Review

The Bile Salt Export Pump: Molecular Structure, Study Models and Small-Molecule Drugs for the Treatment of Inherited BSEP Deficiencies

Muhammad Imran Sohail 1,†, Yaprak Dönmez-Cakil 2,†, Dániel Szöllősi 3, Thomas Stockner 3,* and Peter Chiba 4,*

1 Department of Zoology, Government College University, Lahore 54000, Pakistan; imransohail@gcu.edu.pk
2 Department of Histology and Embryology, Faculty of Medicine, Maltepe University, Maltepe, 34857 Istanbul, Turkey; yaprak.cakil@maltepe.edu.tr
3 Institute of Pharmacology, Center for Physiology and Pharmacology, Medical University of Vienna, Waehringerstrasse, 13A, 1090 Vienna, Austria; daniel.szooloesi@meduniwien.ac.at
4 Institute of Medical Chemistry, Center for Pathobiology and Genetics, Medical University of Vienna, Waehringerstrasse, 10, 1090 Vienna, Austria
* Correspondence: thomas.stockner@meduniwien.ac.at (T.S.); peter.chiba@meduniwien.ac.at (P.C.);
Tel.: +43-1-40160-31215 (T.S.); +43-1-40160-38005 (P.C.)
† Equally contributing first authors.

Abstract: The bile salt export pump (BSEP/ABCB11) is responsible for the transport of bile salts from hepatocytes into bile canaliculi. Malfunction of this transporter results in progressive familial intrahepatic cholestasis type 2 (PFIC2), benign recurrent intrahepatic cholestasis type 2 (BRIC2) and intrahepatic cholestasis of pregnancy (ICP). Over the past few years, several small molecular weight compounds have been identified, which hold the potential to treat these genetic diseases (chaperones and potentiators). As the treatment response is mutation-specific, genetic analysis of the patients and their families is required. Furthermore, some of the mutations are refractory to therapy, with the only remaining treatment option being liver transplantation. In this review, we will focus on the molecular structure of ABCB11, reported mutations involved in cholestasis and current treatment options for inherited BSEP deficiencies.

Keywords: BSEP; ABCB11; bile salts; intrahepatic cholestasis; chaperones; PFIC2; BRIC

1. Introduction

The ATP-binding cassette (ABC) proteins constitute one of the largest families of membrane proteins. They are universally present in all kingdoms of life. In humans, 48 functional genes encode for ABC proteins, which on the basis of structural and sequence similarity are categorized into seven subfamilies, designated as ABCA through G [1]. Most of these proteins transport substrates across cellular membranes. A functional ABC transporter comprises at least four domains: two transmembrane domains (TMDs) and two nucleotide-binding domains (NBDs), as shown in Figure 1. In the ABCB subfamily, each of the TMDs consists of six membrane-spanning helices. Five of these extend into the cytoplasm to form an expansive intracellular domain. The two TMDs are responsible for substrate binding and translocation. The NBDs form two composite nucleotide-binding sites (NBSs) at their interface, which bind and hydrolyze ATP, and thereby provide the energy for substrate transport. These NBSs are formed by the Walker A and Walker B motifs, as well as the A-, Q- and H-loops of one NBD and the signature motif and D-loop of the other NBD [2,3].
The ABCB subfamily is one of the most diverse groups of ABC proteins, as it includes dimeric half transporters, monomers of which are each composed of one TMD and one NBD, but also full-length transporters, in which all four domains are fused into a single polypeptide chain. The former group comprises the homodimeric transporters ABCB6, ABCB7, ABCB8, ABCB9 and ABCB10, and the heterodimeric transporter ABCB2/ABCB3. The four full-length transporters of the ABCB subfamily are ABCB1, ABCB4, ABCB5 and ABCB11. The heterodimeric ABCB2/ABCB3 and full-length ABCB11 differ from the other members as they contain only one canonical NBS rather than two [4].

2. BSEP/ABCB11: Physiological Role

The bile salt export pump (BSEP) is expressed in hepatocytes. While high levels of BSEP mRNA were detected in testes and lower levels were reported in other extrahepatic tissues, including trachea, prostate, lungs, thymus, kidney and colon [5,6], plasma membrane expression of functional protein was found in liver cells only [5-8]. Adjacent hepatocytes form tight junctions to enclose functional structures called bile canaliculi, to which BSEP is targeted. Bile salts undergo an enterohepatic circulation, which depends on active transport systems in the liver and intestine. In the course of this process, newly synthesized and recycled bile salts are secreted from hepatocytes into bile canaliculi by BSEP and via bile ducts reach the duodenum. In the ileum, these bile salts are reabsorbed by the apical sodium-dependent bile salt transporter in intestinal epithelial cells (ASBT/SLC10A2). From the intestine, bile salts return to the liver via the superior mesenteric and portal veins, which carry the blood that feeds liver sinusoids. Uptake into hepatocytes is mediated by the sodium taurocholate co-transporting polypeptide (NTCP/SLC10A1) and organic anion transporters (OATPs). Bile salt transport by BSEP constitutes the rate-limiting step in bile formation and provides the major driving force for enterohepatic circulation [9].

The bile salt pool is recycled from the intestine to the liver six to eight times a day [10], resulting in daily bile salt excretion of about 20–40 g [11]. Impairment of BSEP results in the failure to maintain physiological bile flow, resulting in a clinical condition called intrahepatic cholestasis. BSEP has narrow specificity for its substrate bile salts, but the drugs pravastatin, vinblastine and fexofenadine are reported to be non-physiological substrates [12-14].
3. Transcriptional Regulation

BSEP expression is highly regulated by transcriptional mechanisms, and a wide inter-individual variability has been described at the mRNA and protein levels [15].

Expression of BSEP is regulated by a major ligand-activated transcription factor, farnesoid X receptor (FXR, NR1H4), which forms a signaling-competent nuclear receptor heterodimer with the retinoid X receptor (RXR) (Figure 2). Bile acids, such as chenodeoxycholic acid (CDCA), deoxycholic acid (DCA) and cholic acid (CA), are endogenous ligands of FXR with varying potential for activation [16–19]. Upon ligand binding, the FXR/RXR heterodimer binds to an FXR response element (FXRE) in the promoter region of BSEP, thereby inducing the expression of the transporter [20]. Additionally, components of the activating signal cointegrator-2-containing complex (ASCOM) interact with FXR to enhance BSEP expression. Ananthanarayanan and co-workers [21] showed that the recruitment of ASCOM to the BSEP promoter was disrupted in cholestasis, which was induced by common bile duct ligation. Furthermore, co-activator-associated arginine methyltransferase 1 (CARM1) also regulates FXR/RXR-dependent BSEP transcription [22]. Similarly, steroid receptor co-activator 2 (SRC2) knockout mice showed reduced expression of BSEP [23], indicating its involvement in transcriptional regulation of the transporter.

Hepatocyte-specific liver receptor homolog-1 (LRH-1, NR5A2) is another transcription factor involved in modulation of BSEP expression. LRH-1 plays a supporting role for FXR [24]. The absence of LRH-1 is associated with reduced BSEP expression and an altered BA composition, with disappearance of CA and taurocholic acid (TCA) [25]. BSEP promoter activity is also stimulated by nuclear factor erythroid 2-related factor 2 (Nrf2), a positive transcriptional regulator, which acts as a sensor for oxidative stress. Nrf2 regulates the expression of BSEP, but also that of a number of hepatic phase I and II enzymes and other hepatic efflux transporters such as MRP3 (ABCC3) and MRP4 (ABCC4) [26].
4. Processing and Trafficking of BSEP

Membrane insertion and folding occur at the level of the endoplasmic reticulum (ER) [27]. Insertion into the ER membrane is facilitated by the protein transport protein SEC61, which assists transmembrane portions of nascent proteins to adopt helicity prior to domain folding. Correct positioning of domains or subdomains relative to each other typically occurs late in the folding trajectory of a multidomain membrane protein. Of all ABC proteins, the folding trajectory of cystic fibrosis transmembrane conductance regulator (CFTR, ABCC7) has been studied the most [28,29]. The intracellular loops (ICLs) play a critical role in transporter folding by contributing to the formation of the functionally important TMD/NBD coupling interface [30]. Furthermore, the involvement of molecular chaperons is required, as they sense the presence of hydrophobic helices in the cytosol, and thus contribute to obtaining the folding endpoint [31].

In the ER, newly synthesized and correctly folded BSEP undergoes N-linked core glycosylation. The sugar moieties are added at four conserved asparagine residues in extracellular loop 1 (ECL1), namely Asn109, 116, 122 and 125, and then are subject to subsequent modifications while traveling through the Golgi stacks. N-linked core glycosylation in the ER lumen plays a pivotal role in ER protein folding by mediating interactions with the lectin chaperones calnexin and calreticulin and by increasing the folding efficiency [32]. Only correctly folded proteins are trafficked to the Golgi apparatus in clathrin-coated COPII vesicles. Aberrantly folded proteins are identified by the endoplasmic-reticulum-associated degradation (ERAD) machinery and retro-translocated to the cytoplasm for degradation in the 26S proteasome following ubiquitination [33]. A number of BSEP mutants, including G238V, D482G, G982R, R1153C and R1286Q, are predominantly degraded by ERAD, thus leading to a PFIC2 phenotype [27,34,35]. Different ERAD E3 ubiquitin ligases are thought to recognize and ubiquitinate different mutants of BSEP [27]. Before trafficking to the canalicular membrane, BSEP is fully glycosylated in the Golgi apparatus through trimming to the core structure and extension from the core [34]. Glycosylation directly impacts protein stability and at least two of the four glycans are required for BSEP trafficking to the canalicular membrane [35]. Using enhanced green fluorescent protein (EGFP)-tagged mouse BSEP, it was shown that the partial glycosylation of the PFIC2-related mutant D482G causes an unstable BSEP protein and reduces levels of the mature protein at the canalicular membrane [36].

The majority of integral plasma membrane proteins of polarized hepatic cells are distributed from the basolateral membrane to the appropriate apical cell surface location via transcytosis. In contrast, ABC transporters targeted to the canalicular membrane use the non-transcytotic direct route from the Golgi apparatus via Rab11a-positive apical endosomes [37,38]. Under physiological conditions, the apical pool of BSEP is strictly regulated by the demand for biliary excretion of bile salts. The intracellular endosomal pool is thought to exceed that at the canalicular membrane [39] by at least 6-fold. Internalization of BSEP is mediated by clathrin-coated vesicles and is dependent on the highly conserved endocytic cargo motif (Trp-Lys-Leu-Val) [40]. This trafficking motif is recognized by adaptor protein 2 (AP-2), which modulates the internalization process and expression of cell-surface-resident BSEP through direct interaction [41]. Moreover, trafficking of BSEP through the endosomal system to the canalicular membrane is a microtubule-dependent process and requires the myosin light chain [42], myosin Vb [43] and Rab11a. The latter two components were shown to also be associated with canalicular biogenesis by maintaining proper trafficking of Rab11a–myosin Vb-containing membranes to the canalicular membrane in polarized WIF-B9 cells [43].

Continuous cycling of BSEP between the apical and intracellular pools is disrupted in most human cholestatic liver diseases. Shifting the balance towards endocytic internalization results in impaired bile salt secretion [44]. A causative role of enhanced retrieval into the subapical endosomal compartment was demonstrated for estradiol 17 β-D-glucuronide (E17G)-induced cholestasis, an experimental model for pregnancy-related cholestasis [45,46]. In this model, BSEP was found to co-localize with clathrin, AP-2
and Rab5 as evidence for clathrin-mediated endocytosis [46]. Classical (Ca\textsuperscript{2+}-dependent) protein kinase C (cPKC)–p38, mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinase (PI3K)–ERK1/2 signaling pathways are thought to be involved in (E17G)-induced cholestasis [47–49].

TCA, the major bile acid in mammals, as well as cyclic adenosine monophosphate (cAMP) are known to increase the apical pool of BSEP within minutes by promoting its cellular relocation [37]. Moreover, TCA was demonstrated to induce the formation of bile canaliculi in mice via the liver kinase B1 (LKB1)–AMP-activated protein kinase (AMPK) pathway [50]. A subsequent publication showed that knocking out LKB1, the upstream serine–threonine kinase, which is implicated in regulation of cellular energy metabolism, impairs both canalicular biogenesis and intracellular trafficking of BSEP. On the other hand, cAMP induces BSEP trafficking through a PKA-mediated pathway, which does not involve AMPK activation [51]. Unlike TCA-mediated trafficking, this process is PI3K-independent [52]. Similar to TCA, the conjugated bile salt tauroursodeoxycholate (TUDCA) also promotes the relocation of BSEP to the canalicular membrane through activation of the p38 MAPK [53,54]. Similar to other conjugated bile salts, TUDCA stimulates the ATPase activity of BSEP [55].

Ubiquitination is another modification, which changes the expression of cell-surface-resident BSEP. The half-life of BSEP in the canaliculi is shortened by modification with two to three ubiquitin molecules. This induces the removal of the protein from the cell surface, whereby the rates are governed by the degree of ubiquitination. While the PFIC2-related mutations E297G and D482G cause short-chain ubiquitination, thereby shortening the half-life of cell-surface-resident BSEP, the chemical chaperone 4-phenylbutyrate (4-PB) reduces its degradation rate [56]. In a later study, ubiquitination of canalicular BSEP was shown to act as a signal for internalization by promoting clathrin-mediated endocytosis. After internalization, BSEP is either recycled back to the canalicular membrane in a Rab11-dependent manner or degraded through a ubiquitination-independent pathway [57]. Degradation was suggested to be lysosome-mediated and dependent on a sorting signal from within the endosomal compartment [58].

5. Structural Models of BSEP

Before publication of the cryo-electron microscopy (cryo-EM) structure of BSEP [59], several homology models of the transporter were presented. Kubitz and colleagues [60,61] generated an outward-facing homology model of ABCB11 by using the Sav1866 structure (PDB: 2HYD [62]) as a template. This model was used to show a putative antibody binding site at the long ECL1, as well as to indicate positions of disease-causing mutations. The model compares well at the individual domain level with the cryo-EM structure that has recently become available. Giovannoni et al. [63] created a model based on the corrected mouse ABCB1 structure (PDBID: 4M1M [64]). Here, loops that could not be matched to the template were not modeled (ABCB11 residues 102–120 and 659–728). The model was used to localize positions of disease-causing mutations. Again, this model agrees well with the cryo-EM structure that has recently become available. Giovannoni et al. [63] created a model based on the corrected mouse ABCB1 structure (PDBID: 4M1M [64]). Here, loops that could not be matched to the template were not modeled (ABCB11 residues 102–120 and 659–728). The model was used to localize positions of disease-causing mutations. Again, this model agrees well with the cryo-EM structure within the resolution at which cryo-EM data were presented. Notably, an overall structural alignment of mouse ABCB1 (PDBID: 4M1M [64]) and the cryo-EM structure of ABCB11 results in an RMSD of 0.36 nm, with a better fit of the TMDs. Dröge et al. [65] also used the mouse ABCB1 structure as a template. This model was used to locate the positions of the most commonly occurring PFIC2 missense mutations. Moreover, in the process of structure evaluation, different web services were used to predict the influence of missense mutations on protein function. The authors provided a list of possible effects of newly identified mutations included in their study. However, the accuracy of this prediction was not evaluated. In a different study, Jain and co-workers [66] generated an ABCB11 homology model also using the inward-facing mouse ABCB1 structure as the template (PDB: 4M1M [64]). Extensive docking studies with 405 inhibitor compounds and 807 non-inhibitors were performed in order to explore the interaction with small molecules. Prediction accuracy results of 81% in the training set and 73% in two external
test sets were obtained. In addition to standard scoring functions, the homology model used for docking was validated by molecular dynamics (MD) simulation. The use of MD simulations in protein stability checks is a well-established procedure, however it is computationally expensive. Short simulations were performed for structure validation of membrane-inserted ABCB11 homology models. We also previously [67] generated a homology model based on the X-ray structure of Sav1866 as a template (PDB ID: 2ONJ [62]) to elucidate the NBD-NBD interdomain communication of the transporter. The model allowed us to infer the potential roles of conserved motifs of the nucleotide-binding domains in ATP hydrolysis and the transmission of conformational changes from the NBDs to the TMDs.

In 2020, Wang et al. [59] determined the human BSEP structure using cryo-EM (PDBID: 6LR0). This structure has an average resolution of 0.35 nm, with the TMDs being resolved at 0.33 nm. The protein shows an inward open state in the absence of nucleotides or other small molecules. According to this structure, ABCB11 closely resembles ABCB1, with the most similar structure found in the Protein Data Bank [68] being the apo inward-open ABCB1 structure (PDBID: 6GDI, RMSD: 0.206 nm [69]). Thus, it shares the typical type I exporter fold with the other members of the ABCB subfamily. The domain-swapped transmembrane helices are connected by coupling helices 2 and 4, which are embedded in grooves formed between the core and the helical domain of the NBDs, supporting their crucial role in interdomain communication. Interestingly, inside the central cavity, contiguous electron density was found, into which the N-terminus of the protein could be fitted. Currently, no biochemical data are available with respect to any putative auto-inhibition of BSEP by this N-terminus. The poor resolution of ECL1 (Q101-I134) reflects its highly dynamic nature. It contains the four known glycosylation sites (N109, 116, 122 and 125) [70].

BSEP harbors two ATP-binding sites, one of which is canonical and capable of ATP hydrolysis. When compared to ABCB1, only four amino acids differ in NBS1. These are E502, M584, R1221 and E1223 in BSEP corresponding to S474, E556, G1178 and Q1180 in the ABCB1 protein sequence [71]. The amino acid changes result in a catalytically inactive ATP binding site (NBS1), which is also variably called “degenerate” NBS. It has been suggested that the degenerate site imparts extended functionality to the transporter. The mechanistic details are currently missing, although the role of ATP hydrolysis in each of the two NBDs has been elucidated in greater detail [67].

6. Experimental Model Systems

In vitro and in vivo models have been developed for the study of BSEP function, folding and trafficking, as well as the actions of drug candidates with the potential to treat the malfunction or incorrect cellular routing of the transporter. These model systems are discussed below with respect to their potentials and limitations.

6.1. In Vitro Models

6.1.1. Membrane Vesicles

For the study of substrate transport and inhibition by drugs and metabolites, membrane vesicles represent the most commonly used model system. Vesicles are either prepared from BSEP-transfected insect cell lines (Sf9 and Sf21), which give higher protein yields, or mammalian cell lines (including CHO, HeLa, MDCK, LLC-PK1 and HEK cells). Despite having lower protein expression, mammalian cells are often preferred for functional studies, as insect cells show a different lipid membrane composition and only core glycosylated protein is produced. In order to overcome lower expression levels in mammalian cells, the Bac/Mam gene transfer system has been advocated [72]. For experimental details on the preparation of membrane vesicles, readers are referred to [13,69,73]. As a mixture of inside-out and right-side-out vesicles is obtained, a protocol for increasing the yield of inside-out vesicles has been published [74]. In addition, a protocol for preparation of membrane vesicles from canalicular membranes of rat hepatocytes has been
Int. J. Mol. Sci. 2021, 22, 784

7 of 22

reported [73]. These vesicles were used for the identification and characterization of BSEP substrates and inhibitors [75,76].

High-quality membrane vesicles represent an ideal experimental system for transport and transport inhibition studies, but cannot be used to address aspects of trafficking or cellular metabolism [77]. As in inside-out vesicles, the BSEP NBDs are exposed towards the medium, ATP and substrates can be added into the transport medium and accumulation of substrates in vesicles can be monitored by rapid filtration. We previously used membrane vesicles from plasmid-transfected HEK cells to study the domain interaction and the roles of the canonical and non-canonical NBS in supporting BSEP substrate transport [67].

6.1.2. Polarized Cell Lines Expressing BSEP

Polarized MDCK and LLC-PK1 cells have been used to study BSEP function in intact cells. The experimental system requires double transfection with BSEP and the hepatocyte bile salt uptake transporter NTCP, as BSEP-mediated efflux can only be monitored after bile salt substrates have been taken-up into cells. Physiologically, NTCP enables the reentry of bile salts from the circulation into hepatocytes in the context of the enterohepatic circulation of these compounds between the intestine and the liver. Polarized cells are grown on a permeable membrane in a hang-in assembly. BSEP is localized on the apical surface. The function of BSEP is determined from the ratio of basal-to-apical as compared to apical-to-basal transport of substrates [78,79].

6.1.3. Primary Hepatocyte Cultures

When cultured in the appropriate medium, hepatocytes form tight junctions to generate sealed tube-like structures, which resemble bile canaliculi [80,81]. Therefore, this system provides the possibility of assessing the excretion of drugs and bile components into bile canaliculi [82–84]. Hepatocytes in suspension also provide a suitable option for studying drug metabolism and drug transport [85,86]. One major advantage of hepatocyte suspension cultures is their easy, quick and high-yield preparation. Furthermore, this assay does not require radiolabeled substrates [87]. Therefore, this system is often used for large-scale screening of drugs for assessment of drug-induced liver injury (DILI). It has to be kept in mind, however, that the presence of multiple transporters in primary hepatocytes limits their use for assessing BSEP-specific substrates [88].

6.2. BSEP Knockout Animals

6.2.1. Rodents

In order to investigate the mechanisms involved in innate and acquired intrahepatic cholestasis, BSEP knockout animal models (mice and rat) have been established [89,90]. Recently, the CRISPR/cas9 technology has been employed to knock out the BSEP gene in adult mice [91,92]. In all models, expression of BSEP was strongly reduced, thus providing an alternative experimental model for studying intrahepatic cholestasis and putative therapeutic intervention in rodents. Interestingly, the mouse models do not show signs of severe cholestasis as seen in humans, because these mice produce a large amount of poly-hydroxylated bile acids, which are excreted renally [93]. Wang et al. used this model system to suggest that P-glycoprotein (ABCB1) can act as a compensatory bile salt transporter, which alleviates the severity of cholestasis in BSEP knockout mice [94].

6.2.2. Zebrafish

Recently, Ellis et al. generated an abcb11b knockout zebrafish by using the CRISPR/Cas9 gene editing technology [95]. Abcb11b is the orthologue of the human BSEP gene in zebrafish. The histological and ultrastructural analysis showed a morphological hepatocyte injury pattern similar to that seen in patients with PFIC2. Similar to the situation in humans, BSEP deficiency induced autophagy in zebrafish hepatocytes. Treatment with rapamycin restored bile acid excretion, attenuated hepatocyte damage and extended the life span.
of abcb11b mutant zebrafish. These effects were paralleled by a recovery of the correct canaliclar localization of multidrug resistance protein 1.

Due to the transparency of these fish, the system allows monitoring of the bile flow in the intact animal with fluorescently labeled bile salt analogs. Furthermore, P-glycoprotein, which is reported to play a compensatory role (by transporting bile acids, and thereby protecting the hepatocytes from cholestasis induced injury) in BSEP knockout mice [96], is mislocalized to the hepatocyte cytoplasm in mutant zebrafish.

Animal models play an important role in studying cholestatic liver disease [93]. In contrast, identification of drug candidates for the treatment of folding and functionally deficient BSEP-mutants usually relies on in vitro model systems. For such studies, the patient-specific mutations are generated and expressed in cell lines. The impact of small molecules on the structure and function of BSEP is evaluated. Once the drugs have proven a potential in cell models, they are translated to a clinical setting [97,98].

Hydrodynamic tail vein injection in combination with the CRISPR/Cas9 technology has been used to specifically delete BSEP in mice and to study the consequences of the loss of an enzyme of the urea cycle (argininosuccinate lyase) [91]. In a similar way, such a BSEP knockout model system may be used for the study of mice expressing mutant forms of human BSEP in the liver and to monitor the effects of drug candidates on the folding, trafficking and function of the transporter.

7. Treatment Options for BSEP-Related Diseases

Impairment in the expression or function of BSEP leads to one of three human disease phenotypes of differing severity: PFIC2, BRIC2 and intrahepatic cholestasis of pregnancy (ICP). Several drugs with the potential to enhance the expression and function of the transporter have been reported. Disease-causing mutations and potential correctors are listed in Table 1 and depicted in the ABCB11 cryo-EM structure in Figure 3.

![Figure 3](image-url)

Figure 3. The positions of disease-causing mutations contained in Table 1 are shown in the ABCB11 cryo-EM structure. C-alpha atoms are shown as spheres and colored according to the resulting disease phenotype (PFIC2: red; BRIC2: blue; either PFIC2 or BRIC2: magenta; nonsense mutations: yellow). Outlines and filled colors indicate the domain organization of the transporter in accordance with Figure 1.
Table 1. Synopsis of a subset of disease-causing mutations, for which potential therapeutic interventions have been proposed. The defects, model systems and associated disease phenotypes are indicated. With the exception of the C129Y and G806D variants, the listed mutants represent a subset of a total of 192 disease-associated missense or nonsense mutations that have been identified to date [99]. PFIC2: progressive familial intrahepatic cholestasis type 2, BRIC2: benign recurrent intrahepatic cholestasis type 2, 4-PB: 4-phenylbutyrate, CA: cholic acid, CDCA: chenodeoxycholic acid, DCA: deoxycholic acid, UDCA: ursodeoxycholic acid, FXR: farnesoid X receptor.

| Nucleotide Change | Type of Mutation | Amino Acid | Defect | Potential Corrective Therapy | Cell Line/Organism | Disease | References |
|-------------------|------------------|------------|--------|-------------------------------|--------------------|---------|------------|
| c.386GA           | Missense         | C129Y      | Impaired membrane trafficking, reduced level of mature protein | 4-PB              | HEK293T          | PFIC2   | [100]      |
| c.470AG           | Missense         | Y157C      | Reduced/absent BSEP activity | 4-PB in combination with oxcarbazepine and maralixibat | Patient with 2 heterozygous missense mutations | PFIC2   | [101]      |
| c.3892GA          | Missense         | G1298R     | Reduced/absent BSEP activity | 4-PB in combination with oxcarbazepine and maralixibat | Patient with 2 heterozygous missense mutations | PFIC2   | [101]      |
| c.698TC           | Missense         | L233S      | -       | Methylprednisolone            | Patient with heterozygosity in ABCB11, as well as in CFTR, NPHP4 and A1ATD | BRIC2   | [102]      |
| c.890AG           | Missense         | E297G      | Protein instability, ubiquitin-dependent degradation [103], impaired membrane trafficking, reduced level of mature protein | 4-PB              | Madin-Darby canine kidney (MDCK) II cells and Sprague-Dawley rats | BRIC2   | [104]      |
| c.1211AG          | Missense         | D404G      | Reduced level of mature protein, ER-like distribution | 4-PB              | HEK293T          | BRIC2   | [109]      |
| c.1211AG c.1331TC | Missense         | D404G V444A | Reduced level of mature protein | 4-PB              | Patient compound heterozygous for D404G and homozygous for V444A mutations | BRIC2   | [109]      |
| c.1388CT          | Missense         | T463I      | Impaired ATP-binding, BSEP dysfunction | Ivacaftor         | MDCK II cells   | PFIC2   | [110]      |
| Nucleotide Change | Type of Mutation | Amino Acid | Defect | Potential Corrective Therapy | Cell Line/Organism | Disease | References |
|-------------------|------------------|------------|--------|-----------------------------|--------------------|---------|------------|
| c.1445AG          | Missense         | D482G ²    | Protein instability, ubiquitin-dependent [103], impaired membrane trafficking, reduced level of mature protein, severe differential splicing [105] | 4-PB | MDCK II cells and Sprague–Dawley rats | PFIC2 | [104] |
|                   |                  |            | | Sodium butyrate and 4-PB | HEK293T cells |         | [103] |
|                   |                  |            | | Butyrate and octanoic acid | MDCK II cells |         | [108] |
| c.1708GA          | Missense         | A570T      | Reduced level of mature protein, reduced BSEP activity [105] | UDCA | MDCK II cells | BRIC2 | [111] |
|                   |                  |            | | Glycerol at 28 °C | CHO-K1 cells |         | [105] |
| c.2417GA          | Missense         | G806D      | Reduced level of mature protein, aberrant splicing | 4-PB | BSEP-deficient hepatocyte-like cells | PFIC2 | [112] |
| c.-24CA           | 5'-UTR (five prime untranslated region) | | | Steroid | Patient with compound heterozygosity | PFIC2 | [113] |
| c.2494CT          | Missense         | R832C      | Differential splice products [105] | Steroid | Patient with compound heterozygosity | PFIC2 | [113] |
| c.150+3AC         | Splice-site mutation | | Partial exon skipping [114] | | | | |
| c.2756_2758delCCA | Deletion         | T919del    | Reduced BSEP activity [115] | Steroid | Patient with compound heterozygosity | PFIC2 | [113] |
| c.3703CT          | Nonsense         | R1235X     | Truncated, non-functional transporter [115] | UDCA, 4-PB single agents or in combination | Can 10 cells | PFIC2 | [97] |
| c.2944GA          | Missense         | G982R      | Retention in ER, reduced level of mature protein | 4-PB | Patient with compound heterozygosity | PFIC2 | [97] |
| c.2944GA          | Missense         | G982R      | Retention in ER, reduced level of mature protein | 4-PB | Patient with compound heterozygosity | PFIC2 | [97] |
| c.770CT           | Missense         | A257V      | Normal canalicular expression of BSEP | | | | |
| c.2944GA          | Missense         | G982R      | Retention in ER, reduced level of mature protein | 4-PB | Patient with compound heterozygosity | PFIC2 | [97] |
| c.3003AG          | Silent           | R1001R     | Abnormal splicing [116] | | | | |
| Nucleotide Change | Type of Mutation | Amino Acid | Defect | Potential Corrective Therapy | Cell Line/Organism | Disease | References |
|-------------------|------------------|------------|--------|-----------------------------|--------------------|---------|------------|
| c.3382CT         | Missense         | R1128C     | Retention in ER, reduced level of mature protein, Mild exon skipping [105] | UDCA, 4-PB single agents or in combination | Can 10 cells | PFIC2 | [97] |
|                  |                  |            |        | 4-PB | Patient homozygous for R1128C |
| c.3628AC         | Missense         | T1210P     | Retention in ER, reduced level of mature protein | UDCA, 4-PB single agents or in combination | Can 10 cells | PFIC2 | [97] |
|                  |                  |            |        | 4-PB | Can 10 cells |
|                  |                  |            |        | 4-PB | Patient with homozygous mutation |
| c.3692GA         | Missense         | R1231Q     | Retention in ER [117], no splicing, immature protein [105] | 4-PB | HEK293T cells, McA-RH7777 cells, patient with homozygous mutation |
| c.1062TA         | Nonsense         | Y354X      | Premature termination codon | G418, gentamicin | NIH3T3 cells (increased readthrough) | PFIC2 | [118] |
| c.1243CT         | Nonsense         | R415X      | Premature termination codon | G418, gentamicin | NIH3T3 cells (increased readthrough) | PFIC2 | [118] |
|                  |                  |            |        | Gentamicin | HEK293 cells (production of a full-length BSEP protein) |
| c.1408CT         | Nonsense         | R470X      | Premature termination codon | G418, gentamicin | NIH3T3 cells (increased readthrough) | PFIC2 | [118] |
|                  |                  |            |        | Gentamicin | HEK293 cells (production of a full-length BSEP protein) |
| c.3169CT         | Nonsense         | R1057X     | Premature termination codon | G418, gentamicin | NIH3T3 cells (increased readthrough) | PFIC2 | [118] |
|                  |                  |            |        | Gentamicin | HEK293 cells (production of a full-length BSEP protein) |
| Nucleotide Change | Type of Mutation | Amino Acid | Defect | Potential Corrective Therapy | Cell Line/Organism | Disease | References |
|-------------------|------------------|------------|--------|-----------------------------|-------------------|---------|------------|
| c.3268CT          | Nonsense         | R1090X     | Premature termination codon | G418, gentamicin  | NIH3T3 cells (increased readthrough) | PFIC2    | [118]      |
|                   |                  |            |        | Gentamicin                  | HEK293, Can10 and HepG2 cells (production of a full-length BSEP protein and localization at the PM of HEK293 and at the CM of Can 10 and HepG2 cells) |         |           |
|                   |                  |            |        | Gentamicin treatment with UDCA, 4-PB and UDCA + 4-PB, gentamicin at 27 °C | Can10 cells (increased canalicular expression) |         |           |
|                   |                  |            |        | Gentamicin, gentamicin with 4-PB, gentamicin at 27 °C | NTCP expressing MDCK cells (significantly increased transport of [3H]TC) |         |           |
| c.3904GT          | Nonsense         | E1302X     | Premature termination codon | G418, gentamicin, PTC124 | NIH3T3 cells (increased readthrough) | PFIC2    | [118]      |

Note: ¹ Most frequently reported in Japan. ² E297G and D482G mutations account for 58% of PFIC2 cases in the Western population [119].
7.1. Transcriptional Modulators

FXR is the major ligand-activated transcription factor controlling BSEP expression, which makes it a possible target for therapeutic intervention. Furthermore, 6α-ethyl-CDCA (obeticholic acid, OCA), a derivative of the primary human bile acid CDCA and an FXR agonist, was approved by the FDA for the treatment of primary biliary cholangitis (PBC) either in monotherapy or in combination with UDCA in adults, depending on UDCA responsivity and tolerability [120]. OCA’s 100-fold higher FXR-activating potential (as compared to the natural ligand CDCA) formed the basis for advocating it as a novel therapeutic treatment strategy for PBC [121]. Its long-term efficacy and safety profile were reported recently [122]. OCA was also introduced for the treatment of non-alcoholic steatohepatitis (NASH), whereby the interim analysis from a phase 3 trial demonstrated clinical improvement and partial reversal of histopathological features [123]. Several other steroidal and non-steroidal FXR agonists, such as EDP-305 and tropifexor (LJN4524), are currently being investigated in a clinical setting for the treatment of NASH [124].

Garzel and co-workers evaluated the effects of 30 BSEP inhibitors on BSEP expression and FXR activation in human primary hepatocytes to understand the underlying mechanisms of drug-induced liver injury (DILI) [125]. Among five potent transcriptional repressors, lopinavir and troglitazone were shown to mediate their effects by reducing the FXR activity. The latter drug was previously withdrawn from the market because of DILI [125]. A number of natural FXR agonists or antagonists were reported to modulate FXR activity in a variety of model systems, as reviewed in detail by Hiebl et al. [126]. A natural product, geniposide, was reported to modulate the expression of BSEP via the FXR, as well as via the Nrf2 signaling pathways [127]. On the other hand, 9-cis retinoic acid (9cRA) is an RXR agonist, which when co-administered with CDCA, represses FXR/RXR-mediated expression of BSEP, thus exerting an opposite effects on BSEP transcription [128].

7.2. Ursodeoxycholic Acid (UDCA)

UDCA is one of the most commonly used agents for the treatment of cholestatic disorders. It showed promising results in animal models and in patients by alleviating disease symptoms. Although the exact mechanism of action of UDCA is not known, it was reported that the compound may act by correcting a potential trafficking deficiency of BSEP mutants, as well as by reducing the internalization of the transporter [112,129,130]. Furthermore, UDCA also reduces the overall hydrophobicity of the bile acid pool, thereby protecting hepatocytes from damage [131]. However, in some patients UDCA failed alleviate disease symptoms. Likely this finding reflects the genetic diversity of the underlying disease [129].

A UDCA derivative, norUDCA, is also being used for treatment of BSEP-related diseases. Because of its capacity for cholehepatic shunting (i.e., bypassing the normal enterohepatic circulation), norUDCA counteracts bile duct damage via bicarbonate-rich choleresis. Furthermore, norUDCA has antifibrotic, antiproliferative and anti-inflammatory properties and propagates bile acid detoxification through elimination via the urine [131–133].

7.3. Chemical Correction with 4-PB

A large number of mutations have been reported to interfere with either BSEP folding or its correct trafficking to the canalicular membrane. Among these are the E297G and D482G mutations, which account for approximately 60% of PFIC2 cases in the European population [119]. The underlying hypothesis behind the concept of chemical correction is that these mutants, when rescued to the canalicular membrane, would function normally, and thus the disease phenotype would be alleviated. Furthermore, 4-PB, an FDA-approved drug for the treatment of urea cycle disorders, functions as a chemical chaperone for folding-deficient BSEP variants [56]. Indeed, in vitro studies in HEK293 and MDCKII cell lines indicated that upon treatment with 4-PB, these mutants would show enhanced surface expression, as well as increased TCA transport activity [103,104]. Furthermore, in support
of the validity of this concept, 4-PB treatment also increased the biliary excretion of TCA in animal models [104].

Recently, use of 4-PB showed promising results in a clinical setting. Gonzales and co-workers [97,98] showed that treating PFIC2 patients carrying at least one mutation (out of p.G982R, p.R1128C and p.T1210P) with 4-PB led to an improvement in serum liver parameters, including the serum bile acid concentration, and a reduction in the pruritus score. Similarly, a preterm infant diagnosed with BSEP-related cholestasis was treated with 4-PB and showed an improvement of the disease symptoms [134]. In addition, BRIC2 patients have also been treated successfully with 4-PB [109]. The therapeutic doses ranged from 150 to 500 mg/kg/day. In some patients, a 4-PB dose of \( \leq 350 \text{ mg/kg/day} \) had no beneficial effect, while a high dose regimen (500 mg/kg/day) improved disease symptoms [109,117]. In a recent study, two PFIC II patients were given a combination of 4-PB, oxcarbazepine (a peripheral nerve stabilizer reducing pruritus) and maralixibat (an apical sodium-dependent bile acid transporter inhibitor), which had a beneficial effect on disease markers [101]. Most of these studies did not show apparent side effects, even in the high-dose regimen. However, psychological disorders (bipolar and related disorders) have been connected to the use of 4-PB in a clinical setting [135].

### 7.4. Potentiation with Ivacaftor

UDCA and 4-PB have been shown to correct the misfolding of trafficking-deficient variants. However, several disease-associated mutations (especially those in the NBDs) do not interfere with folding, trafficking and canalicular localization, but rather lead to impairment of transporter function [65,119]. Ivacaftor (VX-770) a potentiator has been approved by the FDA for treatment of some class III (gating deficient) CFTR mutants [136,137]. In these variants, an improvement of respiratory function could be demonstrated. Similar to clinical results in CFTR patients, ivacaftor was shown to rescue the function of missense mutations in the NBDs of ABCB4/MDR3 [138]. Recently, Mareux and co-workers showed that ivacaftor also rescued the function of an NBD missense mutation (T463I) of BSEP [110]. The mechanism of action of ivacaftor presently remains unclear.

### 7.5. Readthrough Therapy with Gentamicin

Nonsense mutations result in an in-frame premature termination codon and the absence of functional protein. This results in severe phenotypes of the disease and an increased risk for the development of hepatocellular carcinoma [139]. The aminoglycoside antibiotic gentamicin binds to ribosomes and induces a translational readthrough at the premature termination codon, thereby leading to fractional restoration of synthesis of the full-length protein [140,141]. In a recent study, Amzal et al. [118] evaluated the impact of gentamicin on six BSEP nonsense mutations (Y354X, R415X, R470X, R1057X, R1090X and E1302X) in vitro. Readthrough results were significantly increased for all mutations. The strongest responses were seen for the R1090X mutation, with partial restoration and correct localization at the plasma membrane of HepG2 and Can 10 cells. The rescued protein was shown to mediate transcellular transport of \( ^3\text{H}\)TC in MDCK cells. Expression of the R1090X mutant was shown to be further enhanced by simultaneous treatment with 4-PB [118].

### 8. Summary and Conclusions

Despite the relative infrequency of the inherited forms of progressive intrahepatic cholestasis, the symptoms are severe, and about half of the patients progress to a stage of the disease that makes them candidates for liver transplantation. Therefore, the quest for identification of causal therapies that go beyond the purely symptomatic treatment of pruritus is an important objective. Attempts to alleviate disease symptoms by transcriptional upregulation are directed towards missense mutations with preserved functionality. Similarly, those mutants that are folding- and consequently trafficking-deficient have successfully been treated with folding correctors. Recent evidence points to yet another
therapeutic path, in which the function of impaired BSEP mutants is potentiated by drugs that were initially developed to treat different disease entities, which are also associated with a malfunction of human ABC proteins. However, BSEP missense or deletion mutations and mutants with compromised functionality will only be amenable to therapy using gene editing. Current advances in gene editing technologies have not been considered in this review, as they are subject to another article in this Special Issue. The availability of the recently published cryo-EM structure of BSEP can be considered an important basis for structure-based drug design. Moreover, structural data, MD-simulations and site-directed mutagenesis studies continuously expand our understanding of the functional biology of BSEP.

We also briefly summarized available in vitro model systems for the functional characterization of BSEP, animal models and case reports discussing emerging clinical therapies. The perspective that treatment regimens combining small molecules with different mechanisms of action will ultimately lead to an improvement in the quality of life and life span of affected individuals appears promising.

Author Contributions: Conceptualization, P.C. and T.S.; writing—original draft preparation, M.I.S., Y.D.-C. and D.S.; writing—review and editing, M.I.S., Y.D.-C., D.S., T.S. and P.C.; visualization, D.S., Y.D.-C., T.S.; project administration, T.S. and P.C.; funding acquisition, P.C. and T.S. All authors have read and agreed to the published version of the manuscript.

Funding: Open Access Funding by the Austrian Science Fund (FWF), grant numbers SFB3509 (to P.C.), SFB3524 (to T.S.) and (P32017 to T.S.).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not available.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

Abbreviations

| Abbreviation | Definition |
|--------------|------------|
| ABC          | ATP-binding cassette |
| AMPK         | AMP-activated protein kinase |
| AP-2         | adaptor protein 2 |
| ASCOM        | activating signal cointegrator-2-containing complex |
| BSEP (ABCB11) | bile salt export pump |
| BRIC2        | benign recurrent intrahepatic cholestasis type 2 |
| CA           | cholic acid |
| cAMP         | cyclic adenosine monophosphate |
| CARM1        | co-activator-associated arginine methyltransferase 1 |
| CFTR (ABCC7) | cystic fibrosis transmembrane conductance regulator |
| CDCA         | chenodeoxycholic acid |
| CM           | canalicular membrane |
| cPKC         | classical (Ca2+-dependent) protein kinase C |
| cryo-EM      | cryo electron microscopy |
| DCA          | deoxycholic acid |
| DILI         | drug-induced liver injury |
| ECL          | extracellular loop |
| Epac         | exchange protein directly activated by cAMP |
| ICL          | intracellular loop |
| ICP          | intrahepatic cholestasis of pregnancy |
| ERAD         | Endoplasmic-reticulum-associated degradation |
| E17G         | estradiol 17 β-D-glucuronide |
| FXR (NR1H4)  | farnesoid X receptor |
| FXRE         | FXR response element |
LKB1 liver kinase B1
LRH-1 (NR5A2) liver receptor homolog-1
MAPK Mitogen-activated protein kinase
MRP3 (ABCC3) Multidrug-resistance-associated protein 3
MRP4 (ABCC4) Multidrug-resistance-associated protein 4
NASH non-alcoholic steatohepatitis
NBD nucleotide-binding domain
NBS nucleotide-binding site
Nrf2 nuclear factor erythroid 2-related factor 2
NTCP (SLC10A1) sodium taurocholate co-transporting polypeptide
OCA 6α-ethyl-CDCA (obeticholic acid)
4-PB 4-phenylbutyrate
PBC primary biliary cholangitis
PFIC2 progressive familial intrahepatic cholestasis type 2
PI3K phosphoinositide 3-kinase
PM plasma membrane
RXR retinoid X receptor
SRC2 steroid receptor co-activator 2
TCA taurocholic acid
TMD transmembrane domain
TUDCA tauroursodeoxycholic acid
UDCA ursodeoxycholic acid

References
1. Dean, M.; Rzhetsky, A.; Allikmets, R. The human ATP-binding cassette (ABC) transporter superfamily. Genome Res. 2001, 11, 1156–1166. [CrossRef] [PubMed]
2. Schmitt, L. Structure and mechanism of ABC transporters. Curr. Opin. Struct. Biol. 2002, 12, 754–760. [CrossRef]
3. Shintre, C.A.; Pike, A.C.W.; Li, Q.; Kim, J.-I.; Barr, A.J.; Goubin, S.; Snesesha, L.; Yang, J.; Berridge, G.; Ross, J.; et al. Structures of ABCB10, a human ATP-binding cassette transporter in apo- and nucleotide-bound states. Proc. Natl. Acad. Sci. USA 2013, 110, 9710–9715. [CrossRef] [PubMed]
4. Procko, E.; O’Mara, M.L.; Bennett, W.F.D.; Tieleman, D.P.; Gaudet, R. The mechanism of ABC transporters: General lessons from structural and functional studies of an antigenic peptide transporter. FASEB J. 2009, 23, 1287–1302. [CrossRef]
5. Langmann, T.; Mauerer, R.; Zahn, A.; Moehle, C.; Probst, M.; Stremmel, W.; Schmitz, G. Real-Time Reverse Transcription-PCR Expression Profiling of the Complete Human ATP-Binding Cassette Transporter Superfamily in Various Tissues. Clin. Chem. 2003, 49, 230–238. [CrossRef]
6. Stieger, B. The Role of the Sodium-Taurocholate Cotransporting Polypeptide (NTCP) and of the Bile Salt Export Pump (BSEP) in Physiology and Pathophysiology of Bile Formation. In Drug Transporters; Springer: Berlin/Heidelberg, Germany, 2011; pp. 205–259.
7. Lagana, S.M.; Salomao, M.; Remotti, H.E.; Knisely, A.S.; Moreira, R.K. Bile salt export pump: A sensitive and specific immunohistochemical marker of hepatocellular carcinoma. Histopathology 2015, 66, 598–602. [CrossRef]
8. Hilgendorf, C.; Ahlin, G.; Seithel, A.; Artursson, P.; Ungell, A.-L.; Karlsson, J. Expression of Thirty-six Drug Transporter Genes in Human Intestine, Liver, Kidney, and Organotypic Cell Lines. Drug Metab. Dispos. 2007, 35, 1333–1340. [CrossRef]
9. Stieger, B.; Beuers, U. The Canalicular Bile Salt Export Pump BSEP (ABCB11) as a Potential Therapeutic Target. Curr. Drug Targets 2011, 12, 661–670. [CrossRef]
10. Trauner, M.; Fuchs, C.D.; Halilbasic, E.; Paumgartner, G. New therapeutic concepts in bile acid transport and signaling for management of cholestasis. Hepatology 2017, 65, 1393–1404. [CrossRef]
11. Trauner, M.; Boyer, J.L. Bile Salt Transporters: Molecular Characterization, Function, and Regulation. Physiol. Rev. 2003, 83, 633–671. [CrossRef]
12. Lecureur, V.; Sun, D.; Hargrove, P.; Schuetz, E.G.; Kim, R.B.; Lan, L.B.; Schuetz, J.D. Cloning and expression of murine sister of P-glycoprotein reveals a more discriminating transporter than MDR1/P-glycoprotein. Mol. Pharmacol. 2000, 57, 24–35. [PubMed]
13. Hirano, M.; Maeda, K.; Hayashi, H.; Kusuhara, H.; Sugiyama, Y. Bile Salt Export Pump (BSEP/ABCB11) Can Transport a Nonbile Acid Substrate, Pravastatin. J. Pharmacol. Exp. Ther. 2005, 314, 876–882. [CrossRef] [PubMed]
14. Matsushima, S.; Maeda, K.; Hayashi, H.; Debori, Y.; Schinkel, A.H.; Schuetz, J.D.; Kusuhara, H.; Sugiyama, Y. Involvement of Multiple Efflux Transporters in Hepatic Disposition of Fexofenadine. Mol. Pharmacol. 2008, 73, 1474–1483. [CrossRef] [PubMed]
15. Ho, R.H.; Leake, B.F.; Kilkenney, D.M.; Meyer zu Schweden, H.E.; Glaeser, H.; Kroetz, D.L.; Kim, R.B. Polymorphic variants in the human bile salt export pump (BSEP; ABCB11): Functional characterization and interindividural variability. Pharmacogenet. Genom. 2010, 20, 45–57. [CrossRef] [PubMed]
16. Makishima, M. Identification of a Nuclear Receptor for Bile Acids. Science 1999, 284, 1362–1365. [CrossRef] [PubMed]
17. Wang, H.; Chen, J.; Hollister, K.; Sowers, L.C.; Forman, B.M. Endogenous Bile Acids Are Ligands for the Nuclear Receptor FXR/BAR. *Mol. Cell* 1999, 3, 543–553. [CrossRef]

18. Parks, D.J. Bile Acids: Natural Ligands for an Orphan Nuclear Receptor. *Science* 1999, 284, 1365–1368. [CrossRef]

19. Baghdasaryan, A.; Chiba, P.; Trauner, M. Clinical application of transcriptional activators of bile salt transporters. *Mol. Aspects Med.* 2014, 37, 57–76. [CrossRef]

20. Plass, J.R.M.; Mol, O.; Heegsma, J.; Geuken, M.; Faber, K.N.; Jansen, P.L.M.; Müller, M. Farnesoid X receptor and bile salts are involved in transcriptional regulation of the gene encoding the human bile salt export pump. *Hepatology* 2002, 35, 589–596. [CrossRef]

21. Ananthanarayanan, M.; Li, Y.; Surapureddi, S.; Balasubramaniyan, N.; Ahn, J.; Goldstein, J.A.; Suchy, F.J. Histone H3K4 trimethylation by ML3 as part of ASCOM complex is critical for NR activation of bile acid transporter genes and is downregulated in cholestasis. *Am. J. Physiol. Liver Physiol.* 2011, 300, G771–G781. [CrossRef]

22. Ananthanarayanan, M.; Li, S.; Balasubramaniyan, N.; Suchy, F.J.; Walsh, M.J. Ligand-dependent Activation of the Farnesoid X-receptor Directs Arginine Methylation of Histone H3 by CARM1. *J. Biol. Chem.* 2004, 279, 54348–54357. [CrossRef] [PubMed]

23. Chopra, A.R.; Kommagani, R.; Saha, P.; Louet, J.-F.; Salazar, C.; Song, J.; Jeong, J.; Finegold, M.; Viollet, B.; DeMayo, F.; et al. Cellular Energy Depletion Resets Whole-Body Energy by Promoting Coactivator-Mediated Dietary Fuel Absorption. *Cell Metab.* 2011, 13, 35–43. [CrossRef] [PubMed]

24. Song, X.; Kaimal, R.; Yan, B.; Deng, R. Liver receptor homolog 1 transcriptionally regulates human bile salt export pump expression. *J. Lipid Res.* 2008, 49, 973–984. [CrossRef]

25. Mataki, C.; Magnier, B.C.; Houten, S.M.; Annicotte, J.-S.; Argmann, C.; Thomas, C.; Overmars, H.; Kulik, W.; Metzger, D.; Auwerx, J.; et al. Compromised Intestinal Lipid Absorption in Mice with a Liver-Specific Deficiency of Liver Receptor Homolog 1. *Mol. Cell. Biol.* 2007, 27, 8330–8339. [CrossRef] [PubMed]

26. Weerachayaphorn, J.; Cai, S.-Y.; Soroka, C.J.; Boyer, J.L. Nuclear factor erythroid 2-related factor 2 is a positive regulator of human liver receptor homolog 1 expression. *Hepatology* 2009, 50, 1588–1596. [CrossRef] [PubMed]

27. Wang, L.; Dong, H.; Soroka, C.J.; Wei, N.; Boyer, J.L.; Hochstrasser, M. Degradation of the bile salt export pump at endoplasmic reticulum in progressive familial intrahepatic cholestasis type II. *Hepatology* 2008, 48, 1558–1569. [CrossRef]

28. Kleizen, B.; van Vlijmen, T.; de Jonge, H.R.; Braakman, I. Folding of CFTR Is Predominantly Cotranslational. *Mol. Cell* 2005, 20, 277–287. [CrossRef]

29. Rudashevskaya, E.L.; Stockner, T.; Trauner, M.; Freissmuth, M.; Chiba, P. Pharmacological correction of misfolding of ABC transporters in human non-alcoholic fatty liver disease. *Drug Discov. Today Technol.* 2014, 12, e87–e94. [CrossRef]

30. Chiba, P.; Freissmuth, M.; Stockner, T. Defining the blanks—Pharmacochaperoning of SLC6 transporters and ABC transporters. *Pharmacol. Res.* 2014, 83, 63–73. [CrossRef]

31. Guna, A.; Hegde, R.S. Transmembrane Domain Recognition during Membrane Protein Biogenesis and Quality Control. *Curr. Biol.* 2018, 28, R498–R511. [CrossRef]

32. Printsev, I.; Curiel, D.; Carraway, K.L. Membrane Protein Quantity Control at the Endoplasmic Reticulum. *J. Membr. Biol.* 2017, 250, 379–392. [CrossRef]

33. Needham, P.G.; Guerriero, C.J.; Brodsky, J.L. Chaperoning Endoplasmic Reticulum–Associated Degradation (ERAD) and Protein Conformational Diseases. *Cold Spring Harb. Perspect. Biol.* 2019, 11, a033928. [CrossRef] [PubMed]

34. Clarke, J.D.; Novak, P.; Lake, A.D.; Hardwick, R.N.; Cherrington, N.J. Impaired N-linked glycosylation of uptake and efflux transporters in human non-alcoholic fatty liver disease. *Liver Int.* 2017, 37, 1074–1081. [CrossRef] [PubMed]

35. Mochizuki, K.; Kagawa, T.; Numari, A.; Harris, M.J.; Itoh, J.; Watanabe, N.; Mine, T.; Arias, I.M. Two N-linked glycans are required for trafficking of the bile salt export protein to the apical membrane in Madin-Darby Canine Kidney Cells. *J. Hepatol.* 2007, 47, 299–310. [CrossRef] [PubMed]

36. Plass, J.R.; Mol, O.; Heegsma, J.; Geuken, M.; de Bruin, J.; Elling, G.; Müller, M.; Faber, K.N.; Jansen, P.L. A progressive familial intrahepatic cholestasis type 2 mutation causes an unstable, temperature-sensitive bile salt export pump. *Hepatology* 2010, 51, 1588–1596. [CrossRef] [PubMed]

37. Kipp, H.; Pichetschote, N.; Arias, I.M. Transporters on Demand. *Mol. Aspects Med.* 2009, 30, 589–596. [CrossRef]

38. Wakabayashi, Y.; Lippincott-Schwartz, J.; Arias, I.M. Intracellular Trafficking of Bile Salt Export Pump (ABCB11) in Polarized Hepatic Cells: Constitutive Cycling between the Canalicular Membrane and rab11-positive Endosomes. *Mol. Biol. Cell* 2010, 21, 973–984. [CrossRef] [PubMed]

39. Lam, P.; Soroka, C.; Boyer, J. The Bile Salt Export Pump: Clinical and Experimental Aspects of Genetic and Acquired Cholestatic Liver Disease. *Semin. Liver Dis.* 2010, 30, 125–133. [CrossRef]

40. Lam, P.; Xu, S.; Soroka, C.J.; Boyer, J.L. A C-terminal tyrosine-based motif in the bile salt export pump directs clathrin-dependent endocytosis. *Hepatology* 2012, 55, 1901–1911. [CrossRef]

41. Hayashi, H.; Inamura, K.; Aida, K.; Naoi, S.; Horikawa, R.; Nagasaki, H.; Takatani, T.; Fukushima, T.; Hattori, A.; Yabuki, T.; et al. AP2 adaptor complex mediates bile salt export pump internalization and modulates its hepatocanonical expression and transport function. *Hepatology* 2012, 55, 1899–1909. [CrossRef]

42. Chan, W.; Calderon, G.; Swift, A.L.; Moseley, J.; Li, S.; Hosoya, H.; Arias, I.M.; Ortiz, D.F. Myosin II Regulatory Light Chain Is Required for Trafficking of Bile Salt Export Protein to the Apical Membrane in Madin-Darby Canine Kidney Cells. *J. Biol. Chem.* 2005, 280, 23741–23747. [CrossRef] [PubMed]
43. Wakabayashi, Y.; Dutt, P.; Lippincott-Schwartz, J.; Arias, I.M. Rab11a and myosin Vb are required for bile canalicular formation in WIF-B9 cells. *Proc. Natl. Acad. Sci. USA* 2005, 102, 15087–15092. [CrossRef] [PubMed]

44. Crocenzi, F.A. Localization status of hepatocellular transporters in cholestasis. *Front. Biosci.* 2012, 17, 1201. [CrossRef] [PubMed]

45. Crocenzi, F.A.; Mottino, A.D.; Cao, J.; Veggi, L.M.; Pozzi, E.S.; Vore, M.; Coleman, R.; Roma, M.G. Estradiol-17β-D-glucuronide induces endocytic internalization of Bsep in rats. *Am. J. Physiol. Liver Physiol.* 2003, 285, G449–G459. [CrossRef]

46. Miszczuk, G.S.; Barosso, I.R.; Larocca, M.C.; Marrone, J.; Marinielli, R.A.; Boaglio, A.C.; Sánchez Pozzi, E.J.; Roma, M.G.; Crocenzi, F.A. Mechanisms of canalicular transporter endocytosis in the cholestatic rat liver. *Biochim. Biophys. Acta Mol. Basis Dis.* 2018, 1864, 1072–1085. [CrossRef]

47. Crocenzi, F.A.; Sánchez Pozzi, E.J.; Ruiz, M.L.; Zucchetti, A.E.; Roma, M.G.; Mottino, A.D.; Vore, M. Ca2+-dependent protein kinase C isoforms are critical to estradiol 17β-D-glucuronide-induced cholestasis in the rat. *Hepatology* 2008, 48, 1885–1895. [CrossRef]

48. Boaglio, A.C.; Zucchetti, A.E.; Sánchez Pozzi, E.J.; Pellegrino, J.M.; Ochoa, J.E.; Mottino, A.D.; Vore, M.; Crocenzi, F.A.; Roma, M.G. Phosphoinositide 3-kinase/protein kinase B signaling pathway is involved in estradiol 17β-D-glucuronide-induced cholestasis: Complementarity with classical protein kinase c. *Hepatology* 2010, 52, 1465–1476. [CrossRef]

49. Boaglio, A.C.; Zucchetti, A.E.; Toledo, F.D.; Barosso, I.R.; Sánchez Pozzi, E.J.; Crocenzi, F.A.; Roma, M.G. ERK1/2 and p38 MAPKs Are Complementarily Involved in Estradiol 17β-D-Glucuronide-Induced Cholestasis: Crossstalk with cPKC and PI3K. *PLoS ONE* 2012, 7, e49255. [CrossRef]

50. Fu, D.; Wakabayashi, Y.; Lippincott-Schwartz, J.; Arias, I.M. Bile acid stimulates hepatocyte polarization through a CAMP-Epac-AMP-PNP pathway. *Proc. Natl. Acad. Sci. USA* 2011, 108, 1403–1408. [CrossRef]

51. Homolya, L.; Fu, D.; Sengupta, P.; Jarnik, M.; Gillet, J.-P.; Vitale-Cross, L.; Gutkind, J.S.; Lippincott-Schwartz, J.; Arias, I.M. LKB1-AMPK and PKA Control ABCB11 Trafficking and Polarization in Hepatocytes. *PLoS ONE* 2014, 9, e91921. [CrossRef]

52. Kurz, A.K.; Graf, D.; Schmitt, M.; Vom Dahl, S.; Häussinger, D. Tauroidosesoxycholate-induced cholestasis involves p38MAPK activation and translocation of the bile salt export pump in rats. *Gastroenterology* 2001, 121, 407–419. [CrossRef] [PubMed]

53. Kubitz, R.; Stüffels, G.; Kühlkamp, T.; Kölling, R.; Häussinger, D. Trafficking of the bile salt export pump from the canalicular membrane vesicles. *Am. J. Physiol. Liver Physiol.* 2003, 285, G316–G324. [CrossRef] [PubMed]

54. Misra, S.; Varticovski, L.; Arias, I.M. Mechanisms by which CAMP increases bile acid secretion in rat liver and canalicular membrane vesicles. *Am. J. Physiol.* 2001, 280, G316–G324. [CrossRef] [PubMed]

55. Crocenzi, F.A. Localization status of hepatocellular transporters in cholestasis. *Am. J. Physiol. Liver Physiol.* 2003, 285, G449–G459. [CrossRef]

56. Wang, L.; Hou, W.-T.; Chen, L.; Jiang, Y.-L.; Xu, D.; Sun, L.; Zhou, C.-Z.; Chen, Y. Cryo-EM structure of human bile salts exporter ABCC11. *FEBS Lett.* 2017, 581, 935–938. [CrossRef] [PubMed]

57. Czuba, L.C.; Hillgren, K.M.; Swaan, P.W. Post-translational modifications of transporters. *Pharmacol. Ther.* 2018, 192, 88–99. [CrossRef]

58. Wang, L.; Hou, W.-T.; Chen, L.; Jiang, Y.-L.; Xu, D.; Sun, L.; Zhou, C.-Z.; Chen, Y. Cryo-EM structure of human bile salts exporter ABCB11. *Cell Res.* 2020, 30, 623–625. [CrossRef] [PubMed]

59. Keitel, V.; Burdelski, M.; Vojnisek, Z.; Schmitt, L.; Häussinger, D.; Kubitz, R. De novo bile salt transporter antibodies as a possible cause of recurrent graft failure after liver transplantation: A novel mechanism of cholestasis. *Hepatology* 2009, 50, 510–517. [CrossRef]

60. Kubitz, R.; Dröge, C.; Stindt, J.; Weissenberger, K.; Häussinger, D. The bile salt export pump (BSEP) in health and disease. *Clin. Res. Hepatol. Gastroenterol.* 2012, 36, 536–553. [CrossRef]

61. Dawson, R.J.P.; Locher, K.P. Structure of the multidrug ABC transporter Sav1866 from Staphylococcus aureus in complex with AMP-PNP. *FEBS Lett.* 2007, 581, 935–938. [CrossRef] [PubMed]

62. Giovannoni, I.; Callea, F.; Bellacchio, E.; Torre, G.; De Ville De Goyet, J.; Francalanci, P. Genetics and Molecular Modeling of New Mutations of Familial Intrahepatic Cholestasis in a Single Italian Center. *PLoS ONE* 2015, 10, e0145021. [CrossRef] [PubMed]

63. Li, J.; Jaimes, K.F.; Aller, S.G. Refined structures of mouse P-glycoprotein. *Protein Sci.* 2014, 23, 34–46. [CrossRef] [PubMed]

64. Dröge, C.; Bonus, M.; Baumann, U.; Klindt, C.; Lainka, E.; Kathemann, S.; Brinkert, F.; Grabhorn, E.; Pfister, E.-D.; Wenning, G.; et al. Sequencing of FIC1, BSEP and MDR3 in a large cohort of patients with cholestasis revealed a high number of different genetic variants. *J. Hepatol.* 2017, 67, 1253–1264. [CrossRef] [PubMed]

65. Jain, S.; Grandits, M.; Richter, L.; Ecker, G.F. Structure based classification for bile salt export pump (BSEP) inhibitors using comparative structural modeling of human BSEP. *J. Comput. Aided. Mol. Des.* 2017, 31, 507–521. [CrossRef] [PubMed]

66. Sohail, M.I.; Schmid, D.; Wlec, K.; Spork, M.; Szakács, G.; Trauner, M.; Stockner, T.; Chiba, P. Molecular Mechanism of Taurolcholate Transport by the Bile Salt Export Pump, an ABC Transporter Associated with Intrahepatic Cholestasis. *Mol. Pharmacol.* 2017, 92, 401–413. [CrossRef]

67. Berman, H.M. The Protein Data Bank. *Nucleic Acids Res.* 2000, 28, 235–242. [CrossRef]

68. Thonghin, N.; Collins, R.F.; Barbieri, A.; Shafi, T.; Siebert, A.; Ford, R.C. Novel features in the structure of P-glycoprotein (ABCB1) in the post-hydrolytic state as determined at 7.9 Å resolution. *BMC Struct. Biol.* 2018, 18, 17. [CrossRef]
70. Kagawa, T.; Watanabe, N.; Mochizuki, K.; Numari, A.; Ikeno, Y.; Itou, J.; Tanaka, H.; Arias, I.M.; Mine, T. Phenotypic differences in PFC2 and BRIC2 correlate with protein stability of mutant Bsep and impaired taurocholate secretion in MDCK II cells. Am. J. Physiol. Liver Physiol. 2008, 294, G58–G67. [CrossRef]

71. Goda, K.; Dönmez-Cakil, Y.; Tarapcsák, S.; Szalóki, G.; Szölősi, D.; Parveen, Z.; Türk, D.; Szakács, G.; Chiba, P.; Stockner, T. Human ABCB1 with an ABCB11-like degenerate nucleotide binding site maintains transport activity by avoiding nucleotide occlusion. PLoS Genet. 2020, 16, e1009016. [CrossRef]

72. Shukla, S.; Schwartz, C.; Kapoor, K.; Kounda, A.; Ambudkar, S.V. Use of Baculovirus BacMam Vectors for Expression of ABC Drug Transporters in Mammalian Cells. Drug Metab. Dispos. 2012, 40, 304–312. [CrossRef] [PubMed]

73. Stieger, B.; Fattinger, K.; Madon, J.; Kullak-Ublick, G.A.; Meier, P.J. Drug- and estrogen-induced cholestasis through inhibition of the hepatocellular bile salt export pump (Bsep) of rat liver. Gastroenterology 2000, 118, 422–430. [CrossRef]

74. Kondo, T.; Dale, G.L.; Beutler, E. Simple and rapid purification of inside-out vesicles from human erythrocytes. Biochim. Biophys. Acta Biomembr. 1980, 602, 127–130. [CrossRef]

75. Guyot, C.; Stieger, B. Interaction of bile salts with rat canalicular membrane vesicles: Evidence for bile salt resistant microdomains. J. Hepatol. 2011, 55, 1368–1376. [CrossRef] [PubMed]

76. Horikawa, M.; Kato, Y.; Tyson, C.A.; Sugiyama, Y. Potential Cholestatic Activity of Various Therapeutic Agents Assessed by Bile Canalicular Membrane Vesicles Isolated from Rats and Humans. Drug Metab. Pharmacokinet. 2003, 18, 16–22. [CrossRef] [PubMed]

77. Stieger, B.; Mahdi, Z.M. Model Systems for Studying the Role of Canalicular Efflux Transporters in Drug-Induced Cholestatic Liver Disease. J. Pharm. Sci. 2017, 106, 2295–2301. [CrossRef] [PubMed]

78. Mita, S.; Suzuki, H.; Akita, H.; Hayashi, H.; Onuki, R.; Hofmann, A.F.; Sugiyama, Y. Vectorial transport of unconjugated and conjugated bile salts by monolayers of LLC-PK1 cells doubly transfected with human NTCP and BSEP or with rat Ntcp and Bsep. Am. J. Physiol. Liver Physiol. 2006, 290, G550–G556. [CrossRef] [PubMed]

79. Montanari, F.; Pinto, M.; Khuneewarepong, N.; Weeke, K.; Sohail, M.I.; Noeske, T.; Boyer, S.; Chiba, P.; Stieger, B.; Kucherl, K.; et al. Flagging Drugs That Inhibit the Bile Salt Export Pump. Mol. Pharm. 2016, 13, 163–171. [CrossRef]

80. Kenna, J.G.; Taskar, K.S.; Battista, C.; Bourdet, D.L.; Brouwer, K.L.R.; Brouwer, K.R.; Dai, D.; Funk, C.; Hafey, M.J.; Lai, Y.; et al. Can Bile Salt Export Pump Inhibition Testing in Drug Discovery and Development Reduce Liver Injury Risk? An International Transponder Consortium Perspective. Clin. Pharmacol. Ther. 2018, 104, 916–932. [CrossRef]

81. Brouwer, K.L.R.; Keppler, D.; Hoffmaster, K.A.; Bow, D.A.J.; Cheng, Y.; Lai, Y.; Palm, J.E.; Stieger, B.; Evers, R. In Vitro Methods to Support Transponder Bile salt export pump (BSEP) inhibition testing in drug development and drug discovery. Clin. Pharmacol. Ther. 2013, 94, 95–112. [CrossRef]

82. Yang, K.; Guo, C.; Woodhead, J.L.; St. Claire, R.L.; Watkins, P.B.; Siler, S.Q.; Howell, B.A.; Brouwer, K.L.R. Sandwich-Cultured Hepatocytes as a Tool to Study Drug Disposition and Drug-Induced Liver Injury. J. Pharm. Sci. 2016, 105, 443–459. [CrossRef] [PubMed]

83. De Bruyn, T.; Chatterjee, S.; Fattah, S.; Keemink, J.; Nicolai, J.; Augustijns, P.; Annaert, P. Sandwich-cultured hepatocytes: Utility for in vitro experimentation of hepatobiliary drug disposition and drug-induced hepatotoxicity. Expert Opin. Drug Metab. Toxicol. 2013, 9, 589–616. [CrossRef] [PubMed]

84. Swift*, B.; Peifer*, N.D.; Brouwer, K.L.R. Sandwich-cultured hepatocytes: An in vitro model to evaluate hepatobiliary transporter-based drug interactions and hepatotoxicity. Drug Metab. Rev. 2010, 42, 446–471. [CrossRef]

85. Li, A.P.; Gorycki, P.D.; Hengstler, J.G.; Kedderis, G.L.; Koebe, H.G.; Rahmani, R.; de Sousas, G.; Silva, J.M.; Skett, P. Present status of the application of cryopreserved hepatocytes in the evaluation of xenobiotics: Consensus of an international expert panel. Chem. Biol. Interact. 1999, 121, 117–123. [CrossRef]

86. Lundquist, P.; Englund, G.; Skogastiaen, C.; Lööf, J.; Johansson, J.; Hoogstraate, J.; Afzelius, L.; Andersson, T.B. Functional ATP-Binding Cassette Drug Efflux Transporters in Isolated Human and Rat Hepatocytes Significantly Affect Assessment of Drug Disposition. Drug Metab. Dispos. 2014, 42, 448–458. [CrossRef] [PubMed]

87. Yucha, R.W.; He, K.; Shi, Q.; Cai, L.; Nakashita, Y.; Xia, C.Q.; Liao, M. In Vitro Drug-Induced Liver Injury Prediction: Criteria Optimization of Efflux Transporter IC50 and Physicochemical Properties. Toxicol. Sci. 2017, 157, 487–499. [CrossRef]

88. Cheng, Y.; Woolf, T.F.; Gan, J.; He, K. In vitro model systems to investigate bile salt export pump (BSEP) activity and drug interactions: A review. Chem. Biol. Interact. 2016, 255, 23–30. [CrossRef]

89. Cheng, Y.; Freedcn, C.; Zhang, Y.; Abraham, P.; Shen, H.; Wescott, D.; Humphreys, W.G.; Gan, J.; Lai, Y. Biliary excretion of pravastatin and taurocholate in rats with bile salt export pump (BSEP) impairment. Biopharm. Drug Dispos. 2016, 37, 276–286. [CrossRef]

90. Wang, R. Targeted inactivation of sister of P-glycoprotein gene (spgp) in mice results in nonprogressive but persistent intrahepatic cholestasis. Proc. Natl. Acad. Sci. USA 2001, 98, 2011–2016. [CrossRef]

91. Pankowicz, F.P.; Barzi, M.; Kim, K.H.; Legras, X.; Martins, C.S.; Wooton-Kee, C.R.; Lagor, W.R.; Marinj, J.C.; Elsea, S.H.; Bissig-Choisat, B.; et al. Rapid Disruption of Genes Specifically in Livers of Mice Using Multiplex CRISPR/Cas9 Editing. Gastroenterology 2018, 155, 1967–1970.e6. [CrossRef]

92. Alves-Bezerra, M.; Furey, N.; Johnson, C.G.; Bissig, K.-D. Using CRISPR/Cas9 to model human liver disease. JHEP Rep. 2019, 1, 392–402. [CrossRef] [PubMed]
93. Fuchs, C.D.; Paumgartner, G.; Wahlström, A.; Schwabl, P.; Reiberger, T.; Leditznig, N.; Stojakovic, T.; Rohr-Udilova, N.; Chiba, P.; Marschall, H.-U.; et al. Metabolic preconditioning protects BSEP/ABCB11−/− mice against cholestatic liver injury. J. Hepatol. 2017, 66, 95–101. [CrossRef] [PubMed]

94. Wang, R.; Chen, H.-L.; Liu, L.; Sheps, J.A.; Phillips, M.J.; Ling, V. Compensatory role of P-glycoproteins in knockout mice lacking the bile salt export pump. Hepatology 2009, 50, 948–956. [CrossRef] [PubMed]

95. Ellis, J.L.; Bove, K.E.; Schuetz, E.G.; Leino, D.; Valencia, C.A.; Schuetz, J.D.; Miethke, A.; Yin, C. Zebrafish abcb11b mutant reveals strategies to restore bile excretion impaired by bile salt export pump deficiency. Hepatology 2018, 67, 1531–1545. [CrossRef] [PubMed]

96. Lam, P.; Wang, R.; Ling, V. Bile Acid Transport in Sister of P-Glycoprotein (ABCB11) Knockout Mice. Biochemistry 2005, 44, 12598–12605. [CrossRef] [PubMed]

97. Gonzales, E.; Grosse, B.; Schuller, B.; Davit-Spraul, A.; Conti, F.; Guettier, C.; Cassio, D.; Jacquemin, E. Targeted pharmacotherapy in progressive familial intrahepatic cholestasis type 2: Evidence for improvement of cholestasis with 4-phenylbutyrate. Hepatology 2015, 62, 558–566. [CrossRef]

98. Gonzales, E.; Grosse, B.; Cassio, D.; Davit-Spraul, A.; Fabre, M.; Jacquemin, E. Successful mutation-specific chaperone therapy with 4-phenylbutyrate in a child with progressive familial intrahepatic cholestasis type 2. J. Hepatol. 2012, 57, 695–698. [CrossRef]

99. The Human Gene Mutation Database. Available online: http://www.hgmd.cf.ac.uk/ac/gene.php?gene=ABCB11 (accessed on 12 November 2020).

100. Imagawa, K.; Hayashi, H.; Sabu, Y.; Tanikawa, K.; Fujishiro, J.; Kajikawa, D.; Wada, H.; Kudo, T.; Kage, M.; Kusuhara, H.; et al. Clinical phenotype and molecular analysis of a homozygous ABCB11 mutation responsible for progressive infantile cholestasis. J. Hum. Genet. 2018, 63, 569–577. [CrossRef]

101. Malatack, J.J.; Doyle, D. A Drug Regimen for Progressive Familial Cholestasis Type 2. Pediatrics 2018, 141, e20163877. [CrossRef]

102. Arthur Lorio, E.; Valadez, D.; Alkhouri, N.; Loo, N. Cholestasis in Benign Recurrent Intrahepatic Cholestasis 2. J. Hum. Genet. 2017, 63, 569–577. [CrossRef]

103. Lam, P.; Pearson, C.L.; Soroka, C.J.; Xu, S.; Mennone, A.; Boyer, J.L. Levels of plasma membrane expression in progressive and benign mutations of the bile salt export pump (Bsep/Abcb11) correlate with severity of cholestatic diseases. Am. J. Physiol. 2007, 293, C1709–C1716. [CrossRef]

104. Hayashi, H.; Sugiyama, Y. 4-phenylbutyrate enhances the transport capacity of wild-type and mutated bile salt export pumps. Hepatology 2007, 45, 1506–1516. [CrossRef] [PubMed]

105. Byrne, J.A.; Strautnieks, S.S.; Ihre, G.; Pagani, F.; Knisely, A.S.; Chiba, P.; Schulz-Jürgensen, M.; et al. Partial external biliary diversion in bile salt export pump deficiency. Association between outcome and mutation. World J. Gastroenterol. 2017, 23, 5295. [CrossRef] [PubMed]
116. Davit-Spraul, A.; Oliveira, C.; Gonzales, E.; Gaignard, P.; Théron, P.; Jacquemin, E. Liver transcript analysis reveals aberrant splicing due to silent and intronic variations in the ABCB1 gene. Mol. Genet. Metab. 2014, 113, 225–229. [CrossRef] [PubMed]

117. Naoi, S.; Hayashi, H.; Inoue, T.; Tanikawa, K.; Igarashi, K.; Nagasaka, H.; Kage, M.; Takikawa, H.; Sugiymara, Y.; Inui, A.; et al. Improved Liver Function and Relieved Pruritus after 4-Phenylbutyrate Therapy in a Patient with Progressive Familial Intrahepatic Cholestasis Type 2. J. Pediatr. 2014, 164, 1219–1227.e3. [CrossRef] [PubMed]

118. Amzal, R.; Thebaut, A.; Lapalus, M.; Almes, M.; Grosse, B.; Mareux, E.; Collado-Hilly, M.; Davit-Spraul, A.; Bidou, L.; Namy, O.; et al. Pharmacological premature termination codon readthrough of ABCB1 in bile salt export pump deficiency: An in vitro study. Hepatology 2020, 70, 3147–3156. [CrossRef]

119. Strautnieks, S.S.; Byrne, J.A.; Pawlikowska, L.; Cebecauerová, D.; Rayner, A.; Dutton, L.; Meier, Y.; Antoniou, A.; Stieger, B.; Arnell, H.; et al. Severe Bile Salt Export Pump Deficiency: 82 Different ABCB11 Mutations in 109 Families. Gastroenterology 2008, 134, 1203–1214.e8. [CrossRef]

120. Highlights of Prescribing Information. Available online: https://www.accessdata.fda.gov/drugsatfda_docs/label/2018/207999s003lbl.pdf (accessed on 6 November 2020).

121. Fiorucci, S.; Antonelli, E.; Rizzo, G.; Renga, B.; Mencarelli, A.; Riccardi, L.; Orlandi, S.; Pellicciari, R.; Morelli, A. The nuclear receptor SHP mediates inhibition of hepatic stellate cells by FXR and protects against liver fibrosis. Gastroenterology 2004, 127, 1497–1512. [CrossRef]

122. Trauner, M.; Nevens, F.; Shiffman, M.L.; Drenth, J.P.H.; Bowlus, C.L.; Vargas, V.; Andreone, P.; Hirschfield, G.M.; Pereck, R.; Malecha, E.S.; et al. Long-term efficacy and safety of obeticholic acid for patients with primary biliary cholangitis: 3-year results of an international open-label extension study. Lancet Gastroenterol. Hepatol. 2019, 4, 445–453. [CrossRef]

123. Younossi, Z.M.; Ratziu, V.; Loomba, R.; Rinella, M.; Anstee, Q.M.; Goodman, Z.; Bedossa, P.; Geier, A.; Beckebaum, S.; Newsome, P.N.; et al. Obeticholic acid for the treatment of non-alcoholic steatohepatitis: interim analysis from a multicentre, randomised, placebo-controlled phase 3 trial. Lancet 2019, 394, 2184–2196. [CrossRef]

124. Fiorucci, S.; Biagioli, M.; Sepe, V.; Zampella, A.; Distrutti, E. Bile acid modulators for the treatment of nonalcoholic steatohepatitis (NASH). Expert Opin. Investig. Drugs 2020, 29, 623–632. [CrossRef]

125. Garzel, B.; Yang, H.; Zhang, L.; Huang, S.-M.; Polli, J.E.; Wang, H. The Role of Bile Salt Export Pump Gene Repression in Drug-Induced Cholestatic Liver Toxicity. Drug Metab. Dispos. 2014, 42, 318–322. [CrossRef]

126. Hiebl, V.; Ladurner, A.; Latkolik, S.; Dirsch, V.M. Natural products as modulators of the nuclear receptors and metabolic sensors LXR, FXR and RXR. Biotechnol. Adv. 2018, 36, 1567–1578. [CrossRef]

127. Wu, G.; Wen, M.; Sun, L.; Li, H.; Liu, Y.; Li, R.; Wu, F.; Yang, R.; Lin, Y. Mechanistic insights into geniposide regulation of bile salt-induced expression of human and mouse hepatic bile salt transporters. Hepatology 2009, 49, 151–159. [CrossRef]

128. Telbisz, A.; Homolya, L. Recent advances in the exploration of the bile salt export pump (BSEP/ABCB11) function. Expert Opin. Ther. Targets 2016, 20, 501–514. [CrossRef]

129. Halibasic, E.; Steinacher, D.; Trauner, M. Nor-Ursodeoxycholic Acid as a Novel Therapeutic Approach for Cholestatic and Metabolic Liver Diseases. Dig. Dis. 2017, 35, 288–292. [CrossRef]

130. Kim, D.J.; Yoon, S.; Ji, S.C.; Yang, J.; Kim, Y.-K.; Lee, S.; Yu, K.-S.; Jang, I.-J.; Chung, J.-Y.; Cho, J.-Y. Ursodeoxycholic Acid Improves Liver Function and Relieved Pruritus after 4-Phenylbutyrate Therapy in a Male Adolescent with Bile Salt Export Pump Deficiency Disease. Psychiatry Investig. 2014, 11, 580. [CrossRef]

131. Hoek, W.; Falck, H.; Hui, J.; Geuken, M.; van Rijnbergen, D.; Baller, J.F.; Kuipers, F.; Moshage, H.; Jansen, P.L.; Faber, K.N. Low retinol levels differentially modulate bile salt-induced expression of human and mouse hepatic bile salt transporters. Hepatology 2009, 49, 1972–1982. [CrossRef]

132. Moustafa, T.; Tickert, P.; Macnug, M.; Guvelly, C.; Thueringer, A.; Frank, S.; Kratky, D.; Sattler, W.; Reicher, H.; Sinner, F.; et al. Alterations in Lipid Metabolism Mediate Inflammation, Fibrosis, and Proliferation in a Mouse Model of Chronic Cholestatic Liver Injury. Gastroenterology 2012, 142, 140–151.e12. [CrossRef]

133. Ito, S.; Hayashi, H.; Sugiura, T.; Ito, K.; Ueda, H.; Togawa, T.; Endo, T.; Tanikawa, K.; Kage, M.; Kusuhara, H.; et al. Effects of 4-phenylbutyrate therapy in a preterm infant with cholestasis and liver fibrosis. Pediatr. Int. 2016, 58, 506–509. [CrossRef]

134. Pitale, G.; Simonetti, G.; Pirillo, M.; Taruschio, G.; Pietro, A. Bipolar and Related Disorders Induced by Sodium 4-Phenylbutyrate in a Male Adolescent with Bile Salt Export Pump Deficiency Disease. Psychiatry Investig. 2016, 13, 580. [CrossRef]

135. Van Goor, F.; Hadida, S.; Grootenhuis, P.D.J.; Burton, B.; Cao, D.; Neuberger, T.; Turnbull, A.; Singh, A.; Jobrann, J.; Hazlewood, A.; et al. Rescue of CF airway epithelial cell function in vitro by a CFTR potentiator, VX-770. Proc. Natl. Acad. Sci. USA 2009, 106, 18825–18830. [CrossRef]

136. De Boeck, K.; Munck, A.; Walker, S.; Faro, A.; Hiatt, P.; Gilmartin, G.; Higgins, M. Efficacy and safety of ivacaftor in patients with cystic fibrosis and a non-G551D gating mutation. J. Cyst. Fibros. 2014, 13, 674–680. [CrossRef]

137. Delaunay, J.; Bruneau, A.; Hoffmann, B.; Durand-Schneider, A.; Barbu, V.; Jacquemin, E.; Maurice, M.; Housset, C.; Cellebaut, I.; Ait-Slimane, T. Functional defect of variants in the adenosine triphosphate–binding sites of ABCB4 and their rescue by the cystic fibrosis transmembrane conductance regulator potentiator, ivacaftor (VX-770). Hepatology 2017, 65, 560–570. [CrossRef]
139. Van Wessel, D.B.E.; Thompson, R.J.; Gonzales, E.; Jankowska, I.; Sokal, E.; Grammatikopoulos, T.; Kadaristiana, A.; Jacquemin, E.; Spraul, A.; Lipiński, P.; et al. Genotype correlates with the natural history of severe bile salt export pump deficiency. *J. Hepatol.* 2020, 73, 84–93. [CrossRef]

140. Dąbrowski, M.; Bukowy-Bierylło, Z.; Zietkiewicz, E. Advances in therapeutic use of a drug-stimulated translational readthrough of premature termination codons. *Mol. Med.* 2018, 24, 25. [CrossRef]

141. Cuyx, S.; De Boeck, K. Treating the Underlying Cystic Fibrosis Transmembrane Conductance Regulator Defect in Patients with Cystic Fibrosis. *Semin. Respir. Crit. Care Med.* 2019, 40, 762–774. [CrossRef]