

Inborn Errors of Bile Acid Metabolism

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ABSTRACT

Bile acids are synthesized by the liver from cholesterol through a complex series of reactions involving at least 14 enzymatic steps. A failure to perform any of these reactions will block bile acid production with failure to produce "normal bile acids" and, instead, result in the accumulation of unusual bile acids and intermediary metabolites. Failure to synthesize bile acids leads to reduced bile flow and decreased intraluminal solubilization of fat and fat-soluble vitamins. In some circumstances, the intermediates created because of blockade in the bile acid biosynthetic pathway may be toxic to hepatocytes. Nine recognized inborn errors of bile acid metabolism have been identified that lead to enzyme deficiencies and impaired bile acid synthesis in infants, children, and adults. Patients may present with neonatal cholestasis, neurologic disease, or fat and fat-soluble vitamin malabsorption. If untreated, progressive liver disease may develop or reduced intestinal bile acid concentrations may lead to serious morbidity or mortality. This review focuses on a description of the disorders of bile acid synthesis that are directly related to single defects in the metabolic pathway, their proposed pathogenesis, treatment, and prognosis.

KEYWORDS: Cholestasis, bile acid, cholic acid, liver

Bile acids have been implicated in the pathogenesis of liver disease; however, their exact role in initiating or perpetuating liver injury has proved difficult to discern because nonspecific alterations in serum, urinary, and biliary bile acid composition are found in infants and children with cholestasis. Specific inherited inborn errors in bile acid biosynthesis have been recently recognized that are causal in the pathogenesis of the idiopathic and familial forms of neonatal hepatitis.¹⁻¹³ The deficiency in activity of specific enzymes involved in bile acid synthesis results in diminished production of the primary bile acids that are essential for promoting bile flow and the concomitant production of atypical bile acids with the potential for causing liver injury.^{14,15} This review outlines the pathways for bile acid synthesis and describes the clinical and biochemical characteristics of inborn errors in bile acid synthesis.

CHEMISTRY AND PHYSIOLOGY

The bile acids are a group of compounds that belong to the steroid class and are classified as acidic sterols.¹⁶ In humans, the principal bile acids synthesized by the liver^{3,17} have hydroxyl groups substituted in the nucleus at the carbon positions C-3, C-7, and C-12. During early development, alternative pathways for bile acid synthesis and metabolism become quantitatively important, as is evident from the findings of relatively high proportions of bile acids hydroxylated at the C-1, C-2, C-4, and C-6 positions of the nucleus.^{18,19} The two principal bile acids synthesized by the liver and referred to as the "primary" bile acids are cholic acid (3 α ,7 α ,12 α -trihydroxy-5 β -cholanoic acid) and chenodeoxycholic acid (3 α ,7 α -dihydroxy-5 β -cholanoic acid). These bile acids are extensively conjugated to the amino acids

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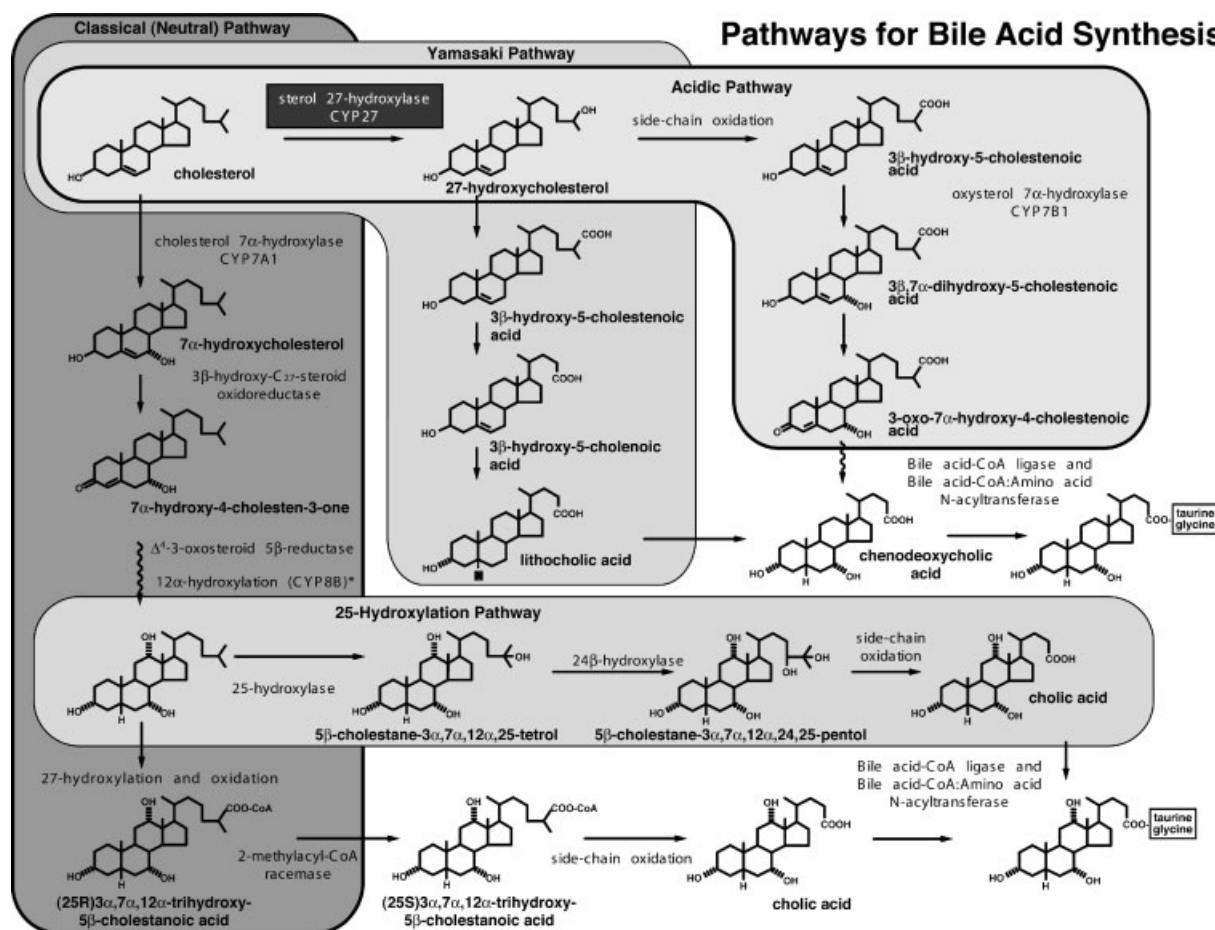


Figure 1 Metabolic pathway for the biosynthesis of the primary bile acids in the classic or “neutral” pathway and the alternative or acidic pathway. Recognized inborn errors are shown in boxes in the pathways. (Reproduced from Bove et al,⁵⁶ with kind permission from Springer Science and Business Media.)

glycine and taurine.²⁰ The biosynthetic pathway for bile acids is depicted in Fig. 1.

Bile acids perform several important functions. Bile acids are the major catabolic pathways for the elimination of cholesterol from the body.^{3,17} Bile acids provide the primary driving force for the promotion and secretion of bile and are essential to the development of the biliary excretory route for the elimination of endogenous and exogenous toxic substances, including bilirubin, xenobiotics, and drug metabolites.¹⁴ Within the intestinal lumen, the detergent action of bile acids facilitates the absorption of fats and fat-soluble vitamins.

Physiologically, the normal bile acid pool size in the adult is 2 to 4 g, but the effectiveness of this pool is increased by an efficient enterohepatic recycling (10 to 12 times/day) stimulated by postprandial gallbladder contraction.²¹ Conservation of the bile acid pool occurs by an efficient reabsorption, principally from the small intestine, and an effective hepatic extraction from the portal venous circulation so that each day less than 5% of the pool is lost in the stool.²² This bile acid loss is compensated for by hepatic synthesis of newly formed bile acids. A fraction of the pool is converted to secondary bile acids

(deoxycholic and lithocholic acid) and deconjugated with most recycled within the enterohepatic circulation and reconstituted in the liver. Although the term and preterm neonate are born with a relatively reduced, size-corrected bile acid pool, rapid expansion of the pool in the first months of life ensures adequate intraluminal concentrations for fat and fat-soluble vitamin absorption and promotion of bile flow.^{23–26}

INBORN ERRORS IN BILE ACID SYNTHESIS

Disorders in bile acid synthesis and metabolism can be broadly classified as primary or secondary. Primary enzyme defects involve congenital deficiencies in enzymes responsible for catalyzing key reactions in the synthesis of cholic and chenodeoxycholic acids. The primary defects include cholesterol 7α-hydroxylase (CYP7A1) deficiency; 3β-hydroxy-C₂₇-steroid oxidoreductase deficiency; Δ⁴-3-oxosteroid 5β-reductase deficiency⁷; oxysterol 7α-hydroxylase deficiency⁹; 27-hydroxylase deficiency, or cerebrotendinous xanthomatosis (CTX); 2-methylacyl-CoA racemase deficiency; trihydroxycholestanic acid CoA oxidase deficiency;

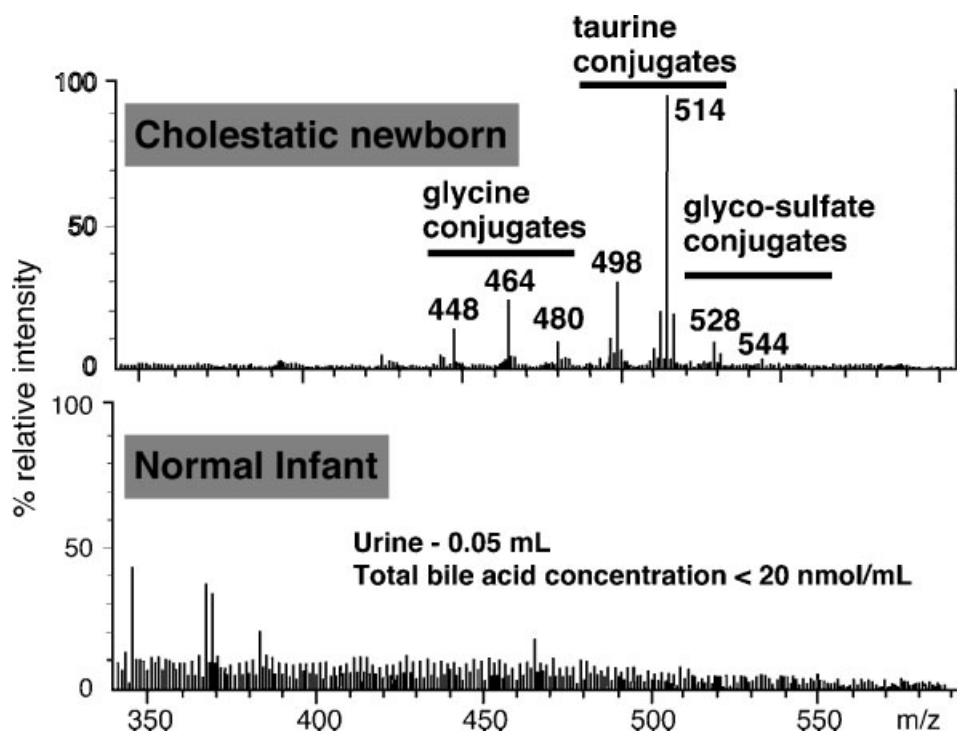


Figure 2 Negative-ion LSIMS mass spectrum analysis of typical urine from cholestatic and normal infants.

amidation defects involving a deficiency in the bile acid-CoA ligase; and side-chain oxidation defect in the 25-hydroxylation pathway for bile acid resulting in an overproduction of bile alcohols.²⁷ Secondary metabolic defects that impact primary bile acid synthesis include peroxisomal disorders such as cerebrotendinous xanthomatosis of Zellweger²⁸ and related disorders²⁹ and Smith-Lemli-Opitz syndrome³⁰ caused by a deficiency of Δ^7 -desaturase. Secondary defects will not be discussed in this review, and excellent reviews may be found elsewhere.³¹

The biochemical presentation of these bile acid synthetic defects includes a markedly reduced or complete lack of cholic and chenodeoxycholic acids in the serum, bile, and urine and greatly elevated concentrations of atypical bile acids and sterols that retain the characteristic structure of the substrates for the deficient enzyme. These signature metabolites are generally not detected by the routine or classic methods for bile acid measurement, and mass spectrometric techniques currently provide the most appropriate means of characterizing defects in bile acid synthesis. Screening procedures using liquid secondary ionization mass spectrometry (LSIMS) indicate that inborn errors in bile acid synthesis probably account for 1% to 2% of the cases of liver disease in infants, children, and adolescents, making this an important and specific category of metabolic liver disease. Typical LCIMS scans for normal and cholestatic infants are shown in Fig. 2. Over a period of 20 years, 128 bile acid synthetic defects have been identified from 7000 samples analyzed in the Mass

Spectrometry Laboratory at Children's Hospital Medical Center (Table 1). An excellent concise review for lay people and professionals is available at <http://rarediseasesnetwork.epi.usf.edu/clic>.

CEREBROTENDINOUS XANTHOMATOSIS

CTX is a rare inherited lipid storage disease with an estimated prevalence of 1 in 70,000.^{4,32} Characteristic features of the disease in adults include progressive neurologic dysfunction, dementia, ataxia, cataracts, and xanthomata in the brain and tendons and in infants with neonatal cholestasis (K.D.R. Setchell, unpublished data, 2003). Biochemically, the disease can be distinguished from other conditions involving xanthomata by (1)

Table 1 Inborn Errors of Bile Acid Metabolism ($n = 128$) Identified at Cincinnati Children's Hospital Medical Center

Diagnosis	Number
3 β -Hydroxy-C ₂₇ -steroid oxidoreductase deficiency	38
Δ^4 -3-Oxosteroid 5 β -reductase deficiency	19
27-Hydroxylase deficiency	13
Amidation defects	11
2-Methylacyl CoA racemase deficiency	2
Oxysterol 7 α -hydroxylase deficiency	1
Peroxisomal defects (Zellweger or neonatal leukodystrophy)	10
Peroxisomal defects (single-enzyme defects)	34

significantly reduced primary bile acid synthesis; (2) elevations in biliary, urinary, and fecal excretion of bile alcohol glucuronides; (3) low plasma cholesterol concentration, with deposition of cholesterol and cholestanol in the tissues; and (4) marked elevations in cholestanol. Point mutations in the gene located on the long arm of chromosome 2 have been identified that lead to inactivation of the sterol 27-hydroxylase.³³

Impaired oxidation of the cholesterol side chain results in accelerated cholesterol synthesis and metabolism that leads to greatly increased production and excretion of bile alcohol glucuronides detectably by LCIMS.^{2,34} The elevation in 5 α -cholestan-3 β -ol (cholestanol) in the nervous system of CTX patients and the high plasma concentrations of this sterol are unique features of the disease.^{35,36} Early diagnosis of this disorder, which is readily achieved by mass spectrometry analysis of the urine, is crucial to prevent the progressive accumulation of cholestanol and cholesterol in tissues in the long term. Recently, we have found several infants that had deficiencies in the sterol 27-hydroxylase owing to mutations in the gene encoding this enzyme but only because of a clinical presentation of elevated serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) and conjugated bilirubin with normal serum γ -glutamyl transpeptidase, which ultimately resolved by \sim 6 months of age presumably because the size of the cholic acid pool expanded with compensatory synthesis via the alternative 25-hydroxylation pathway. The histopathology findings on liver biopsy in these young patients are similar to those observed in idiopathic neonatal hepatitis. Neonatal cholestasis may be the typical early clinical presentation of CTX even though this has never been previously documented.

3 β -HYDROXY-C₂₇-STEROID OXIDOREDUCTASE DEFICIENCY

This was the first metabolic defect to be described involving an early step in the bile acid biosynthetic pathway; the conversion of 7 α -hydroxycholesterol is to 7 α -hydroxy-4-cholesten-3-one, a reaction catalyzed by a 3 β -hydroxy-C₂₇-steroid oxidoreductase. This is the most common of all of the bile acid synthetic defects described to date. Although the clinical presentation of this disorder is somewhat heterogeneous, most patients present as neonates with elevated serum ALT and AST, a conjugated hyperbilirubinemia, and normal serum γ -glutamyl transpeptidase.^{6,37-39} Clinical features include hepatomegaly with or without splenomegaly, fat-soluble vitamin malabsorption, and mild steatorrhea, and in most instances, pruritus is absent. The liver histology shows a generalized hepatitis, the presence of giant cells, and evidence of cholestasis^{6,37,40-42} (Fig. 3). The heterogeneity in clinical course of those with early-onset disease is illustrated by some patients who initially

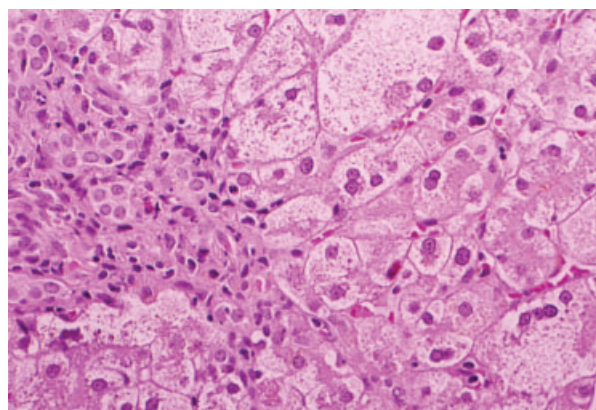


Figure 3 Liver in 3 β -hydroxy-C₂₇-steroid oxidoreductase deficiency with persistent cholestasis in a young infant. Hematoxylin and eosin-stained sections show prominent ballooned multinucleate hepatocytes. Mild portal inflammation is related to bile ductules along limiting plate (magnification, \times 250).

resolve their jaundice and are identified later in life to those with more fulminant disease, eventuating in death or transplantation at an early age. Although the earliest cases were identified in infants, increasingly, idiopathic late-onset chronic cholestasis has been explained by this disorder.^{37,38} In such patients, liver disease is not always evident initially, and patients may have fat-soluble vitamin malabsorption and rickets, which are corrected with vitamin supplementation. Serum liver enzymes that are often normal in the early stages of the disease later show progressive increases with evidence of progressive hepatic fibrosis (Fig. 4). Definitive diagnosis of the 3 β -hydroxy-C₂₇-steroid oxidoreductase deficiency currently requires mass spectrometric analysis of biologic fluids and is readily accomplished by LSIMS (formerly referred to as FAB-MS)^{2,4} or by electrospray and tandem mass spectrometry.⁴³⁻⁴⁶ LSIMS analysis of the urine permits the detection of the sulfate and glycosulfate conjugates of the 3 β -hydroxy- Δ^5 bile acids that are the signature metabolites of this bile acid defect (Fig. 4).

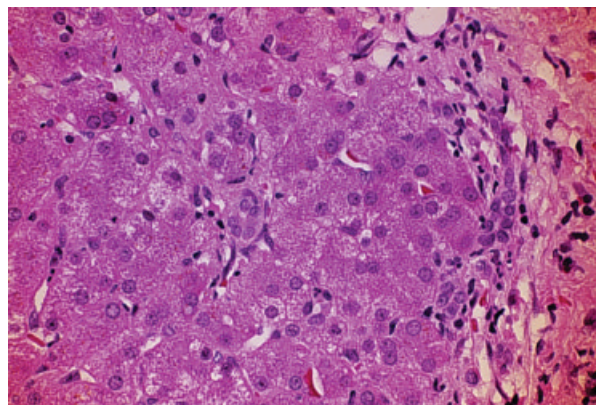


Figure 4 Liver in 3 β -hydroxy-C₂₇-steroid oxidoreductase deficiency with mild hyperbilirubinemia presenting in later childhood. Hematoxylin and eosin-stained slide shows slight disarray of hepatocytes. Portal inflammation and periportal fibrosis is minimal (magnification, \times 250).

Molecular techniques that have led to the cloning of the *HSD3B7* gene encoding 3 β -hydroxy-C₂₇-steroid oxidoreductase now permit the accurate genetic basis of the defect.⁴⁷ Using this approach to confirm the biochemical diagnosis of this enzyme deficiency in 15 patients, from 13 kindreds, 12 different mutations were found to account for the disease.⁴⁸ The mechanism of cholestasis and liver injury is believed to result from failure to synthesize adequate amounts of primary bile acids that are essential to the promotion and secretion of bile and the increased production of unusual bile acids with hepatotoxic potential^{15,49,50}. Treatment with cholic acid leads to gradual resolution of biochemical and histologic abnormalities with an excellent long-term prognosis. In selected older children/adolescents presenting with extensive fibrosis or cirrhosis, cholic acid therapy has prevented progression of disease.

Δ^4 -3-OXOSTEROID 5 β -REDUCTASE DEFICIENCY

Application of LSIMS for urine analysis led to the discovery of a defect in the Δ^4 -3-oxosteroid 5 β -reductase, which catalyzes the conversion of the intermediates 7 α -hydroxy-4-cholesten-3-one and 7 α ,12 α -dihydroxy-4-cholesten-3-one to the corresponding 3-oxo-5 β (H) intermediates.⁷ The clinical presentation of this defect is similar to that of patients with the 3 β -hydroxy-C₂₇-steroid oxidoreductase deficiency; however, in contrast, the γ -glutamyl transpeptidase is

usually elevated, and the average age at diagnosis is lower in patients with Δ^4 -3-oxosteroid 5 β -reductase deficiency. In contrast with 3 β -hydroxy-27-steroid oxidoreductase deficiency, infants with Δ^4 -3-oxosteroid 5 β -reductase deficiency tend to have more severe liver disease with rapid progression to cirrhosis and death without intervention. The Δ^4 -3-oxosteroid 5 β -reductase deficiency has since been found in several patients presenting with neonatal hemochromatosis.⁸ Liver function tests in infants with Δ^4 -3-oxosteroid 5 β -reductase deficiency present with elevations in serum ALT and AST, markedly elevated serum conjugated bilirubin, and coagulopathy. Liver biopsies^{42,51} have revealed marked lobular disarray as a result of giant cell and pseudoacinar transformation of hepatocytes, hepatocellular and canalicular bile stasis, and extramedullary hematopoiesis (Fig. 5). On electron microscopy, bile canaliculi were small and sometimes slit-like in appearance and showed few or absent microvilli containing electron-dense material.⁷

Diagnosis of this defect is possible by LSIMS and gas chromatography-mass spectrometry (GC-MS) analysis of the urine. LSIMS spectra reveal elevated amounts of bile acids with molecular weights consistent with 3-oxo-7 α -hydroxy-4-cholenoic and 3-oxo-7 α ,12 α -dihydroxy-4-cholenoic acids, which can be confirmed with GC-MS. Gallbladder bile contains only traces (less than 2 μ M) of bile acids, and urinary excretion becomes the major route for bile acid loss with Δ^4 -3-oxo bile acids comprising more than 75% of the total

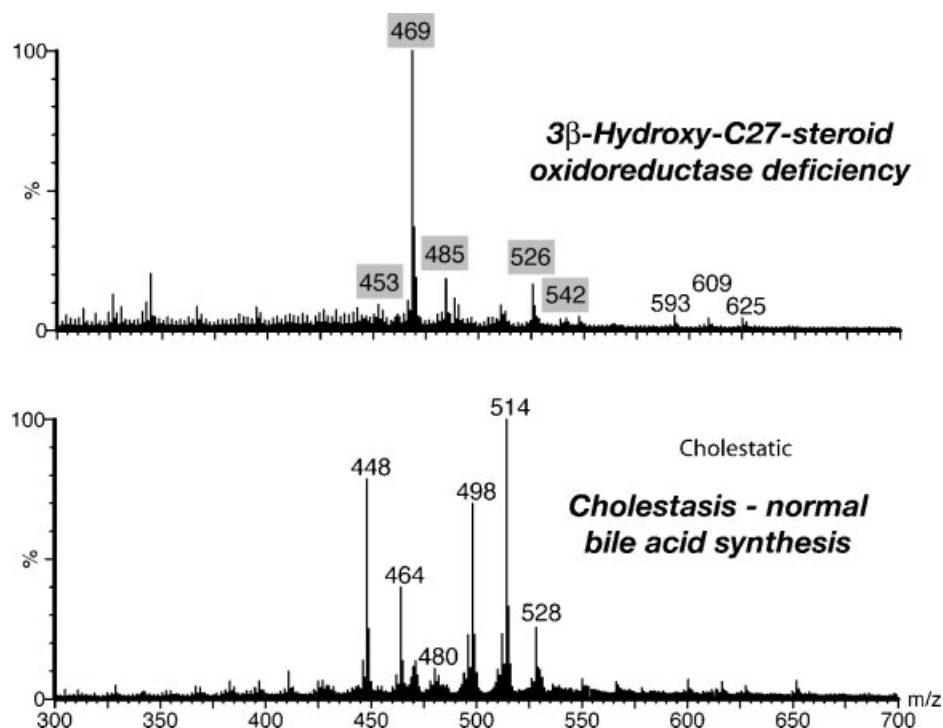


Figure 5 Negative-ion LSIMS mass spectrum analysis of typical urine from a patient with a 3 β -hydroxy-C₂₇-steroid oxidoreductase deficiency.

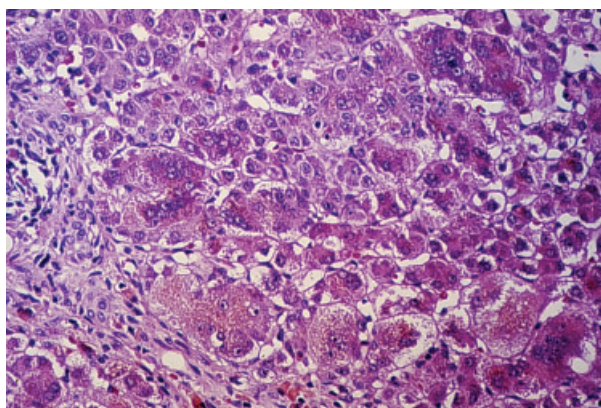


Figure 6 Liver in Δ^4 -3-oxosteroid 5β -reductase deficiency presenting with neonatal liver failure. Hematoxylin and eosin-stained sections show focally prominent cytoplasmic cholestasis and giant cell transformation with minimal portal inflammation. Bile ductules are normal (magnification, $\times 250$).

urinary bile acids. In serum, relatively high concentrations of *allo*-chenodeoxycholic and *allo*-cholic acids are found, which lends support for an active hepatic Δ^4 -3-oxosteroid 5β -reductase catalyzing the conversion of the Δ^4 -3-oxo sterol intermediates to the corresponding 3α -hydroxy- 5α (H) structures.

The Δ^4 -3-oxosteroid 5β -reductase is exclusively of hepatic origin and, unlike 3α -hydroxy- C_{27} -steroid oxidoreductase, is not expressed in fibroblasts. Monoclonal antibodies raised against the rat cytosolic Δ^4 -3-oxosteroid 5β -reductase have been used to demonstrate an absence of the 38-kDa protein in several of these patients and the formation of a truncated protein.³ In 1 patient from Japan who met our previous biochemical criteria for a deficiency in this enzyme, sequence analysis of the gene revealed a single silent mutation in the coding region of the gene,⁵² but the protein was normally expressed when analyzed by immunoblot of the liver homogenate using a monoclonal antibody.⁵³

Increased production of Δ^4 -3-oxo bile acids occurs in patients with severe liver disease⁵⁴ and in infants during the first few weeks of life.⁵⁵ It is important to perform a repeat analysis of urine in the case of a suspected Δ^4 -3-oxosteroid 5β -reductase deficiency because on rare occasions, a resolution of the liver disease occurs and the atypical bile acids disappear.⁵⁶

The liver injury in this defect is presumed to be the consequence of the diminished primary bile acid synthesis and the hepatotoxicity of the accumulated Δ^4 -3-oxo bile acids (Fig. 6). The lack of canalicular secretion can be explained by the relative insolubility of oxo-bile acids, and the cholestatic effects of the taurine conjugate of 7α -dihydroxy-3-oxo-4-cholenoic acid have been demonstrated in rat canalicular plasma membrane vesicles.¹⁵ The unique morphologic findings in these patients⁵¹ may indicate that maturation of the canalicular membrane and the transport system for bile acid secretion may require a threshold concentration of

primary bile acids in early development. Treatment with ursodeoxycholic acid or cholic acid leads to resolution of histologic and biochemical abnormalities with an excellent long-term prognosis.

OXYSTEROL 7α -HYDROXYLASE DEFICIENCY

The recent discovery of a genetic defect in oxysterol 7α -hydroxylase⁹ establishes the acidic pathway as a quantitatively important pathway for bile acid synthesis in early life. In the human, the oxysterol 7α -hydroxylase may be more important than cholesterol 7α -hydroxylase for bile acid synthesis in early life. This defect has been found in only 1 infant, a 10-week-old boy of parents who were first cousins, who presented with severe progressive cholestasis, hepatosplenomegaly, cirrhosis, and liver synthetic failure from early infancy. Serum ALT and AST were markedly elevated, and serum γ -glutamyl transpeptidase was normal. Liver biopsy findings included cholestasis, bridging fibrosis, extensive giant cell transformation, and proliferating bile ductules.⁹ Oral UDCA therapy led to deterioration in liver function tests, and oral cholic acid was therapeutically ineffective. The patient subsequently underwent orthotopic liver transplant at 4½ months of age but died from disseminated Epstein-Barr virus-related lymphoproliferative disease.

Analysis of the urine by LSIMS revealed intense ions in the spectrum at mass-to-charge ratio (m/z) 453 and m/z 510, corresponding with sulfate and glycosulfate conjugates of 3β -hydroxy-5-cholenoic and 3β -hydroxy-5-cholestenoic acids. These accounted for 97% and 86% of the total serum and urinary bile acids, respectively, and primary bile acids were virtually undetectable. Monohydroxy bile acids with the 3β -hydroxy- Δ^5 structure have been previously shown to be extremely cholestatic.^{49,57} Their hepatotoxicity in this patient is presumed to have been exacerbated by the lack of primary bile acids necessary for the maintenance of bile flow.

Oxysterol 7α -hydroxylase mRNA was also not present in this patient's liver tissue, and analysis of the oxysterol 7α -hydroxylase gene revealed a cytosine-to-thymidine transition mutation in exon 5 that converts an arginine codon at position 388 to a stop codon. The patient was homozygous for this nonsense mutation, whereas both parents were heterozygous.⁹ When human embryonic 293 or Chinese hamster ovary cells were transfected with the complementary DNA (cDNA) with the R388* mutation, there was no detectable 7α -hydroxylase activity, and immunoblot analysis confirmed that the mutated gene encoded a truncated and inactive protein.

Unlike the other two nuclear defects in bile acid synthesis, the oxysterol 7α -hydroxylase deficiency is particularly severe and untreatable by primary bile acid

therapy. The characteristic metabolites formed in the genetic defect are some of the most cholestatic bile acids known, and, clearly, oxysterol 7 α -hydroxylase is crucial for protecting the liver against the toxicity of monohydroxy bile acids produced in the acidic pathway.

2-METHYLACYL-CoA RACEMASE DEFICIENCY

2-Methylacyl-CoA racemase is a crucial enzyme that is uniquely responsible for the racemization of (25*R*)THCA-CoA to its (25*S*) enantiomer and also performs the same reaction on the branched-chain fatty acid (2*R*)pristanoyl-CoA. Defects in this enzyme therefore have profound effects on both the bile acid and the fatty acid pathways. Mutations in the gene encoding 2-methylacyl-CoA racemase were first reported in 3 adults who presented with a sensory motor neuropathy⁵⁸ and later in a 10-week-old infant who had severe fat-soluble vitamin deficiencies, hematochezia, and mild cholestatic liver disease.¹³ Liver histologic findings included cholestasis and giant cell transformation with modest inflammation. The infant had the same missense mutation (S52P) as that described in 2 of the adult patients yet was seemingly phenotypically quite different.¹³ Two of the adult patients had neurologic symptoms but were asymptomatic until the fourth decade of life, whereas the other adult was described as having the typical features of Niemann-Pick type C disease at 18 months of age and presumably had some liver dysfunction. The clinical descriptions of these adult patients, in particular the early history, were too scant to draw conclusions about the phenotypic differences between the adult and the early presentation of the 2-methylacyl-CoA racemase. It is therefore possible that these adults could have had undocumented mild liver disease and fat-soluble vitamin absorption early in life that, if undiagnosed in infancy, would probably lead to a neuropathy owing to the tissue accumulation of phytanic and pristanic acids. In the first infant described with the 2-methylacyl-CoA racemase deficiency, the liver from a 5½-month-old sibling, who 2 years previously had died from an intracranial bleed, had been transplanted into a child with end-stage liver disease.¹³ Analysis of the urine from the recipient confirmed the same biosynthetic defect in the donor liver. Diagnosis of the defect in the infant was based on urinary, serum, and biliary bile acid analysis by FAB-MS, GC-MS, and electrospray ionization–tandem mass spectrometry, which revealed subnormal levels of primary bile acids and markedly increased concentrations of cholestanoic (C27) acids, which are characteristically found as major bile acids of the alligator, other reptiles, and amphibians.^{59,60} The mass spectrum and GC profiles in this defect resemble closely those observed in peroxisomal disorders affecting bile acid synthesis, such as Zellweger syndrome. Fibroblast studies can be used to

further confirm a deficiency in peroxisomal 2-methylacyl-CoA racemase.⁶¹ Primary bile acid therapy with cholic acid has proved effective in normalizing liver enzymes and preventing the onset of neurologic symptoms in the infant; additionally, dietary restriction of phytanic acid and pristanic acids is likely to be necessary in the long-term for such patients to prevent neurotoxicity from accumulation of these fatty acids in the brain.

THCA-CoA OXIDASE DEFICIENCY

Several patients have been reported to have side-chain oxidation defects involving the THCA-CoA oxidase.^{12,62–65} The clinical presentation differs among these cases, and although all impact on primary bile acid synthesis, neurologic disease was the main clinical feature.¹² Whether these are primary bile acid defects or secondary to single-enzyme defects in peroxisomal β -oxidation is unclear. Two distinct acyl-CoA oxidases have been identified in humans.⁶⁴ The human acyl-CoA oxidase active on bile acid C₂₇ cholestanoic acid intermediates has been found to be the same enzyme that catalyzes the oxidation of 2-methyl branched-chain fatty acids.⁶⁴ The cDNA of the gene encoding this human enzyme has been cloned.⁶⁶ Of the case reports in the literature of the proposed THCA-CoA oxidase deficiency, interestingly, phytanic and pristanic acids, when measured, were elevated.^{12,32,62–65} All had ataxia as a primary feature of the disease, with its onset occurring at ~3½ years of age. None had evidence of liver disease. It is possible, with the exception of the patient described by Clayton and colleagues,¹² that these patients had a 2-methylacyl-CoA racemase deficiency, but the analysis of the cholestanoic acids was not sufficiently detailed to permit the diastereoisomers of THCA and 3 α ,7 α -dihydroxy-5 β -cholestanoic acid (DHCA) or pristanic acid to be measured, which would have helped in the differential diagnosis. In the case of the patient reported by Clayton and colleagues, we excluded 2-methylacyl-CoA racemase deficiency as an explanation for the clinical presentation.¹² The phenotypic presentation of defects involving the peroxisomal apparatus can present with a wide diversity in symptoms that make it difficult to pinpoint the exact defect involved. In all suspected cases, analysis of peroxisomal enzymes, pristanic and phytanic acids, VLCFAs, and plasmalogens should be performed to complement detailed bile acid analysis.

BILE ACID CoA LIGASE DEFICIENCY AND DEFECTIVE AMIDATION

The final step in bile acid synthesis involves conjugation with the amino acids glycine and taurine.²⁰ Hepatic conjugation is extremely efficient, and negligible amounts of unconjugated bile acids typically appear in

bile under normal and cholestatic conditions⁶⁷ and also when large doses of an unconjugated bile acid such as UDCA are administered.⁶⁸ Two enzymes catalyze the reactions leading to amidation of bile acids. In the first, a CoA thioester is formed by the rate-limiting bile acid–CoA ligase, after which glycine or taurine is coupled in a reaction catalyzed by a cytosolic bile acid–CoA:amino acid *N*-acyltransferase.

A defect in bile acid amidation, presumed to involve the bile acid–CoA ligase, was described in patients presenting with fat and fat-soluble vitamin malabsorption.¹¹ The index case was a 14-year-old boy of Laotian descent who, in the first 3 months of life, presented with conjugated hyperbilirubinemia, elevated serum transaminases, and normal γ -glutamyl transpeptidase. This child also had a form of β -thalassemia. Subsequently, additional patients have been identified who have presented with a history of neonatal cholestasis, growth failure, or fat-soluble vitamin deficiency.

The diagnosis is based on the LSIMS analysis of the urine and serum and bile, which reveals unique negative-ion spectra featuring a major peak of mass (m/z 407) corresponding with unconjugated cholic acid. In addition, ions characterizing sulfate and glucuronide conjugates of dihydroxy and trihydroxy bile acids were present. There was a complete lack of the usual glycine and taurine conjugated bile acids, and this was confirmed after chromatographic separation and GC-MS. Serum and urinary bile acids were markedly elevated and comprised predominately cholic and deoxycholic acids. The initial patients identified have been lost to follow-up, making it impossible to ascertain the molecular genetics of the defect despite the fact that the cDNAs for both conjugating enzymes have been cloned.^{69,70} All recently identified patients with this defect have all been identified with mutations in the bile acid–CoA ligase gene.

Carlton et al have described kindred of Amish descent with mutations in the bile acid–CoA:amino acid *N*-acyltransferase (BAAT).⁷¹ Patients homozygous for the 226G mutation had increased serum bile acids and variable growth failure and coagulopathy without jaundice and normal serum γ -GT concentrations. Homozygotes had only unconjugated bile acids in serum, and heterozygotes had increased amounts of unconjugated serum bile acids.

Administration of conjugates of the primary bile acid, glycocholic acid, to 2 recently identified patients has improved their growth and should correct the fat-soluble vitamin malabsorption in this defect. The recognition that genetic defects in bile acid synthesis are associated with fat-soluble vitamin malabsorption warrants a more concerted effort to explore this type of patient population, particularly as conjugated bile acids in the form of glycocholic acid are available

under a treatment Investigational New Drug application (IND).

SIDE-CHAIN OXIDATION DEFECT IN THE ALTERNATE 25-HYDROXYLATION PATHWAY

A speculative diagnosis of a defect in side-chain oxidation in the 25-hydroxylation pathway^{72–74} was proposed by Clayton and colleagues for a 9-week-old infant presenting with familial giant cell hepatitis and severe intrahepatic cholestasis.²⁷ The rationale for the diagnosis was based on the finding of reduced cholic and chenodeoxycholic acids in the serum, concomitant with high concentrations of bile alcohol glucuronides. These bile alcohols are not normally found in the plasma of infants with liver disease. Bile alcohol glucuronides were also identified as major metabolites in the urine.²⁷ Although the profile resembled that seen in CTX patients, it was concluded on the basis of the liver disease (not previously reported for CTX) that this represented a different side-chain defect and that it was possibly an oxidation defect downstream of the 25-hydroxylation step in this minor pathway for bile acid synthesis. The implications of the findings are that it could indicate that the 25-hydroxylation pathway, considered of negligible importance in adults,⁷⁴ may be an important pathway for infants. This is speculation, and further studies to prove the exact site of the defect are required before this is convincing. The patient was, however, treated with chenodeoxycholic acid and cholic acid, and this led to normalization in serum transaminases and suppression in the production of bile alcohols.

CHOLESTEROL 7 α -HYDROXYLASE DEFICIENCY

Several patients have recently been identified with a homozygous mutation deletion in the *CYP7A1* gene, and when the cDNA of this mutant was expressed in vitro in cultured HEK 293 cells, cholesterol 7 α -hydroxylase was found to be inactive.⁷⁵ Bile acid synthesis was reduced, and upregulation of the alternative sterol 27-hydroxylase pathway presumably compensated for the reduced synthesis of bile acids via absent cholesterol 7 α -hydroxylase activity. Three patients carrying this mutation were found to have abnormal serum lipids, but, in contrast with an infant identified with a mutation in oxysterol 7 α -hydroxylase,⁹ there was no liver dysfunction in these patients. Instead, the clinical phenotype was one of markedly elevated total and low-density lipoprotein (LDL) cholesterol and premature gallstones in 2 patients and premature coronary and peripheral vascular disease in 1 patient. The elevated serum cholesterol concentration was unresponsive to HMG-CoA reductase inhibitor therapy. Interestingly, individuals

who were shown to be heterozygous for this mutation were found to have an above-normal level of serum cholesterol. The phenotype of this deficiency in cholesterol 7 α -hydroxylase differed significantly from that expressed in the CYP7A1 knockout mouse model.⁷⁶

DIAGNOSIS AND TREATMENT OF INBORN ERRORS IN BILE ACID SYNTHESIS

Accurate identification of inborn errors in metabolism requires techniques that afford detailed metabolic profiles, and for the moment, GC-MS continues to be the principal confirmatory analytical tool.⁷⁷⁻⁷⁹ Because of the high cost, technical difficulty, and time-consuming nature of bile acid analysis by GC-MS, the technique is outside the scope of most routine clinical laboratories. Perhaps the most significant advances in mass spectrometry in recent years have been the introduction of FAB-MS and electrospray mass spectrometry, both of which are referred to by the generic term LSIMS. These techniques greatly simplified and extended the scope of mass spectrometry so that many nonvolatile compounds can be analyzed rapidly and directly in biologic samples or simple crude extracts, thereby circumventing the need for extensive and time-consuming sample pretreatments. Intact bile acid conjugates are ideally suited to LSIMS, and negative ionization mass spectra of steroid and bile acid conjugates can be generated from microliter volumes of urine and blood.^{2,77,80-86}

In healthy individuals, urinary bile acid excretion is of negligible quantitative importance; consequently, the mass spectrum obtained is unremarkable, showing only background ions from the matrix and the presence of some steroid hormone metabolites. During cholestasis, urinary bile acid excretion increases and bile acid conjugates can be readily detected by the presence of single intense ions corresponding with the pseudomolecular ([M-H]⁻) ions (Fig. 2).

With cholestasis, and in the absence of an inborn error in bile acid synthesis, the ions corresponding with the glyco- and tauroconjugates of the primary bile acids appear in the mass spectrum, and the intensity of the ions is proportional to the degree of cholestasis.² When bile acid synthesis is impaired, a unique mass spectrum is obtained, revealing ions corresponding in mass to the accumulated intermediates and/or metabolites with structural characteristics of the substrates proximal to the enzyme block. Positive identification of these bile acids generally requires GC-MS analysis after prior hydrolysis of the conjugates and preparation of volatile derivatives, and this is a time-consuming technique. The potential for rapid screening of bile acid defects has been realized with the electrospray ionization-mass spectrometry, and bile acid metabolites can be detected in dried blood spots obtained from newborns for the Guthrie test.⁴⁵ This approach allows fast throughput of samples

for screening, but definitive diagnosis of suspected inborn errors in bile acid synthesis is still likely to be complemented with GC-MS and, for the moment, will be restricted to specialist laboratories. Now that many of the genes encoding the enzymes involved in bile acid synthesis have been cloned, the application of molecular techniques to sequence DNA from patients identified by mass spectrometry as having bile acid synthetic defects is an important complementary tool and should prove of value in prenatal diagnosis in these familial diseases.

Although it is clear that FAB-MS screening may be the best means to identify inborn errors of bile acid metabolism, the turnaround time for the assay is sometimes slow. As a screening test, in assessing infants with conjugated hyperbilirubinemia, it may be practical to measure serum bile acids by a standard laboratory technique that will identify primary and secondary bile acids but not the metabolites typically seen in the inborn errors of bile acid metabolism. If the serum bile acids by this technique are elevated, one can safely assume one has ruled out the more life-threatening defects such as 3 β -hydroxy-C₂₇ steroid oxidoreductase deficiency, Δ^4 -oxo-5 β reductase deficiency, and oxysterol 7 α -hydroxylase deficiency. This simple screen would not necessarily rule out defects of amidation, which typically present with fat and fat-soluble vitamin malabsorption, or 27-hydroxylase deficiency, and FAB-MS would be essential for screening. It is also essential to note that if ursodeoxycholic acid is being administered during the screening with either the urine FAB-MS or conventional serum bile acid methods, the results may be difficult to interpret so all specimens should be collected after a period of ~7 to 10 days off UDCA.

Early diagnosis of inborn errors in bile acid synthesis is important because if untreated, these conditions may be fatal. The possibility of bile acid synthetic defects in older children, and even in some adults with idiopathic forms of liver disease, should also be considered given that many cases of 3 β -hydroxy-C₂₇-steroid oxidoreductase have been found in older children and teenagers presenting with late-onset chronic cholestasis.

The earliest experience with feeding a primary bile acid was for the treatment of CTX,^{87,88} even though this is not a condition that is manifested as liver disease. Long-term treatment with chenodeoxycholic acid (750 mg/day) normalized plasma cholestanol concentrations,^{88,89} markedly reduced the urinary excretion of bile alcohols,^{2,88,90} and improved the clinical condition.⁹⁰⁻⁹² Treatment of these patients may be more effective if bile acid is combined with an HMG-CoA reductase inhibitor because this combination has a greater effect in lowering plasma cholestanol.^{93,94}

Oral bile acid therapy was found to be an effective means of treating patients with the 3 β -hydroxy-C₂₇-steroid oxidoreductase deficiency, Δ^4 -3-oxosteroid 5 β -reductase deficiency, and 2-methylacyl-CoA racemase

deficiency.^{37,95,96} Cholic acid, available under a treatment IND, is the therapy of choice and has been shown to be effective in a dose range of 10 to 15 mg/kg body weight/day. UDCA has proved helpful for some patients with the 3 β -hydroxy-C₂₇-steroid oxidoreductase deficiency, lowering serum transaminases and improving liver histology.³⁸ However, it does not suppress the synthesis of atypical 3 β -hydroxy- Δ^5 bile acids, which over the long-term is important given that these bile acids are cholestatic and interfere with canalicular bile acid transport.^{15,51} When UDCA was used in combination with cholic acid, it was our experience that the effectiveness of cholic acid in downregulating endogenous bile acid synthesis was reduced, and this we believe is because UDCA during its enterohepatic recycling competitively inhibits the ileal uptake of cholic acid.

The success of this therapeutic approach for patients with these 3 defects,^{37,38,95,96} is evident from the few treatment failures, and several patients have avoided the need for orthotopic liver transplant even though they were wait-listed for a donor liver. One notable failure was the treatment of the only patient found to have a mutation in the oxysterol 7 α -hydroxylase gene.⁹ Cholic acid therapy was unable to downregulate the synthesis of the oxysterols and hepatotoxic 3 β -hydroxy- Δ^5 -monohydroxy bile acids, and this patient eventually underwent transplant.⁹

Finally, what can be offered to patients with a bile acid conjugation (amidation) defect¹¹? In these cases, they are able to make unconjugated bile acids, mostly cholic acid, yet they fail to absorb fat-soluble vitamins. Restoring the conjugated bile acid pool seems logical, and this is possible by administration of a conjugated bile acid such as taurocholate or glycocholate. Alternatively, choly sarcosine may also be helpful because this has been shown to improve fat absorption in a patient with short-bowel syndrome.⁹⁷ Recent experience with glycocholic acid suggests that it is an effective therapy for this condition; however it is only available under a treatment IND.⁹⁸

CONCLUSION

Inborn errors in bile acid synthesis represent a specific category of metabolic liver disease. These disorders have a significant effect on gastrointestinal physiology and function because of the key role that bile acids play in maintaining the enterohepatic circulation and in facilitating the absorption of fat and fat-soluble vitamins. At the Cincinnati Children's Hospital Medical Center, more than 130 patients have been identified with defects, accounting for 1% to 2% of the cases of unexplained liver disease in infants and children. Early diagnosis is important because the liver disease and fat-soluble vitamin malabsorption associated with these inborn errors can be successfully treated medically, thereby avoiding ortho-

topic liver transplant in what may otherwise be progressive and fatal conditions when undiagnosed or untreated.

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ABBREVIATIONS

BAAT	bile acid-CoA:amino acid <i>N</i> -acyltransferase
cDNA	complementary DNA
CoA	coenzyme A
CTX	cerebrotendinous xanthomatosis
CYP7A1	cholesterol 7 α -hydroxylase
DHCA	3 α ,7 α -dihydroxy-5 β -cholestanoic acid
FAB-MS	fast atom bombardment-mass spectrometry
γ -GT	gamma-glutamyl transpeptidase
GC-MS	gas chromatography-mass spectrometry
HMG-CoA	hydroxymethylglutaryl-coenzyme A
IND	Investigational New Drug application
LSIMS	liquid secondary ionization mass spectrometry
LDL	low-density lipoprotein
THCA	trihydroxycholestanoic acid
UDCA	ursodeoxycholic acid
VLCFAs	very long chain fatty acids

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