Review

EASL EUROPEAN ASSOCIATION FOR THE STUDY OF THE LIVER | JOURNAL OF HEPATOLOGY

Liver disease associated with canalicular transport defects: Current and future therapies

Janneke M. Stapelbroek[1,2], Karel J. van Erpecum[3], Leo W.J. Klomp[2], Roderick H.J. Houwen[1,*]

[1]*Department of Paediatric Gastroenterology, Wilhelmina Children's Hospital, University Medical Centre Utrecht, Post-Box 85090, 3508 AB, Utrecht, The Netherlands;* [2]*Department of Metabolic and Endocrine Diseases, University Medical Centre Utrecht, and Netherlands Metabolomics Centre, Utrecht, The Netherlands;* [3]*Department of Gastroenterology, University Medical Centre Utrecht, Utrecht, The Netherlands*

Bile formation at the canalicular membrane is a delicate process. This is illustrated by inherited liver diseases due to mutations in *ATP8B1*, *ABCB11*, *ABCB4*, *ABCC2* and *ABCG5/8*, all encoding hepatocanalicular transporters. Effective treatment of these canalicular transport defects is a clinical and scientific challenge that is still ongoing. Current evidence indicates that ursodeoxycholic acid (UDCA) can be effective in selected patients with PFIC3 (ABCB4 deficiency), while rifampicin reduces pruritus in patients with PFIC1 (ATP8B1 deficiency) and PFIC2 (ABCB11 deficiency), and might abort cholestatic episodes in BRIC (mild ATP8B1 or ABCB11 deficiency). Cholestyramine is essential in the treatment of sitosterolemia (ABCG5/8 deficiency). Most patients with PFIC1 and PFIC2 will benefit from partial biliary drainage. Nevertheless liver transplantation is needed in a substantial proportion of these patients, as it is in PFIC3 patients. New developments in the treatment of canalicular transport defects by using nuclear receptors as a target, enhancing the expression of the mutated transporter protein by employing chaperones, or by mutation specific therapy show substantial promise. This review will focus on the therapy that is currently available as well as on those developments that are likely to influence clinical practice in the near future.

Keywords: PFIC; Therapy; Canalicular transport; Hereditary cholestasis.
* Corresponding author. Address: Department of Paediatric Gastroenterology [KE.01.144.3], Wilhelmina Children's Hospital, University Medical Centre Utrecht, Post-Box 85090, 3508 AB, Utrecht, The Netherlands. Tel.: +31 88 75 55555; fax: +31 88 75 55342.
E-mail address: r.houwen@umcutrecht.nl (R.H.J. Houwen).
Abbreviations: ABC, adenosine triphosphate-binding-cassette; PC, phosphatidylcholine; PS, phosphatidylserine; PFIC, progressive familial intrahepatic cholestasis; BRIC, benign recurrent intrahepatic cholestasis; GGT, gamma-glutamyl transpeptidase; ICP, intrahepatic cholestasis of pregnancy; LPAC, low-phospholipid associated cholelithiasis syndrome; DJS, Dubin Johnson syndrome; UDCA, ursodeoxycholic acid; PXR, pregnane X receptor; MARS, extracorporal albumin dialysis; PBD, partial biliary diversion; IB, ileal bypass; PEBD, partial external biliary diversion; PIBD, partial internal biliary diversion; NBD, nasobiliary drainage; FXR, farnesoid X receptor; 6-ECDCA, 6-ethyl chenodeoxycholic acid; PPAR, peroxisome proliferator activator receptor; CAR, constitutive androstane receptor; CF, cystic fibrosis; 4-PBA, 4-phenylbutyrate acid; CFTR, cystic fibrosis transmembrane conductance regulator.

Introduction

The process of primary bile formation occurs at the canalicular membrane predominantly through the action of transporters belonging to the adenosine triphosphate-binding-cassette (ABC) family [1,2] (Fig. 1A). Secretion of bile salts, phosphatidylcholine (PC) and cholesterol is mediated by ABCB11 (BSEP), ABCB4 (MDR3) and ABCG5/8, respectively. Excretion of organic anions is mediated by other members of the ABC-family such as ABCC2 (MRP2). In addition, ATP8B1 (FIC1), a P4 P-type ATPase, is essential for a proper composition of the canalicular membrane, and thus for normal bile flow [3].

Bile formation at the canalicular membrane is a delicate process and any inaccuracy may have devastating consequences. This is illustrated by inherited liver diseases caused by mutations in any of the hepatocanalicular transporters described above. In this review, we give recommendations for the treatment of canalicular transport defects based on current evidence. In addition, this review focuses on the developments that are likely to influence clinical practice in the near future.

Canalicular transport defects in liver disease

Phospholipid-flippases ATP8B1 (FIC1)

ATP8B1 is thought to specifically translocate phosphatidylserine (PS) from the outer to the inner leaflet of plasma membranes, causing the outer leaflet to be enriched in PC, sphingomyelin and cholesterol [4–7]. Cholesterol has a high affinity for sphingomyelin, and both are thought to be preferentially located in laterally separated microdomains (previously called lipid rafts). These microdomains offer protection against the detergent action of bile salts in the canalicular lumen and are essential for normal function of transmembrane transporters [8–10]. Recent evidence indicates that canalicular ABC-transporters are indeed localized within these microdomains [11]; therefore, disruption of lipid asymmetry and reduction of cholesterol content in the apical membrane decreases the function of resident proteins such as the bile salt export pump ABCB11, resulting in cholestasis [9,10]. In addition, the canalicular membrane in both humans and mice with ATP8B1/Atp8b1 deficiency develops a decreased

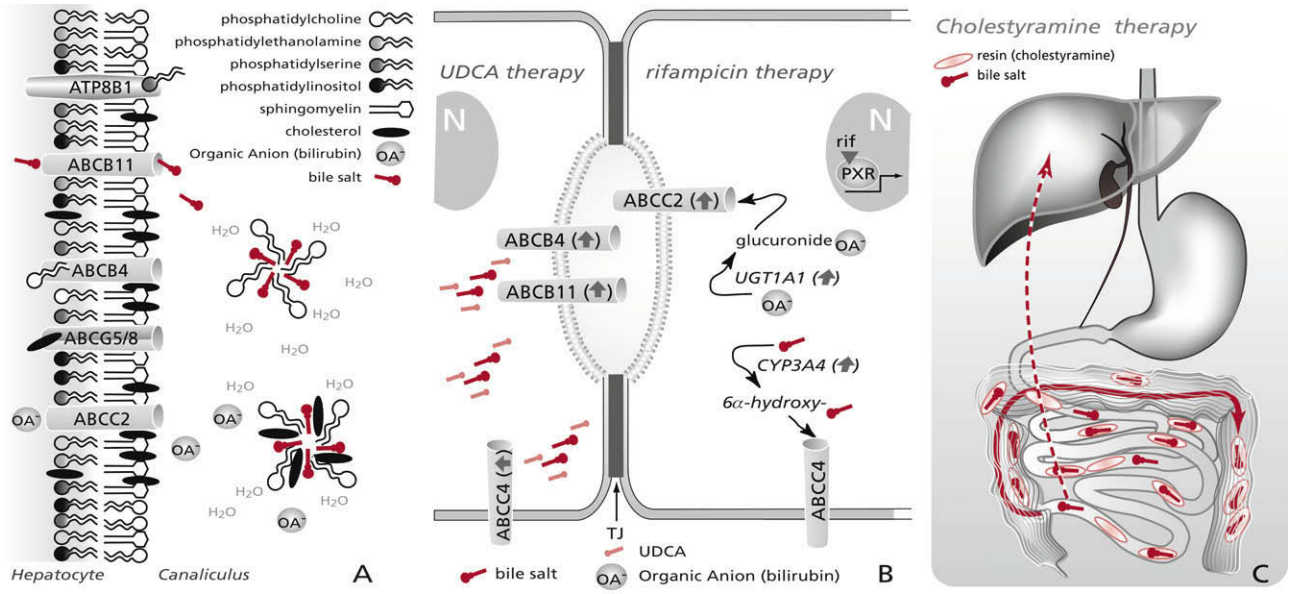

Fig. 1. Medical treatment of canalicular transport defects. (A) Schematic representation of bile formation at the canalicular membrane. ATP8B1 (FIC1) is essential for normal bile flow, probably through maintaining an asymmetric distribution of phospholipids between the inner and outer leaflet of the canalicular membrane. Secretion of bile salts into the canaliculus by the bile salt export pump ABCB11 (BSEP) is the main driving force for bile flow, with water following through osmotic forces. ABCB4 (MDR3) and ABCG5/8 induce secretion of phosphatidylcholine and cholesterol, respectively. These lipids form mixed micelles with the bile salts and protect membranes lining the biliary tract against detergent bile salts. ABCC2 (MRP2) mediates efflux of a broad range of organic anions. As indicated most ABC transporters are probably organized in microdomains enriched in sphingomyelin and cholesterol. (B) Left panel: the effect of UDCA in the hepatocyte. The hydrophilic ursodeoxycholic acid (UDCA) partly replaces the endogenous cytotoxic hydrophobic bile salts. In addition, by inducing expression of ABCB11, and ABCB4, UDCA stimulates hepatobiliary secretion of bile salts and protective phospholipids. The up-regulation of ABCC4 (MRP4) induces the efflux of conjugated bile acids across the basolateral membrane. Right panel: rifampicin (RIF) activates PXR regulated transcription of CYP3A4. This stimulates 6α-hydroxylation of bile salts, which can be excreted at the basolateral membrane via ABCC4 (MRP4), with subsequent excretion in the urine. In addition, the conjugation and excretion of bilirubin is enhanced through induction of UGT1A1 and ABCC2 (MRP2). (C) Cholestyramine binds bile salts in the intestinal lumen and interrupts the enterohepatic circulation of bile salts by reducing re-absorption and stimulating faecal excretion. N, nucleus; TJ, tight junction.

resistance to hydrophobic bile salts, evidenced by an enhanced biliary recovery of phospholipids, cholesterol and canalicular ectoenzymes in bile [9,12,13]. It is likely that this damage to the canalicular membrane adds to the cholestasis.

ATP8B1 deficiency (formerly FIC1 disease) is an autosomal recessive condition characterized by mutations in the *ATP8B1* gene [3,14,15]. Patients with ATP8B1 deficiency may present in infancy or early childhood with progressive familial intrahepatic cholestasis type 1 (PFIC1) [15–18] or later in life with episodes of cholestasis and intractable pruritus: benign recurrent intrahepatic cholestasis type 1 (BRIC1) [19–22]. PFIC1 and BRIC1 are in fact two ends of a clinical spectrum, as is illustrated by patients who initially present with episodic cholestasis but progress to permanent cholestasis in time [14,23,24]. During a cholestatic episode, all patients have low serum concentrations of gamma-glutamyl transpeptidase (GGT) in combination with high serum bile salt levels. Liver biopsies of PFIC1 patients show bland cholestasis with characteristic coarse and granular bile on the ultrastructural level [12,25]. ATP8B1 is localized on the canalicular membrane of the hepatocyte [6,26,27], but its expression is even more abundant in other tissues [3,28], where it can also be found at the apical membranes of polarized cells [26,27,29]. This is consistent with a proposed general cellular function of ATP8B1 and with extrahepatic features such as persistent short stature [16], diarrhoea [16,30,31], pancreatitis [22,32], sensorineural hearing loss [29,33] and an abnormal sweat composition [16,32], which are frequently present in patients with ATP8B1 deficiency. Heterozygous mutations in *ATP8B1* can be found in patients with intrahepatic cholestasis of pregnancy (ICP), a liver disorder that is characterized by pruritus and raised serum bile salt levels during pregnancy or use of oral contraceptives [34,35] (Table 1).

Bile salt transporter ABCB11 (BSEP)

Since bile flow is largely dependent on bile salt excretion, it is not surprising that a deficiency of ABCB11, the main bile salt transporter, can cause a severe autosomal recessive cholestatic syndrome that is hard to distinguish from ATP8B1 deficiency. Patients may present with progressive intrahepatic cholestasis in the first decade of life that rapidly leads to liver failure (PFIC2) [36,37]. However, ABCB11 deficiency also represents a phenotypic spectrum, with episodic cholestasis (BRIC2) as the mild manifestation [38–41]. Biochemically, serum concentrations of bile salts are markedly elevated, but GGT concentrations remain low [25,42]. Histological characteristics of the liver, with portal-tract fibrosis, bile duct proliferation and amorphous canalicular bile may distinguish ABCB11 from ATP8B deficiency [25]. In addition, ABCB11/Abcb11 localization is restricted to the canalicular membrane of hepatocytes [36,43,44] and no extrahepatic symptoms are described. In contrast, cholelithiasis is often observed, probably due to biliary bile salt concentrations that are too low to solubilise all biliary cholesterol [38,41]. Also, hepatocellular carcinoma or cholangiocarcinoma may be a complication of ABCB11 deficiency [45,46]. Heterozygous mutations in *ABCB11*

Table 1. Canalicular transporters and canalicular transport defects.

Canalicular transporter (synonym)	Canalicular transport defect (synonym)	Disease characteristics	Biochemical and histological characteristics	Disease associated with heterozygous canalicular transport defect
ATP8B1 (FIC1)	**ATP8B1 deficiency** (FIC1 disease, PFIC1, Byler disease and Greenland familial cholestasis, BRIC1, Tygstrup-Summerskill and Walshe cholestasis)	Spectrum of intrahepatic cholestasis comprising PFIC1 and BRIC1 **PFIC1**: progressive intrahepatic cholestasis, pruritus and in some patients extrahepatic symptoms **BRIC1**: episodic cholestasis, pruritus and in some patients extrahepatic symptoms. In between episodes no symptoms	High serum bile salts but low GGT concentrations. Liver biopsy: bland cholestasis with coarse and granular bile	ICP
ABCB11 (BSEP)	**ABCB11 deficiency** (PFIC2, BRIC2)	Spectrum of intrahepatic cholestasis comprising PFIC2 and BRIC2 **PFIC2**: progressive intrahepatic cholestasis, pruritus and in some patients cholelithiasis **BRIC2**: episodic cholestasis, pruritus and in some patients cholelithiasis. In between episodes no symptoms	High serum bile salts but low GGT concentrations. Liver biopsy: portal-tract fibrosis, bile duct proliferation and amorphous canalicular bile	ICP, drug induced cholestasis, transient neonatal cholestasis
ABCB4 (MDR3)	**ABCB4 deficiency** (PFIC3)	Progressive intrahepatic cholestasis, high serum GGT concentrations. Pruritus less prominent	High serum bile salts and high GGT concentrations. Liver biopsy: fibrosis and marked bile duct proliferation	ICP, drug induced cholestasis, transient neonatal cholestasis LPAC
ABCC2 (MRP2)	**Dubin Johnson syndrome**	Asymptomatic but in some patients gastrointestinal symptoms	High serum conjugated bilirubin concentrations. Liver biopsy: dark blue or black due to pigmentation	
ABCG5/8	**Sitosterolemia**	Xanthomas, arthralgias and premature coronary artery disease	High serum sitosterols with relatively low cholesterol concentration. Liver biopsy: unknown	

PFIC, progressive familial intrahepatic cholestasis; BRIC, benign recurrent intrahepatic cholestasis; GGT, gamma-glutamyl transpeptidase; ICP, intrahepatic cholestasis of pregnancy; LPAC, low-phospholipid associated cholelithiasis syndrome.

are described in drug induced cholestasis [47], ICP [48–50] and transient neonatal cholestasis [51] (Table 1).

Phosphatidylcholine transporter ABCB4 (MDR3)

ABCB4 is expressed at the apical membrane of the hepatocyte [44,52] and is essential for PC secretion into the bile [53,54]. A defective ABCB4 protein causes an imbalance in the composition of primary bile, with lack of PC and a surplus of bile salts, the latter damaging the canaliculus and small bile ducts, causing chronic and progressive liver disease [55]. Mutations in *ABCB4* are associated with progressive familial intrahepatic cholestasis type 3 (PFIC3) which, like the other PFIC types, inherits in an autosomal recessive pattern [55,56]. In contrast to patients with PFIC1 and PFIC2, serum GGT levels are elevated. Liver histology reveals fibrosis (progressing to cirrhosis) and marked bile duct proliferation [55]. There are no extrahepatic symptoms but heterozygous mutations can be encountered in unexplained cholestasis [57,58] and many milder cholestatic conditions, such as ICP [59,50,60–66], drug induced cholestasis [47], transient neonatal cholestasis [67], and isolated and recurrent intrahepatic cholesterol gallstones, designated as LPAC (low-phospholipid associated cholelithiasis syndrome) [68–71] (Table 1). The latter is characterized by gallstone disease at relatively young age (<40 yrs) persistent after cholecystectomy. The underlying mechanism is an insufficient concentration of PC in bile to form mixed micelles with cholesterol, resulting in cholesterol supersaturation and crystal formation. The wide clinical spectrum of ABCB4 deficiency is illustrated by a patient with a heterozygous mutation in *ABCB4* described by Lucena et al. This patient presented with juvenile cholelithiasis, recurrently manifested ICP and finally developed biliary cirrhosis [72].

Organic anion transporter ABCC2 (MRP2)

ABCC2 expression is found in the liver but also at the apical membranes of other polarized cells [44,73,74]. Its substrate specificity is broad and comprises organic anions, mainly conjugated compounds. ABCC2 has an important role in the excretion of bilirubin into bile and in the excretion of bile salts after their sulfation or glucuronidation [75,76]. Nevertheless, the mild hepatic phenotype and lack of extrahepatic symptoms in ABCC2 deficiency suggests that other transporters can complement its function. ABCC2 deficiency causes Dubin Johnson syndrome (DJS). This syndrome is an autosomal, recessively inherited disorder characterized by chronic or intermittent conjugated hyperbilirubinemia. Although some patients complain about gastrointestinal symptoms and drug metabolism might be different, there are no further symptoms. Plasma concentrations of liver enzymes are usually within the normal range, and there is no permanent liver damage. However, on macroscopic examination the liver itself appears dark blue or black due to pigmentation [77–79] (Table 1). So far, no associations between the heterozygous state and liver or other disease has been found [80].

Cholesterol transporter ABCG5/8

ABCG5/8 is expressed at the apical membrane of liver and intestine [81]. The protein-complex consists of two half transporters, ABCG5 and ABCG8, that heterodimerise in the endoplasmic reticulum to become functionally active [82,83]. The ABCG5/8 transporter has a major role in the biliary and intestinal excretion of cholesterol and plant sterols (mainly sitosterols) [84,85]. Mutations in either *ABCG5* or *ABCG8* cause a rare autosomal recessive disease, sitosterolaemia. This disease is characterized by an

increased retention of sitosterols by the intestine and a failure to secrete sterols into bile, resulting in high plasma sitosterol levels and accumulation of sterols in peripheral tissues and blood [84–87]. Patients consequently present with tendon xanthomas, arthralgias and premature coronary artery disease, despite relatively low plasma levels of cholesterol [88–90] (Table 1). Sporadically, haemolytic abnormalities are mentioned [91]. Except for one patient with chronic active hepatitis and signs of cirrhosis, nothing is known about liver histology [92]. The effect of being a heterozygous carrier for these mutations is not clear yet [93].

Treatment of canalicular transport defects

All liver diseases described above are due to mutations in genes encoding hepatocanalicular transporters. For most of these diseases the response to current medical therapy is either non-existent or of limited duration. Some agents have proven to be effective in specific situations, mainly by providing symptomatic relief.

Nevertheless, most patients with progressive cholestasis eventually need surgical intervention. Even patients with the relative "benign" phenotypes of intrahepatic cholestasis (BRIC) may undergo invasive therapy, purely to improve quality of life [16,22,24].

Current medical treatment

The therapeutic strategies for cholestasis due to canalicular transport defects may target bile composition, bile salt toxicity and the secretion of bile salts. The ideal therapy should have anti-cholestatic, anti-fibrotic and anti-neoplastic properties. Ursodeoxycholic acid (UDCA), rifampicin and cholestyramine are amongst the most commonly used. Sometimes combination therapy is employed, but there is no evidence for any synergistic effect.

UDCA

The main therapeutic target of UDCA is the protection of hepatocytes and cholangiocytes by replacing endogenous, cytotoxic bile salts [94,95]. In addition, UDCA induces expression of functional transporters at transcriptional and post-transcriptional level and enhances bile flow, possibly through cholehepatic shunting [96–101]. A simplified illustration of the effect of UDCA in the hepatocyte can be found in Fig. 1B.

More than half of the patients with high GGT-PFIC or proven PFIC3 responded to UDCA treatment [102–104] (Table 2A). Although in most reports this response was not further clarified we presume that it was characterized by at least partial improvement in serum transaminase levels and pruritus. Interestingly, those with missense mutations generally had a good response to UDCA therapy, while those with a premature stop codon showed no response [103]. Therefore in patients with PFIC3 and a presumed residual function of the ABCB4 protein based on mutational analysis, UDCA is the therapy of choice.

In patients with low GGT-PFIC or an undefined subtype of PFIC, the response to UDCA therapy was much less promising. Although in some reports serum transaminase levels and pruritus improved in about half of the patients upon UDCA [102,104,105], in most studies UDCA was not effective [106–113]. Even in patients with a mild phenotype (BRIC1, BRIC2), UDCA did not prevent or abort a cholestatic attack [19,24,31,39,40,114–116] (Table 2A). Currently it is not possible to predict who would benefit from UDCA in low GGT cholestasis and it is doubtful whether UDCA has a place in the treatment of ATP8B1 or ABCB11 deficiency. Especially for patients with progression to severe liver disease, surgical management is needed as soon as possible.

Table 2A. UDCA treatment in hepatocanalicular transport defects.

Hepatocanalicular defect	Number patients	Outcome (number patients)	Reference
PFIC undefined subtypes	58	Improvement (2) Partial improvement (0) No improvement (56)	[107,111]
Low GGT-PFIC	98	Improvement (22) Partial improvement (12) No improvement (64)	[102,104–106, 108–110,112,113]
High GGT-PFIC	46	Improvement (20) Partial improvement (14) No improvement (12)	[102–104]
BRIC	12	Improvement (0) Partial improvement (2) No improvement (10)	[19,24,31,39, 40,114–116]
DJS	2	Improvement (1) Partial improvement (0) No improvement (1)	[77,117]

"Improvement", indicates (almost complete) normalization of serum transaminases and/or bilirubin concentration and total relief of pruritus.
"Partial improvement", indicates no complete normalization of serum transaminases and/or bilirubin concentration with or without persistent pruritus.
"No improvement", indicates no response or deterioration of the symptoms.
PFIC, progressive familial intrahepatic cholestasis; BRIC, benign recurrent intrahepatic cholestasis; DJS, Dubin Johnson syndrome; GGT, gamma-glutamyl transpeptidase. In the high GGT-PFIC subtype, 11 patient are reported twice [102,103].

For DJS, two case-reports have been published with opposite effect. In one patient, serum bilirubin declined upon UDCA treatment, while in the other patient a combination of rifampicin with UDCA led to a dramatic rise in serum bilirubin and bile salt concentrations, which normalized once again after these medications were discontinued [77,117] (Table 2A). For sitosterolaemia no clinical trials or case-reports have been published.

UDCA treatment is safe, and except for the reversible effect in the DJS patient no serious side-effects have been described.

Recently it was found that shortening of the side chains increases the therapeutic efficacy of UDCA [100]. This modified, so called *nor*UDCA has already been proven to be more effective than the parent compound in a murine model of primary sclerosing cholangitis [118].

Rifampicin

The primary effect of rifampicin is inducing CYP3A4 expression through activation of the xenosensor pregnane X receptor (PXR). This increases 6α-hydroxylation of bile salts, compounds which can subsequently be glucuronidated and excreted in the urine [99,119]. In addition, conjugation and excretion of bilirubin is enhanced through induction of UGT1A1 and ABCC2 [99]. Enhanced expression of the latter might also be important for excretion of other, still unidentified pruritogenic compounds (Fig. 1B).

In patients with low GGT-PFIC, rifampicin did not have any long lasting effect on serum concentration of bilirubin and transaminases, but reduced the pruritus in some patients [108,109, 112,113]. This marginal effect contrasts with the results obtained in patients with BRIC. After starting rifampicin treatment in seven patients, eighteen out of twenty-two episodes were completely aborted within several weeks [24,115,116,120] (Table 2B). Thus

it seems that rifampicin can reduce pruritus in some patients with low GGT-PFIC, but might even induce remission in patients with BRIC. Nevertheless, rifampicin treatment should be used with caution because of its potential hepatotoxic effect.

The use of rifampicin in DJS has been described in one patient, but instead of reducing serum bilirubin concentrations, the conjugated bilirubinemia increased during treatment [77] (Table 2B). It is not known whether rifampicin affects cholesterol homeostasis as well, but so far no experience with sitosterolaemia has been published.

Cholestyramine

Cholestyramine is a negative ion exchange resin that binds bile salts in the intestinal lumen, reduces re-absorption and stimulates faecal excretion of bile salts (Fig. 1C) [121].

Cholestyramine does not seem to be effective in patients with low GGT-PFIC or undefined subtypes of PFIC [107,108,112,122,123]. For patients with BRIC the results are variable, varying from shortening of the cholestatic episodes [24,124,125] to no effect at all [19,24,115,116,126] (Table 2C). Consequently cholestyramine seems to have no place in the treatment of PFIC, but it may be beneficial in patients with BRIC. Given the potentially better tolerability and higher efficacy of the new bile salt resin binders with other polymer structures, such as colesevalam [127], these drugs should be the topic of further investigations.

There is no published evidence for cholestyramine treatment in high GGT cholestasis or DJS. However, extensive experiments are available for sitosterolaemia in which cholestyramine in combination with a diet low in cholesterol reduced the serum levels of plant sterols with improvement of clinical symptoms, such as reduction of xanthomas [88,93,128–132] (Table 2C). Chronic cholestyramine treatment may cause constipation, but no other serious complications have been found.

Invasive treatment

A few BRIC patients have been treated successfully with extracorporal albumin dialysis (MARS) [133,134]. However biliary diversion and liver transplantation are the most commonly used invasive treatments.

Table 2B. Rifampicin treatment in hepatocanalicular transport defects.

Hepatocanalicular defect	Number patients	Outcome (number patients)	Reference
Low GGT-PFIC	17	Improvement (0) Partial improvement (3) No improvement (14)	[108,109,112,113]
BRIC	7	Improvement (5) Partial improvement (0) No improvement (2)	[24,115,116,120]
DJS	1	Improvement (0) Partial improvement (0) No improvement (1)	[77]

"Improvement", indicates the almost complete normalization of serum transaminases and/or bilirubin concentration and total relief of pruritus. For patients with BRIC "improvement" indicates the abortion of a cholestatic attack. "Partial improvement" indicates lack of complete normalization of serum transaminase concentration but improvement of pruritus. "No improvement" indicates lack of response or deterioration of the symptoms.
PFIC, progressive familial intrahepatic cholestasis; BRIC, benign recurrent intrahepatic cholestasis; DJS, Dubin Johnson syndrome; GGT, gamma-glutamyl transpeptidase.

Table 2C. Cholestyramine treatment in hepatocanalicular transport defects.

Hepatocanalicular defect	Number patients	Outcome (number patients)	Reference
PFIC undefined subtypes	23	Improvement (0) Partial improvement (1) No improvement (22)	[107,122,123]
Low GGT-PFIC	34	Improvement (0) Partial improvement (0) No improvement (34)	[108,112]
BRIC	20	Improvement (2) Partial improvement (2) No improvement (16)	[19,24,115,116,124–126]
Sitosterolaemia	13	Improvement (12) Partial improvement (1) No improvement (0)	[88,93,128–132,210]

"Improvement" indicates an almost complete normalization of serum transaminases, bilirubin and/or sterol concentration and total relief of pruritus. In BRIC "improvement" indicates the abortion of a cholestatic attack. "Partial improvement" indicates a lack of complete normalization of serum transaminases, bilirubin and/or sterol concentration with or without persistent pruritus. In patients with BRIC, "partial improvement" means reduction of pruritus. "No improvement" indicates a lack of response or deterioration of the symptoms.
PFIC, progressive familial intrahepatic cholestasis; BRIC, benign recurrent intrahepatic cholestasis; GGT, gamma-glutamyl transpeptidase.

Biliary diversion

Non-transplant surgical intervention can be effective in patients with intrahepatic cholestasis. Partial biliary diversion (PBD) or ileal bypass (IB) are two of these surgical interventions, in which PBD may be achieved by either a jejunal conduit from gallbladder to the abdominal wall (partial external biliary diversion; PEBD) [123], or one that connects the gallbladder to the colon (partial internal biliary diversion; PIBD) [109]. Leaving the common bile duct intact, PBD establish only a partial diversion of bile (about 80%) while the remainder enters the duodenum. In IB, bile salt re-absorption is diminished by bypassing the small intestine at the terminal ileum through an ileocolonic anastomosis (Fig. 2) [135]. Although to a much stronger extent, the working mechanism of PBD is similar to cholestyramine – it reduces the accumulation of toxic bile salts by a reduction of the enterohepatic circulation.

PEBD was initially described by Withington and Withington in 1988 [123]. This innovative technique was quickly adopted by other centres worldwide and so far sixteen additional case-reports/series addressing the effect and technique of PEBD in the treatment of PFIC have been published (Table 3). Except for one small series of five patients in which PEBD did not have any effect [104], all others report normalization or improvement of liver function in 75–100% of the patients with low GGT-PFIC, indicated by at least improved liver tests and reduced pruritus [106,108,110,112,136–143]. The response in patients with an undefined subtype of PFIC seems to be less [111,144]. Liver biopsies post PEBD were performed in some patients and did not show further progression or even a resolution of hepatic morphologic abnormalities in all these patients [112,123,137,141]. Advanced disease and liver cirrhosis were proposed as the main causes of therapeutic failures, indicating that early surgical intervention in PFIC patients is essential. Three BRIC1 patients were treated with PEBD to improve quality of life rather than to prevent disease progression. In these patients PEBD aborted the cholestatic attack immediately but did not always prevent subsequent minor cholestatic episodes [24,116].

Another surgical technique for biliary drainage is IB, first described by Whitington et al. [108] in patients who were not

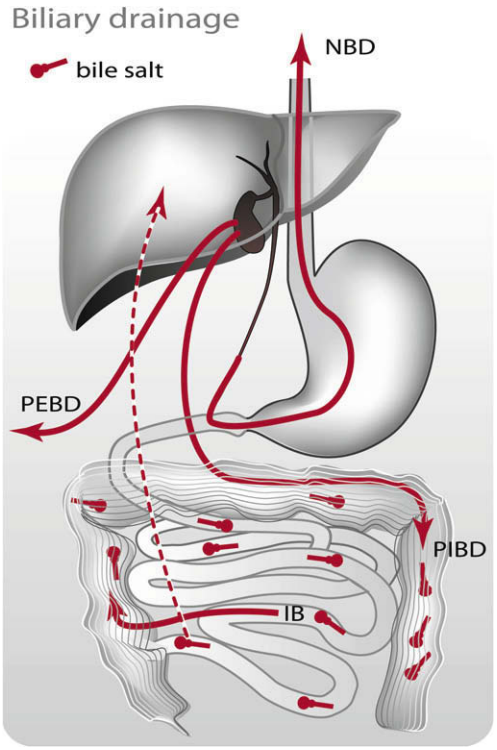

Fig. 2. Biliary drainage. Partial biliary diversion (PBD), or ileal bypass (IB), are two of non-transplant surgical interventions that interrupt the enterohepatic circulation of bile salts and can be effective in the treatment of canalicular transport defects. PBD may be achieved by either a jejunal conduit from gallbladder to the abdominal wall: partial external biliary diversion; PEBD, or one that connects the gallbladder to the colon: partial internal biliary diversion; PIBD. In IB, bile salt re-absorption is diminished by bypassing the terminal ileum through an ileocolonic anastomosis. A cholestatic attack in patients with BRIC may be aborted by endoscopically introducing a nasobiliary drain during a cholestatic episode (NBD).

Table 3. Partial biliary drainage in hepatocanalicular transport defects.

Hepatocanalicular defect	Number treated	Outcome (number patients)	Reference
PFIC undefined subtypes **Treatment by PEBD**	42	Improvement (24) Partial improvement (5) No improvement (13)	[111,123,144]
Low GGT-PFIC **Treatment by PEBD**	94	Improvement (66) Partial improvement (11) No improvement (17)	[104,106,108, 110,112,136–143]
High GGT-PFIC **Treatment by PEBD**	1	Improvement (0) Partial improvement (0) No improvement (1)	[104]
BRIC **Treatment by PEBD**	3	Improvement (1) Partial improvement (2) No improvement (0)	[24,116]
PFIC undefined subtypes **Treatment by IB**	5	Improvement (1) Partial improvement (0) No improvement (4)	[144]
Low GGT-PFIC **Treatment by IB**	7	Improvement (6) Partial improvement (0) No improvement (1)	[108,135]
Low GGT-PFIC **Treatment by PIBD**	2	Improvement (2) Partial improvement (0) No improvement (0)	[109]
BRIC **Treatment by PIBD**	1	Improvement (1) Partial improvement (0) No improvement (0)	[109]

"Improvement" indicates an almost complete normalization of serum transaminases and/or bilirubin concentration and total relief of pruritus. "Partial improvement" indicates a lack of complete normalization of serum transaminases and/or bilirubin concentration and/or persistent pruritus. "No improvement" indicates a lack of response or deterioration of the symptoms.
PFIC, progressive familial intrahepatic cholestasis; BRIC, benign recurrent intrahepatic cholestasis; GGT, gamma-glutamyl transpeptidase; PEBD, partial external biliary diversion; PIBD, partial internal biliary diversion; IB, ileal bypass.
In the low GGT-PFIC subtype, four patient are reported double [108,123].

amenable for PEBD because of a previous cholecystectomy. An additional advantage of this procedure is the lack of an external fistula. However, after a short initial response, clinical symptoms recurred in half of the treated patients with low GGT- or an undefined subtype of PFIC within a year [135,144] (Table 3). This is probably due to secondary adaptation of the ileum to the resection and it was therefore concluded that IB is inferior to PEBD in patients with low GGT cholestasis.

Recently, PIBD has been described in two teenage patients with PFIC and low GGT cholestasis. This technique combines the advantages of external drainage and ileal bypassing by partially interrupting enterohepatic circulation without an external biliary fistula. The initial clinical and laboratory results were very promising, but long-term follow-up is necessary to evaluate late results and complications [109] (Table 3).

In the few BRIC patients treated with PEBD, drainage immediately reduced pruritus and relieved cholestasis. Moreover, in the follow-up period just a few very short additional episodes were noticed [24,116] (Table 3). However the permanent character of PEBD makes it less appropriate to be used in a disorder that is only episodic. Based on these results we developed a temporary intervention: nasobiliary drainage (NBD) for which the first results were published in 2006 [145]. Until now, in our centre a total of twelve cholestatic attacks in five BRIC1 patients were treated by NBD. This was established by endoscopically introducing a nasobiliary drain during a cholestatic episode (Fig. 2). In eight out of twelve treatments, pruritus totally resolved within 48 h and serum bile salt levels returned to normal or near normal levels. Failure of NBD was either due to practical difficulties when introducing the drain (one treatment) or progression of liver disease (three treatments in two patients who are now doing well after PEBD). Thus, for most cholestatic episodes in BRIC1, NBD is an effective therapy. Because in some patients there is a transition from episodic to progressive cholestasis, PEBD should be considered when NBD fails to resolve a cholestatic episode.

Given the current evidence, PBD is the therapy of choice in patients with low GGT-PFIC, and should be performed as soon as possible after diagnosis to prevent liver damage. At present, large multicenter studies are in progress that investigate PBD results stratified for patients with ATP8B1 vs ABCB11 deficiency and in subpopulations with different mutations. It is to be expected that the current recommendations can be refined upon publication of these results. It is also important to realize that PBD induces the loss of considerable amounts of fluids and electrolytes, and patients might become dehydrated. Adequate and individualized electrolyte supplementations and fluid is mandatory in all patients with PBD. Finally complications from intestinal surgery as stoma prolaps and intestinal obstruction have been described.

There is no evidence of the effect of non-transplant surgery in patients with other canalicular transport defects, except for one

patient with ABCB4 deficiency who was unsuccessfully treated with PEBD [104].

Liver transplantation

Because of the high risk of complications and the life-long need for immune-suppressive therapy, liver transplantation should be reserved for patients who have established liver cirrhosis at the time of presentation or who have progressive liver disease despite treatment. Unfortunately, for many patients with the severe form of ATP8B1, ABCB11 and ABCB4 deficiency, liver transplantation is still the only option.

Independent of the subtype of PFIC, the survival rate after transplantation ranges from 61–92% in the 80ths and early 90ths [108,137,146] to 75–100% more recently [31,104,106,111,147–154]. However, most follow-up periods do not yet exceed 3 years. Transplantation improved cholestasis-related symptoms like itching, malnutrition and liver function in almost all surviving patients. Due to the lack of cadaver donors, living-related liver transplantation is often used in patients with PFIC. Although it was feared that the heterozygous status of the parent donor would affect the results unfavourably, complications and survival rates of these types of transplantation in PFIC patients are similar to living-related transplantations for non-genetic liver diseases such as biliary atresia [149,151]. However, in some PFIC patients, symptoms of cholestasis may recur after several years. Apart from the usual long-term complications after liver transplantation, such as chronic rejection and conduit stricture, in PFIC this may also be due to allo-immunization of the recipient against the ATP8B1, ABCB11 or ABCB4 protein located in the (heterozygous) donor liver [155].

The ATP8B1 protein is abundantly expressed outside the liver, e.g. in the intestine [3,27] and correction of the liver defect by transplantation will not cure the extrahepatic symptoms, such as diarrhoea. Indeed, in a substantial proportion of PFIC1 patients, diarrhoea exacerbates when biliary bile salt secretion is restored after liver transplantation. In these patients, liver biopsies revealed severe steatosis [31,147,148,150,154]. In one case, by constructing a total biliary diversion after transplantation, all bile salts were diverted from the intestine and diarrhoea resolved [153].

The ABCG5/8 half transporters are also expressed in both intestine and liver but, in contrast to ATP8B1 deficiency, liver transplantation was completely effective in normalization of sterol plasma concentration in a patient with sitosterolaemia, suggesting that adequate excretion of sterols by the liver is sufficient for keeping levels of xenosterols low, even when absorption in the intestine remains increased [92].

Recently, transplantation of human hepatocytes has been used for treatment of liver-based metabolic conditions. The injected hepatocytes would in theory have a selective growth advantage over the patient's own defective hepatocytes and should (partly) repopulate the native liver, as has already been shown in a mouse model for PFIC [156]. Unfortunately, for the two PFIC2 patients treated so far there was no clear benefit, probably due to pre-existing fibrosis that precluded proper engraftment of hepatocytes into the liver [157,158].

Future treatment options

As previously described, mutations in the canalicular transporters cause defective bile formation and retention of substances which are normally secreted into the bile. This may be due to a decreased transporter expression at the plasma membrane, due to a functional defect of the protein itself or a combination of both possibilities. It follows that ameliorating the expression of functional protein at the canalicular membrane could theoretically restore bile flow and improve liver disease in some patients.

Nuclear receptors as therapeutic target

Bile formation is highly regulated by nuclear receptors such as the bile salt sensor farnesoid X receptor (FXR). FXR is activated by naturally occurring (chenodeoxycholic acid) or synthetic (GW4064 and 6-ethyl chenodeoxycholic acid (6-ECDCA)) ligands. Upon activation it transactivates a number of genes that co-ordinately reduce hepatic bile salt uptake and *neo*synthesis and stimulate bile salt detoxification and secretion of both bile salts and the protective phospholipids [159–162] (Fig. 3). Synthetic ligands for FXR and other nuclear receptors are increasingly recognized as possible therapeutic options in cholestatic syndromes [163]. The use of FXR as a target for drug-therapy has already been studied in some detail in several rat models for cholestasis. For example, treatment with potent synthetic FXR ligands protected rats against oestrogen-induced cholestasis [164]. The latter condition has been linked to reduced activity and/or diminished expression of several transporters at the canalicular membrane, including Abcb11 and Abcc2 [165,166]. This new class of drugs is currently investigated in patients with primary biliary cirrhosis [163]. We propose that this treatment would also positively affect the cholestasis associated with the canalicular transport defects reviewed in this article.

ATP8B1 is not a known target gene for any of the nuclear receptors but its activity was described to influence FXR function. Although controversial [7,167,168], ATP8B1 deficiency might directly or indirectly reduce FXR expression and activity in liver and intestine [169–173]. The resulting down-regulation of *ABCB11* would be an explanation for the cholestatic phenotype in ATP8B1 deficient patients. Hence, if synthetic FXR ligands could counteract the FXR down-regulation this would induce *BSEP* expression and improve canalicular transport of bile salts. However, until now this concept has only been tested in cell culture [173].

In addition to FXR ligands, ligands of other nuclear receptors have been proven to affect the expression of canalicular transporters as well. As the classic ligands for peroxisome proliferator activator receptor alpha (PPARα), fibrates directly increase the expression of Abcb4/ABCB4 at the canalicular membrane and induce PC secretion [174,175]. For fibrates, clinical trials in cholestatic diseases such as primary sclerosing cholangitis, primary biliary cirrhosis and chronic hepatitis C have been started, and initial results are promising [176–179]. The nuclear constitutive androstane receptor (Car) and Pxr share the same response element in the rat *Abcc2* promotor with Fxr. Ligands for Car and PXR, such as phenobarbital and rifampicin, induce *Abcc2/ABCC2* expression [99,161]. These nuclear receptor ligands are already used to treat liver disease due to canalicular transport defects with variable results as discussed above for rifampicin.

Mutation specific therapy

Inserting a non-mutated gene or correcting a mutation at the DNA level was long considered to be the Holy Grail in the treatment of genetic diseases. Unfortunately, safe and effective gene therapy turned out to be much harder to accomplish than expected. Some of the early trials in humans resulted in mortality and enthusiasm for this approach decreased [180]. However, recent advances in our understanding of transcription, transla-

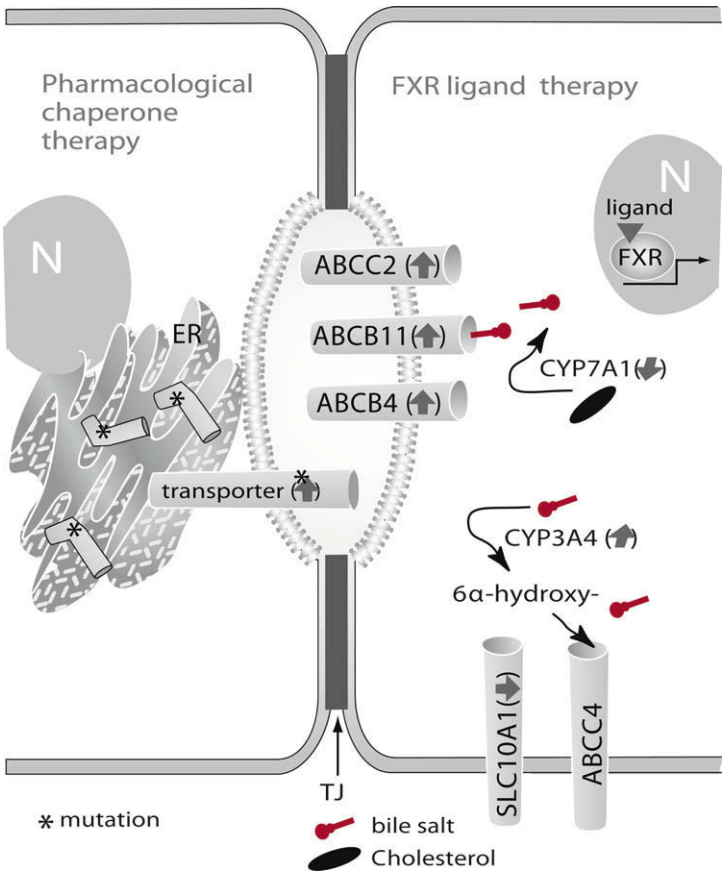

Fig. 3. Future treatment of canalicular transport defects. Left panel: many missense mutations influence protein processing, causing the abnormal but potentially functional protein to be misfolded, trapped in the ER and subsequently degraded. Pharmacological chaperones (such as 4-phenylbutyrate acid (4-PBA)) are small molecular weight compounds that help stabilize these abnormal proteins and enhance the expression of transporters at the canalicular membrane. Right panel: artificial ligands (such as GW4064) activate FXR. FXR transactivates a number of genes that together coordinate bile salt homeostasis. Reduced transcription of the sodium/bile acid co-transporter SLC10A1 (NTCP) decreases the bile salt uptake at the basolateral membrane. *Neo*synthesis of bile salts is reduced by inhibition of the transcription of the rate limiting enzymes (for example CYP7A1) in the conversion of cholesterol to bile salts. CYP3A4 is induced, enhancing 6α-hydroxylation of bile salts and subsequent excretion through ABCC4. Finally, FXR activation causes increased secretion of bile salts (through induced ABCB11 (BSEP) expression), phospholipids (through induced ABCB4 (MDR3) expression) and bilirubin (through induced ABCC2 (MRP2) expression). N, nucleus; ER, endoplasmic reticulum; TJ, tight junction.

tion and the subsequent processing of proteins have opened the possibility to obtain functional protein at the right place, even when mutations are still present in the DNA. This exciting new development has recently moved from bench to bedside in some early phase I and II trials, mainly in cystic fibrosis (CF). This approach should be applicable to other genetic diseases, including canalicular transport defects.

It has been known for many years that aminoglycosides, in addition to their antimicrobial activity, can suppress premature termination codons. [181,182]. Results from clinical trials with gentamicin in CF patients with premature stop codons due to nonsense mutations were promising [183,184]. However, the potential toxicities and its insufficient oral absorption precluded wide spread use of this antibiotic. As it was already clear that premature stop codons could be suppressed, a large number of compounds were screened to find such a drug. The first of this new group of drugs to become available was PTC124 [185]. After supportive results in a phase-I study in healthy volunteers [186], a phase-II study was recently started in CF patients, which shows normalization of chloride transport in about half of all patients [187]. We expect that PTC124 can also ameliorate the phenotype in patients with hepatocanalicular transport defects. This treatment would however be restricted to a subpopulation of patients in whom the disease is caused by specific premature stopcodons (e.g. UGA).

When missense mutations are present at an ATP binding site, or in a functional domain, the resulting protein is generally dysfunctional. However, many missense mutations influence protein processing, causing the abnormal but potentially functional protein to be misfolded, trapped in the endoplasmic reticulum and subsequently degraded. Pharmacological chaperones are small molecular weight compounds that help stabilize these abnormal proteins (Fig. 3) [188]. 4-Phenylbutyrate acid (4-PBA) is such a pharmacological chaperone which is already approved by the US Food and Drug Administration for use in urea-cycle disorders, where it acts as an ammonia scavenger [189,190]. The working mechanism probably involves interfering of 4-PBA with degradation and maturation of the mutated proteins, by modulation of the heat shock protein expression [191–193] (Fig. 3). The application of 4-PBA to protein-misfolding diseases was first studied for the deltaF508 mutation in the cystic fibrosis transmembrane conductance regulator (*CFTR)* gene. *In vitro* treatment of nasal- and bronchial epithelial cell lines resulted in an increased expression of mature CFTR at the plasma membrane and restoration of chloride secretion [194]. Clinical trials

Table 4. Recommendations for treatment of hepatocanalicular transport defects.

Hepatocanalicular defect	Recommendation for treatment
PFIC1–2 (low GGT-cholestasis)	PEBD should be performed directly after diagnosis. Consider liver transplantation when this treatment fails
PFIC3 (high GGT-cholestasis)	Start UDCA treatment as soon as possible. Consider liver transplantation when this treatment fails
BRIC 1–2	Start with rifampicin early at the beginning of an episode (cave hepatotoxicity). For some patients the addition of cholestyramine may be favourable. When no improvement in serum bile salts levels and pruritus is seen within 4–8 weeks perform NBD. If both medical therapy and NBD are not effective, consider PEBD
DJS	No specific therapy. Drug metabolism might be different in DJS
Sitosterolemia	Low-sterol-diet in combination with cholestyramine

PFIC, progressive familial intrahepatic cholestasis; BRIC, benign recurrent intrahepatic cholestasis; DJS, Dubin Johnson syndrome; GGT, gamma-glutamyl transpeptidase; PEBD, partial biliary drainage; UDCA, ursodeoxycholic acid; NBD, nasobiliary drainage.

with 4-PBA (Buphenyl) in patients with a homozygous deltaF508 mutation in the *CFTR* gene show improvement of chloride transport in the nasal epithelia [195,196]. Several mutations in *ATP8B1* [197], *ABCB11* [198] and *ABCB4* [199] have been shown to influence proper protein folding *in vitro*. These findings indicate that strategies to stabilize the mutant protein at the canalicular membrane by 4-PBA or other pharmacological chaperones may be therapeutic in patients with hepatocanalicular transport defects as well. 4-PBA has been tested *in vitro* for the E297G and D482G mutations frequently found in ABCB11 deficiency. Treatment reduced the protein ubiquitination and increased the cell surface expression of mature ABCB11 [200–202]. Plasma membrane expression of mutated ATP8B1 could also be induced by 4-PBA [197].

Mutation specific therapy of splice-site mutations can be divided into two groups. Some splice-site mutations generate both aberrantly and correctly spliced transcripts; if the latter are present the resulting disease is generally not severe [203,204]. The variability of splicing patterns is regulated through the interaction of a complex repertoire of splicing factors [205] which implies molecular targets for mutation specific therapy. Indeed, compounds like sodium butyrate can enhance the expression of the full-length transcripts in the presence of splice-site mutations [206]. Next, *in vivo* studies should confirm the benefit of splicing factor inducers in monogenetic diseases. The other group of splicing mutations completely abolish exon recognition. Although some early *in vitro* work shows that this category of mutations might also be amenable to mutation specific treatment, many issues have still to be solved [207–209].

Conclusion

The hepatocanalicular transport defects underscore the essential role of these transporters in bile formation. At present, surgery (PBD or liver transplantation) is the only effective therapy in most patients. Although medical treatment seems to be effective in some patients this is not true for others. Based on current evidence [211] and our own experience we propose different treatment options for the specific canalicular transport defects as depicted in Table 4. It should be clear that a universally effective and non-invasive treatment for patients afflicted by canalicular transport defects still has to be developed. However, with the scientific progress in the field of nuclear receptor ligands and mutation specific therapy, this might be accomplished within the next decade.

Acknowledgement

The authors who have taken part in his study declared that they do not have anything to declare regarding funding from industry or conflict of interest with respect to this manuscript.

References

[1] Oldham ML, Davidson AL, Chen J. Structural insights into ABC transporter mechanism. Curr Opin Struct Biol 2008;18:726–733.
[2] Borst P, Elferink RO. Mammalian ABC transporters in health and disease. Annu Rev Biochem 2002;71:537–592.
[3] Bull LN, van Eijk MJ, Pawlikowska L, DeYoung JA, Juijn JA, Liao M, et al. A gene encoding a P-type ATPase mutated in two forms of hereditary cholestasis. Nat Genet 1998;18:219–224.
[4] Paulusma CC, Folmer DE, Ho-Mok KS, de Waart DR, Hilarius PM, Verhoeven AJ, et al. ATP8B1 requires an accessory protein for endoplasmic reticulum exit and plasma membrane lipid flippase activity. Hepatology 2008;47:268–278.
[5] Pomorski T, Lombardi R, Riezman H, Devaux PF, van MG, Holthuis JC. Drs2p-related P-type ATPases Dnf1p and Dnf2p are required for phospholipid translocation across the yeast plasma membrane and serve a role in endocytosis. Mol Biol Cell 2003;14:1240–1254.
[6] Ujhazy P, Ortiz D, Misra S, Li S, Moseley J, Jones H, et al. Familial intrahepatic cholestasis 1: studies of localization and function. Hepatology 2001;34:768–775.
[7] Cai SY, Gautam S, Nguyen T, Soroka CJ, Rahner C, Boyer JL. ATP8B1 deficiency disrupts the bile canalicular membrane bilayer structure in hepatocytes, but FXR expression and activity are maintained. Gastroenterology 2009;136:1060–1069.
[8] Amigo L, Mendoza H, Zanlungo S, Miquel JF, Rigotti A, Gonzalez S, et al. Enrichment of canalicular membrane with cholesterol and sphingomyelin prevents bile salt-induced hepatic damage. J Lipid Res 1999;40:533–542.
[9] Paulusma CC, Groen A, Kunne C, Ho-Mok KS, Spijkerboer AL, Rudi de WD, et al. Atp8b1 deficiency in mice reduces resistance of the canalicular membrane to hydrophobic bile salts and impairs bile salt transport. Hepatology 2006;44:195–204.
[10] Paulusma CC, Dewaart DR, Kunne C, Mok KS, Oude Elferink RP. Activity of the bile salt export pump (ABCB11) is critically dependent on canalicular membrane cholesterol content. J Biol Chem 2009.
[11] Ismair MG, Hausler S, Stuermer CA, Guyot C, Meier PJ, Roth J, et al. ABC-transporters are localized in caveolin-1-positive and reggie-1-negative and reggie-2-negative microdomains of the canalicular membrane in rat hepatocytes. Hepatology 2009;49:1673–1682.
[12] Bull LN, Carlton VE, Stricker NL, Baharloo S, DeYoung JA, Freimer NB, et al. Genetic and morphological findings in progressive familial intrahepatic cholestasis (Byler disease [PFIC-1] and Byler syndrome): evidence for heterogeneity. Hepatology 1997;26:155–164.
[13] Groen A, Kunne C, Jongsma G, van den OK, Mok KS, Petruzzelli M, et al. Abcg5/8 independent biliary cholesterol excretion in Atp8b1-deficient mice. Gastroenterology 2008;134:2091–2100.

[14] Klomp LW, Vargas JC, van Mil SW, Pawlikowska L, Strautnieks SS, van Eijk MJ, et al. Characterization of mutations in ATP8B1 associated with hereditary cholestasis. Hepatology 2004;40:27–38.
[15] Klomp LW, Bull LN, Knisely AS, van Der Doelen MA, Juijn JA, Berger R, et al. A missense mutation in FIC1 is associated with Greenland familial cholestasis. Hepatology 2000;32:1337–1341.
[16] Bourke B, Goggin N, Walsh D, Kennedy S, Setchell KD, Drumm B. Byler-like familial cholestasis in an extended kindred. Arch Dis Child 1996;75:223–227.
[17] Clayton RJ, Iber FL, Ruebner BH, McKusick VA. Byler disease. Fatal familial intrahepatic cholestasis in an Amish kindred. Am J Dis Child 1969;117: 112–124.
[18] Nielsen IM, Ornvold K, Jacobsen BB, Ranek L. Fatal familial cholestatic syndrome in Greenland Eskimo children. Acta Paediatr Scand 1986;75:1010–1016.
[19] Brenard R, Geubel AP, Benhamou JP. Benign recurrent intrahepatic cholestasis. A report of 26 cases. J Clin Gastroenterol 1989;11:546–551.
[20] De Koning TJ, Sandkuijl LA, De Schryver JE, Hennekam EA, Beemer FA, Houwen RH. Autosomal-recessive inheritance of benign recurrent intrahepatic cholestasis. Am J Med Genet 1995;57:479–482.
[21] Summerskill WH, Walshe JM. Benign recurrent intrahepatic "obstructive" jaundice. Lancet 1959;2:686–690.
[22] Tygstrup N, Steig BA, Juijn JA, Bull LN, Houwen RH. Recurrent familial intrahepatic cholestasis in the Faeroe Islands. Phenotypic heterogeneity but genetic homogeneity. Hepatology 1999;29:506–508.
[23] van Mil SW, Klomp LW, Bull LN, Houwen RH. FIC1 disease: a spectrum of intrahepatic cholestatic disorders. Semin Liver Dis 2001;21:535–544.
[24] van Ooteghem NA, Klomp LW, van Berge-Henegouwen GP, Houwen RH. Benign recurrent intrahepatic cholestasis progressing to progressive familial intrahepatic cholestasis: low GGT cholestasis is a clinical continuum. J Hepatol 2002;36:439–443.
[25] Knisely AS. Progressive familial intrahepatic cholestasis: a personal perspective. Pediatr Dev Pathol 2000;3:113–125.
[26] Eppens EF, van Mil SW, De Vree JM, Mok KS, Juijn JA, Oude Elferink RP, et al. FIC1, the protein affected in two forms of hereditary cholestasis, is localized in the cholangiocyte and the canalicular membrane of the hepatocyte. J Hepatol 2001;35:436–443.
[27] van Mil SW, van Oort MM, van dB I, Berger R, Houwen RH, Klomp LW. Fic1 is expressed at apical membranes of different epithelial cells in the digestive tract and is induced in the small intestine during postnatal development of mice. Pediatr Res 2004;56:981–987.
[28] Harris MJ, Arias IM. FIC1, a P-type ATPase linked to cholestatic liver disease, has homologues (ATP8B2 and ATP8B3) expressed throughout the body. Biochim Biophys Acta 2003;1633:127–131.
[29] Stapelbroek JM, Peters TA, van Beurden DH, Curfs JH, Joosten A, Beynon AJ, et al. ATP8B1 is essential for maintaining normal hearing. Proc Natl Acad Sci USA 2009.
[30] Chen HL, Chang PS, Hsu HC, Ni YH, Hsu HY, Lee JH, et al. FIC1 and BSEP defects in Taiwanese patients with chronic intrahepatic cholestasis with low gamma-glutamyltranspeptidase levels. J Pediatr 2002;140:119–124.
[31] Lykavieris P, van MS, Cresteil D, Fabre M, Hadchouel M, Klomp L, et al. Progressive familial intrahepatic cholestasis type 1 and extrahepatic features: no catch-up of stature growth, exacerbation of diarrhea, and appearance of liver steatosis after liver transplantation. J Hepatol 2003;39:447–452.
[32] Knisely AS, Agostini RM, Zitelli BJ, Kocoshis SA, Boyle JT. Byler's syndrome. Arch Dis Child 1997;77:276–277.
[33] Oshima T, Ikeda K, Takasaka T. Sensorineural hearing loss associated with Byler disease. Tohoku J Exp Med 1999;187:83–88.
[34] Mullenbach R, Bennett A, Tetlow N, Patel N, Hamilton G, Cheng F, et al. ATP8B1 mutations in British cases with intrahepatic cholestasis of pregnancy. Gut 2005;54:829–834.
[35] Painter JN, Savander M, Ropponen A, Nupponen N, Riikonen S, Ylikorkala O, et al. Sequence variation in the ATP8B1 gene and intrahepatic cholestasis of pregnancy. Eur J Hum Genet 2005;13:435–439.
[36] Strautnieks SS, Bull LN, Knisely AS, Kocoshis SA, Dahl N, Arnell H, et al. A gene encoding a liver-specific ABC transporter is mutated in progressive familial intrahepatic cholestasis. Nat Genet 1998;20:233–238.
[37] Strautnieks SS, Byrne JA, Pawlikowska L, Cebecauerova D, Rayner A, Dutton L, et al. Severe bile salt export pump deficiency: 82 different ABCB11 mutations in 109 families. Gastroenterology 2008;134:1203–1214.
[38] Jansen PL, Strautnieks SS, Jacquemin E, Hadchouel M, Sokal EM, Hooiveld GJ, et al. Hepatocanalicular bile salt export pump deficiency in patients with progressive familial intrahepatic cholestasis. Gastroenterology 1999;117:1370–1379.
[39] Lam CW, Cheung KM, Tsui MS, Yan MS, Lee CY, Tong SF. A patient with novel ABCB11 gene mutations with phenotypic transition between BRIC2 and PFIC2. J Hepatol 2006;44:240–242.
[40] Takahashi A, Hasegawa M, Sumazaki R, Suzuki M, Toki F, Suehiro T, et al. Gradual improvement of liver function after administration of ursodeoxycholic acid in an infant with a novel ABCB11 gene mutation with phenotypic continuum between BRIC2 and PFIC2. Eur J Gastroenterol Hepatol 2007;19:942–946.
[41] van Mil SW, van der Woerd WL, van der BG, Sturm E, Jansen PL, Bull LN, et al. Benign recurrent intrahepatic cholestasis type 2 is caused by mutations in ABCB11. Gastroenterology 2004;127:379–384.
[42] Thompson R, Strautnieks S. BSEP: function and role in progressive familial intrahepatic cholestasis. Semin Liver Dis 2001;21:545–550.
[43] Gerloff T, Stieger B, Hagenbuch B, Madon J, Landmann L, Roth J, et al. The sister of P-glycoprotein represents the canalicular bile salt export pump of mammalian liver. J Biol Chem 1998;273:10046–10050.
[44] Keitel V, Burdelski M, Warskulat U, Kuhlkamp T, Keppler D, Haussinger D, et al. Expression and localization of hepatobiliary transport proteins in progressive familial intrahepatic cholestasis. Hepatology 2005;41: 1160–1172.
[45] Knisely AS, Strautnieks SS, Meier Y, Stieger B, Byrne JA, Portmann BC, et al. Hepatocellular carcinoma in ten children under five years of age with bile salt export pump deficiency. Hepatology 2006;44:478–486.
[46] Scheimann AO, Strautnieks SS, Knisely AS, Byrne JA, Thompson RJ, Finegold MJ. Mutations in bile salt export pump (ABCB11) in two children with progressive familial intrahepatic cholestasis and cholangiocarcinoma. J Pediatr 2007;150:556–559.
[47] Lang C, Meier Y, Stieger B, Beuers U, Lang T, Kerb R, et al. Mutations and polymorphisms in the bile salt export pump and the multidrug resistance protein 3 associated with drug-induced liver injury. Pharmacogenet Genomics 2007;17:47–60.
[48] Dixon PH, van Mil SW, Chambers J, Strautnieks S, Thompson RJ, Lammert F, et al. Contribution of variant alleles of ABCB11 to susceptibility to intrahepatic cholestasis of pregnancy. Gut 2009;58:537–544.
[49] Meier Y, Zodan T, Lang C, Zimmermann R, Kullak-Ublick GA, Meier PJ, et al. Increased susceptibility for intrahepatic cholestasis of pregnancy and contraceptive-induced cholestasis in carriers of the 1331T>C polymorphism in the bile salt export pump. World J Gastroenterol 2008;14:38–45.
[50] Pauli-Magnus C, Lang T, Meier Y, Zodan-Marin T, Jung D, Breymann C, et al. Sequence analysis of bile salt export pump (ABCB11) and multidrug resistance p-glycoprotein 3 (ABCB4, MDR3) in patients with intrahepatic cholestasis of pregnancy. Pharmacogenetics 2004;14:91–102.
[51] Hermeziu B, Sanlaville D, Girard M, Leonard C, Lyonnet S, Jacquemin E. Heterozygous bile salt export pump deficiency: a possible genetic predisposition to transient neonatal cholestasis. J Pediatr Gastroenterol Nutr 2006;42:114–116.
[52] Smit JJ, Schinkel AH, Mol CA, Majoor D, Mooi WJ, Jongsma AP, et al. Tissue distribution of the human MDR3 P-glycoprotein. Lab Invest 1994;71: 638–649.
[53] Smith AJ, Timmermans-Hereijgers JL, Roelofsen B, Wirtz KW, van Blitterswijk WJ, Smit JJ, et al. The human MDR3 P-glycoprotein promotes translocation of phosphatidylcholine through the plasma membrane of fibroblasts from transgenic mice. FEBS Lett 1994;354:263–266.
[54] van Helvoort A, Smith AJ, Sprong H, Fritzsche I, Schinkel AH, Borst P, et al. MDR1 P-glycoprotein is a lipid translocase of broad specificity, while MDR3 P-glycoprotein specifically translocates phosphatidylcholine. Cell 1996;87:507–517.
[55] De Vree JM, Jacquemin E, Sturm E, Cresteil D, Bosma PJ, Aten J, et al. Mutations in the MDR3 gene cause progressive familial intrahepatic cholestasis. Proc Natl Acad Sci USA 1998;95:282–287.
[56] Degiorgio D, Colombo C, Seia M, Porcaro L, Costantino L, Zazzeron L, et al. Molecular characterization and structural implications of 25 new ABCB4 mutations in progressive familial intrahepatic cholestasis type 3 (PFIC3). Eur J Hum Genet 2007;15:1230–1238.
[57] Gotthardt D, Runz H, Keitel V, Fischer C, Flechtenmacher C, Wirtenberger M, et al. A mutation in the canalicular phospholipid transporter gene, ABCB4, is associated with cholestasis, ductopenia, and cirrhosis in adults. Hepatology 2008;48:1157–1166.
[58] Ziol M, Barbu V, Rosmorduc O, Frassati-Biaggi A, Barget N, Hermelin B, et al. ABCB4 heterozygous gene mutations associated with fibrosing cholestatic liver disease in adults. Gastroenterology 2008;135:131–141.
[59] Dixon PH, Weerasekera N, Linton KJ, Donaldson O, Chambers J, Egginton E, et al. Heterozygous MDR3 missense mutation associated with intrahepatic cholestasis of pregnancy: evidence for a defect in protein trafficking. Hum Mol Genet 2000;9:1209–1217.

[60] Schneider G, Paus TC, Kullak-Ublick GA, Meier PJ, Wienker TF, Lang T, et al. Linkage between a new splicing site mutation in the MDR3 alias ABCB4 gene and intrahepatic cholestasis of pregnancy. Hepatology 2007;45:150–158.
[61] Floreani A, Carderi I, Paternoster D, Soardo G, Azzaroli F, Esposito W, et al. Intrahepatic cholestasis of pregnancy: three novel MDR3 gene mutations. Aliment Pharmacol Ther 2006;23:1649–1653.
[62] Floreani A, Carderi I, Paternoster D, Soardo G, Azzaroli F, Esposito W, et al. Hepatobiliary phospholipid transporter ABCB4, MDR3 gene variants in a large cohort of Italian women with intrahepatic cholestasis of pregnancy. Dig Liver Dis 2008;40:366–370.
[63] Gendrot C, Bacq Y, Brechot MC, Lansac J, Andres C. A second heterozygous MDR3 nonsense mutation associated with intrahepatic cholestasis of pregnancy. J Med Genet 2003;40:e32.
[64] Jacquemin E, Cresteil D, Manouvrier S, Boute O, Hadchouel M. Heterozygous non-sense mutation of the MDR3 gene in familial intrahepatic cholestasis of pregnancy. Lancet 1999;353:210–211.
[65] Mullenbach R, Linton KJ, Wiltshire S, Weerasekera N, Chambers J, Elias E, et al. ABCB4 gene sequence variation in women with intrahepatic cholestasis of pregnancy. J Med Genet 2003;40:e70.
[66] Wasmuth HE, Glantz A, Keppeler H, Simon E, Bartz C, Rath W, et al. Intrahepatic cholestasis of pregnancy: the severe form is associated with common variants of the hepatobiliary phospholipid transporter ABCB4 gene. Gut 2007;56:265–270.
[67] Jung C, Driancourt C, Baussan C, Zater M, Hadchouel M, Meunier-Rotival M, et al. Prenatal molecular diagnosis of inherited cholestatic diseases. J Pediatr Gastroenterol Nutr 2007;44:453–458.
[68] Nakken KE, Labori KJ, Rodningen OK, Nakken S, Berge KE, Eiklid K, et al. ABCB4 sequence variations in young adults with cholesterol gallstone disease. Liver Int 2009;29:743–747.
[69] Rosmorduc O, Hermelin B, Poupon R. MDR3 gene defect in adults with symptomatic intrahepatic and gallbladder cholesterol cholelithiasis. Gastroenterology 2001;120:1459–1467.
[70] Rosmorduc O, Hermelin B, Boelle PY, Parc R, Taboury J, Poupon R. ABCB4 gene mutation-associated cholelithiasis in adults. Gastroenterology 2003;125:452–459.
[71] Rosmorduc O, Poupon R. Low phospholipid associated cholelithiasis: association with mutation in the MDR3/ABCB4 gene. Orphanet J Rare Dis 2007;2:29.
[72] Lucena JF, Herrero JI, Quiroga J, Sangro B, Garcia-Foncillas J, Zabalegui N, et al. A multidrug resistance 3 gene mutation causing cholelithiasis, cholestasis of pregnancy, and adulthood biliary cirrhosis. Gastroenterology 2003;124:1037–1042.
[73] Sandusky GE, Mintze KS, Pratt SE, Dantzig AH. Expression of multidrug resistance-associated protein 2 (MRP2) in normal human tissues and carcinomas using tissue microarrays. Histopathology 2002;41:65–74.
[74] Schaub TP, Kartenbeck J, Konig J, Spring H, Dorsam J, Staehler G, et al. Expression of the MRP2 gene-encoded conjugate export pump in human kidney proximal tubules and in renal cell carcinoma. J Am Soc Nephrol 1999;10:1159–1169.
[75] Kamisako T, Leier I, Cui Y, Konig J, Buchholz U, Hummel-Eisenbeiss J, et al. Transport of monoglucuronosyl and bisglucuronosyl bilirubin by recombinant human and rat multidrug resistance protein 2. Hepatology 1999;30:485–490.
[76] Akita H, Suzuki H, Ito K, Kinoshita S, Sato N, Takikawa H, et al. Characterization of bile acid transport mediated by multidrug resistance associated protein 2 and bile salt export pump. Biochim Biophys Acta 2001;1511:7–16.
[77] Corpechot C, Ping C, Wendum D, Matsuda F, Barbu V, Poupon R. Identification of a novel 974C → G nonsense mutation of the MRP2/ABCC2 gene in a patient with Dubin-Johnson syndrome and analysis of the effects of rifampicin and ursodeoxycholic acid on serum bilirubin and bile acids. Am J Gastroenterol 2006;101:2427–2432.
[78] Dubin IN, Johnson FB. Chronic idiopathic jaundice with unidentified pigment in liver cells; a new clinicopathologic entity with a report of 12 cases. Medicine (Baltimore) 1954;33:155–197.
[79] Rastogi A, Krishnani N, Pandey R. Dubin-Johnson syndrome – a clinicopathologic study of twenty cases. Indian J Pathol Microbiol 2006;49:500–504.
[80] Nies AT, Keppler D. The apical conjugate efflux pump ABCC2 (MRP2). Pflugers Arch 2007;453:643–659.
[81] Klett EL, Lee MH, Adams DB, Chavin KD, Patel SB. Localization of ABCG5 and ABCG8 proteins in human liver, gall bladder and intestine. BMC Gastroenterol 2004;4:21.
[82] Graf GA, Li WP, Gerard RD, Gelissen I, White A, Cohen JC, et al. Coexpression of ATP-binding cassette proteins ABCG5 and ABCG8 permits their transport to the apical surface. J Clin Invest 2002;110:659–669.
[83] Graf GA, Yu L, Li WP, Gerard R, Tuma PL, Cohen JC, et al. ABCG5 and ABCG8 are obligate heterodimers for protein trafficking and biliary cholesterol excretion. J Biol Chem 2003;278:48275–48282.
[84] Berge KE, Tian H, Graf GA, Yu L, Grishin NV, Schultz J, et al. Accumulation of dietary cholesterol in sitosterolemia caused by mutations in adjacent ABC transporters. Science 2000;290:1771–1775.
[85] Lee MH, Lu K, Hazard S, Yu H, Shulenin S, Hidaka H, et al. Identification of a gene, ABCG5, important in the regulation of dietary cholesterol absorption. Nat Genet 2001;27:79–83.
[86] Lam CW, Cheng AW, Tong SF, Chan YW. Novel donor splice site mutation of ABCG5 gene in sitosterolemia. Mol Genet Metab 2002;75:178–180.
[87] Heimerl S, Langmann T, Moehle C, Mauerer R, Dean M, Beil FU, et al. Mutations in the human ATP-binding cassette transporters ABCG5 and ABCG8 in sitosterolemia. Hum Mutat 2002;20:151.
[88] Alam M, Garzon MC, Salen G, Starc TJ. Tuberous xanthomas in sitosterolemia. Pediatr Dermatol 2000;17:447–449.
[89] Katayama T, Satoh T, Yagi T, Hirose N, Kurita Y, Anzai T, et al. A 19-year-old man with myocardial infarction and sitosterolemia. Intern Med 2003;42:591–594.
[90] Kolovou G, Voudris V, Drogari E, Palatianos G, Cokkinos DV. Coronary bypass grafts in a young girl with sitosterolemia. Eur Heart J 1996;17:965–966.
[91] Rees DC, Iolascon A, Carella M, O'marcaigh AS, Kendra JR, Jowitt SN, et al. Stomatocytic haemolysis and macrothrombocytopenia (Mediterranean stomatocytosis/macrothrombocytopenia) is the haematological presentation of phytosterolaemia. Br J Haematol 2005;130:297–309.
[92] Miettinen TA, Klett EL, Gylling H, Isoniemi H, Patel SB. Liver transplantation in a patient with sitosterolemia and cirrhosis. Gastroenterology 2006;130:542–547.
[93] Hidaka H, Nakamura T, Aoki T, Kojima H, Nakajima Y, Kosugi K, et al. Increased plasma plant sterol levels in heterozygotes with sitosterolemia and xanthomatosis. J Lipid Res 1990;31:881–888.
[94] Lazaridis KN, Gores GJ, Lindor KD. Ursodeoxycholic acid 'mechanisms of action and clinical use in hepatobiliary disorders'. J Hepatol 2001;35:134–146.
[95] Paumgartner G, Beuers U. Ursodeoxycholic acid in cholestatic liver disease: mechanisms of action and therapeutic use revisited. Hepatology 2002;36:525–531.
[96] Beuers U, Bilzer M, Chittattu A, Kullak-Ublick GA, Keppler D, Paumgartner G, et al. Tauroursodeoxycholic acid inserts the apical conjugate export pump, Mrp2, into canalicular membranes and stimulates organic anion secretion by protein kinase C-dependent mechanisms in cholestatic rat liver. Hepatology 2001;33:1206–1216.
[97] Dumont M, Emmanuel J, Serge E. Effect of ursodeoxycholic acid on the expression of the hepatocellular bile acid transporters (Ntcp and bsep) in rats with estrogen-induced cholestasis. J Pediatr Gastroenterol Nutr 2002;35:185–191.
[98] Fickert P, Zollner G, Fuchsbichler A, Stumptner C, Pojer C, Zenz R, et al. Effects of ursodeoxycholic and cholic acid feeding on hepatocellular transporter expression in mouse liver. Gastroenterology 2001;121:170–183.
[99] Marschall HU, Wagner M, Zollner G, Fickert P, Diczfalusy U, Gumhold J, et al. Complementary stimulation of hepatobiliary transport and detoxification systems by rifampicin and ursodeoxycholic acid in humans. Gastroenterology 2005;129:476–485.
[100] Hofmann AF, Zakko SF, Lira M, Clerici C, Hagey LR, Lambert KK, et al. Novel biotransformation and physiological properties of norursodeoxycholic acid in humans. Hepatology 2005;42:1391–1398.
[101] van de Meeberg PC, van Erpecum KJ, van Berge-Henegouwen GP. Therapy with ursodeoxycholic acid in cholestatic liver disease. Scand J Gastroenterol Suppl 1993;200:15–20.
[102] Jacquemin E, Hermans D, Myara A, Habes D, Debray D, Hadchouel M, et al. Ursodeoxycholic acid therapy in pediatric patients with progressive familial intrahepatic cholestasis. Hepatology 1997;25:519–523.
[103] Jacquemin E, De Vree JM, Cresteil D, Sokal EM, Sturm E, Dumont M, et al. The wide spectrum of multidrug resistance 3 deficiency: from neonatal cholestasis to cirrhosis of adulthood. Gastroenterology 2001;120:1448–1458.
[104] Wanty C, Joomye R, Van HN, Paul K, Otte JB, Reding R, et al. Fifteen years single center experience in the management of progressive familial intrahepatic cholestasis of infancy. Acta Gastroenterol Belg 2004;67:313–319.
[105] Morton DH, Salen G, Batta AK, Shefer S, Tint GS, Belchis D, et al. Abnormal hepatic sinusoidal bile acid transport in an Amish kindred is not linked to FIC1 and is improved by ursodiol. Gastroenterology 2000;119:188–195.

[106] Ismail H, Kalicinski P, Markiewicz M, Jankowska I, Pawlowska J, Kluge P, et al. Treatment of progressive familial intrahepatic cholestasis: liver transplantation or partial external biliary diversion. Pediatr Transplant 1999;3:219–224.
[107] Naveh Y, Bassan L, Rosenthal E, Berkowitz D, Jaffe M, Mandel H, et al. Progressive familial intrahepatic cholestasis among the Arab population in Israel. J Pediatr Gastroenterol Nutr 1997;24:548–554.
[108] Whitington PF, Freese DK, Alonso EM, Schwarzenberg SJ, Sharp HL. Clinical and biochemical findings in progressive familial intrahepatic cholestasis. J Pediatr Gastroenterol Nutr 1994;18:134–141.
[109] Bustorff-Silva J, Sbraggia NL, Olimpio H, de Alcantara RV, Matsushima E, De Tommaso AM, et al. Partial internal biliary diversion through a cholecystojejunocolonic anastomosis – a novel surgical approach for patients with progressive familial intrahepatic cholestasis: a preliminary report. J Pediatr Surg 2007;42:1337–1340.
[110] Emerick KM, Elias MS, Melin-Aldana H, Strautnieks S, Thompson RJ, Bull LN, et al. Bile composition in alagille syndrome and PFIC patients having partial external biliary diversion. BMC Gastroenterol 2008;8:47.
[111] Englert C, Grabhorn E, Richter A, Rogiers X, Burdelski M, Ganschow R. Liver transplantation in children with progressive familial intrahepatic cholestasis. Transplantation 2007;84:1361–1363.
[112] Kurbegov AC, Setchell KD, Haas JE, Mierau GW, Narkewicz M, Bancroft JD, et al. Biliary diversion for progressive familial intrahepatic cholestasis: improved liver morphology and bile acid profile. Gastroenterology 2003;125:1227–1234.
[113] Yerushalmi B, Sokol RJ, Narkewicz MR, Smith D, Karrer FM. Use of rifampin for severe pruritus in children with chronic cholestasis. J Pediatr Gastroenterol Nutr 1999;29:442–447.
[114] Crosignani A, Podda M, Bertolini E, Battezzati PM, Zuin M, Setchell KD. Failure of ursodeoxycholic acid to prevent a cholestatic episode in a patient with benign recurrent intrahepatic cholestasis: a study of bile acid metabolism. Hepatology 1991;13:1076–1083.
[115] Cancado EL, Leitao RM, Carrilho FJ, Laudanna AA. Unexpected clinical remission of cholestasis after rifampicin therapy in patients with normal or slightly increased levels of gamma-glutamyl transpeptidase. Am J Gastroenterol 1998;93:1510–1517.
[116] Metzelder ML, Petersen C, Melter M, Ure BM. Modified laparoscopic external biliary diversion for benign recurrent intrahepatic cholestasis in obese adolescents. Pediatr Surg Int 2006;22:551–553.
[117] Regev RH, Stolar O, Raz A, Dolfin T. Treatment of severe cholestasis in neonatal Dubin-Johnson syndrome with ursodeoxycholic acid. J Perinat Med 2002;30:185–187.
[118] Fickert P, Wagner M, Marschall HU, Fuchsbichler A, Zollner G, Tsybrovskyy O, et al. 24-norUrsodeoxycholic acid is superior to ursodeoxycholic acid in the treatment of sclerosing cholangitis in Mdr2 (Abcb4) knockout mice. Gastroenterology 2006;130:465–481.
[119] Wietholtz H, Marschall HU, Sjovall J, Matern S. Stimulation of bile acid 6 alpha-hydroxylation by rifampin. J Hepatol 1996;24:713–718.
[120] Balsells F, Wyllie R, Steffen R, Kay M. Benign recurrent intrahepatic cholestasis: improvement of pruritus and shortening of the symptomatic phase with rifampin therapy: a case report. Clin Pediatr (Phila) 1997;36:483–485.
[121] Garbutt JT, Kenney TJ. Effect of cholestyramine on bile acid metabolism in normal man. J Clin Invest 1972;51:2781–2789.
[122] Nakagawa M, Tazawa Y, Kobayashi Y, Yamada M, Suzuki H, Konno T, et al. Familial intrahepatic cholestasis associated with progressive neuromuscular disease and vitamin E deficiency. J Pediatr Gastroenterol Nutr 1984;3:385–389.
[123] Whitington PF, Whitington GL. Partial external diversion of bile for the treatment of intractable pruritus associated with intrahepatic cholestasis. Gastroenterology 1988;95:130–136.
[124] Uegaki S, Tanaka A, Mori Y, Kodama H, Fukusato T, Takikawa H. Successful treatment with colestimide for a bout of cholestasis in a Japanese patient with benign recurrent intrahepatic cholestasis caused by ATP8B1 mutation. Intern Med 2008;47:599–602.
[125] Al Drees K, Al ZA, Al AA, Abdulla A. Benign recurrent intrahepatic cholestasis in a Saudi child. Ann Trop Paediatr 1999;19:215–217.
[126] Odievre M, Gautier M, Hadchouel M, Alagille D. Severe familial intrahepatic cholestasis. Arch Dis Child 1973;48:806–812.
[127] Bays HE, Goldberg RB. The 'forgotten' bile acid sequestrants: is now a good time to remember? Am J Ther 2007;14:567–580.
[128] Belamarich PF, Deckelbaum RJ, Starc TJ, Dobrin BE, Tint GS, Salen G. Response to diet and cholestyramine in a patient with sitosterolemia. Pediatrics 1990;86:977–981.
[129] Nguyen LB, Cobb M, Shefer S, Salen G, Ness GC, Tint GS. Regulation of cholesterol biosynthesis in sitosterolemia: effects of lovastatin, cholestyramine, and dietary sterol restriction. J Lipid Res 1991;32:1941–1948.
[130] Gregg RE, Connor WE, Lin DS, Brewer Jr HB. Abnormal metabolism of shellfish sterols in a patient with sitosterolemia and xanthomatosis. J Clin Invest 1986;77:1864–1872.
[131] Salen G, Kwiterovich Jr PO, Shefer S, Tint GS, Horak I, Shore V, et al. Increased plasma cholestanol and 5 alpha-saturated plant sterol derivatives in subjects with sitosterolemia and xanthomatosis. J Lipid Res 1985;26:203–209.
[132] Cobb MM, Salen G, Tint GS, Greenspan J, Nguyen LB. Sitosterolemia: opposing effects of cholestyramine and lovastatin on plasma sterol levels in a homozygous girl and her heterozygous father. Metabolism 1996;45:673–679.
[133] Saich R, Collins P, Ala A, Standish R, Hodgson H. Benign recurrent intrahepatic cholestasis with secondary renal impairment treated with extracorporeal albumin dialysis. Eur J Gastroenterol Hepatol 2005;17:585–588.
[134] Sturm E, Franssen CF, Gouw A, Staels B, Boverhof R, De Knegt RJ, et al. Extracorporal albumin dialysis (MARS) improves cholestasis and normalizes low apo A-I levels in a patient with benign recurrent intrahepatic cholestasis (BRIC). Liver 2002;22:72–75.
[135] Hollands CM, Rivera-Pedrogo FJ, Gonzalez-Vallina R, Loret-de-Mola O, Nahmad M, Burnweit CA. Ileal exclusion for Byler's disease: an alternative surgical approach with promising early results for pruritus. J Pediatr Surg 1998;33:220–224.
[136] Ekinci S, Karnak I, Gurakan F, Yuce A, Senocak ME, Cahit TF, et al. Partial external biliary diversion for the treatment of intractable pruritus in children with progressive familial intrahepatic cholestasis: report of two cases. Surg Today 2008;38:726–730.
[137] Emond JC, Whitington PF. Selective surgical management of progressive familial intrahepatic cholestasis (Byler's disease). J Pediatr Surg 1995;30:1635–1641.
[138] Melter M, Rodeck B, Kardorff R, Hoyer PF, Petersen C, Ballauff A, et al. Progressive familial intrahepatic cholestasis: partial biliary diversion normalizes serum lipids and improves growth in noncirrhotic patients. Am J Gastroenterol 2000;95:3522–3528.
[139] Ng VL, Ryckman FC, Porta G, Miura IK, de CE, Servidoni MF, et al. Long-term outcome after partial external biliary diversion for intractable pruritus in patients with intrahepatic cholestasis. J Pediatr Gastroenterol Nutr 2000;30:152–156.
[140] Rebhandl W, Felberbauer FX, Turnbull J, Paya K, Barcik U, Huber WD, et al. Biliary diversion by use of the appendix (cholecystoappendicostomy) in progressive familial intrahepatic cholestasis. J Pediatr Gastroenterol Nutr 1999;28:217–219.
[141] Arnell H, Bergdahl S, Papadogiannakis N, Nemeth A, Fischler B. Preoperative observations and short-term outcome after partial external biliary diversion in 13 patients with progressive familial intrahepatic cholestasis. J Pediatr Surg 2008;43:1312–1320.
[142] Metzelder ML, Bottlander M, Melter M, Petersen C, Ure BM. Laparoscopic partial external biliary diversion procedure in progressive familial intrahepatic cholestasis: a new approach. Surg Endosc 2005;19:1641–1643.
[143] Yang H, Porte RJ, Verkade HJ, De Langen ZJ, Hulscher JB. Partial external biliary diversion in children with progressive familial intrahepatic cholestasis and alagille disease. J Pediatr Gastroenterol Nutr 2009.
[144] Kalicinski PJ, Ismail H, Jankowska I, Kaminski A, Pawlowska J, Drewniak T, et al. Surgical treatment of progressive familial intrahepatic cholestasis: comparison of partial external biliary diversion and ileal bypass. Eur J Pediatr Surg 2003;13:307–311.
[145] Stapelbroek JM, van Erpecum KJ, Klomp LW, Venneman NG, Schwartz TP, van Berge-Henegouwen GP, et al. Nasobiliary drainage induces long-lasting remission in benign recurrent intrahepatic cholestasis. Hepatology 2006;43:51–53.
[146] Soubrane O, Gauthier F, Devictor D, Bernard O, Valayer J, Houssin D, et al. Orthotopic liver transplantation for Byler disease. Transplantation 1990;50:804–806.
[147] Aydogdu S, Cakir M, Arikan C, Tumgor G, Yuksekkaya HA, Yilmaz F, et al. Liver transplantation for progressive familial intrahepatic cholestasis: clinical and histopathological findings, outcome and impact on growth. Pediatr Transplant 2007;11:634–640.
[148] Bassas A, Chehab M, Hebby H, Al SM, Al HH, Al ZA, et al. Living related liver transplantation in 13 cases of progressive familial intrahepatic cholestasis. Transplant Proc 2003;35:3003–3005.

[149] Cutillo L, Najimi M, Smets F, Janssen M, Reding R, de Ville de GJ, et al. Safety of living-related liver transplantation for progressive familial intrahepatic cholestasis. Pediatr Transplant 2006;10:570–574.
[150] Egawa H, Yorifuji T, Sumazaki R, Kimura A, Hasegawa M, Tanaka K. Intractable diarrhea after liver transplantation for Byler's disease: successful treatment with bile adsorptive resin. Liver Transpl 2002;8:714–716.
[151] Khan I, Al-Shaqrani MA, Arain ZB, Al-Hebbi HA, Wali SH, Bassas AF. One hundred and thirty-seven living donor pediatric liver transplants at Riyadh Military Hospital. Results and outlook for future. Saudi Med J 2009;30:403–408.
[152] D'Antiga L, Moniz C, Buxton-Thomas M, Cheeseman P, Gray B, Abraha H, et al. Bone mineral density and height gain in children with chronic cholestatic liver disease undergoing transplantation. Transplantation 2002;73:1788–1793.
[153] Usui M, Isaji S, Das BC, Kobayashi M, Osawa I, Iida T, et al. Liver retransplantation with external biliary diversion for progressive familial intrahepatic cholestasis type 1: a case report. Pediatr Transplant 2008.
[154] Miyagawa-Hayashino A, Egawa H, Yorifuji T, Hasegawa M, Haga H, Tsuruyama T, et al. Allograft steatohepatitis in progressive familial intrahepatic cholestasis type 1 after living donor liver transplantation. Liver Transpl 2009;15:610–618.
[155] Davit-Spraul A, Gonzales E, Baussan C, Jacquemin E. Progressive familial intrahepatic cholestasis. Orphanet J Rare Dis 2009;4:1.
[156] De Vree JM, Ottenhoff R, Bosma PJ, Smith AJ, Aten J, Oude Elferink RP. Correction of liver disease by hepatocyte transplantation in a mouse model of progressive familial intrahepatic cholestasis. Gastroenterology 2000;119:1720–1730.
[157] Quaglia A, Lehec SC, Hughes RD, Mitry RR, Knisely AS, Devereaux S, et al. Liver after hepatocyte transplantation for liver-based metabolic disorders in children. Cell Transplant 2008;17:1403–1414.
[158] Dhawan A, Mitry RR, Hughes RD. Hepatocyte transplantation for liver-based metabolic disorders. J Inherit Metab Dis 2006;29:431–435.
[159] Eloranta JJ, Meier PJ, Kullak-Ublick GA. Coordinate transcriptional regulation of transport and metabolism. Methods Enzymol 2005;400:511–530.
[160] Huang L, Zhao A, Lew JL, Zhang T, Hrywna Y, Thompson JR, et al. Farnesoid X receptor activates transcription of the phospholipid pump MDR3. J Biol Chem 2003;278:51085–51090.
[161] Kast HR, Goodwin B, Tarr PT, Jones SA, Anisfeld AM, Stoltz CM, et al. Regulation of multidrug resistance-associated protein 2 (ABCC2) by the nuclear receptors pregnane X receptor, farnesoid X-activated receptor, and constitutive androstane receptor. J Biol Chem 2002;277:2908–2915.
[162] Plass JR, Mol O, Heegsma J, Geuken M, Faber KN, Jansen PL, et al. 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.
[163] Zollner G, Trauner M. Nuclear receptors as therapeutic targets in cholestatic liver diseases. Br J Pharmacol 2009;156:7–27.
[164] Fiorucci S, Clerici C, Antonelli E, Orlandi S, Goodwin B, Sadeghpour BM, et al. Protective effects of 6-ethyl chenodeoxycholic acid, a farnesoid X receptor ligand, in estrogen-induced cholestasis. J Pharmacol Exp Ther 2005;313:604–612.
[165] Mottino AD, Cao J, Veggi LM, Crocenzi F, Roma MG, Vore M. Altered localization and activity of canalicular Mrp2 in estradiol-17beta-D-glucuronide-induced cholestasis. Hepatology 2002;35:1409–1419.
[166] Stieger B, Fattinger K, Madon J, Kullak-Ublick GA, Meier PJ. Drug- and estrogen-induced cholestasis through inhibition of the hepatocellular bile salt export pump (Bsep) of rat liver. Gastroenterology 2000;118:422–430.
[167] Demeilliers C, Jacquemin E, Barbu V, Mergey M, Paye F, Fouassier L, et al. Altered hepatobiliary gene expressions in PFIC1: ATP8B1 gene defect is associated with CFTR downregulation. Hepatology 2006;43:1125–1134.
[168] Pawlikowska L, Groen A, Eppens EF, Kunne C, Ottenhoff R, Looije N, et al. A mouse genetic model for familial cholestasis caused by ATP8B1 mutations reveals perturbed bile salt homeostasis but no impairment in bile secretion. Hum Mol Genet 2004;13:881–892.
[169] Alvarez L, Jara P, Sanchez-Sabate E, Hierro L, Larrauri J, Diaz MC, et al. Reduced hepatic expression of farnesoid X receptor in hereditary cholestasis associated to mutation in ATP8B1. Hum Mol Genet 2004;13:2451–2460.
[170] Chen F, Ananthanarayanan M, Emre S, Neimark E, Bull LN, Knisely AS, et al. Progressive familial intrahepatic cholestasis, type 1, is associated with decreased farnesoid X receptor activity. Gastroenterology 2004;126:756–764.
[171] Frankenberg T, Miloh T, Chen FY, Ananthanarayanan M, Sun AQ, Balasubramaniyan N, et al. The membrane protein ATPase class I type 8B member 1 signals through protein kinase C zeta to activate the farnesoid X receptor. Hepatology 2008;48:1896–1905.
[172] Koh S, Takada T, Kukuu I, Suzuki H. FIC1-mediated stimulation of FXR activity is decreased with PFIC1 mutations in HepG2 cells. J Gastroenterol 2009.
[173] Martinez-Fernandez P, Hierro L, Jara P, Alvarez L. Knockdown of ATP8B1 expression leads to specific downregulation of the bile acid sensor FXR in HepG2 cells: effect of the FXR agonist GW4064. Am J Physiol Gastrointest Liver Physiol 2009;296:G1119–G1129.
[174] Shoda J, Okada K, Inada Y, Kusama H, Utsunomiya H, Oda K, et al. Bezafibrate induces multidrug-resistance P-Glycoprotein 3 expression in cultured human hepatocytes and humanized livers of chimeric mice. Hepatol Res 2007;37:548–556.
[175] Chianale J, Vollrath V, Wielandt AM, Amigo L, Rigotti A, Nervi F, et al. Fibrates induce mdr2 gene expression and biliary phospholipid secretion in the mouse. Biochem J 1996;314:781–786.
[176] Kurihara T, Niimi A, Maeda A, Shigemoto M, Yamashita K. Study of effectiveness of bezafibrate in the treatment of chronic hepatitis C. Am J Gastroenterol 2001;96:1659–1660.
[177] Kurihara T, Maeda A, Shigemoto M, Yamashita K, Hashimoto E. Investigation into the efficacy of bezafibrate against primary biliary cirrhosis, with histological references from cases receiving long term monotherapy. Am J Gastroenterol 2002;97:212–214.
[178] Kurihara T, Maeda A, Shigemoto M, Yamashita K, Kamatani N. Efficacy of bezafibrate in a patient with primary sclerosing cholangitis. J Gastroenterol 2003;38:300–301.
[179] Kurihara T, Niimi A, Maeda A, Shigemoto M, Yamashita K. Bezafibrate in the treatment of primary biliary cirrhosis: comparison with ursodeoxycholic acid. Am J Gastroenterol 2000;95:2990–2992.
[180] Hacein-Bey-Abina S, Von KC, Schmidt M, Le DF, Wulffraat N, McIntyre E, et al. A serious adverse event after successful gene therapy for X-linked severe combined immunodeficiency. N Engl J Med 2003;348:255–256.
[181] Luzzatto L, Apirion D, Schlessinger D. Mechanism of action of streptomycin in *E. coli*: interruption of the ribosome cycle at the initiation of protein synthesis. Proc Natl Acad Sci USA 1968;60:873–880.
[182] Ruusala T, Kurland CG. Streptomycin preferentially perturbs ribosomal proofreading. Mol Gen Genet 1984;198:100–104.
[183] Clancy JP, Bebok Z, Ruiz F, King C, Jones J, Walker L, et al. Evidence that systemic gentamicin suppresses premature stop mutations in patients with cystic fibrosis. Am J Respir Crit Care Med 2001;163:1683–1692.
[184] Wilschanski M, Yahav Y, Yaacov Y, Blau H, Bentur L, Rivlin J, et al. Gentamicin-induced correction of CFTR function in patients with cystic fibrosis and CFTR stop mutations. N Engl J Med 2003;349:1433–1441.
[185] Welch EM, Barton ER, Zhuo J, Tomizawa Y, Friesen WJ, Trifillis P, et al. PTC124 targets genetic disorders caused by nonsense mutations. Nature 2007;447:87–91.
[186] Hirawat S, Welch EM, Elfring GL, Northcutt VJ, Paushkin S, Hwang S, et al. Safety, tolerability, and pharmacokinetics of PTC124, a nonaminoglycoside nonsense mutation suppressor, following single- and multiple-dose administration to healthy male and female adult volunteers. J Clin Pharmacol 2007;47:430–444.
[187] Kerem E, Hirawat S, Armoni S, Yaakov Y, Shoseyov D, Cohen M, et al. Effectiveness of PTC124 treatment of cystic fibrosis caused by nonsense mutations: a prospective phase II trial. Lancet 2008;372:719–727.
[188] Welch WJ. Role of quality control pathways in human diseases involving protein misfolding. Semin Cell Dev Biol 2004;15:31–38.
[189] Dover GJ, Brusilow S, Samid D. Increased fetal hemoglobin in patients receiving sodium 4-phenylbutyrate. N Engl J Med 1992;327:569–570.
[190] Maestri NE, Brusilow SW, Clissold DB, Bassett SS. Long-term treatment of girls with ornithine transcarbamylase deficiency. N Engl J Med 1996;335:855–859.
[191] Rubenstein RC, Zeitlin PL. Sodium 4-phenylbutyrate downregulates Hsc70: implications for intracellular trafficking of DeltaF508-CFTR. Am J Physiol Cell Physiol 2000;278:C259–C267.
[192] Rubenstein RC, Lyons BM. Sodium 4-phenylbutyrate downregulates HSC70 expression by facilitating mRNA degradation. Am J Physiol Lung Cell Mol Physiol 2001;281:L43–L51.
[193] Choo-Kang LR, Zeitlin PL. Induction of HSP70 promotes DeltaF508 CFTR trafficking. Am J Physiol Lung Cell Mol Physiol 2001;281:L58–L68.
[194] Rubenstein RC, Egan ME, Zeitlin PL. In vitro pharmacologic restoration of CFTR-mediated chloride transport with sodium 4-phenylbutyrate in cystic fibrosis epithelial cells containing delta F508-CFTR. J Clin Invest 1997;100:2457–2465.
[195] Rubenstein RC, Zeitlin PL. A pilot clinical trial of oral sodium 4-phenylbutyrate (buphenyl) in deltaF508-homozygous cystic fibrosis patients: partial

restoration of nasal epithelial CFTR function. Am J Respir Crit Care Med 1998;157:484–490.
[196] Zeitlin PL, Ener-West M, Rubenstein RC, Boyle MP, Lee CK, Brass-Ernst L. Evidence of CFTR function in cystic fibrosis after systemic administration of 4-phenylbutyrate. Mol Ther 2002;6:119–126.
[197] van der Velden LM, Stapelbroek JM, Krieger E, van den Berghe PV, Berger R, Verhulst PM, et al. Folding defects in ATP8B1 associated with hereditary cholestasis are ameliorated by 4-phenylbutyrate. Hepatology 2009 sep 4. [Epub ahead of print].
[198] Wang L, Dong H, Soroka CJ, Wei N, Boyer JL, Hochstrasser M. Degradation of the bile salt export pump at endoplasmic reticulum in progressive familial intrahepatic cholestasis type II. Hepatology 2008;48:1558–1569.
[199] Delaunay JL, Durand-Schneider AM, Delautier D, Rada A, Gautherot J, Jacquemin E, et al. A missense mutation in ABCB4 gene involved in progressive familial intrahepatic cholestasis type 3 leads to a folding defect that can be rescued by low temperature. Hepatology 2008.
[200] Hayashi H, Sugiyama Y. 4-phenylbutyrate enhances the cell surface expression and the transport capacity of wild-type and mutated bile salt export pumps. Hepatology 2007;45:1506–1516.
[201] Hayashi H, Sugiyama Y. Short-chain ubiquitination is associated with the degradation rate of a cell-surface-resident bile salt export pump (BSEP/ABCB11). Mol Pharmacol 2009;75:143–150.
[202] Lam P, Pearson CL, Soroka CJ, Xu S, Mennone A, Boyer JL. 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 Cell Physiol 2007;293:C1709–C1716.
[203] Nissim-Rafinia M, Kerem B. Splicing modulation as a modifier of the CFTR function. Prog Mol Subcell Biol 2006;44:233–254.
[204] Aznarez I, Chan EM, Zielenski J, Blencowe BJ, Tsui LC. Characterization of disease-associated mutations affecting an exonic splicing enhancer and two cryptic splice sites in exon 13 of the cystic fibrosis transmembrane conductance regulator gene. Hum Mol Genet 2003;12:2031–2040.
[205] Black DL. Mechanisms of alternative pre-messenger RNA splicing. Annu Rev Biochem 2003;72:291–336.
[206] Nissim-Rafinia M, Aviram M, Randell SH, Shushi L, Ozeri E, Chiba-Falek O, et al. Restoration of the cystic fibrosis transmembrane conductance regulator function by splicing modulation. EMBO Rep 2004;5:1071–1077.
[207] Madden HR, Fletcher S, Davis MR, Wilton SD. Characterization of a complex Duchenne muscular dystrophy-causing dystrophin gene inversion and restoration of the reading frame by induced exon skipping. Hum Mutat 2009;30:22–28.
[208] Mitrpant C, Adams AM, Meloni PL, Muntoni F, Fletcher S, Wilton SD. Rational design of antisense oligomers to induce dystrophin exon skipping. Mol Ther 2009.
[209] Pros E, Fernandez-Rodriguez J, Canet B, Benito L, Sanchez A, Benavides A, et al. Antisense therapeutics for neurofibromatosis type 1 caused by deep intronic mutations. Hum Mutat 2009;30:454–462.
[210] Salen G, Starc T, Sisk CM, Patel SB. Intestinal cholesterol absorption inhibitor ezetimibe added to cholestyramine for sitosterolemia and xanthomatosis. Gastroenterology 2006;130:1853–1857.
[211] Beuers U, Boberg KM, Chapman RW, Chazouillères O, Invernizzi P, Jones DEJ, et al. EASL clinical practice guidelines: management of cholestatic liver diseases. J Hepatol 2009;51:237–267.