'neutral' pathway starts with conversion of cholesterol to 7**α**-hydroxycholesterol and the 'acidic' pathway with formation of 27-hydroxycholesterol. Defined inborn errors are highlighted with *crossed arrows*. Enzymatic steps thus far not associated with known deficiencies are numbered: (*1*) 12**α**-hydroxylase, (*2*) sterol 27-hydroxylase catalyses both 27-hdroxylation and further oxidation to a carboxylic acid, (*3*) 3**α**-hydroxysteroid dehydrogenase, (*4*) very long chain acyl-CoA synthase (VLCS)/di-/trihydroxycholestanoic acid-CoA ligase and (*5*) intraluminal bacterial deconjugation

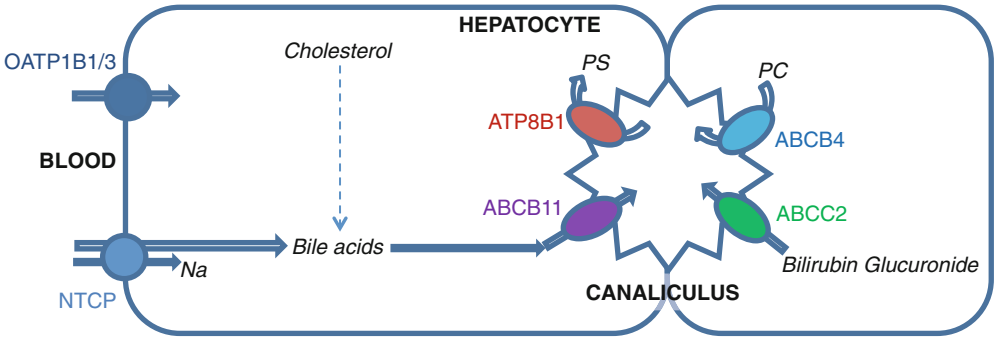


**Fig. 34.2** Diagram illustrating the transporters involved in the generation of bile. ATP8B1, a member of the type 4 subfamily of P-type ATPases, is present in the apical membrane of many epithelial cells including hepatocytes and enterocytes. ATP8B1 appears to translocate aminophospholipids such as phosphatidylserine (PS) from the outer to the inner leaflet of the plasma membrane bilayer but also has other functions such as facilitating polarised expression of other apical membrane proteins. *ABCB11* encodes the bile salt export pump (BSEP), which is responsible for the ATP-dependent transport of taurine and glycine-conjugated primary BA across the canalicular membrane. BSEP is a member of the P-glycoprotein/multidrug resistance (MDR/ABCB) subfamily of transporters. ABCB4, or multidrug resistance protein 3 (MDR3), is a P-glycoprotein that translocates phospholipids from internal to external leaflet of the canalicular membrane. ABCB4 deficiency results in impaired excretion of phosphatidylcholine (*PC*) into bile and can result in a spectrum of cholestatic disorders including neonatal hepatitis and biliary cirrhosis. As PC is a major component of the mixed micelles into which salts of bile acids are emulsified, deficiency of ABCB4 leads to hepatocyte and cholangiocyte damage by bile acids. The protein encoded by *ABCC2* (ABCC2 or MRP2) is a member of the multidrug resistance protein subfamily. It exports anionic glutathione and glucuronate conjugates (including bilirubin) from hepatocytes into canaliculi. ABCC2 is expressed on apical membranes of many epithelial cells including hepatocytes, proximal renal tubules, gallbladder, small intestine, bronchi and placenta. OATP1B1 and OATP1B3 localise to the sinusoidal membrane of hepatocytes and mediate sodium-independent cellular uptake of highly diverse compounds that include bilirubin glucuronide, bile acids, steroid and thyroid hormones, and numerous drugs

## 34.4 Signs and Symptoms

**Table 34.1** 3β-Hydroxy-Δ5-C27-steroid dehydrogenase/isomerase deficiency

| System | Symptom | Neonatal | Infancy | Childhood | Adolescence | Adulthood |
|---|---|---|---|---|---|---|
| Cardiovascular | **Vit K responsive bleeding** | ± | ± | ± | | |
| CNS | **Seizures/tetany, hypocalcaemic** | | ± | ± | | |
| Dermatological | **Itching** | N/A | ± | ± | | |
| Digestive | Cholestasis | + | ± | ± | | ± |
| | Giant cell hepatitis | + | + | | | |
| | **Jaundice** | + | ± | ± | | ± |
| | Liver cirrhosis | | ± | ± | | ± |
| | **Steatorrhoea** | + | + | + | | |
| Musculoskeletal | **Rickets** | + | + | + | | |
| Routine laboratory | Albumin (P) | n | n | n | | n-↓ |
| | Alkaline phosphatase (P) | ↑ | ↑ | ↑ | | n-↑ |
| | ASAT/ALAT (P) | ↑ | ↑ | ↑ | | ↑ |
| | Bilirubin conjugated (P) | ↑ | n-↑ | n-↑ | | |
| | Calcium (P) | ↓-n | ↓-n | ↓-n | | |
| | Cholesterol (S) | ↓-n | ↓-n | ↓-n | | |
| | Gamma-glutamyl transpeptidase (GGT) (P) | n | n | n | | n |
| | Prothrombin ratio | n-↑ | n-↑ | n-↑ | | n-↑ |

(continued)

**Table 34.1** (continued)

| System | Symptom | Neonatal | Infancy | Childhood | Adolescence | Adulthood |
|---|---|---|---|---|---|---|
| Special laboratory | 25-Hydroxy-vitamin D (P) | ↓ | ↓ | ↓ | | |
| | 7α-Hydroxycholesterol dehydrogenase (FB) | + | + | + | | + |
| | Chenodeoxycholic acid (P) | ↓ | ↓ | ↓ | | |
| | HSD3*B7* gene sequencing | + | + | + | | + |
| | LSI-MS for sulphate/glycine-conjugated di/trihydroxy-5-cholenoic acids (m/z 469,485,526,542)-negative ion mode | + | + | + | | + |
| | Periportal inflammation | + | + | + | | |
| | Bridging fibrosis | | + | + | | |
| | Sulphated 3β,7α-dihydroxy and 3β,7α, 12α-trihydroxy-5-cholenoic acids (P,U) | + | + | + | | |
| | Vitamin A (P) | ↓-n | ↓-n | ↓-n | | |
| | Vitamin E (P) | ↓ | ↓ | ↓ | | |

[a]Described modes of presentation include, in order of frequency, neonatal cholestasis, rickets and symptomatic hypocalcaemia (Clayton et al. 1987; Subramaniam et al. 2010). Two asymptomatic patients have been described that were identified on family screening of affected siblings. It is not clear whether these children would have progressed to disease state as they started on prophylactic treatment (Subramaniam et al. 2010)
[b]Some patients have been described with an ESI-MS pattern that is predominated by non-sulphated metabolites including glycine-conjugated/unconjugated compounds (m/z 405,446,462). This may reflect different endogenous sulphation of compounds, although certain experimental conditions may promote the formation of doubly charged sulphate conjugates (m/z 234, 263), and these may not be detected if the low mass/charge end of the urine spectrum is not included (Clayton et al. 2011)

**Table 34.2** Δ4-3-Oxosteroid-5β-reductase deficiency

| System | Symptom | Neonatal | Infancy | Childhood | Adolescence | Adulthood |
|---|---|---|---|---|---|---|
| Digestive | **Ascites, oedema** | ± | ± | | | |
| | Cholestasis | + | + | | | |
| | Giant cell hepatitis | + | + | | | |
| | **Hepatosplenomegaly** | ± | ± | | | |
| | **Jaundice** | + | ± | | | |
| Others | Pseudoacinar transformation | + | ± | | | |
| Routine laboratory | Albumin (P) | ↓-n | ↓-n | | | |
| | Alkaline phosphatase (P) | n-↑ | n-↑ | | | |
| | ASAT/ALAT (P) | ↑ | ↑ | | | |
| | Bilirubin conjugated (P) | ↑ | n-↑ | | | |
| | Cholesterol (S) | ↓-n | ↓-n | | | |
| | Gamma-glutamyl transpeptidase (GGT) (P) | n-↑ | n-↑ | | | |
| | Prothrombin ratio | ↑ | n-↑ | | | |
| Special laboratory | 7α-Hydroxy-3-oxo and 7α, 12α-dihydroxy-3-oxo-4-cholenoic acids (U) | + | + | + | + | |
| | *AKR1D1* gene | + | + | | | |
| | Allocholic and allochenodeoxycholic acid (P) | + | + | + | + | |
| | Cortisol metabolites increased 5αH/5βH ratio (U) | | + | + | + | |
| | Periportal and lobular inflammation | ± | ± | | | |
| | Small caniculae, few microvilli | + | ± | | | |

There is no biochemical test currently available that can confidently distinguish between a patient that has reduced activity of 5β-reductase as a result of mutations in *AKR1D1* and a patient that has reduced activity of the enzyme (resulting in excretion of 3-oxo-$\Delta^4$ bile acids) as a non-specific consequence of severe liver damage in infancy/childhood (Clayton 1994). Only patients with a proven genetic abnormality of 5β-reductase have been included in this chapter (Lemonde et al. 2003; Clayton 2011)

**Table 34.3** Oxysterol 7α-hydroxylase deficiency

| System | Symptom | Neonatal | Infancy | Childhood | Adolescence | Adulthood |
|---|---|---|---|---|---|---|
| Cardiovascular | **Vit K responsive bleeding** | | ± | | | |
| CNS | Ataxia, cerebellar | | | | | ± |
| | Progressive spastic paraplegia | | | ± | ± | ± |
| Digestive | Bile duct proliferation | | + | | | |
| | Cholestasis | | + | | | |
| | Giant cell hepatitis | | + | | | |
| | **Hepatosplenomegaly** | | + | | | |
| | **Jaundice** | ± | + | | | |
| Eye | Cataract | | | | | ± |
| | Optic atrophy | | | | | ± |
| Routine laboratory | Alkaline phosphatase (P) | | ↑ | | | |
| | ASAT/ALAT (P) | | ↑ | | | |
| | Bridging fibrosis | | + | | | |
| | Bilirubin-total/direct (P) | | ↑ | | | |
| | Cholesterol (S) | | n | | | |
| | Gamma-glutamyl transpeptidase (GGT) (P) | | n | | | |
| | Glucose (P) | | ↓ | | | |
| | Prothrombin ratio | | n-↑ | | | |
| Special laboratory | 27-Hydroxycholesterol and 3β-hydroxy-5-cholestenoic acid (P) | | ↑ | ↑ | ↑ | ↑ |
| | 3β-Hydroxy-5-cholenoic acids (U) | | + | ↑ | ↑ | ↑ |
| | *CYP7B1* gene | + | + | + | + | + |
| | Periportal inflammation | | + | | | |
| | Vitamin E (P) | | ↓ | | | |
| | White matter abnormalities | | | | | + |

Oxysterol 7α-hydroxylase deficiency can present with neonatal cholestasis (Setchell et al. 1998), but, interestingly, mutations of *CYP7B1* have been found in a number of cases of hereditary spastic paraplegia (SPG5 [Spastic paraplegia group 5]), who present later in life with a neurological presentation without clinical history of liver disease (Arnoldi et al. 2012)

**Table 34.4** Cholesterol 7α-hydroxylase deficiency

| System | Symptom | Neonatal | Infancy | Childhood | Adolescence | Adulthood |
|---|---|---|---|---|---|---|
| Cardiovascular | **Statin-resistant hyperlipidaemia** | | | | | ± |
| Digestive | Gallstones | | | | | ± |

Only three patients (siblings) have been described with a deficiency in this enzyme (Pullinger et al. 2002). They were identified in the sixth decade of life, amongst patients in a hyperlipidaemia clinic, by screening the *CYP7A1* gene in patients who had significant statin-resistant hyperlipidaemia and gallstone disease. Total production of bile acids was shown to be reduced, but no hepatic or neurological pathology was identified. One sibling was found to have significant atherosclerotic disease

**Table 34.5** Sterol 27-hydroxylase deficiency

| System | Symptom | Neonatal | Infancy | Childhood | Adolescence | Adulthood |
|---|---|---|---|---|---|---|
| Cardiovascular | Ischaemic heart disease, angina/myocardial infarct | | | | ± | ± |
| CNS | Ataxia | | | | ± | ± |
| | Demyelination and lipid deposition on MRI | | | | ± | |
| | Developmental delay | | | ± | ± | ± |
| | Expressive dysphasia | | | | ± | ± |
| | Neuropathy | | | | | ± |
| | Parkinsonism | | | | ± | |
| | Regression, dementia | | | | ± | ± |
| | Seizures | | | ± | ± | ± |
| | Spastic paresis/pyramidal signs | | | ± | ± | ± |
| | Spinal cord, myelopathy | | | | | ± |
| Dermatological | **Xanthomas, tendon** | | | | ± | ± |

(continued)

**Table 34.5** (continued)

| System | Symptom | Neonatal | Infancy | Childhood | Adolescence | Adulthood |
|---|---|---|---|---|---|---|
| Digestive | Diarrhoea | | ± | ± | | |
| | Gallstones | | | | | ± |
| | Jaundice | ± | ± | ± | | |
| | Pigment granules in liver biopsy | | | | | ± |
| Endocrine | Adrenal insufficiency | | | | | ± |
| | Hypo or hyperthyroidism | | | | | ± |
| | Pituitary/hypothalamic dysfunction | | | | | ± |
| Eye | Cataract | | | ± | ± | ± |
| Musculoskeletal | Osteoporosis | | | | | ± |
| | Pes cavus | | | | | ± |
| Respiratory | Failure | | | | | ± |
| Routine laboratory | Cholesterol (S) | | | | n-↑ | n-↑ |
| | **EEG: +/– evoked potentials, abnormal** | | | ± | ± | ± |
| Special laboratory | 25-Hydroxy-Vitamin D (P) | | | | ↓-n | ↓-n |
| | 27-Hydroxylase (FB) | ↓ | ↓ | ↓ | ↓ | ↓ |
| | Cholestane pentol glucuronides (U) | + | + | + | + | + |
| | Cholestanol (P) | ↑ | ↑ | ↑ | ↑ | ↑ |
| | *CYP27A1* gene | + | + | + | + | + |

(Verrips et al. 2000; Clayton et al. 2002)

**Table 34.6** α-Methylacyl-CoA racemase deficiency

| System | Symptom | Neonatal | Infancy | Childhood | Adolescence | Adulthood |
|---|---|---|---|---|---|---|
| Cardiovascular | **Vit K responsive bleeding** | ± | | | | |
| CNS | Cognitive decline | | | | | ± |
| | Developmental delay | | ± | | | |
| | Epilepsy +/– encephalopathy | | | ± | ± | ± |
| | Headache | | | | ± | ± |
| | Neuropathy | | | | | ± |
| | Spastic paraparesis | | | | | ± |
| | Tremor | | | | | ± |
| Digestive | Cholestasis | ↑ | | | | |
| | **Gallstones** | | | ± | ± | ± |
| | Giant cell hepatitis | + | | | | |
| | **Jaundice** | ± | ± | | | |
| Endocrine | Hypothyroidism | | | | | ± |
| Eye | Pigmentary retinopathy | | | | ± | ± |
| | Vision, progressive loss | | | | ± | ± |
| Routine laboratory | Gamma-glutamyl transpeptidase (GGT) (P) | ↑ | | | | |
| | Transaminases (P) | ↑ | | | | |
| Special laboratory | (25R)-3α,7α,12α-trihydroxy-5β-cholestan-26-oic acid (THCA) (P) | ↑ | ↑ | ↑ | ↑ | ↑ |
| | 25-Hydroxy-vitamin D (P) | ↓ | | | | |
| | *AMACR* gene | + | + | + | + | + |
| | Taurotetra-[tri/penta]-hydroxycholestanoic acids (U) [m/z 556,572,588 on LSI-MS] | ↑ | ↑ | ↑ | ↑ | ↑ |
| | Vitamin E (P) | ↓ | | | | |

[a]Five patients have been described with an AMACR deficiency. Four presented in later life with neurological symptoms (Ferdinandusse et al. 2000; Thompson et al. 2008) and one in the neonatal period with cholestasis (Setchell et al. 2003)

**Table 34.7** Bile acid-CoA/amino acid N-acyltransferase deficiency

| System | Symptom | Neonatal | Infancy | Childhood | Adolescence | Adulthood |
|---|---|---|---|---|---|---|
| Dermatological | **Itching** | | ± | ± | ± | |
| Digestive | **Failure to thrive** | | ± | ± | | |
| | **Jaundice** | ± | ± | ± | ± | |
| | **Steatorrhoea** | | ± | ± | | |
| Musculoskeletal | **Rickets** | | ± | ± | | |
| Routine laboratory | Bilirubin (P) | n-↑ | n-↑ | n-↑ | n-↑ | |
| | Prothrombin ratio | | n-↑ | | | |
| | Transaminases (P) | | n-↑ | n-↑ | | |
| Special laboratory | *BAAT* gene | + | + | + | + | + |
| | LSI-MS showing unamidated bile acids (m/z 407,471,487,567,583)-negative ion mode | | ↑ | ↑ | | |
| | Vitamin A (P) | | ↓-n | ↓-n | ↓-n | |
| | Vitamin D (P) | | ↓-n | ↓-n | ↓-n | |
| | Vitamin E (P) | | ↓-n | ↓-n | ↓-n | |

**Table 34.8** Bile acid-CoA ligase deficiency

| System | Symptom | Neonatal | Infancy | Childhood | Adolescence | Adulthood |
|---|---|---|---|---|---|---|
| Digestive | Bile duct proliferation | ± | | | | |
| | **Jaundice** | ± | | | | |
| | Liver cirrhosis | ± | | | | |
| Other | Bridging fibrosis | ± | | | | |
| Routine laboratory | Bilirubin conjugated (P) | n-↑ | | | | |
| | Gamma-glutamyl transpeptidase (GGT) (P) | n | | | | |
| | Transaminases (P) | n-↑ | | | | |
| Special laboratory | LSI-MS showing unamidated bile acids (m/z 407,471,487,567,583)-negative ion mode | ↑ | ↑ | | | |
| | *SLC27A5* gene | + | + | + | + | + |

Only two siblings have been described with bile acid-CoA ligase deficiency. One has remained asymptomatic, whilst the other with cholestatic liver disease was identified after a premature birth and prolonged period of parenteral feeding and also had BSEP mutations. It is unclear to what extent the clinical findings were as a result of the bile acid-CoA ligase deficiency, TPN-related cholestasis, BSEP deficiency or indeed a combination of all three

**Table 34.9** ATP8B1 deficiency

| System | Symptom | Neonatal | Infancy | Childhood | Adolescence | Adulthood |
|---|---|---|---|---|---|---|
| Digestive | Bland centrilobular cholestasis | | ± | ± | | |
| | **Diarrhoea** | | ± | ± | ± | |
| | **Failure to thrive** | ± | ± | ± | ± | |
| | **Jaundice** | ± | ± | ± | ± | |
| | Liver fibrosis | | | ± | ± | |
| | **Pancreatitis** | | ± | ± | ± | |
| | **Steatorrhoea** | | ± | ± | ± | |
| Ear | **Deafness, sensorineural** | | ± | ± | ± | |
| Genitourinary | **Delayed puberty** | | | | ± | |
| Respiratory | **Pneumonia** | | ± | ± | | |
| Routine laboratory | Bile acids (enzyme assay) (P) | ↑ | ↑ | ↑ | ↑ | |
| | Chloride (sweat) | | ↑ | ↑ | | |
| | **Fat-soluble vitamin deficiency** | ± | ± | ± | ± | |
| | Gamma-glutamyl transpeptidase (GGT) (P) | n | n | n | n | |
| Special laboratory | *ATP8B1* gene | + | + | + | + | |
| | Bile acids, normal | ↑ | ↑ | ↑ | ↑ | |
| | Coarse granular bile on electron microscopy | | ± | ± | | |

PFIC1 may be suspected because of the extrahepatic manifestations (not often seen in PFIC2). The extrahepatic manifestations do not resolve after liver transplantation. At presentation patients with PFIC1 have higher alkaline phosphatase, but lower plasma bile acids, transaminases and albumin than PFIC2 patients (Pawlikowska et al. 2010)

**Table 34.10** ABCB11 deficiency

| System | Symptom | Neonatal | Infancy | Childhood | Adolescence | Adulthood |
|---|---|---|---|---|---|---|
| Dermatological | **Itching** | | + | + | + | |
| Digestive | **Failure to thrive** | | ± | ± | ± | |
| | **Gallstones** | | + | + | + | |
| | Giant cell hepatitis | + | + | | | |
| | **Jaundice** | + | + | + | + | |
| | Liver cirrhosis | | ± | ± | ± | |
| | **Risk of malignant hepatoma** | | | + | + | |
| Routine laboratory | Bile acids (enzyme assay) (P) | ↑↑ | ↑↑ | ↑↑ | ↑↑ | |
| | Chloride (sweat) | | ↑ | ↑ | | |
| | Gamma-glutamyl transpeptidase (GGT) (P) | n | n | n | n | |
| Special laboratory | *ABCB11* gene | + | + | + | + | |
| | Bile acids, normal | ↑ | ↑ | ↑ | ↑ | |
| | Filamentous bile on electron microscopy | ± | ± | ± | ± | |

Patients with BSEP deficiency (PFIC2) are more likely to have gallstones and portal hypertension than PFIC1 patients (Pawlikowska et al. 2010). Patients with the D482G mutation have less rapidly progressive PFIC than those with other mutations

**Table 34.11** ABCB4 deficiency

| System | Symptom | Neonatal | Infancy | Childhood | Adolescence | Adulthood |
|---|---|---|---|---|---|---|
| Digestive | **Gallstones** | | ± | ± | ± | |
| | **Jaundice, cholestatic** | + | + | + | + | |
| | **Liver dysfunction** | ± | ± | ± | ± | |
| | Liver fibrosis/biliary cirrhosis | ± | ± | + | + | |
| Haematological | **Splenomegaly** | | | ± | ± | |
| Routine laboratory | Bile acids (enzyme assay) (P) | ↑ | ↑ | ↑ | ↑ | |
| | Gamma-glutamyl transpeptidase (GGT) (P) | ↑ | ↑ | ↑ | ↑ | |
| Special laboratory | *ABCB4* gene | + | + | + | + | |
| | Bile acids, normal | ↑ | ↑ | ↑ | ↑ | |
| | Portal inflammation with ductular proliferation | ± | ± | ± | ± | |

**Table 34.12** OATP1B1 and OATP1B3 disease

| System | Symptom | Neonatal | Infancy | Childhood | Adolescence | Adulthood |
|---|---|---|---|---|---|---|
| Digestive | **Jaundice** | + | + | + | + | |
| Routine laboratory | Bilirubin conjugated (P) | ↑ | ↑ | ↑ | ↑ | |
| | LFTs not including conjugated bilirubin | n | n | n | n | |
| Special laboratory | Clearance of unconjugated bromsulphthalein (P) | ↓ | ↓ | ↓ | ↓ | |
| | Coproporphyrin I (U) | ↑↑ | ↑↑ | ↑↑ | ↑↑ | |
| | Normal liver biopsy, no pigment | + | + | + | + | |
| | *SLCO1B1* and *SLCO1B3* genes | + | + | + | + | |

(Van de Steeg et al. 2012)

**Table 34.13** ABCC2 deficiency

| System | Symptom | Neonatal | Infancy | Childhood | Adolescence | Adulthood |
|---|---|---|---|---|---|---|
| Digestive | **Jaundice, episodic** | + | + | + | + | |
| Routine laboratory | Bilirubin conjugated (P) | n-↑ | n-↑ | n-↑ | n-↑ | |
| | LFTs not including conjugated bilirubin | n | n | n | n | |
| Special laboratory | *ABCC2* gene | + | + | + | + | |
| | Clearance of unconjugated bromsulphthalein (P) | n | n | n | n | |
| | Coproporphyrin I (U) | n | n | n | n | |
| | Pigment granules in liver biopsy | + | + | + | + | |

It is thought that the mild phenotype of ABCC2 deficiency is explained by upregulation of other transporters such as ABCC3

**Carriers for Mutations in Hepatocyte Transporter Proteins**

Carriers of *ATP8B1*, *ABCB11* and *ABCB4* mutations are predisposed to intrahepatic cholestasis of pregnancy (ICP), which is a third-trimester disorder that is characterised by pruritus and elevated serum concentrations of bile acids (van der Woerd et al. 2010). It seems that the subtype with low serum γGT values occurs in *ABCB11* and *ATP8B1* mutation carriers, whilst carriers of *ABCB4* typically have high γGT values. ICP is associated with fetal disease, fetal distress, premature birth and stillbirth. Cholestasis associated with the administration of oral contraceptives is also more frequent in carriers of *ABCB11* mutations. Low-phospholipid-associated cholelithiasis (LPAC) is a form of gallstone disease that occurs in younger patients which is associated with *ABCB4* mutations, recurs after cholecystectomy and appears to respond well to UDCA. Mutations in *ABCB11* and *ABCB4* have been associated with drug-induced cholestasis (DIC) following administration of amoxicillin, clavulanic acid and risperidone.

## 34.5 Reference Values

**Table 34.14** Determination of urinary cholanoid (bile acid and bile alcohol) profile by ESI-MS (electrospray ionisation mass spectrometry)

| Ion | Identity | Normal | Cholestasis |
|---|---|---|---|
| 234 | $[M-2H]^{2-}$ 3β,7α-Dihydroxy-5-cholenoic acid ($SO_4$) | – | – |
| 263 | $[M-2H]^{2-}$ 3β,7α-Dihydroxy-5-cholenoic acid (Gly,$SO_4$) or isomer | – | ± |
| 391 | Dihydroxycholanoic acids (e.g. chenodeoxycholic acid) | – | ± |
| 405 | 3β,7α,12α-Trihydroxy-5-cholenoic acid | – | – |
| 407 | Trihydroxy-cholanoic acids (e.g. cholic acid) | ± | ± |
| 444 | 7α-Hydroxy-3-oxo-4-cholenoic acid (Gly) | – | ± |
| 446 | 3β,7α-Dihydroxy-5-cholenoic acid (Gly) | – | – |
| 448 | Dihydroxycholanoic acids (e.g. chenodeoxycholic acid) (Gly) | ± | ±/↑ |
| 453 | 3β-Hydroxy-5-cholenoic acid ($SO_4$) | – | ± |
| 460 | 7α,12α-Dihydroxy-3-oxo-4-cholenoic acid (Gly) | – | ± |
| 462 | 3β,7α,12α-Trihydroxy-5-cholenoic acid (Gly) | – | – |
| 464 | Trihydroxy-cholanoic acids (e.g. cholic acid) (Gly) | ± | ±/↑ |
| 469 | 3β,7α-Dihydroxy-5-cholenoic acid ($SO_4$) | – | – |
| | Also steroid sulphate[a] | ± | – |
| 471 | Dihydroxycholanoic acid(s) ($SO_4$) | – | ±[c] |
| 480 | 3β-Hydroxy-5-cholenoic acid (Tau) | – | ± |
| | Tetrahydroxy-cholanoic acids (Gly) | ± | ± |
| 485 | 3β,7α,12α-Trihydroxy-5-cholenoic acid ($SO_4$) | – | – |
| 494 | 7α-Hydroxy-3-oxo-4-cholenoic acid (Tau) | – | ± |
| 498 | Dihydroxycholanoic acids (e.g. chenodeoxycholic acid) (Tau) | ± | ±/↑ |
| 510 | 7α,12α-Dihydroxy-3-oxo-4-cholenoic acid (Tau), 3β-Hydroxy-5-cholenoic acid (Gly,$SO_4$) | ± | ± |
| 514 | Trihydroxy-cholanoic acids (e.g. cholic acid) (Tau) | ± | ±/↑ |
| 526 | 3β,7α-Dihydroxy-5-cholenoic acid (Gly,$SO_4$) | – | ± |
| 528 | Dihydroxycholanoic acids (e.g. chenodeoxycholic acid) (Gly,$SO_4$) | ± | ±/↑ |
| 530 | Tetrahydroxy-cholanoic acids (Tau) | ± | ±/↑ |
| 542 | 3β,7α,12α-Tihydroxy-5-cholenoic acid (Gly,$SO_4$) | – | – |
| 567 | Dihydroxycholanoic acids (Gluc) | – | ±[c] |
| 572 | Tetrahydroxycholestanoic acids (Tau) | – | – |
| 583 | Trihydroxy-cholanoic acids (Gluc) | – | ±[c] |
| 611 | Cholestanetetrols (Gluc) | ± | ± |
| 613 | 27-Nor-cholestanepentol($SO_4$) (Gluc) | ±/↑[b] | ± |
| 627 | Cholestanepentols (Gluc) | ±/↑ | ± |
| 643 | Cholestanehexols (Gluc) | ±/↑ | ± |
| 651 | Dihydroxycholanoic acids (Gly,GlcNAc) | – | ± |
| 701 | Dihydroxycholanoic acids (Tau, GlcNAc) | – | ± |

Cholanoid conjugates are abbreviated; *Gly* glycine conjugate, *Tau* taurine conjugate, *$SO_4$* sulphate, *Gluc* glucuronide, *GlcNAc* N-acetylglucosamine conjugate

[a]An ion of mass/charge ratio 469 can occur in urine samples from patients who do not have 3β-HSDH deficiency; the other ions which are characteristic of 3β-HSDH (485,526,542) are not present, and GC-MS fails to show increased excretion of 3β,7α-dihydroxy-5-cholenoic acid

[b]In normal neonates and young infants m/z 613 or 627 can be the base peak. However, m/z 627 is much less intense than in patients with CTX

[c]These peaks are prominent in patients with cholestasis who are receiving ursodeoxycholic acid; spectra from patients on ursodeoxycholic acid treatment are very difficult to interpret

The mass spectrometer scans negative ions over the range m/z 350–700, or sometimes 200–800, and draws a spectrum with the largest peak as 100 % intensity. In Table 34.14, indicates that the peak is not detectable above the background, ± indicates undetectable to 20 % of the largest peak and ↑ indicates 20–100 % intensity of largest peak. Daughter ions generated in a collision cell can be used to help confirm peak identities, e.g. m/z 74 for glycine conjugates, m/z 80 for taurine conjugates, m/z 97 for sulphates and m/z 85 for glucuronides.

The values below refer to total plasma concentration determined by GC-MS analysis following hydrolysis of cholestanol esters.

**Table 34.15** Urinary cholanoid excretions determined by GC-MS

| Cholanoid | μmol/mmol creatinine | % Total bile acid excretion |
|---|---|---|
| 3β,7α-diOH-5-cholenoic acid[a] | <0.1 | <2 % |
| 3β,7α,12α-triOH-5-cholenoic acid[a] | <0.1 | <2 % |
| 7αOH-3-oxo-4-cholenoic acid[b] | Trace[d] | [d] |
| 7α,12α-triOH-3-oxo-4-cholenoic acid[b] | Trace[d] | [d] |
| Cholestanepentols[c] | <1.0 | |

[a]Following mild solvolysis and enzymatic hydrolysis of glycine conjugates
[b]Following enzymatic hydrolysis of glycine and taurine conjugates
[c]Following hydrolysis with glucuronidase
[d]Amount of urinary bile acids in healthy neonates, including 3-oxo-$\Delta^4$ bile acids, has been shown to be elevated in the first month of life – 7α,12α diOH-3-oxo-cholenoic acid <13.0 pmol/mmol creatinine (<30 % total bile acid excretion) and 7α-OH-3-oxo-cholenoic acid <0.4 pmol/mmol creatinine (<1 % total bile acid excretion). See Kimura et al. (1999)

**Table 34.16** Plasma cholanoid concentrations

| Plasma cholanoid | Concentration (μmol/l) | |
|---|---|---|
| | Normal | Cholestatic |
| Chenodeoxycholic acid | 0.22–12.4 | 25–359 |
| Cholic acid | 0.05–4.55 | 7–317 |
| Other cholanoids of diagnostic significance[a] | <0.25 | <0.25 |

The data below refers to results obtained by GC-MS following hydrolysis of glycine and taurine conjugates with cholylglycine hydrolase. Normal plasma bile acid concentrations are higher in the postprandial period (½–3 h following a fat-containing meal) than in the fasting state. They are also higher in the neonatal period than later in infancy. For the purposes of diagnosis of inborn errors, these differences are not of great importance and have not been included in the reference data
[a]3β,7α-Dihydroxy-5-cholenoic, 3β,7α,12α-trihydroxy-5-cholenoic, 7α-hydroxy-3-oxo-4-cholenoic, 7α,12α-diOH-3-oxo-4-cholenoic, allocholic, allochenodeoxycholic and 3α,7α, 12α-trihydroxy-5β-cholestanoic acid (THCA)

**Table 34.17** Plasma cholestanol concentrations

| Age | <15 years | >15 years | Cholestatic |
|---|---|---|---|
| Cholestanol (P) (μmol/l) | 1–9 | 4–18 | 4–50 |

## 34.6 Pathological Values

Urinary cholanoid (bile acid and alcohol) profile by ESI-MS

| Ion | Identity | **34.1** 3β-HSDH | **34.2** 5β-reductase[a] | **34.5** Sterol 27-hydroxylase (CTX) | **34.4** Oxysterol 7α-hydroxylase | **34.6** AMACR (+other peroxisome disorders) | **34.7** BAAT | **32.8** Bile acid-CoA ligase |
|---|---|---|---|---|---|---|---|---|
| 234 | $[M-2H]^{2-}$ 3β,7α-Dihydroxy-5-cholenoic acid ($SO_4$) | ±/↑ | | | | | | |
| 263 | $[M-2H]^{2-}$ 3β,7α-Dihydroxy-5-cholenoic acid (Gly,$SO_4$) | ±/↑ | | | | | | |
| 405 | 3β,7α,12α-Trihydroxy-5-cholenoic acid | ±/↑ | | | | | | |
| 407 | Unconjugated cholic acid | | | | | | ↑ | ↑ |
| 444 | 7α-Hydroxy-3-oxo-4-cholenoic acid (Gly) | | ↑ | | | | | |
| 446 | 3β,7α-Dihydroxy-5-cholenoic acid (Gly) | ±/↑ | | | | | | |
| 448 | Dihydroxycholanoic acids (e.g. chenodeoxycholic acid) (Gly) | | –/± | | | ± | – | – |
| 453 | 3β-Hydroxy-5-cholenoic acid ($SO_4$) | | | | ↑ | | | |
| 460 | 7α,12α-Dihydroxy-3-oxo-4-cholenoic acid (Gly) | | ↑ | | | | | |
| 462 | 3β,7α,12α-Trihydroxy-5-cholenoic acid (Gly) | ±/↑ | | | | | | |
| 464 | Trihydroxy-cholanoic acids (e.g. cholic acid) (Gly) | | –/± | | | ± | – | – |
| 469 | 3β,7α-Dihydroxy-5-cholenoic acid ($SO_4$) | ↑ | | | | | | |
| | ?also steroid sulphate | | | | | | | |
| 471 | Chenodeoxycholic acid ($SO_4$) | | | | | | ±/↑ | ↑ |
| 480 | 3β-Hydroxy-5-cholenoic acid (Tau) | | | | ↑ | | | |
| 485 | 3β,7α,12α-Trihydroxy-5-cholenoic acid ($SO_4$) | ↑ | | | | | | |
| 487 | Cholic acid ($SO_4$) | | | | | | ±/↑ | ↑ |
| 494 | 7α-Hydroxy-3-oxo-4-cholenoic acid (Tau) | | ↑ | | | | | |
| 498 | Dihydroxycholanoic acids (e.g. chenodeoxycholic acid) (Tau) | | –/± | | | ± | – | – |
| 510 | 7α,12α-Dihydroxy-3-oxo-4-cholenoic acid (Tau) or 3β-Hydroxy-5-cholenoic acid (Gly, $SO_4$) | | ↑ | | ↑ | | | |
| 514 | Trihydroxy-cholanoic acids (e.g. cholic acid) (Tau) | | –/± | | | ± | – | – |
| 526 | 3β,7α-Dihydroxy-5-cholenoic acid (Gly, $SO_4$) | ↑ | | | | | | |
| 528 | Dihydroxycholanoic acids (e.g. chenodeoxycholic acid) (Gly, $SO_4$) | | | | | ± | | |
| 530 | Tetrahydroxy-cholanoic acids (Tau) | | | | | ± | | |
| 542 | 3β,7α,12α-Trihydroxy-5-cholenoic acid (Gly, $SO_4$) | ↑ | | | | | | |
| 567 | Chenodeoxycholic acid (Gluc) | | | | | | ±/↑ | ↑ |

(continued)

| Ion | Identity | **34.1** 3β-HSDH | **34.2** 5β-reductase[a] | **34.5** Sterol 27-hydroxylase (CTX) | **34.4** Oxysterol 7α-hydroxylase | **34.6** AMACR (+other peroxisome disorders) | **34.7** BAAT | **32.8** Bile acid-CoA ligase |
|---|---|---|---|---|---|---|---|---|
| 572 | Tetrahydroxycholestanoic acids (Tau) | | | | | ±/↑[b] | | |
| 583 | Cholic acid (Gluc) | | | | | ± | ±/↑ | ↑ |
| 611 | Cholestane terols (Gluc) | | | ↑ | | | | |
| 613 | 27-Nor-cholestanepentol(s) (Gluc) | | | ±/↑ | | | | |
| 627 | Cholestanepentols (Gluc) | | | ↑ | | | | |
| 643 | Cholestanehexols (Gluc) | | | ±/↑ | | | | |

[a]In patients considered to have a genetic deficiency of 5β-reductase, the ESI-MS spectrum shows peaks due to 3-oxo-$\Delta^4$ bile acids that are four to five times larger than those due to the corresponding saturated bile acids (i.e. 444>448,460>464,494>498, 514>510). The saturated bile acids may not be detectable above the background. By contrast an ESI-MS spectrum that shows 3-oxo-$\Delta^4$ peaks of similar size to the corresponding saturated bile acid (i.e. 444≡448) indicates severe hepatocyte damage due to something other than genetic 5β-reductase deficiency. In these patients the excretion of 3-oxo-$\Delta^4$ bile acids will disappear when the hepatocyte function improves

[b]In patients with peroxisomal biogenesis, defects over the age of 18 months the ESI-MS analysis may give a negative result

Further analysis of plasma cholanoid profile by GC-MS

| Plasma cholanoids (μmol/l) | **34.1** 3β- HSDH | **34.2** **5**β-reductase | **32.5** Sterol 27-hydroxylase (CTX) | **32.3** Oxysterol 7α-hydroxylase | **32.6** α-Methylacyl-CoA racemase | Peroxisomal disorders |
|---|---|---|---|---|---|---|
| Chenodeoxycholic acid | ↓(<0.1) | ↑/↓(0–25) | ↓ | n | n | n/↑ |
| Cholic acid | n/↓(0–4.5) | n/↓ | n/↓ | n | n | n/↓ |
| 3β,7α-Dihydroxy-5-cholenoic[a] | ↑↑(1–80) | | | | | |
| 3β,7α,12α-Trihydroxy-5-cholenoic[a] | ↑(0.05–30) | | | | | |
| 3β,7α-Dihydroxy-5-cholestenoic | ↑↑(3–40) | | | | | |
| 7α-Hydroxy-3-oxo-4-cholenoic | | ↑(0.8–10.0)[b] | | | | |
| 7α,12α-Dihydroxy-3-oxo-4-cholenoic | | ↑(0.3–2.0)[b] | | | | |
| Allochenodeoxycholic acid | | ↑(0.5–10.0)[c] | | | | |
| Allocholic acid | | ↑(0.5–8.0)[c] | | | | |
| 3β-Hydroxy-5-cholenoic acid | | | | ↑↑(1.4–87) | | |
| 3β-Hydroxy-5-cholestenoic acid | | | | ↑↑(7–24) | | |
| 3α,7α-Dihydroxy-5β-cholestan-26-oic acid [DHCA] | | | | | ↑↑(0.1–0.8)[g] | ↑↑(0.5–12) |
| 3α,7α,12α-Trihydroxy-5β-cholestan-26-oic acid [THCA] | | | | | ↑↑(0.7–23.8)[g] | ↑↑(0.8–30) |
| 5β-Cholestane-3α,7α,12α,25-tetrol | | | ↑↑ | | | |
| Other compounds detected in cholanoid profile | | | [d] | [f] | | [e] |
| Cholestanol | | | ↑(19–400) | | | |

[a]These compounds are present almost entirely as sulphates in the plasma of patients with 3β-HSDH deficiency and will not be detected unless plasma is subjected to a mild solvolysis procedure

[b]3-Oxo-$\Delta^4$-bile acids constitute >10 % of total plasma bile acids

[c]Allo-bile acids constitute >20 % of total

[d]7α-Hydroxycholesterol, 7α-hydroxy-cholest-4-en-3-one, 7α,12α-dihydroxy-cholest-4-en-3-one, 5β-cholestane-3α,7α,12α-triol

[e]$C_{29}$-dicarboxylic acid and tetrahydroxycholestanoic acids in disorders of peroxisome biogenesis. Varanic acid in disorders of D-bifunctional protein and thiolase

[f]27-Hydroxycholesterol

[g]These compounds are [25R]-DHCA/THCA acid stereoisomers

GC-MS analysis is used to confirm the identities of ions in the ESI-MS urine spectrum and to show that the excretion of abnormal cholanoids is >20 times normal. In the case of 5β-reductase deficiency GC-MS analysis should show that 3-oxo-$\Delta^4$ bile acids account for >70 % of the total urinary bile acid excretion. In the case of sterol 27-hydroxylase deficiency (CTX), GC-MS analysis should indicate that the major cholestane pentols in the urine are 3, 7, 12, 22, 25 and 3, 7, 12, 23, 25 pentols. Tandem mass spectrometry (e.g. liquid secondary ion tandem MS[LSI-MS/MS]) is an alternative method to GC-MS and can rapidly confirm the identity of a number of diagnostic ions that are found in the LSI-MS/ESI-MS spectrum of urine. These include sulphated and taurine-conjugated abnormal metabolites such as those observed in 3β-HSDH deficiency (**34.1**), 5β-reductase deficiency (**34.2**), oxysterol 7α-hydroxylase deficiency (**34.3**) and peroxisomal disorders.

## 34.7 Diagnostic Flow Charts

There are also some syndromes which include low γGT cholestasis as well as extrahepatic features, e.g. the arthrogryposis, renal dysfunction and cholestasis (ARC) and Åagenaes (cholestasis lymphoedema) syndromes.

Immunostaining can detect the absence of proteins involved in bile acid metabolism or biliary secretion in a liver biopsy.

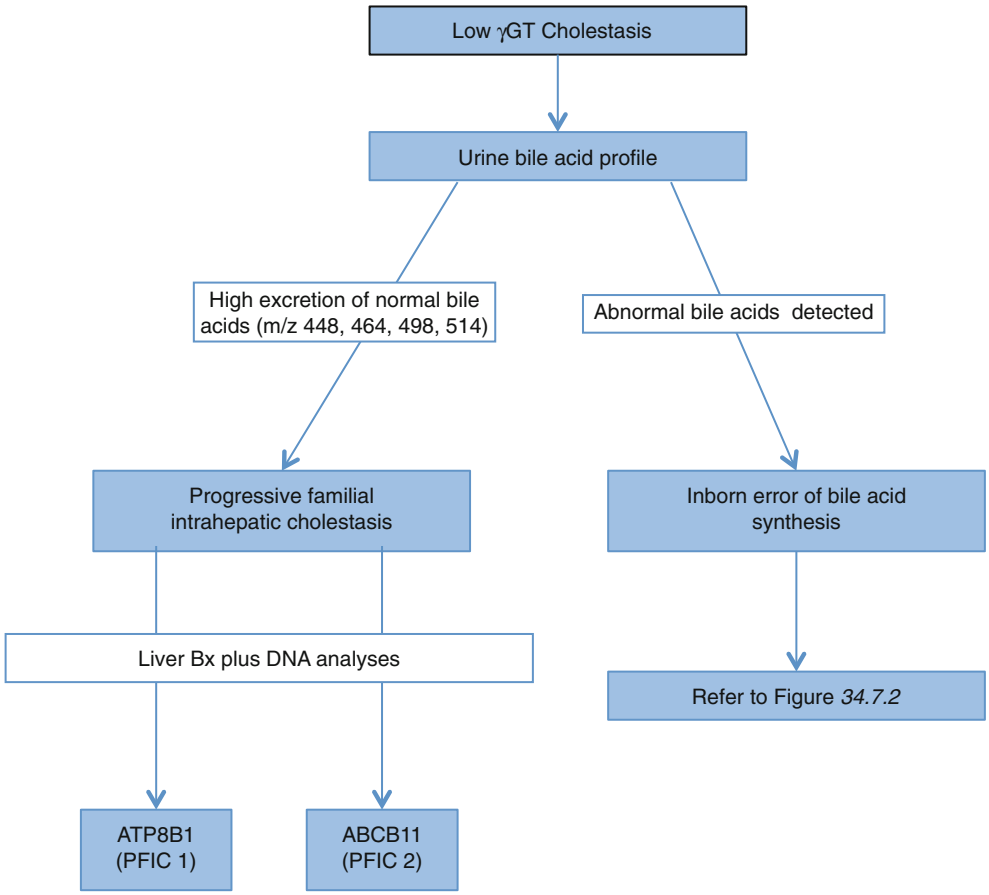


**Fig. 34.3** Diagnostic flowchart for low γGT cholestasis

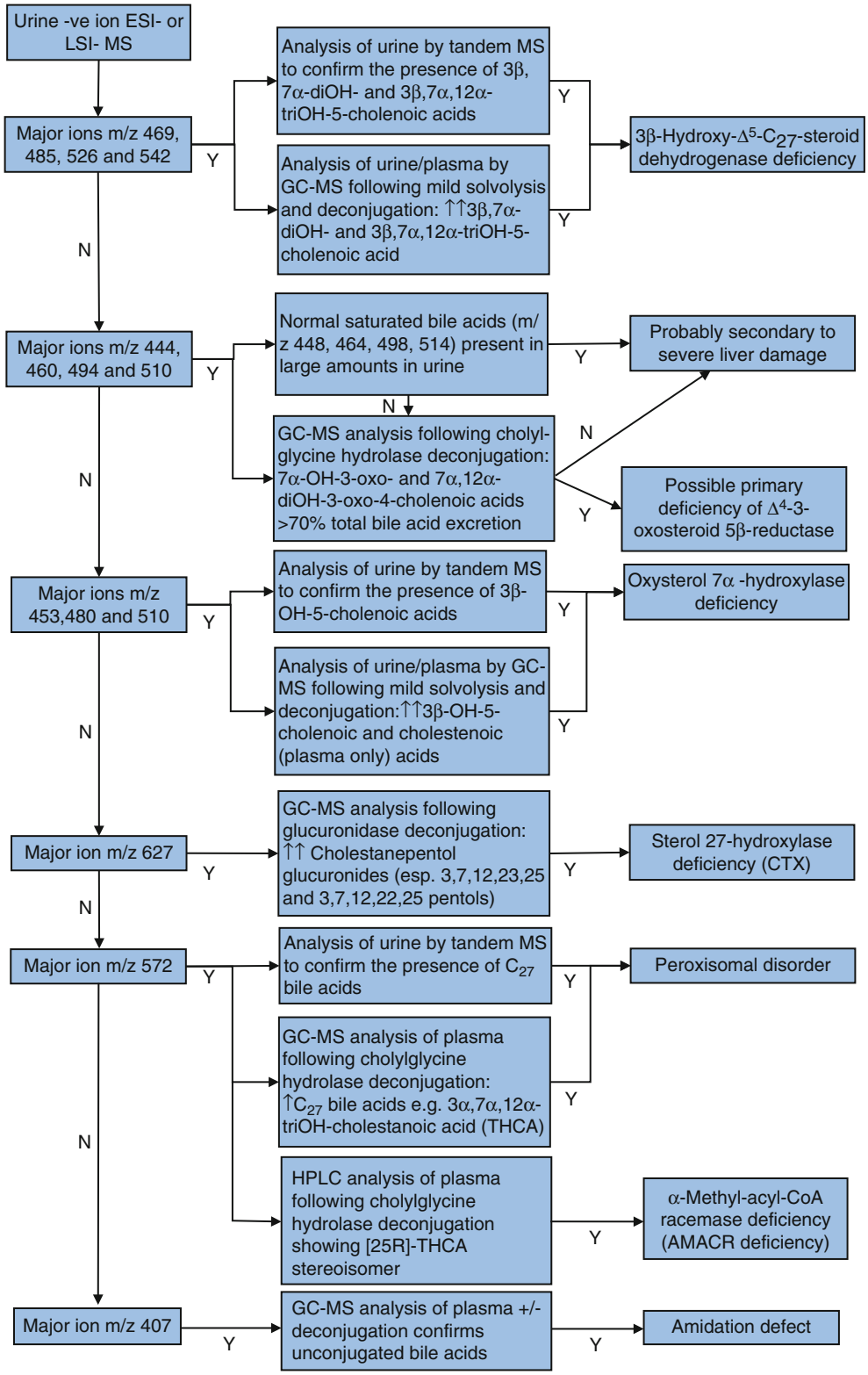


**Fig. 34.4** Diagnostic flow chart for disorders of bile acid synthesis

## 34.8 Specimen Collection

| Test | Conditions | Material | Handling | Pitfalls |
|---|---|---|---|---|
| Urine cholanoid profile by LSI-MS or ESI-MS | No bile acid therapy | Urine ≥0.5 ml | Ambient temp. 12 h, 4 °C for 48 h, −20 °C for >6 months | Drugs and radiographic contrast media may produce large peaks on the LSI-MS spectrum |
| | | Cholanoids from urine adsorbed on $C_{18}$ cartridge (volume of urine and creatinine recorded) | Ambient temp. 48 h | |
| Further analysis of urinary cholanoids by GC-MS | No bile acid therapy | Urine ≥2.0 ml (can be sent on $C_{18}$ cartridge as above) | As above | |
| Plasma bile acids | No bile acid therapy | Plasma/serum 0.5–2.0 ml | Ambient temp. 12 h, 4 °C for 48 h, −20 °C for >6 months | |
| Plasma cholestanol | No bile acid therapy | Plasma/serum 0.2–1.0 ml | As above | |

## 34.9 Prenatal Diagnosis and DNA Testing

Routine specific DNA testing for the inborn errors of bile acid metabolism and biliary secretion is not generally available and is conducted on a research basis only. The advent of whole-exome sequencing will potentially increase the number of patients identified with these disorders. Prenatal diagnosis can be undertaken using DNA analysis.

## 34.10 Treatment

**Summary**

Treatment for most of the synthesis disorders is simple and, if instituted before the onset of significant hepatic damage, effective. Liver function tests and biopsy appearances can be normalised by treatment with oral chenodeoxycholic acid and/or cholic acid. These bile acids enter the enterohepatic circulation and drive bile flow and also inhibit the endogenous production of abnormal bile acids. In some, however, liver damage progresses requiring liver transplantation. Treatment of the consequences of acute vitamin K deficiency is important and can be immediately life saving, especially in the case of hypocalcaemic seizures and haemorrhage secondary to vitamin K deficiency. Not only can the treatment of cholestasis be successful, but the treatment can improve neurological sequelae in these conditions. In CTX, treatment with chenodeoxycholic acid reduces the rate of synthesis of cholestanol and the urinary excretion of bile alcohols and can reverse the patient's neurological disability, with clearing of the dementia, improved orientation, a rise in intelligence quotient and enhanced strength and independence (Berginer et al. 1984).

Patients with PFIC usually require treatment for fat-soluble vitamin deficiency. They often have severe pruritus which is difficult to treat but may respond to drugs such as rifampicin, cholestyramine and ursodeoxycholic acid. Rifampicin has been shown to inhibit the expression of the enzyme autotaxin which is involved in the origin of pruritus (Kremer et al. 2012). No treatments are yet available that can correct the underlying transport defect. Ursodeoxycholic acid promotes bile flow and can probably protect biliary epithelial cells and hepatocytes from damage during cholestasis. It is of proven benefit in ABCB4 deficiency, but, in ATP8B1 and ABCB11 deficiencies, there are conflicting reports of any benefit. Some patients with ATP8B1 and ABCB11 deficiencies have benefitted from partial external biliary diversion or ileal exclusion surgery. However, in all three PFIC disorders, liver damage is progressive and most children ultimately require liver transplantation.

The Dubin-Johnson and Rotor syndromes generally produce mild (and often intermittent) conjugated hyperbilirubinaemia which has no important consequences and no progressive liver disease. So treatment is not required except for severe neonatal cases.

**Initial Management of the Cholestatic Infant with a Bile Acid Synthesis Defect**

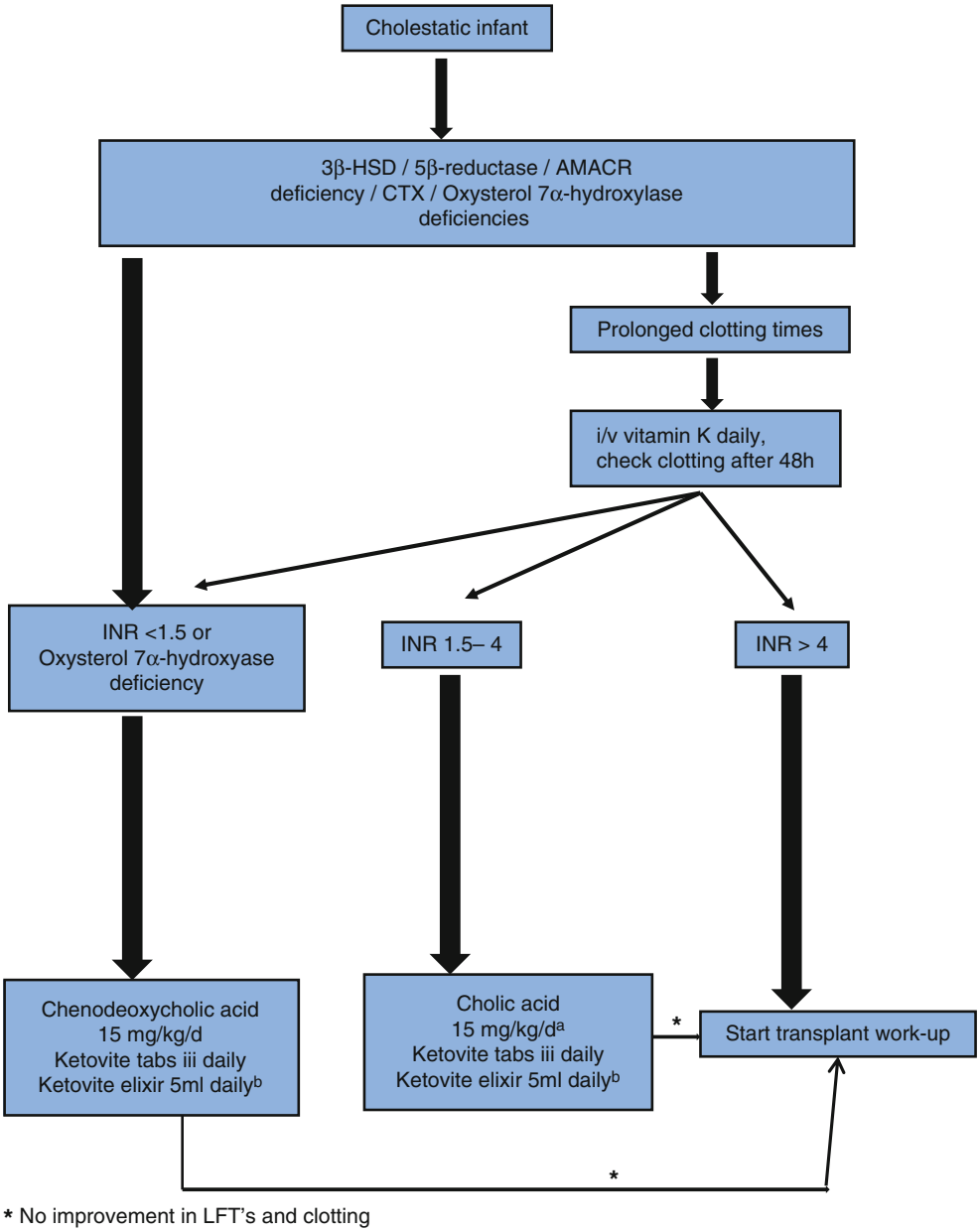


**Fig. 34.5** Initial management of the cholestatic infant with a bile acid synthesis defect. [a]or cholic acid 7.5 mg/kg/day plus chenodeoxycholic acid 7.5 mg/kg/day. [b]rapid healing of rickets may require more vitamin D (as 1,25-dihydroxycholecalciferol) and a calcium supplement

### Treatment of the Consequences of Fat-Soluble Vitamin Malabsorption

Once coagulopathy has been corrected and rickets healed, bile acid replacement therapy should be adequate to prevent any manifestations of fat-soluble vitamin malabsorption; however, it is wise to continue for ca.3 months after starting treatment with a vitamin supplement containing all four fat-soluble vitamins, e.g. Ketovite tabs, iii daily (provides 15 mg α-tocopheryl acetate and 1.5 mg acetomenaphthone), plus Ketovite elixir 5 ml daily (provides 2,500 units of vitamin A and 400 units ergocalciferol).

| Treatment for | Medication | Dose | Route | Target |
|---|---|---|---|---|
| Vitamin K deficient bleeding | Vitamin K (phytomenadione) | 1 mg daily | i/v slowly[a] | Normal clotting times |
| Hypocalcaemia (fits, tetany) | 10 % Calcium gluconate (plus 1,25-dihydroxycholecacliferol, see below) | 0.1–0.3 ml/kg/dose | i/v slowly | Normal ionised calcium |
| Rickets | 1,25-Dihydroxycholecalciferol | 0.25–1.00 μg/day | Oral | Normal calcium, healing of rickets. Avoidance of hypercalcaemia |
| Vitamin E deficiency | Alpha-tocopherol acetate or water-soluble/ miscible vit E preparation | 50 mg | Oral | Normal plasma vitamin E |
| Vitamin A deficiency | Vitamin A or water miscible analogue | 2,500 Units e.g. Ketovite elixir 5 ml daily | Oral | Normal plasma vitamin A |
| Basic defect | Chenodeoxycholic acid and/or cholic acid | See individual disorders | Oral | Normal liver function tests, etc. |

[a]Immediate treatment of a coagulopathy caused by vitamin K deficiency may be life saving but intravenous phytomenadione can cause anaphylaxis

### Specific Treatment Strategies

3β-Hydroxysteroid-$\Delta^5$-$C_{27}$-steroid dehydrogenase deficiency

| Treatment for | Medication | Dose | Route | Target |
|---|---|---|---|---|
| Basic defect | Chenodeoxycholic acid Initial dose<br>After 2 months<br>*or*<br>Chenodeoxycholic acid<br>plus<br>Cholic acid | 12–18 mg/kg/day<br>9–12 mg/kg/day<br><br>7 mg/kg/day<br><br>7 mg/kg/day | Oral<br>Oral<br><br>Oral<br><br>Oral | Normalisation of liver function tests, prevention of fat-soluble vitamin malabsorption |
| Consequences of fat-soluble vitamin malabsorption | See above | See above | See above | See above |

$\Delta^4$-3-Oxosteroid-5β-reductase deficiency

| Treatment for | Medication | Dose | Route | Target |
|---|---|---|---|---|
| Basic defect | Chenodeoxycholic acid<br>plus<br>Cholic acid<br>or<br>Cholic acid alone | 8 mg/kg/day<br><br>8 mg/kg/day<br><br>15 mg/kg/day | Oral<br><br>Oral<br><br>Oral | Normal liver function tests |
| Consequences of fat-soluble vitamin malabsorption | See above | See above | See above | See above |
| Failure to respond to bile acid replacement | Liver transplant | | | |

(Clayton et al. 1996; Clayton 2011)

Patients with 5$\beta$-reductase deficiency usually present with cholestatic liver disease in infancy. It is important to distinguish patients with mutations in the 5$\beta$-reductase gene from patients in whom excretion of 3-oxo-$\Delta^4$ bile acids is secondary to severe liver damage caused by another genetic disorder (e.g. tyrosinaemia) or an acquired disorder (e.g. hepatitis B).

Oxysterol-7α-hydroxylase deficiency

| Treatment for | Medication | Dose | Route | Target |
|---|---|---|---|---|
| Basic defect | Chenodeoxycholic acid | 11 mg/kg/day | Oral | Normal liver function tests |
| Consequences of fat-soluble vitamin malabsorption | See above | See above | See above | See above |
| Failure to respond to bile acid replacement | Liver transplant | | | |

The three patients described initially were treated with ursodeoxycholic acid +/− liver transplantation, either as a result of initial severe liver disease or lack of chenodeoxycholic acid available for therapy. One recently described patient, postulated to have a deficiency of oxysterol 7α-hydroxylase, responded well to chenodeoxycholic acid therapy (Chong et al. 2010). If the liver disease does not respond to bile acid treatment, cholestasis will persist until liver transplantation can be undertaken (Mizuochi et al. 2011). Therefore, these children require forms of the fat-soluble vitamins that are water soluble or can be given by injection.

Cholesterol 7α-hydroxylase deficiency

| Treatment for | Medication | Dose | Route | Targets |
|---|---|---|---|---|
| Hyperlipidaemia | Atorvastatin<br>plus<br>Niacin | 40–80 mg/day (adult dose)<br>4–7 g/day (adult dose) | Oral<br>Oral | Normal cholesterol |

Sterol 27-hydroxlase deficiency (CTX)

| Treatment for | Medication | Dose | Route | Target |
|---|---|---|---|---|
| Cholestasis in infancy | Cholic acid | 7–15 mg/kg/day | Oral | Normal liver function tests |
| Consequences of fat-soluble vitamin malabsorption | See above | See above | See above | See above |
| Dementia, neurological disease, xanthomata | Chenodeoxycholic acid | 750 mg daily (adult dose) | Oral | Reduced bile alcohol excretion, plasma cholestanol, improved neurology |

α-Methylacyl-CoA racemase deficiency

| Treatment for | Medication | Dose | Route | Target |
|---|---|---|---|---|
| Cholestasis in infancy | Cholic acid | 15 mg/kg/day | Oral | Normal liver function tests |
| Consequences of fat-soluble vitamin malabsorption | See above | See above | See above | See above |
| Pristanic acid accumulation ?contributing to neurological disease | Low phytanate diet | | | Prevention/amelioration of neurological disease |

Bile acid-CoA: amino acid N-acyltransferase deficiency (BAAT deficiency)

| Treatment for | Medication | Dose | Route | Target |
|---|---|---|---|---|
| Cholestasis in infancy | Ursodeoxycholic acid[a]<br>Glycocholic acid[b] | 30 mg/kg/day (3 doses)<br>15 mg/kg/day | Oral<br>Oral | Normal liver function tests and jaundice |
| Consequences of fat-soluble vitamin malabsorption | See above | See above | See above | See above |

[a]One UK patient has been described who showed a good response to ursodeoxycholic acid
[b]An initial report of 6 US patients suggests glycocholic therapy improves vitamin D absorption and helps normalise growth (Heubi et al. 2009)

Bile acid-Co ligase deficiency

| Treatment for | Medication | Dose | Route | Target |
|---|---|---|---|---|
| Cholestasis in infancy | Ursodeoxycholic acid | 30 mg/kg/day (3 doses) | Oral | Normal liver function tests |
| Consequences of fat-soluble vitamin malabsorption | See above | See above | See above | See above |

The requirement for treatment in this condition is unclear. Only two siblings have been described with a bile acid-CoA ligase deficiency, one of whom was asymptomatic and did not require treatment. Another sibling was treated with ursodeoxycholic acid for presumed TPN-related cholestasis, and only after resolution of cholestasis and termination of therapy was a diagnosis made. The patient remains well and has not required further therapy.

ATP8B1 disease (PFIC1)

| Treatment for | Medication | Dose | Route | Target |
|---|---|---|---|---|
| Consequences of fat-soluble vitamin malabsorption | See above | See above | See above | See above |
| Pruritus | Cholestyramine | 1 month–1 year: 1–9 g/day<br>1–6 years: 2–18 g/day<br>6–12 years: 4–24 g/day<br>12–18 years: 4–36 g/day | Oral[a] | Relief of pruritus<br>Monitor for folate deficiency; supplement if necessary |
| | Rifampicin | 5 mg/kg/day | | Relief of pruritus<br>Monitor LFT's and stop if serious rise in transaminase |
| Pruritus and progression of liver disease | Ursodeoxycholic acid<br>Partial external biliary diversion | 30 mg/kg/day (3 doses) | Oral | Relief of pruritus |
| Cirrhosis /liver failure | Liver transplantation[b] | | | |

[a]For lower doses, a single dose in the morning can be used; higher doses need three times daily regimen
[b]Liver transplantation does not cure the extrahepatic problems

ABCB11 disease (PFIC2)

| Treatment for | Medication | Dose | Route | Target |
|---|---|---|---|---|
| Consequences of fat-soluble vitamin malabsorption | See above | See above | See above | See above |
| Pruritus | Cholestyramine<br>Rifampicin | See above | See above | See above |
| Pruritus and progression of liver disease | Ursodeoxycholic acid<br>Partial external biliary diversion | | | |
| Cirrhosis /liver failure | Liver transplantation | | | |

ABCB4 disease (PFIC3)

| Treatment for | Medication | Dose | Route | Target |
|---|---|---|---|---|
| Progressive damage to bile ducts, cirrhosis | Ursodeoxycholic acid | 30 mg/kg/day (3 doses) | Oral | |
| Cirrhosis, liver failure | Liver transplantation | | | |

OATPB1 and OATPB3 disease (Rotor syndrome)

| Treatment for | Medication | Dose | Route | Target |
|---|---|---|---|---|
| Jaundice | Outside neonatal period not necessary. Severe neonatal jaundice; see below, Table for ABCC2 disease | | | |
| Risk of drug toxicity | Avoid drugs unless there is a strong indication[a] | | | |

[a]The OATPB1 and OATPB3 organic anion transporters are involved in the hepatic uptake and metabolism of many drugs. Defective metabolism could lead to increased toxicity so all drugs should be used with caution and only when there is a strong indication

ABCC2 disease (Dubin-Johnson syndrome)

| Treatment for | Medication | Dose | Route | Target |
|---|---|---|---|---|
| Jaundice | Outside neonatal period not necessary | | | |
| Severe neonatal jaundice | Phenobarbitone | 5 mg/kg/day in 2 doses | Oral | Reduction in plasma conjugated bilirubin |
| | Ursodeoxycholic acid | 30 mg/kg/day (3 doses) | Oral | Reduction in plasma conjugated bilirubin |

(Kimura et al. 1991; Regev et al. 2002)

### Alternative Therapies/Experiment Treatment

Cerebrotendinous xanthomatosis

| Mode of treatment | Dose | Route | Comment |
|---|---|---|---|
| Lovastatin (mevinolin) [HMG-CoA reductase inhibitor] | 6.25 mg twice daily (adult dose) | Oral | Insufficient data to assess efficacy |
| Low density lipoprotein apheresis | | | Insufficient data to assess efficacy |

(Lewis et al. 1983)

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