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## Mechanisms of Disease: inborn errors of bile acid synthesis

Shikha S Sundaram, Kevin E Bove, Mark A Lovell, and Ronald J Sokol\*

SS Sundaram is an Assistant Professor of Pediatrics in the Section of Pediatric Gastroenterology, Hepatology and Nutrition, MA Lovell is Associate Professor of Pathology and Medical Director of the Molecular Diagnostics Laboratory and RJ Sokol is Professor and Vice Chair of Pediatrics, Chief of the Section of Pediatric Gastroenterology, Hepatology and Nutrition, and Director of the Pediatric Clinical and Translational Research Center, all at the University of Colorado School of Medicine and The Children's Hospital, CO, USA. KE Bove is Professor of Pathology and Pediatrics at the University of Cincinnati College of Medicine and a Staff Pathologist at the Cincinnati Children's Hospital Medical Center, OH, USA

### Summary

Inborn errors of bile acid synthesis are rare genetic disorders that can present as neonatal cholestasis, neurologic disease or fat-soluble-vitamin deficiencies. There are nine known defects of bile acid synthesis, including oxysterol 7 $\alpha$ -hydroxylase deficiency,  $\Delta^4$ -3-oxosteroid-5 $\beta$ -reductase deficiency, 3 $\beta$ -hydroxy- $\Delta^5$ -C<sub>27</sub>-steroid dehydrogenase deficiency, cerebrotendinous xanthomatosis (also known as sterol 27-hydroxylase deficiency),  $\alpha$ -methylacyl-CoA racemase deficiency, and Zellweger syndrome (also known as cerebrotendinous xanthomatosis). These diseases are characterized by a failure to produce normal bile acids and an accumulation of unusual bile acids and bile acid intermediaries. Individuals with inborn errors of bile acid synthesis generally present with the hallmark features of normal or low serum bile acid concentrations, normal  $\gamma$ -glutamyl transpeptidase concentrations and the absence of pruritus. Failure to diagnose any of these conditions can result in liver failure or progressive chronic liver disease. If recognized early, many patients can have a remarkable clinical response to oral bile acid therapy.

### Keywords

bile acid; cholestasis; fat soluble vitamins; liver failure; ursodeoxycholic acid

### Introduction

Inborn errors of bile acid synthesis constitute an expanding category of rare genetic disorders that are responsible for up to 1–2% of cases of neonatal cholestasis,<sup>1</sup> a condition defined by the development of conjugated or direct hyperbilirubinemia within the first few months of life. Most patients with inborn errors of bile acid synthesis have a remarkable clinical response to oral bile acid therapy.

The primary bile acids cholic acid and chenodeoxycholic acid are synthesized by a sequence of enzymatic modifications to cholesterol that involves at least 14 enzymes, multiple subcellular compartments and two complementary chemical pathways (Figure 1). The

classic 'neutral' pathway is considered the main pathway for bile acid synthesis and produces both cholic acid and chenodeoxycholic acid in approximately equal amounts. In this pathway, the rate-limiting enzymatic step is the steroid nucleus modification that takes place in hepatic microsomes and is catalyzed by cholesterol 7 $\alpha$ -hydroxylase (the *CYP7A1* gene product). The farnesoid X receptor (FXR) is vital for the regulation of *CYP7A1* by bile acids. FXR is a member of the superfamily of ligand-activated transcription factors that acts on target genes as both a monomer and as a heterodimer with the retinoid X receptor (RXR).<sup>2-4</sup> Bile acids are physiologic FXR ligands, with chenodeoxycholic acid the most potent activator of human FXR.<sup>5-7</sup> FXR activation by primary bile acids leads to upregulation of the atypical nuclear receptor small heterodimer protein (SHP), which interacts with liver receptor homolog 1 (LRH-1) and inhibits the ability of LRH-1 to activate *CYP7A1*.<sup>8</sup> LRH-1 is also important for SHP gene expression because formation of the SHP-LRH-1 complex reduces SHP expression, thereby decreasing the negative feedback signal.<sup>9</sup>

An alternative 'acidic' pathway has also been described, in which C27-hydroxylation of cholesterol by sterol 27-hydroxylase is the initial step, followed by C7 $\alpha$ -hydroxylation by oxysterol 7 $\alpha$ -hydroxylase. This pathway leads to the production of primarily chenodeoxycholic acid. In both the classic and alternative pathways, side-chain modification takes place after steroid nucleus modification and is initiated in other organelles: C27-hydroxylation occurs in mitochondria, but further side-chain modification requires functioning peroxisomes. The final step in primary bile acid synthesis is the conjugation of cholic acid and chenodeoxycholic acid to taurine or glycine.<sup>10,11</sup>

In the past 20 years, deficiencies have been identified in the genes encoding the multiple enzymes involved in the bile acid synthesis pathways (Table 1). Such defects are proposed to cause liver disease in two ways. First, impaired hepatocyte production of primary bile acids reduces canalicular bile acid secretion, thereby hindering bile acid dependent bile flow. Second, potentially hepatotoxic atypical bile acid precursors accumulate in hepatocytes and cause cellular injury.<sup>12,13</sup> These perturbations most commonly manifest in infants as cholestasis that can mimic other neonatal liver diseases, including biliary atresia.

## Clinical Features

The clinical presentation of each of the nine known bile acid synthesis defects has been characterized, along with the liver histopathology, diagnostic procedures of choice and response to therapy (Table 1). Bile acid synthesis defects share three important clinical features that should raise clinical suspicion for these disorders. First, other cholestatic liver diseases are associated with elevated total serum bile acid concentrations, but levels are generally normal or low in infants with bile acid synthesis defects. Second, the serum level of  $\gamma$ -glutamyl transpeptidase (GGTP), which is often elevated in patients with other cholestatic liver diseases, is characteristically normal or minimally elevated in those with inborn errors of bile acid synthesis. Third, pruritus, which is a common and distressing feature of chronic cholestasis, is usually absent in infants with bile acid synthesis defects. A high index of suspicion is required to prevent the diagnosis of a bile acid synthesis defect being overlooked, as the clinical presentations of these rare disorders can be similar to those of other causes of neonatal liver failure, neonatal cholestasis and chronic liver disease. Importantly, many bile acid synthesis defects are readily treatable and therefore have an excellent prognosis if recognized and treated early in life. Moreover, these disorders represent 'accidents of nature' that have led to a more thorough understanding of basic biochemistry and biology, which can be applied to normal liver physiology and the pathophysiology of other diseases. In this Review, the current state of our understanding of bile acid synthesis defects is characterized, with emphasis on the translational and clinical investigations that have led to the emergence of this relatively new field within hepatology.

It should be noted that because of the rarity of these diseases, many of the treatment recommendations mentioned are based on expert opinion rather than on firm data.

### Oxysterol 7 $\alpha$ -Hydroxylase Deficiency

A deficiency in the enzyme oxysterol 7 $\alpha$ -hydroxylase, which is encoded by the *CYP7B* gene, interrupts the alternative 'acidic' pathway for synthesis of the bile acid steroid nucleus. A single reported case of oxysterol 7 $\alpha$ -hydroxylase deficiency has been described in the literature.<sup>14</sup> The infant, the second child of a consanguineous Mexican couple, presented with cholestasis, acholic stools, liver synthetic failure, and hepatosplenomegaly. Liver biopsy showed lobular disarray with giant cell transformation, moderate portal inflammation and fibrosis, extramedullary hematopoiesis, and canalicular and bile duct plugging (Figure 2). Although the infant was initially believed to have biliary atresia, the findings of a normal serum GGTP level and low concentrations of total serum bile acids led to the diagnosis of a bile acid synthesis defect being considered.

Fast-atom-bombardment mass spectrometry (FAB-MS) of the patient's urine revealed a lack of primary bile acids and high amounts of sulfate or glycosulfate conjugates of monohydroxycholeonic acids. Gas-chromatography mass spectrometry (GC-MS) analysis found that 96% of serum bile acids were 3- $\beta$ -hydroxy-5-choleonic acid and 3- $\beta$ -hydroxy-5-cholestenoic acid. Serum analysis showed extremely high levels of 27-hydroxycholesterol and the absence of 7 $\alpha$ -hydroxylated sterols. These features suggested a block at the level of oxysterol 7 $\alpha$ -hydroxylase, which was found to be absent from the infant's liver tissue. Subsequent genetic evaluation of the patient revealed homozygosity for a cytosine to thymidine transition mutation at position 388 in exon 5 of *CYP7B*, and heterozygosity was demonstrated in both parents.<sup>14</sup>

Treatment with ursodeoxycholic acid worsened liver function test results, and oral cholic acid therapy was ineffective in reversing the liver failure. The baby underwent orthotopic liver transplantation, but subsequently died of a fulminant post-transplant lymphoproliferative disorder.<sup>14</sup> It has been speculated that additional patients with oxysterol 7 $\alpha$ -hydroxylase deficiency have not been identified because the severity of this disease potentially results in prenatal or early neonatal death. If additional patients are identified, early liver transplantation might be the only possible therapeutic option available.<sup>14</sup>

This case, combined with results from animal studies, has shed considerable light on the intricacies of the different pathways involved in the modification of cholesterol to bile acids. The expression of oxysterol 7 $\alpha$ -hydroxylase, which is active in the alternative acidic pathway, is developmentally regulated in rodents.<sup>15-18</sup> Mice in which the classic pathway is rendered nonfunctional by knockout of cholesterol 7 $\alpha$ -hydroxylase (*Cyp7a1*<sup>-/-</sup>) are normal at birth but rapidly become ill; death occurs in the early postnatal period unless the mice are given supplemental vitamins and bile acids.<sup>19</sup> Schwarz *et al.* found that adult *Cyp7a1*<sup>-/-</sup> mice can fully compensate for the lack of cholesterol 7 $\alpha$ -hydroxylase by synthesizing 7 $\alpha$ -hydroxylated bile acid products via the alternative acidic pathway.<sup>15</sup> In rats in which cholesterol 7 $\alpha$ -hydroxylase had been chemically ablated, 43% of expected bile acid synthesis still occurred, again supporting the importance of the alternative acidic pathway.<sup>17</sup> Examination of the single reported case of human oxysterol 7 $\alpha$ -hydroxylase deficiency similarly helped to establish the importance of the alternative acidic pathway for the synthesis of primary bile acids in humans, especially during infancy.<sup>14</sup>

### $\Delta^4$ -3-Oxosteroid-5 $\beta$ -Reductase Deficiency

Deficiency of  $\Delta^4$ -3-oxosteroid-5 $\beta$ -reductase (also known as 5 $\beta$ -reductase deficiency) is an autosomal recessive condition that causes defective bile acid steroid nucleus synthesis.<sup>20,21</sup>

The enzyme  $\Delta^4$ -3-oxosteroid-5 $\beta$ -reductase, which is encoded by the gene *AKR1D1* (previously known as *SRD5B1*), converts 7 $\alpha$ -hydroxy-4-cholesten-3-one and 7 $\alpha$ ,12 $\alpha$ -dihydroxy-4-cholesten-3-one into 3-oxo-5 $\beta$  analogs. Deficiency of this enzyme impairs the reduction of the double bond between C4 and C5 of the steroid nucleus. Consequently, low levels of normal primary bile acids are present in the urine and serum of affected patients, while intermediate products of bile acid synthesis accumulate and are readily detectable by FAB-MS.<sup>1</sup>

The 5 $\beta$ -reductase deficiency was first described by Setchell *et al.* in 1988 in a set of identical twins.<sup>22</sup> The typical presentation is neonatal cholestasis and is characterized by increased concentrations of aminotransferases, a normal GGTP concentration, conjugated hyperbilirubinemia, and coagulopathy that worsens with disease progression.<sup>1,22,23</sup> Without treatment, liver failure rapidly ensues; there is, therefore, a 50% mortality rate in infants for whom diagnosis is delayed.<sup>1</sup> An alternative clinical presentation, neonatal liver failure resembling neonatal hemochromatosis, has also been described in patients with 5 $\beta$ -reductase deficiency, although none of these individuals were shown to have mutations in the *AKR1D1* gene.<sup>24,25</sup> The phenotypic overlap with neonatal hemochromatosis is postulated to be caused by an impairment in iron excretion, which is usually enhanced by bile acids.<sup>24,26,27</sup>

The enzyme  $\Delta^4$ -3-oxosteroid-5 $\beta$ -reductase is not expressed in fibroblasts or leukocytes, so cell-based enzyme studies are not useful for establishing the diagnosis of 5 $\beta$ -reductase deficiency. Instead, spot urine samples are examined by FAB-MS and GC-MS for the presence of characteristic mass spectra of  $\Delta^4$ -3-oxo bile acids.<sup>1,23</sup> High levels of  $\Delta^4$ -3-oxo bile acids are, however, present in various advanced liver diseases and might be nonspecific. Thorough evaluation of patients for advanced liver disease that can result in a secondary 5 $\beta$ -reductase deficiency is, therefore, important.<sup>28,29</sup> In addition, molecular analysis of *AKR1D1* to determine the presence of mutations is available in research laboratories and can be helpful in firmly establishing the diagnosis of primary 5 $\beta$ -reductase deficiency.<sup>20,30</sup>

The histopathology of 5 $\beta$ -reductase deficiency is typical of that of neonatal hepatitis, with findings of giant cell hepatitis, pseudoacinar transformation, hepatocellular and canalicular cholestasis, and extramedullary hematopoiesis (Figure 3). Electron microscopy shows a mix of normal and abnormal bile canaliculi, without dilation. The abnormal canaliculi have been described as having diverticuli, and many contain dense granular material.<sup>30,31</sup>

Therapy for 5 $\beta$ -reductase deficiency is directed at the replacement of primary bile acids to stimulate bile flow and limit the production of toxic bile acid precursors through feedback inhibition.<sup>13,26</sup> Initially, both chenodeoxycholic acid and cholic acid were used as therapy.<sup>26</sup> Although treatment trials in humans are lacking, concerns over potential hepatotoxicity in animal models of the disease have curbed the use of chenodeoxycholic acid.<sup>1,32,33</sup> Treatment with cholic acid (10–20 mg/kg daily) should be titrated to ensure that urinary excretion of  $\Delta^4$ -3-oxo bile acids ceases. Ursodeoxycholic acid has also been used as therapy in this setting because of its choleric and hepatoprotective properties. Although ursodeoxycholic acid does help to stimulate bile flow, it does not, however, inhibit the first step in bile acid synthesis, which is mediated by cholesterol 7 $\alpha$ -hydroxylase, and is, therefore, ineffective as sole therapy for this condition.<sup>1,26</sup> Overall treatment response is good if the diagnosis of  $\Delta^4$ -3-oxosteroid-5 $\beta$ -reductase deficiency is made early in the course of the disease.<sup>31</sup>

## 3 $\beta$ -hydroxy- $\Delta^5$ -C<sub>27</sub>-Steroid Dehydrogenase Deficiency

The most commonly reported defect of bile acid synthesis, 3 $\beta$ -hydroxy- $\Delta^5$ -C<sub>27</sub>-steroid dehydrogenase (3 $\beta$ HSD) deficiency, is an autosomal recessive condition in which modification of the steroid nucleus is defective. The enzyme 3 $\beta$ HSD is necessary for the oxido-reduction of the 3- $\beta$ -hydroxyl moiety of 7 $\alpha$ -hydroxycholesterol, converting it to 7 $\alpha$ -hydroxy-4-cholesten-3-one. A defect in this enzyme results in accumulation of both 7 $\alpha$ -hydroxycholesterol and abnormal C<sub>24</sub> bile acids that retain the 3- $\beta$ -hydroxyl- $\Delta^5$  structure.<sup>34</sup> Hepatic injury occurs as a consequence of the inadequate synthesis of the normal bile acids necessary to stimulate bile flow and the accumulation of abnormal toxic bile acids.

Most patients with 3 $\beta$ HSD deficiency present as neonates, although age at onset can be quite variable (3 months to 14 years), as can the clinical presentation.<sup>1</sup> Typical features in the neonate include progressive jaundice, increased aminotransferase levels, a normal GGTP level, low or normal total serum bile acid concentrations, and conjugated hyperbilirubinemia.<sup>23,35,36</sup> Hepatomegaly, with or without splenomegaly, is common, and pruritis can be present. In addition, malabsorption can occur, with resultant steatorrhea, fat-soluble-vitamin deficiency, poor growth, and rickets.<sup>23,36,37</sup>

Although total serum bile acid levels are quantitatively normal in this setting, they are qualitatively abnormal.<sup>1</sup> The diagnosis of 3 $\beta$ HSD deficiency can be made on the basis of FAB-MS analysis of a spot urine sample. Urine bile acids are abnormal, with a lack of the normal glycine and taurine conjugates of primary bile acids and the presence of sulfate and glycosulfate conjugates of dihydroxy and trihydroxy choleonic acids, and levels are elevated.<sup>23,35</sup> These abnormal bile acids are poorly transported across the canalicular membrane and interfere with ATP-dependent transport of conjugated bile acids.<sup>36</sup> As 3 $\beta$ HSD is also expressed in fibroblasts, enzyme activity can be measured in cultured skin fibroblasts by using 7 $\alpha$ -hydroxycholesterol as the substrate. No detectable enzyme activity is observed in affected patients, while heterozygotes have decreased but measurable enzyme activity.<sup>37</sup> Identification of mutations in the causative gene, *HSD3B7*, on chromosome 16p11.2–12 also allows for genetic diagnosis.<sup>38,39</sup>

The histopathology of 3 $\beta$ HSD deficiency varies with patient age and correlates with the mode of presentation and rate of disease progression. Liver histology in the infant includes giant cell hepatitis, canalicular bile plugs, hepatocyte bile stasis, and portal tract inflammation with variable fibrosis (Figure 4).<sup>35,40–42</sup> Liver biopsy samples from older infants and children can show less striking features of giant cell transformation and cholestasis; however, fibrosis becomes more prominent in the portal and periportal areas, and cirrhosis has been described.<sup>1,35,40,42</sup> Variable levels of inflammation with lymphocytic predominance can be seen, mimicking chronic hepatitis. Although no specific ultrastructural changes have been described, findings include partial or complete loss of canalicular microvilli and granular or membranous material in canaliculi that is distinct from the granular material seen in progressive familial intrahepatic cholestasis type 1 (also known as FIC1 deficiency or Byler disease).<sup>1,42</sup>

Treatment of 3 $\beta$ HSD deficiency is targeted at stimulating bile flow and downregulating 7 $\alpha$ -hydroxylase activity to diminish or eliminate the production of hepatotoxic bile acids.<sup>12,13,41</sup> We believe that this result is accomplished by administration of oral primary bile acid therapy combined with proper monitoring of the suppression of abnormal urine metabolites. In our opinion, oral bile acid therapy probably decreases the production of hepatotoxic bile acids by inducing negative feedback inhibition of 7 $\alpha$ -hydroxylase transcription through FXR and SHP. Treatment with cholic acid (10–15 mg/kg daily) leads to improved liver function test results and resolution of jaundice.<sup>1</sup> If advanced fibrosis is present, prognosis is

not as good. Ursodeoxycholic acid has been used as a treatment for  $3\beta$ HSD deficiency, but is ineffective without concomitant cholic acid therapy.<sup>43</sup>

## Cerebrotendinous Xanthomatosis

Cerebrotendinous xanthomatosis (CTX) is a rare autosomal recessive lipid-storage disorder that results from abnormal side-chain modification of bile acids, which is caused by mitochondrial sterol 27-hydroxylase deficiency.<sup>44</sup> Impaired oxidation of the cholesterol side chain causes accelerated cholesterol synthesis and metabolism, increased excretion of bile alcohol glucuronides, and abnormal cholestanol and cholesterol deposition in tissue, particularly in the nervous system and cardiovascular system.<sup>45–47</sup> Various mutations in the sterol 27-hydroxylase gene *CYP27A1*, which is on the long arm of chromosome 2, cause CTX.<sup>48</sup>

CTX is typically detected in patients in the second or third decade of life as part of a neurologic evaluation, although young pediatric cases have been reported.<sup>49,50</sup> The estimated prevalence of CTX is 1 in 70,000.<sup>51</sup> Classic findings include progressive neurologic dysfunction, ataxia, dementia, cataracts, xanthomas of the brain, and atherosclerosis.<sup>44,45,52,53</sup> Chronic diarrhea coupled with early bilateral juvenile cataracts can suggest the diagnosis of CTX before the onset of overt neurologic symptoms.<sup>49,50,54,55</sup> Isolated neonatal cholestasis without neurologic symptoms, diarrhea or cataracts is a rare but reported presentation.<sup>23,56,57</sup> Affected infants have a normal GGTP level and increased levels of aminotransferases and conjugated bilirubin, which gradually normalize by 6 months of age.<sup>23,57</sup> One reported case of CTX was associated with fatal cholestasis in an infant.<sup>56</sup> Early diagnosis is essential to limit or prevent the development of neurologic and cardiovascular complications.<sup>58</sup>

CTX can be differentiated from other conditions associated with xanthomata on the basis of serum cholesterol concentrations, which are generally elevated in other types of xanthomata-associated disorders.<sup>51</sup> In patients with CTX, primary bile acid synthesis is reduced (chenodeoxycholic acid synthesis is affected more than cholic acid synthesis), bile alcohol glucuronide excretion in bile, urine and stools is increased, serum or plasma cholestanol levels are low or normal, plasma cholestanol levels are markedly elevated, and plasma cholestanol:cholesterol ratios are increased. The diagnosis of CTX is established by finding a greatly increased plasma cholestanol:cholesterol ratio or characteristic metabolites in urine (e.g. increased bile alcohol glucuronides detected by FAB-MS), followed by DNA sequencing of *CYP27A1*. Affected individuals have tissue (including central nervous system) deposition of cholesterol and cholestanol, which is responsible for neurologic injury and the associated symptomatology.<sup>23</sup>

The primary treatment of CTX is oral bile acid administration. Chenodeoxycholic acid (750 mg daily in adults) decreases plasma cholestanol levels and reduces urinary bile alcohol glucuronide excretion through feedback inhibition of cholesterol 7 $\alpha$ -hydroxylase.<sup>59,60</sup> Ursodeoxycholic acid, which does not inhibit cholesterol 7 $\alpha$ -hydroxylase, is ineffective as a treatment.<sup>61</sup> Cholic acid therapy, another treatment option, also decreases plasma cholestanol levels.<sup>62</sup> Studies using 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase inhibitors, which lower cholesterol levels by inhibiting a rate-limiting step of endogenous cholesterol synthesis, have yielded mixed results for the treatment of CTX.<sup>61,63–65</sup> A combination of oral bile acid therapy and an HMG-CoA reductase inhibitor, however, might be more effective at reducing plasma cholestanol concentrations than HMG-CoA reductase inhibitors alone.<sup>65,66</sup> Concern has been raised that HMG-CoA reductase inhibitors could potentially worsen CTX by increasing LDL uptake by enhancing LDL receptor activity.<sup>44</sup>

## Alpha Methylacyl-Coa Racemase Deficiency

Alpha methylacyl-CoA racemase (AMACR) deficiency is an autosomal recessive defect that inhibits cholesterol side-chain oxidation.<sup>1</sup> AMACR is necessary for the racemization of trihydroxycholestanoic acid and pristanic acid into their stereoisomers.<sup>67</sup> Conversion to these stereoisomers is necessary for the subsequent step of peroxisomal  $\beta$ -oxidation of the C27 bile acid side chain.<sup>1,68</sup> Deficiency of AMACR leads to an accumulation of plasma pristanic acid and the bile acid intermediates dihydroxycholestanoic acid and trihydroxycholestanoic acid; therefore, AMACR deficiency affects both the bile acid and fatty acid synthesis pathways.<sup>69,70</sup>

Only five patients with this disorder have been described in the literature.<sup>71,72</sup> Three of these patients were reported by Ferdinandusse *et al.* in 2000.<sup>71</sup> Two patients were asymptomatic as children and presented with adult-onset peripheral neuropathy; one of these patients also had retinitis pigmentosa. A third patient had symptoms at 18 months of age that were consistent with Niemann-Pick disease type C. All three patients had a complete absence of AMACR enzyme activity in cultured fibroblasts. Subsequently, two mutations in the *AMACR* gene were identified that were present in all three patients.

Two additional patients with AMACR deficiency were later identified and described by Setchell and co-workers.<sup>72</sup> The first was a 2-week-old infant with mild cholestasis, coagulopathy, and fat-soluble-vitamin deficiency. The infant's liver biopsy sample revealed the presence of giant cell transformation, moderate intralobular cholestasis, scattered necrotic hepatocytes, and areas with multinucleated hepatocytes. Electron microscopy showed a low number of peroxisomes, with those identified being described as abnormally small and mildly pleomorphic.<sup>1</sup> A missense mutation in the *AMACR* gene was confirmed in this patient,<sup>72</sup> and fibroblast studies also confirmed AMACR deficiency.<sup>73</sup> Treatment with cholic acid therapy (15 mg/kg daily) and fat-soluble-vitamin supplementation normalized the infant's liver enzyme levels.<sup>72</sup> Interestingly, the infant had a sibling who died at 5.5 months of age because of intracranial hemorrhage secondary to vitamin K deficiency, and who had become a liver transplant donor.<sup>72</sup> AMACR deficiency was later confirmed in the recipient of the liver from this sibling, on the basis of an abnormal urine bile acid profile and an increased pristanic acid level.<sup>72</sup> The liver transplant recipient, the second of the two additional patients, is receiving oral bile acid therapy.<sup>72</sup>

## Zellweger Syndrome

Zellweger syndrome (also known as cerebrohepatorenal syndrome) is an autosomal recessive disorder that disrupts peroxisomal biogenesis, affecting bile acid synthesis pathways as well as other peroxisomal functions. The final steps of bile acid synthesis occur in peroxisomes, including beta-oxidation of the 2-methyl branched side chain of the C27 bile acids dihydroxycholestanoic acid and trihydroxycholestanoic acid, forming the C24 primary bile acids cholic acid and chenodeoxycholic acid.<sup>74,75</sup> Mutations in 12 genes have been associated with Zellweger syndrome in humans: *PEX1*; *PEX2*; *PEX3*; *PEX5*; *PEX6*; *PEX10*; *PEX12*; *PEX13*; *PEX14*; *PEX16*; *PEX19*; and *PEX26*. *PEX1* mutations are the most commonly identified mutations in individuals with Zellweger syndrome.<sup>76</sup> The *PEX* family of genes encode proteins, called peroxins, that are essential for the proper assembly of functional peroxisomes.<sup>76</sup>

Zellweger syndrome is the most severe of the peroxisome biogenesis disorders; patients with neonatal adrenoleukodystrophy or infantile Refsum disease have less severe disease. Patients with neonatal adrenoleukodystrophy have deafness and progressive psychomotor delay, and commonly have seizures and hypotonia.<sup>76</sup> Some patients have dysmorphic

features. The liver disease associated with this disorder is very mild. Patients with Refsum disease are also universally deaf and have progressive psychomotor delay. Hypotonia and craniofacial abnormalities can occur. Hepatomegaly, neonatal cholestasis and abnormal liver tests are more common in patients with Refsum disease than in those with neonatal adrenoleukodystrophy.<sup>77</sup>

Zellweger syndrome manifests in multiple ways, reflecting the paramount role of peroxisomes throughout the body. Craniofacial abnormalities associated with the condition include a wide anterior fontanelle, a prominent forehead, epicanthal folds and midface hypoplasia.<sup>76</sup> Patients have neuronal migration defects, with profound neurologic complications including hypotonia, areflexia, seizures, and little spontaneous movement. Affected individuals have a poor suck, with resultant difficulty feeding and failure to thrive. Polycystic kidneys are present and eye abnormalities (abnormal electroretinogram, cataracts, glaucoma, and optic atrophy) have been described. Bony abnormalities include characteristic stippling of the patella and other bones, and dislocated hips.<sup>76,78,79</sup> Chronic liver disease is typical and manifests as hepatomegaly, conjugated hyperbilirubinemia, and abnormal liver function test results and, in some patients, as splenomegaly, cirrhosis, and portal hypertension.<sup>1,76,78</sup> The progression of such liver disease is quite variable. Zellweger syndrome is usually fatal in the first 2 years of life; in most patients death occurs before the development of the serious complication of portal hypertension.<sup>1,80</sup>

The diagnosis of Zellweger syndrome is multitiered. Initial screening includes evaluating the patient for the presence of increased plasma concentrations of very long chain fatty acids and for low levels of erythrocyte plasmalogens.<sup>80,81</sup> If the level of either of these is abnormal, further investigations can be made by checking the concentrations of plasma phytanic acid, pristanic acid, pipercolic acid and bile acids.<sup>80,81</sup> Hyperpipercolic acidemia and increased levels of monohydroxy, dihydroxy and trihydroxy bile acids with elongated side chains (such as trihydroxycholestanic acid) are characteristic of Zellweger syndrome.<sup>80,81</sup> If the level of one of these types of bile acids is abnormal, confirmatory testing can include assessment of plasmalogen synthesis in cultured fibroblasts.<sup>80,81</sup> Studies of peroxisome function in cultured fibroblasts can be helpful in determining whether genetic studies are needed. *PEX* gene mutation analysis is available for cases in which the biochemical or clinical profile is not classic for Zellweger syndrome.<sup>76</sup> Prenatal diagnosis of Zellweger syndrome is also possible through either molecular or biochemical analysis.<sup>76</sup>

Histologic findings of Zellweger syndrome include an increase in the size of the hepatic stores of iron, mild cholestasis, and hepatocellular injury. Lesions of the cholangioles are also present and were originally termed biliary dysgenesis.<sup>1</sup> Small plugs of inspissated bile can be seen in cholangioles, portal tracts, and small interlobular bile ducts.<sup>30</sup> The biliary epithelium can be swollen, thin or absent in areas and bile duct proliferation can be present. Various nonspecific findings characteristic of metabolic liver diseases might be present, including canalicular and cytoplasmic cholestasis, sinusoidal fibrosis, and pseudoacinar transformation of hepatocytes. Intralobular and/or portal fibrosis can progress to cirrhosis in some patients.<sup>1</sup> The hepatic ultrastructure in patients with this disorder has also been studied in great detail. Most striking is the absence of peroxisomes, which is virtually diagnostic of a peroxisomal biogenesis disorder and is one of the reasons to consider electron microscopy of liver biopsy samples when evaluating infants with cholestasis. Mitochondria can contain increased numbers of electrondense tubular cristae, enlarged matrix crystalloids and granules, or can be normal.<sup>1,82</sup>

Treatment of this fatal condition is largely supportive and palliative, and includes therapy for seizures and providing developmental support. Diets low in phytanic acid have been suggested, but have not proven helpful.<sup>76,78</sup> Patients with Zellweger syndrome have been



found to have particularly low concentrations of docosahexaenoic acid.<sup>83</sup> Clinical trials of docosahexaenoic acid supplementation have yielded mixed results, although small series have shown improvements in eye abnormalities and liver function tests.<sup>83–85</sup> Oral primary bile acid therapy has also been attempted with limited success and is not recommended for the treatment of Zellweger syndrome.<sup>86,87</sup>

## D-Bifunctional Protein Deficiency

Deficiency of D-bifunctional protein causes abnormal peroxisomal fatty acid oxidation.<sup>88</sup> The D-bifunctional protein is composed of two units, D-3-hydroxyacyl-CoA-dehydratase and D-3-hydroxyacyl-CoA-dehydrogenase. There are three variants of D-bifunctional protein deficiency, depending on which protein subunit is affected.<sup>89</sup> Patients with the type I defect are deficient in both the dehydratase and the dehydrogenase subunits. Patients with the type II deficiency are deficient in the dehydratase subunit and those with the type III variant are deficient in the dehydrogenase subunit.<sup>89</sup> The disease is caused by mutations in the *HSD17B4* gene.<sup>90</sup> Both peroxisomal beta-oxidation and oxidation of bile acid precursors are abnormal in patients with D-bifunctional protein deficiency.<sup>91</sup> Most patients present with neonatal hypotonia, seizures and craniofacial abnormalities, and can have visual impairment and psychomotor retardation.<sup>91–93</sup> Liver disease has also been reported in affected patients.<sup>92</sup>

## Bile Acid Conjugation Defects

Conjugation of cholic acid and chenodeoxycholic acid to taurine or glycine is the final step in primary bile acid synthesis.<sup>10,11</sup> The two enzymes that catalyze these final reactions are bile acid CoA ligase and bile acid CoA:amino acid *N*-acyltransferase,<sup>94–96</sup> which are encoded by the genes *BAL* and *BAAT*.<sup>97,98</sup> 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, which leads to the inability to form mixed micelles because of rapid small intestinal absorption of the unconjugated bile acids.<sup>96</sup> Conjugated hyperbilirubinemia, severe cholestasis, and liver failure have also been described in patients with bile acid conjugation defects.<sup>1,51</sup> Similar to other bile acid synthesis defects, conjugation defects can be diagnosed using FAB-MS analysis of urine and serum.<sup>51</sup> Administration of oral primary conjugated bile acids is a potential treatment for these disorders. There is not enough evidence available, however, to support a firm recommendation for the therapeutic use of these bile acids, and further data are needed.

## Conclusions

Defects in bile acid synthesis comprise an expanding group of important hepatic disorders. Clinically, these conditions resemble many other causes of neonatal cholestasis and chronic liver disease, and a high index of clinical suspicion is required when making a diagnosis. Early diagnosis is important because most of these disorders can be treated effectively with bile acid replacement therapy. The current gold standard for definitive diagnosis, FAB-MS and GC-MS analyses of serum and urine, is technically demanding and only available in a few specialized referral laboratories. Our improved understanding of these disorders attests to the triumph of combining modern biochemical and molecular techniques for unraveling the etiology of rare liver diseases.

## Note added in proof

A case report published in April 2008 has identified a second case of oxysterol 7 $\alpha$ -hydroxylase deficiency, which presented as neonatal cholestatic cirrhosis in a Taiwanese infant who died of liver failure at 11 months of age.<sup>99</sup>

## Acknowledgments

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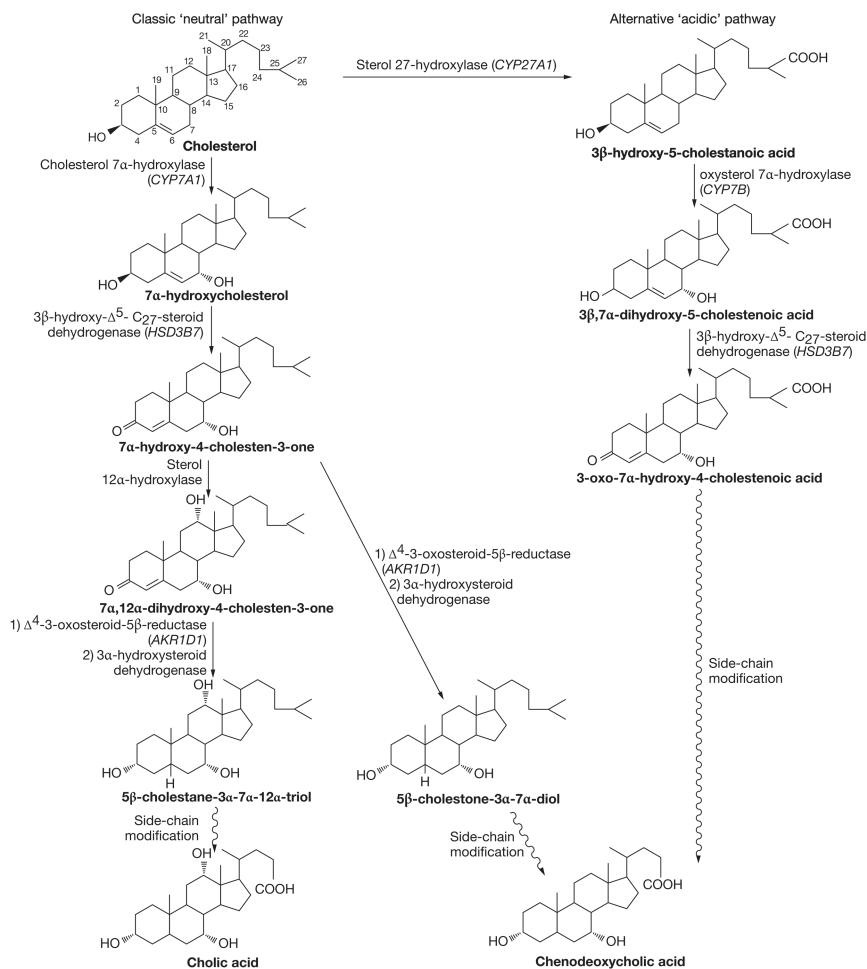
### Review Criteria

The literature cited in this article was located by searching PubMed using the terms “bile acid”, “bile acid synthesis”, “bile acid synthetic defect”, “ neonatal hepatitis”, “oxysterol 7 alpha hydroxylase deficiency”, “5 beta reductase deficiency”, “3 beta hydroxyl steroid dehydrogenase”, “alpha methyl coA racemase”, “Zellweger syndrome”, “cerebrotendinous xanthomatosis”, and “conjugation defect”, alone or in combination. Only full papers published in English language journals were considered. Reviews have been cited where appropriate to limit the number of references. The original search was performed through December 2007 and the reference list was updated in March 2008.

### Key Points

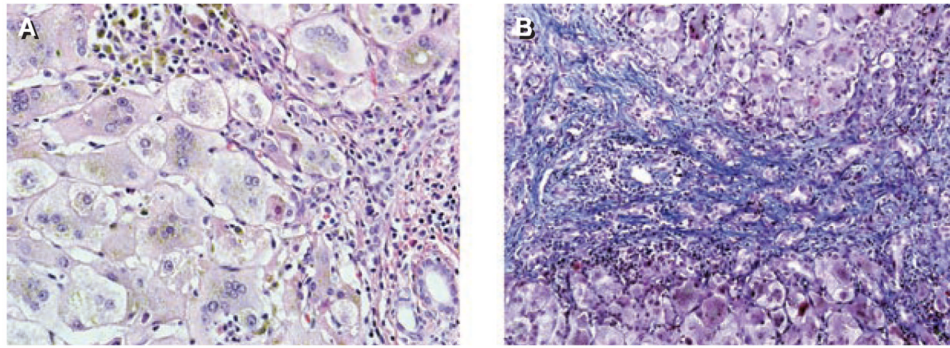
- Defects in bile acid synthesis most commonly present as neonatal cholestasis or neonatal hepatitis, but can present as chronic liver disease in older children
- Unlike most cholestatic diseases, patients with bile acid synthesis defects generally have the hallmark features of low or normal serum bile acid levels, normal or minimally increased  $\gamma$ -glutamyl transpeptidase levels and lack of pruritus
- Deficiency of  $\Delta^4$ -3-oxosteroid-5 $\beta$ -reductase can present as either neonatal cholestasis or as liver failure that resembles neonatal hemochromatosis; it has a 50% mortality in infants when diagnosis is delayed
- Deficiency of 3 $\beta$ -hydroxy- $\Delta^5$ -C27-steroid dehydrogenase is the most common defect of bile acid synthesis, presenting both as neonatal cholestasis and as chronic liver disease in older patients
- Cerebrotendinous xanthomatosis is a lipid storage disorder that presents with symptoms of progressive neurologic dysfunction in the second or third decade of life and occasionally as neonatal cholestasis; it can be treated with oral bile acids
- Early recognition and diagnosis of bile acid synthesis defects is important as these disorders are often readily treatable with oral bile acid therapy



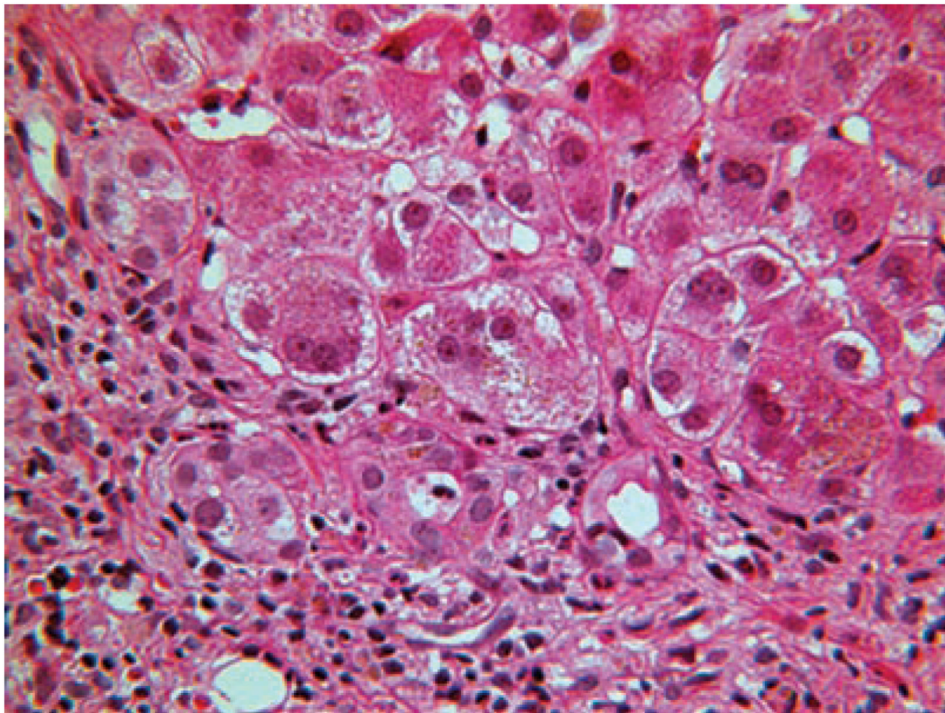


**Figure 1.**

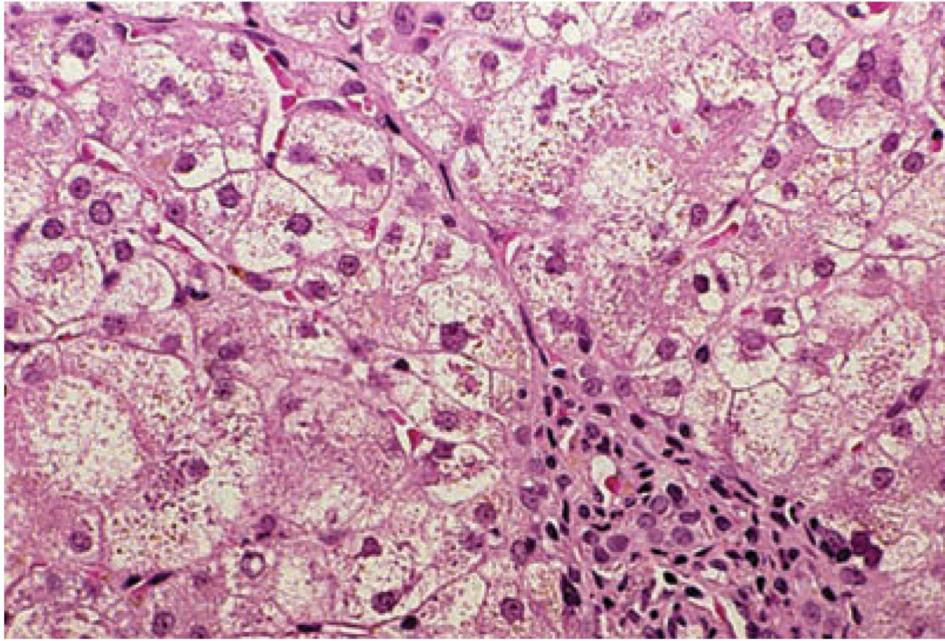
Bile acid synthesis occurs by two pathways, the classic 'neutral' pathway and the alternative 'acidic' pathway. In the classic 'neutral' pathway, the rate-limiting step in bile acid formation is the conversion of cholesterol to 7 $\alpha$ -hydroxycholesterol by cholesterol 7 $\alpha$ -hydroxylase. Multiple sequential steps that modify both the steroid nucleus and the side chain produce cholic acid and chenodeoxycholic acid. The main enzymes in this process include cholesterol 7 $\alpha$ -hydroxylase, 3 $\beta$ -hydroxy- $\Delta^5$ -C<sub>27</sub>-steroid dehydrogenase (encoded by *HSD3B7*), sterol 12 $\alpha$ -hydroxylase,  $\Delta^4$ -3-oxosteroid-5 $\beta$ -reductase (encoded by *AKR1D1* [previously known as *SRD5B1*]) and 3 $\alpha$ -hydroxysteroid dehydrogenase. A series of side-chain modifications then occurs to yield cholic and chenodeoxycholic acid. Alternatively, via the alternative 'acidic' pathway, cholesterol is converted to 3 $\beta$ -hydroxy-5-cholestanic acid by sterol 27-hydroxylase (encoded by *CYP27A1*). This is followed by conversion to 3 $\beta$ ,7 $\alpha$ -dihydroxy-5-cholestanic acid by oxysterol 7 $\alpha$ -hydroxylase (encoded by *CYP7B*).



**Figure 2.** Liver histology in a patient aged 12 weeks with oxysterol 7 $\alpha$ -hydroxylase deficiency. **(A)** Giant cell transformation with intrahepatocytic and focal canalicular cholestasis and bile-laden Kupffer cells are associated with moderate portal inflammation. Hematoxylin and eosin stain, magnification  $\times$  400. **(B)** Bile ductular proliferation associated with portal-to-portal fibrosis, highlighted in blue, is flanked by regenerative nodules. Masson trichrome stain, magnification  $\times$  200.



**Figure 3.** Liver histology in a patient aged 6 weeks with  $\Delta^4$ -3-oxosteroid  $5\beta$ -reductase deficiency. Multiple small ducts near the margin of the portal area are lined with degenerated epithelium and are accompanied by mild pericholangitis, a presumed toxic reaction to monohydroxy bile acids. The larger ducts are normal. The hepatocytes are irregularly ballooned. Hematoxylin and eosin stain, magnification  $\times 250$ .



**Figure 4.** Liver histology in a patient aged 6 months with  $3\beta$ -hydroxy- $\Delta^5$ -C<sub>27</sub> steroid dehydrogenase deficiency. Focal giant cell transformation of hepatocytes is seen in Zone 1 adjacent to the portal area. Hematoxylin and eosin stain, magnification  $\times 250$ .

Table 1

Genetic, biochemical and clinical features of bile acid synthesis defects.

Enzyme defect	Gene encoding the affected enzyme (reference)	Urine bile acid profile	serum bile acid profile	Clinical features
Oxysterol 7 $\alpha$ -hydroxylase deficiency	<i>CYP7B1</i> <sup>14</sup>	↑ Sulfate and glycosulfate conjugates of 3 $\beta$ - $\delta$ 5-monohydroxy bile acids Absence of primary bile acids	Extremely high levels of bile acids, primarily 3 $\beta$ - $\delta$ 5-monohydroxy bile acids	Neonatal hepatitis (single reported case; unrecognized cases could be due to prenatal or early-postnatal death)
$\Delta^4$ -3-oxosteroid-5 $\beta$ -reductase deficiency	<i>AKR1D1(SRD5B1)</i> <sup>20</sup>	↑ 3-oxo- $\delta$ 4 bile acids ↑ Allo bile acids ↓ Primary bile acids	↑ 3-oxo- $\delta$ 4 bile acids ↑ Allo bile acids ↓ Primary bile acids	Neonatal hepatitis with rapid progression to liver failure Neonatal hemochromatosis
3 $\beta$ -hydroxy- $\Delta^5$ -C <sub>27</sub> -steroid dehydrogenase deficiency	<i>HSD3B7</i> <sup>38,39</sup>	↑ Dihydroxy & trihydroxy cholenoic acids ↓ Primary bile acids	↓ or absence of primary bile acids	Neonatal hepatitis Late-onset liver disease Malabsorption
Cerebrotendinous xanthomatosis (sterol 27-hydroxylase deficiency)	<i>CYP27A1</i> <sup>48</sup>	↑ Plasma cholestanol: cholesterol ratio	↑ Bile alcohol glucuronides	Progressive neurologic dysfunction in 2 <sup>nd</sup> -3 <sup>rd</sup> decade of life Chronic diarrhea Bilateral juvenile cataracts Neonatal cholestasis
Alpha methylacyl-CoA racemase deficiency	<i>AMACR</i> gene on chromosome 5p13.2-q11.1 <sup>71</sup>	↑ C27 trihydroxycholestanic and pristanic acid ↓ Primary bile acids	↑ C27 trihydroxycholestanic and pristanic acid ↓ Primary bile acids Normal long-chain fatty acids and phytanic acid	Adult onset peripheral neuropathy Neonatal cholestasis with considerable fat-soluble-vitamin deficiency
Zellweger syndrome (cerebrohepato renal syndrome)	12 <i>PEX</i> gene mutations; <i>PEX1</i> mutations are the most common.	Atypical monohydroxy, dihydroxy and trihydroxy C27 bile acids ↓ Primary bile acids	↑ Long-chain fatty acids ↑ Cholestanic and piperolic acid ↑ C29 dicarboxylic acid ↓ Primary bile acids	Craniofacial abnormalities Neuronal migration defects Polycystic kidneys Chronic liver disease Bony abnormalities
Bile acid conjugation defects	<i>BAAT</i> and <i>BAL</i> <sup>97,98</sup>	Absence of glycine and/or taurine conjugates. Presence of cholic glucuronidates and/or sulfates and unconjugated cholic acid	↑ Unconjugated cholic and deoxycholic acid Absence of chenodeoxycholic acid	Transient neonatal cholestasis Fat-soluble-vitamin deficiencies

This table provides a summary of the enzymatic defects that occur in bile acid synthetic defects, along with the associated genes, urine and serum bile acid profiles and salient clinical features.<sup>1,30</sup>