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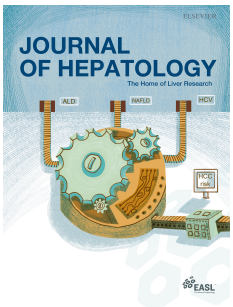

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Variants in *ABCB4* (MDR3) across the spectrum of cholestatic liver diseases in adults

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Abbreviations: *ABCB4* – ATP binding cassette subfamily B member 4, *ABCB11* – ATP binding cassette subfamily B member 11, BRIC – benign recurrent intrahepatic cholestasis, BSEP – bile salt export pump, CIC – contraceptive-induced intrahepatic cholestasis, DILI – drug-induced liver injury, FIC1 – familial intrahepatic cholestasis 1 protein, ICP – intrahepatic cholestasis of pregnancy, LPAC – low phospholipid associated cholelithiasis, MDR3 – multidrug resistance (gene) 3, PFIC – progressive familial intrahepatic cholestasis, UDCA – ursodeoxycholic acid

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M.T. served as a speaker and/or consultant and/or advisory board member for Albireo, Boehringer Ingelheim, Bristol-Myers Squibb, Falk, Genfit, Gilead, Intercept, MSD, Novartis, Phenex, Regulus and Shire, and received travel support from AbbVie, Falk, Gilead, and Intercept, as well as grants/research support from Albireo, Cymabay, Falk, Gilead, Intercept, MSD, and Takeda. He is also co-inventor of patents on the medical use of 24-norursodeoxycholic acid

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A.F.S. writing of the manuscript, literature research; E.H., F.W., P.F. literature research, critical revision of the manuscript for important intellectual content; M.T. literature research, outlining and writing of the manuscript

KEY POINTS

- several variants in *ABCB4* have been identified accounting for a disease spectrum ranging from progressive familial intrahepatic cholestasis 3 (PFIC-3) to less severe forms like low phospholipid associated cholelithiasis (LPAC), intrahepatic cholestasis of pregnancy (ICP) or drug induced liver injury (DILI)
- clinical presentation can vary widely and clear genotype-phenotype correlations are lacking so far
- PFIC-3 the most severe form is characterized by cholestasis with jaundice and pruritus presenting in late infancy, adolescence or sometimes even young adulthood and progresses to cirrhosis and end-stage liver disease
- diagnosis should be considered in patients with otherwise unexplained chronic cholestatic liver disease or hepatobiliary disease in younger ages, positive family history or consanguineous background
- diagnosis of ABCB4 deficiency associated disease is based on the exclusion of other causes of cholestatic liver disease by laboratory assessment, imaging studies, liver histology and genetic testing
- ursodeoxycholic acid is the most commonly used medical treatment, but large controlled studies on its benefit are lacking

- stimulation/restoration of residual function by chaperones or induction of transcription by FXR or PPAR agonists may be promising future therapeutic options
- in patients with end-stage liver disease and/or intractable pruritus orthotopic liver transplantation remains the last and often only therapeutic option

ABSTRACT

The ATP binding cassette subfamily B member 4 (*ABCB4*) gene on chromosome 7 encodes the ABCB4 protein (alias multi drug resistance 3 protein [MDR3]), a P-glycoprotein in the canalicular membrane of the hepatocytes that acts as a translocator of phospholipids into bile. Several variants in *ABCB4* have been identified so far that lead to ABCB4 deficiency accounting for a disease spectrum ranging from progressive familial cholestasis type 3 (PFIC-3) to less severe forms presenting as low phospholipid associated cholelithiasis (LPAC), intrahepatic cholestasis of pregnancy (ICP) or drug-induced liver injury (DILI). Furthermore, whole genome sequencing identified *ABCB4* variants to be associated with an increased incidence of gallstone disease, gallbladder and cholangiocarcinoma, liver cirrhosis or elevated liver function tests. Diagnosis of ABCB4 deficiency related diseases is based on clinical presentation, serum biomarkers, imaging techniques, liver histology and genetic testing. Nevertheless, the clinical presentation can vary widely and clear genotype-phenotype correlations are lacking so far. Ursodeoxycholic acid is the most commonly used medical treatment, but confirmation of its benefit by large controlled clinical studies is still lacking. Future pharmacological options may include stimulation/restoration of residual function by chaperones (e.g. 4-phenyl butyric acid, curcumin) or induction of *ABCB4* transcription by farnesoid X receptor (FXR) agonists or PPARα-ligands/fibrates. In cirrhotic patients with end stage liver disease or patients with intractable pruritus orthotopic liver transplantation remains the last and often only therapeutic option.

CLINICAL VIGNETTE

In 1995, a 19-year-old male, asymptomatic patient (body weight: 70 kg, height: 176 cm, BMI: 22.6 kg/m^2) was referred for evaluation of abnormal elevated liver tests detected at a routine laboratory check-up (alkaline phosphatase 406 U/L [AP; normal: 35-105 U/l], γ-glutamyltransferase: 270 U/L, [γGT; <40 U/l]; ALT: 188 U/L [<35 U/l], AST: 88 U/L [<35 U/l]). Total bilirubin, prothrombin time and albumin were in normal range. Platelet count was 171 G/L (normal range: 150-400 G/L), suggesting absence of portal hypertension. Viral and autoimmune hepatitis, Wilson disease, α_1-antitrypsin deficiency were excluded by appropriate serologic and laboratory tests. PBC specific anti-mitochondrial antibodies (AMA-M2) and antinuclear antibodies (ANA: sp100, gp210) were negative. He denied drinking alcohol and had no drug history. No intake of hepatotoxic agents or herbal supplements was reported. By sonography cholelithiasis or cholangiectasia were excluded. A percutaneous liver biopsy showed portal fibrosis with incipient bridging, scarce lymphomononuclear cell infiltration in the portal field, bile duct proliferation, but absence of cholestasis or any hepatocellular damage (figure 1a-c). Staining for cytokeratin 7 revealed that bile ducts were present in 13 of 18 portal fields thus excluding ductopenia. An endoscopic retrograde cholangiography at that time (1995) showed no large bile duct abnormalities.

No final diagnosis was made; nevertheless, treatment with ursodeoxycholic acid (UDCA) was initiated. He was lost to follow-up until 2014, when he deteriorated clinically presenting with jaundice and pruritus. A second liver biopsy showed liver cirrhosis with scarce lymphomononuclear cell infiltration (figure 1d-f). Preexisting bile ducts showed mild secondary alterations including reactive epithelial proliferation and segments of nuclear loss. No mechanical obstruction could be observed. Residual liver parenchyma showed pericellular fibrosis, reactive lymphocytic infiltrates, and Mallory-Denk bodies. Intracytoplasmic bile pigment was present in some of the

hepatocytes. Hepatic copper content was 632 µg/g dry weight (normal: <50 µg/g), and the 24-hr urinary copper excretion was 229 µg/day (both findings are common in chronic cholestatic diseases), but *ATP7B* mutation analysis revealed no evidence for Wilson disease. Further genetic testing identified a previously unknown homozygous variant in exon 28 in *ABCB4* – c.3768_3769delAG (p.R1256Sfs*39; reference sequence #NM_018849.2) – a frameshift mutation resulting in a consecutive prolongation of 39 additional amino acids of the protein. According to *in-silico* testing (MutationTaster, Mutalyzer) the mutation was rated as disease causing. Furthermore, a well-known *per se* non-disease causing homozygous variant in *ABCB11* (c.1331T>C [p.V444A]) could be identified.

Due to decompensated liver disease (MELD score 24) with ascites and jaundice the patient was listed for orthotopic liver transplantation. Subsequently, the patient further deteriorated with esophageal variceal hemorrhage, ascites and acute kidney injury (MELD score at admission: 31). He died several days later as a result of multi organ failure.

Family screening identified his clinically asymptomatic sister (34 years at diagnosis) as homozygous carrier of the same variant in *ABCB4*. All routine liver parameters were in the normal range (AP: 60 U/L, γGT: 26 U/L, AST 15 U/L [<35 U/l], ALT 24 U/L [< 35 U/l]) at diagnosis. Total fasting bile acids were slightly elevated: 13 µmol/L (<10 µmol/L). Treatment with UDCA was initiated. She is now on regular follow up for five years. The patient is in stable condition under UDCA treatment, with normal cholestasis markers but mildly elevated transaminases. Fasting bile acids were elevated over time and showed a fluctuating course (2017: 24.5 µmol/L, peak in 2018: 204.1 µmol/L, 2019: 34.0 µmol/L), but the patient did not complain of pruritus. Repeated MR-imaging presented no alterations of the intrahepatic bile ducts (figure

2a-b). Repeated transient elastography showed a liver stiffness consistent with absence of fibrosis (2016: 4.0 kPa; 2017: 3.5 kPa; 2018: 4.3 kPa).

These two cases highlight the phenotypic diversity of *ABCB4* variants. No clear diagnosis was made at the time of referral of the index patient (age 19). Within 11 years, he progressed from cholestatic liver disease with portal fibrosis to decompensated cirrhosis. The final diagnosis was made by genetic testing. However, his sister (now at the age of 40) with the same homozygous variant has no evidence of advanced liver disease so far.

INTRODUCTION

Over the past two decades, modern genetic studies yielded major novel insights into our understanding of the molecular-biological mechanisms and pathophysiology of liver diseases. The fundamental technologies for genetic analysis provided positional cloning for so far unidentified disease genes and simple gene tests for known single nucleotide polymorphisms (SNP). Further, genome wide association studies (GWAS) compared genotype frequencies across the whole genome between disease and control groups and thus identified new genetic risk factors. Next generation sequencing (NGS) made it possible to identify different variants within the exome (whole exome sequencing, WES) or even the whole genome (whole genome sequencing, WGS) in individual patients[1,2]. However, despite the possibilities and limitations of state-of-the-art computational methods, the assignment of certain variants to pathogenicity remains difficult[3].

A heterogenous group of cholestatic liver diseases with autosomal-recessive inheritance was first described by Clayton et al. in 1969 as Byler's disease in a population of Amish kindred[4]. Later, based on the underlying genetic defect of hepatobiliary transport systems the disease has been reclassified in three types and renamed into progressive familial intrahepatic cholestasis (PFIC) type 1, 2 and 3[5,6,7] (see overview in table 1). Although, the exact prevalence is unknown, PFIC are rare diseases with an overall estimated incidence of 1 per 50,000 to 1 per 100,000 births[8]. Nevertheless, they account for up to 10-15 % of neonatal cholestasis syndromes and are the cause for liver transplantation requirement in almost 10-15 % in childhood[9].

PFIC-1 (formerly known as Byler's disease) is associated with genetic defects in *ATP8B1* on chromosome 18 (18q21) which encodes familial intrahepatic cholestasis 1 protein (FIC1), a member of the type 4 subfamily of P type adenosine triphosphatase (ATPase)[5]. ATP8B1 is a multispan transmembrane protein with

flippase activity, that translocates aminophospholipids from the outer (exoplasmic) to the inner (endoplasmic) leaflet of the biological canalicular membrane in hepatocytes[10]. PFIC-2, which was formerly known as Byler's syndrome, is caused by gene defect in *ABCB11*. *ABCB11* (ATP binding cassette subfamily B member 11) resides on chromosome 2 (2q31) and encodes the canalicular bile salt export pump (BSEP)[11] as the main transporter of bile acids from hepatocytes into bile.

PFIC-3 is caused by variants in *ABCB4* (ATP binding cassette subfamily B member 4) located on chromosome 7 (7q21) encoding for the ABCB4 protein[12]. Recently, further gene defects were detected in patients with PFIC (PFIC4: *TJP2*, PFIC5: *FXR*, PFIC6: *MYO5B*; figure 3)[13,14,15].

The ABCB4 protein (also known as multi drug resistance 3 protein [MDR3]) is a P-glycoprotein that acts as a phospholipid translocator in the canalicular membrane of hepatocytes responsible for secretion of phospholipids, predominantly phosphatidylcholine, into bile[16,17].

In contrast to patients with PFIC-1 or -2, who present symptoms very early in childhood (4-5 months of age), PFIC-3 patients usually develop cholestasis in late infancy or adolescent age group. In some cases of PFIC-3 signs of cirrhosis and portal hypertension (e.g. gastrointestinal bleeding) might appear as the first symptom in older children or even young adults[8] (table 1).

Genetic changes (e.g. missense variations in the coding sequence, insertion, deletion, variations in the splicing area) in the *ABCB4* gene can result in either missense or nonsense variants leading to premature stop with loss of function, truncation or even complete failure of production of the protein, reflecting the wide clinical spectrum of *ABCB4* associated diseases. PFIC-3 is associated with homozygous or compound-heterozygous variants with severe gene defects causing usually either a premature truncation of the protein or a total failure of protein

production, while heterozygous *ABCB4* variants result in less severe clinical patterns. Milder phenotypes of PFIC-3 may present as ICP[18], cholesterol gallstone disease (low phospholipid-associated cholelithiasis, LPAC)[19], drug-induced cholestasis or liver injury[20], adult idiopathic/cryptogenic cirrhosis[21,22] or transient neonatal cholestasis[23].

Whole genome sequencing in a large-scale Icelandic population[24] identified an association of *ABCB4* mutations with higher risk of gallstone diseases, gallbladder and bile duct carcinoma, liver cirrhosis and even higher serum levels of liver-related biomarkers like aspartate transaminase (AST), alanine transaminase (ALT) and γ-glutamyl transpeptidase (γGT). *ABCB4* is now on the map of the most critical mutations that aggravate the progression to chronic liver disease[25].

Aim of this review is to give a concise synopsis on the spectrum of diseases of the hepatobiliary system related to mutations in the *ABCB4* gene.

PATHOPHYSIOLOGY AND CLINICAL PRESENTATION

The *ABCB4* gene resides on chromosome 7 (7q21) and encodes the ABCB4 protein[12], a P-glycoprotein which is expressed in the canalicular membrane of hepatocytes functioning as a phospholipid translocator. It acts as a floppase transporting lipids from the inner to the outer leaflet of the membrane and thereby is responsible for phospholipid secretion – predominantly phosphatidylcholine – into the bile[26]. The ABCB4 protein consists of two cytoplasmic nucleotide binding domains (NBDs) and two transmembrane domains, each with six transmembrane segments. The NBDs are large domains that include highly conserved sequences for ATP binding like the Walker A and B motifs, the signature sequence and the A-, D-, H- and Q-loop[27].

Biliary phospholipids are responsible for neutralization of the detergent effects of hydrophobic bile salts by formation of mixed micelles[28]. Thus, intrahepatic cholestasis in liver diseases associated with ABCB4 protein defects is a result of injury of the biliary epithelium and canalicular membrane facing the highest bile acid concentrations in the setting of a “toxic” bile composition[29,17]. Moreover, low phospholipid levels lead to destabilization of micelles and thus promote cholesterol crystallization increasing biliary lithogenicity. Increased lithogenicity in turn facilitates liver damage by obstruction of small bile ducts[9]. In contrast to cholestasis associated with variants in *ATP8B1* and *ABCB11*, ABCB4 deficiency related cholestasis is generally characterized by higher levels of γ-glutamyl transpeptidase (high γGT cholestasis), which might reflect direct toxic effects to the epithelium of the bile ducts due to relatively higher free bile salt concentrations compared to lower phospholipid levels[23,29]. Nevertheless, a study by Schneider et al.[30] showed that γGT is not a reliable marker to discriminate between cholestasis attributed to ATP8B1, ABCB11 or

ABCB4 associated disease. Some patients with disease causing variants in *ABCB4* may present with low or even normal γGT levels (see case described in figure 4).

Delaunay et al.[31] proposed a classification of *ABCB4* variations in PFIC-3 as either nonsense variations (I) or missense variations affecting primarily the maturation (II), the activity (III) or the stability (IV) of the protein, and mutations without detectable defect (V) similar to classifications proposed initially for cystic fibrosis[32]. While patients with PFIC-3 are predominantly homozygous or compound-heterozygous with nonsense mutations, less severe clinical phenotypes of cholestatic liver disease like intrahepatic cholestasis of pregnancy (ICP) or contraceptive induced cholestasis (CIC), low phospholipid-associated cholelithiasis (LPAC), transient neonatal cholestasis, drug-induced liver injury (DILI) or adult idiopathic cirrhosis[33] are linked to heterozygous variants of *ABCB4* or are homozygous with at least partially preserved protein function[34] (table 2). Several studies have been published so far to address genotype-phenotype correlations in patients with *ABCB4* associated cholestatic liver diseases[23,31,35,36]. Compound-heterozygous or homozygous carriers of disease-causing mutations had higher rates of progression to cirrhosis and end-stage liver disease, whereas heterozygotes were generally associated with less severe disease or even absence of symptoms[37]. In LPAC patients most subjects were heterozygous and had missense mutations; subjects harboring a truncating variant had an earlier disease onset than those with missense mutations, but nevertheless the occurrence of severe liver disease was independent of the genotype[38].

Progressive familial intrahepatic cholestasis type 3 (PFIC-3)

Cholestasis, characterized by jaundice and pruritus is the clinical hallmark of all types of PFIC. While patients with PFIC-1 and -2 develop symptoms in very early infancy (PFIC-1) or even during the neonatal period (PFIC-2), the age of onset in PFIC-3 is generally later in late infancy (around one third of cases) or even in adolescence or

young adulthood[9] (table 1). Pruritus is generally less severe in PFIC-3 than in PFIC-1 or -2. Complications due to cirrhosis and portal hypertension like gastrointestinal bleeding can often be the first clinical presentation in older children and young adults. PFIC-3 usually progresses from chronic cholestasis with or without jaundice to portal hypertension and end-stage liver disease within the first to second decade of life. Patients suffering from PFIC-3 are also at an increased risk for development of cholesterol stones in intrahepatic bile ducts as well as in the gall bladder[38]. Furthermore, the risk for the occurrence of hepatocellular carcinoma (HCC) in these patients is mildly increased. In contrast to PFIC-1, extrahepatic disease manifestations (diarrhea, pancreatitis, pneumonia, hearing loss, short stature) are generally absent. In female subjects, cholestasis and jaundice may present first in early adulthood due to hormonal changes (e.g. intake of oral contraceptives containing estrogenes or progesteron, pregnancy) and thus may be misinterpreted as intrahepatic cholestasis in pregnancy (ICP) or contraceptive induced cholestasis (CIC)[39]. Laboratory findings show elevated γGT activity which is a typical finding in PFIC-3 (high γGT cholestasis), but usually not in other types of PFIC. Serum ALT and AST levels are usually only mildly increased. Serum bile acids are raised but not as high as in patients with PFIC-1 or -2.

Low phospholipid-associated cholelithiasis (LPAC)

LPAC is a rare form of intrahepatic cholelithiasis occurring in young adults and is associated with mutations in the *ABCB4* gene. It was first described by Rosmorduc et al.[19] in 2001 as a peculiar form of intrahepatic cholelithiasis and gallbladder cholesterol stones in two men and four women (age between 26 and 55 years). In all six patients, a mutation in *ABCB4* was found, and all responded well to UDCA treatment. In a second study[40] by the same authors, 32 predominantly female

subjects confirmed these observations in 2003 and several cases have been published since.

LPAC should be suspected in patients with cholesterol gallbladder stones and at least two of the following characteristics[41]: (1) onset of symptoms before the age of 40 years, (2) intrahepatic hyperechogenic foci, sludge or microlithiasis, (3) recurrence of biliary symptoms (jaundice, biliary colic, cholangitis, acute pancreatitis) after cholecystectomy. In a large study on patients fulfilling the diagnostic criteria of LPAC half of them harbored a variant in *ABCB4* (predominantly heterozygous missense variants)[35]. Often, a family history of gallstones in first-degree relatives can be found. Furthermore, LPAC is more prevalent in women and shows a strong association with ICP[35]. In a small study[42] in young female patients almost 25 % admitted for symptomatic cholelithiasis had LPAC, particularly those with normal body weight and onset under 18 years.

Again, low phospholipid concentrations in the bile lead to a bile composition that is supersaturated with cholesterol and hence highly lithogenic leading to the formation of macroscopic stones. Moreover, cholesterol crystals precipitate in the bile and damage the cholangiocyte epithelium together with free (non-micellar bound) bile acids. Importantly, biliary lesions induce cholestasis with elevated γGT levels.

Intrahepatic cholestasis in pregnancy (ICP)

ICP is defined as an acquired form of cholestasis during pregnancy in otherwise healthy women with normal medical history. It usually occurs during the second or third trimester of pregnancy, while serum concentrations of estrogens and progesterone reach their peak. ICP represents the most common pregnancy-related liver disease[43]. Its incidence varies widely across different geographical regions and ethnical background between 0.05 to more than 20 %[44]. The highest incidence rates were reported from Chile in women with Araucanian Indian descent. In Europe, the

prevalence of ICP is around 2 %, 15 % of which are attributable to ABCB4 deficiency[45].

Clinical symptoms quickly resolve after delivery (typically within 12 weeks *post-partum*), which supports the key role of female sex hormones in the pathophysiology. Women, affected by ICP, do also show a higher susceptibility to develop cholestasis under oral contraception (contraceptive induced cholestasis, CIC)[23]. Furthermore, environmental co-factors seem to play a pivotal role in ICP as it is indicated by its wide variation in incidence according to different geographic regions. Besides hormonal and most likely environmental factors, genetic susceptibility accounts for higher risk for ICP. Association with transporter mutations in *ATP8B1*, *ABCB11* and *ABCB4*[43], were observed, so far and more than 10 % of women with ICP harbor a mutated allele in *ABCB4*[46]. A pathogenic role of *ABCB4* was first discussed in a large consanguineous family with an index case suffering from PFIC-3 and several female family members with recurrent ICP episodes[47].

ICP is characterized by pruritus, raised bile salt and transaminases levels and rarely elevated concentrations of serum bilirubin in about 25 % of cases. Elevation of fasted serum bile acids above 10 µmol/L is often the first and sometimes even the only laboratory abnormality in ICP. In ICP associated with ABCB4 deficiency serum γGT levels are elevated and may distinguish it from other forms of ICP[43]. Other laboratory findings include elevation of total and direct bilirubin and transaminases (2- to 10-fold increase).

ICP is associated with increased risk for perinatal complications, including premature delivery, respiratory distress, meconium staining of the amniotic fluid, low Apgar scores, and even stillbirth[48]. A recent meta-analysis showed that risk of stillbirth significantly increased with a maternal serum bile acid level >100 µmol/L[49]. As serum bile acid levels are generally <100 µmol/L in ICP the risk of stillbirth in these women

is probably comparable to the general population, provided serum bile testing is performed until delivery.

Although, ICP is reversible after delivery, it may unmask an underlying defect associated with mutations in *ABCB4* which predispose to hepatobiliary diseases, cirrhosis and hepatobiliary malignancies (HCC and CCC). Regular follow-up and genetic counselling should be discussed in women with ICP due to ABCB4 deficiency or other genetic background as ICP may have adverse long-term consequences (an increased risk of gallstones, liver cirrhosis, hepatocellular cancer, autoimmune-mediated and cardiovascular diseases was reported)[50,51,52].

Drug-induced liver injury (DILI)

Multiple lines of evidence suggest that ABCB4 deficiency predisposes to cholestatic DILI (e.g., oral contraceptive-induced cholestasis [CIC][39]): molecular evidence from tissue cultures suggests that xenobiotics inhibiting P-glycoproteins including ABCB4 can also induce cholestasis in predisposed patients with *ABCB4* aberrations[53].

Patients with four different nonsynonymous variants in *ABCB4* were found to have up to threefold increased risk of cholestatic DILI from psychotropic drugs, proton pump inhibitors, or selected antibiotics and and oral contraceptives[20]. ABCB4 deficiency confers genetic predisposition for the development of DILI in general and CIC in particular. Treatment with drugs that potentially inhibit the function or expression of ABCB4 (e.g. sirolimus cyclosporine, verapamil or vinblastine) may impair biliary phosphatidylcholine excretion in subjects with genetically determined ABCB4 deficiency that is otherwise clinically silent. However, canalicular ABC transporter expression is profoundly disturbed in most cases of cholestatic DILI irrespective of *ABCB4* variants[54]. Besides, despite the potential role of different *ABCB4* variants in DILI, it has to be emphasized that HLA haplotypes are more relevant for the pathogenesis of DILI[55].

DIAGNOSTIC ASSESSMENT

Although syndromes associated with *ABCB4* variants (or other transporters in the canalicular membrane of hepatocytes) are rare, they should be considered in patients with otherwise unexplained hepatobiliary disease, consanguineous family background, or with a family history of liver disease. Furthermore, ICP, drug-induced liver injury with cholestasis or occurrence of (intrahepatic) cholelithiasis in younger ages (possibly indicative of LPAC) should draw the attention of clinicians to a possible genetic background of the disease.

Differential diagnosis primarily depends on the clinical setting. In pediatric patients Alagille syndrome and biliary atresia should be excluded. In pregnant women, other causes for intrahepatic cholestasis or acute fatty liver of pregnancy, viral hepatitis with cholestasis or HELLP syndrome (hemolysis, elevated liver enzymes, low platelets) should be considered. Differential diagnosis in adult patients includes primary biliary cholangitis (PBC) and primary sclerosing cholangitis (PSC). Besides, variants in *ABCB4* may have a pivotal role as disease modifiers in patients with adult cholestatic liver disease[56]. In young adults with otherwise unexplained cholestatic liver disease hepatic copper accumulation (like in the index patient of the clinical vignette) may mislead to the diagnosis of Wilson disease[57].

Laboratory assessment

Laboratory assessment should include routine blood tests for transaminases (ALT and AST), panels of cholestasis (AP, γGT, serum bilirubin and serum bile acids). Further, liver synthesis parameter (coagulation and serum albumin) need to be checked. Other causes of cholestatic liver disease need to be ruled out by appropriate laboratory (e.g. primary biliary cholangitis, IgG_4 associated disease, viral hepatitis), imaging testing or histological assessment. Cholestasis associated with *ABCB4* variants may show markedly higher serum levels of γGT than other

cholestatic syndromes most likely reflecting bile duct injury due to toxic biliary constituents and/or cholesterol crystals[58]. Nevertheless, as stated above[30], some patients with ABCB4 deficiency present with normal or only mildly elevated γGT levels.

Imaging investigations

Abdominal ultrasound is the first examination in patients presenting with clinical signs of cholestasis. Although, in many patients findings are normal or unspecific (e.g. signs of advanced disease like cirrhosis or portal hypertension) it helps to exclude other causes of cholestasis, especially extrahepatic cholestasis. In LPAC ultrasonography may show intrahepatic hyperechogenic foci or real gallstones with dorsal acoustic shadow. Color Doppler examination reveals “twinkling” artifacts associated with stones, which may also correspond to the “comet tail” signs formed by small stones not detected in normal ultrasound[19]. Nevertheless, it needs to be pointed out, that intrahepatic hyperechogenic foci, sludge or microlithiasis suggestive for LPAC are observed in only up to 85 % of cases in patients with established diagnosis of LPAC.

Magnetic resonance cholangiopancreaticography (MRCP) may help to rule out primary and secondary sclerosing cholangitis or extrahepatic causes for cholestasis. MRCP and computed tomography (CT) can be used to detect intrahepatic stones and to document markedly dilated bile ducts (figure 2c-f).

Liver histology

Portal fibrosis and true bile ductular proliferation can be seen in PFIC-3 at disease onset as well as mild giant cell hepatitis. Both, giant cell hepatitis, ductular proliferation and portal fibrosis are absent in PFIC-1, whereas liver histology in PFIC-2 shows lobular and portal fibrosis and pronounced hepatocellular necrosis and giant cell hepatitis[59]. In later disease stages of PFIC-3 marked portal fibrosis and biliary

cirrhosis can be observed. Disease causing variants in *ABCB4* lead to a defect in hepatic biliary phospholipid secretion with increased cholesterol saturation. Cholesterol precipitation may be seen in liver histology as lipid crystals[60] and may be suggestive but not specific for ABCB4 deficiency. As all these histological findings are non-specific, biopsy specimen should be subjected to immunohistochemical staining with antibodies against ABCB11 and ABCB4 (standardized antibodies for immunhistochemistry staining against ATP8B1 are so far not available). Though absent or faint immunostaining is diagnostic for PFIC-2 or -3 respectively, normal immunostaining cannot exclude the diagnosis of PFIC as some mutations in *ABCB4* or *ABCB11* are associated with only a functional defect of the ABCB11 or MDR3 gene product and shows otherwise normal synthesis and expression[29]. Further, in a series of heterozygous patients with *ABCB4* variants[56] immunostaining on paraffin section was not efficient, as all these patients had a strong diffuse canalicular staining. Thus, immunostaining cannot be used to rule out ABCB4 deficiency.

Presence of subtle bile duct changes in liver biopsy such as epithelial injury (with nuclear loss see figure 1), atrophy and cholesterol clefts should raise the suspicion of clinicians for the diagnosis of ABCB4 deficiency.

Genetic testing

Genetic testing is still the gold standard in diagnosis of ABCB4 deficiency and other inherited syndromes of intrahepatic cholestasis. So far, more than 500 missense and 24 loss-of-function variants in *ABCB4* are known (genome aggregation database, gnomAD: https://gnomad.broadinstitute.org). The large gene size and the lack of highly predominant mutational hotspots form obstacles in exploration of gene-gene interactions and genotype-phenotype correlations. A commercially available customized resequencing gene chip was developed that reads five of the most common genes (*SERPINA1* [α_1-antitrypsin], *JAG1*, *ATP8B1*, *ABCB11* and *ABCB4*)

responsible for intrahepatic cholestasis simultaneously in a single assay with high call rate and accuracy[61]. Recently several studies showed that in up to half of the patients, in whom cholestatic liver disease remained unexplained after standard diagnostic work-up, mutations in genes responsible for PFIC could be detected by genetic examination (sometime in heterozygous status)[62,63,64]. Thus, patients with otherwise unexplained cholestasis (see clinical vignette above) despite extensive diagnostic work-up (laboratory assessment, MRCP, liver histology) need to undergo genetic sequencing in *ABCB4* and other genes associated with cholestatic liver disease[2].

THERAPY

Medical treatment

Medical therapy is the first line treatment in all patients with any kind of PFIC or *ABCB4* mutation associated syndrome. The main objective is to provide symptom relief in case of pruritus, to improve the nutritional status of the patient and to treat or prevent complications due to cirrhosis and portal hypertension like ascites or gastro-esophageal hemorrhage. Simple procedures like moisturizing the skin or trimming nails may be helpful against pruritus. The total daily caloric intake should be 125 % of the recommended daily allowance (RDA). Dietary fat should be provided as medium chain triglycerides, which do not require bile salts for absorption. Water and fat soluble vitamins should be supplemented appropriately (1-2 times RDA). Furthermore, adequate exposition to sunlight and calcium supplementation is mandatory[65]. Hepatic osteodystrophy caused by calcium and vitamin D malabsorption leading to secondary hyperparathyreoidism, rickets or osteomalacia is a significant extrahepatic expression of chronic cholestatic liver disease[66]. Besides, preclinical studies[67,68,69] in *Abcb4*$^{-/-}$ mice indicate that vitamin D modulates biliary injury and fibrogenesis, and vitamin D deficiency seems to aggravate liver fibrosis in this animal model. Although, beneficial effects of vitamin D do not fully protect against liver fibrosis the authors speculate that adequate supplementation may abate hepatic injury and confer antifibrotic effects.

Ursodeoxycholic acid (UDCA) is the most commonly used medical treatment in patients with PFIC-3, LPAC or ICP. It is a hydrophilic bile acid that replaces toxic hydrophobic bile salts. Under long-term treatment it may amount to up to 40 % of total bile salt serum concentration[70]. Another postulated mechanism is the induction of expression of ABCB11 and ABCB4 and thus increased secretion of bile acids and phospholipids into the bile[71]. In PFIC-3 around 70 % of patients respond to UDCA[72];

nevertheless, in patients with low γGT cholestasis (approximately 35-40 %)[73] and in patients with a total defect in ABCB4 expression a response to UDCA is unlikely[23]. UDCA is a safe drug with no major side effects or teratogenicity allowing its use during pregnancy in patients with ICP. In a meta-analysis[74], it showed to be effective against maternal pruritus and in improving liver tests in women with ICP. On the other hand, a recent randomized placebo-controlled trial testing UDCA in ICP (PITCHES) observed no difference between UDCA or placebo treated women regarding adverse perinatal outcomes (perinatal death, preterm delivery, or neonatal unit admission) [75]. Thus, the authors concluded that its use needs to be reconsidered in ICP. However, it is possible that the PITCHES trial population included also participants who did not suffer from ICP according to the EASL CPG, but had either pruritus without cholestasis or with an underlying chronic liver disease different from ICP, since in some included patients pruritus, serum bile acids and ALT tended to decrease rather than increase which is unexpected, considering the fact that in patients with ICP pruritus usually worsens and ALT and serum bile acids usually increase during the third trimester[76]. Based on these uncertainties in PITCHES trial UDCA treatment may be useless in pregnant women with pruritus during pregnancy who do not have an underlying secretory defect of the liver, but its benefit in ICP has to be evaluated in further trials.

Rifampicin and cholestyramine are used for symptomatic treatment of cholestasis-associated pruritus. Rifampicin is the strongest inductor of CYP3A4 expression increasing the 6-α hydroxylation of bile salts, which are thereafter glucuronidated and excreted in the urine. It induces the uridine diphosphate (UDP)-glucuronosyl transferase (UGT1A1) leading to increased conjugation and excretion of bilirubin. Enhanced detoxification of bile acids and bilirubin conjugation by rifampicin together with stimulation of hepatobiliary transport systems by UDCA seem to have

independent but complementary effects in patients with cholestatic diseases[71] and may also benefit in patients with ABCB4 deficiency.

Rifampicin may be used as second-line treatment in patients who failed UDCA treatment and a recent study showed successful decline in serum bile acids in women with severe ICP[77]. Besides, cholestyramine, dexamethasone and S-adenosyl-L-methionine have been used in ICP so far, but due to varying results and lack of data none of them can be considered as first-line treatment[71].

Fibrates, peroxisome proliferator-activated receptor (PPAR) α ligands, have been successfully used in patients with cholestasis (mostly PBC) for the last two decades[78]. Bezafibrate has been recently proved beneficial in a randomized placebo-controlled trial in patients with PBC not responding to UDCA[79] and in patients with cholestatic pruritus[80]. One of the postulated mechanisms of action of bezafibrate is a PPARα dependent increase in ABCB4 expression in human and murine cells[81] and biliary phospholipid excretion as shown in bezafibrate treated patients undergoing percutaneous biliary drainage[82]. Similarly, fenofibrate, another PPARα ligand, also upregulated ABCB4 in primary cultured human hepatocytes and stimulated its activity in HepG2 cells. Further, fenofibrate increased phosphatidylcholine excretion into bile canaliculi in cultured rat hepatocytes[83]. In regard with these findings, fibrates could represent an interesting novel therapeutic strategy in patients with PFIC-3 and the entire spectrum of ABCB4 deficiency.

The bile acid-activated nuclear farnesoid X receptor (FXR) is considered the master regulator in bile acid metabolism in the liver. FXR induces the transcription of *ABCB4* and *ABCB11* and thereby export of bile acids and phospholipids from the hepatocyte into the canaliculus (figure 3)[84]. This makes FXR a potential target for treatment in patients with chronic cholestatic liver disease as reflected by its recent approval as second line treatment in PBC[85]. In a recent study the FXR agonist obeticholic acid

(OCA) significantly induced *ABCB4* in human precision cut liver slices[86]. Another study in an *Abcb4*$^{-/-}$ mouse model showed that dual FXR/TGR5 (membrane G protein-coupled receptor) activation improved liver injury by reduction of biliary bile acid output and promotion of HCO_3^--rich bile secretion[87].

A recent study[88] uncovered an additional pathway of *ABCB4* regulation in mice. It showed that ABCB4 is hormonally regulated at a transcriptional level by ligands for the liver specific thyroid hormone receptor β (currently also developed as therapeutic target for NASH) possibly opening additional therapeutic opportunities for ABCB4-related liver disease.

Within the past years several studies showed promising results to ameliorate the consequences of disease causing variants in *ABCB4*. Drugs with chaperone-like activity like curcumin and 4-phenylbutyric acid (4-PBA) may enhance targeting of misfolded and intracellularly trapped mutant proteins to the plasma membrane[89,90,91,92] leading to enhanced phospholipid efflux activity, but nevertheless this effect was mutant-specific. In a study[93] with four PFIC-2 patients with at least one mistrafficking variant in *ABCB11* 4-PBA led to a decrease in serum bile acids and pruritus. The improvement may be a result of the ability of 4-PBA to retarget mutated ABCB11. 4-PBA (sodium phenylbutyrate) is a drug that is approved for urea cycle disorders and has a wide spectrum of side effects[94]: irregular menstrual cycles was the most common adverse effect followed by appetite loss, body odor and dysgeusia. Besides, laboratory alterations like acidosis or alkalosis, electrolyte changes, hypoalbuminemia and increased transaminases or AP were observed. On the other hand, in a small study with seven patients with PFIC-1 and -2 treated with 4-PBA no adverse events even after dose escalation were reported[95].

Correction and restoration of the activity of ABCB4 variants, although *in vitro*, provide interesting leads in the context of personalized medicine. Recently, in functional

defects in the ATP-binding site of ABCB4 the cystic fibrosis transmembrane conductance regulator (CFTR) potentiator, ivacaftor showed its ability to rescue phosphatidylcholine secretion activity[96]. Similarly, another study showed that structural analogues of roscovitine (a molecule used in clinical trials in cystic fibrosis correcting the F508del variant in CFTR) were able to correct intracellular traffic of ER-retained ABCB4 variants in HepG cells[97].

Surgical treatment

Liver transplantation is the last therapeutic option for patients with end stage liver disease, hepatocellular carcinoma (HCC) or those with low quality of life due to intractable pruritus despite optimal medical management. Liver transplantation improves cholestasis associated symptoms in more than 75 % irrespective of PFIC subtype over a short-term follow-up of 3-5 years[98,99].

In patients with LPAC, cholecystectomy may be necessary in cases with recurrent biliary colic or acute cholecystitis and cholelithiasis. Nevertheless, cholecystectomy should be deferred in those subjects without macroscopic gallbladder stones and only symptomatic biliary sludge. Instead, UDCA treatment should be attempted prior to all surgical interventions and continued after cholecystectomy. In rare cases with intrahepatic concrements and bile duct dilations, biliary drainage or even hepatic resection may be necessary[100].

SUMMARY

Mutations of *ABCB4* are associated with a broad spectrum of disease entities ranging from mild elevated liver enzymes or cholestasis to progressive liver disease with decompensated biliary cirrhosis, and just recently, evidence emerges on the association of *ABCB4* with hepatobiliary malignancies[24,34]. Due to the low prevalence and the overlapping disease manifestation diagnosis is often delayed for many years. Although, genotype-phenotype correlations are lacking, testing for mutations in *ABCB4* should be considered in case of chronic (cholestatic) liver disease in order to counsel individual patients and their families. In this regard treating physicians should be aware of a standardized approach for diagnosis including laboratory, imaging, histological and molecular genetic techniques.

So far, therapeutic options for patients are limited. Besides medical treatment with UDCA, cholestyramine or rifampicin, orthotopic liver transplantation remains the only treatment in patients with end stage liver disease or intractable pruritus. Nevertheless, large and especially prospective data are lacking so far. Especially in severe forms of ABCB4 deficiency like PFIC-3 UDCA can often only delay biliary cirrhosis and hepatic decompensation. As effective treatment options are currently lacking, the search for new treatment approaches and identification of molecular targets for therapeutic interventions are of high interest.

LEGENDS TO FIGURES AND TABLES

Table 1: Overview of clinical, biochemical and histological features of different types of progressive familial intrahepatic cholestasis (PFIC)

Table 2: Summary of different disease manifestations associated with *ABCB4* variants (AAT: α_1-antitrypsin, HCC: hepatocellular carcinoma, HELLP: hemolysis, elevated liver enzymes, low platelets, OLT: orthotopic liver transplantation, UDCA: ursodeoxycholic acid)

Figure 1: **Histological findings in liver biopsies of a patient with *ABCB4* homozygous mutation**

a: Percutaneous liver biopsy at the time of first presentation shows periportal fibrosis with incipient bridging fibrosis (chromatrope-aniline staining). **b**: Scarce lymphomononuclear cell infiltration in the portal tracts. The preexisting bile ducts are regularly configurated with discrete signs of unspecific alteration represented by segmental nuclear loss of the epithelium (black arrow, detail showed in higher magnification). **c**: The liver parenchyma shows slight regenerative changes, without any inflammatory infiltrate and cholestasis.

d: The liver biopsy performed 18 years later at the time of the decompensation shows cirrhotic liver tissue (chromatrope-aniline staining) with scarce lymphomononuclear cell infiltration. **e**: Preexisting bile ducts are significantly altered by reactive epithelial proliferation and segments of nuclear loss, an intraluminal cholestasis cannot be seen. Residual liver parenchyma presented in HE staining. **f**: areas of pericellular fibrosis and Mallory-Denk bodies (red arrow). Intracytoplasmic bile pigment is present in some of the hepatocytes (red asterisk).

Figure 2: Imaging findings of the clinically asymptomatic sister of the index patient (a and b) and of a 44-year old female patient with LPAC (c-f)

a: MR-cholangiography **b:** T1 weighted sequence after contrast application (Primovist®), both without any pathological findings

(c-f) Imaging findings of another patient (not related) that became symptomatic at the age of 20 with recurring cholelithiasis. Cholecystectomy was performed at the age of 23. She underwent two ERCPs due to recurring choledocholithiasis (6 and 10 years after cholecystectomy, respectively) and was then set on UDCA treatment. Since then she is clinically asymptomatic with normal transaminases and cholestasis parameters. Nevertheless, repeated MRI showed progressive segmental cholangitis in segment VIII as well as dilated bile ducts and hepatolithiasis in segments II, III and VIII. Genetic work-up (performed at age of 40) identified a heterozygous variant (c.1634G>A) in *ABCB4* and a non-disease-causing variant (c.1331T>C [p.V444A]) in *ABCB11*. **c**: diffusion weighted MRI sequence showing segmental cholangitis in segment VIII (white circle). **d**: T2 weighted haste sequence centrally showing intrahepatic cholelithiasis (white arrow) **e:** T1 weighted MRI sequence after contrast application (Primovist®) centrally showing intrahepatic cholelithiasis (white arrow). **f**: ultrasound study showing intrahepatic gallstones with a proximal bile duct dilatation (presenting as cystic formation with intraluminal hyperechoic foci measuring 1.4 cm in diameter, white arrow).

Figure 3: Molecular mechanisms underlying cholestasis associated with ABCB4 deficiency

ABCB4 (ATP binding cassette subfamily B member 4) is a transmembrane P-glycoprotein acting as a floppase transporting phosphatidylcholine (PC) from the inner to the outer leaflet resulting in PC secretion into the bile. Variants in *ABCB4*

leading to inadequate phospholipid secretion may cause injury of the biliary epithelium and canalicular membrane by toxic bile acid concentrations, destabilization of micelles and thus promote cholesterol crystallization and increased biliary lithogenicity. Additionally, increased lithogenicity in turn facilitates liver damage by obstruction of small bile ducts. (ABCB11: ATP binding cassette subfamily B member 4, ATP8B1: ATPase class I, type 8B, member 1, FXR: farnesoid X receptor, MYO5B: myosin 5 B, TJP2: tight junction protein 2. Inspired and adapted from a figure of J. Stindt presented by V. Keitel at the Postgraduate Course of The Liver Meeting of the AASLD, November 9th, 2019 in Boston, MA)

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REFERENCES

[1] Karlsen TH, Lammert F, Thompson RJ. Genetics of liver disease: from pathophysiology to clinical practice. J Hepatol 2015; 62: 6-14

[2] Hakim A, Zhang X, DeLisle A, Oral EA, Dykas D, Drzewiecki K, et al. Clinical utility of genomic analysis in adults with idiopathic liver disease. J Hepatol 2019; 70: 1214-1221

[3] Eichler EE. Genetic variation, comparative genomics and the diagnosis of disease. N Engl J Med 2019; 381: 64-74

[4] Clayton RJ, Iber FI, Ruebner BH, McKusick VA. Byler disease: fatal intrahepatic cholestasis in an Amish kindred. Am J Dis Child 1969; 117: 112-124

[5] Bull LN, Carlton VEH, 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; 1: 155-164

[6] Bull LN, van Eijk MJ, Pawlikowska L, DeYoung JA, Jujin 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

[7] Trauner M, Meier PJ, Boyer JL. Molecular pathogenesis of cholestasis. N Engl J Med 1998; 339: 1217-1227

[8] Srivastava A. Progressive familial intrahepatic cholestasis. J Clin Exp Hepatol 2014; 4: 25-36

[9] Jacquemin E. Progressive familial intrahepatic cholestasis. Clin Res Hepatol Gastroenterol 2012; 36 (suppl 1): 26-35

[10] Paulusma CC, Oude Elferink RPJ, Jansen PLM. Progressive familial intrahepatic cholestasis type 1. Semin Liver Dis 2010; 30: 117-124

[11] Strautnieks SS, Kagalwalla AF, Tanner MS, Knisely AS, Bull L, Freimer N, et al. Identification of a locus for progressive familial intrahepatic cholestasis PFIC2 on chromosome 2q24. Am J Hum Genet 1997; 61: 630-633

[12] van der Bliek AM, Kooiman PM, Schneider C, Borst P. Sequence of mdr3 cDNA encoding a human p-glycoprotein. Gene 1988; 71: 401-411

[13] Vitale G, Gitto S, Vukotic R, Raimondi F, Andreone P. Familial intrahepatic cholestasis: new and wide perspectives. Dig Liver Dis 2019; 51: 922-933

[14] Gomez-Ospina N, Potter CJ, Xiao R, Manickam K, Kim MS, Kim KH, et al. Mutations in the nuclear bile acid receptor FXR cause progressive familial intrahepatic cholestasis. Nat Commun 2016; Feb 18; 7: 10713

[15] Gonzalez E, Taylor SA, Davit-Spraul A, Thebaut A, Thomassin N, Guettier C, et al. MYO5B mutations cause cholestasis with normal serum gamma-glutamyl transferase activity in children without microvillous inclusion disease. Hepatology 2017; 65: 164-173

[16] Oude Elferink RP, Paulusma CC. Function and pathophysiological importance of ABCB4 (MDR3 P -glycoprotein). Pflugers Arch. 2007; 453: 601-610

[17] Smit JJ, Schinkel AH, Oude Elferink RP, Groen AK, Wagenaar E, van Deemter L, et al. Homozygous disruption of the murine mdr2 P-glycoprotein gene leads to a complete absence of phospholipid from bile and to liver disease. Cell 1993; 75: 451-462

[18] 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 multi drug resistance p-glycoprotein 2 (ABCB4, MDR3) in patients with intrahepatic cholestasis of pregnancy. Pharmacogenetics 2004; 14: 91-102

[19] Rosmorduc O, Hermelin B, Poupon R. MDR3 gene defects in adults with symptomatic intrahepatic and gallbladder cholesterol cholelithiasis. Gastroenterology 2001; 120: 1459-1467

[20] 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

[21] Gotthard D, Runz H, Keitel V, Flechtenmacher C, Wirtenberger M, Weiss KH, 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

[22] Ziol M, Barbu V, Rosmorduc O, Frassati-Biaggi A, Barget N, Hermelin B, et al. ABCB4 heterozygous mutations associated with fibrosing cholestatic liver disease in adults. Gastroenterology 2008; 135: 131-141

[23] 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 in adulthood. Gastroenterology 2001; 120: 1448-1458

[24] Gudbjartsson DF, Helgasson H, Gudjonsson SA, Zink F, Oddson A, Gylfason A, et al. Large-scale whole genome sequencing of the Icelandic population. Nat Genet 2015; 47: 435-444

[25] Lammert F, Hochrath K. A letter on ABCB4 from Iceland: on the highway to liver disease. Clin Res Hepatol Gastroenterol 2015; 39: 655-658
[26] Smit AJ, Timmermans-Hereijgers JL, Roelofsen B, Wirtz KW, van Bitterswijk 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
[27] Zolnerciks JK, Andress EJ, Nicolao M, Linton KJ. Structure of ABC transporters. Essays Biochem 2011; 50: 43-61
[28] Hofmann AF. Bile acid secretion, bile flow and biliary lipid secretion in humans. Hepatology 1990; 12: 17-22
[29] De Vree JM, Jacquemin E, Sturm E, Cresteil D, Bosma PJ, Aten J, et al. Mutations in MDR3 gene cause progressive familial intrahepatic cholestasis. Proc Natl Acad Sci USA 1998; 95: 282-287
[30] 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
[31] Delaunay JL, Durand-Schneider AM, Dossier C, Falguieres T, Gautherot J, Davit-Spraul A, et al. A functional classification of ABCB4 variations causing progressive familial intrahepatic cholestasis type 3. Hepatology 2016; 63: 1620-1631
[32] Wilschanski M, Durie PR. Patterns of GI disease in adulthood associated with mutations in the CFTR gene. Gut 2007; 56: 1153-1163
[33] Kubitz R, Bode J, Erhardt A, Graf D, Kircheis G, Müller-Stöver I, et al. Cholestatic liver disease from child to adult: the diversity of MDR3 disease. Z Gastroenterol 2011; 49: 728-736
[34] Reichert MC, Lammert F. ABCB4 gene aberrations in human liver disease: an evolving spectrum. Semin Liver Dis 2018; 38: 299-307
[35] Poupon R, Rosmorduc O, Boelle BY, Chretien Y, Corpechot C, Chazouielleres C, et al. Genotype-phenotype relationships in the low-phospholipid-associated cholelithiasis syndrome: a study of 156 consecutive patients. Hepatology 2013; 58: 1105-1110
[36] Colombo C, Vajro P, Degiorgio D, Coviello DA, Constantino L, Tornillo L, et al. Clinical features and genotype-phenotype correlations in children with progressive familial intrahepatic cholestasis type 3 related to ABCB4 mutations. J Pediatr Gastroenterol Nutr 2011; 52: 73-83
[37] Schatz SB, Jüngst C, Keitel-Anselmo V, Kubitz R, Becker C, Gerner P, et al. Phenotypic spectrum and diagnostic pitfall of ABCB4 deficiency depending on age of onset. Hepatology Communications 2018; 2: 504-514
[38] Davit-Spraul A, Gonzales E, Baussan C, Jacquemin E. The spectrum of liver diseases related to ABCB4 gene mutations: Pathophysiology and clinical aspects. Semin Liv Dis 2010; 30: 134-146
[39] Ganne-Carrié N, Baussan C, Grando V, Gaudelus J, Cresteil D, Jacquemin E. Progressive familial intrahepatic cholestasis type 3 revealed by oral contraceptive pills. J Hepatol 2003; 38: 693-694
[40] Rosmorduc O, Hermelin B, Boelle PY, Parc R, Taboury J, Poupon R. ABCB4 gene mutation-associated cholelithiasis in adults. Gastroenterology 2003; 125: 452-459
[41] Rosmorduc O, Poupon R. Low phospholipid associated cholelithiasis: association with mutation in the MDR3/ABCB4 gene. Orphanet J Rare Dis 2007; 29: 29
[42] Condat B, Zanditenas D, Barbu V, Hauuy MP, Parfait B, El Naggar A, et al. Prevalence of low phospholipid-associated cholelithiasis in young female patients. Dig Liver Dis 2013; 45: 915-919
[43] Pauli-Magnus C, Meier PJ, Stieger B. Genetic determinants of drug-induced cholestasis and intrahepatic cholestasis of pregnancy. Semin Liv Dis 2010; 30: 147-159
[44] Geenes V, Williamson C. Intrahepatic cholestasis of pregnancy. World J Gastroenterol 2009; 15: 2049-2066
[45] Bacq Y. Liver diseases unique to pregnancy: a 2010 update. Clin Res Hepatol Gastroenterol 2011; 35: 182-193
[46] Dixon PH, Williamson C. The pathophysiology of intrahepatic cholestasis of pregnancy. Clin Res Hepatol Gastroenterol 2016; 40: 141-153
[47] 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
[48] Keitel V, Dröge C, Stepanow S, Fehm T, Mayatepek E, Köhrer K, et al. Intrahepatic cholestasis of pregnancy (ICP): case report and review of literature. Z Gastroenterol 2016; 54: 1327-1333
[49] Ovadia C, Seed PT, Sklavounos A, Geenes V, Di Ilio C, Chambers J, et al. Association of adverse perinatal outcomes of intrahepatic cholestasis of pregnancy with biochemical markers: results of aggregate and individual patient data meta-analyses. Lancet 2019; 393: 899-909
[50] Ropponen A, Sund R, Riikonen S, Ylikorkala O, Aittomäki K. Intrahepatic cholestasis of pregnancy as an indicator of liver and biliary diseases: a population-based study. Hepatology 2006; 43: 723-728

[51] Wikström Shemer EA, Stephansson O, Thuresson M, Thorsell M,Ludvigsson JF, Marschall HU. Intrahepatic cholestasis of pregnancy and cancer, immune-mediated and cardiovascular diseases: A population-based cohort study. J Hepatol 2015; 63: 456-461
[52] Westbrook RH, Dusheiko G, Williamson C. Pregnancy and liver disease. J Hepatol 2016; 64: 933-945
[53] Smith AJ, van Helvoort A, van Meer G, Szabo K, Welker E, Szakacs G, et al. MDR3 P-glycoprotein, a phosphatidylcholine translocase, transports several cytotoxic drugs and directly interacts with drugs as judged by interference with nucleotide trapping. J Biol Chem 2000; 275: 23530-23539
[54] Zollner G, Thueringer A, Lackner C, Fickert P, Trauner M. Alterations of canalicular ATP-binding cassette transporter expression in drug-induced liver injury. Digestion 2014; 90: 81-88
[55] EASL Clinical Practice Guidelines: Drug-induced liver injury. J Hepatol. 2019; 70:1222-1261
[56] Trauner M, Fickert P, Wagner M. MDR3 (ABCB4) defects: a paradigm for the genetics of adult cholestatic syndromes. Sem Liv Dis 2007; 27: 77-98
[57] Ramraj R, Finegold MJ, Karpen SJ. Progressive familial intrahepatic cholestasis type 3: overlapping presentation with Wilson disease. Clin Pediatr (Phila). 2012; 51:689-691
[58] Trauner M, Fickert P, Halilbasic E, Moustafa T. Lessons from the toxic bile concept for the pathogenesis and treatment of cholestatic liver diseases. Wien Med Wochenschr 2008; 158: 542-548
[59] Morotti RA, Suchy FJ, Magid MS. Progressive familial intrahepatic cholestasis (PFIC) type 1, 2, and 3: a review of the liver pathology findings. Semin Liv Dis 2011; 31: 3-10
[60] Wendum D, Barbu V, Rosmorduc O, Arrive L, Flejou JF, Poupon R. Aspects of liver pathology in adult patients with MDR3/ABCB4 gene mutations. Virchows Arch. 2012; 460: 291-298
[61] Liu C, Aronow BJ, Jegga AJ, Wang N, Miethke A, Mourya R, et al. Novel resequencing chip to diagnose mutations in patients with inherited syndromes of intrahepatic cholestasis. Gastroenterology 2007; 132: 119-126
[62] Aamann L, Ørntoft N, Vogel I, Groenbaek H, Becher N, Vilstrup H, et al. Unexplained cholestasis in adults and adolescents: diagnostic benefit of genetic examination. Scand J Gastroenterol. 2018; 53: 305-311
[63] Dröge C, Bonus M, Baumann U, Klindt C, Lainka E, Kathemann S, et al. Sequencing of FIC1, BSEP and MDR3 in a large cohort of patients with cholestasis revealed a high number of different genetic variants. J Hepatol. 2017; 67:1253-1264
[64] Vitale G, Gitto S, Raimondi F, Mattiaccio A, Mantovani V, Vukotic R, et al. Cryptogenic cholestasis in young and adults: ATP8B1, ABCB11, ABCB4, and TJP2 gene variants analysis by high-throughput sequencing. J Gastroenterol. 2018; 53: 945-958
[65] Feranchak AP, Ramirez RO, Sokol RJ. Medical and nutritional management of cholestasis. In Such FJ, Sokol RJ, Ballistreri WF (editors). Liver disease in children, 2nd edition. Lippincott, Williams and Wilkins 2001: 195-238 [chapter 10]
[66] Gasser RW. Cholestasis and metabolic bone disease - a clinical review. Wien Med Wochenschr 2008; 158: 553-557
[67] Hochrath K, Stokes CS, Geisel J, Pollheimer MJ, Fickert P, Dooley S, et al. Vitamin D modulates biliary fibrosis in ABCB4-deficient mice. Hepatol Int 2014; 8: 443-452
[68] Reiter FP, Hohenester S, Nagel JM, Wimmer R, Artmann R, Wottke L, et al. 1,25-$(OH)_2$-vitamin D_3 prevents activation of hepatic stellate cells in vitro and ameliorates inflammatory liver damage but not fibrosis in the Abcb4(-/-) model. Biochem Biophys Res Commun 2015; 459: 227-233
[69] Hochrath K, Ehnert S, Ackert-Bicknell CL, Lau Y, Schmid A, Krawczyk M, et al. Modeling hepatic osteodystrophy in Abcb4 deficient mice. Bone. 2013; 55: 501-511
[70] Poupon R. Intrahepatic cholestasis of pregnancy: from bedside to bench to bedside. Liv Int 2005; 25: 467-468
[71] Marschall HU, Wagner M, Zollner G, Diczfalusy U, Gumhold J, Silbert D, et al. Complementary stimulation of hepatobiliary transport and detoxification systems by rifampicin and ursodeoxycholic acid in humans. Gastroenterology. 2005; 129: 476-485
[72] 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
[73] Paumgartner G, Beuers U. Ursodeoxycholic acid in cholestatic liver disease: mechanism of action and therapeutic use revisited. Hepatology 2002; 36: 525-531
[74] Bacq Y, Sentilhes L, Reyes HB, Glantz A, Kondrackiene J, Binder T, et al. Efficacy of ursodeoxycholic acid in treating intrahepatic cholestasis of pregnancy: a meta-analysis. Gastroenterology 2012; 143: 1492-1501

[75] Chappell LC, Bell JL, Smith A, Linsell L, Juszczak E, Dixon PH, et al. Ursodeoxycholic acid versus placebo in women with intrahepatic cholestasis of pregnancy (PITCHES): a randomised controlled trial. Lancet 2019; 394: 849-860
[76] Beuers U, de Vries E. Reply to: "UDCA therapy in intrahepatic cholestasis of pregnancy?". J Hepatol. 2020; 72: 587-588
[77] Geenes V, Chambers J, Khurana R, Wilksrom Shemer E, Sia W, Mandair D, et al. Rifampicin in the treatment of severe intrahepatic cholestasis of pregnancy. Eur J Obstet Gynecol Reprod Biol 2015; 189: 59-63
[78] Cuperus FJ, Halilbasic E, Trauner M. Fibrate treatment for primary biliary cirrhosis. Curr Opin Gastroenterol. 2014; 30:279-286
[79] Corpechot C, Chazouillères O, Rousseau A, Le Gruyer A, Habersetzer F, Mathurin P, et al. A Placebo-Controlled Trial of Bezafibrate in Primary Biliary Cholangitis. N Engl J Med. 2018; 378: 2171-2181
[80] De Vries E, Bolier R, Goet J et al. Bezafibrate is more effective then placebo in pruritus of chronic cholestasis: the FITCH trial. Hepatology 2019; 70; S1: 9A, Abstract
[81] 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
[82] Nakamuta M, Fujino T, Yada R, Yasutake K, Yoshimoto T, Harada N, et al. Therapeutic effect of bezafibrate against biliary damage: a study of phospholipid secretion via the PPARalpha-MDR3 pathway. Int J Clin Pharmacol Ther. 2010; 48: 22-28
[83] Ghonem NS, Ananthanarayanan M, Soroka CJ, Boyer JL. Peroxisome proliferator-activated receptor α activates human multidrug resistance transporter 3/ATP-binding cassette protein subfamily B4 transcription and increases rat biliary phosphatidylcholine secretion. Hepatology 2014; 59: 1030-1042
[84] 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
[85] Nevens F, Andreone P, Mazzella G, Strasser SI, Bowlus C, Invernizzi P, et al. A placebo-controlled trial of obeticholic acid in primary biliary cholangitis. N Engl J Med 2016; 375: 631-643
[86] Ijssennagger N, Janssen AWF, Milona A, Ramos Pittol JM, Hollmann DAA, Mokry M, et al. Gene expression profiling in human precision cut liver slices in response to the FXR agonist obeticholic acid. J Hepatol 2016; 64: 1158-1166
[87] Baghdasaryan A, Claudel T, Gumhold J, Silbert D, Adorini L, Roda A, et al. Dual farnesoid X receptor/TGR5 agonist INT-767 reduces liver injury in the Mdr2-/- (Abcb4-/-) mouse cholangiopathy model by promoting biliary HCO^-_3 output. Hepatology 2011; 54: 1303-1312
[88] Gautherot J, Claudel T, Cuperus F, Fuchs CD, Falguières T, Trauner M. Thyroid hormone receptor β1 stimulates ABCB4 to increase biliary phosphatidylcholine excretion in mice. J Lipid Res 2018; 59: 1610-1619
[89] 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
[90] van den Berghe PV, Stapelbroek JM, Krieger E, de Bie P, van de Graaf SFJ, de Groot REA, et al. Reduced expression of ATP7B affected by Wilson disease-causing mutations is rescued by pharmacological folding chaperones 4-phenylbutyrate and curcumin. Hepatology 2009; 50: 1783-1795
[91] van der Velden LM, Stapelbroek JM, Krieger E, van den Berghe PVE, Berger R, Verhulst PM, et al. Folding defects in P-type ATP8B1 associated with hereditary cholestasis are ameliorated by 4-phenylbutyrate. Hepatology 2010; 51: 286-296
[92] Gordo-Gilart R, Andueza S, Hierro L, Jara P, Alvarez L. Functional rescue of trafficking-impaired ABCB4 mutants by chemical chaperons. PLOS One 2016; 11(2): e0150098.
[93] Gonzales E, Grosse B, Schuller B, Davit-Spraul A, Conti F, Guettier C, et al. Targeted pharmacotherapy in progressive familial intrahepatic cholestasis type 2: Evidence for improvement of cholestasis with 4-phenylbutyrate. Hepatology 2015; 62: 558-566
[94] https://www.accessdata.fda.gov/drugsatfda_docs/label/2009/020572s016,020573s015lbl.pdf
[95] Nakano S, Osaka S, Sabu Y, Minowa K, Hirai S, Kondou H, et al. Effect of food on the pharmacokinetics and therapeutic efficacy of 4-phenylbutyrate in progressive familial intrahepatic cholestasis. Sci Rep. 2019; 9: 17075
[96] Delaunay JL, Bruneau A, Hoffmann B, Durand-Schneider AM, Barbu V, Jacquemin E, et al. Functional defect of variants in the adenosine triphosphate-binding sites of ABCB4 and their rescue by the cystic fibrosis transmembrane conductance regulator potentiator, ivacaftor (VX-770). Hepatology. 2017; 65: 560-570

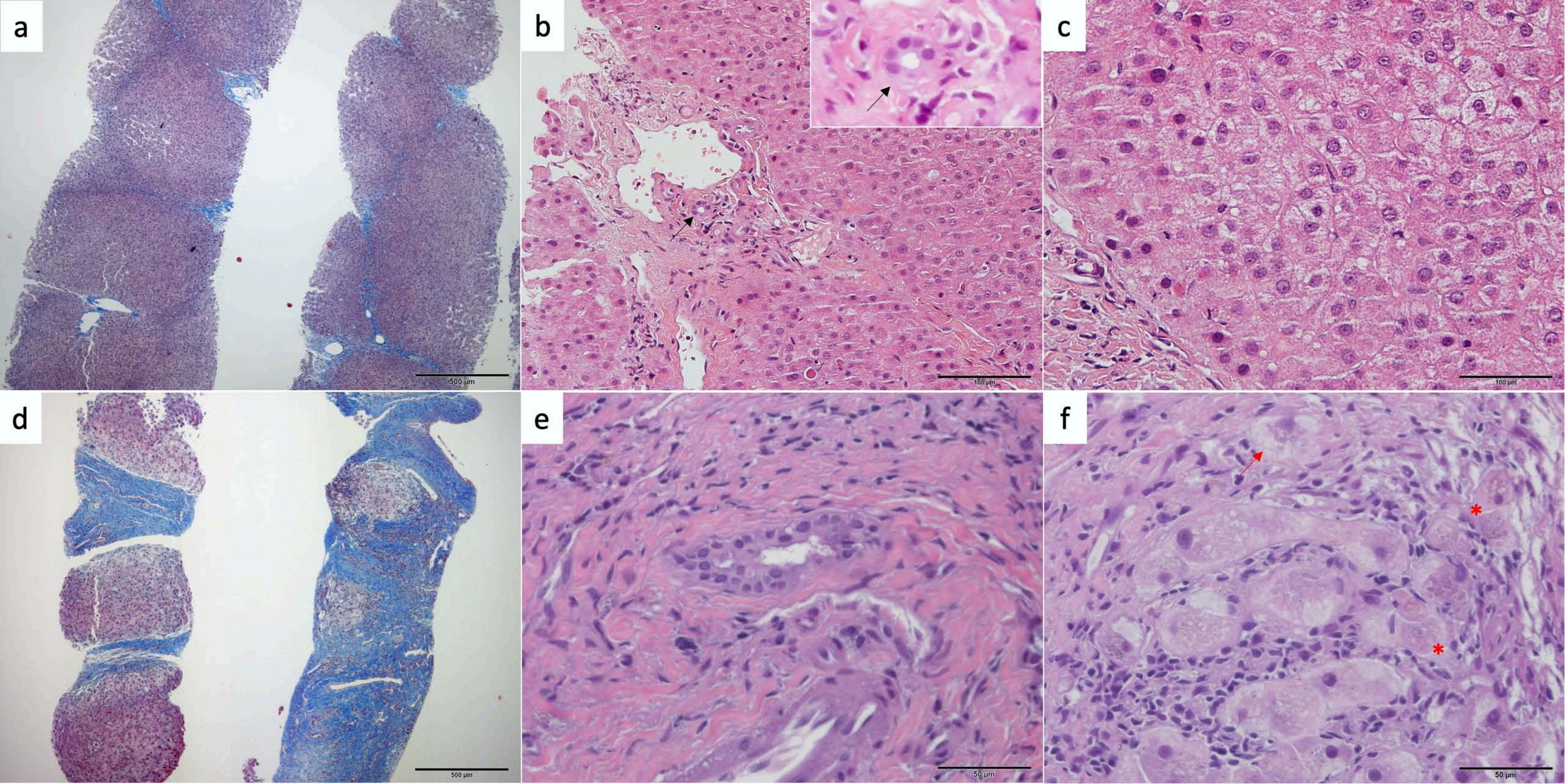
[97] Vauthier V, Ben Saad A, Elie J, Oumata N, Durand-Schneider AM, Bruneau A, et al. Structural analogues of roscovitine rescue the intracellular traffic and the function of ER-retained ABCB4 variants in cell models. Sci Rep. 2019; 9: 6653
[98] Englert C, Grabhorn E, Richter A, Rogiers X, Burdelski M, Ganschow R, et al. Liver transplantation in children with progressive familial intrahepatic cholestasis. Transplantation 2007; 84: 1361-1363
[99] Hori T, Egawa H, Takada Y, Ueda M, Oike F, Ogura Y, et al. Progressive familial intrahepatic cholestasis: a single-centre experience of living-donor liver transplantation during two decades in Japan. Clin Transplant 2011; 25: 776-785
[100] Erlinger S. Low phospholipid-associated cholestasis and cholelithiasis. Clin Res Hepatol Gastroenterol 2012; 36: 36-40

	PFIC-1	PFIC-2	PFIC-3	PFIC-4	PFIC-5	PFIC-6
gene	*ATP8B1*	*ABCB11*	*ABCB4*	*TJP2*	*NR1H4*	*MYO5B*
chromosomal location	18q21.31	2q31.1	7q21.12	9q21.11	12q23.1	18q21.1
gene product	FIC1 (familial intrahepatic cholestasis 1 protein)	BSEP (bile salt export pump)	MDR3 (multidrug resistance 3)	TJP2 (tight junction protein 2)	FXR (farnesoid X receptor)	MYO5B (myosin 5b)
disease onset	infancy	neonates, early infancy	late infancy, adolescence, early adulthood	neonates	neonates	neonates, early infancy
disease course	moderate	severe	insidious	severe	severe	mild/moderate
end stage liver disease	1^{st} decade	infancy	1^{st}-2^{nd} decade	infancy	infancy	unknown
extrahepatic disease manifestation	diarrhea, pancreatitis, hearing loss, short stature	absent	absent	unknown	unknown	diarrhea, neurologic symptoms(?)
pruritus	++	+++	(+)	++	unknown	+++
risk of cholelithiasis	-	+	++	unknown	unknown	unknown
risk of liver tumors	-	++	+	++	unknown	unknown
serum ALT or AST	↑	↑↑	↑	normal/↑	↑↑	normal/↑
serum γGT	normal	normal	↑↑	normal	normal	normal
serum bile acids	↑↑	↑↑↑	↑	↑↑(↑)	↑↑(↑)	↑↑(↑)
liver histology	canalicular cholestasis, lobular fibrosis	canalicular cholestasis, giant-cell hepatitis, hepatocellular necrosis, portal fibrosis	biliary fibrosis, ductular proliferation, macrophagic infiltration of portal tracts, ductopenia, cholesterol crystals	giant cell transformation, canalicular cholestasis	ductular reaction, diffuse giant cell transformation, hepatocyte ballooning, intralobular cholestasis	giant cell transformation, canalicular cholestasis

Table 1

	PFIC-3	LPAC	ICP
underlying genetic defect in *ABCB4*	homozygous, compound-heterozygous	heterozygous	heterozygous
age at presentation	infancy, adolescence, (early adulthood)	early adulthood (<40 years)	pregnancy (2^{nd}/3^{rd} trimester)
clinical presentation	pruritus	cholelithiasis, biliary colic	gestational pruritus
disease course	insidious	benign	benign
complications	biliary cirrhosis, HCC, cholangiocarcinoma	jaundice, cholangitis, biliary pancreatitis, intrahepatic stones	premature birth, fetal asphyxia, meconium-stained amniotic fluid
treatment	UDCA, (cholestyramine, rifampicin, fibrates), OLT	UDCA, cholecystectomy	UDCA
differential diagnosis	all causes of neonatal cholestasis, Alagille's syndrome, sclerosing cholangitis (primary/secondary), AAT-deficiency	primary sclerosing cholangitis, Caroli's disease (congenital dilation of intrahepatic bile ducts)	acute fatty liver of pregnancy, HELLP syndrome, Budd-Chiari-syndrome

Table 2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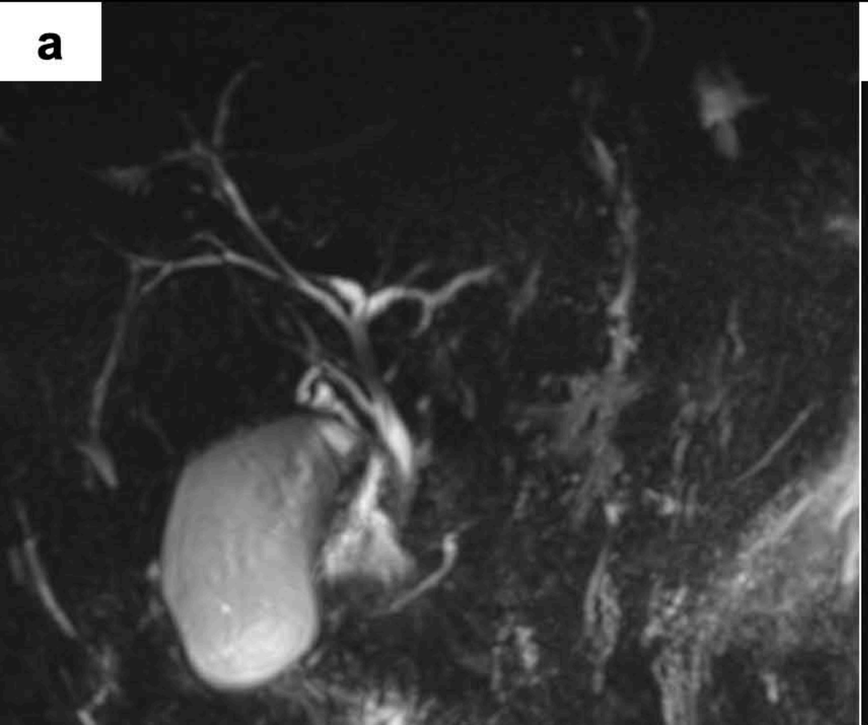
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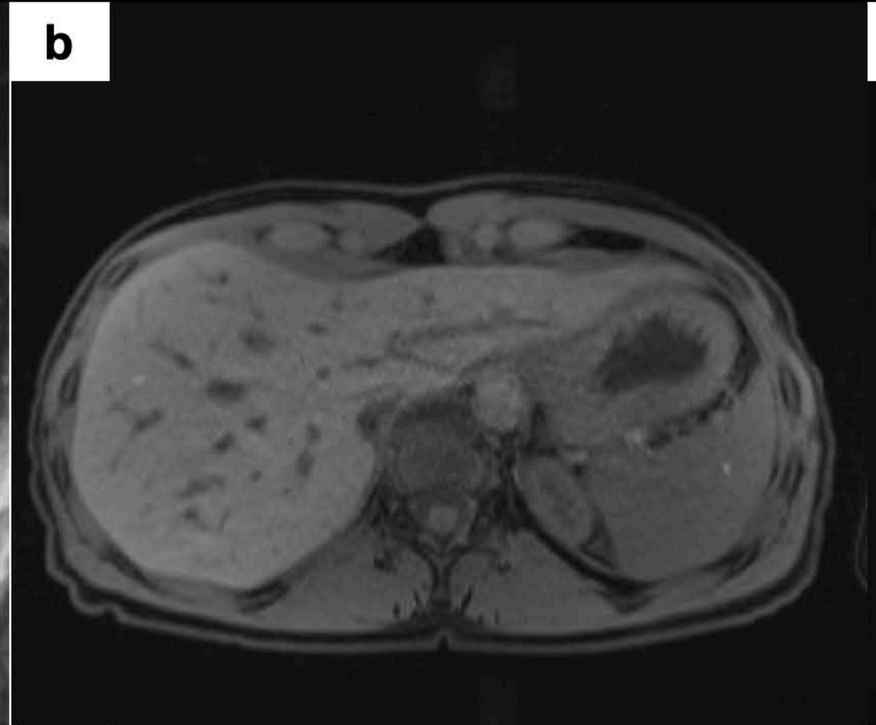
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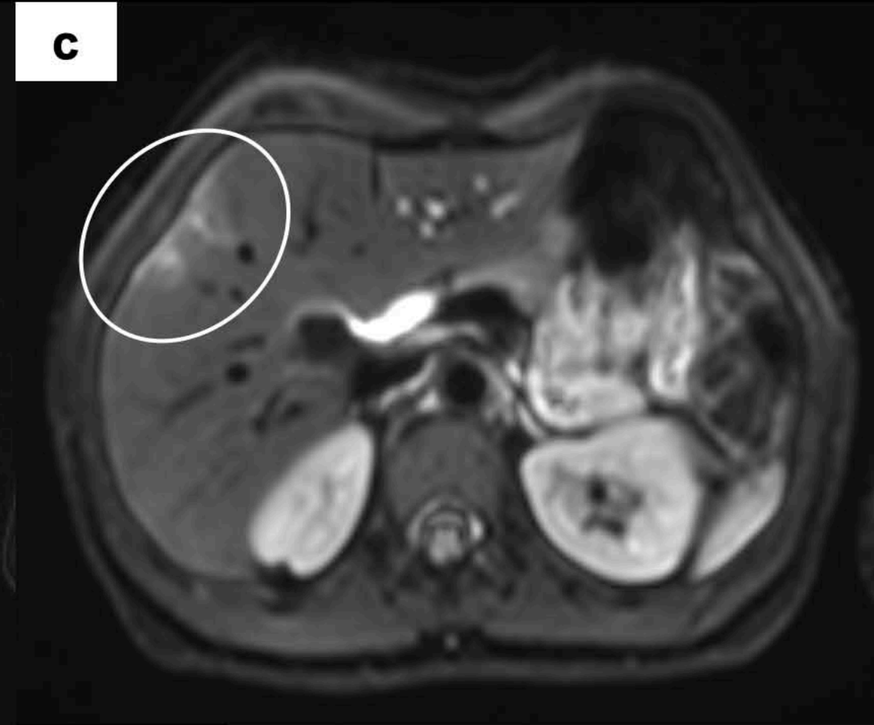
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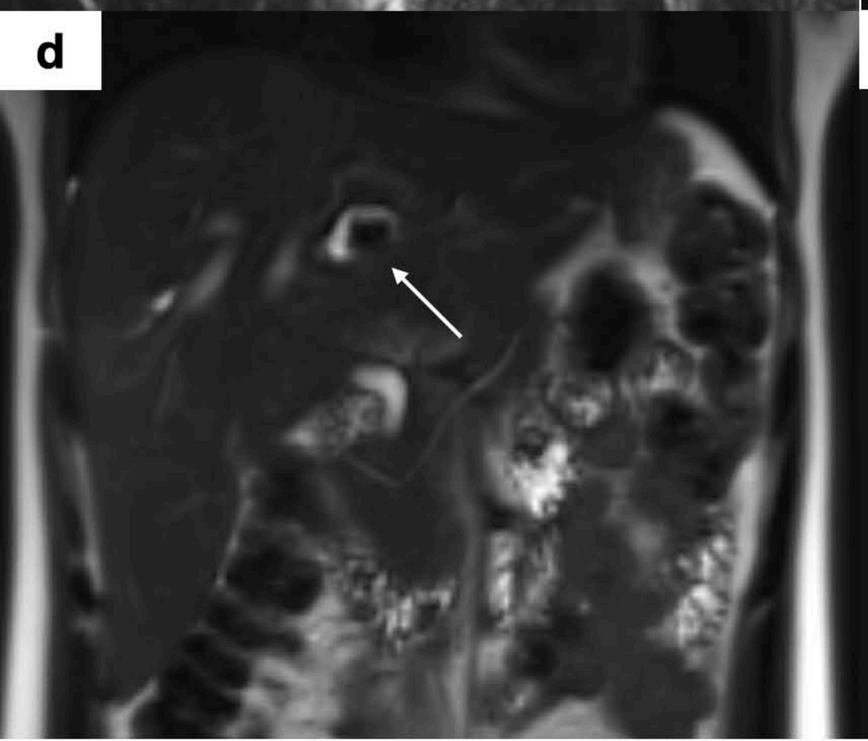
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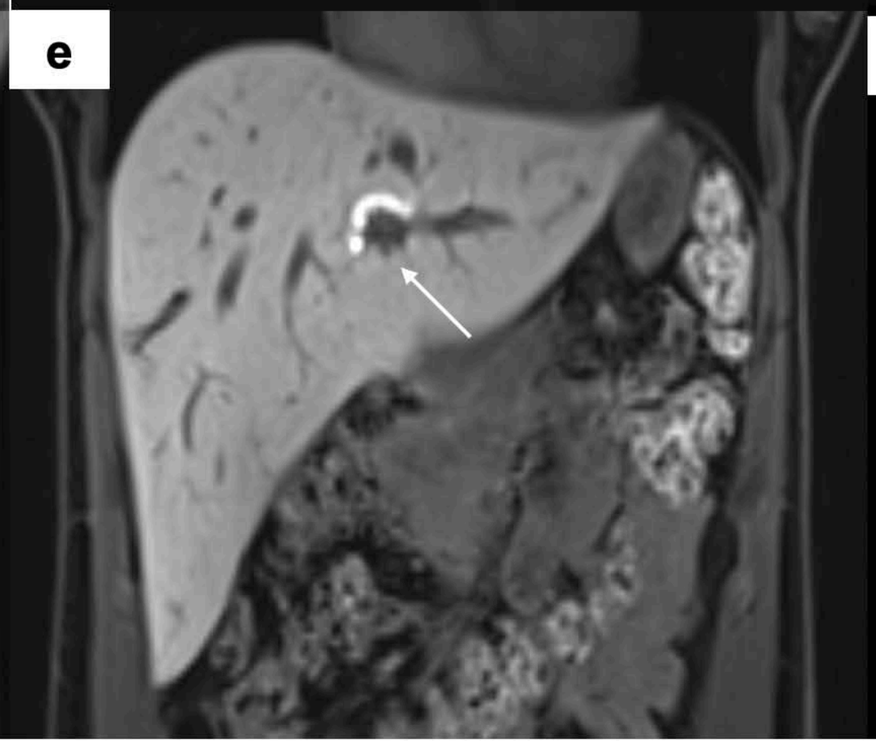
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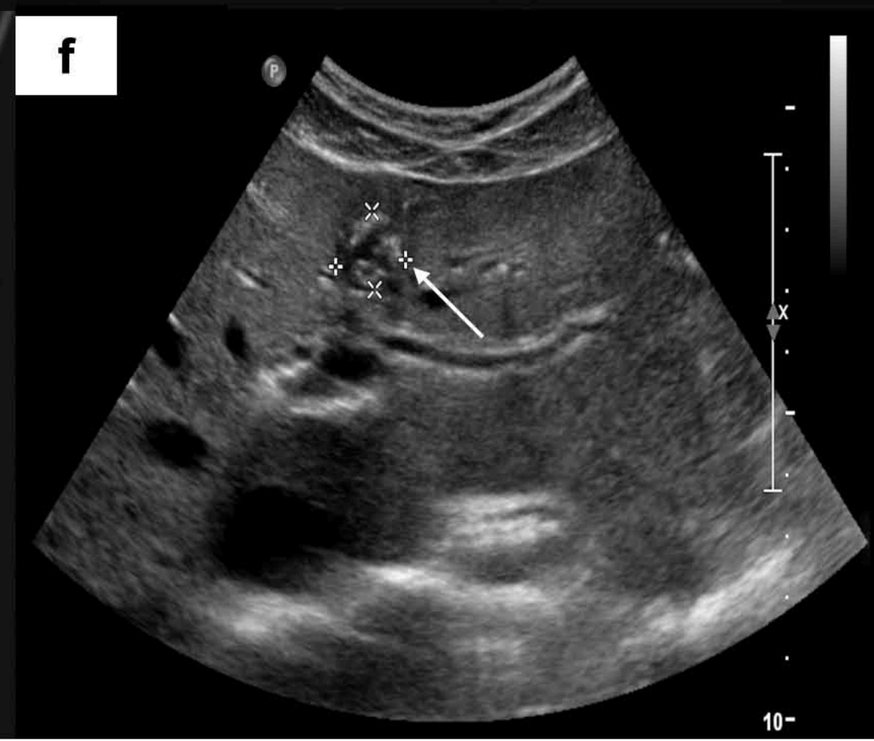
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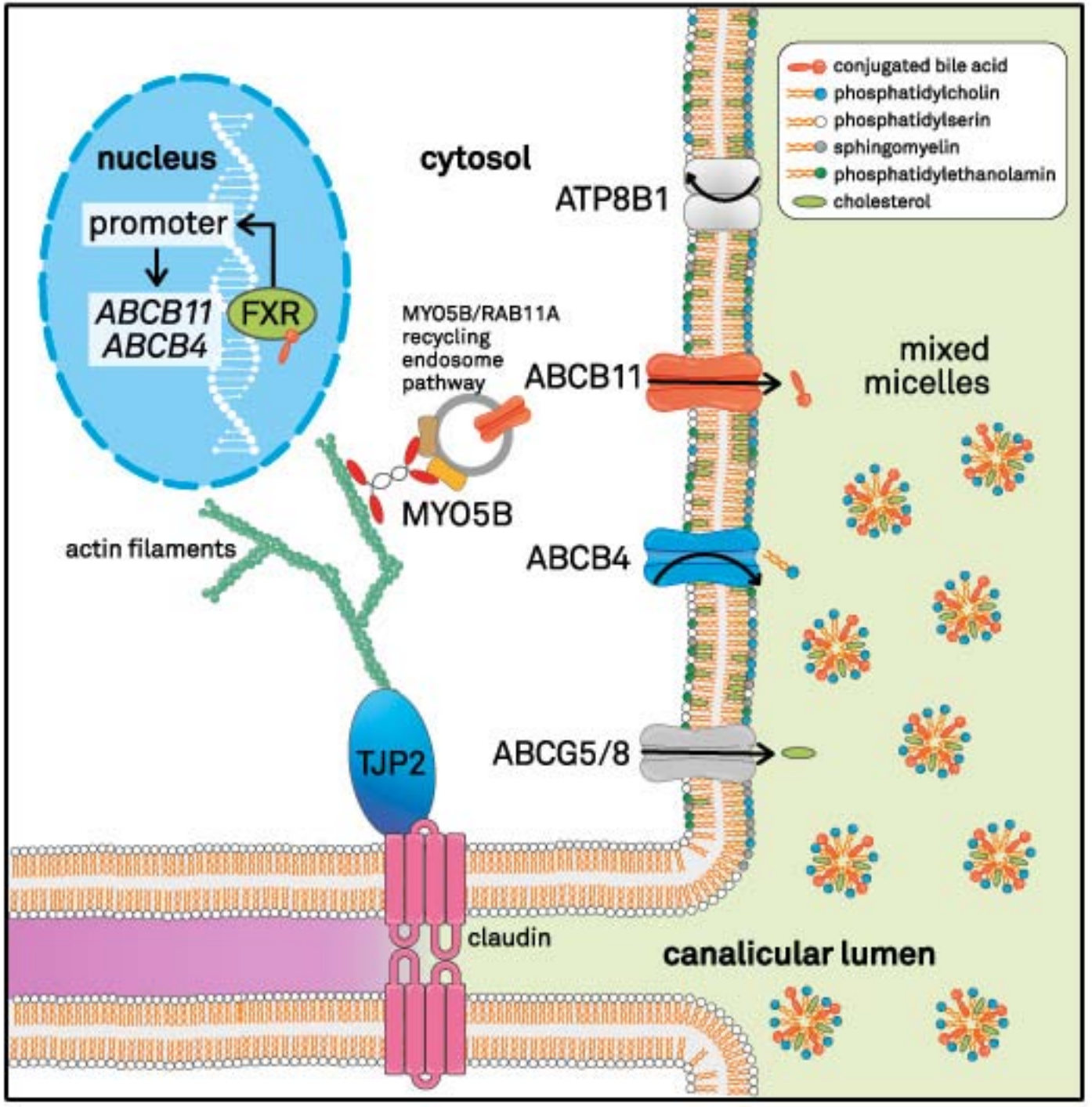
conjugated bile acid
phosphatidylcholin
phosphatidylserin
sphingomyelin
phosphatidylethanolamin
cholesterol
nucleus
promoter
ABCB11
ABCB4
FXR
cytosol
ATP8B1
MYO5B/RAB11A
recycling
endosome
pathway
ABCB11
mixed
micelles
MYO5B
actin filaments
ABCB4
TJP2
ABCG5/8
claudin
canalicular lumen