Phenotypic Spectrum and Diagnostic Pitfalls of ABCB4 Deficiency Depending On Age of Onset

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Genetic variants in the adenosine triphosphate-binding cassette subfamily B member 4 (*ABCB4*) gene, which encodes hepatocanalicular phosphatidylcholine floppase, can lead to different phenotypes, such as progressive familial intrahepatic cholestasis (PFIC) type 3, low phospholipid-associated cholelithiasis, and intrahepatic cholestasis of pregnancy. The aim of this multicenter project was to collect information on onset and progression of this entity in different age groups and to assess the relevance of this disease for the differential diagnosis of chronic liver disease. Clinical and laboratory data of 38 patients (17 males, 21 females, from 29 families) with homozygous or (compound) heterozygous *ABCB4* mutations were retrospectively collected. For further analysis, patients were grouped according to the age at clinical diagnosis of *ABCB4*-associated liver disease into younger age (<18 years) or adult age (≥18 years). All 26 patients diagnosed in childhood presented with pruritus (median age 1 year). Hepatomegaly and splenomegaly were present in 85% and 96% of these patients, respectively, followed by jaundice (62%) and portal hypertension (69%). Initial symptoms preceded diagnosis by 1 year, and 13 patients received a liver transplant (median age 6.9 years). Of note, 9 patients were misdiagnosed as biliary atresia, Alagille syndrome, or PFIC type 1. In the 12 patients with diagnosis in adulthood, the clinical phenotype was generally less severe, including intrahepatic cholestasis of pregnancy, low phospholipid-associated cholelithiasis, or (non)cirrhotic PFIC3. *Conclusion:* ABCB4 deficiency with onset in younger patients caused a more severe PFIC type 3 phenotype with the need for liver transplantation in half the children. Patients with milder phenotypes are often not diagnosed before adulthood. One third of the children with PFIC type 3 were initially misdiagnosed, indicating the need for better diagnostic tools and medical education. (*Hepatology Communications* 2018;2:504-514)

Adenosine triphosphate-binding cassette subfamily B member 4 (*ABCB4*)-associated liver disease is caused by an impaired biliary phospholipid secretion due to mutations of the *ABCB4* gene, which encodes the hepatocanalicular phosphatidylcholine transporter (also known as multidrug resistance p-glycoprotein 3 [MDR3]).[1,2] Mutations in the *ABCB4* gene constitute a heterogeneous group of autosomal recessive disorders usually presenting in childhood with a classical phenotype of progressive familial intrahepatic cholestasis (PFIC). ABCB4 disease can also manifest itself anytime later during life. Clinical presentation includes cholestasis, pruritus, and jaundice. In contrast to other forms of PFIC, serum

Abbreviations: ABCB4, adenosine triphosphate-binding cassette subfamily B member 4; GGT, γ-glutamyltransferase; ICP, intrahepatic cholestasis of pregnancy; LPAC, low phospholipid-associated cholelithiasis syndrome; OLT, orthotopic liver transplantation; PFIC, progressive familial intrahepatic cholestasis; UDCA, ursodeoxycholic acid.

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γ-glutamyltransferase (GGT) activity is elevated in ABCB4 disease.

To date, about 200 patients with *ABCB4*-associated liver disease have been described in the literature, mostly in smaller case reports (Table 1). Historically, patients with familial cholestasis and high GGT were depicted to have a PFIC3 phenotype leading to liver fibrosis, cirrhosis, and portal hypertension in infancy or childhood; however, *ABCB4* mutations could be detected only in a subgroup of patients with this phenotype.[3,4] Adult patients carrying *ABCB4* mutations were identified through screening of cohorts with unexplained cholestasis.[5] Patients with low phospholipid-associated cholelithiasis syndrome (LPAC) have been associated with (in most cases) heterozygous or less severe *ABCB4* mutations.[6] *ABCB4* mutations are also involved in intrahepatic cholestasis of pregnancy (ICP), in particular the subgroup of severe ICP with markedly elevated bile acid levels.[7-9]

The aim of this study was to provide national survey data on ABCB4 disease correlating genotypic information of homozygous or compound heterozygous carriers with features of the clinical and laboratory phenotype. In addition, we explored aspects of onset and course of ABCB4 deficiency. The results of the study illustrate not only the tremendous phenotypic spectrum of this disease but also pitfalls in differential diagnosis.

Patients and Methods

PATIENT RECRUITMENT

Due to the rare nature of mutations in the *ABCB4* gene, cases with genetically proven *ABCB4* gene mutations were systematically collected in a multicenter study with 10 participating hospitals in Germany. Patient files and electronic health care records of 38 patients, all of whom presented with one of the clinical phenotypes (PFIC3, ICP, or LPAC syndrome), were retrospectively reviewed. For further analysis, the patients were assigned to two groups according to age at clinical diagnosis: younger age (<18 years) or adults (≥18 years). Data were collected using a standardized questionnaire.

DATA COMPILATION

Systematic data collection was facilitated by a purpose-built electronic database. The following clinical parameters were documented: age at onset of symptoms of pruritus, jaundice, hepatomegaly, splenomegaly, liver cirrhosis, growth retardation, portal hypertension, and/or gallstones. Laboratory parameters collected at the first available documentation were alanine aminotransferase, aspartate aminotransferase, GGT, serum bilirubin, and bile acid concentrations. Age at genetic and clinical diagnosis was recorded as well as laboratory values at these time points.

OUTCOME AND TREATMENT

Outcome was analyzed regarding treatment with ursodeoxycholic acid (UDCA), rifampicin, or phenobarbital and was assessed by liver function tests and development of pruritus. A positive response to UDCA was defined as normalization of aminotransferase activities and total bilirubin; no improvement constituted a negative response. An intermediate response was defined as a reduction of liver enzymes without reaching normal values or with ongoing symptoms. Rifampicin

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TABLE 1. VARIETY OF ONSET AND CLINICAL MANIFESTATIONS OF PFIC3 IN CHILDREN AND ADULTS

Authors	No.	Age at Diagnosis (Onset)[§]	Pruritus	Cholestasis	Hepatomegaly	Splenomegaly	Advanced Disease	Others	OLT (Age)
De Vree et al. 1998[21]	2	6 years, 9 years (3 months-8 months)	2	2	2	2	C (n = 2), PH (n = 2)	Diarrhea, fever	2 (3.5 years, 9 years)
Jacquemin et al. 2001[3]	31[¶]	n.a. (1 month-20.5 years)	14	12	27	27	PH (n = 27)	Gallstones (n = 4)	18 (7.5 years)
Keitel et al. 2005[34]	5	n.a. (birth –7 years)	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	5 (3-11 years)
Jung et al. 2007[35]	2*	Prenatal (1 patient, 3.5 months)	n.a.	1	1	n.a.	0	Transient neonatal cholestasis (n = 1)	0
Englert et al. 2007[19]	16[‖]	2 months-11 years	14	12	n.a.	n.a.	C (n = 13)	Growth retardation (n = 9)	6
Ziol et al. 2008[5]	11*	16-57 years	n.a.	11	n.a.	n.a.	0	Gallstones (n = 4) ICP (n = 3)	0
Crupi 2010[36]	2[†]	birth-4 years (birth, 1 year)	1	n.a.	n.a.	n.a.	n.a.	Growth retardation (n = 1), prolonged neonatal jaundice (n = 1)	0
Anheim et al. 2010[37]	1	40 years (17 years)	n.a.	1	n.a.	1	C, PH	Gallstones, combination with chorea-acanthocytosis	1 (40 years)
Denk et al. 2010[38]	3*,[†]	13-n.a.	n.a.	n.a.	n.a.	n.a.	C (n = 1)	Gallstones (n = 3)	0
Matte et al. 2010[39]	1[‡]	1.5 years	n.a.	1	n.a.	n.a.	n.a.	n.a.	0
Colombo et al. 2011[14]	28	n.a. (1-204 months)	14	12	14	n.a.	C (n = 15), PH (n = 10)	Growth retardation (n = 3), gallstones (n = 1)	5 (6-17 years)
Kubitz et al. 2011[40]	8	1-45 years (<1 year-14 years)	4		2	2	PH (n = 1)	Growth retardation (n = 2), gallstones (n = 2), ICP (n = 2)	0
Giovannoni et al. 2011[41]	2	5 years, 9 years (3 years, 4 years)	n.a.	2	1	1	C (n = 1)	n.a.	1 (7 years)
Schukfeh et al. 2012[42]	1	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	
Tougeron et al. 2012[33]	*	23-55 years (13-55 years)	2	4	n.a.	n.a.	PH (n = 1)	Gallstones (n = 2), CCa (n = 2)	0
Fang et al. 2012[13]	3[‡]	n.a. (1 month-4.5 years)	3	3	3	3	C (n = 1), PH (n = 3)	Growth retardation	0
Anzivino et al. 2013[43]	5*	pregnant women	5	5	n.a.	n.a.	n.a.	Gallstones (n = 1), ICP (n = 5)	
Poupon et al. 2013[6]	79[#]	n.a. (20-34 years)	n.a.	n.a.	n.a.	n.a.	C (n = 2)	Gallstones (n = 75), ICP (n = 41), intrahepatic CCa (n = 1), LPAC (n = 75)	
Giovanoni et al. 2015[44]	3	19 months-5 years	3	3				Liver failure (n = 1)	2

*Heterozygous mutations; [†]patients of same family; [‡]compound heterozygous; [§]in brackets: age at onset; [‖]genetics performed only in 12 patients; [¶]genetics performed only in 22 patients; [#]mostly heterozygous variants.

Abbreviations: C, liver cirrhosis; CCa, cholangiocarcinoma; n.a., not applicable; No., number of patients; PH, portal hypertension.

and phenobarbital medication were regarded as effective if pruritus was absent or improving by patient or parental assessment (intermediate improvement). Age at listing for orthotopic liver transplantation (OLT), age at OLT, and outcome were also documented. Complications were collected as reported in patient files.

ABCB4 GENE ANALYSIS

All exons and exon–intron boundaries of the *ABCB4* gene were sequenced as described.[10,11]

STATISTICAL ANALYSIS AND INSTITUTIONAL BOARD APPROVAL

Quantitative variables were expressed as median and range. We used Excel 2007 and Access (Microsoft Corporation, Redmond, WA) for data collection and IBM SPSS Statistics version 23 for calculations. The study was conducted according to the guidelines of the Declaration of Helsinki and was reviewed and approved by the local ethics committee of Hannover Medical School (1645-2012).

Results

Overall, 38 patients (17 males, 21 females, from 29 families) with homozygous or (compound) heterozygous mutations of the *ABCB4* gene were enrolled in this multicenter study. Twenty-six patients had been clinically diagnosed before the age of 18 years, and in 12 patients the diagnosis was established in adulthood.

CLINICAL CHARACTERISTICS OF PATIENTS <18 YEARS AT DIAGNOSIS OF ABCB4 DEFICIENCY

To date, 24 of the 26 patients (92%) are alive and have a median age of 8.9 years (range, 1.9-25.3 years). The family background revealed consanguinity in six of 19 families. A history of gallstones could be recorded in first-degree relatives in three of 19 families, and ICP was reported by two mothers of the patients. In one family, the brother of a patient had died several years before due to transplant-related complications after OLT for liver disease of, at that time, unknown etiology. Due to lack of data, this relative was not included in this cohort.

An overview of the clinical characteristics is presented in Table 2. All patients developed pruritus, and 16 (61.5%) patients presented with jaundice. Hepatomegaly was observed in 22 (84.6%) and splenomegaly in 25 patients (96.1%). Other clinical signs of portal hypertension were described in 18 patients (69%). Gallstones at any time were detected in only 4 patients (15%). Detailed data of the onset of disease were available for 23 patients; for the 3 other patients, clinical and laboratory data were only available after the age of 4 and 8 years for two siblings and 1 patient died at the age of 2 years after OLT. The onset of disease in these 3 patients occurred much earlier, but a detailed disease course could not be reconstructed; thus, these 3 patients were excluded from all subsequent analyses. The median age at onset of symptoms was 0.4 year (range, 0.1-13.7 years). Pruritus appeared at a median age of 1.0 year (0.1-15.0 years), hepatomegaly at 0.7 year (0.1-13.3 years), and splenomegaly at 1.2 years (0.2-13.3 years). Of note, jaundice did not belong to the first symptoms; the median age for jaundice onset was 2.9 years (0.1-15.0 years). Disease progression, as indicated by signs of portal hypertension, appeared at about 6.5 years (0.4-15.0 years).

Comorbidities

None of the patients suffered from chronic diarrhea. Growth retardation was reported in 10 patients; reduced bone density was detected in 4 patients. Central nervous system involvement, mostly concentration disturbances, was reported by the parents of 9 patients. At the time of the study, 18 children were still attending kindergarten or elementary school. No child has gone on to higher education, while 2 children did attend a special needs school. A lower secondary school education was reported by 3 children, and comprehensive school education by 2 children.

Laboratory Values

The first laboratory values were determined at a median age of 0.85 year, (range, 0.1-14.6 years). The tests often revealed elevated liver enzymes, with median aspartate aminotransferase 130 U/L (range, 26-693 U/L), alanine aminotransferase 140 U/L (21-540 U/L), and GGT 197 U/L (10-535 U/L). GGT in the first blood sample was elevated in all but 1 patient, but GGT also fluctuated in many patients over time. Serum bilirubin concentrations were often low (median 20 μmol/L) but were elevated in 12 patients. At the time of diagnosis, only 7 patients had elevated serum bilirubin concentrations.

TABLE 2. CLINICAL CHARACTERISTICS OF THE STUDY COHORT WITH *ABCB4* MUTATIONS

Patients (Sex)	Age at Clinical Diagnosis	Phenotype	Jaundice	Cholelithiasis/ Recurrence After CHE	Cirrhosis, Portal Hypertension	GGT ALT [U/L]	UDCA Treatment/ Response	OLT (Age)	Family History
Patients <18 years at clinical diagnosis of ABCB4 deficiency									
1 (m)	5 months	PFIC3	–	–/n.a.	–	308 166	+/(+)	–	brother of 6 & 12
2 (f)	7 months	PFIC3	–	–/n.a.	–	210 54	+/n.d.	–	–
3 (m)	8 months	PFIC3	+	–/n.a.	–	82 171	+/–	2 years 6 months	–
4 (m)	9 months	PFIC3	+	–/n.a.	+	183 124	+/(+)	1 year 3 months	–
5 (f)	11 months	PFIC3	+	–/n.a.	+	n.d. n.d.	+/–	3 months	–
6 (f)	11.5 months	PFIC3	–	+/n.a.	+	309 69	+/(+)	–	sister of 1 & 12
7 (m)	11.6 months	PFIC3	+	–/n.a.	+	81 68	+/(+)	on list	–
8 (f)	1 year 6 months	PFIC3	–	–/n.a.	+	232 140	+/(+)	–	cousin of 16 & 17
9 (f)	1 year 6 months	PFIC3	+	–/n.a.	–	34 39	+/(+)	11 years 6 months	sister of 15
10 (m)	1 year 9 months	PFIC3	+	–/n.a.	+	168 290	+ / (+)	8 years 10 months	brother of 14
11 (m)	1 year 10 months	PFIC3	+	–/n.a.	+	n.d. n.d.	+/n.d.	2 years 2 months	–
12 (f)	2 years 2 months	PFIC3	–	–/n.a.	+	577 320	+/(+)	–	sister of 1 & 6
13 (f)	2 years 6 months	PFIC3	–	–/n.a.	–	8 17	+/+	–	–
14 (f)	2 years 8 months	PFIC3	+	–/n.a.	+	228 194	+/(+)	5 years10 months	sister of 10
15 (m)	3 years 3 months	PFIC3	+	–/n.a.	+	92 41	+/(+)	13 years 2 months	brother of 9
16 (m)	3 years 7 months	PFIC3	–	–/n.a.	–	92 106	+/–	–	brother of 17
17 (f)	3 years 7 months	PFIC3	–	–/n.a.	–	258 300	+/(+)	–	sister of 16
18 (m)	2 years 1 month	PFIC3	+	–/n.a.	+	98 123	+/–	4 years 7 months	–
19 (f)	5 years	PFIC3	+	+/n.a.	–	8 20	+/(+)	–	–
20 (m)	6 years	PFIC3	+	–/n.a.	+	103 164	+/(+)	6 years 7 months	–
21 (f)	6 years 2 months	PFIC3	+	–/n.a.	+	139	+/(+)	on list	–

TABLE 2. *CONTINUED*

Patients (Sex)	Age at Clinical Diagnosis	Phenotype	Jaundice	Cholelithiasis/ Recurrence After CHE	Cirrhosis, Portal Hypertension	GGT ALT [U/L]	UDCA Treatment/ Response	OLT (Age)	Family History
22 (f)	9 years 9 months	PFIC3	+	–/n.a.	+	116 n.d. n.d.	+/(+)	11 years 10 months	sister of 24
23 (f)	13 years 8 months	PFIC3	–	+/n.a.	+	146 78	–/n.a.	on list	sister of 25
24 (m)	13 years 10 months	PFIC3	+	–/n.a.	+	n.d. n.d.	+/(+)	16 years 1 month	brother of 22
25 (f)	14 years 8 months	PFIC3	–	–/n.a.	+	244 108	–/n.a.	16 years	sister of 23
26 (m)	15 years	PFIC3	+	+/n.a.	+	268 106	+/(+)	–	–
Patients >18 years at clinical diagnosis of ABCB4 deficiency									
27 (m)	19 years 3 months	PFIC3	–	+/n.a.	+	428 258	+/(+)	–	brother of 28
28 (f)	19 years 10 months	PFIC3	–	+/n.a.	–	24 136	+/(+)	–	sister of 27
29 (f)	20 years 5 months	PFIC3	–	+/n.a.	–	78 56	+/+	–	–
30 (f)	21 years 5 months	PFIC3	+	–/n.a.	–	156 190	–/n.a.	–	sister of 31
31 (m)	21 years10 months	PFIC3	–	–/n.a.	–	122 178	–/n.a.	–	brother of 30
32 (f)	26 years 7 months	ICP	+	–/–	–	27 1540	+/+	–	–
33 (f)	29 years 2 months	ICP	+	–/–	–	224 998	+/(+)	–	–
34 (f)	35 years 3 months	LPAC	–	+/+	–	545 67	+/(+)	–	–
35 (m)	36 years 7 months	Bile duct stones	+	+/n.a.	–	208 103	+/+	–	–
36 (m)	37 years 2 months	LPAC	+	+/+*	–	74 137	+/(+)	–	–
37 (m)	38 years 7 months	LPAC	–	+/+	–	38 16	n.d./n.d.	–	–
38 (f)	40 years 8 months	LPAC	+	+/+	–	217 51	+/(+)	–	–

*recurrent microlithiasis; "–", no or no response; "+", yes or full response; "(+)", partial response.
Abbreviations: ALT, alanine aminotransferase (values at time of genetic diagnosis); CHE, cholecystectomy; (f), female; GGT, γ-glutamyltransferase (values at time of genetic diagnosis); (m), male; n.a., not applicable; n.d., no data.

There is a period of latency between the onset of symptoms and diagnosis. The onset of disease occurred at a median age of 0.4 year (range, 0.1-13.7 years), and the median age at genetic diagnosis was 4.8 years (0.6-23.5 years). Clinical diagnosis followed symptoms 1.6 years (0-4.9 years) later. Diagnosis was delayed by 1 year when considering only the index patients and not the second affected child in the families.

In the 13 patients who required a liver transplant, 8 patients were previously suspected incorrectly of having other diseases, among them 3 patients with a diagnosis of biliary atresia leading to a Kasai operation in all 3; 1 patient with suspected Alagille syndrome; 1 patient with presumed PFIC1, who underwent a biliary diversion; 1 patient diagnosed with primary biliary cholangitis; and 2 patients with presumed viral infection (cytomegalovirus, Epstein-Barr virus).

Therapy

In total, 24 patients received UDCA treatment (doses of 11-30 mg/kg body weight per day). The effect of therapy was judged as intermediate in 17 children; 4 patients showed no response to UDCA. Additional treatment of pruritus was initiated in 9 children, using rifampicin (n = 7) and phenobarbital (n = 6). Rifampicin (4-11 mg/kg/day) seemed to stop in 2 patients or ameliorate pruritus in 3 patients. Phenobarbital was not as effective in the cohort, with 4 patients reporting no benefit and 1 patient describing intermediate benefit.

With progression of disease, 16 out of 26 children were listed for liver transplantation at a median age of 6.8 years. Thirteen patients underwent liver transplantation 4.2 years (range, 0.5-12.9 years) after the onset of symptoms at a median age of 6.9 years (0.6-16.1 years); among those, 6 patients split liver transplants. The indications for OLT were severe liver failure and pruritus. Of the children having a liver transplant, 2 died due to OLT-related complications (sepsis and primary graft failure). Acute biopsy-proven rejection was observed in 4 patients, and biliary complications occurred in 2 patients (bilioma, revision of biliodigestive anastomosis).

CLINICAL CHARACTERISTICS OF PATIENTS ≥18 YEARS AT DIAGNOSIS OF ABCB4 DEFICIENCY

In 12 patients of the study cohort, the diagnosis of ABCB4 deficiency was made in adulthood. An overview of their clinical characteristics is provided in Table 2.

The 12 patients originate from 10 different families. Five of these 12 patients presented with biliary fibrosis and/or cirrhosis corresponding to (non)cirrhotic PFIC3. The PFIC3 phenotype of these patients was generally less severe than in patients <18 years with diagnosis of ABCB4 deficiency. Only 1 patient had symptoms of portal hypertension, namely esophageal varices grade I without a bleeding episode. One patient also developed LPAC syndrome. Liver transplantation was not necessary in these patients. Four patients presented with LPAC syndrome, which was defined by at least two of the following features: age ≤40 years at the first onset of biliary symptoms; intrahepatic hyperechoic foci, sludge, or microlithiasis; recurrence of biliary symptoms after cholecystectomy.[6] Two patients were clinically diagnosed with ICP. One patient had recurrent gallstones within the common bile duct, which were repeatedly extracted by endoscopic retrograde cholangiopancreaticography. In total, gallstones were found in 8 of the 12 patients (67%), and 6 of 12 patients (50%) had been jaundiced. GGT at the time of diagnosis was elevated in 9 of 12 patients (75%), with the median level being 139 U/L (range, 24-545 U/L).

UDCA therapy was conducted in 9 of 11 patients (81.8%; 1 patient had no available data). One patient with PFIC3 completely responded to therapy with normalization of laboratory parameters, and 2 patients with PFIC3 showed a partial response. Treatment in 1 of the patients with ICP resulted in a complete response while pregnant, and the second patient responded partially. For 3 of the 4 patients with LPAC, data were available, and all patients partially responded to UDCA therapy. In 1 of the patients with LPAC, only partial removal of intrahepatic bile duct stones was feasible despite multiple approaches by endoscopic retrograde cholangiopancreaticography and percutaneous transhepatic cholangiography. Due to relapsing episodes of biliary pain and cholangitis, right-sided hepatectomy was carried out.

ABCB4 GENE VARIANTS

In total, all 38 patients were analyzed for *ABCB4* variants. A detailed overview of the genetic characteristics is provided in Table 3.

In patients with disease onset before the age of 18 years, 20 different mutations were found. Patient number 18 was genetically diagnosed at an adult age, but severe disease manifestation, including OLT, occurred at an early age, so this patient was considered for

TABLE 3. *ABCB4* GENE VARIANTS IN THE STUDY COHORT

Patient	ABCB4 Variant 1				ABCB4 Variant 2			
	Location And Nucleotide Change	Predicted Effect	Status	Type	Location And Nucleotide Change	Predicted Effect	Status	Type
Patients <18 years at clinical diagnosis of ABCB4 deficiency								
1	c.2177C>T	p.Pro726Leu	hom	missense				
2	c.2165G>C	p.Gly722Ala	het	missense	c.3486+5G>A	splicing variant	het	splicing
3	c.1769G>A	p.Arg590Gln	hom	missense				
4	c.1584G>C	p.Glu528Asp	het	missense	c.1954A>G	p.Arg652Gly	het	missense
5	c.523A>G	p.Thr175Ala	het	missense	c.1954A>G	p.Arg652Gly	het	missense
6	c.2177C>T	p.Pro726Leu	hom	missense				
7	c.67-68insAC	p.Leu23Hisfs*16	het	frameshift	c.79A>G	p.Ser27Gly	het	missense
8	c.2177C>T	p.Pro726Leu	hom	missense				
9	c.3633+1G>T	splicing variant	hom	splicing				
10	c.2858C>A	p.Ala953Asp	hom	missense				
11	c.2858C>A	p.Ala953Asp	hom	missense				
12	c.2177C>T	p.Pro726Leu	hom	missense				
13	c.286+1G>A	splicing variant	het	splicing	c.3541C>G	p.Gln1181Glu	het	missense
14	c.2858C>A	p.Ala953Asp	het	missense	c.504C>T	p.Asn168Asn	het	synonymous
15	c.3633+1G>T	splicing variant	hom	splicing				
16	c.2177C>T	p.Pro726Leu	hom	missense				
17	c.2177C>T	p.Pro726Leu	hom	missense				
18	c.2380G>C	p.Ala794Pro	hom	missense				
19	c.504C>T	p.Asn168Asn	hom	synonymous	c.523A>G	p.Thr175Ala	het	missense
20	c.2783+1G>C	splicing variant	hom	splicing				
21	c.3257A>C	p.Tyr1086Ser	hom	missense				
22	c.283C>T	p.Pro95Ser	hom	missense				
23	c.79A>G	p.Ser27Gly	het	missense	c.959C>T	p.Ser320Phe	het	missense
24	c.283C>T	p.Pro95Ser	hom	missense				
25	c.79A>G	p.Ser27Gly	het	missense	c.959C>T	p.Ser320Phe	het	missense
26	c.2950G>A	p.Ala984Thr	het	missense				
Patients >18 years at clinical diagnosis of ABCB4 deficiency								
27	c.139C>T	p.Arg47X	het	nonsense	c.959C>T	p.Ser320Phe	het	missense
28	c.139C>T	p.Arg47X	het	nonsense	c.959C>T	p.Ser320Phe	het	missense
29	c.140G>A	p.Arg47Gln	het	missense				
30	c.2165G>C	p.Gly722Ala	het	missense	c.2860G>A	p.Gly954Ser	het	missense
31	c.2165G>C	p.Gly722Ala	het	missense	c.2860G>A	p.Gly954Ser	het	missense
32	c.959C>T	p.Ser320Phe	het	missense				
33	c.672C>A	p.Ser224Arg	het	missense				
34	c.1769G>A	p.Arg590Gln	het	missense	c.2507C>A	p.Ala836Glu	het	missense
35	c.2380G>C	p.Ala794Pro	het	missense				
36	c.1731G>A	splicing variant	het	splicing				
37	c.523A>G	p.Thr175Ala	het	missense	c.1744C>T	p.Arg582Trp	het	missense
38	c.523A>G	p.Thr175Ala	het	missense				

Abbreviations: het, heterozygous; hom, homozygous.

clinical and genetic evaluation in the pediatric cohort. In the majority of cases, the 20 different mutations, which were mainly homozygous, were missense mutations (n = 14) alongside one synonymous variant, one frameshift mutation, and four splicing defects.

In the 12 patients with clinical and genetic diagnosis at ≥18 years, *ABCB4* gene sequencing revealed 12 distinct mutations. Compound heterozygous mutations were detected in 6 patients. Of the 12 mutations, mainly missense mutations were present (n = 10, 83.3%), along with one nonsense mutation leading to a premature stop of translation and one mutation leading to a splicing defect.

Discussion

Because *ABCB4* mutations and resulting phenotypes (PFIC3, LPAC, ICP) are rare, multicenter studies are necessary to gather data on the clinical course of these peculiar diseases. Epidemiologic data for PFIC3 do not exist. The estimated frequency according to an

Italian study group is 1:50,000.[12] Only small cohorts of pediatric patients with PFIC3 have been published (Table 1).[3,12-16] In earlier studies, not all patients were genetically tested[17-19] as the first genetically identified patients date to the late 1990s.[20,21] To date, more than 100 different *ABCB4* mutations, reflecting the spectrum of disease, have been reported.[22] In the present study, we were able to compile a cohort of 38 patients, all with genetically determined *ABCB4* mutations, allowing us to provide an insight on the clinical course of the disease in pediatric patients and adults.

The pathophysiologic relevance is not clear for all *ABCB4* variants.[23] A minor allele frequency of >10% of the variant p.R652G suggests no pathogenicity, but the clinical presentation of patients 4 and 5 with at least one pathogenic variant indicates a pathogenic effect of this variant. It cannot be ruled out that another complex variant has been missed or that functional effects occur in combination with other variants. Additionally, there is only limited knowledge on alternative splicing of *ABCB4* and its functional relevance. Further functional analyses are needed to address these questions. Factors or modifiers responsible for symptoms in heterozygous carriers are unknown. There might be an underrepresentation of heterozygotes due to the broad disease spectrum not leading to medical contact in a subgroup of these individuals.

Of note, in the pediatric patients, 9 children were initially misdiagnosed as having other cholestatic diseases and 7 underwent transplantation. These incorrect diagnoses might have been due to a delay in genetic diagnosis as testing of *ABCB4* variants was performed in the pediatric patients more than 4 years (median) after initial clinical symptoms. Fluctuating activities of transaminases and even normal GGT or bilirubin levels might contribute to the delay in correct diagnosis. Young patients of the cohort were misdiagnosed with biliary atresia as a common differential diagnosis at this age, but no obvious risk factors could be identified. Similarly, Wilson disease has been misdiagnosed at an older age.[24] These findings illustrate the need for increased awareness for ABCB4 disease in different age groups combined with broadened diagnostic strategies.

Cognitive development disorders have not been described in patients with PFIC3. Although no higher education was reported in our pediatric cohort, statistic confounders and the patient's young age or socioeconomic background must be considered. Children surviving liver transplantation are at risk of prolonged cognitive and academic deficits.[25,26]

Patients with an initial diagnosis of ABCB4 disease in adulthood may display a clinical phenotype with a later onset, such as ICP; alternatively, clinical or laboratory signs of the disease could have been misinterpreted or overlooked. These patients typically present with a heterogeneous clinical picture.[27] In the series, the phenotypic presentation ranged from ICP and LPAC syndrome to (non)cirrhotic PFIC3. In the study, *ABCB4* mutations were all heterozygous in these patients, a finding that has also been reported in previous series; however, some patients may have homozygous mutations.[5,6] Genetic analysis in patients with cryptogenic cirrhosis might lead to detection of *ABCB4* mutations.[28] Of interest, genetic analysis in 1 of 2 siblings (patients 27 and 28) revealed the same compound heterozygous mutations, whereas the clinical PFIC3 phenotype was more severe in the male patient. This finding points to the impact of further disease modifiers, such as genetic variants of other hepatocanalicular transporter genes, hormones, or environmental factors.

As a treatment strategy, UDCA has been used in most patients soon after diagnosis. UDCA is more hydrophilic and less hepatotoxic compared to the primary bile acids, competes with them for re-uptake in the intestine, and suppresses their *de novo* synthesis.[29] The response rate to UDCA was satisfactory in PFIC3 patients but mostly with an intermediate response. For pruritus relief in 9 children, additional drugs had to be used. When the outcome of UDCA and rifampicin/phenobarbital was unsuccessful, children usually progressed to end-stage liver disease and were assessed for OLT. In fact, the majority of children were listed for OLT, and children frequently had a liver transplant within the first decade of life even when initial symptomatic response to UDCA was noted. In the group with a diagnosis of ABCB4 deficiency in childhood, the combination of medical and surgical therapies resulted in 92% of patients being alive at a median age of 8.9 years. However, current medical therapy is not satisfactory for the majority of patients in the long term. Only recently, cell culture-based studies demonstrated that a disturbed ABCB4 function in selected missense variants leading to defective membrane trafficking can be restored by treatment with chaperone drugs.[30,31] In the series, the vast majority of patients harbored missense mutations. These data support the relevance of further functional studies of *ABCB4* variants and early genetic testing to provide specific individualized medical therapy in the future to prevent development of cirrhosis.

An increased risk for hepatic malignancies has been reported in adult patients with PFIC,[32] in particular, in *ABCB4* gene mutations and cholangiocarcinoma.[33] No malignancy has been reported so far in our cohort, but prospective follow-up is necessary to determine and monitor the carcinoma risk.

One of the limitations of the present study is that data could only be gathered retrospectively from different hospitals. Moreover, histopathologic changes were not assessed systematically due to the heterogeneity of biopsy sampling at different time points, when performed, reflecting various stages of disease progression. Despite the retrospective and multicenter nature of this study, its strength is the large number of patients who were included and treated successfully.

This series highlights the severe and early manifestation of PFIC3 that leads to the necessity of liver transplantation in almost 50% of patients, frequently in the first decade of life. When clinical onset of symptoms in ABCB4 deficiency appear in adulthood, the clinical phenotype and genetic alterations are generally less severe; however, the clinical picture is variable, and patients may also present with liver cirrhosis and signs of portal hypertension or complex intrahepatic bile duct stones. Sometimes, even severe *ABCB4* mutations become evident only in adults because they only affect one allele. To eliminate the reporting bias of retrospective analyses, more comprehensive clinical data and a longer follow-up in prospective studies are needed to establish genotype–phenotype correlations, which are suggested by functional analyses that relate clinical outcomes to the degree of phosphatidylcholine floppase activity.[15] Early genetic testing during the course of liver disease will be even more relevant in the future when individualized pharmacotherapy might become available for selected missense variants.

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