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Not all (bile acids) who wander are lost: The 1st report of a patient with an isolated NTCP defect

Saul J. Karpen[1] and **Paul A. Dawson**[1]

[1]Department of Pediatrics, Division of Pediatric Gastroenterology, Hepatology and Nutrition, Emory University School of Medicine, Children's Healthcare of Atlanta, Atlanta, Georgia

Keywords

Liver; pruritus; hypercholanemia; mutation; pediatric

The modern post-genome sequencing era is becoming increasingly replete with genetic assignments for disease. Often in relation to liver, the findings can be complex, necessitating evaluations of very large datasets to deliver insights to the molecular mechanisms. However, there also are times when a single patient yields significant new insights to physiology and disease. Such an example is the recent work by a multicenter group of talented Dutch investigators in the current issue of HEPATOLOGY[1]. These investigators pursued the unexpected clinical finding of very high plasma bile acid levels in a child without apparent liver disease, ultimately leading to the identification of a new condition affecting hepatic transport and enterohepatic cycling of bile acids.

Hepatic bile formation depends on the synthesis, canalicular secretion and efficient intestinal reabsorption of bile acids. In fact, only about 5% of the biliary bile acids are newly synthesized, and most have already been secreted by the liver into small intestine and returned via the enterohepatic circulation. Many of the players important for the enterohepatic circulation of bile acids are known and inherited defects in those genes can have profound effects on hepatic function (Figure 1; clinical characteristics summarized in Table 1). The list of genes (and associated disease states) includes: *ATP8B1*/FIC1 (Progressive familial intrahepatic cholestasis type 1; PFIC1), *ABCB11*/BSEP (PFIC2), *ABCB4*/MDR3 (PFIC3), *SLCO1B1*/OATP1B1 and *SLCO1B3*/OATP1B3 (Rotor Syndrome), and *SLC10A2*/ASBT (Primary bile acid malabsorption; PBAM).[2] Notably absent from this list is *SLC10A1*/NTCP, the presumed primary transporter for hepatic uptake of conjugated bile acids[3]. Polymorphisms in *SLC10A1* that interfere with bile acid transport have been characterized *in vitro*, but clinical data was unavailable for subjects carrying these variants.[4] Loss of NTCP is predicted to result in increased plasma conjugated bile acid levels

Correspondence to: Saul J. Karpen, M.D., Ph.D., Division Chief, Pediatric Gastroenterology, Hepatology and Nutrition, Emory University School of Medicine, Children's Healthcare of Atlanta, Health Sciences Research Building, 1760 Haygood Drive, NE, Suite 200E, Atlanta, Georgia 30322. skarpen@emory.edu; or Paul A. Dawson, Ph.D., Pediatric Gastroenterology, Hepatology and Nutrition, Emory University School of Medicine, Health Sciences Research Building, 1760 Haygood Drive, NE, Suite 200E, Atlanta, Georgia 30322. Tel: 404-727-7083; paul.dawson@emory.edu.

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(hypercholanemia), yet previous reports of hypercholanemia-associated inherited disorders found causal mutations in genes other than *SLC10A1*.[5,6] These negative findings, coupled with fact that the hepatocyte sinusoidal membrane also expresses other sodium-independent bile acid transporters, raised the possibility that an isolated NTCP gene defect may be asymptomatic, with limited impact on bile acid homeostasis. The present identification and characterization of a phenotypic loss-of-function *SLC10A1* mutation addresses those lingering doubts and clearly establishes a primary role for NTCP in hepatic bile acid clearance.

In brief, the authors describe the 4th child of consanguineous Afghan parents, who presented in the first year of life with growth retardation, hypotonia, and substantial impairments in achieving developmental milestones. Starting at approximately 9 months of age, this anicteric child underwent a comprehensive workup for her developmental delay and neurocognitive impairments, which excluded a variety of potential diagnoses but did not reveal a cause. Abdominal imaging was normal and her lab work, including liver function tests, was unremarkable. The only substantive lab abnormalities were a low 25-hydroxy vitamin D level, which was associated with reduced bone density, and mildly reduced levels of fat-soluble vitamins, A and K (as evidenced by a mildly prolonged PT). However as part of this workup, total and fractionated bile acids were measured in plasma. This yielded the surprising finding of markedly elevated plasma bile acid levels, 445 μM (normal <16 μM), nearly all of which were conjugated primary bile acids. By 2 years of age, the patient's plasma bile acid levels had risen to 1531 μM, yet during this time the child was without jaundice, pruritus, or steatorrhea. Additional plasma bile acid measurements were obtained at 3, 4, and 5 years of age, over which time the total bile acid levels tended downward to 494 μM, and the proportion of conjugated secondary bile acids increased. Urine bile acid levels were also higher than normal, but not specifically quantitated. Plasma levels of C4 (7α-hydroxy-4-cholesten-3-one), a marker of hepatic bile acid synthesis, were normal, as were plasma levels of Fibroblast growth factor-19 (FGF19), an ileal-derived enterokine involved in regulating hepatic bile acid synthesis. Levels of autotaxin activity, a marker for pruritus in cholestasis, were also normal in this patient.

At 3 years of age, the authors sequenced the NTCP gene and identified a homozygous nonsynonymous variant (NTCP^{R252H}) that could explain the conjugated hypercholanemia in this patient. This rare single nucleotide polymorphism (SNP, rs147226818) has been identified previously, and is present in less than 0.1% of European and African ancestry alleles (Exome variant server, evs.gs.washington.edu). The R252 residue is highly conserved in NTCP. *In silico* analysis predicted that R252H is likely a damaging variant (PolyPhen2 score, 0.975). This was directly validated using cell-based assays, demonstrating that the NTCP^{R252H} variant is poorly trafficked to the plasma membrane (even after treatment with known molecular chaperones), reducing taurocholate uptake by more than 9-fold.

This first clinical description of an isolated NTCP-deficiency delivers unique insights to human physiology and the fate of "wandering bile acids". Those include: *i*) Conclusive support for the long presumed primary role of NTCP in hepatic bile acid clearance, as evidenced by the striking elevation of plasma bile acids in the absence of apparent liver disease. *ii*) Support for a limited role of alternative hepatic bile acid uptake mechanisms.

Although non-NTCP hepatic uptake mechanisms are sufficient to maintain normal enterohepatic cycling of bile acids in some lower vertebrates[7], that was not the case in this patient. Persistent but low levels of hepatic uptake and enterohepatic cycling of bile acids were apparently maintained, as evidenced by the downward trend in plasma bile acid levels over time, without apparent changes in synthesis, and increased proportion of conjugated secondary bile acids. Candidates for this residual hepatic activity include OATP1B1/1B3, which transport both conjugated and unconjugated bile acids. With a functioning NTCP, OATP1B1/1B3-deficiency does not affect plasma bile acid levels[8], yet in the absence of NTCP, OATP1B1/1B3 may be play a larger but still limited role in bile acid clearance. The other candidate for the residual hepatic uptake activity is OSTα-OSTβ, which can function as a bidirectional transporter. The findings suggest that the transport capacity of these alternative systems is insufficient to compensate for loss of NTCP, although alterations in their expression or activity secondary to altered hepatic bile acid homeostasis in this patient cannot be excluded. *iii*) Insights regarding the regulation of hepatic bile acid synthesis in humans. Reducing hepatic uptake of bile acids by blocking their return from the intestine, for example by use of a bile acid sequestrant, typically leads to a dramatic induction of *de novo* bile acid synthesis. Remarkably, reducing hepatic uptake of bile acids at the sinusoidal membrane appeared to have little effect on their synthesis in this patient. Although hepatic bile acid levels were not measured, these findings support the concept that mechanisms other than bile acid return to the liver regulates synthesis in humans, such as signaling via the FGF19-FGFR4 pathway.[9,10] *iv*) The etiology of cholestasis-associated pruritus. The striking absence of pruritus in this patient further supports the argument that a factor other than conjugated bile acids, such as lysophosphatidic acid (a product of the circulating enzyme autotaxin), is the offending pruritogen in cholestatic patients.[11]

The study also raises many new questions, not least of which is whether hypercholanemia is a disease or not. With regard to the health of the liver in this patient, we do not know if liver histology is normal or if hepatic secretion of other biliary constituents such as cholesterol, phospholipids or conjugated xenobiotics is impacted. One may also postulate that the undiagnosed extrahepatic manifestations in this child (muscular weakness, neurocognitive impairments) may have a basis in the high bile acid levels in the circulation, and presumably in her developing brain. Several studies suggest that cholestasis during the newborn period results in substantial impairments in neurocognitive function, including expressive language, more so in girls than boys.[12,13] The relationship between isolated conjugated hypercholanemia and the spectrum of this child's growth and cognitive impairments is unclear and perhaps unrelated, but should be explored as a rational new area for investigation.

Plasma bile acid levels are often elevated in cholestatic liver disease. However, little is known regarding the long-term clinical consequences of conjugated hypercholanemia in the absence of liver disease. In addition to their potentially cytotoxic detergent properties, bile acids act as metabolic regulators and activate a variety of nuclear and G protein-coupled receptors in tissues beyond the liver and gastrointestinal tract.[14,15] Kidney[16], heart[17,18], vascular[19], and endocrine tissues[20,21] are but a few of the extrahepatic organs and systems whose functions could be adversely affected by persistently high circulating levels of bile

acids. Careful follow-up of this index patient and any future cases is warranted and will provide additional insights.

The authors should be applauded for pursuing this unexpected finding of severe hypercholanemia and ultimately identifying the likely cause. With characterization of the molecular defect in this patient, the authors confirm that NTCP is critical for efficient hepatic clearance of bile acids, but not necessarily for hepatic function. This report of a solitary patient with a case of wandering bile acids advances our understanding of both normal physiology and disease, and will complement advances emerging from large scale genome and exome sequencing efforts of patients with hepatobiliary disorders. These findings are an important step in the long journey towards understanding the broader role for bile acids in health and disease.

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Abbreviations

ABC	ATP-binding cassette
ASBT	apical sodium-dependent bile acid transporter
BSEP	bile salt export pump
FIC1	P-type ATPase mutated in progressive familial intrahepatic cholestasis type 1
MDR	multidrug resistance protein
NTCP	Na+-taurocholate cotransporting polypeptide
OATP	organic anion transporting polypeptide
OST	organic solute transporter
PBAM	primary bile acid malabsorption
PFIC	progressive familial intrahepatic cholestasis

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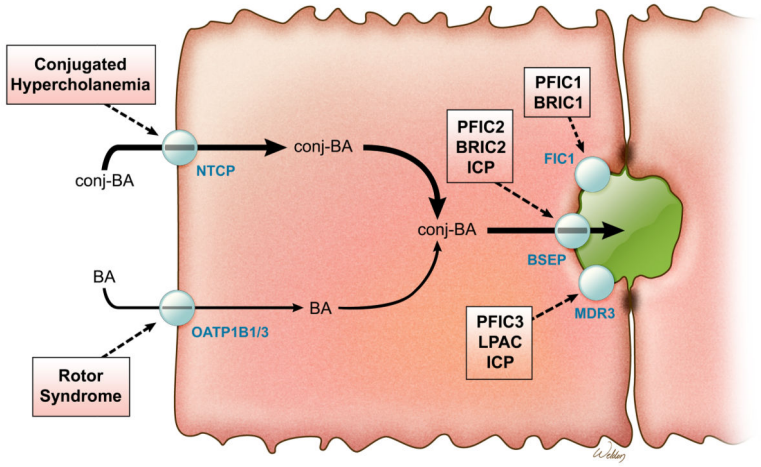

Fig 1.

Schematic view of transporters involved in inherited hypercholanemic disorders. BA, bile acid; BSEP, bile salt export pump; FIC1, P-type ATPase mutated in progressive familial intrahepatic cholestasis type 1; ICP, intrahepatic cholestasis of pregnancy; LPAC, low-phospholipid-associated cholelithiasis; MDR, multidrug resistance protein; NTCP, Na+-taurocholate cotransporting polypeptide; PFIC, progressive intrahepatic cholestasis.

Table 1

Inherited Disorders Associated with Altered Transport of Bile Acids

Protein (gene symbol)	Function	Disease	Clinical Presentation
Liver			
NTCP (*SLC10A1*)	Sinusoidal membrane uptake of bile acids	**Conjugated Hypercholanemia**	Index patient in this report—no liver phenotype.
OATP1B1 (*SLCO1B1*) **OATP1B3 (*SLCO1B3*)**	Sinusoidal membrane uptake of organic anion conjugates and unconjugated bile acids	**Rotor Syndrome**	Jaundice, benign conjugated hyperbilirubinemia, delayed clearance of cholephilic anions such as BSP, ICG
FIC1 (*ATP8B1*)	Canalicular membrane aminophospholipid flipping	**PFIC1**	Progressive cholestasis, elevated serum bile acids, pruritus, normal serum GGT, pancreatitis, intestinal malabsorption, hearing loss
		BRIC1	Periodic attacks of cholestasis, cholelithiasis, normal serum GGT, hepatosplenomegaly
BSEP (*ABCB11*)	Canalicular export of bile acids	**PFIC2**	Progressive cholestasis, pruritus, jaundice, giant cell formation, lobular and portal fibrosis, hepatobiliary malignancy, normal serum GGT
		BRIC2	Periodic attacks of cholestasis, cholelithiasis, normal serum GGT, hepatosplenomegaly
		ICP	Cholestasis in third trimester of pregnancy, pruritus, fetal loss and prematurity, normal serum GGT
MDR3 (*ABCB4*)	Canalicular export of phosphatidylcholine	**PFIC3**	Cholestasis, extensive bile duct proliferation and periportal fibrosis, elevated serum GGT
		LPAC	Cholelithiasis, intrahepatic hyperechoic foci, biliary cirrhosis, elevated serum GGT
		ICP	Cholestasis in third trimester of pregnancy, fetal loss and prematurity, elevated serum GGT
MRP2 (*ABCC2*)	Canalicular export of organic anion conjugates	**Dubin-Johnson Syndrome**	Jaundice, benign conjugated hyperbilirubinemia
TJP2	Maintain tight cellular junctions	**Progressive cholestatic liver disease**	Severe progressive cholestasis, normal GGT
Intestinal			
ASBT (*SLC10A2*)	Apical membrane uptake of bile acids	**PBAM**	Chronic diarrhea, steatorrhea, fat-soluble vitamin malabsorption

ABC, ATP-binding cassette; ASBT, apical sodium-dependent bile acid transporter; BSEP, bile salt export pump; BSP, bromsulphalein; FIC1, P-type ATPase mutated in progressive familial intrahepatic cholestasis type 1; GGT, gamma glutamyl transpeptidase; ICG, indocyanine green; ICP, intrahepatic cholestasis of pregnancy; LPAC, low-phospholipid-associated cholelithiasis; MDR, multidrug resistance protein; MRP, multidrug resistance–associated protein; NTCP, Na+-taurocholate cotransporting polypeptide; PBAM, primary bile acid malabsorption; TJP2, tight junction protein 2.