Targeting the Four Pillars of Enterohepatic Bile Salt Cycling; Lessons From Genetics and Pharmacology

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Bile salts play a pivotal role in lipid homeostasis, are sensed by specialized receptors, and have been implicated in various disorders affecting the gut or liver. They may play a role either as culprit or as potential panacea. Four very efficient transporters mediate most of the hepatic and intestinal bile salt uptake and efflux, and are each essential for the efficient enterohepatic circulation of bile salts. Starting from the intestinal lumen, conjugated bile salts cross the otherwise impermeable lipid bilayer of (primarily terminal ileal) enterocytes through the apical sodium–dependent bile acid transporter (gene SLC10A2) and leave the enterocyte through the basolateral heteromeric organic solute transporter, which consists of an alpha and beta subunit (encoded by SLC51A and SLC51B). The Na+-taurocholate cotransporting polypeptide (gene SLC10A) efficiently clears the portal circulation of bile salts, and the apical bile salt export pump (gene ABCB11) pumps the bile salts out of the hepatocyte into primary bile, against a very steep concentration gradient. Recently, individuals lacking either functional Na+-taurocholate cotransporting polypeptide or organic solute transporter have been described, completing the quarter of bile acid transport deficiencies, as apical sodium–dependent bile acid transporter and bile salt export pump deficiencies were already known for years. Novel pathophysiological insights have been obtained from knockout mice lacking functional expression of these genes and from pharmacological transporter inhibition in mice or humans. Conclusion: We provide a concise overview of the four main bile salt transport pathways and of their status as possible targets of interventions in cholestatic or metabolic disorders. (HEPATOLOGY 2021;73:2577–2585).

The enterohepatic circulation is a very efficient recycling system for bile salts containing most of the bile salt present in the body.¹ The entire bile salt pool circulates multiple times with each contraction of the gallbladder to the duodenum, through the ileum back to the liver and the biliary tract, to eventually be stored again for release during the next contraction (mostly after a meal) (Fig. 1). This implies that at least 20 g of bile salt passes the small intestinal every day, of which only less than 1 g/day is lost through fecal excretion (2%-5% per cycle). Some of the bile salts (mostly unconjugated) passively pass the epithelial lining of the colon, thereby escaping fecal excretion. Most of the bile salt, however, is actively taken up already in the ileum through the apical sodium–dependent bile acid transporter (ASBT, also referred to as the ileal bile acid transporter [IBAT]). We label this as the first of four pillars of the enterohepatic circulation, complemented by the second, the organic solute transporter (OST), facilitating efflux from enterocytes, the third, the Na+-taurocholate cotransporting polypeptide (NTCP), mediating hepatic uptake from the portal circulation, and finally the fourth, the bile salt export pump (BSEP), extruding bile salts from the hepatocyte into the canalliculi.
ASBT and BSEP deficiencies were identified in patients in 1997\(^{(2)}\) and 1998\(^{(3)}\) respectively, but only recently individuals lacking functional NTCP\(^{(4)}\) or OST\(^{(5,6)}\) were described. Genetic deficiencies of these transporters, either in humans or mice, have provided pathophysiological insights into the enterohepatic circulation of bile salts, and in their roles in health and disease. Apart from lipid solubilization in bile and intestine, bile salts also play a pivotal role as signaling molecules, both within and outside the enterohepatic circulation.\(^{(7,8)}\) This has led to the idea that pharmacological inhibition of some of these transporters could be used to ameliorate metabolic or cholestatic disorders. Here, we discuss the potential risks/limitations and advantages of these strategies.

**ASBT**

Oelkers et al. were the first to describe the consequences of genetic deficiency of ASBT, by identification of inactivating mutations in \(SLC10A2\), the gene encoding ASBT, in patients with primary bile
acid malabsorption.\(^{(2)}\) These patients show defective conjugated bile salt absorption from the small intestine, leading to an increased spillover into the colon, causing diarrhea.\(^{(9)}\) This disease can reliably be diagnosed by determining retention of \(^{75}\)Seleno-homotaurocholic acid in the body, but this procedure is not universally available. The pivotal role of ASBT in the enterohepatic cycling of bile salts is confirmed in ASBT knockout (KO) mice,\(^{(10)}\) enabling the use of this model in studies on possible value of pharmacological ASBT inhibition as remedy. The first and most obvious disorder to mention is constipation, in which ASBT inhibition provided benefit.\(^{(11,12)}\) Second, ASBT inhibition was also successfully tested as therapy for hypercholesterolemia, as it results in stimulation of bile salt synthesis and therefore cholesterol catabolism,\(^{(13)}\) and in strongly reduced transintestinal cholesterol excretion.\(^{(14)}\) Note that similar mechanisms were evoked by surgically bypassing the ileum, a procedure designed in the 1960s to treat familial hypercholesterolemia.\(^{(15)}\) In addition, increased levels of bile salts in the (large) intestine stimulates secretion of the incretin hormone glucagon-like peptide 1 (GLP-1), suggesting that ASBT inhibition could also have value in type 2 diabetes.\(^{(16)}\) Furthermore, the increase in bile salts in the colon following ASBT inhibition stimulates the modification of bile salts by bacteria, increasing the (relative) amount of secondary bile salt species. The altered bile salt composition affects farnesoid X receptor (FXR) signaling. The combined effects of ASBT inhibition on lipid and glucose metabolism and signaling triggered the concept of using this approach to treat NAFLD. Studies in mice have suggested that an intestine-restricted ASBT inhibitor improves multiple aspects of NAFLD with restored glucose tolerance, reduced hepatic triglyceride, and total cholesterol concentrations.\(^{(17)}\) Unfortunately, in a recent phase 2 trial in adults with NASH, this strategy failed to lower the amount of fat in the liver, or to have a beneficial effect on liver injury, while the main side effect of ASBT inhibition, diarrhea, was present in most tested individuals.\(^{(18)}\)

Cholestatic disorders form a final set of conditions in which pharmacological ASBT inhibition could be beneficial, as treatment will reduce the hepatic bile salt load, leading to reduced liver injury. In multidrug resistance protein (Mdr) 2 KO mice, an animal model for bile salt–induced cholangiopathy, intestine-restricted pharmacological ASBT inhibition reduced cholestatic liver and bile duct damage.\(^{(19,20)}\) In humans, a trial was performed using similar inhibitors in patients with primary biliary cholangitis (PBC). Like in other cholestatic disorders, patients with PBC frequently suffer from chronic pruritus. Patients with PBC are commonly treated with ursodeoxycholate, but this is unfortunately largely ineffective against itch. The results of a phase 2 trial, with a reduction in itch as primary endpoint, were promising, as ASBT inhibition using GSK2330672 resulted in an approximate 35% reduction in pruritus, as assessed using the 5-D itch score, which was significantly different from the approximate 15% reduction in the placebo group.\(^{(21)}\) ASBT inhibition is now also proposed as a nonsurgical intervention for other cholestatic disorders and has already been shown to reduce itch intensity in patients with Alagille syndrome.\(^{(22,23)}\) Unfortunately, diarrhea is reported as an adverse effect of ASBT inhibition in some, but not all, studies with patients with Alagille syndrome (ClinicalTrials.gov: NCT01903460).\(^{(22,23)}\) We recently proposed combination therapy of ASBT inhibitors with FXR agonism to reduce bile acid synthesis and potentially lower the risk of diarrhea.\(^{(24)}\) Another strategy to reduce intestinal bile salt uptake with lowered incidence of diarrhea is the use of bile acid binding resins such as colesevelam. This increased fecal bile salt excretion and lowered cholestatic liver damage in Abcb4 KO mice.\(^{(25)}\)

**BSEP**

The work of Bull and Thompson\(^{(26)}\) and many others has been pivotal in the elucidation of genetic defects resulting in genetic forms of cholestasis, including progressive familial intrahepatic cholestasis (PFIC) type 2, where BSEP is mutated. BSEP deficiency has a wide spectrum of disease presentations varying from a mild or late-onset phenotype to early and severe liver damage. The incidence of HCC is increased and life expectation decreased when BSEP is nonfunctional or absent, compared with patients with residual BSEP function.\(^{(27)}\) BSEP activity in patients with PFIC type 2 with specific mutations in the \(ABCB11\) gene can partially be corrected using 4-phenylbutyrate\(^{(28)}\) or ivacaftor (for specific missense mutations)\(^{(29)}\) or agents inducing readthrough (for nonsense mutations).\(^{(30)}\) Not only genetic defects, but also pharmacological (off-target) inhibition of BSEP transport function can cause cholestasis, and this leads to drug-induced liver injury.\(^{(31)}\) Interestingly,
the phenotype of the BSEP KO mouse model is rather mild, with impaired mitochondrial fatty acid β-oxidation, but no signs of cholestasis, probably due to compensatory transport of (hydrophilic) bile salts through MDR1α/1b and to the more hydrophilic bile acid composition of murine bile compared with human bile. The canalicular targeting of MDR1 is impaired in BSEP deficiency, as was shown in Abcb11b mutant zebrafish and in a patient lacking BSEP protein due to nonsense mutations in ABCB11, suggesting that strategies aimed at up-regulation or restoration of alternative transport routes could be beneficial in BSEP deficiency. A challenge with a bile duct ligation revealed that BSEP KO mice are largely protected against the liver injury. This may be explained by the enhanced synthesis of tetrahydroxy and pentahydroxy bile acids in these mice. This dramatically lowers the hydrophobicity of the bile salt pool, which decreases its detergent activity and mitigates damage to the hepatocytes and the biliary epithelium.

**NTCP**

The first individuals carrying mutations in SLC10A1 leading to NTCP deficiency were described by Vaz et al. The most prominent biochemical feature was the extremely elevated total bile salt level in plasma (up to 1,500 μM; hypercholanemia), whereas clinical signs of jaundice, pruritus, or liver dysfunction were absent. Subsequently, multiple other NTCP-deficient individuals have been reported with isolated hypercholanemia as the common denominator, showing mild and transient neonatal hyperbilirubinemia and gallbladder anomalies but with no apparent long-term clinical consequences. Pruritus was notably absent in these individuals, suggesting that isolated hypercholanemia may not be so detrimental as once thought. Interestingly, the p.Ser267Phe mutation in SLC10A1 (rs2296651), leading to transporter inactivation, is highly prevalent in East Asia, with an allele frequency of 8%–12% in individuals in Southern China. The authors of the latter study suggest that NTCP deficiency leading to “hidden” hypercholanemia affects 0.64% of the Southern Han, 1.44% of the Dai Chinese population, and 1.21% of the Vietnamese population, indicating that a clinical presentation has a low penetrance, and NTCP deficiency is mostly asymptomatic. In contrast, SLC10A1 inactivity is likely even protective in certain conditions, and this variant may have exerted selection pressure in Southeast Asia. NTCP is the cell surface receptor for both HBV as well as HDV, and the p.Ser267Phe variant may provide protection against both HBV/HDV hepatocytic uptake and infection and HCC, which has been associated with these infections. Myrcludex B (also called bulevirtide), a therapeutic peptide similar to the pre-S1 domain of the HBV envelope, competes with viral binding to NTCP and blocks both viral entry and hepatic bile salt uptake. Myrcludex B has been tested in clinical trials in patients infected with HDV: It induces isolated hypercholanemia lasting for >12 hours per day without signs of pruritus. Myrcludex B treatment also decreased HDV-RNA serum levels and induced alanine aminotransferase normalization under monotherapy in a phase 1b/2 trial. In a phase 2 trial, 10 mg daily Myrcludex B combined with tenofovir resulted in a HDV-RNA negativation or decrease by ≥2 log10 from baseline in 77% of participants versus 4% in the tenofovir–only arm of the study (trial NCT03546621). The European Medicines Agency recently provided conditional marketing authorization for bulevirtide intended for the treatment of chronic HDV infection in adult patients with compensated liver disease, and indicated that no concerns exist on potential long-term safety/efficacy issues in relation to pediatric use in children infected with HDV. Preclinical studies in mice offer promise for additional applications of NTCP inhibitors in conditions of hypercholesterolemia, obesity, and cholestasis.

**NTCP-DEFICIENT MICE**

NTCP-deficient mice, first described by Slijepcevic et al., showed that some redundancy exists in hepatocytic uptake systems for bile salts in mice, due to the expression of other transporters of the organic anion transporting polypeptide (OATP) 1a/1b family. Most mice displayed no elevated bile salt levels at all, whereas a subset of these KO mice exhibited strong hypercholanemia. This was independently confirmed by Mao et al. This remarkable interindividual difference may be explained by a feed-forward mechanism in which elevated bile salt levels in plasma induce intestinal expression of FGF15. This represses gene expression of Oatp1a/1b members in hepatocytes,
leading to an almost complete block of hepatic bile salt uptake and therefore hypercholanemia, despite FGF15-induced dampening of bile acid synthesis.(53) Elevated levels of FGF15 may also explain the gallbladder abnormalities found in hyperchol- emic 4-week-old NTCP KO mice.(39) In addition, NTCP deficiency induces bile salt sulfation, leading to increased elimination through urine, thus dampening the hypercholanemia. (55)

PROTECTIVE ROLE OF NTCP DEFICIENCY IN METABOLIC DISEASE

NTCP deficiency may lead to partial protection against the deleterious effects of a high-calorie diet, as NTCP KO mice have a lower body weight gain and reduced hepatosteatosis on a high-fat diet (HFD).(51) This phenotype was explained by a reduced intestinal fat absorption and increased thermogenesis due to activation of brown adipose tissue. (51) Interestingly, NTCP/TGR5 (or GPBAR1, G protein-coupled bile acid receptor) double-KO mice revealed that the bile salt receptor TGR5 is completely dispensable for this dampened body weight gain, suggesting the presence of distinct bile salt sensory mechanisms. (51) Pharmacological inhibition of NTCP using Myrcludex B lowers body weight, dampens liver fat content, and induces GLP-1 in a Oat1p1a/1b-deficient mouse HFD model for obesity. (52) Interestingly, NTCP expression and hepatic bile salt uptake are down-regulated following Roux-en-Y gastric bypass, suggesting that this could contribute to some of the consequences of bariatric surgery on body weight and glucose handling. (56) Additionally, both NTCP deficiency and pharmacological NTCP inhibition reduce cholesterol levels in plasma in mice and humans. (44,51,52) In mice, NTCP inhibition leads to an enhanced biliary lipid/bile salt ratio. (57) Whether enhanced biliary cholesterol excretion contributes to the cholesterol lowering in plasma following NTCP deficiency awaits further exploration.

NTCP INHIBITION IS HEPATOPROTECTIVE IN CHOLESTATIC CONDITIONS

Pharmacological NTCP inhibition using daily injections with Myrcludex B is hepatoprotective in various animal models for choles tat is. (50,58) NTCP inhibition induced hypercholanemia, indicating effective reduction in hepatic bile salt uptake. This was paralleled by a relative increase in biliary phospholipid excretion (increased phospholipid/bile salt ratio), reduced liver enzymes, and lower expression of genes involved in inflammation and fibrosis in various cholestasis models. Mdr2 KO mice treated with Myrcludex B did not show any hypercholanemia and no hepatoprotective effect, likely due to relatively less repression of Oatp1a/1b-mediated bile salt uptake in this choles tat is model. (50)

Together, these studies suggest that pharmacological targeting of NTCP could have multiple applications in viral hepatitis and metabolic and cholestatic conditions. Furthermore, they suggest that the beneficial effects of NTCP inhibition are not only due to keeping bile salts out of the hepatocytes, but also attributable to the prolonged elevated bile salt levels in plasma.

OSTα-OSTβ

The heteromeric OSTαβ was identified in 2001 by Ballatori et al. (59) In a remarkable expression-cloning approach, two distinct nonhomologous subunits were identified that need to be co-expressed to form a functional bile salt transporter. (59) OSTα and OSTβ are encoded by two genes on separate chromosomes (SLC51A and SLC51B on Chr3 and Chr15, respectively). SLC51B deficiency was identified in 2018 in 2 brothers, clinically characterized by recessive inheritance, chronic diarrhea, severe fat-soluble vitamin deficiency, and features of cholestatic liver disease including elevated liver enzymes, particularly serum gamma-glutamyltransferase activity in serum. (5) In 1 of the siblings, a liver biopsy was taken that revealed mild portal fibrosis without steatosis or inflammation. Stool frequency declined with age. No additional patients have subsequently been identified yet. A KO animal model for SLC51B-deficiency is not yet published, so little is known about this novel genetic bile salt transport disorder. Only recently was a single patient with SLC51A deficiency identified. (6) A liver biopsy from this boy revealed a lobular architecture with periportal fibrosis, suggestive of early cirrhosis and minimal inflammation. His clinical presentation was more severe compared with the two cases of SLC51B deficiency, with easy bruising, two episodes
of prolonged bleeding that required blood transfusions (each likely attributable to malabsorption of fat-soluble vitamin K), and failure to thrive. OSTα and OSTβ deficiency appears to have in common that patients display chronic malabsorptive diarrhea, but the number of cases are still too low to provide comprehensive disease insight. OSTαβ does not exclusively transport bile salts, and its deficiency may affect transport of multiple compounds and drugs. An animal model for OSTα deficiency was generated by two groups. Intestinal bile salt reabsorption is greatly reduced in these mice. OSTα KO mice have a much smaller bile salt pool size, related to strongly increased intestinal FXR activation. They display a clear intestinal phenotype, with decreased villus length and a longer and thicker small intestine. The morphological phenotype with enterocyte damage is not due to FXR activation, but restored following ASBT deletion, indicating that OSTαβ is pivotal to protect enterocytes from intracellular bile salt accumulation. Whether hepatic OSTαβ is similarly critical to protect hepatocytes is less obvious. Both genes are expressed at a low level in normal conditions but up-regulated in cholestasis, in an FXR-dependent manner, suggesting a protective role. However, challenging OSTα KO mice with a bile duct ligation or cholate feeding revealed that these mice are largely protected against cholestatic liver damage. The authors suggest that pharmacological OSTαβ inhibition may be beneficial under cholestatic conditions, as it leads to increased urinary bile salt excretion and reduced intestinal uptake, inducing fecal bile salts excretion in early cholestasis. Furthermore, pharmacological OSTαβ inhibition induces intestine-specific FXR activation, potentially leading to a hepatoprotective reduction of the bile salt pool and to target metabolic-syndrome associated diseases. Nevertheless, OSTαβ up-regulation following FXR activation will counteract some of its inhibition and the clinical phenotype of SLC51A/
SLC51B deficiency with combined malabsorptive and cholestatic characteristics further argues against this approach.

Conclusions

The quartet of human bile acid transport deficiencies, the various animal models for these deficiencies, and the studies with pharmacological inhibition have yielded considerable novel pathophysiological insights into the enterohepatic circulation and (adaptive) consequences of disruptions at the four positions (Table 1). Nevertheless, multiple scientific questions remain. For example, can the benefits of NTCP inhibition seen in mice be transferred to the human situation? Why does OST deficiency appear to lead to cholestasis in humans, while both OSTα and OSTβ are normally only expressed at low levels in liver? Also illustrated by the multiple novel drugs targeting ASBT or NTCP in clinical trials, the field clearly remains highly dynamic.

Author Contributions: All authors have contributed to the manuscript and agree with its content.

REFERENCES

1) Heaton KW. The importance of keeping bile salts in their place. Gut 1969;10:857-863.
2) Oelkers P, Kirby LC, Heubi JE, Dawson PA. Primary bile acid malabsorption caused by mutations in the ileal sodium-dependent bile acid transporter gene (SLC10A2). J Clin Invest 1997;99:1880-1887.
3) Strautnieks SS, Bull LN, Knisely AS, Kocoshis SA, Dahl N, Arnell H, et al. A gene encoding a liver-specific ABC transporter is mutated in progressive familial intrahepatic cholestasis. Nat Genet 1998;20:233-238.
4) Vaz FM, Paulusma CC, Huidekoper H, de Ru M, Lim C, Koster J, et al. Sodium taurocholate cotransporting polypeptide (SLC10A1) deficiency: conjugated hypercholanemia without a clear clinical phenotype. Hepatology 2015;61:260-267.
5) Sultan M, Rao A, Elpeleg O, Vaz FM, Abu-Libdeh B, Karpen SJ, et al. Organic solute transporter-beta (SLC51B) deficiency in two brothers with congenital diarrhea and features of cholestasis. Hepatology 2018;68:590-598.
6) Gao E, Cheema H, Waheed N, Mushtaq I, Erden N, Nelson-Williams C, et al. Organic solute transporter alpha deficiency: a disorder with cholestasis, liver fibrosis, and congenital diarrhea. Hepatology 2020;71:1879-1882.
7) Li T, Chiang JY. Bile acid signaling in metabolic disease and drug therapy. Pharmacol Rev 2014;66:948-983.
8) Thomas C, Pellicciani R, Pruzanski M, Auwerx J, Schoonjans K. Targeting bile-acid signalling for metabolic diseases. Nat Rev Drug Discov 2008;7:678-693.
9) Hegyi P, Maleth J, Walters JR, Hofmann AF, Keely SJ. Guts and gall: bile acids in regulation of intestinal epithelial function in health and disease. Physiol Rev 2018;98:1983-2023.
10) Dawson PA, Haywood J, Craddock AL, Wilson M, Tietjen M, Khackman K, et al. Targeted deletion of the ileal bile acid transporter eliminates enterohepatic cycling of bile acids in mice. J Biol Chem 2003;278:33920-33927.
11) Chey WD, Camilleri M, Chang L, Rikner L, Graffner H. A randomized placebo-controlled phase IIb trial of a3309, a bile acid transporter inhibitor, for chronic idiopathic constipation. Am J Gastroenterol 2011;106:1803-1812.
12) Nakajima A, Seki M, Yanaguchi S, Ohra A, Gillberg PG, Mattsson JP, et al. Safety and efficacy of elobixibat for chronic constipation: results from a randomised, double-blind, placebo-controlled, phase 3 trial and an open-label, single-arm, phase 3 trial. Lancet Gastroenterol Hepatol 2018;3:537-547.
13) Hijiki J, Harai S, Takasu N, Tonda K, Miyata K, Shike T, et al. Inhibition of ileal Na+/bile acid cotransporter by S-8921 reduces serum cholesterol and prevents atherosclerosis in rabbits. Arterioscler Thromb Vasc Biol 1998;18:1304-1311.
14) van de Peppel JP, Bertolini A, van Dijk TH, Groen AK, Jonker JW, Verkade HJ. Efficient reabsorption of transintestinally exerted cholesterol is a strong determinant for cholesterol disposal in mice. J Lipid Res 2019;60:1562-1572.
15) Thompson GR, Gotto AM, Jr. Ileal bypass in the treatment of hyperlipoproteinemia. Lancet 1973;2:355-356.
16) Chen L, Yao X, Young A, McNulty J, Anderson D, Liu Y, et al. Inhibition of apical sodium-dependent bile acid transporter as a novel treatment for diabetes. Am J Physiol Endocrinol Metab 2012;302:E68-E76.
17) Rao A, Kosters A, Mells JE, Zhang W, Setchell KD, Amano AM, et al. Inhibition of ileal bile acid uptake protects against non-alcoholic fatty liver disease in high-fat diet-fed mice. Sci Transl Med 2016;8:357ra122.
18) Newsome PN, Palmer M, Freilich B, Sheikh MY, Sheikh A, Sarles H, et al. Volibixat in adults with non-alcoholic steatohepatitis: 24-week interim analysis from a randomized, phase II study. J Hepatol 2020;73:231-240.
19) Baghdasaryan A, Fuchs CD, Oelkers P, Kirby LC, Heubi JE, Dawson PA. Primary bile acid malabsorption caused by mutations in the ileal sodium-dependent bile acid transporter gene (SLC10A2). J Clin Invest 1997;99:1880-1887.
20) Strautnieks SS, Bull LN, Knisely AS, Kocoshis SA, Dahl N, Arnell H, et al. A gene encoding a liver-specific ABC transporter is mutated in progressive familial intrahepatic cholestasis. Nat Genet 1998;20:233-238.
26) Bull LN, Thompson RJ. Progressive familial intrahepatic cholestasis. Clin Liver Dis 2018;22:657-669.
27) van Wessel DBE, Thompson RJ, Gonzales E, Jankowska I, Sokal E, Grammatikopoulos T, et al. Genotype correlates with the natural history of severe bile salt export pump deficiency. J Hepatol 2020;73:84-93.
28) Gonzales E, Grosse B, Schuller B, Davit-Sraul A, Conti F, Guettier C, et al. Targeted pharmacotherapy in progressive familial intrahepatic cholestasis type 2: evidence for improvement of cholestasis with 4-phenylbutyrate. Hepatology 2015;62:558-566.
30) Mareux E, Lepalus M, Amzal R, Almes M, Ait-Slimane T, Dejaunay JL, et al. Functional rescue of an ABCB11 mutant by ivacaftor: a new targeted pharmacotherapy approach in bile salt export pump deficiency. Liver Int 2020.
31) Amzal R, Thibault A, Lepalus M, Almes M, Grosse B, Mareux E, et al. Pharmacological premature termination codon readthrough of ABCB11 in bile salt export pump deficiency: an in vitro study. Hepatology 2020 Jul 23. https://doi.org/10.1002/hep.31476. [Epub ahead of print]
32) Zhang Y, Li F, Patterson AD, Wang Y, Krausz KW, Neale G, et al. Abcb11 deficiency induces cholestasis coupled to impaired beta-fatty acid oxidation in mice. J Biol Chem 2012;287:24784-24794.
33) Wang R, Chen HL, Liu L, Sheps JA, Phillips MJ, Ling V. Compensatory role of P-glycoproteins in knockout mice lacking the bile salt export pump. Hepatology 2009;50:948-956.
34) Lam P, Wang R, Ling V. Bile acid transport in sister of P-glycoprotein (ABCB11) knockout mice. Biochemistry 2005;44:12598-12605.
35) Ellis JL, Bove KE, Schuetz EG, Leino D, Valencia CA, Schuetz JD, et al. Zebrafish abcb11b mutant reveals strategies to restore bile excretion impaired by bile salt export pump deficiency. Hepatology 2018;67:1531-1545.
36) Fuchs CD, Paumgartner G, Wahlstrom A, Schwabl P, Reiberger T, Leditzng N, et al. Metabolic preconditioning protects BSEP/ABCB11(-/-) mice against cholestatic liver injury. J Hepatol 2017;66:95-101.
37) Wang R, Sheps JA, Liu L, Han J, Chen PSK, Lamontagne J, et al. Hydrophilic bile acids prevent liver damage caused by lack of biliary phospholipid in Mdr2(-/-) mice. J Lipid Res 2019;60:85-97.
38) Yant YY, Wang MX, Gong JY, Liu LL, Setchell KDR, Xie XB, et al. Abnormal bilirubin metabolism in patients with sodium taurocholate transporting polypeptide deficiency. J Pediatr Gastroenterol Nutr 2020 Aug 10. https://doi.org/10.1097/MPG.0000000000002862. [Epub ahead of print]
39) Mao F, Wang MX, Hou X, Zhou Z, Yan YY, Fang LJ, et al. NTCP Deficiency causes gallbladder abnormalities in mouse and human beings. Cell Mol Gastroenterol Hepatol 2020 Sep 9. https://doi.org/10.1016/j.jcmgh.2020.09.001. [Epub ahead of print]
40) Dong C, Zhang BP, Wang H, Xu H, Zhang C, Cai ZS, et al. Clinical and histopathologic features of sodium taurocholate co-transporting polypeptide deficiency in pediatric patients. Medicine (Baltimore) 2019;98:e17305.
41) Liu R, Chen C, Xia X, Liao Q, Wang Q, Newcombe PJ, et al. Homozygous p.Ser267Phe in SLC10A1 is associated with a new type of hypercholesterolaemia and implications for personalized medicine. Sci Rep 2017;7:9214.
42) Ni YJ, Lemp FA, Mehrle S, Nkongolo S, Kaufman C, Felth M, et al. Hepatitis B and D viruses exploit sodium taurocholate co-transporting polypeptide for species-specific entry into hepatocytes. Gastroenterology 2014;146:1070-1083.
43) Yan H, Zhong G, Xu G, He W, Jing Z, Gao Z, et al. Sodium taurocholate co-transporting polypeptide is a functional receptor for human hepatitis B and D virus. eLife 2012;1:e00049.
44) Cheng X, Wang Y, Tian J, Zhou L, Chen X, Guo H, et al. SLC10A1 S267F variant influences susceptibility to HBV infection and reduces cholesterol level by impairing bile acid uptake. J Viral Hepat 2019;26:1178-1185.
45) Hu HH, Liu J, Lin YL, Luo WS, Chu YJ, Chang CL, et al. The rs2296651 (S267F) variant on NTCP (SLC10A1) is inversely associated with chronic hepatitis B and progression to cirrhosis and hepatocellular carcinoma in patients with chronic hepatitis B. Gut 2016;65:1514-1521.
46) Peng L, Zhao Q, Li Q, Li M, Li C, Xu T, et al. The p.Ser267Phe variant in SLC10A1 is associated with resistance to chronic hepatitis B. Hepatology 2015;61:1251-1260.
47) Blank A, Eidam A, Haag M, Hohmann N, Buhrenne J, Schwab M, et al. The NTCP-inhibitor myrcludex B: effects on bile acid disposition and tenofovir pharmacokinetics. Clin Pharmacol Ther 2018;103:341-348.
48) Blank A, Markert C, Hohmann N, Carsl A, Mikus G, Lehr T, et al. First-in-human application of the novel hepatitis B and hepatitis D virus entry inhibitor myrcludex B. J Hepatol 2016;65:483-489.
49) Bogomolov P, Alexandrov A, Voronkova N, Macievich M, Kokina K, Petrachenkov M, et al. Treatment of chronic hepatitis D with the entry inhibitor myrcludex B: first results of a phase Ib/Ia study. J Hepatol 2016;65:490-498.
50) Slijepec D, Roscam Abbing RLP, Fuchs CD, Haazen LCM, Beuers U, Trauner M, et al. Na(+)-taurocholate co-transporting polypeptide inhibition has hepatoprotective effects in cholestasis in mice. Hepatology 2018;68:1057-1069.
51) Donkers JM, Kooijman S, Slijepec D, Kunst RF, Roscam Abbing RL, Haazen L, et al. NTCP deficiency in mice protects against obesity and hepatosteatosis. JCI Insight 2019;5:e127197.
52) Donkers JM, Roscam Abbing RL, van Weeghel M, Levels JHM, Boelen A, Schinkel AH, et al. Inhibition of hepatic bile acid uptake by myrcludex B promotes glucagon-like peptide-1 release and reduces obesity. Cell Mol Gastroenterol Hepatol 2020;10:451-466.
53) Slijepec D, Roscam Abbing RLP, Katafuchi T, Blank A, Donkers JM, van Hoppe S, et al. Hepatic uptake of conjugated bile acids is mediated by both sodium taurocholate co-transporting polypeptide and organic anion transporting polypeptides and modulated by intestinal sensing of plasma bile acid levels in mice. Hepatology 2017;66:1631-1643.
54) Slijepec D, Kaufman C, Wichters CG, Gilgion E, Lemp FA, Duijst S, et al. Impaired uptake of conjugated bile acids and hepatitis B virus pres1-binding in na(+) -taurocholate co-transporting polypeptide knockout mice. Hepatology 2015;62:207-219.
55) Mao F, Liu T, Hou X, Zhao H, He W, Li C, et al. Increased sulfation of bile acids in mice and human subjects with sodium taurocholate co-transporting polypeptide deficiency. J Biol Chem 2019;294:11853-11862.
56) Chavez-Talavera O, Baud G, Spinelli V, Daoudi M, Kouach M, Goossens JF, et al. Roux-en-Y gastric bypass increases systemic but not portal bile acid concentrations by decreasing hepatic bile acid uptake in minipigs. JCI Insight 2019;5:e127197.
57) Roscam Abbing RLP, Slijepec D, Donkers JM, Havinga R, Duijst S, Paulusma CC, et al. Blocking sodium-taurocholate co-transporting polypeptide stimulates biliary cholesterol and phospholipid secretion in mice. Hepatology 2020;71:247-258.
58) Cai SY, Ouyang X, Chen Y, Soroka C, Wang J, Mennone A, et al. Bile acids initiate cholestatic liver injury by triggering a hepatocyte-specific inflammatory response. JCI Insight 2017;2:e90780.
59) Wang W, Seward DJ, Li L, Boyer JL, Ballatori N. Expression cloning of two genes that together mediate organic solute and steroid transport in the liver of a marine vertebrate. Proc Natl Acad Sci U S A 2001;98:9431-9436.

60) Beaudoin JJ, Brouwer KLR, Malinen MM. Novel insights into the organic solute transporter alpha/beta, OSTalpha/beta: from the bench to the bedside. Pharmacol Ther 2020;211:107542.

61) Rao A, Haywood J, Craddock AL, Belinsky MG, Kruh GD, Dawson PA. The organic solute transporter alpha-beta, Ostalpha-Ostbeta, is essential for intestinal bile acid transport and homeostasis. Proc Natl Acad Sci U S A 2008;105:3891-3896.

62) Ballatori N, Fang F, Christian WV, Li N, Hammond CL. Ostalpha-Ostbeta is required for bile acid and conjugated steroid disposition in the intestine, kidney, and liver. Am J Physiol Gastrointest Liver Physiol 2008;295:G179-G186.

63) Lan T, Rao A, Haywood J, Kock ND, Dawson PA. Mouse organic solute transporter alpha deficiency alters FGF15 expression and bile acid metabolism. J Hepatol 2012;57:359-365.

64) Ferrebee CB, Li J, Haywood J, Pachura K, Robinson BS, Hinrichs BH, et al. Organic solute transporter alpha-beta protects ileal enterocytes from bile acid-induced injury. Cell Mol Gastroenterol Hepatol 2018;5:499-522.

65) Soroka CJ, Mennone A, Hagey LR, Ballatori N, Boyer JL. Mouse organic solute transporter alpha deficiency enhances renal excretion of bile acids and attenuates cholestasis. Hepatology 2010;51:181-190.

66) Soroka CJ, Velazquez H, Mennone A, Ballatori N, Boyer JL. Ostalpha depletion protects liver from oral bile acid load. Am J Physiol Gastrointest Liver Physiol 2011;301:G574-G579.

67) van de Wiel SMW, de Waart DR, Oude Elferink RPJ, van de Graaf SFJ. Intestinal farnesoid X receptor activation by pharmacologic inhibition of the organic solute transporter alpha-beta. Cell Mol Gastroenterol Hepatol 2018;5:223-237.

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