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The spectrum of Progressive Familial Intrahepatic Cholestasis diseases: Update on pathophysiology and emerging treatments

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ABSTRACT

The Progressive Familial Intrahepatic Cholestasis (PFIC) disease spectrum encompasses a variety of genetic diseases that affect the bile production and the secretion of bile acids. Typically, the first presentation of these diseases is in early childhood, frequently followed by a severe course necessitating liver transplantation before adulthood. Except for transplantation, treatment modalities have been rather limited and frequently only aim at the symptoms of cholestasis, such as cholestatic pruritus. In recent years, progress has been made in understanding the pathophysiology of these diseases and new treatment modalities have been emerging. Herewith we summarize the latest developments in the field and formulate the current key questions and opportunities for further progress.

1. Introduction

Progressive familial intrahepatic cholestasis (PFIC) has become a term for a group of genetic liver diseases with an estimated incidence ranging from 1/50.000–1/100.000 (Davit-Spraul et al., 2009). The different PFIC diseases usually lead to varying degrees of cholestasis and progressive liver injury, up until the point of end-stage liver disease and the need for liver transplantation (LTx) (van Wessel et al., 2020). Classically, three forms of PFIC have been identified, type 1, 2 and 3. More recently several other genetic diseases have been characterized that also have a phenotype of progressive cholestasis in childhood, three of them have sometimes been referred to as PFIC type 4, 5 and 6. Others, especially the latest PFIC related diseases that genetically have been identified, have only been referred to by their gene or protein defect. Rather than trying to dissolve disputes on nomenclature or on how extended (or not) the family of PFIC diseases should be, we feel it more helpful to name the diseases by the deficiency of the respective gene products. The classic types of PFIC include PFIC1 with functional deficiency of FIC1 protein encoded by the *ATP8B1(ATPase Phospholipid Transporting 8B1)* gene, PFIC2 with loss of functionality of the canalicular bile salt export pump (BSEP) protein, due to mutations in *ABCB11 (ATP Binding Cassette Subfamily B Member 11)*, and PFIC3 with multi-drug-resistance-protein 3 (MDR3) defects, caused by mutations in *ABCB4 (ATP Binding Cassette Subfamily B Member 4)*, (see Fig. 2). We prefer to refer to these diseases as BSEP deficiency, FIC1 deficiency, and MDR3 deficiency, respectively. Examples of other diseases in this spectrum include mutations in tight junction protein 2 gene (*TJP2*; TJP2 deficiency), mutations in the *NR1H4 (Nuclear Receptor Subfamily 1 Group H Member 4)* gene, leading to defective Farsenoid X Receptor (FXR deficiency) and more recently, defects in the *Myosin 5 B* gene, which not only can lead to the intestinal phenotype of microvillus inclusion disease, but has also been associated with a cholestatic phenotype (MYO5B; MYO5B deficiency) (Henkel et al., 2019), (see Fig. 2). Among the latest discovered and least investigated diseases are defects in the Ubiquitin Specific Peptidase 53 gene (USP53; USP53 deficiency), Lipolysis stimulated lipoprotein receptor gene (LSR; LSR deficiency) and the WD Repeat Domain 83 Opposite Strand gene (*WDR83OS;* WDR83OS deficiency) (Maddirevula et al., 2019), (see Fig. 2).

Current therapies for these diseases include off-label treatments such as ursodeoxycholic acid (UDCA) or rifampicin, aimed at either the cholestasis (particularly in MDR3-deficiency) or the frequently associated cholestatic pruritus, as well as invasive approaches with different kinds of surgical biliary diversion (SBD) techniques (see Fig. 1). LTx is commonly considered as ultimate treatment for these diseases, be it for intractable pruritus, for the end-stage liver disease and/or for its associated complications, such as hepatocellular carcinoma (van Wessel et al., 2020). However, LTx involves major surgery and comes with the price of lifelong immunosuppression. Therefore, alternative treatment

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strategies to prevent or delay the need for LTx are warranted.

Since there have recently been several preclinical and clinical promising developments for these diseases, we considered it appropriate to provide an update. We herewith review the recent developments and discuss the expectations and some of the remaining challenges. We have chosen to review this for the forms of genetic cholestasis mentioned above and have concentrated on their disease expression in childhood.

1.1. FIC1 deficiency

FIC1 deficiency is caused by mutations in the *ATP8B1* gene. The FIC1 protein is a P-type ATPase acting as a phospholipid flippase. FIC1 maintains a phospholipid asymmetry across membranes including the bile canalicular membrane (Andersen et al., 2016; Mehl et al., 2016). *ATP8B1* mutations are thought to disrupt the membrane structure, impairing the activity of membrane proteins, including that of the bile salt export pump (BSEP). Severe FIC1 deficiency usually presents within the first year of life with symptoms of cholestasis manifested by pruritus and jaundice (van Wessel et al., 2021). Secondary manifestations including vitamin K deficiency, malabsorption, and poor weight gain can already become evident before the age of three months. The natural history of the disease includes cirrhosis and end-stage liver disease, usually during child age (van Wessel et al., 2021; Knisley S et al., 2001). The *FIC1* gene is also expressed in extrahepatic tissues, such as the intestinal epithelium, pancreas and intra-auricular cochlear hair cells (Stapelbroek et al., 2009). Indeed, FIC1 disease has been associated with symptoms as diarrhea, pancreatitis, and hearing loss (Pawlikowska et al., 2010). FIC1 deficiency has, in many cases, been refractory to medical interventions. Nevertheless, medical treatments such as UDCA and rifampicin are generally regarded as primary treatment before surgical interventions are considered, such as SBD or LTx. FIC1 deficiency patients can undergo SBD procedures to partially interrupt the enterohepatic circulation (EHC) and to mitigate the cholestatic symptoms. Recent epidemiological analyses indicated that a subfraction of FIC1 deficiency patients benefit from SBD, namely those patients in whom serum bile acids strongly decrease by the procedure (van Wessel et al., 2021). Nonetheless, most patients will proceed to need an LTx for therapy-resistant pruritus or less frequently, for progressive liver disease (van Wessel et al., 2021). In FIC1 deficiency, however, LTx should not be considered as definitive treatment due to the extrahepatic tissue expression of the disease (Bull et al., 2018). FIC1 deficiency patients may experience severe complications after LTx such as persistent secretory diarrhea and steatohepatitis of the graft that even can necessitate one or more re-transplantations (Mehl et al., 2016; Pawlikowska et al., 2010; Bull et al., 2018).

1.2. BSEP deficiency

Bile Salt Export Pump (BSEP) deficiency, results from mutations in the *ABCB11* (ATP Binding Cassette Subfamily B Member 11) gene. The BSEP protein transports conjugated bile acids from the hepatocyte, across the bile canalicular membrane, into the bile canaliculus. Severe BSEP deficiency patients often progress to end-stage liver disease and the need for LTx. Two common *ABCB11* mutations (p.D482G and p.E297G) are thought to have residual bile acid transport function and have been associated with a relatively mild phenotype (van Wessel et al., 2020; Hayashi et al., 2005). On the other hand, a genotype with two predicted protein truncating mutations (PPTMs) has been associated with a more severe phenotype. It has been hypothesized that the amount of residual bile acid transport activity of the two mutated alleles determines the phenotype and the responsiveness to therapeutic interventions (van Wessel et al., 2020; Strautnieks et al., 2008; Felzen et al., 2020). However, the large amount of different *ABCB11* mutations, who have only partly been characterized towards functional

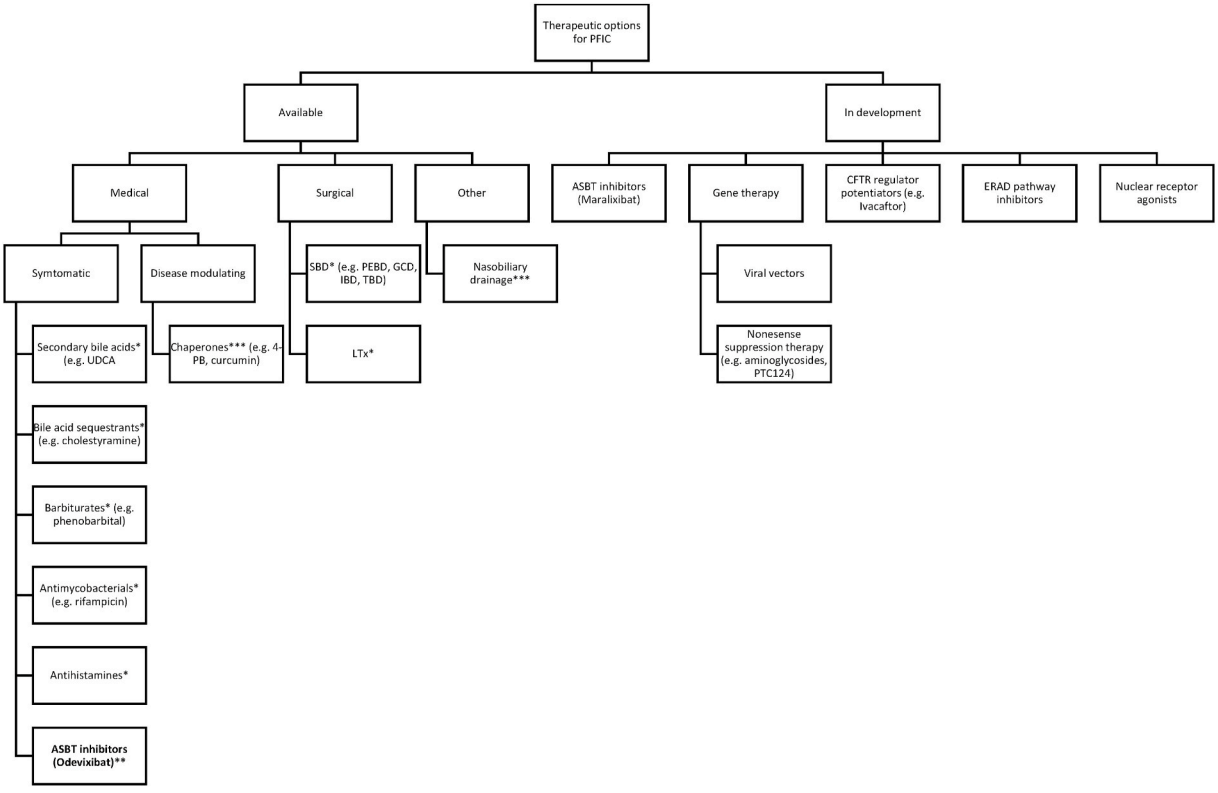

Fig. 1. Therapeutic options for PFIC, both available and in development. *current treatment of PFIC; **FDA approved for the use in PFIC; ***experimental treatment of PFIC; UDCA, ursodeoxycholic acid; 4-PB, 4-phenylbutyrate; SBD, surgical biliary diversion; PEBD, partial external biliary diversion; GCD, gallbladder-colon diversion; IBD, internal biliary diversion; TBD, total biliary diversion; ASBT, apical sodium-dependent bile acid transporter; CFTR, cystic fibrosis transmembrane conductance regulator; ERAD, endoplasmic reticulum-associated protein degradation (Gonzales and Jacquemin, 2010).

consequences, has prevented to predict the degree of residual function in all individual patients.

Patients with severe BSEP deficiency commonly have jaundice, pruritus, high serum bile acids (sBA) and high transaminases, and present in early childhood. Some patients may respond (temporarily) to medical interventions such as UDCA. An SBD in severe BSEP deficiency patients is associated with improved native liver survival (NLS) in many cases. However, those with two PPTM mutations do not respond (van Wessel et al., 2020). The amount of residual function conferred by missense mutations is difficult to predict, and adding to this, most patients are compound heterozygotes, what further complicates assessment of residual activity at the level of individual patients. Even patients with one PPTM and one of the common mutations p.D482G or p.E297G are not always responsive to SBD (Felzen et al., 2020). SBD aims to partially interrupt the enterohepatic circulation, thereby decreasing the amount of bile acids available for reuptake in the terminal ileum and reducing the bile acid accumulation in the body. Patients in which the SBD results in a substantial decrease in systemic bile acid accumulation will be able to avoid LTx (van Wessel et al., 2020; Verkade et al., 2020). Patients in whom SBD fails to reduce the bile salt accumulation will usually need an LTx later in childhood (van Wessel et al., 2020). We know from the global BSEP deficiency registry (NAPPED) that only about one third of severe BSEP deficiency patients reach adulthood with their native liver (van Wessel et al., 2020). Even though LTx offers an often curative approach for these patients, it is not a panacea. There are cases (mostly in patients with no native BSEP expression, such as caused by PPTMs) in which alloimmunization against the donor BSEP developed after LTx, causing phenotypical BSEP deficiency disease recurrence that required immunosuppressive treatment intensification and sometimes even re-transplantation (Kubitz et al., 2015; Krebs-Schmitt et al., 2019). BSEP deficiency has also been associated with the development of hepatocellular carcinoma (HCC) often before 5 years, a complication which occurs in up to 34% of PFIC2 patients with two PPTM in *ABCB11* (van Wessel et al., 2020).

1.3. MDR3 deficiency (PFIC3)

MDR3 deficiency is caused by mutations in the *ABCB4* (ATP Binding Cassette Subfamily B Member 4) gene. Differently from FIC1 and BSEP deficiency, MDR3 deficiency does not only have a phenotype upon homozygous or compound heterozygous mutations in the MDR3 gene. Rather, patients with a single *ABCB4* mutation can have a clinical phenotype, either in childhood (e.g., cholelithiasis) or in adulthood (e.g., intrahepatic cholestasis of pregnancy) (Mehl et al., 2016; Sundaram and Sokol, 2007). The MDR3 protein is responsible for bile acid-induced phospholipid translocation from the hepatocyte into the bile (Sundaram and Sokol, 2007). MDR3 deficiency has a later onset of symptoms than FIC1 and BSEP deficiencies, generally after the first year of life. An enhanced tendency for cholelithiasis, intrahepatic cholestasis of pregnancy, or drug-induced cholestasis when only one of the ABCB4 alleles is mutated may become apparent in the family history of pediatric patients with severe cholestasis secondary to bi-allelic mutations (Sundaram and Sokol, 2007).

In contrast to FIC1 and BSEP deficiency, MDR3 deficiency patients have a markedly elevated serum gamma glutamyl transferase (GGT). MDR3 deficiency patients can develop progressive peri-portal inflammation and biliary cirrhosis leading to portal hypertension, but the progression of disease towards the need for transplantation tends to be slower than in FIC1 and BSEP deficiency (Sundaram and Sokol, 2007).

UDCA is the primary treatment for MDR3 deficiency and is thought to decrease the detergent activity of biliary bile acids by changing its composition in favor of hydrophilic bile acids, which are less toxic to the bile duct epithelium. Partial biliary diversion, a proven therapy for PFIC types 1 and 2, has only scarcely been applied in patients with MDR3 deficiency (Lemoine et al., 2017), although theoretically one would expect a clinical benefit. LTx seems to provide the best therapeutic option for patients who are unresponsive to UDCA or have progressive disease despite an initially positive response. The use of partial grafts from living related donors is theoretically not ideal, because of the reported clinical consequences of MDR3 deficiencies with monoallelic mutations. Yet, the heterozygote *ABCB4* carrier state in donors may not necessarily have major adverse effects on the short-term outcome of transplanted patients (Sundaram and Sokol, 2007).

1.4. TJP2 deficiency

TJP2 deficiency is even more uncommon than the classical PFIC types 1–3. The *TJP2* gene codes for the tight junction protein 2 which is part of the membrane-associated guanylate cyclase family. *TJP2* can stabilize tight junctions by binding to junctional transmembrane

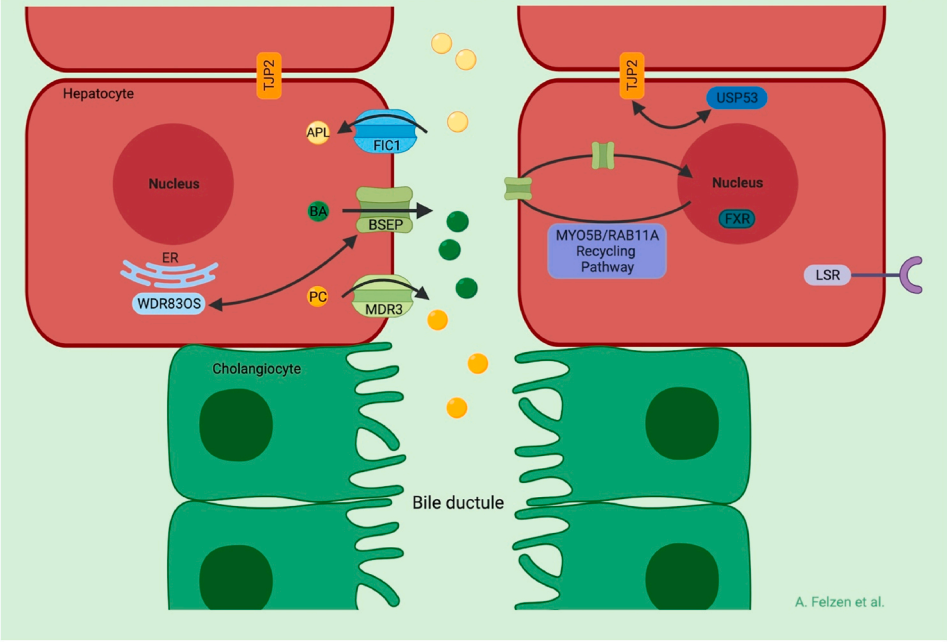

Fig. 2. Progressive familial intrahepatic cholestasis associated proteins. *FIC1, familial intrahepatic cholestasis protein 1; BSEP, Bile Salt Export Pump; MDR3, multi-drug-resistance-protein 3; TJP2, Tight Junction Protein 2; FXR, Farsenoid X Receptor; MYO5B, Myosin 5 B; RAB11A, Ras-related protein Rab-11A; USP53, Ubiquitin Specific Peptidase 53; LSR, Lipolysis stimulated lipoprotein receptor; WDR83OS, WD Repeat Domain 83 Opposite Strand; APL, Aminophospholipids; BA, Bile Acids; PC, phosphadylcholine; ER, Endoplasmatic Reticulum. Created with BioRender.com.*

proteins (Wei et al., 2020). It is expressed at the cytoplasmic side of tight junctions in epithelial and endothelial cells and thus present in many different types of organs (Sambrotta and Thompson, 2015).

The hepatocyte membranes at the junction of the bile duct canaliculi and the hepatocyte basal membrane surface form a tight junction essential for the separation of bile from plasma. Loss-of-function variants in *TJP2* are thought to affect the composition and functional integrity of the tight junction complex between the cells and lead to severe cholestatic liver disease, along with extrahepatic manifestations such as hearing loss, deafness, neurological and respiratory disorders (Wei et al., 2020; Sambrotta and Thompson, 2015). Yet, the liver seems to be the main organ affected in *TJP2* deficiency. Sambrotta et al. hypothesized that other organs may have more effective compensatory mechanisms than the liver (Sambrotta and Thompson, 2015). Currently, treatment is largely and individually based on the severity of this rare disease and includes liver transplantation upon the development of end stage liver disease.

1.5. FXR deficiency

Defects in FXR caused by mutations in the *NR1H4* gene can cause a phenotype with similarities to FIC1 or BSEP deficiency. Gomez-Ozpina et al. reported four patients with neonatal cholestasis and mutations found in the *NR1H4* gene (Gomez-Ospina et al., 2016). FXR is a nuclear hormone receptor, predominantly expressed in liver and intestine, for which bile acids are identified as the endogenous ligands. FXR serves many functions, including feedback regulation of hepatic bile acid synthesis. The presentation of FXR deficiency patients involved a neonatal onset of cholestasis, that subsequently persisted and rapidly progressed to end-stage liver disease. Frequently, these patients showed signs of vitamin K independent coagulopathy, normal GGT and undetectable BSEP expression (Gomez-Ospina et al., 2016).

1.6. MYO5B deficiency

A deficiency in the *MYO5B* gene can cause a progressive form of cholestasis in early life. Thus far there is only limited information regarding the pathophysiology and natural course of disease. Mutations in *MYO5B* can cause (intestinal) microvillus inclusion disease (MVID) which is a congenital disorder of the enterocyte causing a secretory diarrhea (Gonzales et al., 2017). In MYO5B deficient patients with the hepatic phenotype the cholestasis may result from an impaired MYO5B/RAB11A interaction, altering the targeting of the BSEP to the canalicular membrane of hepatocytes (Gonzales et al., 2017). Patients with MYO5B deficiency also show a low-normal GGT cholestasis. Furthermore, Gonzales et al. showed data of 5 patients which suggested that MYO5B deficiency may also lead to isolated cholestasis without a clinically evident intestinal phenotype (Gonzales et al., 2017).

1.7. Recently discovered gene loci

In a 2019 study by Maddirevula et al. three other gene loci among the PFIC spectrum and their corresponding disease phenotypes were described, namely the USP53, LSR and the WDR83OS gene (Maddirevula et al., 2019). The USP53 gene is thought to interact with TJP2 causing a defective tight junction complex, resulting in a similar phenotype to TJP2 deficiency. In the affected patients it leads to cholestasis, pruritus, hypocalcemia, and hearing loss (Maddirevula et al., 2019). Another study in 7 patients with USP53 deficiency found a highly variable age of onset ranging from early infancy to 15 years of age and reported heart failure as an extrahepatic manifestation in one patient (Bull et al., 2021). LSR has a role in liver development and deficiency of LSR also caused decreased liver size in different animal models (Mesli et al., 2004; Dokmanovic-Chouinard et al., 2008). In the affected family it was striking that apart from typical cholestatic features there were also prominent extrahepatic manifestations including severe fine motor discoordination, intellectual disability and short stature. The WDR83OS gene seems to interact with the BSEP although the exact mechanism remains to be understood. Patients with a WDR83OS defect also displayed extrahepatic manifestations among which were microcephaly, dysmorphic facies and genital abnormalities (Table 1) (Maddirevula et al., 2019).

2. Novel developments

2.1. ASBT inhibitors

Bile acid absorption in the terminal ileum is mediated by the apical sodium-dependent bile acid transporter (ASBT, synonymous with ileal bile acid transporter, IBAT) (van der Woerd et al., 2017). Recently, ASBT inhibition has become a therapeutic target of intervention in patients with progressive cholestatic disease. Surgical interruption of the enterohepatic circulation by external or internal bile diversion had been demonstrated to reduce serum bile acid concentration, to improve pruritus and to prolong native liver survival. The approach to inhibit intestinal ASBT activity aims to interrupt the enterohepatic circulation in a non-surgical, titratable, and reversible fashion. Recently, the results of the first randomized controlled ASBT inhibitor trial in FIC1 and BSEP deficient patients (Odevixibat, NCT03566238) have become available, demonstrating a significant reduction of pruritus and serum bile acid concentration, compared with placebo (Kamath et al., 2020). Another study, with a different ASBT inhibitor, is presently ongoing (Maralixibat, NCT03905330), see Table 2.

The presently available data on ASBT inhibition holds promise for its future application in patients with other forms of progressive cholestasis. BSEP deficiency patients with two PPTMs in the *ABCB11* gene were generally neither responsive to ASBT inhibition nor to SBD. Interestingly, FIC1 deficiency or BSEP deficiency patients with other (non-PPTM) mutations are not guaranteed to be responsive to ASBT inhibition or SBD either. It is presently still rather unclear which genetic and/or environmental factors determine the responsiveness of treatment. Recently, (July 2021), the ABST inhibitor Odevixibat was approved for use in PFIC patients by the European Medicine Agency and the Food and Drug Administration regulatory authorities.

2.2. Chaperone therapy

A wide variety of genetic diseases is mechanistically due to improper folding of the encoded protein, usually due to a missense mutation in the respective gene, which prevents its passage through the intracellular transport machinery and results in decreased amounts of biologically active proteins at the physiological site of action. Molecular and chemical chaperones have been developed, aiming to stabilize and correct protein folding defects, thereby allowing passage through and beyond intracellular quality control machinery, instead of premature degradation (Welch and Brown, 1996; Brown et al., 1996). This approach has specifically been successful in patients with cystic fibrosis where it (partially) restored the cystic fibrosis transmembrane conductance regulator (CFTR) membrane expression and transport function (Brown et al., 1996). In the progressive genetic cholestatic diseases, chaperone treatment has also been suggested as effective methodology to enhance cell surface expression and transport capacity (Hayashi and Sugiyama, 2007). Gonzales et al. reported a case study of a BSEP deficiency patient, suggesting that the chaperone 4-phenyl butyric acid (4-PB) could reduce serum bile acid concentration and pruritus and improve serum liver function tests (Gonzales et al., 2012). Clinical improvement sustained, and no severe side effects were observed after a follow-up up to 19 months. After 3 months of 4-PB treatment, a canalicular BSEP immunostaining on liver biopsy, that had not been detected before starting treatment, suggested that 4-PB treatment had stimulated the successful intracellular transport of proteins to the bile canalicular membrane (Gonzales et al., 2012).

Table 1
Progressive familial intrahepatic cholestasis associated diseases (van Wessel et al., 2020, 2021; Maddirevula et al., 2019; Goldberg and Mack, 2020).

	FIC1 deficiency	BSEP deficiency	MDR3 deficiency	TJP2 deficiency	FXR deficiency	MYO5B deficiency	USP53 deficiency	LSR deficiency	WDR83OS deficiency
Affected gene	ATP8B1	ABCB11	ABCB4	TJP2	NR1H4	MYO5B	USP53	LSR	WDR83OS
Affected protein	FIC1	BSEP	MDR3	TJP2	FXR	Myosin 5B	USP53	LSR	WDR83OS
Protein function	Phosphatidylserine flippase translocates phospholipids from the outer to the inner leaflet of the bile canalicular membrane	ATP-dependent transport of bile acids against concentration gradient	Transport of phosphatidylcholine from the inner to the outer leaflet of the bile canaliculus and subsequent bile-acid dependent secretion	Regulates tight junction integrity and regulated passage of molecules between hepatocytes and prevents bile acid reflux	Nuclear BA receptor and regulator of BA metabolism	Cell polarization and trafficking of bile canalicular proteins, including BSEP	Interacts with TJP2	Regulation of liver development	Interacts with BSEP
Phenotype	• Low GGT cholestasis • Hearing loss • Pancreatic insufficiency • BRIC1 • ICP1	• Low GGT cholestasis • Early onset liver cirrhosis • HCC • BRIC2 • ICP2	• High GGT cholestasis • Late onset • Gallstones • HCC risk • ICP3	• Low or high GGT cholestasis • Rapid progression • HCC risk	• Normal GGT cholestasis • Rapid progression	• Normal GGT, episodic cholestasis • MVID	• Normal GGT cholestasis • Pruritus • Hypocalcemia • Hearing loss	• Normal GGT cholestasis • Pruritus • Hypocalcemia • Severe fine motor discoordination • Intellectual disability • Short stature	• Hypercholanemia • Pruritus • Intellectual disability • Short stature • Microcephaly • Dysmorphic facies • Genital abnormalities
Therapy	Medical (see Fig. 1) SBD, odevixibat LTx	Medical (see Fig. 1) SBD, odevixibat LTx	Medical (see Fig. 1) UDCA, LTx	Medical (see Fig. 1) LTx	Medical (see Fig. 1) LTx	Medical (see Fig. 1) LTx	UDCA Rifampicin LTx	UDCA Rifampicin	Not reported
Prognosis	NLS: 44% at 18y	NLS: 3.5–20.4 years depending on subclass	Variable, from LTx in childhood to medically controlled disease in adulthood	Poor	Poor	Unknown	One LTx at 6y One alive with NL at 15y One alive with NL at 1y	Unknown	Unknown

FIC1, familial intrahepatic cholestasis protein 1; BSEP, Bile Salt Export Pump; MDR3, multi-drug-resistance-protein 3; TJP2, Tight Junction Protein 2; FXR, Farsenoid X Receptor; MYO5B, Myosin 5 B; USP53, Ubiquitin Specific Peptidase 53; LSR, Lipolysis stimulated lipoprotein receptor; WDR83OS, WD Repeat Domain 83 Opposite Strand; ATP8B1, ATPase Phospholipid Transporting 8B1; ABCB11, ATP Binding Cassette Subfamily B Member 11; ABCB4, ATP Binding Cassette Subfamily B Member 4; NR1H4, Nuclear Receptor Subfamily 1 Group H Member 4; BA, Bile Acid; ICP, Intrahepatic Cholestasis of Pregnancy; GGT, Gamma-glutamyl transferase; MVID, Microvillus Inclusion Disease; SBD, Surgical Biliary Diversion; LTx, Liver Transplantation; UDCA, Ursodeoxycholic Acid; NLS, Native Liver Survival; y, years; NL, native liver.

Table 2
Current clinical trials on ASBT/IBAT inhibitors.

	Odevixibat (A4250)	Maralixibat (Formerly LUM001 and SHP625)
Trial indication	Efficacy and safety, PFIC1 and PFIC2	Efficacy and safety in PFIC (primary cohort PFIC2; supplemental cohort with PFIC1, PFIC3 and PFIC4)
Primary endpoints	Change in pruritus severity Bile acid reduction	Change in pruritus severity
Current phase	Double-Blind, Randomized, Placebo-controlled trial finished. Approval for use in PFIC disease (EMA, FDA; July 2021)	Phase 3 Double-blind, Randomized Placebo-controlled trial (on-going)
Company/ sponsor	Albireo	Mirum Pharmaceuticals

ASBT, Apical Sodium-dependent Bile acid Transporter; IBAT, Ileal Bile Acid Transporter; PFIC, Progressive Familial Intrahepatic Cholestasis.

Thus, molecular chaperone therapy is potentially effective in patients with mutations causing misfolding of BSEP proteins. In FIC1 deficiency, a study by Van der Woerd et al. showed that several CFTR correctors were able to improve trafficking of FIC1 to the plasma membrane *in vitro* (van der Woerd et al., 2016). The approach may also be applicable to other progressive cholestatic diseases due to defective intracellular protein targeting.

However, caution is warranted before its widespread use: a case study in a FIC1 deficiency patient has suggested that 4-PB could lead to phenylacetate hepatotoxicity (Gonzales et al., 2015). Discontinuation of rifampicin therapy, which had been used to treat pruritus, resulted in severe acute liver injury, luckily reversible, that was potentially the result of phenylacetate toxicity. In this context, the authors cautioned that interactions between 4-PB and cytochrome P450 enzymes should be considered in the use of this agent with special attention to potential phenylacetate toxicity (Shneider et al., 2016).

2.3. Gene therapy

Another promising approach for treating PFIC diseases is gene therapy using viral vectors. Siew et al. developed a hybrid vector system to facilitate stable human *ABCB4* expression *in vivo* and to correct juvenile-onset chronic liver disease in a murine model of PFIC3 (Siew et al., 2019). A single dose of hybrid vector at birth led to life-long restoration of bile composition, prevention of biliary cirrhosis, and a substantial reduction in tumorigenesis in this mouse model. (Siew et al., 2019). Similar results have also been reported in other studies investigating gene therapy for MDR3 deficiency with long lasting positive results (Aronson et al., 2019; Weber et al., 2019). Theoretically, gene therapy, with further refinements, has potential for translation into the clinic, provided the inherent vector methodology needs to conquer the development of antibodies and the long-term dilution of expression of the corrected gene, upon initial administration to a small child (and therefore, to a small liver).

A different approach is nonsense suppression therapy (also called "readthrough" therapy) which aims to suppress translation termination at in-frame premature termination codons known as nonsense mutations, thereby restoring the translation and subsequent functional expression of the protein. Amzal et al. have studied this mechanism in an *in vitro* model of BSEP deficiency and demonstrated in a proof-of-concept study that a combination of aminoglycosides and chaperone drugs like 4-PB increased readthrough and partially restored the transport function of BSEP in selected mutations. However, it should be noted that the successful induction of canalicular expression does not guarantee actual stimulation of BSEP transport activity, which still could be suboptimal (Amzal et al., 2021).

3. Remaining questions

3.1. FIC1 deficiency

Many pathophysiological aspects of FIC1 deficiency have not been elucidated yet. The interactions between its deficient function in maintaining bilayer asymmetry of phospholipids and the clinical consequences have only partly been resolved. One recent illustration is the (epidemiologically) similar phenotype in FIC1 deficiency patients with two, one or zero PPTMs in FIC1 gene (van Wessel et al., 2021). As already indicated above, liver transplantation does not always cure the complete phenotype of the disease, but may even elicit symptoms, both in the extrahepatic organs (with mutated FIC1 expression) as well as in the transplanted liver graft. Limited information has become available that decreasing or completely preventing intestinal exposure to bile products, including bile acids, may mitigate post-transplant intestinal symptoms like persistent diarrhea. It seems reasonable to assume that first epidemiological studies are warranted to assess in more detail which factors are associated with hepatic and extrahepatic manifestations of FIC1 disease, both before and after LTx.

3.2. BSEP deficiency

The recent epidemiological and pharmaceutical data on patients with BSEP deficiency have indicated that interruption of the EHC, either surgical or medical, relieves symptoms of cholestasis and pruritus and, at least upon surgical interruption, extends survival with native liver. However, interruption of EHC does seem only effective in a fraction of BSEP deficiency patients. Apart from patients with 2 PPTMs being generally unresponsive, the underlying mechanism of (non-) responsiveness is still unclear and, so far, rather unpredictable. Jannone et al. used biliary bile acid levels in BSEP deficiency patients as a guide to decide whether or not a SBD would be successful, based on a prior nasobiliary drainage procedure (Jannone et al., 2020). There remains a clear need to better understand the pathophysiology of the disease and the (un)responsiveness to surgical or medical interruption of the enterohepatic circulation.

Upon characterization of the genetics underlying BSEP deficiency, a multitude of pathological *ABCB11* mutations has been identified of which only a few have been characterized with respect to their consequences on BSEP trafficking and/or transport functionality (Strautnieks et al., 2008). More *in vitro* studies in which the different mutations are expressed in cell systems will be necessary to improve our insights of the specific mutations at (sub-)cellular level. The same cell systems could theoretically also be used to assess the potential therapeutic action of small molecules, including compounds that have been successful in treatment of other genetic diseases, including cystic fibrosis (Brown et al., 1996; Gonzales et al., 2012; Mareux et al., 2020). The large variability in the specific *ABCB11* mutations in BSEP deficiency patients may warrant the need for individualized model systems to test *in vitro* responsiveness.

Finally, the relevance of specific single nucleotide polymorphisms (SNPs), especially of p.V444A, in the phenotype of patients with one or more pathological *ABCB11* mutations is rather unexplored. It has thus far been demonstrated that the V444A-responsible SNP on a single allele seems not sufficiently pathogenic to cause a clinically severe phenotype of BSEP deficiency but rather is associated with drug-induced liver injury and intrahepatic cholestasis of pregnancy (Ali et al., 2019). Presently, however, insights are lacking about its possible effects when the second allele carries a known pathogenic BSEP variant. The analysis of mutation combinations in which one of the alleles has the p.V444A-responsible SNP could help to advance in insights into the full spectrum of BSEP deficiency.

3.3. Other forms of progressive genetic cholestasis

Where FIC1 deficiency and BSEP deficiency have been investigated in more detail the last years, the other mentioned progressive forms of cholestasis are still lagging behind when it comes to the exploration of the natural course of disease, reaction to different therapeutical interventions and predictability of the disease outcome. More studies both *in vitro* and in cohorts are needed to better understand the disease mechanism of these diseases, their clinical phenotype, and their natural history.

4. Conclusion

Although significant progress has been made for several forms of progressive genetic cholestatic disease, the majority of patients still is likely to need an LTx during their lifetime. Yet, recent developments in medical strategies (ASBT inhibition, protein folding correctors, gene therapy) may change the current treatment strategies and may at least delay or even prevent the need for LTx.

The wide variety in clinical expressions and underlying genetic mutations, together with the low incidence of these diseases, remain a challenge towards improving the perspectives for the individual patient and the academic understanding of the pathophysiology. Lack in basic understanding of the natural history and pathophysiology is particularly prominent in the more recently discovered types of TJP2 deficiency, FXR deficiency, MYO5B deficiency, USP53 deficiency, LSR deficiency and WDR83OS deficiency and warrants further investigation.

Author agreement

I herewith certify that both authors have seen and approved the final version of the manuscript being submitted.

The article is our original work, hasn't received prior publication and isn't under consideration for publication elsewhere.

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