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INVITED REVIEW

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Function and pathophysiological importance of ABCB4 (MDR3 P-glycoprotein)

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Abstract Like several other ATP-binding cassette (ABC) transporters, ABCB4 is a lipid translocator. It translocates phosphatidylcholine (PC) from the inner to the outer leaflet of the canalicular membrane of the hepatocyte. Its function is quite crucial as evidenced by a severe liver disease, progressive familial intrahepatic cholestasis type 3, which develops in persons with ABCB4 deficiency. Translocation of PC makes the phospholipid available for extraction into the canalicular lumen by bile salts. The primary function of biliary phospholipid excretion is to protect the membranes of cells facing the biliary tree against these bile salts: the uptake of PC in bile salt micelles reduces the detergent activity of these micelles. In this review, we will discuss the functional aspects of ABCB4 and the regulation of its expression. Furthermore, we will describe the clinical and biochemical consequences of complete and partial deficiency of ABCB4 function.

Keywords Bile formation · Cholestasis · ABC transporter · Phospholipid · Cholesterol · Bile salt

Introduction

Two types of intramembrane lipid translocators can be defined: flippases, which translocate lipids from the outer leaflet to the inner leaflet of the membrane and floppases that mediate the reverse process, i.e., translocation from the inner to the outer leaflet of the membrane [1]. ABCB4 is a floppase for phosphatidylcholine (PC). Its function is thought to be limited to the apical (canalicular) membrane of the hepatocyte [2]. ABCB4 basically flops PC from the inner to the outer leaflet of the canalicular membrane to make this phospholipid available for extraction into the canalicular lumen by bile salts. The primary function of biliary phospholipid excretion is to protect the membranes of cells facing the biliary tree against these bile salts: the uptake of PC in bile salt micelles reduces the detergent activity of these micelles (for extensive review, see [3]).

Nomenclature of this protein has been extremely confusing initially because several different names have been given to the orthologs in different species at a time that the function of this protein was not yet understood. In man, this protein was called MDR3 P-glycoprotein, while in rodents the ortholog was called Mdr2 P-glycoprotein. Confusion was even greater because rats and mice have two homologous *Mdr1* (*Abcb1*) genes, *Mdr1a* and *Mdr1b*, the first of which has also been called *Mdr3* (and sometimes, it still is). The new nomenclature of ABC transporters fortunately resolves this confusion, and this protein should now be uniformly called ABCB4 in humans and Abcb4 in non-human species.

We will give a short introduction into the mechanism of bile formation, but for this purpose, we also refer to the article in this volume on the function of ABCB11, the bile salt export pump BSEP. For a more extensive review of the mechanisms of bile formation, we refer to several reviews on this subject [4–6].

Primary bile is formed in the canaliculi, small (1 μm) tubules formed by the apical membranes of adjacent hepatocytes. The arrangement of hepatocytes in plates allows these canalicular tubules to drain into the connecting bile duct. Bile formation is an osmotic process that is primarily driven by the active excretion of biliary solutes by ABC transporters across the canalicular membrane of these hepatocytes. The concentration gradient generated by this process causes an osmotic force that attracts water through tight junctions and via aquaporins in the membrane. The most important biliary solute is bile salt, which is pumped into the canaliculus by ABCB11 (BSEP). The action of the organic anion transporter ABCC2 (MRP2) also contributes to bile flow by the excretion of considerable amounts of glutathione. The lipid floppases ABCB4 (phospholipid) and ABCG5/G8 (sterols) drive the excretion of lipids across the canalicular membrane. Finally, the

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drug transporters ABCB1 (MDR1 P-glycoprotein), BCRP (ABCG2), and MRP2 (ABCC2) fulfill the important hepatic task of drug elimination into bile (for an overview of the relevant transporters, see Fig. 1).

Functional characteristics of ABCB4

The physiological function of ABCB4 is clear: mice with a disrupted *Abcb4* gene do not excrete any phosphatidylcholine into bile, while wild-type mice of the same genetic background excrete considerable amounts of PC [7, 8]. From these observations, it was suggested that Abcb4 flops PC within the membrane. *Abcb4*$^{-/-}$ mice that were made transgenic for the human *ABCB4* gene were completely rescued, demonstrating that these two orthologous genes have the same physiological function [9]. Despite the consensus on the physiological function of ABCB4, its molecular characteristics have not been extensively studied. The reason for this is the fact that it is quite difficult to develop reliable assays for the molecular mechanism of lipid translocators. In most studies, phospholipid analogs that have a fluorescent (NBD) group and a shortened fatty acid chain are used to increase their extractability from membranes. Polarized cells transduced with *ABCB4* have increased rates of NBD-PC translocation to the outer leaflet [10]. In contrast, NBD-labeled phosphatidylethanolamine and phosphatidylsphingomyelin are not translocated, suggesting that ABCB4 has preference for phosphatidylcholine. However, the significance of these findings is limited, as the same study demonstrated that ABCB1 was able to flop all these NBD-labeled phospholipid analogs, including that of PC. Although ABCB1 has been shown to influence the asymmetric distribution of endogenous phosphatidylserine in plasma membranes of cells that overexpress this transporter, it is unlikely that ABCB1 is also capable of flopping natural phospholipids at high rates. This is due to the fact that *Abcb4*$^{-/-}$ mice do not excrete any phospholipid into bile, irrespective of high expression of *Abcb1* in hepatocytes of these animals. Similarly, ABCC1 (MRP1) was found to mediate the translocation of NBD-labeled phospholipids [11–13], and possibly even natural PC [13]. In this case too, the question rises whether these translocation rates are physiologically important. ABCC2 has an almost identical substrate specificity as ABCC1 and is abundantly present in the canalicular membrane of the hepatocyte but is not able to rescue the PC translocation function in the absence of ABCB4. It may be assumed that the artificial NBD-labeled phospholipids are handled by these ABC transporters as amphiphilic drugs (that insert in the membrane) rather than as natural phospholipids [14, 15].

To date, the translocation of natural PC has been analyzed in only one study [16]. In these experiments, PC translocation was studied in fibroblasts overexpressing *ABCB4* by metabolic labeling with radioactive choline. By addition of phosphatidylcholine-transfer protein to the medium of these cells, radioactive PC could be extracted from the membrane; the amount of radioactive PC in the extract was taken as a measure of floppase activity. Indeed, these studies demonstrated enhanced translocation of endogenous PC in the presence of *ABCB4* expression.

Excretion of phosphatidylcholine involves not only translocation but also extraction from the membrane

As described above, it is generally accepted that ABCB4 flops phosphatidylcholine (PC) from the inner to the outer leaflet, but the mechanism of the subsequent step, i.e., extraction from the membrane, is largely unknown. With the discovery of the function of the two half-transporters ABCG5 and ABCG8, which translocate sterols, it became clear that translocation of the lipid molecule per se may not be the only important event in the excretion process. Although controversy exists on the spontaneous flip-flop

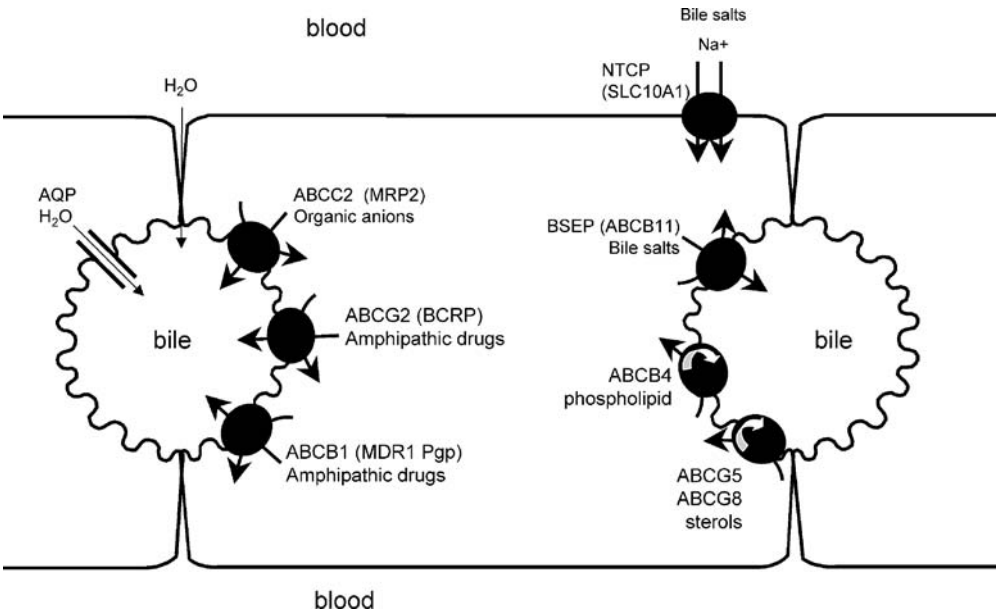

Fig. 1 Schematic representation of the most important transport processes in the canalicular membrane of hepatocytes mediated by ABC transporters. *AQP* aquaporin, *NTCP* Na^+-dependent taurocholate transporter

rates of cholesterol in membranes, it is generally considered to be fast (half-times less than 1 s have been reported [17]). Nevertheless, in the absence of Abcg5/8, biliary cholesterol excretion in mice is reduced by about 75% [18, 19]. Small [20] suggested that not only translocation but also subsequent exposition of the cholesterol molecule out of the membrane bilayer by the transporter (termed "lifting") is an essential step in the excretion (see also [21]). The exposed cholesterol molecule may then be transferred to mixed micelles of bile salts and phospholipids. Such a lifting or exposure mechanism may in fact also play a role in ABCB4-mediated phospholipid excretion. It has been known for many years that phospholipid and cholesterol excretion are driven by micelle forming bile salts. In the past, we have proposed that this involves vesiculation from the outer leaflet of the membrane, induced by local translocation of PC to the outer leaflet, combined with destabilization of the membrane by luminal bile salts [3]. Indeed, membrane-adherent vesicles have been detected in careful electron microscopic studies [22, 23]. Mechanistically it remains difficult, however, to envision a vesiculation process from a single (outer) leaflet of the membrane, as this would invoke highly unstable structures in the membrane. In the presence of high concentrations of bile salts, this might present a rather unfavorable situation. The discovery of ABCG5/8 as a cholesterol translocator demonstrated that PC and cholesterol excretion involve largely independent mechanisms; in the absence of either Abcg5 or Abcg8 (or both), cholesterol excretion in mice is strongly reduced, while PC excretion is much less affected [18, 19]. Cholesterol excretion is completely impaired in the Abcb4$^{-/-}$ mouse, but this is caused by the absence of PC in bile, which renders the bile salt micelles as very poor cholesterol solubilizers [24]. Indeed, if more hydrophobic bile salts, such as taurodeoxycholate (which are better cholesterol solubilizers in the absence of PC), are infused in Abcb4$^{-/-}$ mice, cholesterol excretion can be restored [24]. Hence, the excretion machineries of PC and cholesterol are more or less independent. It is difficult to envision a general lipid vesiculation mechanism from the outer leaflet driven by ABCB4-mediated PC-flopping that does not involve simultaneous excretion of PC and cholesterol. It may therefore be suggested that PC excretion does not take place via vesiculation, but rather by translocation followed by exposition of the PC molecule by ABCB4, which subsequently allows extraction by bile salt micelles. This generates mixed micelles of bile salt and PC, which can subsequently act as acceptor for cholesterol that is exposed by ABCG5/8 (for an overview of our current model of biliary lipid excretion, see Fig. 2).

Regulation of *ABCB4* expression

Expression of *ABCB4* is largely restricted to the hepatocytes in the liver. Although low levels of mRNA transcripts have been detected in the adrenal gland, muscle,

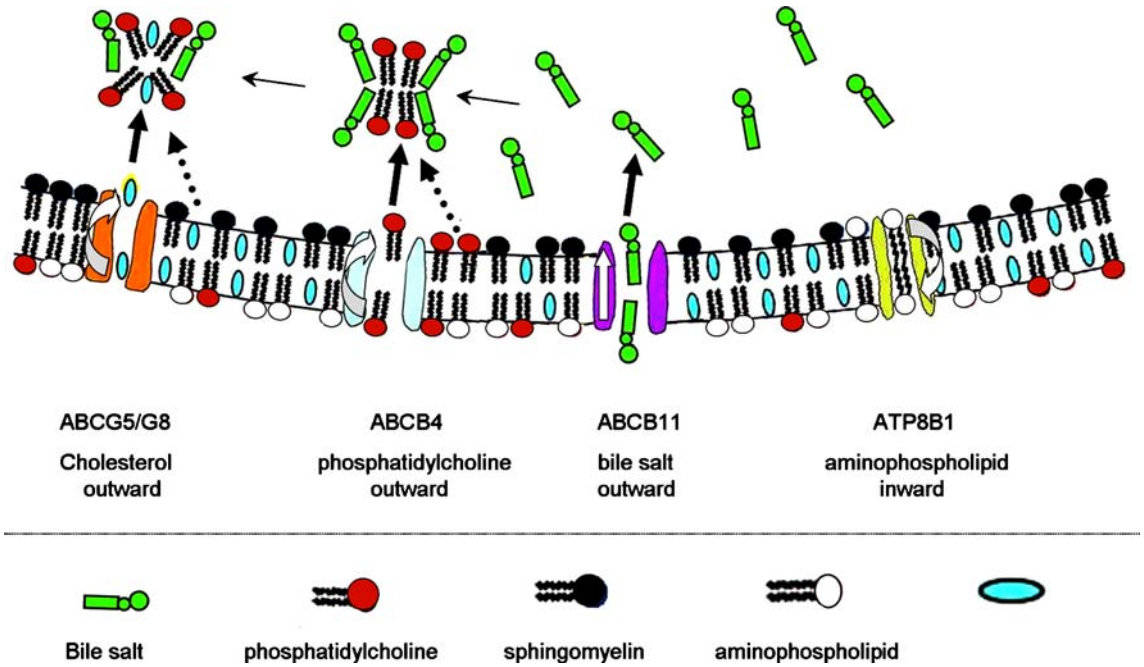

Fig. 2 Hypothetical mechanism of canalicular lipid excretion. Lipid excretion starts with bile salt excretion via ABCB11. This creates canalicular bile salt concentrations above the critical micellar concentration. ABCB4 flops PC from the inner to the outer leaflet of the canalicular membrane and exposes it in such a fashion that PC can be taken up in bile salt micelles to form mixed micelles. Subsequently, these mixed micelles can accept cholesterol that is flopped and exposed by ABCG5/8. ATP8B1 flips aminophospholipids from the outer to the inner leaflet, thereby increasing the relative content of sphingomyelin and cholesterol in the outer leaflet, which makes the membrane resistant against bile salts (figure modified from Small [21])

tonsil, spleen [25], in placenta [26], testis (Sertoli cells), and ileum [27], the protein has not been demonstrated outside the liver. Nevertheless, it is still possible that ABCB4 serves a similar, hitherto unnoticed, function in other cell types. Interestingly, ectopic overexpression of *ABCB4* was found to cause pathology in neurons and in the eye. Smit et al. [28] produced mice with transgenic, vimentin promotor-driven, expression of *ABCB4* and observed peripheral neuropathy with slowed motor nerve conduction and dismyelinization. The mechanism of this pathology has not been elucidated but may be caused by the disturbing effect of high levels of the protein in the highly organized myelinated sheets. On the other hand, translocation of PC to the outer leaflet of these myelinated sheets may also have a disturbing effect. It was also reported that overexpression of *ABCB4* in the eye lens generates ultrastructural abnormalities [29].

It has been established that *Abcb4* expression in rodents is induced by bile salts [30, 31]. Interestingly, this induction occurred with cholate feeding but not with ursodeoxycholate (UDCA) feeding. This observation fits with the more recent findings that *Abcb4* is regulated by the nuclear hormone receptor Fxr. UDCA is not a FXR ligand, while cholate and chenodeoxycholate are weak and strong activators, respectively. Liu et al. [32] demonstrated induction of *Abcb4*/*ABCB4* expression in rats and in cultured human hepatocytes after treatment with the artificial FXR agonist, GW4064. In addition, the human *ABCB4* gene was found to contain a functional FXR response element [33]. Marschall et al. [34] observed that patients undergoing UDCA treatment (1 g/day) did not have increased hepatic *ABCB4* mRNA levels. Although this was expected (because UDCA is not an FXR agonist), they did find an increase in hepatic ABCB4 protein levels; this most likely involves a different, posttranslational event [35].

It has also been established by various groups that fibrates induce the expression of *Abcb4* in mice [36, 37]. This most likely involves activation of the nuclear hormone Ppar-α because *Abcb4* expression was inert to fibrate treatment in Ppar-$\alpha^{-/-}$ mice [38]. The induction of ABCB4 by fibrates (with increased biliary phospholipid excretion as a consequence) has been suggested to underlie the observed beneficial effect of bezafibrate in patients with primary biliary cirrhosis (PBC) [39, 40]. However, in humans, PPAR-α displays a different activation pattern toward fibrates than in mice. Indeed, in a patient group treated with bezafibrate, no induction of ABCB4 expression could be observed [41]. In line with these observations, Shoda et al. [42] observed only a minor induction of ABCB4 expression in HepG2 cells; however, they did find a significant increase in the rate of NBD-labeled PC excretion into the pseudocanaliculi of these cells, suggesting that phospholipid excretion is enhanced. They attributed this phenomenon to an enhanced mobilization of ABCB4 from intracellular vesicles to the canalicular membrane.

The ontogenesis of *ABCB4* expression has not been thoroughly studied. Chen et al. [43] analyzed the expression of several transporters relevant for bile formation in fetal liver from 14 to 20 weeks of gestation. While mRNA levels of *ABCB11* and *ABCC2* were two- to threefold lower than in adult liver, the *ABCB4* mRNA was 16-fold lower, suggesting that ABCB4 expression develops late in gestation or even postnatally. It was suggested that this late development may contribute to the cholestatic problems encountered with total parenteral nutrition (TPN) and infection in neonates. Patients receiving TPN are prone to develop cholestasis, and this holds especially for newborn children. In a mouse model, Tazuke et al. [44] recently showed that *Abcb4* expression was reduced in mice on TPN, while *Abcb11* expression was increased. In humans, de Vree et al. [45] showed that the absence of enteral nutrition was found to cause a generalized reduction of bile secretion, with a particular decrease in the function of ABCB4.

Mutations in the *ABCB4* gene cause progressive familial intrahepatic cholestasis type 3

The canalicular membrane has to withstand very high detergent concentrations. Bile salts, excreted via ABCB11 (BSEP), reach concentrations in the canaliculus well above the critical micellar concentration. This represents a condition, which in principle, leads to solubilization of membranes, followed by immediate cell death. One mechanism of protection is the organized excretion of phospholipid via ABCB4. Phospholipids associate with simple bile salt micelles to form mixed micelles (containing both phospholipid and bile salt). This association strongly reduces the concentration of toxic bile salt monomers and simple micelles (together called the intermicellar concentration) [46]. The resulting mixed micelles have a much lower capacity to take up more phospholipid from the membrane. The importance of this mechanism is underscored by the deleterious consequences of the absence of biliary phospholipid excretion in patients who have mutations in the *ABCB4* gene and develop progressive familial intrahepatic cholestasis type 3 (PFIC3) [47–49]. These patients usually present at a few years of age with chronic and progressive cholestasis. Progressive familial intrahepatic cholestasis describes a group of similar, inherited liver diseases that are characterized by the diagnosis of an inherited cholestasis that often progresses to end-stage liver disease [4]. Apart from PFIC3, there are also patients with PFIC type 1, who have mutations in the *ATP8B1*gene (see below) and patients with PFIC type 2, who have mutations in the *ABCB11* gene. In PFIC3, liver histology reveals fibrosis (progressing into cirrhosis) with portal inflammation and, in contrast to the other forms of PFIC, strong bile duct proliferation. Another difference with the two other forms of PFIC is a characteristic high serum α-glutamyl transpeptidase (GGT) activity. As expected, PFIC3 patients have reduced concentration of PC in bile, although it must be stressed that relatively few data are available on the composition of bile in these patients [49]. About 50% of the patients need a liver transplantation, while the other half may benefit from treatment with UDCA [49]. The rationale

of this treatment is to replace endogenous, cytotoxic, bile salts with UDCA, which is a much less cytotoxic bile salt. Mice with a disruption of the *Abcb4* gene develop a very similar, but less severe, liver disease. This is caused by the fact that mice have a less cytotoxic bile salt composition.

More recently, it has become clear that mutations in the *ABCB4* gene that may reduce but not eliminate activity of the protein can cause a variety of milder PFIC3 phenotypes. This also applies in case of heterozygosity for mutations that eliminate transporter activity [50]. Mutations in the *ABCB4* gene have been detected in patients with symptoms of primary biliary cirrhosis (PBC) [51]. Such patients may be primarily, if not exclusively, found in the subgroup of PBC patients without antimitochondrial antibodies (AMA-negative), i.e., patients with symptoms of PBC that may not be caused by autoimmunity. Lucena et al. [51] and Thompson et al. [52] reported on AMA-negative patients with mutations in the *ABCB4* gene, while Pauli-Magnus et al. [53] could not find *ABCB4* mutations in a group of PBC patients with antimitochondrial antibodies (AMA-positive). Furthermore, *ABCB4* mutations have been reported in patients with intrahepatic cholesterol gallstones (see below) [51, 54].

Canalicular lipid transport defects can cause gallstone formation

Cholesterol supersaturation of bile, which occurs in a large proportion of humans, leads to the formation of cholesterol

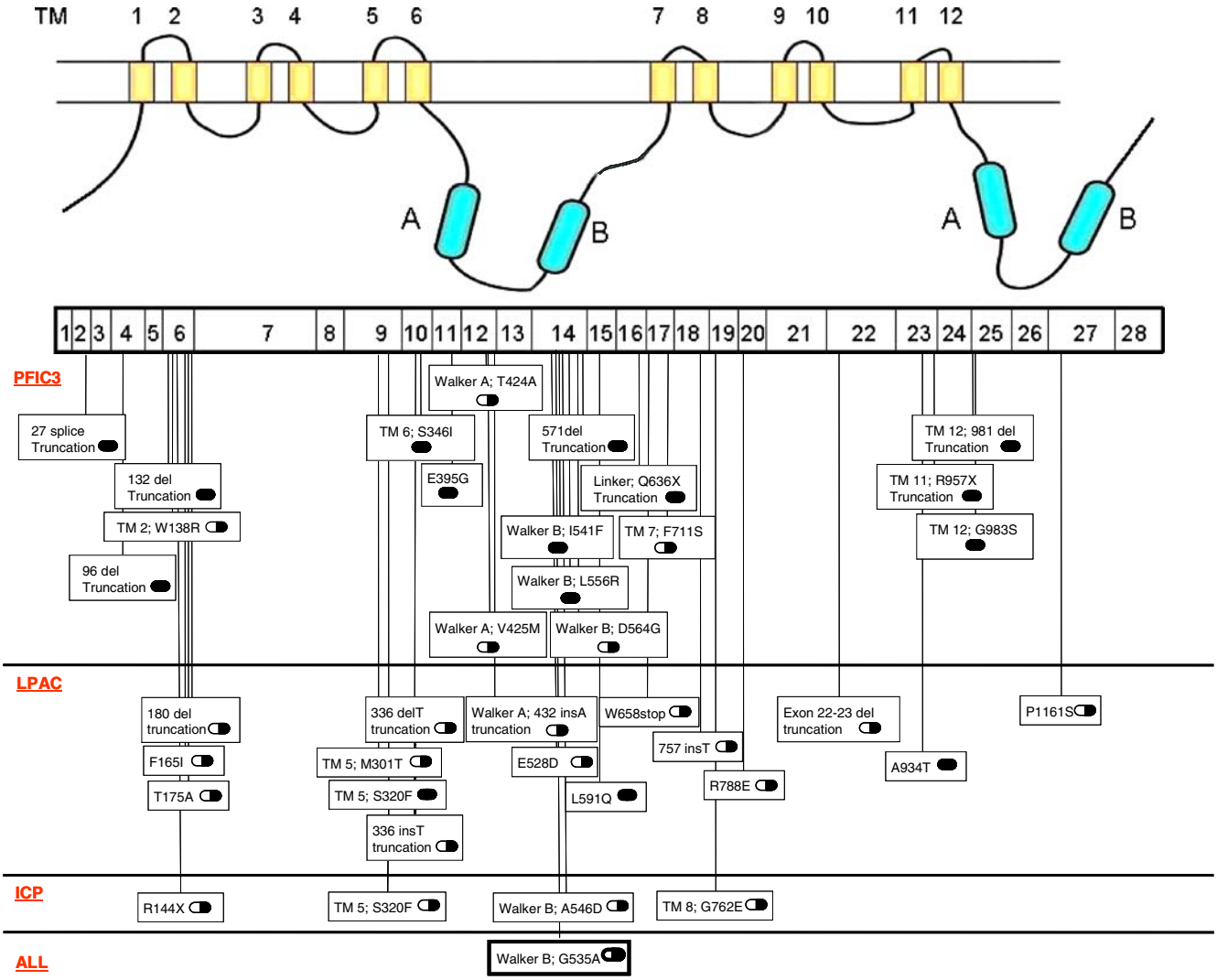

Fig. 3 Summary of the known mutations and their localization in the protein, as identified in patients with PFIC type 3, LPAC syndrome (intrahepatic gallstone formation), and intrahepatic cholestasis of pregnancy (ICP). If the mutation is in an essential domain, this is also indicated (*TM* transmembrane domain; Walker A and Walker B are the consensus motifs for the nucleotide binding domain). A *filled oval* indicates that the mutation was homozygous; a *half-filled oval* means that the mutation was heterozygous. In all cases, the codon has been indicated in which the mutation is present or in which the mutation starts (in case of larger deletions). Some of the patients described combine more than one of the symptoms (such as the combination of LPAC syndrome and ICP [54]). In one patient all three syndromes have been explicitly described (designated as ALL) [51]. The indicated mutations have been described in [47–51, 54, 63, 64, 71, 90, 91]

gallstones. Biliary cholesterol solubilization depends not only on the concentration of the sterol itself but also on the bile salt and phospholipid concentration [55]. Mixed micelles of bile salts and phospholipids solubilize cholesterol more efficiently than simple bile salt micelles. Hence, the rate of phospholipid excretion can be expected to be an important factor in the prevention of gallstone formation. This concept was validated by the finding that *Abcb4*$^{-/-}$ mice, which have a complete defect in phospholipid excretion, develop cholesterol gallstones [56]. These mice, subjected to a control diet, excrete very little cholesterol into bile because the endogenous bile salts are insufficiently strong detergents to solubilize cholesterol in the absence of phospholipid. However, when the bile salt pool is largely replaced by taurocholate (upon cholate feeding), these mice start to excrete cholesterol, and abundant cholesterol crystal formation is observed [24]. In normal humans, biliary cholesterol saturation is already high. One might therefore expect that also partial defects in phospholipid secretion may cause cholesterol gallstone formation. This has, indeed, been shown. Individuals with mutations in *ABCB4* are particularly prone to cholesterol gallstone formation. Gallstone formation was observed in several PFIC3 patients [49] and, conversely, mutations in *ABCB4* were observed in patients with intrahepatic gallstones [51, 54]. Importantly, the latter group of patients was not diagnosed as PFIC3 patients. As these patients were adults with symptoms different from PFIC3, it was hypothesized that this concerned a mild form of PFIC3 caused by mutations in *ABCB4* that leave residual activity of the transporter. Rosmorduc et al. [54] designated this as low-phospholipid associated cholelithiasis (LPAC) syndrome.

More recently, it was reported that cholelithiasis is also frequently observed in patients with PFIC type 2 [57] and in patients with benign recurrent intrahepatic cholestasis type 2 [58], who both have mutations in the gene encoding the major bile salt transporter, ABCB11.

Association between canalicular transport defects and cholestasis of pregnancy

Intrahepatic cholestasis of pregnancy (ICP) is a reversible form of cholestasis that may develop in the third trimester of pregnancy and usually rapidly resolves after delivery. The incidence of ICP lies between 10 and 100 cases per 10,000 pregnancies, but there is a strong ethnical background to this phenomenon. Notably, in the Chilean population, ICP develops in as much as 16% of pregnancies, and within the Araucanian Indian subpopulation it is as high as 28% [59]. The main symptoms are pruritus and, to a lesser extent, jaundice. Serum bile salt levels are increased [60, 61]. Increased incidence of fetal distress, premature birth, and even stillbirth in association with ICP has been reported (for a review, see [62]). It is generally accepted that women who have suffered from ICP are also susceptible to the development of cholestasis upon the use of oral contraceptives.

Several reports have shown that ICP can be associated with mutations in the *ABCB4* gene [50, 63–65]. In all cases, the reported mutations were shown not to occur in control populations. More recently, ICP has also been associated with mutations in the *ATP8B1* gene [66, 67]. Indeed, ICP patients can be divided in a group with low serum GGT (about 70%) and a group with high serum GGT activity (about 30%) [68]. In most cases described, the cholestatic symptoms resolved upon delivery. Thus, the observed mutations in *ABCB4* and *ATP8B1* do not cause transporter deficiencies that are strong enough to induce symptoms of PFIC. Most likely, mutations in ICP patients reduce transporter activity partly and only give rise to clinical symptoms during pregnancy. It can therefore be hypothesized that during the third trimester of pregnancy, there is a generalized impairment of bile formation. The mechanism of this phenomenon has not been completely revealed. It is thought that estrogens play an important role; it is known that estradiol-glucuronide is excreted into bile and, indeed, treatment of rodents with estrogens induces cholestasis [69]. It must be stressed that the present data suggest that the manifestations caused by mutations in canalicular transporter genes are present only in a minority of ICP patients [70–72].

Figure 3 depicts the mutations that have thus far been identified in patients with PFIC3, LPAC syndrome (cholelithiasis), and in intrahepatic cholestasis of pregnancy. One patient (bottom of the figure) who had gallstone formation, ICP, as well as symptoms of primary biliary cirrhosis has been described.

Lipid asymmetry in the canalicular membrane is also essential for protection against bile salts

Biliary phospholipid excretion is a relatively young evolutionary development in the protection against bile salt toxicity. Certain mammalian species, such as the guinea pig [73], excrete very little phospholipid into bile; in fish, reptiles, and most birds, biliary phospholipid levels can be extremely low [74]. Nevertheless, the bile salt species excreted by these animals can be strong detergents. As an example, the little skate (a cartilaginous fish) excretes a conjugated bile alcohol (scymnol sulfate) that is at least as strong a detergent as taurocholate, while there is no phospholipid excretion in this species [75]. Nevertheless, the canalicular membrane of the little skate is resistant against this bile alcohol. Hence, there must be a second mechanism of protection against high bile salt concentration in the canalicular lumen. Indeed, even in the absence of canalicular phospholipid excretion (as in *Abcb4*$^{-/-}$ mice and PFIC3 patients), the hepatocytes are largely resistant to the extremely high bile salt concentrations as the damage is limited to a chronically increased cell turnover without acute necrosis. Most likely, this second mechanism of protection involves the asymmetric distribution of lipids in the membrane bilayer, with a high content of sphingomyelin and cholesterol in the outer leaflet. In vitro experiments have demonstrated that the combination of these two lipids

is the only way by which membranes can be rendered virtually detergent-insoluble [76]. Although direct proof that such a mechanism is responsible for the detergent resistance of the canalicular membrane is lacking, the observed high sphingomyelin content of these membranes isolated from various species makes this hypothesis plausible [77–79]. We speculate that the P-type ATPase ATP8B1 plays a role in this process. This flippase is present in the canalicular membrane (as well as the apical membrane of several other epithelia including cholangiocytes, gallbladder, pancreas, and intestine) and translocates aminophospholipids from the outer to the inner leaflet of the bilayer [80]. The net effect of this translocation is that the membrane becomes enriched in sphingomyelin and cholesterol. Patients with mutation in the *ATP8B1* develop a chronic progressive cholestatic liver disease (PFIC type 1) that resembles PFIC3 (for a more extensive review, see [81]).

The *Abcb4*$^{-/-}$ mouse as a model for chronic liver diseases in humans

As described above, the *Abcb4*$^{-/-}$ mouse suffers from a cholestatic syndrome with increased hepatocyte turnover. It has been shown that in such conditions, the liver can be repopulated by transplanted hepatocytes, provided that these transplanted cells do not have the defect and, therefore, have a growth advantage over the defective cells. This principle has been demonstrated in several mouse and rat models with hepatic damage such as the *Fah*$^{-/-}$ mouse, which is an animal model for tyrosinemia type 1 and which is deficient for fumarylacetoacetate hydrolase, an enzyme in the catabolic pathway of tyrosine and an animal model for tyrosinemia type 1 (for review, see [82]). It was shown by de Vree et al. [83] that transplantation of healthy syngeneic hepatocytes into *Abcb4*$^{-/-}$ mice led to repopulation of the liver, especially if the liver damage was aggravated by feeding the animals a cholate-supplemented diet. These experiments demonstrated that mild liver damage is already sufficient to drive the repopulation with transplanted cells. This opens the possibility of correction of patients with several forms of PFIC by hepatocyte transplantation.

The *Abcb4*$^{-/-}$ mouse does not only suffer from increased hepatocyte turnover. As mentioned above, there is also portal inflammation and bile duct injury accompanied by fibrosis. The histological picture resembles that of primary sclerosing cholangitis in humans. Fickert et al. [84] performed an elaborate study on the pathology of *Abcb4*$^{-/-}$ mice and noted that bile duct epithelial cells are particularly affected by the toxic bile that is excreted by these animals. Disruption of the tight junctions and basement membranes of the small bile ducts leads to bile leakage and a subsequent inflammatory response.

Recently, Popov et al. [85] used the *Abcb4*$^{-/-}$ model to analyze the development of fibrosis in these animals. The authors could demonstrate a temporal change in expression of many genes involved in hepatic fibrogenesis and also fibrolysis, suggesting that a balance between the two is the main determinant in the extent of collagen disposition. Importantly, fibrosis in *Abcb4*$^{-/-}$ mice bears more resemblance to human biliary fibrosis than any other rat or mouse model, which involves the administration of chemicals.

Finally, the *Abcb4*$^{-/-}$ mouse has provided a means to elucidate the mechanism of formation of lipoprotein X (LpX). This aberrant lipoprotein is characteristically found in patients with various forms of cholestasis. In contrast to normal lipoproteins (which have a single outer lipid layer of phospholipid and cholesterol with a core filled with neutral lipid), it consists of a phospholipid/cholesterol bilayer with an aqueous lumen (liposome) [86]. It has been postulated in the past that the LpX particles, in fact, represent biliary lipid vesicles that regurgitate into blood when bile flow is compromised. In line with this contention, it was found that LpX is completely absent in cholestatic *Abcb4*$^{-/-}$ mice [87]. In addition, in mice with different expression levels of *Abcb4*, it was found that the plasma concentration of LpX during cholestasis (bile duct ligation) correlated exactly with the expression level of *Abcb4* [87]. These data strongly suggest that, indeed, LpX is generated by the canalicular excretion machinery of lipid transporters. How this lipoprotein travels from the canalicular lumen to the blood compartment during cholestasis remains to be determined, but this may involve transcytosis through the hepatocyte. In line with the observations in the *Abcb4*$^{-/-}$ mouse, plasma cholesterol levels are characteristically low in patients with different forms of PFIC as opposed to other forms of cholestasis [88, 89].

Conclusion

ABCB4 has proven to be a physiologically very important ABC transporter. Complete absence of this transporter leads to a very serious liver disease. In addition, more and more clinical phenotypes, which are caused by *ABCB4* mutations that cause reduced activity, are discovered. Hence, expression and activity of ABCB4 has to move within a rather narrow window. This is conceivable because on one hand, absence of this function leads to pathology, while too high activity would create a waste of energy; not only in terms of energy spent on transport but also in terms of loss of valuable phospholipid that will be degraded in the intestine. Although a lot of information has been gained on the physiological function of this ABC transporter, more information is needed on the molecular mechanism of this highly specialized and peculiar transport protein.

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