Chapter 9
Bile Acids and Cholestatic Liver Disease 3: Inborn Errors of Bile Acid Synthesis

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Abstract Inborn errors of bile acid synthesis are rare genetic disorders that can present as neonatal cholestasis and fat-soluble vitamin deficiency. Though rare, these diseases account for some patients with cholestasis of unknown etiology. Seven known defects of bile acid synthesis occur in children. These defects may be categorized as deficiencies in activity of enzymes catalyzing reactions affecting the steroid nucleus or the side chain. Defects in reactions involving the steroid nucleus include cholesterol 7α-hydroxylase deficiency, 3β-hydroxysteroid-Δ^5-C_{27}-steroid dehydrogenase/isomerase deficiency, Δ^4-3-oxosteroid 5β-reductase deficiency, and oxysterol 7α-hydroxylase deficiency. Defects in reactions involving side-chain modification are sterol 27-hydroxylase deficiency, α-methyl-CoA racemase deficiency, disorders of peroxisomal β-oxidation, bile acid-CoA: amino acid N-acyltransferase deficiency, and bile acid-CoA ligase deficiency. Cholesterol 7α-hydroxylase deficiency and disorders of peroxisomal β-oxidation are not considered here, since cholesterol 7α-hydroxylase deficiency is not known to occur in children and disorders of peroxisomal β-oxidation represent disease of peroxisomes. When identified early, many patients with inborn errors of bile acid synthesis have a favorable clinical response to oral primary bile acid therapy. These inborn errors characteristically result in elevated serum bilirubin and aminotransferase concentrations but produce no abnormalities of serum γ-glutamyltransferase or of total bile acid concentrations detectable by enzymatic methods. Screening for

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inborn errors of bile acid synthesis using fast atom bombardment ionization mass spectrometry, gas chromatography-mass spectroscopy, and liquid chromatography-electrospray ionization tandem mass spectrometry is useful. Genetic analysis is available for a definitive diagnosis. Disorders of bile acid synthesis account for some 2–3% of screened cases of cholestatic liver disease in infants and children.

Keywords Inborn error of bile acid synthesis (IEBAS) • Cholestasis • Giant cell hepatitis • γ-Glutamyl-transpeptidase (GGT) • Oral primary bile acid therapy

9.1 Two Main Pathways from Cholesterol to Primary Bile Acids [1, 2]

The primary bile acids, cholic acid (CA) and chenodeoxycholic acid (CDCA), are synthesized by sequential enzymatic modifications to cholesterol that involve at least 14 enzymes, multiple subcellular compartments, and two complementary chemical pathways (Fig. 9.1). There are two pathways of 7α-hydroxylation that include a neutral pathway in which cholesterol is hydroxylated by cholesterol 7α-hydroxylase and an acidic pathway in which cholesterol is hydroxylated and oxidized at position 27 and then hydroxylated by oxysterol 7α-hydroxylase. Nine defects in bile acid synthesis show a phenotype of familial and progressive infantile or late-onset cholestasis or fat-soluble vitamin deficiency. In this chapter, however, cholesterol 7α-hydroxylase (CYP7A1) deficiency and disorder of peroxisomal β-oxidation are not considered, because CYP7A1 deficiency has not been found to occur in children and disorders of peroxisomal β-oxidation are disease of peroxisomes, representing a separate category.

9.2 Clinical Features and Diagnosis of Inborn Errors of Bile Acid Synthesis [3]

Although inborn errors of bile acid synthesis (IEBAS) show cholestasis, serum total bile acid (TBA) concentrations are normal when measured by enzymatic methods. Serum γ-glutamyltransferase (GGT) concentrations also are normal. Even though the patient shows obstructive jaundice, pruritus is absent. Histopathologic findings associated with defects involving reactions affecting the steroid nucleus vary with patient age and rate of disease progression. Specimens from infants with impaired steroid nucleus modification show giant cell hepatitis, canalicular bile plugs, hepatocyte bile stasis, and portal tract inflammation, with variable severity of fibrosis. Generally, urinary screening for IEBAS uses fast atom bombardment ionization mass spectrometry (FAB-MS) in the USA and Europe and gas chromatography-mass spectroscopy (GC-MS) or liquid chromatography-electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS) in Japan [4] (Table 9.1). Genetic analysis is available for definitive diagnosis [5].

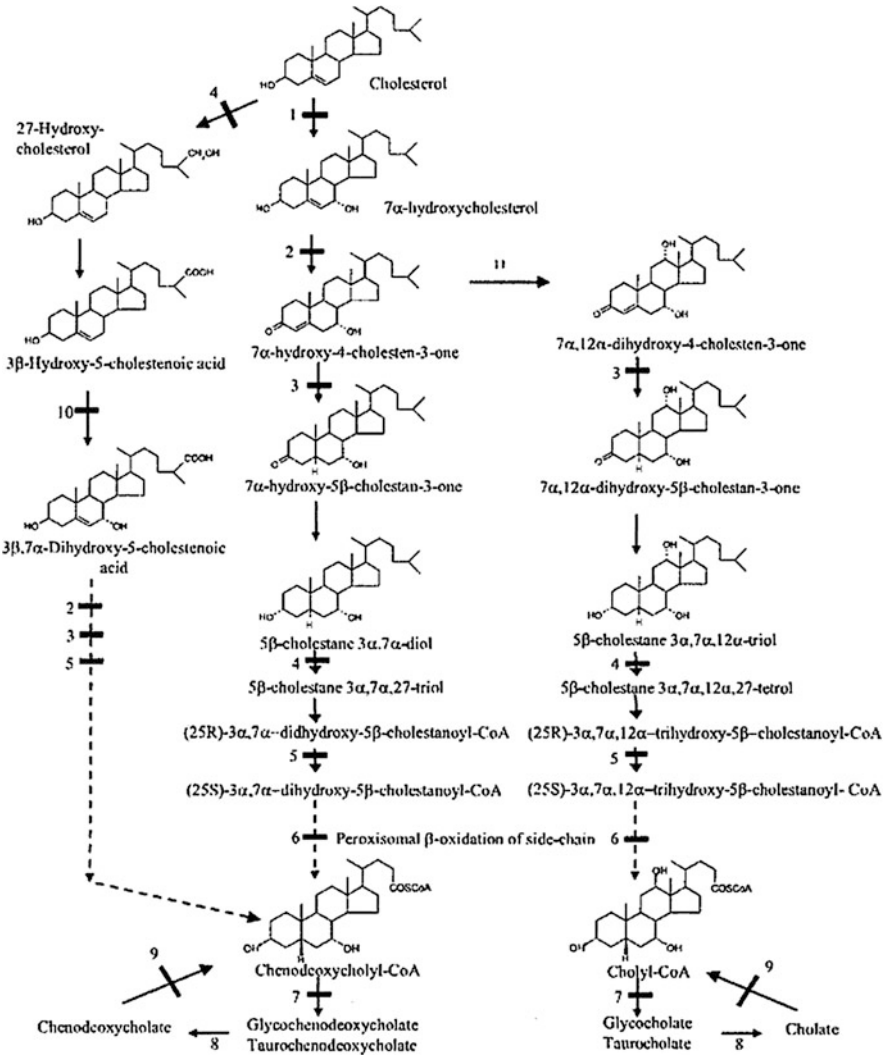

Fig. 9.1 Simplified scheme of the two major pathways for the synthesis of bile acids from cholesterol and for their recycling. The "neutral" pathway starts with conversion of cholesterol to 7α-hydroxycholesterol, while the "acidic" pathway begins with formation of 27-hydroxycholesterol. Numbered bars indicate blockades imposed by enzymatic defects. (*1*) cholesterol 7α-hydroxylase; (*2*) 3β-hydroxy-Δ^5-C_{27}-steroid dehydrogenase/isomerase; (*3*) Δ^4-3-oxosteroid 5β-reductase; (*4*) sterol 27-hydroxylase; (*5*) α-methylacyl CoA racemase; (*6*) proteins involved in peroxisomal biogenesis and β-oxidation; (*7*) bile acid-CoA: amino acid N-acyl transferase; (*8*) bacterial deconjugation in the gut; (*9*) bile acid-CoA ligase; (*10*) oxysterol 7α-hydroxylase; (11) sterol 12α-hydroxylase. Known enzyme defects are depicted by solid bars across the arrows (Adapted from [1])

Table 9.1 Bile acid analysis of the urine using GC/MS in three patients with inborn errors of bile acid synthesis

Patient	*HSD3B7* deficiency (F, 22years)				*SRD5B1* deficiency (M, 6 months)				*CYP7B1* deficiency (F, 6 months)			
	Before		After 3 years		Before		After 4 years		Before		After 1 month	
Treatment	mmol/molCre (%)		mmol/molCre (%)		mmol/molCre (%)		mmol/molCre (%)		mmol/molCre (%)		mmol/molCre (%)	
Total bile acids	115.4		0.8		125.9		6.2		109.5		11.4	
Usual bile acids	2.2	(9.8)	0.2	(30.2)	1.2	(1.0)	0.5	(8.7)	4.9	(9.7)	2.7	(92.9)
UDCA	93.0	–	0.1	–	0.0	–	0.0	–	59.2	–	8.5	–
3-Oxo-Δ^4-bile acids	0.0	(0.0)	0.0	(0.0)	123.9	(98.4)	5.4	(87.0)	2.0	(3.9)	0.0	(1.4)
Monohydroxy-Δ^5-bile acids	0.0	(0.0)	0.1	(11.1)	0.0	(0.0)	0.1	(1.3)	41.7	(82.9)	0.0	(0.0)
Dihydroxy-Δ^5-bile acids	5.0	(22.5)	0.1	(12.7)	0.0	(0.0)	0.0	(0.3)	0.2	(0.3)	0.0	(0.0)
Trihydroxy-Δ^5-bile acids	15.2	(67.7)	0.3	(44.4)	0.0	(0.0)	0.0	(0.0)	0.0	(0.0)	0.0	(0.0)
Others	0.0	(0.0)	0.0	(1.6)	0.8	(0.6)	0.2	(2.7)	1.6	(3.1)	0.2	(5.8)

Two patients, with 3β-hydroxysteroid-Δ^5-C_{27}-steroid dehydrogenase/isomerase (*HSD3B7*) deficiency and Δ^4-3-oxosteroid 5β-reductase (*SRD5B1*) deficiency, underwent oral chenodeoxycholic acid treatment. A patient with oxysterol-7α-hydroxylase (*CYP7B1*) deficiency underwent living donor liver transplantation

9.3 Inborn Errors of Bile Acid Synthesis [3]

9.3.1 Defects Involving Reactions Affecting the Steroid Nucleus

9.3.1.1 3β-Hydroxy-Δ^5-C_{27}-Steroid Dehydrogenase/Isomerase (3βHSD, *HSD3B7*) Deficiency [6]

3βHSD deficiency, the most common bile acid synthetic defect, is caused by mutation in the *HSD3B7* gene on chromosome 16p. The inheritance pattern is autosomal recessive. The major bile acids present in serum and excreted as sulfate esters in the urine are Δ^5-3β,7α-dihydroxy-5-cholenoic and Δ^5-3β,7α,12α-trihydroxy-5-cholenoic acids. About 50 patients with this disorder have been reported. Even adults with this disease have been reported reflecting its relatively mild nature. Some treated patients with this disease have had normal children [7]. Orally administered primary bile acids (CA and/or CDCA) represent an effective treatment that may normalize and growth and development. However, ursodeoxycholic acid (UDCA) is not effective. Bile acid profiles in serum and urine after bile acid therapy show a marked decrease in amounts of unusual bile acids but no decrease in their percentages.

9.3.1.2 Δ^4-3-Oxosteroid 5β-Reductase (5β-Reductase, *SRD5B1*, *AKR1D1*) Deficiency [8, 9]

5β-Reductase deficiency is an autosomal recessive disorder. The affected enzyme, Δ^4-3-oxosteroid-5β-reductase, is encoded by the gene *AKR1D1* (or *SRD5B1*) and converts 7α-hydroxy-4-cholesten-3-one and 7α,12α-dihydroxy-4-cholesten-3-one into 3-oxo-5β analogues. Excretion of large amounts of 3-oxo-Δ^4-bile acids also may be detected in urine from children with severe liver disease arising from causes other than primary defects in bile acid biosynthesis. An alternative clinical presentation, neonatal liver failure resembling neonatal hemochromatosis, also has been described in patients with 5β-reductase deficiency, although no patient with this presentation has been shown to have mutations in the *AKR1D1* gene. Histopathology in patients with 5β-reductase deficiency is typical for neonatal hepatitis, with findings of giant cell hepatitis, pseudoacinar transformation, hepatocellular and canalicular cholestasis, and extramedullary hematopoiesis. Investigation of the urinary steroid profile of patients with this disease showed that tetrahydrocortisone, whose synthesis is catalyzed by 5β-reductase activity in the liver, is decreased; however, no symptoms of adrenal dysfunctional arise, because of compensation by 5β-reductase [10].

9.3.1.3 Oxysterol 7α-Hydroxylase (*CYP7B1*) Deficiency [11]

Deficiency involving the enzyme oxysterol 7α-hydroxylase, which is enclosed by the *CYP7B1* gene, interrupts the alternative acidic pathway for synthesis of the bile acid steroid nucleus. Liver function tests show elevated alanine aminotransferase, but serum TBA and GGT are within the normal range. Large amounts of 3β-monohydroxy-Δ^5 bile acids are detected in serum and urine. Several patients have been reported to be homozygous for nonsense mutations in the *CYP7B1* gene encoding oxysterol 7α-hydroxylase. Further information concerning possible consequences of oxysterol 7α-hydroxylase deficiency has been uncovered by a gene mapping study of hereditary spastic paraplegia [12]. Patients with oxysterol 7α-hydroxylase deficiency have a marked bleeding tendency and are severely ill. Recently, two patients rescued by liver transplantation [13] or CDCA therapy (11 mg/kg/day) [14] have been reported.

9.3.2 Defects Involving Reactions Leading to Side-Chain Modification

9.3.2.1 Sterol 27-Hydroxylase (*CYP27A1*) Deficiency

CYP27A1 deficiency has two possible phenotypes. One is a cholestatic disorder with neonatal [15]. Another is cerebrotendinous xanthomatosis (CTX), which develops in adolescence [16].

A defect in side-chain oxidation via the 25-hydroxylation pathway has been in a reported 9-week-old infant who presented with familial giant cell hepatitis and severe intrahepatic cholestasis. Diagnosis was based upon findings of reduced primary bile acid concentrations and elevated concentrations of specific bile alcohol glucuronides in serum. This boy had consistently normal aminotransferase concentrations. Bile alcohol production was suppressed by primary bile acids, CDCA and CA. Subsequently the patient was diagnosed with CYP27A1 deficiency by demonstrational a mutation in *CYP27A1* [17], after which similar cholestatic disease has been reported in neonates [18].

CTX is a rare inherited lipid storage disease characterized by progressive neurologic dysfunction, dementia, ataxia, and cataracts. This disorder results from abnormal side-chain modification of bile acids, which is caused by mitochondrial sterol 27-hydroxylase deficiency. In patients with CTX, primary bile acid synthesis is reduced, while bile alcohol glucuronide excretion in bile, urine, and stools is increased. Serum and plasma cholesterol concentrations are low or normal, while plasma cholestanol concentrations are markedly elevated. In early childhood, CTX may present with chronic diarrhea and cataracts or with developmental delay/regression. Later in childhood, CTX may present with tendon xanthomata, low IQ, or psychiatric illness. Diagnosis of CTX is established by the finding of a greatly increased plasma cholestanol/cholesterol ratio or a characteristic metabolite

in urine, followed by DNA sequencing of *CYP27A1*. Oral CDCA therapy is effective.

9.3.2.2 α-Methylacyl-CoA Racemase (AMACR) Deficiency [19]

AMACR deficiency is an autosomal recessive disorder in which cholesterol side-chain oxidation is inhibited. AMACR is necessary for racemization of trihydroxycholestenoic acid and pristanic acid into their stereoisomers. Conversion of these stereoisomers is necessary for the subsequent step of peroxisomal β-oxidation of the C27 bile acid side chain. AMACR deficiency affects both bile acid and fatty acid synthesis pathways. Histopathologic findings in infants with this disease include giant cell transformation, moderate intralobular cholestasis, scattered necrotic hepatocytes, and foci with multinucleated hepatocytes. Affected infants' liver enzyme activities were normalized by treatment with CA therapy (15 mg/kg/day) and fat-soluble vitamin supplements [19].

9.3.3 Bile Acid Conjugation Defects

Conjugation of CA and CDCA to taurine or glycine, the final step in primary bile acid synthesis, is catalyzed by bile acid-CoA: amino acid N-acyltransferase (BAAT) in the liver, after which the conjugate is excreted via the intestine, where bacterial deconjugation occurs. Reconjugation in the course of the enterohepatic circulation requires two enzymes, bile acid-CoA ligase (*SLC27A5*) and BAAT, in the liver (Fig. 9.1).

9.3.3.1 Bile Acid-CoA: Amino Acid N-Acyltransferase (BAAT) Deficiency [20]

Patients affected by defects in bile acid conjugation present with marked malabsorption and deficiencies of fat-soluble vitamins. These symptoms occur as a consequence of decreased biliary secretion of conjugated bile acids. Severe cholestasis and liver failure also have been described in patients with bile acid conjugation defects. Oral administration of conjugated primary bile acid, such as glycocholic acid (15 mg/kg/day), is a potential treatment for these patients [21].

9.3.3.2 Bile Acid-CoA Ligase (*SLC27A5*) Deficiency [22]

A patient with this disorder developed conjugated hyperbilirubinemia which persisted until the age of 12 months but was unaccompanied by cholestasis. A liver biopsy specimen showed portal-to-portal bridging fibrosis. Analysis of urinary

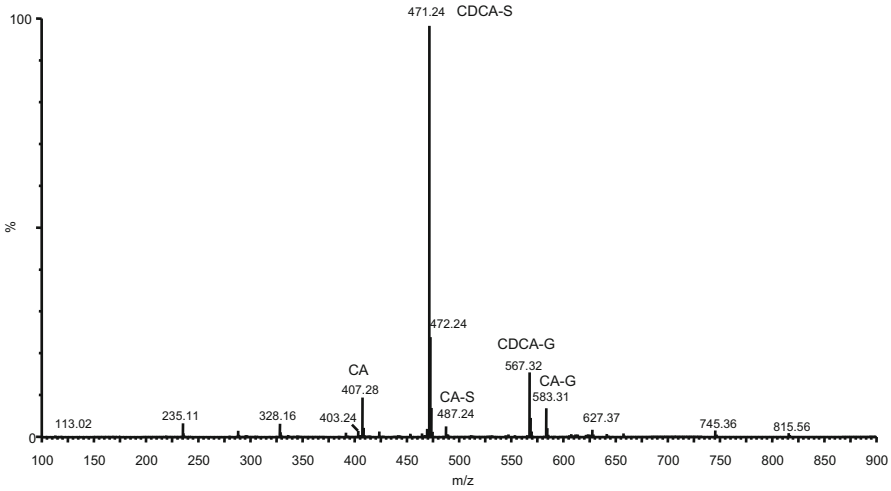

Fig. 9.2 Bile acid electrospray ionization mass spectra in urine of a patient with bile acid amidation defect. Abscissa, mass-charge ratio (m/z); ordinate, intensity of ion current as percentage of intensity of most abundant peak in spectrum (%). Principal peak sites and the species represented are unamidated cholic acid (CA), 407; unamidated chenodeoxycholic acid sulfate (CDCA-S), 471; unamidated cholic acid sulfate CA-S), 487; unamidated chenodeoxycholic acid glucuronide (CDCA-G), 567; unamidated cholic acid glucuronide (CA-G), 583

cholanoids by negative ion electrospray ionization mass spectrometry showed the major cholanoids to be similar to those seen in BAAT deficiency, with the major peak representing unconjugated CA. Most serum bile acids were unconjugated. Sequencing of the BAAT gene showed no mutation, but sequencing of the *SLC27A5* gene, which encodes bile acid-CoA ligase, detected homozygous mutations in a histidine residue. The treatment given was oral UDCA and fat-soluble vitamins. Interestingly, this patient had a homozygous mutation in the bile salt export pump (BSEP, *ABCB11*).

Recently, we encountered a patient with an amidation defect. The specimen dried urine drops on filter paper was sent from Thailand to our institution. Results of bile acid analysis in this material showed a likely amidation defect; almost all bile acids were conjugated to sulfate or glucuronide (Fig. 9.2). Unfortunately, no genetic analysis was performed in this patient.

References

1. Clayton PT. Disorders of bile acid synthesis. J Inherit Metab Dis. 2011;34:593–604.
2. Sundaram S, Bove KE, Lovell MA, Sokol RJ. Mechanisms of disease: inborn errors of bile acid synthesis. Nat Clin Pract Gastroenterol Hepatol. 2008;5:456–68.

3. Setchell KDR, O'Connell NC. Disorders of bile acid synthesis and metabolism: a metabolic basis for liver disease. In: Suchy FJ, Sokol RJ, Balistereri WF, editors. Liver disease in children. New York: Cambridge University Press; 2007. p. 736–66.
4. Muto A, Takei H, Unno A, Murai T, Kurosawa T, Ogawa S, et al. Detection of Δ^4-3-oxo-steroid 5β-reductase deficiency by LC-ESI-MS/MS measurement of urinary bile acids. J Chromatogr B. 2012;900:24–31.
5. Seki Y, Mizuochi T, Kimura A, Takahashi T, Ohtake A, Hayashi S-I, et al. Two neonatal cholestasis patients with mutations in the SRD5B1 (*AKR1D1*) gene: diagnosis and bile acid profiles during chenodeoxycholic acid treatment. J Inherit Metab Dis. 2013;36:565–73.
6. Clayton PT, Leonard JV, Lawson AM, Setchell KDR, Andersson S, Egestad B, et al. Familial giant cell hepatitis associated with synthesis of 3β,7α-dihydroxy- and 3β, 7α,12α–trihydroxy-5-cholenoic acids. J Clin Invest. 1987;79:1031–8.
7. Cheng JB, Jacquemin E, Gerhardt M, Nazer H, Cresteil D, Heubi JE, et al. Molecular genetics of 3β-hydroxy-Δ^5-C_{27}-steroid oxidoreductase deficiency in 16 patients with loss of bile acid synthesis and liver disease. J Clin Endocrinol Metab. 2003;88:1833–41.
8. Setchell KDR, Suchy FJ, Welsh MB, Zimmer-Nechemias L, Heubi JE, Balistreri WF. Δ^4-3-oxosteroid 5β-reductase deficiency described in identical twins with neonatal hepatitis. A new inborn error in bile acid synthesis. J Clin Invest. 1988;82:2148–57.
9. Ueki I, Kimura A, Chen H-L, Yorifuji T, Mori J, Itoh S, et al. SRD5B1 gene analysis needed for the accurate diagnosis of primary 3-oxo-Δ^4-steroid 5β-reductase deficiency. J Gastroenterol Hepatol. 2009;24:776–85.
10. Yanagi T, Mizuochi T, Homma K, Ueki I, Seki Y, Hasegawa T, et al. Distinguishing primary from secondary Δ^4-3-oxosteroid 5β-reductase (*SRD5B1, AKR1D1*) deficiency by urinary steroid analysis. Clin Endocrinol. 2015;82:346–51.
11. Setchell KDR, Schwarz M, O'Connell NC, Lund EG, Davis DL, Lathe R, et al. Identification of a new inborn error in bile acid synthesis: mutation of the oxysterol 7α-hydroxylase gene causes severe neonatal liver disease. J Clin Invest 1998; 102:1690–703.
12. Tsaousidou MK, Ouahchi K, Warner TT, Yang Y, Simpson MA, Laing NG, et al. Sequence alterations within CYP7B1 implicate defective cholesterol homeostasis in motor-neuron degeneration. Am J Hum Genet. 2008;82:510–5.
13. Mizuochi T, Kimura A, Suzuki M, Ueki I, Takei H, Nittono H, et al. Successful heterozygous living donor liver transplantation for an oxysterol 7α-hydroxylase deficiency in a Japanese patient. Liver Transpl. 2011;17:1059–65.
14. Dai D, Mills PB, Footitt E, Gissen P, McClean P, Stahlschmidt J, et al. Liver disease in infancy caused by oxysterol 7α-hydroxylase deficiency: successful treatment with chenodeoxycholic acid. J Inherit Metab Dis. 2014;37:851–61.
15. Clayton PT, Casteels M, Mieli-Vergani G, Lawson AM. Familial giant cell hepatitis with low bile acid concentrations and increased urinary excretion of specific bile alcohols: a new inborn error of bile acid synthesis? Pediatr Res. 1995;37:424–31.
16. Cali JJ, Hsieh CL, Francke U, Russell DW. Mutations in the bile acid biosynthetic enzyme sterol 27-hydroxylase underlie cerebrotendinous xanthomatosis. J Biol Chem. 1991;266:7779–83.
17. Clayton PT, Verrips A, Sistermans E, Mann A, Mieli-Vergani G, Wevers R. Mutations in the sterol 27-hydroxylase gene (CYP27A) cause hepatitis of infancy as well as cerebrotendinous xanthomatosis. J Inherit Metab Dis. 2002;25:501–13.
18. von Bahr S, Björkhem I, van't Hooft F, Alvelius G, Nemeth A, Sjövall J, et al. Mutation in the sterol 27-hydroxylase gene associated with fatal cholestasis in infancy. J Pediatr Gastroenterol Nutr. 2005;40:481–6.
19. Setchell KDR, Heubi JE, Bove KE, O'Connell NC, Brewsaugh T, Steinberg SJ, et al. Liver disease caused by failure to racemize trihydroxycholestanoic acid: gene mutation and effect of bile acid therapy. Gastroenterology. 2003;124:217–32.

20. Carlton VEH, Harris BZ, Puffenberger EG, Batta AK, Knisely AS, Robinson DL, et al. Complex inheritance of familial hypocholanemia with associated mutations in TJP2 and BAAT. Nat Genet. 2003;34:91–6.
21. Heubi JE, Setchell KDR, Jha P, Buckley D, Zhang W, Rosenthal P, et al. Treatment of bile acid amidation defect with glycocholic acid. Hepatology. 2015;61:268–74.
22. Chong CPK, Mills PB, McClean P, Gissen P, Bruce C, Stahlschmidt J, et al. Bile acid-CoA ligase deficiency-a new inborn error of bile acid metabolism. J Inherit Metab Dis. 2012;35:521–30.