



Review article: liver disease in adults with variants in the cholestasis-related genes *ABCB11*, *ABCB4* and *ATP8B1*

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Summary

Background: Children with intrahepatic cholestasis and genetic variants which result in the disruption of the formation and maintenance of bile (*ABCB11*, *ABCB4* and *ATP8B1*) generally have a rapidly progressive clinical course. Adults with different phenotypes of cholestasis are increasingly being evaluated for variants in these genes associated with childhood diseases.

Aims: To review the literature with respect to the presence of variants in cholestasis-related genes in adults with various liver phenotypes, and provide clinical implications of the findings.

Methods: A search of the literature on variants in specific cholestasis-related genes in adults was conducted.

Results: The common variant p.Val444Ala in *ABCB11* confers increased risk of drug-induced liver injury and intrahepatic cholestasis of pregnancy (ICP). Individuals with variants in *ABCB4* are at risk of ICP and low phospholipid-associated cholelithiasis. Variants in *ABCB4*, and possibly *ABCB11* and *ATP8B1*, can be identified in up to a third of patients with cryptogenic chronic cholestasis.

Conclusions: Individuals with variants in *ABCB11* rarely develop cholestasis until BSEP function dips below a threshold, which is also affected by other factors (e.g., drugs, hormones). However, variants in *ABCB4* and consequent reduction in MDR3 protein, have a more linear dose-response curve. In individuals with an *ABCB11* variant, medications known to reduce BSEP activity should be used cautiously; they should be monitored during pregnancy for ICP; and first-degree relatives should be counselled and screened. No proven management strategy exists, although ursodeoxycholic acid may be beneficial. Further work is needed to define the genotype-phenotype correlation and natural history, and to evaluate the penetrance.

Joint Senior Authorship: Deepak Joshi and Richard J. Thompson.

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1 | INTRODUCTION

Cholestatic liver diseases are a heterogeneous group of conditions with an array of overlapping clinical manifestations. Early-onset disease is frequently genetic in origin. A particular focus on patients with progressive familial intrahepatic cholestasis (PFIC) has identified an increasing catalogue of variants in genes vital to the transport of biliary constituents and the stability of the canalicular membrane. PFIC patients present in childhood with intrahepatic cholestasis and frequently progress to end-stage liver disease before adulthood.¹ The three major proteins affected in PFIC are the bile salt export pump (BSEP) encoded by *ABCB11*, the major phospholipid transporter (multidrug resistance protein 3, MDR3) encoded by *ABCB4*, and a protein contributing to membrane lipid composition (FIC1) encoded by *ATP8B1* (Figure 1). The ABC transporters are a superfamily of transporters, mostly with two membrane spanning domains and two nucleotide binding sites, the latter of which are highly conserved through the family.² *ATP8B1* belongs to a subfamily of aminophospholipid-transporting P-type ATPases.³

The consequences of a particular variant are only to some extent predictable. This information has been partly derived from *in vitro* analysis, and *in silico* prediction, but mainly from the behaviour of previous patients with disease.⁴⁻⁶ Severe deficiencies in any of these proteins, particularly where there is a complete loss of function from both alleles, is almost always associated with severe early-onset disease.⁷⁻⁹ The major PFICs follow a Mendelian inheritance in a recessive pattern, and can present with end-stage liver

disease and liver cancer. Missense changes are associated with a spectrum of effects on proteins. Some missense changes may cause a complete loss of function and be just as severe as a non-sense variant; others are much better tolerated and may be of little or no functional consequence; while the majority represent a more intermediate loss of function. There can be a delay in onset of clinical symptoms of years, or even decades. In those with late-onset disease, most have a single variant, others have variants with milder reduction in protein function, or a combination of both.¹⁰ It is unlikely that these variants are behaving like true recessive Mendelian inheritance alleles and it is predicted that reduced protein levels manifest as a slower progression of liver disease or a predisposition to further hepatic insult.

A key aspect to the process of performing genetic testing is the clinical interpretation of detected variants. The American College of Medical Genetics and Genomics and the Association for Molecular Pathology published updated guidelines which are used as a framework to classify variants as: 'pathogenic', 'likely pathogenic', 'uncertain significance', 'likely benign' or 'benign'.¹¹ Multiple criteria are incorporated in the final interpretation of a variant, the end result is quite conservative in ascribing pathogenicity to a variant. The guidelines are intended for variants with a definitive role in a Mendelian disorder. In liver disease involving variants in cholestasis-related genes, the need for a second insult is often required and the degree of damage attributable to genetics is not always clear.^{4,10} Variants in these genes which are categorised as 'likely benign' or 'benign' are not likely to be the sole cause of disease but may confer an increased risk to developing

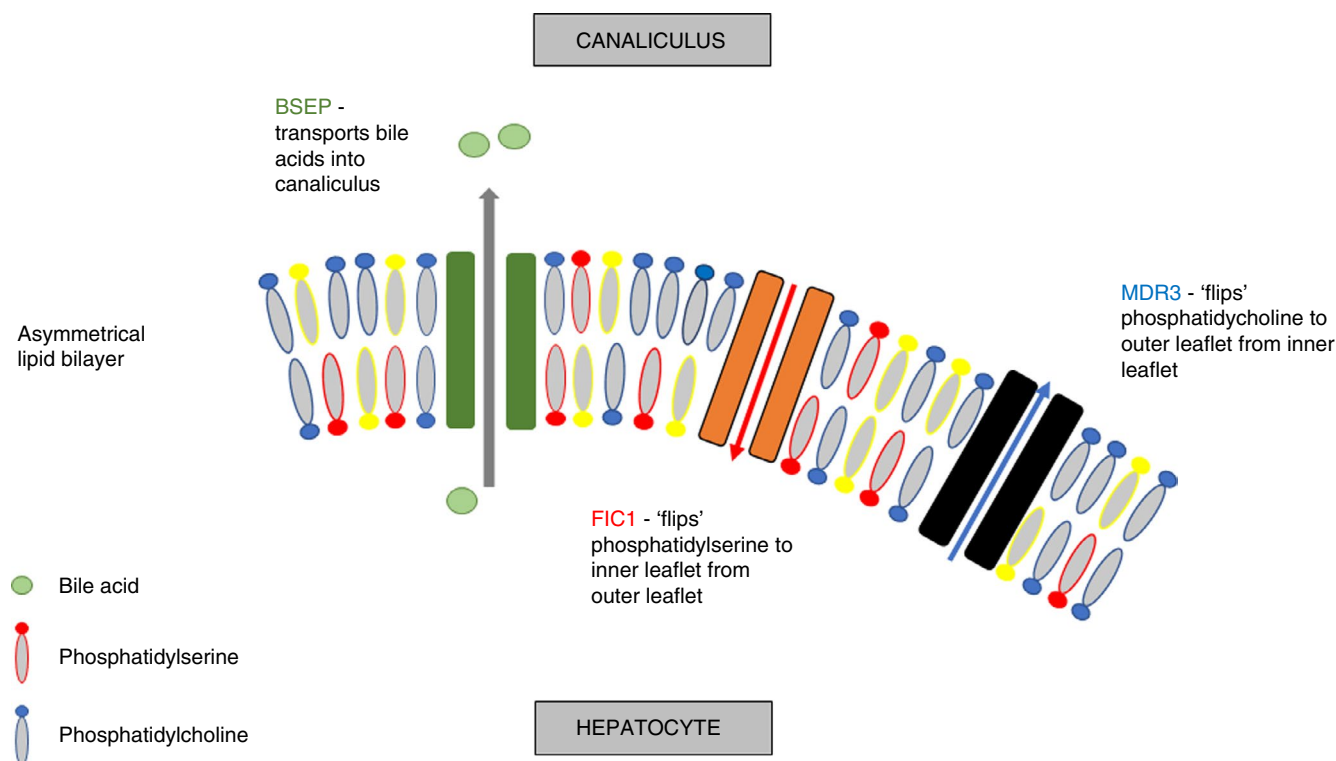


FIGURE 1 Diagram of biliary transporters and representation of asymmetrical lipid bilayer. BSEP transports bile acid into the canalculus. FIC1 'flips' phosphatidylserine from the outer leaflet to the inner leaflet. MDR3 'flips' phosphatidylcholine to the outer leaflet

cholestasis. These latter two categories of variants are often not included in diagnostic reports.

This review summarises the published data on variants in *ABCB11*, *ABCB4* and *ATP8B1* identified in adults, in a range of cholestatic syndromes.

2 | DRUG-INDUCED LIVER INJURY

Drug-induced liver injury (DILI) is a common cause of terminating new drugs during clinical trials and the leading cause for post-marketing withdrawals.¹² The incidence of DILI in general populations is estimated at 13.9 per 100 000.¹³ Large studies assessing the outcomes of patients with DILI, identified mortality of up to 8%, and need for liver transplantation in 2%.^{14,15} The development of DILI is unpredictable. The three main patterns of DILI are hepatocellular, cholestatic and mixed, which are based on the first laboratory tests available after the clinical event and involve the alanine aminotransferase (ALT), alkaline phosphatase (ALP) and ratios between the two values.¹⁶ The only clear genetic association with DILI is between flucloxacillin and HLA-B*5701.¹⁷ Several drugs are thought to cause cholestasis by inhibiting BSEP function, such as cyclosporine, bosentan and troglitazone.^{18,19} In vitro work to determine the effect on BSEP in a large set of drugs, and correlate it with known DILI associations and taurocholate transport, identified a strong correlation between BSEP inhibitors and DILI.²⁰ Over half the BSEP inhibitors were associated with severe DILI, in particular those which reduced taurocholate transport, while those BSEP inhibitors with less severe or no DILI had minimal effect on taurocholate transport.

A cohort of 36 patients with DILI (23 drug-induced cholestasis, 13 drug-induced hepatocellular injury) underwent genetic sequencing for known variants in *ABCB11* and *ABCB4*, in comparison to healthy controls.²¹ Four variants specific for DILI were discovered: two in *ABCB11* for drug-induced cholestasis (p.Asp676Tyr and p.Gly855Arg); one in *ABCB4* for drug-induced cholestasis (p.Ile764Leu); one in *ABCB4* for drug-induced hepatocellular injury (p.Leu1082Gln). In addition, the common *ABCB11* variant p.Val444Ala, which is known to reduce protein levels by half compared to wild type, was observed more frequently in patients with drug-induced cholestasis than drug-induced

hepatocellular injury and healthy controls. Homozygosity for p.Val444Ala was seen in 61% of drug-induced cholestasis, compared to 31% and 32% respectively in drug-induced hepatocellular injury and healthy controls. Functional analysis of the p.Gly855Arg *ABCB11* variant identified 20% expression of reference BSEP, and the authors concluded that patients heterozygous for this variant have enough functional BSEP protein in normal healthy state, however, there is insufficient activity following drug or hormonal challenge, resulting in cholestasis. A similar causative role for the p.Val444Ala *ABCB11* as a susceptibility factor, as a result of reduced hepatic BSEP expression compared to wild type, was also proposed.

The Spanish DILI Registry study of 188 patients (51 drug-induced cholestasis, 48 mixed liver injury, 89 drug-induced hepatocellular injury), sequenced patients and healthy controls for *ABCB11* p.Val444Ala.²² In keeping with previous data, homozygotes for p.Val444Ala were more frequent in DILI than controls (OR 1.6, 1.1–2.4, $P = 0.01$). Causative drugs were checked for the presence of specific chemical moieties with BSEP-mediated taurocholate transport inhibiting properties. In the drugs which cause >50% BSEP inhibition, homozygous p.Val444Ala did not affect the DILI risk, but in drugs with <50% BSEP inhibition, homozygous p.Val444Ala was significantly associated with DILI ($P = 0.01$). The authors hypothesise that drugs which do not cause sufficient change in BSEP activity (ie <50% BSEP inhibition) require a contributing factor, in this case homozygous *ABCB11* p.Val444Ala, to drop below the threshold of functional BSEP level, with resultant hepatotoxicity. The association within this cohort was with drug-induced hepatocellular injury not drug-induced cholestasis, however, it is worth considering that in the early stages of BSEP-mediated hepatotoxicity the elevated transaminases are a consequence of bile acid-related hepatocyte injury, which does not manifest with the classical cholestatic pattern (raised ALP) used in the definitions of DILI.

Further work from this group using the Spanish DILI Registry, performed extended genetic sequencing for common variants in *ABCB1*, *ABCB4* and *ABCC2*, in 141 patients with DILI and compared these to healthy controls. Results detected no significant difference in common variants for these genes between the groups.²³

Where functional BSEP work has been carried out, DILI is particularly evident in BSEP inhibiting drugs which reduce taurocholate

TABLE 1 Results of selected studies testing genetics in drug-induced liver injury

Author	Type of study	Number of patients	Genes tested	Association
Lang 2007 ²¹	Cohort of DILI compared to healthy controls	36 (95 controls)	<i>ABCB11</i> & <i>ABCB4</i>	Two <i>ABCB11</i> variants and two <i>ABCB4</i> variants in DILI cohort. <i>ABCB11</i> p.Val444Ala common variant significantly associated with drug-induced cholestasis
Ullzurrun 2013 ²²	Registry compared to healthy controls	188 (219 controls)	<i>ABCB11</i> p.Val444Ala	<i>ABCB11</i> p.Val444Ala more in DILI than controls
Ullzurrun 2014 ²³	Registry compared to healthy controls	141 (161 controls)	<i>ABCB4</i> common variants	No difference

Abbreviation: DILI, drug-induced liver injury.

transport. From the limited data available (Table 1) on DILI and genetic variants for proteins involved in biliary transport, the signal is of an association with common variants in *ABCB11* gene. Reduced levels of BSEP are generally sufficient, but following a further insult with medications, intrahepatic cholestasis may occur. In individuals with variants this is most commonly seen with medications with a more modest reduction in BSEP activity (ie <50% BSEP inhibition). The biochemical picture of patients with BSEP related DILI does not usually fit the definition of drug-induced cholestasis, as the bile acid-induced hepatocyte injury predominantly presents with raised transaminases, and will not have a ratio of serum activity of ALT to ALP of 2 or less.¹⁶

3 | INTRAHEPATIC CHOLESTASIS OF PREGNANCY

Intrahepatic cholestasis of pregnancy (ICP) affects 0.7% of pregnancies in the United Kingdom.²⁴ The pathogenesis has been extensively studied with an apparent key role thought to be played by reproductive hormones and abnormal bile acid homeostasis.²⁵ Progesterone sulphates are elevated in patients with ICP, and from experimental work these have been shown to function as a farnesoid X receptor partial agonist, with aberrant gene expression including reduced BSEP expression.²⁶ A similar mechanism of action has been demonstrated with 17 β -estradiol.²⁷ The genetic predisposition to ICP, and the causative or contributory role of variants in cholestasis genes, has been assessed by describing clinical events in patients and families with known *ABCB4* variants, and by sequencing patients with ICP.

Investigation of relatives of a proband with PFIC, who was homozygous for a highly deleterious variant in *ABCB4* (c.1712delT), and had undergone liver transplantation in childhood, was the first link between *ABCB4* and ICP.²⁸ Six females from the family had at least one episode of ICP, and the four in whom genetic data were available were all found to be heterozygous for the *ABCB4* variant. Subsequent analysis of 31 patients with PFIC secondary to *ABCB4* variants revealed three mothers with ICP, all of whom were heterozygous for *ABCB4* variants (p.Arg652Gly, p.Arg957Ter, c.1744delT).⁹ In a family with multiple members with *ABCB4* variant p.Arg788Trp and chronic liver disease, all nine female members who survived to adulthood suffered from ICP, including both homozygotes and heterozygotes.²⁹ In five of them their first presentation of liver disease was with ICP. These findings suggest that there is a predisposition to ICP in patients with a family history of liver disease who are heterozygous for *ABCB4* variants.

The results of data from patients with ICP being screened for variants are more variable. A cohort of patients with ICP is combined with those with oral contraceptive-induced cholestasis for analysis in a study testing for *ABCB4* variants.³⁰ Of this subgroup of 59 patients, 27% were heterozygous for variants in *ABCB4*. In small studies (21 and 33 patients respectively) with ICP patients sequenced for *ABCB4* and *ABCB11*, there was an increase in genetic variation, particularly in *ABCB4*, compared to healthy controls.²⁵

The variants were thought to play a causative role in a third of patients with ICP.³¹

High-throughput sequencing in 147 patients with ICP, for an extended panel of genes (including *ABCB4*, *ABCB11*, *ATP8B1*), detected 12 *ABCB4* variants, 4 *ABCB11* variants and 5 *ATP8B1* variants.³² Two similar sized studies sequenced *ATP8B1* in patients with ICP (176 and 182 patients) and identified variants in a minority of patients.^{33,34} Although not a major gene contributing to occurrence of ICP, variants in *ATP8B1* are likely to predispose to the development of cholestasis through a relative rise in biliary phospholipid.

A sequencing study that included 491 women with ICP, demonstrated that heterozygotes for the common *ABCB11* p.Val444Ala variant were more likely to have ICP with an odds ratio of 1.7 (CI 1.4-2.1, $P < 0.001$) for ICP, and p.Val444Ala homozygous individuals were at highest risk compared to wild type, with OR 2.8 (CI 1.7-44, $P < 0.0001$).²⁴ Although other *ABCB11* variants are present in a minority of cases of ICP, the p.Val444Ala variant is a susceptibility locus for ICP and a significant predisposing factor for development of the condition. A genotyping study looking at the influence of common genetic variation on susceptibility to ICP in 563 cases and 642 controls used the HapMap dataset together with the Tagger algorithm (Haploview 4.1 build 22) to select 83 common variants around six candidate genes that included *ABCB11*, *ABCB4* and *ATP8B1*.³⁵ Six variants in *ABCB11* (including p.Val444Ala) and six variants in *ABCB4* were significantly associated with ICP.

A recent study of 310 women with severe early-onset ICP (defined by presentation before 33 weeks' gestation with serum bile acid concentrations ≥ 40 $\mu\text{mol/L}$) that utilised whole-genome sequencing, demonstrated that 20% had pathogenic variants in the *ABCB4* or *ABCB11*.³⁶

The data suggest that patients with *ABCB4*, *ABCB11* and *ATP8B1* variants, both homozygous and heterozygous, may be at an increased risk for ICP (Table 2). Considering the association with severe early-onset ICP, patients with known variants should receive specialist preconception care. However, these patients make up the minority of all patients with ICP, and the common *ABCB11* p.Val444Ala variant is a more common risk factor. The reduced level of BSEP function in *ABCB11* variants (including p.Val444Ala) may confer susceptibility to ICP.²⁴ It is likely that women who are heterozygous for *ABCB4* variants, or have common susceptibility variants, have a transient decompensation in protein function during pregnancy related to elevated concentrations of 17 β -estradiol²⁷ or progesterone sulphates,²⁶ resulting in ICP.⁹ Similarly there may be abnormalities of canalicular membrane lipids during pregnancy in those with *ATP8B1* variants.³³

4 | CHRONIC LIVER DISEASE

In a proportion of patients with chronic cholestatic liver disease, the aetiology is not clear and does not fit clinically, serologically or radiologically with well recognised conditions such as primary biliary cholangitis or primary sclerosing cholangitis.³⁷ Genetic testing in this cohort of unexplained chronic liver disease has been described in case

TABLE 2 Results of selected studies testing genetics in intrahepatic cholestasis of pregnancy

Author	Type of study	Number of patients	Gene	Association
Jacquemin 1999 ²⁸	Case study	6	<i>ABCB4</i>	All tested were heterozygous for <i>ABCB4</i>
Gotthardt 2008 ²⁹	Case study	9	<i>ABCB4</i>	All were heterozygous or homozygous for <i>ABCB4</i>
Pasmant 2012 ³⁰	Cohort of ICP combined with CIC	59	<i>ABCB4</i>	27% (combined data) heterozygous for <i>ABCB4</i>
Anzivino 2013 ³¹	Cohort of ICP compared to healthy controls	33 (100 controls)	<i>ABCB4</i> & <i>ABCB11</i>	33% had a variant (5 <i>ABCB4</i> , 6 <i>ABCB11</i>), none in controls
Pauli-Magnus 2004 ²⁵	Cohort of ICP compared to healthy controls	21 (40 controls)	<i>ABCB4</i> and <i>ABCB11</i>	Genetic variation in <i>ABCB4</i> and <i>ABCB11</i> in ICP patients
Dixon 2017 ³²	Cohort of ICP	147	<i>ABCB11</i> , <i>ABCB4</i> and <i>ATP8B1</i>	14% had a variant (12 <i>ABCB4</i> , 4 <i>ABCB11</i> , 5 <i>ATP8B1</i>)
Mullenbach 2005 ³³	Cohort of ICP compared to healthy controls	182 (120 controls)	<i>ATP8B1</i>	3% had variant in <i>ATP8B1</i> , none in controls
Painter 2005 ³⁴	Cohort of ICP compared to healthy controls	176 (100 controls)	<i>ATP8B1</i>	3.5% had variant in <i>ATP8B1</i> , none in controls
Dixon 2009 ²⁴	Cohort of ICP compared to healthy controls	491 (261 controls)	<i>ABCB11</i>	1.4% had <i>ABCB11</i> variant. p.Val444Ala common variant significant association
Dixon 2014 ³⁵	Cohort of ICP compared to healthy controls	563 (642 controls)	Common variants in <i>ABCB11</i> , <i>ABCB4</i> and <i>ATP8B1</i>	Association with common variants in <i>ABCB11</i> and <i>ABCB4</i>
Turro 2020 ³⁶	Whole-genome sequencing of severe early-onset ICP	310	<i>ABCB11</i> and <i>ABCB4</i>	20% had variants in <i>ABCB11</i> or <i>ABCB4</i>

Abbreviations: CIC, oral contraceptive-induced cholestasis; ICP, intrahepatic cholestasis of pregnancy.

reports incorporating family data, and screening studies of larger cohorts of patients with liver disease.

Case reports describing patients, a 47-year-old female and a 38-year-old male, who presented with adult onset advanced cholestatic liver disease of unknown cause, identified they were heterozygous for variants in *ABCB4* (p.Gly535Asp and p.Val1068Glu).^{38,39} The first patient underwent cholecystectomy aged 19, had pruritus during two pregnancies, and had a daughter who was also heterozygous for the same variant in *ABCB4* and developed ICP. The second case had also previously undergone cholecystectomy, and his daughter and mother were both heterozygous for the same *ABCB4* variant and had cholecystectomy aged 13 and 34 respectively. In both cases the aetiology of chronic liver disease was attributed to the *ABCB4* variant, and their heterozygosity is felt to have resulted in a milder functional deficit in *MDR3* and therefore presented with cirrhosis later in life.³⁸ Another case report describes two brothers who presented with biliary cirrhosis in adulthood on the background of chronic cholestasis since adolescence, and were found to be compound heterozygous for variants in *ABCB4* gene (p.Ser320Phe and c.2682+1G>A).⁴⁰

A more extensive genotype and phenotype characterisation is available from a family of 11 siblings whose parents were 4th cousins, which highlights the difference in clinical presentation in homozygotes and heterozygotes.²⁹ The proband was a 34-year-old female with idiopathic biliary cirrhosis requiring liver transplantation, who was homozygous for a missense variant in *ABCB4* (p.Arg788Trp). Her

mother suffered from ICP in all her pregnancies. Of her siblings, six were deemed severely affected (two childhood cirrhosis, four liver failure in adulthood), and five were mildly affected. The three severely affected who underwent sequencing were all also homozygous, while the mother and the mildly affected siblings were heterozygous, for *ABCB4* variants.

A 20-year-old lady with persistent jaundice attributed to the oral contraceptive pill and had evidence of advanced fibrosis on liver biopsy, was found to be homozygous for a missense variant in *ABCB11* (p.Ala523Gly).⁴¹ Her sister was also affected by episodic cholestasis and has the same variant in homozygous form. A 25 years old diagnosed with intrahepatic cholestasis and cirrhosis, which required liver transplantation, was found to be heterozygous for a missense variant in *ATP8B1* (p.Arg600Trp) as well as homozygous for the common p.Val444Ala variant in *ABCB11*.⁴² A multi-genic cause for his presentation with advanced chronic liver disease in early adulthood was proposed.

In a study of 32 adults with a histological diagnosis of cholestatic liver disease and common causes of chronic liver disease excluded, genetic sequencing of *ABCB4* gene was performed.⁴³ Thirty-four per cent of this cohort was found to be heterozygous for disease-associated *ABCB4* variants, defined as those that led to premature protein truncation, small deletions, insertions and missense variants. There were no differences in clinical or biochemical profile between those with and without variants, but there was a significantly higher fibrosis grade (Ishak score) in those

with *ABCB4* variants, with a third having F3 or F4 disease. The majority of patients with *ABCB4* variants had a significant biochemical response to 15 mg/kg/day of ursodeoxycholic acid (UDCA). Similarly, when genetic sequencing of the *ABCB4* gene was performed prospectively in 90 patients (80 with chronic cholestasis, 10 with prior history of cholestasis [ICP, juvenile cholestasis]) out of 2602 consecutive hepatobiliary referrals screened for chronic cholestasis; heterozygous variants in *ABCB4* gene, predominantly missense variants, were identified in 20% of those tested, including those with primary biliary cholangitis or primary sclerosing cholangitis.⁴⁴

In a series of six young adults (aged 17-22) with chronic liver disease, the patients and their first-degree relatives were sequenced for a number of genes (including *ABCB11* and *ATP8B1*), and found two patients to be heterozygous for variants in *ATP8B1* (p.Asn45Thr, p.Ile349Thr).⁴⁵ In 33 patients with unexplained cholestasis an 8 gene panel was performed (including *ABCB4*, *ABCB11* and *ATP8B1*), and 18% had a major variant in *ABCB4* or *ABCB11*, while others had variants of unclear significance.⁴⁶ A similar gene panel (including *ABCB4*, *ABCB11* and *ATP8B1*) was performed on 48 patients with cryptogenic cholestasis, and likely pathogenic variants were seen in 21% of patients: 2 in *ATP8B1* (p.Asp1219His, p.Pro23Leu); 2 in *ABCB4* (p.Lys672Ter, p.Ala364Val); and 6

in *ABCB11* (p.Tyr93Ser, p.Val597Leu, p.Arg1128Cys, p.Ala523Gly—reported previously,⁴⁰ p.Ser1100Glnfs*38, p.Glu135Lys).⁴⁷

Recent data from sequencing for variants in a combination of *ATP8B1*, *ABCB11* and *ABCB4* genes in 427 paediatric and adult patients with chronic cholestasis, included a large subgroup of adult patients (48 for *ATP8B1*; 67 for *ABCB11*; and 116 for *ABCB4*).⁴⁸ A third of all patients carried at least one disease causing variant, and although the majority of variants were seen in children, 18% were diagnosed in adults with cholestasis. In those with adult onset disease *ATP8B1* variants were identified in 6 patients, *ABCB11* in 16 and *ABCB4* in 15 patients. A significant proportion of adult patients without disease causing variants were found to have common variants in *ATP8B1*, *ABCB11* and *ABCB4*.

As part of a large nationwide genome-wide association study in Iceland, analysis of common *ABCB4* variants was performed and correlated with the presence of liver disease.⁴⁹ Two rare variants, a missense variant and a frameshift insertion variant, were found to have a large effect on the presence of gallstone disease, cholestasis in pregnancy, cirrhosis, abnormal liver enzymes, liver and gallbladder cancer. Further sequencing was performed around the *ABCB4* gene, and the authors did not feel additional variants were missed from the population.

TABLE 3 Results of selected studies testing genetics in chronic cholestasis and chronic liver disease in adults

Author	Type of study	Number of patients	Gene	Association
Lucena 2003 ³⁸	Case study	2	<i>ABCB4</i>	Heterozygous <i>ABCB4</i> (CLD and ICP), daughter heterozygous and ICP
Denk 2010 ³⁹	Case study	3	<i>ABCB4</i>	Heterozygous <i>ABCB4</i> (CLD), daughter and mother heterozygous and had cholecystectomy <40 y of age
Oliveira 2016 ⁴⁰	Case study	2	<i>ABCB4</i>	Siblings both compound heterozygous <i>ABCB4</i> (CLD)
Gotthardt 2008 ²⁹	Case study	12	<i>ABCB4</i>	Homozygous <i>ABCB4</i> (CLD), siblings all homozygous or heterozygous (CLD, ICP), mother heterozygous (ICP)
Vitale 2016 ⁴¹	Case study	2	<i>ABCB11</i>	Homozygous <i>ABCB11</i> (CLD), sibling homozygous (episodic cholestasis)
Hsu 2009 ⁴²	Case study	1	<i>ATP8B1</i> and <i>ABCB11</i>	Heterozygous <i>ATP8B1</i> and p.Val444Ala <i>ABCB11</i> (CLD)
Ziol 2008 ⁴³	Cohort of chronic cholestasis	32	<i>ABCB4</i>	34% heterozygous for <i>ABCB4</i> variants
Degiorgio 2016 ⁴⁴	Cohort of chronic cholestasis	90	<i>ABCB4</i>	20% heterozygous for <i>ABCB4</i> variants
Kulecka 2017 ⁴⁵	Cohort of chronic liver disease	6	<i>ATP8B1</i> and <i>ABCB11</i>	Two heterozygous for <i>ATP8B1</i> variants
Aamann 2018 ⁴⁶	Cohort of cholestasis	33	<i>ABCB4</i> , <i>ABCB11</i> and <i>ATP8B1</i>	18% had a major variant (5 <i>ABCB4</i> , 1 <i>ABCB11</i>)
Vitale 2018 ⁴⁷	Cohort of cholestasis	48	<i>ABCB4</i> , <i>ABCB11</i> and <i>ATP8B1</i>	21% heterozygous for variants (2 <i>ABCB4</i> , 6 <i>ABCB11</i> , 2 <i>ATP8B1</i>)
Droge 2017 ⁴⁸	Cohort of cholestasis	427 (including paediatric)	<i>ABCB4</i> , <i>ABCB11</i> and <i>ATP8B1</i>	15 patients <i>ABCB4</i> (13% of tested), 16 patients <i>ABCB11</i> (22% of tested), 6 patients <i>ATP8B1</i> (13% of tested)
Gudbjartsson 2015 ⁴⁹	Genome-wide association study		<i>ABCB4</i> common variants	Two <i>ABCB4</i> common variants associated with gallstones, ICP, cirrhosis, liver cancer, gallbladder cancer

Abbreviations: CLD, chronic liver disease; ICP, intrahepatic cholestasis of pregnancy.

Case and family studies of patients with idiopathic biliary cirrhosis have revealed a collection of patients with variants in *ABCB4* (homozygous, heterozygous and compound heterozygous) with onset of disease in adulthood and various phenotypes of liver disease in first-degree relatives (Table 3). There are fewer data for *ABCB11* and *ATP8B1* in adults with chronic liver disease, but variants have been detected in a series of cases. The studies sequencing larger cohorts of adult patients with cholestatic liver disease have identified up to 34% as having heterozygous variants in *ABCB4* gene, and there is increasing data on heterozygous variants in *ATP8B1* and *ABCB11* genes. The later onset of disease in these patients could be related to a milder functional deficit, either as a result of the variants being less damaging on a functional level, or because patients are heterozygotes. The only genome-wide association study in the field, suggests that common variants in *ABCB4* are associated with a range of phenotypes of liver disease, including hepatobiliary malignancy. International guidelines recommend considering genetic testing in patients where common cholestatic syndromes have been excluded and it is clinically appropriate.³⁷

It is important to recognise that some of the patients identified with variants had a diagnosis of recognised liver disease syndrome. Testing should therefore be considered in these cases, especially in the presence of atypical features. It is unclear to what extent the presence of genetic variants is acting as disease modifiers in these patients.

5 | LOW PHOSPHOLIPID-ASSOCIATED CHOLESTASIS

Although biliary cholelithiasis is a very common clinical condition, work has been undertaken in a subgroup of such patients evaluating the role of genetic variants. Genetic sequencing of *ABCB4* to assess for an underlying genetic factor was undertaken in six adults with atypical presentations of cholelithiasis: including the presence of cholesterol gallstones and intrahepatic sludge; the recurrence of symptoms after cholecystectomy; and the prevention of recurrence with UDCA.⁵⁰ They were aged 22–55 years, and included three females who had worsening of biliary symptoms during pregnancy or after oral contraception was used. All patients were found to have variants in *ABCB4*, including one patient who was homozygous (p.Ser320Phe, p.Thr175Val, p.Pro1161Ser, 1327insA) which was thought to underpin

their clinical presentation with cholelithiasis. The group then characterised these patients as having a condition called *low phospholipid-associated cholestasis* (LPAC). They performed subsequent genetic analysis on 32 patients with LPAC, 28 with symptomatic gallstones alone and 33 controls without cholelithiasis.⁵¹ Of those with LPAC, 56% were heterozygous or homozygous for variants in *ABCB4* gene, and no variants were detected in the control groups. They concluded that variants in *ABCB4* were a significant risk factor for symptomatic and recurrent cholelithiasis in young adults. Work from a different group included 43 patients with LPAC as part of a larger study evaluating *ABCB4*, and 37% were heterozygous for *ABCB4* variants.³⁰

The largest analysis of *ABCB4* in LPAC was from 156 consecutive patients, and 51% were heterozygous or homozygous for variants.⁵² Although there was no difference in clinical characteristics between those with and without variants in *ABCB4*, analysis of those with *ABCB4* variants identified an earlier onset of symptoms in individuals with truncating variants to those with missense variants (26.5 vs 33.9 years). Interestingly of the patients with *ABCB4* variants, two had biliary cirrhosis and one developed an intrahepatic cholangiocarcinoma.

These findings, however, were not reproduced in a study of 104 patients with symptomatic cholelithiasis under the age of 40 who underwent laparoscopic cholecystectomy and had *ABCB4* sequencing.⁵³ From this cohort less than 2% had variants in *ABCB4*, however, it must be noted that these patients did not necessarily fit the criteria for LPAC as outlined in the original study.

The total number of patients with LPAC who have been tested for variants in *ABCB4* remains relatively small (Table 4). However, there does appear to be an association with *ABCB4* variants in those with the whole clinical syndrome, which only makes up a minority of all patients with cholelithiasis. From the family studies of chronic liver disease in patients with *ABCB4* variants, those with variants frequently had undergone cholecystectomy by early adulthood, adding further weight to the role of *ABCB4* variants in early cholelithiasis.^{38,39}

6 | PRACTICAL RECOMMENDATIONS

It is reasonable to perform genetic testing in adults with unexplained cholestasis and cholestatic chronic liver disease,^{37,47} particularly in those with a family history of liver disease or ICP. Individuals who

TABLE 4 Results of selected studies testing genetics in low phospholipid-associated cholelithiasis

Author	Type of study	Number of patients	Gene	Association
Rosmorduc 2001 ⁵⁰	Cohort of atypical cholelithiasis	6	<i>ABCB4</i>	All <i>ABCB4</i> variants (5 heterozygous, 1 homozygous)
Rosmorduc 2003 ⁵¹	Cohort of LPAC, compared to cholelithiasis and healthy controls	32	<i>ABCB4</i>	56% of LPAC were heterozygous or homozygous for <i>ABCB4</i> , no variants in control
Pasman 2012 ³⁰	Cohort study, subgroup with LPAC	43	<i>ABCB4</i>	37% <i>ABCB4</i> variants (heterozygous)
Nakken 2009 ⁵³	Cohort of cholelithiasis <40 y with cholecystectomy	104	<i>ABCB4</i>	<2% <i>ABCB4</i> variants
Poupon 2013 ⁵²	Cohort of LPAC	156	<i>ABCB4</i>	51% of LPAC were heterozygous or homozygous for <i>ABCB4</i> variants

Abbreviation: LPAC, low phospholipid-associated cholelithiasis.

TABLE 5 A list of commonly used drugs associated with drug-induced liver injury in patients with *ABCB11* variants

Drugs
Non-steroidal anti-inflammatory
Amoxicillin/clavulanic acid
Piperacillin/tazobactam
Flucloxacillin
Oral contraceptive pill
Androgenic anabolic steroids
Fluvastatin

develop DILI, particularly related to medications known to reduce BSEP function (Table 5) or if it is severe DILI, should undergo testing, without too much focus on pattern of biochemical abnormality as bile acid-induced hepatocyte damage presents with raised transaminases. All individuals with ICP do not require testing, but those with other associated liver manifestations, severe ICP (onset before 33 weeks' gestation and serum bile acids >40 $\mu\text{mol/L}$) or with persistent cholestasis post-partum, could be referred for testing. Genetic testing for patients who fit the criteria for LPAC appears to have a high yield with *ABCB4* variants. Although their clinical characteristics may not differ, there is a signal that those with variants need investigation for underlying chronic liver disease.

This article concentrates on variants in the three genes (*ABCB11*, *ABCB4* and *ATP8B1*) most commonly associated with early-onset cholestasis, which have also been most extensively described in adults. Clinical testing of cholestasis-related genes is mainly performed on gene panels which include a wide range of additional genes, that have been derived from childhood liver disease. These genes account for a smaller proportion of childhood liver disease, however, it is unclear if they are equally rare in adult liver disease. Although variants in these genes, such as *TJP2*, *CFTR*, *NR1H4*, *MYO5B* and *USP53*, may contribute to liver disease in adults, data are currently limited to *TJP2*⁴⁷ and *NR1H4*⁵⁴ in a small numbers of patients, and the strength of the associations is not clear. With increased testing of adults, further data on the prevalence of variants will become available, and when combined with accurate clinical phenotype data, will allow conclusions to be drawn on their causative role and contribution to cholestasis. We recommend that details on these variants are submitted to ClinVar (ncbi.nlm.nih.gov/clinvar/), which is a freely accessible public archive of variants with phenotype, interpretation and supporting evidence.⁵⁵ When rare variants are identified in any

of cholestasis-related genes, particularly if the variant is of uncertain significance, it is crucial that the clinical phenotype is explored thoroughly to see if it fits with the pathological pattern of disease. The final clinical interpretation should be in collaboration with geneticists and pathologists, and genetic input into multi-disciplinary meetings may become more commonplace.

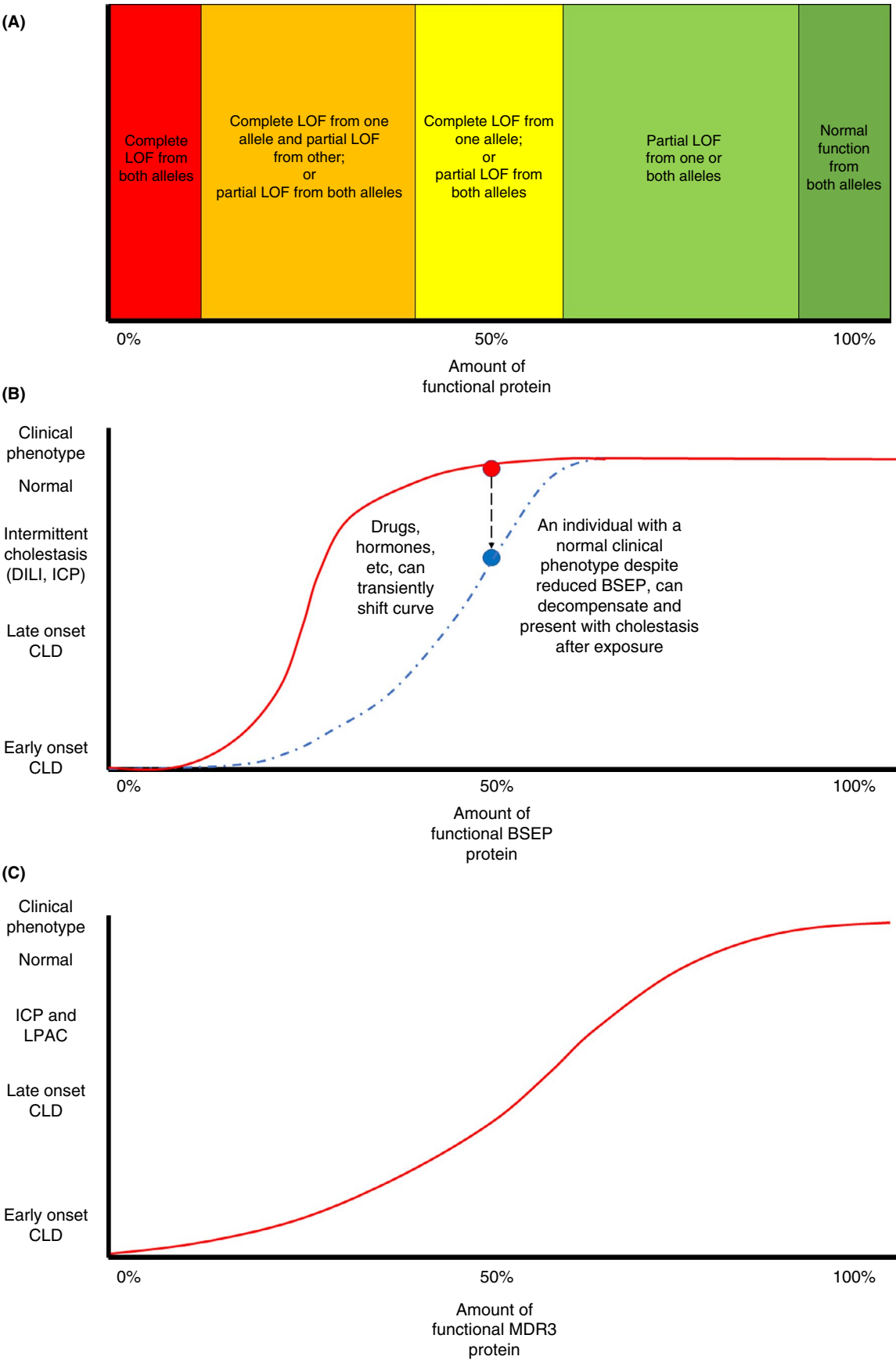
For people with previous DILI and a known variant, medications which reduce BSEP activity should either be avoided or utilised with caution and closer monitoring (Table 5). Those with *ABCB4* variants are at an increased risk of ICP, and should be counselled prior to conception and monitored closely during pregnancy for the development of ICP. Although there are no proven therapeutic interventions for patients with chronic liver disease and these variants, there may be a role for treatment with UDCA. In children with PFIC who received 20-30 mg/kg/day of UDCA there was a significant improvement in liver biochemistry and pruritus, and a suggestion it may prevent evolution to cirrhosis.⁵⁶ A benefit from UDCA has been described in preventing symptom recurrence post-cholecystectomy in patients with LPAC, and a biochemical response to 15 mg/kg/day UDCA has been reported in a cohort of patients with *ABCB4* variant-associated chronic liver disease.⁴³ Although this is promising, further evaluation is needed to determine if this is replicated with histological and clinical endpoints.

Until further information on the natural history of patients with genetic variants is available it will prove difficult to understand the risk to patients of progression to chronic liver disease, or to offer specific prognosis in patients with chronic liver disease associated with these variants, outwith of standard liver prognostic models. In a similar vein their risk of hepatocellular carcinoma and cholangiocarcinoma development is unknown and therefore surveillance should be undertaken according to existing guidelines. These are important considerations prior to testing unaffected first-degree relatives, and it is advisable that where first-degree relatives undergo genetic screening they have access to a genetic counsellor.

7 | CONCLUSIONS AND FUTURE DIRECTIONS

The findings from studies evaluating the presence of variants of genes associated with biliary homeostasis in adults have been summarised, and these encompass acute and chronic phenotypes of liver disease. In patients with acute, transient presentations of cholestasis, such as DILI and ICP, there appears to be an association with variants, especially

FIGURE 2 Relationship between types of genetic variants, amount of functional protein and clinical phenotypes. (A) Predicted amount of functional protein produced with different allelic combinations. (B) BSEP levels and the association with clinical phenotype. There is a threshold amount of functional BSEP protein which is sufficient for bile acid transport in health, which seems to be about 25%. There is a dynamic relationship between functional BSEP protein and clinical phenotype, with drugs and hormones shifting the curve transiently. (C) MDR3 levels and the association with clinical phenotype. There is a spectrum of reduced functional MDR3 protein in individuals with *ABCB4* variants, which is linked to the genotype and is reflected in the clinical phenotype. Those with significant deficiencies of MDR3 present in childhood with complications of end-stage liver disease, whilst those with milder deficiencies have a more insidious onset disease which can present in adulthood. CLD, chronic liver disease; DILI, drug-induced liver injury; ICP, intrahepatic cholestasis or pregnancy; LOF, loss of function; LPAC, low phospholipid-associated cholestasis



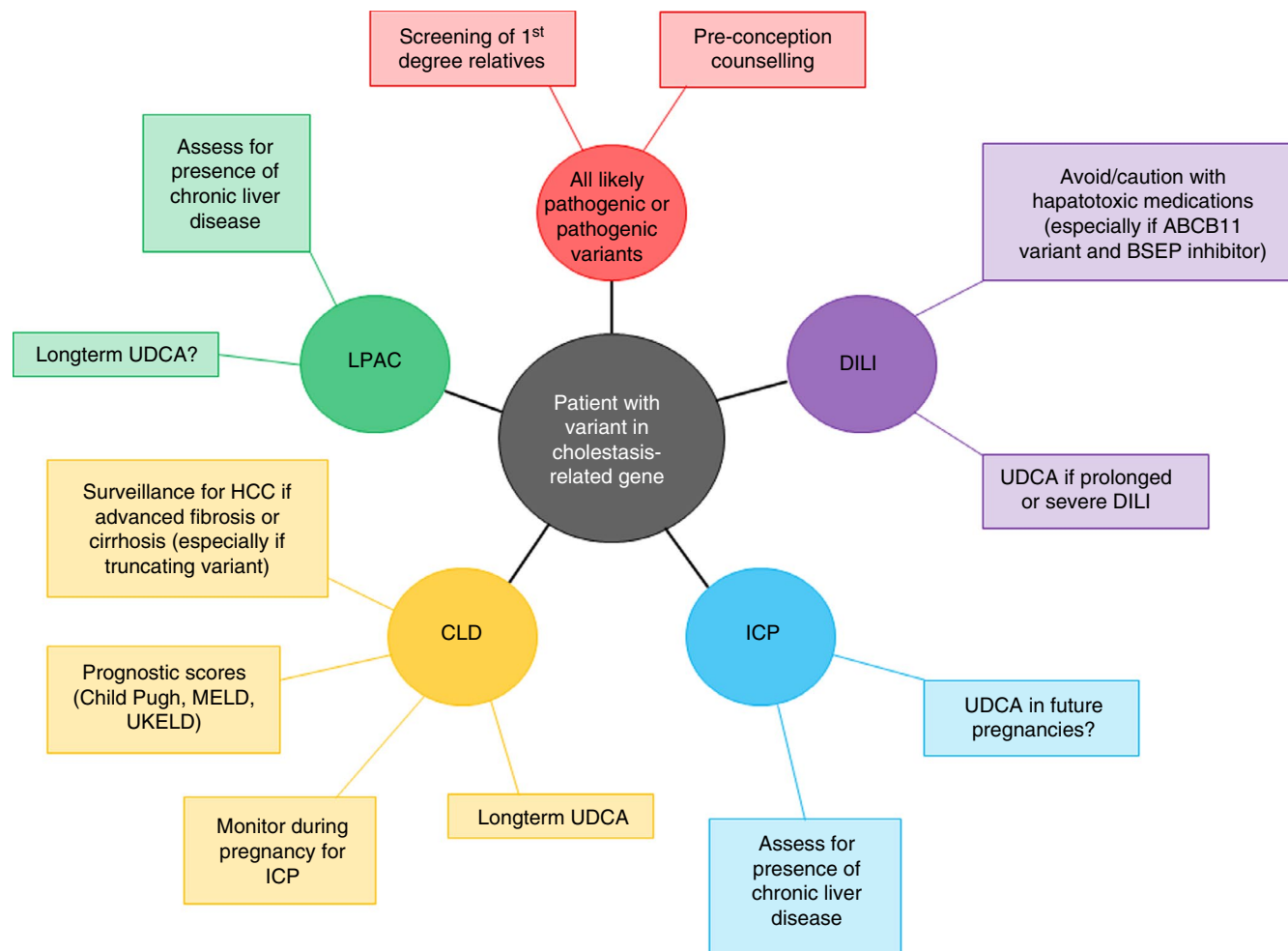


FIGURE 3 Management considerations in patients with variants in cholestasis-related genes and different clinical phenotypes. CLD, chronic liver disease; DILI, drug-induced liver injury; HCC, hepatocellular carcinoma; ICP, intrahepatic cholestasis or pregnancy; LPAC, low phospholipid-associated cholestasis; MELD, model for end-stage liver disease; UDCA, ursodeoxycholic acid; UKELD, United Kingdom Model for End-Stage Liver Disease

common variants, in *ABCB11*. In health, these patients are likely to have reduced, but sufficient, BSEP levels for normal bile acid transport, which a threshold level of approximately 25% of functional BSEP protein (Figure 2). Following a second insult, from drugs or hormones, to an already genetically vulnerable liver, there may be a reduction in BSEP below this threshold level which results in cholestasis. Variants in *ABCB4* have been described in patients with chronic liver disease presenting in adulthood. In these patients the variants may be associated with production of a higher levels of the MDR3 protein than seen in those with severe early-onset disease, as a result of a less damaging variant or being heterozygotes. There is, however, a sufficient reduction in MDR3 protein compared to healthy controls, which results in a persistent reduction in micelle formation, with associated bile acid toxicity and subsequent chronic cholangiopathy. The relationship between amount of functional MDR3 protein and clinical phenotype is likely to take a more linear dose-response relationship (Figure 2).

Although targeted therapies for the genetic defects or the associated specific liver phenotypes are not currently available, there are

potentials for future development. The nature of BSEP deficiency secondary to *ABCB11* variants, and the low level of corrections which could result in clinical improvement, means that it may be suited for novel therapies, including gene and protein manipulation.⁵ Improvements in function seen in patients with cystic fibrosis and specific gating variants in *CFTR* gene (also an ABC transporter) treated with Ivacaftor, a potentiator of CFTR, has seen encouraging early work in its use in *ABCB4* variants, resulting in a significant in vitro increase in phosphatidylcholine secretion.²

More detailed natural history of adults with variants in these cholestasis-related genes is crucial to permit a more accurate genotype-phenotype correlation, and an individualised approach to these patients. Unanswered questions for clinicians and patients relate to the risk of progression of liver disease, the development of complications such as liver cancer, and the management of first-degree relatives. While this information is awaited, we recommend employing supportive management and use of UDCA as outlined in Figure 3.

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REFERENCES

- Davit-Spraul A, et al. Progressive familial intrahepatic cholestasis. *Orphanet J Rare Dis*. 2009;4:1.
- Delaunay J-L, Bruneau A, Hoffmann B, 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.
- Bull LN, van Eijk MJT, Pawlikowska L, et al. A gene encoding a P-type ATPase mutated in two forms of hereditary cholestasis. *Nat Genet*. 1998;18:219-224.
- Lang T, Haberl M, Jung D, et al. Genetic variability, haplotype structures, and ethnic diversity of hepatic transporters MDR3 (ABCB4) and bile salt export pump (ABCB11). *Drug Metab Dispos*. 2006;34:1582-1599.
- Byrne JA, Strautnieks SS, Ihrke G, et al. Missense mutations and single nucleotide polymorphisms in ABCB11 impair bile salt export pump processing and function or disrupt pre-messenger RNA splicing. *Hepatology*. 2009;49:553-567.
- Folmer DE, van der Mark VA, Ho-Mok KS, et al. Differential effects of progressive familial intrahepatic cholestasis type 1 and benign recurrent intrahepatic cholestasis type 1 mutations on canalicular localization of ATP8B1. *Hepatology*. 2009;50:1597-1605.
- Klomp LW, Vargas JC, van Mil SW, et al. Characterization of mutations in ATP8B1 associated with hereditary cholestasis. *Hepatology*. 2004;40:27-38.
- Strautnieks SS, Byrne JA, Pawlikowska L, et al. Severe bile salt export pump deficiency: 82 different ABCB11 mutations in 109 families. *Gastroenterology*. 2008;134:1203-1214.
- Jacquemin E, Bernard O, Hadchouel M, et al. The wide spectrum of multidrug resistance 3 deficiency: from neonatal cholestasis to cirrhosis of adulthood. *Gastroenterology*. 2001;120:1448-1458.
- Schatz SB, Jüngst C, Keitel-Anselmo V, et al. Phenotypic spectrum and diagnostic pitfalls of ABCB4 deficiency depending on age of onset. *Hepatol Commun*. 2018;2:504-514.
- Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*. 2015;17:405-424.
- Mann RD, Andrews EB. *Pharmacovigilance*. John Wiley & Sons; 2007.
- Chalasani N, Björnsson E. Risk factors for idiosyncratic drug-induced liver injury. *Gastroenterology*. 2010;138:2246-2259.
- Chalasani N, Fontana RJ, Bonkovsky HL, et al. Causes, clinical features, and outcomes from a prospective study of drug-induced liver injury in the United States. *Gastroenterology*. 2008;135:1924-1934, 1934.e1-4.
- Andrade R, Lucena M, Fernandez M, et al. Drug-induced liver injury: an analysis of 461 incidences submitted to the Spanish Registry over a 10-year period. *Gastroenterology*. 2005;129:512-521.
- European Association for the Study of the Liver. Electronic address: easloffice@easloffice.eu; Clinical Practice Guideline Panel: Chair; Panel members; EASL Governing Board representative. EASL Clinical Practice Guidelines: drug-induced liver injury. *J Hepatol*. 2019;70:1222-1261.
- Daly AK, Donaldson PT, Bhatnagar P, et al. HLA-B*5701 genotype is a major determinant of drug-induced liver injury due to flucloxacillin. *Nat Genet*. 2009;41:816-819.
- Pauli-Magnus C, Meier PJ. Hepatobiliary transporters and drug-induced cholestasis. *Hepatology*. 2006;44:778-787.
- Dawson S, Stahl S, Paul N, et al. In vitro inhibition of the bile salt export pump correlates with risk of cholestatic drug-induced liver injury in humans. *Drug Metab Dispos*. 2012;40:130-138.
- Pedersen JM, Matsson P, Bergström CAS, et al. Early identification of clinically relevant drug interactions with the human bile salt export pump (BSEP/ABCB11). *Toxicol Sci*. 2013;136:328-343.
- Lang C, Meier Y, Stieger B, 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.
- Ulzurrún E, Stephens C, Crespo E, et al. Role of chemical structures and the 1331T>C bile salt export pump polymorphism in idiosyncratic drug-induced liver injury. *Liver Int*. 2013;33:1378-1385.
- Ulzurrún E, Stephens C, Ruiz-Cabello F, et al. Selected ABCB1, ABCB4 and ABCC2 polymorphisms do not enhance the risk of drug-induced hepatotoxicity in a Spanish cohort. *PLoS One*. 2014;9:e94675.
- Dixon PH, van Mil SWC, Chambers J, et al. Contribution of variant alleles of ABCB11 to susceptibility to intrahepatic cholestasis of pregnancy. *Gut*. 2009;58:537-544.
- Pauli-Magnus C, Lang T, Meier Y, et al. Sequence analysis of bile salt export pump (ABCB11) and multidrug resistance p-glycoprotein 3 (ABCB4, MDR3) in patients with intrahepatic cholestasis of pregnancy. *Pharmacogenetics*. 2004;14:91-102.
- Abu-Hayyeh S, Papacleovoulou G, Lövgren-Sandblom A, et al. Intrahepatic cholestasis of pregnancy levels of sulfated progesterone metabolites inhibit farnesoid X receptor resulting in a cholestatic phenotype. *Hepatology*. 2013;57:716-726.
- Song X, Vasilenko A, Chen Y, et al. Transcriptional dynamics of bile salt export pump during pregnancy: mechanisms and implications in intrahepatic cholestasis of pregnancy. *Hepatology*. 2014;60:1993-2007.
- Jacquemin E, Cresteil D, Manouvrier S, et al. Heterozygous nonsense mutation of the MDR3 gene in familial intrahepatic cholestasis of pregnancy. *Lancet*. 1999;353:210-211.
- Gotthardt D, Runz H, Keitel V, 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.
- Pasmant E, Goussard P, Baranes L, et al. First description of ABCB4 gene deletions in familial low phospholipid-associated cholelithiasis and oral contraceptives-induced cholestasis. *Eur J Hum Genet*. 2012;20:277-282.
- Anzivino C, Odoardi MR, Meschiari E, et al. ABCB4 and ABCB11 mutations in intrahepatic cholestasis of pregnancy in an Italian population. *Dig Liver Dis*. 2013;45:226-232.

32. Dixon PH, Sambrotta M, Chambers J, et al. An expanded role for heterozygous mutations of ABCB4, ABCB11, ATP8B1, ABCB2 and TJP2 in intrahepatic cholestasis of pregnancy. *Sci Rep*. 2017;7:11823.
33. Mullenbach R, Bennett A, Tetlow N et al. ATP8B1 mutations in British cases with intrahepatic cholestasis of pregnancy. *Gut*. 2005;54:829-834.
34. Painter JN, Savander M, Ropponen A, et al. Sequence variation in the ATP8B1 gene and intrahepatic cholestasis of pregnancy. *Eur J Hum Genet*. 2005;13:435-439.
35. Dixon PH, Wadsworth CA, Chambers J, et al. A comprehensive analysis of common genetic variation around six candidate loci for intrahepatic cholestasis of pregnancy. *Am J Gastroenterol*. 2014;109:76-84.
36. Turro E, Astle WJ, Megy K, et al. Whole-genome sequencing of patients with rare diseases in a national health system. *Nature*. 2020;24:1-9.
37. European Association for the Study of the Liver. Electronic address: easloffice@easloffice.eu; European Association for the Study of the Liver. EASL Clinical Practice Guidelines: the diagnosis and management of patients with primary biliary cholangitis. *J Hepatol*. 2017;67:145-172.
38. Lucena J-F, Herrero JI, Jnacio, Quiroga J, et al. A multidrug resistance 3 gene mutation causing cholelithiasis, cholestasis of pregnancy, and adulthood biliary cirrhosis. *Gastroenterology*. 2003;124:1037-1042.
39. Denk GU, Bikker H, Lekan dit Deprez RH, et al. ABCB4 deficiency: a family saga of early onset cholelithiasis, sclerosing cholangitis and cirrhosis and a novel mutation in the ABCB4 gene. *Hepatol Res*. 2010;40:937-941.
40. Oliveira HM, Pereira C, Silva ES, Pinto-Basto J, Miranda HP. Elevation of gamma-glutamyl transferase in adult: Should we think about progressive familial intrahepatic cholestasis? *Dig Liver Dis*. 2016;48:203-205.
41. Vitale G, Pirillo M, Mantovani V, et al. Bile salt export pump deficiency disease: two novel, late onset, ABCB11 mutations identified by next generation sequencing. *Ann Hepatol*. 2016;15:795-800.
42. Hsu YC, Chen HL, Wu MZ, et al. Adult progressive intrahepatic cholestasis associated with genetic variations in ATP8B1 and ABCB11. *Hepatol Res*. 2009;39:625-631.
43. Ziol M, Barbu V, Rosmorduc O, et al. ABCB4 heterozygous gene mutations associated with fibrosing cholestatic liver disease in adults. *Gastroenterology*. 2008;135:131-141.
44. Degiorgio D, Crosignani A, Colombo C, et al. ABCB4 mutations in adult patients with cholestatic liver disease: impact and phenotypic expression. *J Gastroenterol*. 2016;51:271-280.
45. Kulecka M, Habior A, Paziewska A, et al. Clinical applicability of whole-exome sequencing exemplified by a study in young adults with the advanced cryptogenic cholestatic liver diseases. *Gastroenterol Res Pract*. 2017;2017:4761962.
46. Aamann L, Ørntoft N, Vogel I, et al. Unexplained cholestasis in adults and adolescents: diagnostic benefit of genetic examination. *Scand J Gastroenterol*. 2018;53:305-311.
47. Vitale G, Gitto S, Raimondi F, 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.
48. Dröge C, Bonus M, Baumann U, 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.
49. Gudbjartsson DF, Helgason H, Gudjonsson SA, et al. Large-scale whole-genome sequencing of the Icelandic population. *Nat Genet*. 2015;47:435-444.
50. Rosmorduc O, Hermelin B, Poupon R. MDR3 gene defect in adults with symptomatic intrahepatic and gallbladder cholesterol cholelithiasis. *Gastroenterology*. 2001;120:1459-1467.
51. Rosmorduc O, Hermelin B, Boelle P, et al. ABCB4 gene mutation-associated cholelithiasis in adults. *Gastroenterology*. 2003;125:452-459.
52. Poupon R, Rosmorduc O, Boëlle PY, et al. Genotype-phenotype relationships in the low-phospholipid-associated cholelithiasis syndrome: a study of 156 consecutive patients. *Hepatology*. 2013;58:1105-1110.
53. Nakken KE, Labori KJ, Rødningen OK, et al. ABCB4 sequence variations in young adults with cholesterol gallstone disease. *Liver Int*. 2009;29:743-747.
54. van Mil SWC, Milona A, Dixon PH, et al. Functional variants of the central bile acid sensor FXR identified in intrahepatic cholestasis of pregnancy. *Gastroenterology*. 2007;133:507-516.
55. Landrum MJ, Lee JM, Benson M, et al. ClinVar: improving access to variant interpretations and supporting evidence. *Nucleic Acids Res*. 2018;46:D1062-D1067.
56. Jacquemin E, Hermans D, Myara A, et al. Ursodeoxycholic acid therapy in pediatric patients with progressive familial intrahepatic cholestasis. *Hepatology*. 1997;25:519-523.

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