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INVITED REVIEW

The bile salt export pump

Bruno Stieger · Yvonne Meier · Peter J. Meier

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Abstract Canalicular secretion of bile salts mediated by the bile salt export pump Bsep constitutes the major driving force for the generation of bile flow. Bsep is a member of the B-family of the super family of ATP-binding cassette transporters and is classified as ABCB11. Bsep has a narrow substrate specificity, which is largely restricted to bile salts. Bsep is extensively regulated at the transcriptional and posttranscriptional level, which directly modulates canalicular bile formation. Pathophysiological alterations of Bsep by either inherited mutations or acquired processes such as inhibition by drugs or disease-related down regulation may lead to a wide spectrum of mild to severe forms of liver disease. Furthermore, many genetic variants of Bsep are known, some of which potentially render individuals susceptible to acquired forms of liver disease.

Keywords Bile formation · Bile acid transport · ATP-binding cassette transporter · Mutation · Polymorphism

Introduction

Bile formation is one of the key functions of mammalian liver. It involves vectorial secretion of bile acids and other cholephilic compounds across hepatocytes from the sinusoidal blood plasma into bile canaliculi [50, 61, 78, 91] (Fig. 1). Thereby, bile acids or bile salts are concentrated more than 500-fold in bile as compared to blood plasma. This concentrative process is dependent on ATP [87] and mainly driven by the canalicular bile salt export pump BSEP [61]. BSEP is a member of the B-family of the ATB-binding cassette (ABC) superfamily of transporters and is classified as ABCB11.

In 1995, a close relative to MDR1 (ABCB1 or P-glycoprotein) was identified in pig and was named as the sister of P-glycoprotein (spgp) [14]. In a panel of mRNAs from different rat tissues, only the liver was positive by Northern blotting. Based on the hypothesis that the canalicular bile salt export system should be a member of the ABC-transporter superfamily and predominantly, if not exclusively expressed in the liver, the rat isoform of spgp was cloned from liver. After its expression in insect Sf9 cells with the baculovirus system, it could be demonstrated that spgp represents an ATP-dependent bile salt transporter [33]. Based on its function, spgp was renamed as "bile salt export pump" or "Bsep". Positional cloning of the defective gene in patients with progressive familial intrahepatic cholestasis type 2 (PFIC2) led to the identification of the human *BSEP* gene [88], indicating that PFIC2 was actually a BSEP deficiency syndrome [78]. The selective absence of bile salts in the bile of all PFIC2 patients further confirmed the bile salt transport function of BSEP [45]. Bsep is evolutionary relatively old, and a Bsep orthologue is already present in skate (*Raja erinacea*) liver [10]. Furthermore, partial Bsep sequences have been described in the teleost winter flounder (*Pseudopleuronectes americanus*) [14]. Of note, ATP-dependent bile salt transport activity has also been demonstrated in plant vacuoles [42] and in yeast, where the responsible

B. Stieger (✉) · Y. Meier
Department of Medicine, Institute of Clinical Pharmacology and Toxicology, University Hospital,
8091 Zürich, Switzerland
e-mail: bstieger@kpt.unizh.ch

P. J. Meier
University of Basel,
Basel, Switzerland

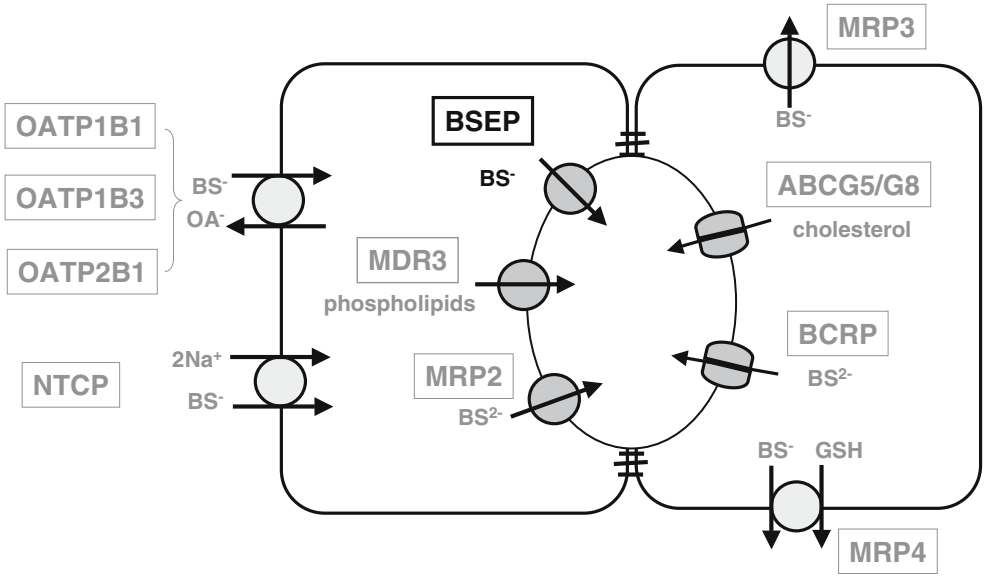

Fig. 1 Principle mechanisms of bile formation. Bile salts are taken up into hepatocytes in a sodium-dependent manner by the sodium-taurocholate transporting polypeptide (*NTCP*) and, to a lesser extent, in a sodium-independent manner by the organic anion transporting polypeptides (*OATPs*) [61]. Bile salts are secreted across the canalicular plasma membrane by the bile salt export pump (*BSEP*), from where they promote in concert with the multidrug-resistance protein 3 (*MDR3*) the release of phospholipids from the canalicular plasma membrane (see article by Oude Elferink in this issue). Phospholipids form together with bile salt-mixed micelles, which act as a vehicle for cholesterol that is exported into the canaliculus by ABCG5/G8 (see article by Patel in this issue). In addition, multidrug resistance protein 2 (*MRP2*) and breast cancer resistance protein (*ABCG2*) mediate canalicular secretion of organic anions and divalent bile salts (see articles by Keppler and Sugiyama, respectively, in this issue). The basolateral exporters MRP3 and MRP4 may act as salvage systems to lower cytoplasmic levels of potentially hepatotoxic compounds (see article by Borst in this issue)

transport protein (BAT1) is also a member of the ABC-transporter superfamily [74].

Functional characteristics

The *BSEP* gene is located on chromosomes 2q24–31 [88]. Its 28 exons encode a protein of 1,321 amino acids [9, 72]. Rat and mouse Bsep consist of 1,321 amino acids and exhibit molecular weights of 160 kDa [33, 35, 54, 69]. Based on comparison of the molecular weight of rat Bsep expressed in Sf9 cells in comparison to rat canalicular membrane vesicles, it is very likely that Bsep is a glycoprotein [33]. Extensive functional comparison in isolated rat canalicular plasma membrane vesicles and in Sf9 cell vesicles revealed similar kinetic properties of rat Bsep with K_m values of 2.1 μM for taurocholate, 3.8 μM for glycocholate, 3.6 μM for taurochenodeoxycholate, and 6.2 μM for tauroursodeoxycholate [86]. These findings indicate that expression in the Sf9 cell systems is suitable for functional characterization of various Bsep orthologues from different species. In fact, comparison of the intrinsic clearances of mouse, rat, and human Bsep/BSEP revealed the same rank order of transporter preferences for different bile salts and comparable K_m values [72]. These close functional relationships between Bsep of different species are paralleled by amino acid sequence similarities of more than 80%, indicating that functional studies with rat Bsep in isolated canalicular liver plasma membrane vesicles can be extrapolated to human BSEP. In rats, the specificity of Bsep is restricted to monovalent bile salts, whereas no transport of taurolithosulfocholate, leukotriene C4, DNP-SG, estradiol-17β-glucuronide, or GSSG was observed [86]. Recently, rat and human Bsep/BSEP were compared after expression in HEK293 cells and were found to have qualitative comparable transport properties with one exception: Human BSEP transported taurolithocholate 3-sulfate significantly, whereas virtually no transport was observed with rat Bsep [38]. This difference may reflect the fact that the human bile salt pool contains higher amounts of taurolithocholate 3-sulfate than the rodent bile salt pool. Despite the close structural relationship between bile salts to cholesterol, rat Bsep seems not to be able to transport sitosterol [2]. Nevertheless, the substrate specificity of Bsep/BSEP seems not to be entirely restricted to bile salts since transfection of the ovary cancer cell line SKOV3 with rat Bsep induced a slight resistance to taxol [15]. In addition, mouse Bsep expressed in LLC-PK_1 cells has been shown to mediate the transport of vinblastine and the fluorescent Mdr1 substrate calcein AM [54]. Interestingly, in vesicles isolated from transfected HEK 293 cells, ATP-dependent pravastatin transport could be demonstrated for

both rat Bsep and human BSEP [40]. Hence, although monovalent bile salts represent the predominant transport substrate class for all Bsep isoforms, some additional compounds may also be transported by Bsep/BSEP. Nevertheless, the fact that the absence of functional BSEP in the canalicular membrane due to mutations in the *BSEP* gene leads to severe forms of intrahepatic cholestasis in childhood and to bile salt levels <1% of normal in bile supports the concept that the major physiological role of the bile salt export pump is ATP-dependent bile salt transport [45].

Tissue distribution

The partial sequence of Bsep from pigs was used by Childs and coworkers to determine its tissue distribution by Northern blotting. These data revealed a predominant, if not exclusive, expression of pig Bsep in the liver [14]. This tissue distribution was confirmed after a full-length sequencing of Bsep in the rat [33] and in mouse [35, 54]. However, using a PCR-based approach, Bsep mRNA was also identified in brain cortex and small and large intestine in the rat [90]. Based on a real-time PCR approach, expression of BSEP was not only found in human liver but also at very high levels in human testis, an organ which had not been previously investigated for BSEP expression [53]. The same study detected some BSEP mRNA also in several other tissues. The observed predominant expression of Bsep in the liver is preserved during evolution since in the primitive elasmobranch vertebrate, little skate, Bsep is exclusively expressed in the liver [10].

Ontogenesis and regulation

Ontogenesis of Bsep has been most extensively studied in rat liver. Bsep mRNA could be detected at day 15 in developing mouse liver [54] and, although at low levels, at embryonic day 20 in rat liver [30, 89, 104]. In rats, positive canalicular protein expression is observed at low levels at embryonic day 20. After embryonic day 20, the expression of rat Bsep at the protein and mRNA levels rapidly increases up to almost adult levels within the first week after birth [30, 89, 104]. This sequence of expression coincides well with the development of the major bile salt uptake system Ntcp in rat liver as well as with the development of the enterohepatic circulation of bile salts [30]. In humans, the *BSEP* gene is expressed at mid-gestation at mRNA levels much lower than in the adult liver [13]. Immunohistochemical analysis demonstrated BSEP expression in the fetal liver both at the bile canaliculi and within hepatocytes.

Regulation of Bsep occurs at two main levels: first, at the transcriptional level and, second, at the posttranscriptional level by insertion and retrieval of Bsep into the canalicular plasma membrane and/or posttranslational protein modifications. The latter two mechanisms can act at very short-time scales, whereas regulation at the transcriptional level has a more long-term impact.

Transcriptional regulation of Bsep The predominant transcription factor involved in the transcriptional regulation of Bsep expression is the farnesoid X receptor (FXR) [23, 50]. This transcription factor acts as an intracellular bile salt sensor in hepatocytes and, upon activation by bile salts, binds as a heterodimer with retinoid X receptor (RXR) to the Bsep promoter. This leads to an upregulation of Bsep expression and consequently to increased canalicular bile salt secretion [3, 32]. These data are supported by a reduced expression of Bsep in mice lacking the gene for FXR [85]. This regulatory mechanism seems to be more strongly affected by transhepatocellular bile salt flux than by steady-state bile salt levels [102]. Since intracellular bile salts are elevated in cholestatic liver disease, this regulatory pathway represents an important protective mechanism for hepatocytes [23]. Recently, it has been shown that the vitamin D receptor (VDR) directly interacts with FXR and, upon binding of vitamin D3, inhibits FXR-mediated transactivation of *BSEP* [41]. In primary cultured rat hepatocytes, mRNA and Bsep protein levels are partially maintained for approximately 72 h of culture time [82]. Interestingly, hyperosmolarity of the culture medium leads to upregulation and hypoosmolarity to downregulation of Bsep mRNA expression [100]. Furthermore, maintenance of Bsep mRNA expression was reported to be critically dependent on the presence of dexamethasone in the culture medium [100]. However, in other studies using sandwich cultured primary rat hepatocytes, the expression of Bsep on both the mRNA and protein levels was less sensitive to the presence of dexamethasone [57, 92], although very low dexamethasone concentration influenced Bsep expression levels also in primary hepatocytes cultured in a collagen-sandwich configuration [92]. Taken together, these results indicate that not only the bile acid sensor FXR is crucial for the control of Bsep expression, but more complex regulatory cascades involving for example steroid receptors need to be considered as well. This is supported by the observation that patients with biliary atresia improve their bile flow rate after surgery with high-dose steroid treatment [64].

Short-term posttranscriptional regulation of Bsep There is considerable evidence from animal models that the canalicular localization of Bsep can be regulated by endo- and

exocytotic mechanisms [4, 96]. Studies in isolated perfused rat livers showed a stimulation of biliary excretion of bile salts concomitant with a stimulation of vesicular transport processes in hepatocytes [36]. Similar experiments in isolated rat hepatocyte couplets resulted in an enhanced secretion of a fluorescent bile acid derivative into the canalicular vacuole [8]. This stimulation of bile salt secretion is independent from protein synthesis but sensitive to the microtubule disrupting agent colchicine, suggesting the presence of an intracellular regulatory pool of Bsep transporters. Treatment of rats with intravenous taurochcholate or dibutyryl cAMP resulted in a fast increase in functional Bsep insertion into canalicular plasma membranes [31]. Inhibition of this process by colchicine confirmed the postulated presence of an intracellular vesicular pool of Bsep, which upon stimulation is incorporated into the canalicular plasma membrane in a microtubule-dependent process. In addition, cAMP-dependent Bsep incorporation into the canalicular plasma membrane was inhibited by wortmannin indicating an important role of PI 3-kinase [66]. These regulatory mechanisms are independent from protein biosynthesis and hence relate to posttranscriptional events. In isolated rat canalicular plasma membrane vesicles, products of PI 3-kinase action exerted a direct effect on the transport activity of Bsep, indicating that PI 3-kinase modulates bile salt transport activity both by Bsep density and Bsep activity in the canalicular plasma membrane [65]. Biosynthetic studies indicate a direct targeting of Bsep from the Golgi to the canalicular plasma membrane. Furthermore, treating rats simultaneously with cAMP and with taurocholate resulted in additive incorporation of Bsep into the canalicular membrane to levels higher than with a single modulator. Based on all these observations, it has been postulated that two separate intracellular pools exist within hepatocytes, from which Bsep can be recruited and inserted into the canalicular plasma membrane under the conditions of increased bile flow rates [47]. In addition to these pathways, the mitogen-activated kinase cascades are also involved in the posttranscriptional regulation of Bsep expression: Induction of choleresis by tauroursodeoxycholic acid or alteration of bile flow by anisosomolarity leads to canalicular insertion/retrieval of Bsep, a process that is critically dependent on mitogen-activated kinases [83]. In addition, studies in polarized cell lines expressing Bsep revealed that HAX-1 and myosin II regulatory light chain are required for the trafficking of Bsep to the apical membrane as well as in the regulation of its abundance in the apical plasma membrane of MDCK cells [12, 73]. Studies in the polarized hepatic WIF-B9 cell line indicated that Bsep undergoes a constitutive cycling between a rab11 positive compartment and the canalicular plasma membrane domain [95]. Bsep and, hence, the capacity of canalicuar bile salt secretion may be regulated not only via carrier density in the canalicular plasma membrane but also by posttranslational modifications of the Bsep protein. Mouse Bsep, if coexpressed with the α-isoform of protein kinase C in Sf9 cells, is phosphorylated, which could affect the functional properties of Bsep [69, 71]. Posttranslational processes occur on short-time scales may be needed to adapt canalicular bile salt secretion to a short-term increase in hepatocellular bile salt load, such as, for example, in a postprandial state. In contrast, the half-life of Bsep in rats is 4 to 6 days, which is in line with the half-life of other rat hepatocellular plasma membrane proteins [48]. To our knowledge, factors regulating the half-life of Bsep have not yet been investigated. Such events may, however, turn out to be important in chronic alterations of liver functions, such as various forms of liver disease.

Pathophysiological consequences of altered Bsep function and expression

Inhibition of Bsep/BSEP leads to a reduced bile salt secretion and decreased bile flow, a process called cholestasis. This has been shown in the perfused rat liver, for example, for cyclosporine A [6]. After the cloning of rat Bsep, it was possible to directly test the inhibition of Bsep expressed in Sf9 cells in the absence of other organic anion transporters. These studies showed that cyclosporine A as well as glybenclamide, rifampicin, and rifamycin are competitive inhibitors of Bsep [86]. The same drugs also inhibit human BSEP [9, 72]. Other drugs that have been shown to inhibit BSEP are bosentan [25], troglitazone [29], and fluvastatin (C. Lang, Y. Meier, and C. Pauli-Magnus, submitted for publication). Such drugs may, in susceptible humans, cause acquired cholestasis, which rapidly resolves after the withdrawal of the drug. Alteration of Bsep-mediated bile salt secretion can also be caused indirectly. For example, the Bsep inhibitor bosentan leads to a hypercholeresis in rats, which depends on the canalicular presence of functional Mrp2 [28]. This canalicular hypercholeresis may lower the bile salt concentration in the canalicular lumen and consequently reduce canalicular lipid secretion [28]. Such a mechanism could in turn lead, via secondary inhibition of Bsep, to the observed accumulation of serum bile acids in rats after administration of bosentan [25, 60]. Studies with mouse Bsep demonstrated that some drugs inhibit not only the transport activity of Bsep but also its ATPase activity [69]. Since the substrate binding site(s) of the various Bsep isoforms is unknown at present, it cannot be excluded that inhibitory drugs are also potential transport substrates of Bsep. Very interestingly, estradiol-17β-glucuronide, a cholestat-

ic metabolite of estrogen, requires coexpression of Mrps for inhibition of Bsep [1, 86]. This has been interpreted as so-called trans-inhibition since estradiol-17β-glucucronide needs to be transported into the canalicular lumen before it can exert its cholestatic action on Bsep. Most importantly, this result has been confirmed for progesterone-sulfate, a key metabolite that increases during pregnancy [93].

Although inhibition of Bsep by drugs and other substances is rapid and reversible, other pathophysiological situations are associated with more pronounced effects on Bsep expression. Sepsis in patients with gram-negative infections may be associated with cholestasis [67]. The causative agent for induction is the endotoxin lipopolysaccharide secreted by gram-negative bacteria. In experimental septic models, Bsep mRNA and protein levels were reduced [55, 94]. In addition, such models of sepsis have provided evidence for a relocation of canalicular Bsep to a percicanalicular vesicular compartment [7, 103]. These effects on Bsep expression could be prevented with the osmolyte taurine [68]. Treatment of human liver slices with lipopolysaccharide also induced the downregulation of BSEP at the protein level, suggesting similar mechanisms in human liver as in animal models [22].

Obstructive cholestasis or cholestasis induced by ethinylestradiol treatment of rats leads to a modest reduction in the expression of Bsep in the canalicular membrane [55] and thus resembles the moderate downregulation of Bsep in primary cultured rat hepatocytes [82]. In the rat model of cholestasis associated with pregnancy (treatment of rats with estradiol), a relocation of Bsep into subapical vesicles is observed [18]. If these rats are treated with 6-ethyl chenodeoxycholate, a potent ligand for FXR, Bsep is induced and the cholestasis induced by estradiol is reversed [27]. And finally, treatment of rats with the cholestatic bile acids lithocholate or taurolithocholate leads to a retrieval of Bsep from the canalicular plasma membrane [19], which can be prevented by the administration of cAMP [19], silibinin [17], or tauroursodeoxy cholic acid [21].

In patients with obstructive cholestasis who underwent biliary drainage, BSEP mRNA levels were reduced in individuals with poor drainage as compared to controls. Additionally, BSEP staining appeared fuzzy, indicating altered expression as a consequence of cholestasis [84]. Another cholestatic liver disease is primary biliary cirrhosis. In such patients, the expression of BSEP is preserved in contrast to the basolateral uptake systems [105, 106]. Patients with inflammation-induced icteric cholestasis due to alcoholic hepatitis have a reduced expression of BSEP mRNA and protein [106]. Frequently, cholestatic liver disease is treated with ursodeoxycholic acid [5, 79]. In a recent study, it was observed that treatment of patients with ursodeoxycholic acid leads to a stimulation of BSEP expression [59]. Additionally, after liver transplantation, the bile flow rate is initially low and recovers with time. This recovery of bile flow rate is paralleled by an increased expression of BSEP mRNA in liver biopsies from transplanted livers [34].

Genetics

Mutations in canalicular transporter genes and in particular in the *BSEP* gene that lead to hereditary forms of liver disease are well known. The BSEP deficiency syndrome is caused by mutations in the *BSEP* gene and leads to functional defects of BSEP-mediated canalicular bile salt secretion [78]. Depending on the clinical course of this syndrome, two different clinical entities of the BSEP deficiency syndrome are known: a mild form also called benign recurrent intrahepatic cholestasis type 2 (BRIC2) and a severe form also called progressive familial intrahepatic cholestasis type 2 (PFIC2). These two diseases represent a heterogeneous group of cholestatic conditions, and BRIC 2 may develop into PFIC2 [51, 78]. So far, more than 30 mutations in the *BSEP* gene have been identified in patients with BSEP deficiency syndrome [78] (Fig. 2). The majority of these mutations affect evolutionary conserved amino acid residues, and the affected amino acids tend to be located in the large cytoplasmic loops of BSEP. In many patients with severe BSEP deficiency syndrome, BSEP staining was absent on liver sections [45, 46]. Direct investigation of the functional consequences of the BSEP mutations in human liver is impossible. Therefore, studies using heterologous expression systems of mutated forms of BSEP were used to subsidize for this lack of in vivo data. Expression of various BSEP mutations affecting conserved amino acids in a rat or mouse background in polarized cells such as MDCK or HepG2 cells has resulted in defects of apical trafficking and/or functional impairment of BSEP as well as in temperature-sensitive, unstable forms of BSEP [81, 97]. It should, however, be noted that such studies on the functional impact of the human mutations based on heterologous expression systems may lead to conflicting results suggesting species-specific effects on transport function [81, 97]. Therefore, directly testing the human mutated forms of BSEP in vivo seems to be an important prerequisite to obtain insights into the functional consequences of BSEP mutations [37, 70].

Although inherited mutations in the *BSEP* gene are very rare [75, 77], acquired forms of cholestasis such as, for example, primary biliary cirrhosis, primary sclerosing cholangitis, cholestasis associated with pregnancy, or drug-induced cholestasis are more frequent [62, 77]. A recent genetic screen of patients identified three non-synonymous *ABCB11* variants to be specifically associated

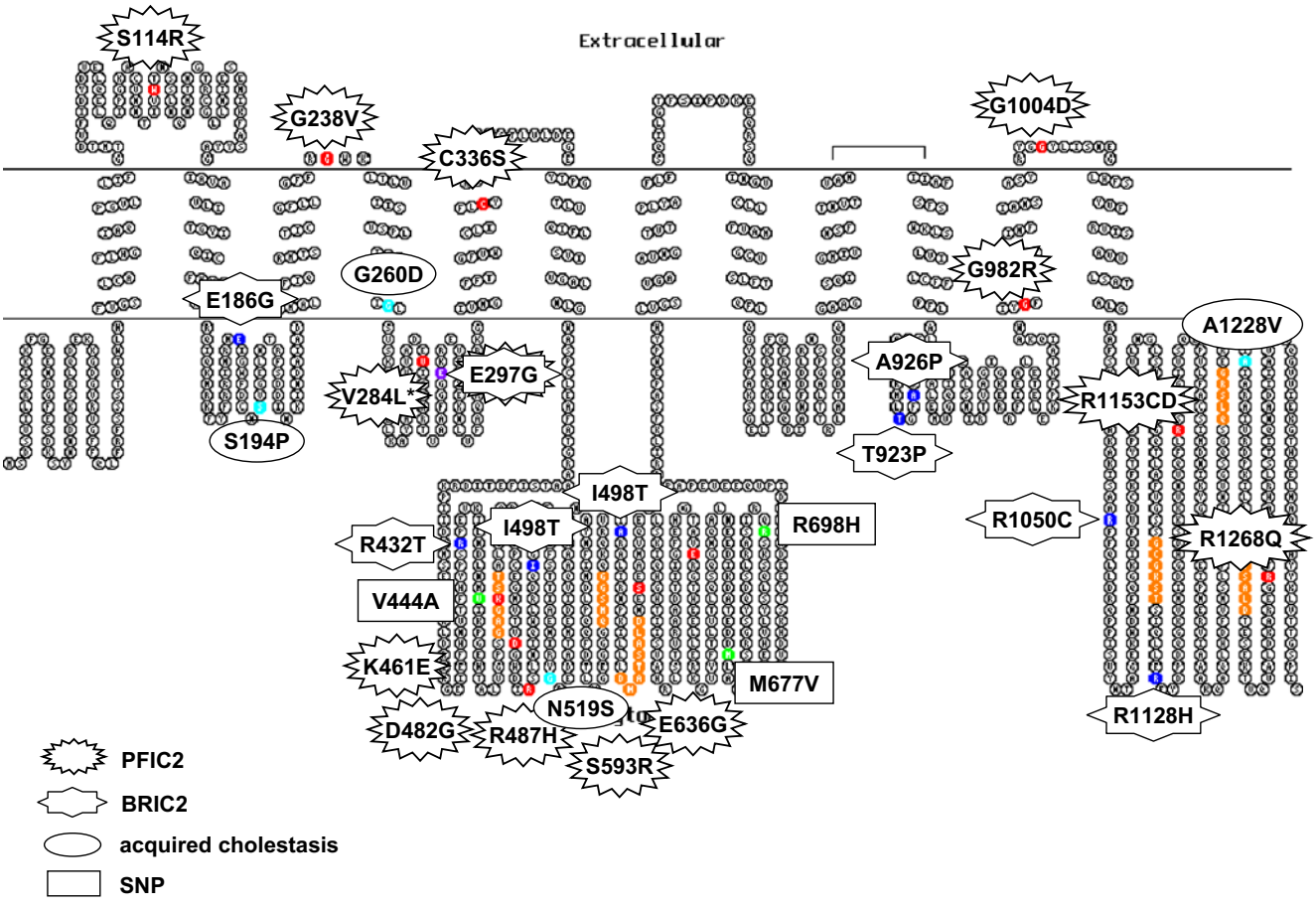

Fig. 2 Putative secondary structure of Bsep (NT-005403) generated with the TOPO program (http://www.sacs.ucsf.edu/TOPO-run/wtopo.pl). Walker A motif, family signature, and Walker B motif [20] are shown in *orange*. Genetic variants and mutations (taken from [51, 78]) are represented as *green* for single nucleotide polymorphisms (*SNP*), *red* for mutations identified in patients with progressive familial intrahepatic cholestasis type 2 (*PFIC1*), and *blue* for mutations identified in patients with benign recurrent intrahepatic cholestasis type 2 (*BRIC2*). *purple* E297G was found in PFIC2 and BRIC2 patients, respectively. Amino acids highlighted in *cyan* were associated acquired forms of cholestasis including intrahepatic cholestasis of pregnancy, primary sclerosing cholangitis, and primary biliary cirrhosis. (*) The mutation V284L was found in a patient with PFIC2, whereas the SNP V284A occurs in healthy individuals

with primary biliary cirrhosis and primary sclerosing cholangitis [76]. In cholestasis associated with pregnancy, the situation seems to be controversial [24, 78]. Consequences of such variants potentially include altered expression and or altered function of BSEP.

Cholestasis induced by BSEP inhibition, for example, by drugs, is a rare event observed in patients. Hence, there need to be underlying mechanisms rendering such patients susceptible to BSEP inhibition. A strong candidate is the genetic background. Recently, the variation of BSEP protein expression levels was studied in 110 healthy liver specimens by Western blotting [63]. BSEP expression levels of these individuals were found to display a unimodal distribution and showed no correlation with, for example, sex or medication. Importantly, 17% of the individuals were found to have a low or very low expression level. In these individuals, low BSEP expression level was found to be associated with the nonsynonymous 1457C allele (444A) of *ABCB11*. Individuals with low or very low expression levels of BSEP could be at risk of developing acquired forms of cholestasis, for example, by drugs or pregnancy.

Mice with genetically altered Bsep expression

In contrast to humans, functional inactivation of the *Bsep* gene (also called sister of P-glycoprotein gene, *spgp*) does not lead to a severe, progressive cholestasis in mice [99]. Although the knockout animals are viable and fertile, they present with mild growth retardation. Knockout animals have no abnormalities in their serum liver parameters. Their bile flow rate is slightly but not significantly lower in comparison to controls, but the total bile salt output into bile is massively reduced and their liver bile salt concen-

trations are increased accordingly. Biliary secretion of cholate and its conjugates is 6% of normal, supporting the key role of Bsep in canalicular taurocholate secretion. Most interestingly, these knockout mice secrete a novel tetrahydroxylated bile acid into bile [80]. The Bsep knockout animals have substantial increases of malondialdehyde levels in brain and heart, indicating an increased lipid peroxidation as a consequence of elevated serum bile acid levels [56]. If Bsep knockout animals are fed with a diet containing cholate, they become severely cholestatic and have a high mortality rate, which is more pronounced in males than females [98]. Unexpectedly, cholate-fed knockout mice secrete significant amounts of cholate conjugate into bile, indicating a salvage transport system for canalicular bile salt secretion. Comparative analysis of gene expression profiles in control and knockout-mice after cholate feeding revealed a striking upregulation of Mdr1a [98]. This observation may indicate that mouse Mdr1a could act as a salvage transporter for bile salts in conditions of high intracellular bile salt concentration. Transport studies using canalicular plasma membrane vesicles from Bsep knockout animals revealed a residual ATP-dependent transport activity for taurocholate [52]. Plasma membrane vesicles isolated from a drug-resistant Chinese hamster ovary cell line expressing high levels (about 15% of total plasma membrane proteins) of class I P-glycoprotein also exhibit ATP-dependent bile salt transport, albeit with lower affinity than Bsep [52]. Hence, Mdr1 may act as a salvage system for bile salts in case of nonfunctional or impaired Bsep. Since the affinity of Mdr1 for bile salts is much lower, intracellular bile salts may rise to toxic levels in hepatocytes and cause liver injury (see article by V. Ling, this issue). The situation in patients with PFIC2 may differ to some extent from the knockout animals since analysis of four PFIC2 patients showed no significant upregulation of MDR1 mRNA [46].

Overexpression of Bsep in the liver transgenic animals leads to an increased biliary bile salt output concomitant with an increase in bile flow rate and biliary lipid secretion [26]. Fecal loss of bile salts is normal in these animals. If these mice are fed a lithogenic diet, they have a markedly reduced hepatic steatosis in comparison to controls. These findings highlight the role of Bsep in the maintenance of hepatocellular lipid homeostasis.

Outlook

The gene for the bile salt export pump Bsep was initially identified as a close relative of the gene encoding P-glycoprotein. Bsep was subsequently shown to be an ATP-driven bile salt pump located to the canalicular plasma membrane of hepatocytes. In this strategic position, Bsep is a key transporter involved in canalicular bile formation and constitutes the main driving force for enterohepatic circulation of bile salts. The regulation of Bsep is set up such that bile salt concentrations in hepatocytes are kept constant at low levels. On the basis that elevated bile salt concentrations are toxic to hepatocytes [49], Bsep may act as a "vacuum cleaner" [39] for bile salts in hepatocytes. Proper function of Bsep is critical for maintaining bile salt concentration in systemic circulation in a tight range at low levels. In the light of recent findings that bile salt-mediated FXR signaling controls liver regeneration and may even control liver size [44], Bsep may move up to a key protein controlling many vital processes in liver. Furthermore, evidence that the bile acid sensor FXR is controlling glucose and lipid metabolism is rapidly accumulating [11, 16, 58]. Since FXR requires bile salt binding to act to control expression target genes, Bsep may, by controlling bile salt concentration in systemic circulation, become an important control element in body energy and lipid homeostasis. And finally, bile salts have recently been shown to control energy expenditure by controlling intracellular thyroid hormone action by binding to the G protein-coupled receptor TGR5 [43, 101].

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