ISSN 2472-1972

Bile Acid Synthesis Disorder Masquerading as Intractable Vitamin D-Deficiency Rickets

Osman Ahmad,[1] Janaina Nogueira,[1] James E. Heubi,[2,3] Kenneth D. R. Setchell,[2,3] and Ambika P. Ashraf[4]

[1]Division of Gastroenterology, Department of Pediatrics, University of Alabama at Birmingham, Birmingham, Alabama 35233; [2]Department of Gastroenterology and Nutrition, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio 45229; [3]Department of Pathology and Laboratory Medicine, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio 45229; and [4]Division of Pediatric Endocrinology and Metabolism, Children's of Alabama, University of Alabama at Birmingham, Birmingham, Alabama 35233

ORCiD numbers: 0000-0002-3560-1848 (O. Ahmad); 0000-0003-0692-6624 (A. P. Ashraf).

Vitamin D-deficiency rickets, not responding to large treatment doses of oral vitamin D, suggest rare receptor mutations, malabsorption, or hepatobiliary dysfunction. We present a set of twins of Hispanic origin who presented with refractory vitamin D-deficiency rickets and failure to thrive (FTT) at 6 months of age. On follow-up, mild elevations in serum alanine transaminases and normal aspartate aminotransferase were noted. Subsequently, patients manifested fat-soluble vitamin deficiencies. More targeted evaluations revealed a diagnosis of 3β-hydroxy-Δ^5-C_{27}-steroid oxidoreductase deficiency. Treatment with oral bile acid replacement with cholic acid resolved rickets and promoted weight gain. Bile acid synthesis disorders should be suspected in refractory rickets in infancy, particularly in a clinical setting of FTT, even in the absence of substantial abnormalities in liver-function tests.

Vitamin D-deficiency rickets is biochemically characterized by a low-serum 25-hydroxy vitamin D (25OHD) level, normal- or low-serum calcium, low-serum phosphorous, elevated serum alkaline phosphatase, and increased parathyroid hormone (PTH). Vitamin D is solublized by intraluminal bile acids and is absorbed through the brush border of the intestinal enterocytes, mainly in the jejunum and ileum. Intestinal absorption of vitamin D is impaired in patients with intestinal, pancreatic, and hepatobiliary disorders and disorders of bile acid synthesis (BASD) or secretion.

BASD are a group of autosomal recessively inherited metabolic disorders that impair the hepatic synthesis of primary bile acids from cholesterol. Bile acids synthesized in the liver play a pivotal role in the absorption of fats and fat-soluble vitamins and in the elimination of cholesterol from the body [1, 2]. BASD present with disparate phenotypes, including cholestasis, failure to thrive (FTT), steatorrhea, hepatosplenomegaly, malabsorption of fats and fat-soluble vitamins in infants and children, and advanced liver disease in later life [1, 3, 4]. If undiagnosed, life-threatening complications, such as liver cirrhosis and eventually, liver failure, can occur.

We present a set of twins, presenting at 6-months of age, with refractory vitamin D-deficiency rickets and FTT caused by a BASD. Their cousins, who are affected by the same disorder, only manifested hepatobiliary abnormalities.

Abbreviations: 25OHD, 25-hydroxy vitamin D; ALT, alanine transaminase; BASD, bile acid synthesis disorders; FAB, fast atom bombardment; FTT, failure to thrive; GGT, gamma glutamyl transferase; HSD3B7, 3β-hydroxy-Δ^5-C_{27}-steroid oxidoreductase; INR, international normalized ratio; MS, mass spectrometry; PT, prothrombin time; PTH, parathyroid hormone.

Received 28 September 2018
Accepted 26 December 2018
First Published Online 31 December 2018

1. Case Report

A. Patients 1 and 2

A 6-month-old girl of Hispanic origin presented to a regional hospital with respiratory syncytial virus bronchiolitis and hypocalcemia. Physical examination was substantial for open anterior fontanelle, widening of wrists, and prominent costochondral junctions. The patient had a body weight of 4 kg (less than the fifth percentile), serum calcium concentration of 7.7 mg/dL, serum phosphorous of 1.4 mg/dL, elevated PTH of 510 pg/mL, and undetectable 25OHD. Her twin sister was also evaluated. The twin sister also had FTT (weight 4.9 kg), hypocalcemia (serum calcium 7.7 mg/dL), hypophosphatemia (serum phosphorous 1.4 mg/dL), and undetectable 25OHD. Both had rachitic changes in the skeletal survey (Fig. 1). They were born full term, small for gestational age, and with birth weights of 2.2 and 1.8 kg. They were fed mostly on "Similac Advance" formula, in addition to breast milk. There was no history of prolonged neonatal jaundice that required evaluation. There was no family history of rickets or metabolic bone diseases. Parents are nonconsanguineous, from Guatemala. The mother did not receive prenatal care and was not on any vitamin supplements during pregnancy.

Both children were initially started on oral calcium 100 mg/kg/day in four divided doses and ergocalciferol 800 IU daily. Subsequently, at the university hospital, the dose of vitamin D was increased to 2000 IU daily, because the infants continued to have persistent rickets (Table 1). Furthermore, the twins were not gaining appropriate weight or length, despite formula feeds and Stage 2 baby foods. Both patients had mild hypotonia and delayed motor development. Parents did not report acholic stools, jaundice, dark-colored urine, or pruritus. There was no hepatomegaly. Their transaminases were intermittently elevated, less than

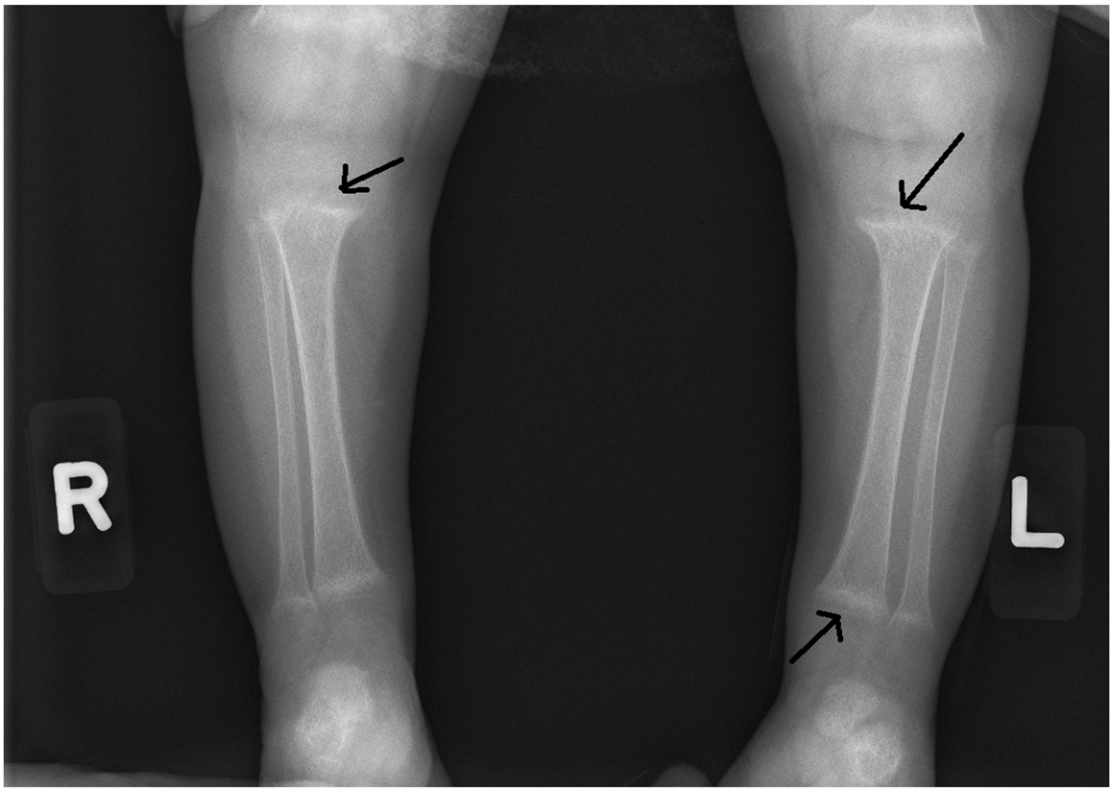

Figure 1. Rickets. X-Ray tibia of one of the twins. Arrows indicate metaphyseal fraying and cupping and loss of the zone of provisional calcification consistent with rickets at the upper and lower end of the tibia.

Table 1. Clinical Course of the Patients

Age	Ca, mg/dL (8.7–9.8)	P, mg/dL (3.9–6.5)	Mg, mg/dL (1.6–2.6)	Alk Phos, U/L (60–330)	Albumin, g/dL (3.4–4.2)	25OHD, ng/mL	1,25(OH)$_2$D, pg/mL	PTH, pg/mL (15–65)	ALT, U/L (5–45)	AST, U/L (20–60)	Total/Direct Bilirubin, mg/dL (0–1.1)	Medications
6 Mo												
#1	7.4	1.4	2.2	1810	4.3	<4[a]	59[a]	510	81	61	0.7	Ergocalciferol 800 IU daily
#2	7.7	1.3	2.2	1316	4.3	<4[a]		963		67	1.8	Calcium carbonate 100 mg/kg/d
8 Mo												
#1	9.3	4.3	1.6	1388	5.3	5[b]		904	73	57	0.7	Ergocalciferol 8000 IU daily
#2	9.7	4.9	1.6	1658	5.3	6[b]		714	142	104	1.9	Calcium carbonate 100 mg/kg/d
11 Mo												
#1	10	4.3			4.7	6[b]		535				Ergocalciferol 16,000 IU daily
#2	9.5	4.3			4.2	7[b]		425				Calcium carbonate 100 mg/kg/d
15 Mo												
#1	8.1	2.7	2.2	799	4.2	<4[a]	76[a]	436	50	49		Ergocalciferol 50,000 IU daily, calcium carbonate
#2	8.9	4.3	2.2	804	4.8	<4[a]	71[a]	574	81	67		Vitamin K 2.5 mg, ursodeoxycholic acid, vitamins A and E
16 Mo												Ergocalciferol 50,000 IU daily
#1	9.6			1172		<4[a]	86[a]		58	63	1.5/1.1	Calcium carbonate
#2	9.6			804		<4[a]	85[a]	698	70	70	1.3/0.9	Vitamin K 2.5 mg, ursodeoxycholic acid, vitamins A and E cholic acid
18 Mo												Cholic acid only
#1	10.0			296	4.2	>96[b]		58	46	46	0.4	
#2	9.9			289	4.3	>96[b]		38	61	60	0.4	
19 Mo												Cholic acid only
#1	10.2			281	4.6	>96[b]		38	61	52	0.5	
#2	10.0			296	4.8	>96[b]		44	65	42	0.9	
3 Y												Cholic acid only
#1	9.6	5.1	1.6	278	4.6	41[a]	74[a]	38	45	47	0.7	
#2	9.4	5.1	1.7	269	4.8	41[a]	66[a]	55	30	35	0.6	

Abbreviations: #1 and #2, Patients 1 and 2 (set of twins); 25OHD, 25-hydroxy vitamin D; 1,25(OH)$_2$D, 1,25-dihydroxy vitamin D; Alk Phos, serum alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; Ca, serum calcium; Mg, serum magnesium; P, serum phosphorus.

[a]The 25OHD and 1,25(H)$_2$D assays were obtained commercially (Quest Diagnostics Nichols Institute, San Juan Capistrano, CA), using liquid chromatography-tandem mass spectrometry (MS/MS) methodology.

[b]The 25OHD was measured by the Abbott Architect Assay by chemiluminescent microparticle immunoassay through the hospital laboratory.

doi: 10.1210/js.2018-00314 | Journal of the Endocrine Society | 399

two times the upper limit of normal. As a result of persistent vitamin D-deficiency, the oral vitamin D doses were increased at each clinic visit.

Given the severe FTT, refractory vitamin D-deficiency rickets unresponsive to massive doses of vitamin D and calcium, and mildly elevated liver enzymes, a hepatobiliary disorder was suspected. The twins had normal serum prealbumin, albumin, ammonia, gamma glutamyl transferase (GGT) levels, celiac panel, as well as MM α-1-phenotype, hepatitis B and C panels, lipid profile, and fecal elastase. Fecal fat and qualitative fat examination results were not available. The serum bilirubin increased slowly (see Table 1) in both infants, and by the age of 15 months, the twins developed scleral icterus and hepatomegaly. They reported feeding intolerance and acholic stools at that time. Both twins had delayed motor development and had not yet started walking. The laboratory evaluation revealed low-serum concentrations of fat-soluble vitamins [vitamin A 16 μg/dL (20–43 μg/dL), vitamin E 0.2 mg/L (2.9–16.6 mg/L), and vitamin K1 < 72 pg/mL (normal 80–160 pg/mL)]. They also had elevated prothrombin time (PT; 17.8 and 17.6 seconds, respectively), partial thromboplastin time (42.0 and 43.1 seconds), and the international normalized ratio (INR; 1.4 in both). They had normal serum zinc, copper, and vitamin B12 concentrations. Abdominal ultrasound revealed mild hepatomegaly with the absence of focal hepatic lesions or intrahepatic biliary dilation. Treatment consisted of oral ursodeoxycholic acid (15 mg/kg/day), vitamin K 2.5 mg daily, and vitamins A and E, in addition to high doses of vitamin D (ergocalciferol 50,000 IU daily) and oral calcium (100 mg/kg/day).

Subsequent evaluations revealed normal serum bile acids analyzed by liquid chromatography. Fast atom bombardment ionization mass spectrometry (FAB-MS) revealed abnormal urine bile acid profile in both twins with the characteristic atypical bile acids that are the biomarkers for a 3β-hydroxy-Δ^5-C_{27}-steroid oxidoreductase (HSD3B7) deficiency (Fig. 2). Plasma analysis by gas chromatography/MS revealed very low concentrations of cholic and chenodeoxycholic acid and high concentrations of 3β,7α-dihydroxy-5-cholenoic and 3β,7α,12α-trihydroxy-5-cholenoic acids. A neonatal and adult cholestasis sequencing panel (Emory Genetics Laboratory, Tucker, GA) identified previously unreported homozygous pathogenic variant nucleotide change at c.890delT. Both babies received treatment with cholic acid at a dose of 10 mg/kg/day at age 16 months, and within 3 months, serum aminotransferases, coagulopathy, and fat-soluble vitamin deficiencies resolved. Parents reported that the twins started walking within 1 month of starting cholic acid.

Subsequent to the diagnosis of Patients 1 and 2 with HSD3B7 deficiency, information regarding two of their first cousins was obtained.

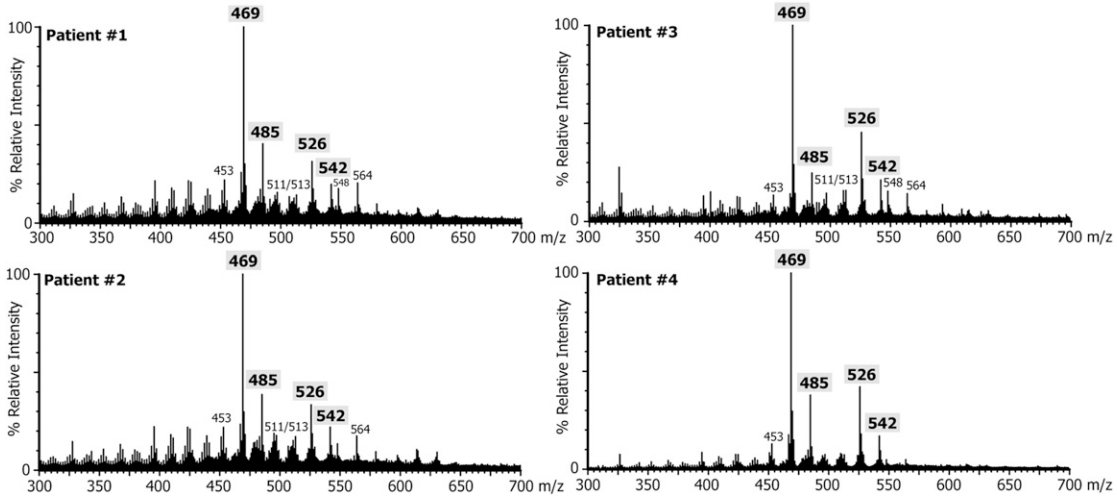

Figure 2. FAB-MS. The negative ion mass spectrum was remarkable for the absence of the usual primary bile acid conjugates, and instead, ions of mass/charge (m/z) 453, 469, 485, 526, and 542 were dominant that correspond to the atypical sulfate and glycosulfate conjugates of the unsaturated bile acids and dihydroxy- and trihydroxy-cholenoic acids.

B. Patients 3 and 4

Patient 3 presented with FTT, with weight below the first percentile, diarrhea, firm hepatomegaly, and prolonged PT at age 23 months. She had cholestasis and portal fibrosis on a liver biopsy with serum total bilirubin 1.8 mg/dL, direct bilirubin 1.2 mg/dL, GGT 19 U/L, alanine transaminase (ALT) 66 U/L, aspartate aminotransferase 65 U/L, PT 17 seconds, and INR 1.3. There was no evidence of rickets on a skeletal survey. Urine FAB-MS, performed at age 24 months, had findings consistent with HSD3B7 deficiency. She responded to treatment with cholic acid with weight gain and resolution of laboratory abnormalities. Patient 4 was a sibling of Patient 3 who was evaluated after Patient 3 was diagnosed. His weight was below the first percentile, ultrasound indicated hepatic steatosis, and he was found to have a total serum bilirubin of 1.0 mg/dL, direct bilirubin of 0.6 mg/dL, normal GGT, ALT of 120 U/L, PT of 17.2 seconds, and an INR of 1.3. He also responded well to treatment with cholic acid. These patients did not have hypotonia or developmental delays. At age 12 years, Patient 3 had a weight in the 50th percentile and Patient 4 in the 67th percentile.

2. Discussion

We describe a BASD in a set of twins presenting with refractory vitamin D-deficiency rickets with concomitant minimal clinical or biochemical features of cholestasis or history of prolonged neonatal hyperbilirubinemia or conjugated hyperbilirubinemia. Even though the motor delay and hypotonia can be seen in vitamin D-deficiency [5, 6], as a result of its known impact on muscle function, the refractory rickets not responding to mega doses of vitamin D [6] and persistent, severe FTT indicated gastrointestinal tract malabsorption or a hepatobiliary disorder. These cases illustrate a diagnostic conundrum as a result of the presence of trivial liver dysfunction and because the typical gastrointestinal workup was negative. The twins have a newly described mutation of a nucleotide change at c.890delT leading to a HSD3B7 deficiency, resulting in characteristic urinary and serum bile acid profiles. Mutation of the *HSD3B7* gene on the short arm of chromosome 16 (16p11.2) is the most prevalent type of the BASD thus far described [7]. Patients with BASD have not been reported to have small for gestational age [8] but can present with FTT in infancy as a result of fat malabsorption from cholestasis and chronic liver disease.

This defect impairs the conversion of 7α-hydroxycholesterol to 7α-hydroxy-4-cholesten-3-one in the pathway of bile acid synthesis from cholesterol [9]. Even though the serum bile acids are quantitatively normal, the bile acid intermediates, produced because of the blockade in the pathway, are cholestatic and believed hepatotoxic [10]. In the absence of normal primary bile acid synthesis, there is reduced bile flow, and these atypical bile acids lead to hepatic injury with cholestasis. Cholic acid suppresses the endogenous synthesis of the atypical 3β-hydroxy-Δ^5 bile acids through feedback inhibition and facilitates bile flow and micellar solubilization of fats and fat-soluble vitamins [2].

Often beginning in infancy, these inherited mutations affect the hepatic synthesis of cholic acid and chenodeoxy cholic acid from cholesterol, leading to fat and fat-soluble vitamin malabsorption [7, 11]. The information regarding the cousins provides additional context for the hereditary nature of the disease and phenotypic spectrum of the condition. It is important to recognize that FTT and rickets may manifest before clinical features of the chronic liver disease in patients with BASD. With the failure of prompt recognition, patients can develop progressive cholestasis and cirrhosis from the accumulation of abnormal bile acid intermediaries. Although patients with the HSD3B7 deficiency present in the neonatal period, it may often be the cause of late-onset chronic cholestasis in older children and even adults [8, 12].

3. Conclusion

We report a case of an enzyme deficiency caused by a new mutation from a nucleotide change at c.890delT in the *HSD3B7* gene, causing HSD3B7 deficiency and leading to refractory

vitamin D rickets. BASD are a rare group of disorders in which manifestations of the liver disease may be absent or inconspicuous yet should be considered as an underlying cause of some cases of refractory rickets and FTT.

Acknowledgments

Author Contributions: O.A. and A.A. conceived the study. O.A., A.A., and J.E.H. collected data from electronic medical records. O.A. wrote the manuscript. All authors (O.A., J.N., J.E.H., K.D.R.S., and A.A.) were involved in reviewing/editing the manuscript, approved the final submitted versions, and take full responsibility for the work as a whole and the decision to submit and publish the manuscript.

Correspondence: Ambika Ashraf, MD, Division of Pediatric Endocrinology and Metabolism, Children's of Alabama, University of Alabama at Birmingham, 1601, 4th Avenue S., CPP M30, Birmingham, Alabama 35233. E-mail: AAshraf@peds.uab.edu.

Disclosure Summary: J.E.H. and K.D.R.S. disclose equity in Asklepion Pharmaceuticals and are consultants with Retrophin. The other authors have nothing to disclose.

References and Notes

1. Heubi JE, Setchell KD, Bove KE. Inborn errors of bile acid metabolism. *Semin Liver Dis*. 2007;**27**(3): 282–294.
2. Gonzales E, Gerhardt MF, Fabre M, Setchell KD, Davit-Spraul A, Vincent I, Heubi JE, Bernard O, Jacquemin E. Oral cholic acid for hereditary defects of primary bile acid synthesis: a safe and effective long-term therapy. *Gastroenterology*. 2009;**137**(4):1310–1320.e3.
3. Cheng JB, Jacquemin E, Gerhardt M, Nazer H, Cresteil D, Heubi JE, Setchell KD, Russell DW. Molecular genetics of 3beta-hydroxy-Delta5-C27-steroid oxidoreductase deficiency in 16 patients with loss of bile acid synthesis and liver disease. *J Clin Endocrinol Metab*. 2003;**88**(4):1833–1841.
4. Akobeng AK, Clayton PT, Miller V, Super M, Thomas AG. An inborn error of bile acid synthesis (3beta-hydroxy-delta5-C27-steroid dehydrogenase deficiency) presenting as malabsorption leading to rickets. *Arch Dis Child*. 1999;**80**(5):463–465. PubMed PMID: 10208955; PubMed Central PMCID: PMCPMC1717927.
5. Gordon CM, Williams AL, Feldman HA, May J, Sinclair L, Vasquez A, Cox JE. Treatment of hypovitaminosis D in infants and toddlers. *J Clin Endocrinol Metab*. 2008;**93**(7):2716–2721. doi: . PubMed PMID: 18413426; PubMed Central PMCID: PMCPMC2729207.
6. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, Murad MH, Weaver CM; Endocrine Society. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab*. 2011;**96**(7):1911–1930.
7. Setchell KD, Heubi JE, Shah S, Lavine JE, Suskind D, Al-Edreesi M, Potter C, Russell DW, O'Connell NC, Wolfe B, Jha P, Zhang W, Bove KE, Knisely AS, Hofmann AF, Rosenthal P, Bull LN. Genetic defects in bile acid conjugation cause fat-soluble vitamin deficiency. *Gastroenterology*. 2013;**144**(5): 945–955.e6; quiz e14–15. doi: . PubMed PMID: 23415802; PubMed Central PMCID: PMCPMC4175397.
8. Heubi JE, Bove KE, Setchell KDR. Oral cholic acid is efficacious and well tolerated in patients with bile acid synthesis and Zellweger spectrum disorders. *J Pediatr Gastroenterol Nutr*. 2017;**65**(3):321–326. doi: . PubMed PMID: 28644367; PubMed Central PMCID: PMCPMC5559188.
9. Sundaram SS, Bove KE, Lovell MA, Sokol RJ. Mechanisms of disease: Inborn errors of bile acid synthesis. *Nat Clin Pract Gastroenterol Hepatol*. 2008;**5**(8):456–468. doi: . PubMed PMID: 18577977; PubMed Central PMCID: PMCPMC3888787.
10. Stieger B, Zhang J, O'Neill B, Sjövall J, Meier PJ. Differential interaction of bile acids from patients with inborn errors of bile acid synthesis with hepatocellular bile acid transporters. *Eur J Biochem*. 1997;**244**(1):39–44.
11. Bove KE, Heubi JE, Balistreri WF, Setchell KD. Bile acid synthetic defects and liver disease: a comprehensive review. *Pediatr Dev Pathol*. 2004;**7**(4):315–334.
12. Fischler B, Bodin K, Stjernman H, Olin M, Hansson M, Sjövall J, Björkhem I. Cholestatic liver disease in adults may be due to an inherited defect in bile acid biosynthesis. *J Intern Med*. 2007;**262**(2):254–262.