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**Expanding the mutational spectrum of the *ABCB4* gene in inherited adult cholestatic liver disorders with four novel pathogenic variants: case reports**

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*Ethics statement: This work is not clinical research and is considered as routine clinical care.*

**ABSTRACT**

Low phospholipid-associated cholelithiasis and intrahepatic cholestasis of pregnancy are two MDR3-related inherited liver disorders caused by biallelic or monoallelic *ABCB4* loss-of-function variants. Low phospholipid-associated cholelithiasis is clinically characterized by the early onset of symptomatic cholelithiasis in young adults while intrahepatic cholestasis of pregnancy is a distinct clinical entity associated with adverse fetal outcomes. Of note, patients carrying *ABCB4* sequence variations commonly exhibit phenotypic expression over a wide continuum due to

environmental and hormonal contributing factors and genetic modifiers. Patients with an early diagnosis of MDR3-related diseases could benefit from ursodeoxycholic acid treatment in order to prevent acute and chronic complications as well as adverse pregnancy outcomes. We herein report five patients with an overlapping phenotype from low phospholipid-associated cholelithiasis to intrahepatic cholestasis of pregnancy, harboring five *ABCB4* missense variants, four of which were novel. Our study highlights the phenotypic and genetic heterogeneity of inherited cholestatic liver diseases and also expands the mutation spectrum of *ABCB4* sequence variations in adult cholestatic liver diseases.

**Key words:** Low phospholipid-associated cholelithiasis syndrome. Intrahepatic cholestasis of pregnancy. Novel *ABCB4* loss-of-function variants. Targeted next-generation sequencing.

## INTRODUCTION

*ABCB4* (MIM \*171060) encodes a phospholipid floppase that translocates phosphatidylcholine from the inner to the outer leaflet of the apical (canalicular) membrane of the hepatocyte. Low phospholipid-associated cholelithiasis syndrome (LPAC syndrome) (MIM #600803) is a recent entity of liver disorders characterized by a) the onset of biliary symptoms before the age of 40, b) microlithiasis or intrahepatic sludge on imaging and c) the recurrence of symptoms after cholecystectomy. The syndrome is caused by biallelic or monoallelic *ABCB4* “loss-of-function” variants. Severe biliary complications including acute pancreatitis, recurrent cholangitis, segmental spindle-shape dilatation of the biliary tree filled with gallstones, secondary sclerosing cholangitis and adverse fetal complications in intrahepatic cholestasis of pregnancy (ICP), which are commonly observed in patients with LPAC syndrome (1,2). ICP is characterized by jaundice and pruritus associated with abnormal values of hepatobiliary-injury biomarkers, including raised serum bile acid concentrations. Adverse pregnancy outcomes include spontaneous preterm labor, fetal distress, fetal asphyxia events and third trimester intrauterine death. Furthermore, the genetic heterogeneity of ICP could hamper the etiological diagnosis and clinical management. ICP (MIM #614972) is caused by heterozygous sequence variations of at least five different genes including *ABCB4*, *ABCB11*, *ATP8B1*, *NRIH4* and *TJP2* (3). We report

herein five patients with an overlapping phenotype, from LPAC syndrome to ICP. Targeted next-generation sequencing identified five *ABCB4* missense variants, four of which were novel. After having reviewed the literature, a molecular analysis and genotype-phenotype correlation were established.

## CASE REPORT

*P1* is a 27-year-old female patient who experienced ICP. Her mother was diagnosed with ICP at the age of 20 and symptomatic biliary gallstones at the age of 30. The proband began to experience episodes of biliary colic at the age of 18 and underwent endoscopic biliary sphincterotomy. Moreover, she presented pruritus associated with a disturbance of the liver function while taking oral contraceptives, which quickly resolved after drug discontinuation at the age of 25. She exhibited intense pruritus with elevated transaminases (AST 108 UI/l, ALT 164 UI/l, GGT 253 UI/l, total bilirubin 28  $\mu\text{mol/l}$ , ALP 385 UI/l and bile acids 85  $\mu\text{mol/l}$ ) at 20 weeks of gestational age and received continued ursodeoxycholic acid treatment until delivery. Itching was persistent and less severe after delivery, predominantly on the trunk and lower limbs at night.

*P2* is a 21-year-old female who was diagnosed with ICP at 24 weeks of gestational age. She presented with intense pruritus at night, mainly on her trunk and lower limbs. Her liver function tests showed elevated levels of AST of 75 UI/l, ALT at 110 UI/l, GGT at 95 UI/l, PAL at 155 UI/l and bile acids of at 50  $\mu\text{mol/l}$ . Her mother (*P3*) had a history of LPAC syndrome and ICP, she also had a family history of gallstone disease; her mother, two sisters and two uncles underwent a cholecystectomy. The proband was referred to our clinic due to intrahepatic microlithiasis and dilatation of the bile ducts in the left liver; a gallstone of 14 mm in the common bile duct was also identified via endoscopic ultrasound. She exhibited anicteric cholestasis, recurrent right upper quadrant abdominal pain over the last two years, which was relieved by paracetamol treatment. She had no pruritus and angiocholitis. Her initial liver function tests showed increased serum levels of ALAT (110 UI/l), GGT (225 UI/l) and ALP (320 UI/l) but a normal bilirubin level. She underwent biliary sphincterotomy for the removal of biliary gallstones and cholecystectomy and also had a positive response to ursodeoxycholic acid

treatment.

*P4* is a 34-year-old female who was hospitalized in the Gastroenterology Service due to diarrhea and abdominal pain. She had a history of cholestasis, nephroblastoma with hepatic metastasis and ICP. She underwent a nephrectomy at the age of 16, a partial hepatectomy (segment IV) due to nephroblastoma metastasis at the age of 17 and a cholecystectomy at the age of 27. The index-patient had multiple episodes of epigastric pain after meals, vomiting and nausea. Abdominal ultrasound identified multiple intrahepatic microlithiasis. Her biological liver function tests showed levels of GGT at 157 UI/l, PAL at 140 UI/l and total bilirubin at 19  $\mu$ mol/l. On clinical physical examination, her abdomen was soft and the epigastric and right hypochondrium regions were sensitive with palpation. She was diagnosed with LPAC syndrome and underwent ursodeoxycholic acid treatment with dose of 10 mg/kg per day.

*P5* is a 23-year-old female with symptomatic gallstone disease. She underwent a cholecystectomy at the age of 24 and had recurrent epigastric pain radiating to the right hypochondrium, which increased after meals. Her liver function tests showed an increased level of ASAT at 150 UI/l, ALAT of 110 UI/l, GGT of 320 UI/l and ALP of 245 UI/l but normal total bilirubin levels. Abdominal ultrasound identified an image of comet-tails and moderate dilatation of intrahepatic bile ducts, particularly the common bile duct. She was treated with ursodeoxycholic acid at 2 x 250 mg per day.

## RESULTS

Targeted next-generation sequencing identified five *ABCB4* missense variants (NM\_000443), four of which were novel. Of interest, one patient (*P3*) with LPAC syndrome and ICP carried compound heterozygous *ABCB4* missense variants c.2540T>C p.Ile847Thr and c.140G>A p.Arg47Gln (Fig. 1), while her daughter (*P2*) was heterozygous for the c.2540T>C p.Ile847Thr variant. Three patients (*P1*, *P4* and *P5*) with LPAC syndrome and ICP carried three novel *ABCB4* missense variants, c.1280T>C p.Leu427Pro, c.1766A>C p.His589Pro and c.832A>G p.Arg278Gly, respectively (Table 1 and Fig. 2). Moreover, heterozygous *ABCG8* c.55G>C p.Asp19His (rs11887534) and homozygous *ABCB11* c.1331T>C p.Val444Ala functional polymorphisms were also detected in *P1* (4-6).

## DISCUSSION

LPAC syndrome and ICP are two common hereditary liver diseases in young adults caused by *ABCB4* “loss-of-function” variants. However, the phenotypic and genetic heterogeneity of liver diseases and the wide phenotypic expression often hamper the clinical diagnosis. We report five patients with LPAC syndrome and ICP carrying *ABCB4* sequence variations. Most patients had a family history of gallstone disease among first-degree relatives. We identified three novel missense variants, c.1280T>C p.Leu427Pro, c.1766A>C p.His589Pro and c.832A>G p.Arg278Gly, in three unrelated patients with ICP and LPAC syndrome (P1, P4, P5). These variants were all predicted to be detrimental (SIFT, Polyphen-2, Mutation Taster). Two missense variants, c.1280T>C p.Leu427Pro and c.1766A>C p.His589Pro, are located in the NBD1 domain (Nucleotide Binding Domain 1), which is highly conserved and important for MDR3 protein function. In addition, one family with cholestatic liver disorders including LPAC syndrome and ICP was observed. The mother (P3) had LPAC syndrome and ICP and she was compound heterozygous for *ABCB4* missense variants, c.2540T>C p.Ile847Thr and c.140G>A p.Arg47Gln. The rare variant, c.2540T>C p.Ile847Thr (rs769944852) has a MAF of  $8.248 \times 10^{-6}$  (1/121240) according to the ExAc databases and was predicted to be deleterious by Polyphen-2 and Mutation Taster. Of interest, another patient with severe LPAC syndrome and *ABCB4* compound heterozygous variants c.140G>A p.Arg47Gln and c.1217G>A p.Arg406Gln was previously recorded (7). It is widely agreed that *ABCB4* missense variants could cause a milder or variable phenotype spectrum due to the residual protein activity. Consequently, some patients with LPAC syndrome carry compound heterozygous *ABCB4* missense variants and one could argue that the synergetic effects of two missense variants would lead to a reduced MDR3 protein activity below a critical threshold to cause the clinical phenotype (8). Moreover, the presence of additional genetic modifiers, such as heterozygous *ABCG8* c.55G>C p.Asp19His and homozygous *ABCB11* c.1331T>C p.Val444Ala, also contribute to the variable clinical phenotype and the disease severity. In conclusion, our study reports five patients with cholestatic liver disease carrying four novel *ABCB4* sequence variants and expands the mutational spectrum of *ABCB4* sequence variations in LPAC syndrome and ICP. Of note, our report highlights the wide



phenotypic variations of inherited liver disorders (9) as well as the contribution of targeted next-generation sequencing for the diagnostic work-up in patients with cholestatic liver diseases. *ABCB4*-mutated patients could benefit from early ursodeoxycholic treatment to prevent acute, chronic and adverse fetal complications.

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**Table 1. Novel and potential *ABCB4* disease-associated sequence variations identified in patients with cholestatic liver disease**

*ABCB4* disease-associated sequence variations

Patient No.	cDNA	Protein nomenclature	<i>In silico</i> prediction			Functional polymorphism	
			SIFT	Polyphen-2	Mutation Taster	<i>ABCG8</i> p.D19H	<i>ABCB11</i> p.Val444Ala
P1	c.1280T>C	p.Leu427Pro	Deleterious	Probably damaging	Disease causing	Yes	Yes
P2	c.2540T>C	p.Ile847Thr	Tolerated	Probably damaging	Disease causing	//	//
P3	c.2540T>C	p.Ile847Thr	Tolerated	Probably damaging	Disease causing	//	//
	c.140G>A	p.Arg47Gln	Deleterious	Probably damaging	Disease causing	//	//
P4	c.1766A>C	p.His589Pro	Deleterious	Probably damaging	Disease causing	//	//
P5	c.832A>G	p.Arg278Gly	Deleterious	Probably damaging	Disease causing	//	//

Figure 1

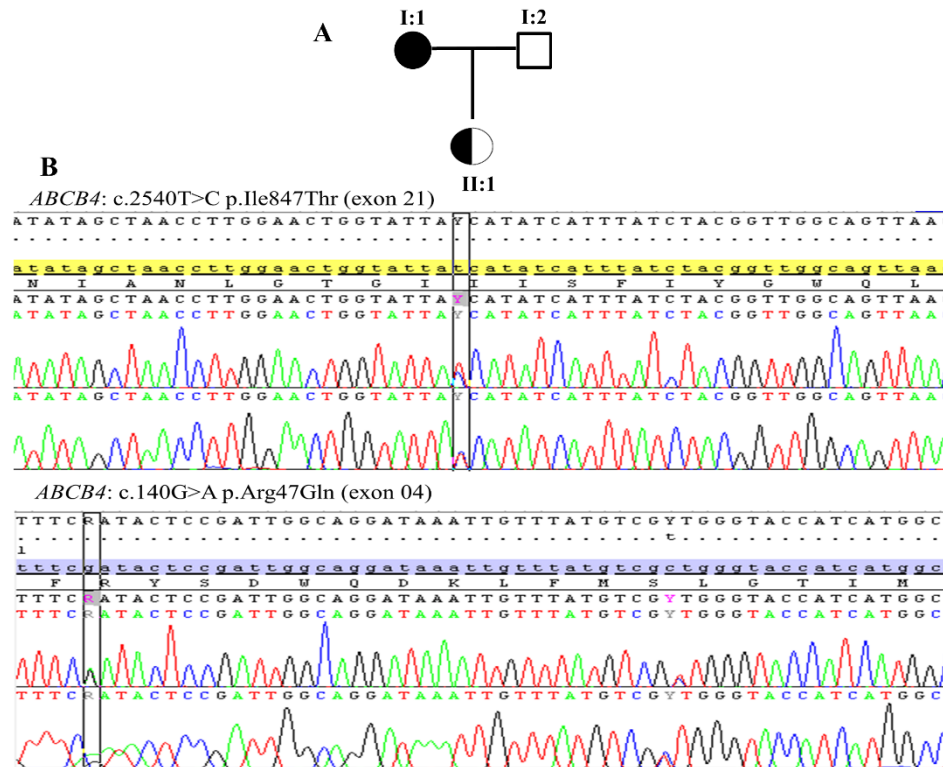


Fig. 1. A. Familial pedigree: mother (P3) was compound heterozygous for *ABCB4* missense variants and her daughter (P2) was heterozygous for an *ABCB4* missense variant. B. Sanger sequencing showing compound heterozygous *ABCB4* missense variants, c.2540T>C p.Ile847Thr and c.140G>A p.Arg47Gln in the mother.

Figure 2

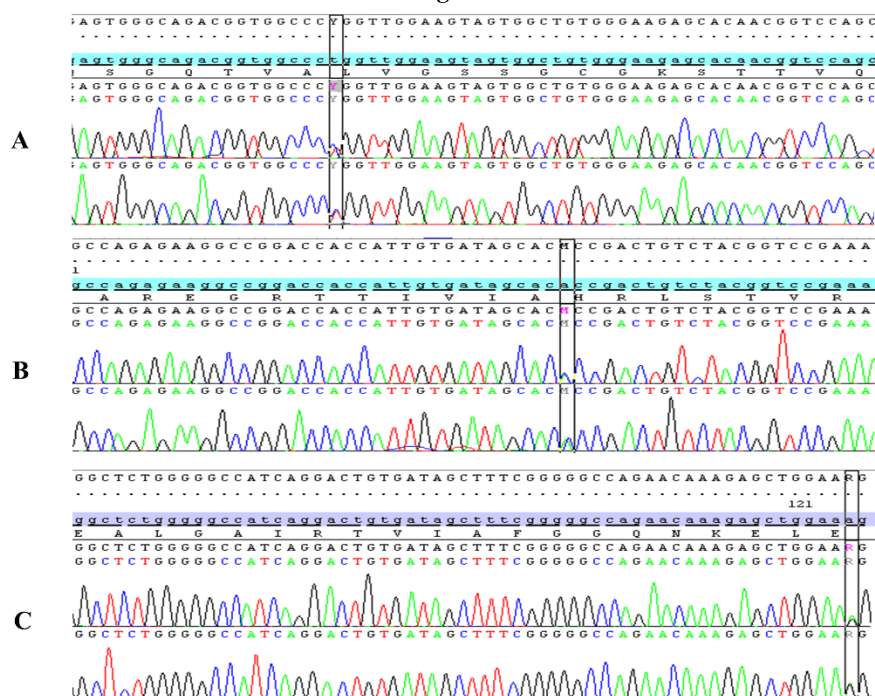


Fig. 2. Sanger sequencing showing three novel *ABCB4* disease-associated sequence variants. A. c.1280T>C p.Leu427Pro (P1). B. c.1766A>C p.His589Pro (P4). C. c.832A>G p.Arg278Gly (P5).