ATP8B1, *ABCB11*, and *ABCB4* Genes Defects: Novel Mutations Associated with Cholestasis with Different Phenotypes and Outcomes

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Objectives To characterize the clinical, laboratory, histologic, molecular features, and outcome of gene-confirmed progressive familial intrahepatic cholestasis (PFIC) 1-3 among Arabs and to evaluate for "genotype–phenotype" correlations.

Study design We retrospectively reviewed charts of 65 children (*ATP8B1* defect = 5, *ABCB11* = 35, *ABCB4* = 25) who presented between 2008 and 2019 with cholestasis. The clinical phenotype of a disease was categorized based on response of cholestasis and itching to ursodeoxycholic acid and ultimate outcome, into mild (complete response), intermediate (partial response, nonprogressive), and severe (progression to end-stage liver disease).

Results Overall, 27 different mutations were identified (*ATP8B1*, n = 5; *ABCB11,* n = 11; *ABCB4,* n = 11), comprising 10 novel ones. Six patients with heterozygous missense mutations (*ATP8B1*, n = 2; *ABCB11*, n = 4) had transient cholestasis. Of the remaining 3 patients with PFIC1, 2 developed severe phenotype (splicing and frameshift mutations). Of the remaining 31 patients with PFIC2, 25 developed severe disease (15 due to frameshift and splicing mutations). Of 25 patients with PFIC3, 10 developed a severe phenotype (1 splicing and 3 frameshift mutations; 6 missense). Patients with PFIC2 had significantly shorter survival time and more rapid disease progression than patients with PFIC3 ($P < .001$). Patients with frameshift mutations in ABCB11 gene (p.Thr127Hisfs*6) and ABCB4 gene (p.Phe210Serfs*5) had significantly shorter survival time than missense mutations ($P = .011$; $P = .0039$, respectively).

Conclusions We identified genotype–phenotype correlations among mutations in *ABCB11* and *ABCB4* genes, which underscore the prognostic value of early genetic diagnosis. The disease course in patients with PFIC3 could be favorably modified by ursodeoxycholic acid therapy. *(J Pediatr 2021;236:113-23)*.

Advancement in molecular testing has allowed the identification of 3 main forms of progressive familial intrahepatic cholestasis (PFIC 1-3) due to defects in specific transport proteins at the hepatocanalicular membrane: familial intrahepatic cholestasis 1, bile salt export pump (BSEP), and multidrug resistance p-glycoprotein 3 encoded by *ATP8B1*, *ABCB11*, and *ABCB4*, respectively. The literature on gene-confirmed PFIC 1-3 is mainly based on reports from populations in Europe and North America and fewer reports from East Asia and India.[1] These reports showed genetic heterogeneity underlying PFIC 1-3, partly attributed to geographic heterogeneity. Variations in the DNA sequence contribute to a large part of the phenotypic differences between individuals. The impact of a specific gene mutation on the clinical phenotype depends on the degree of impairment of the function of protein encoded by that gene.[2-5] Determination of a specific gene mutation may predict disease course but also predict response to ursodeoxycholic acid (UDCA) therapy and success of biliary diversion procedures to treat intractable pruritus.[6]

The available local data indicated that PFIC and biliary atresia were the 2 main indications for pediatric liver transplantation in 2 of 4 liver transplantation centers in Saudi Arabia (29%-33%); however, no further details were given on the clinical and molecular characteristics of these patients with PFIC.[7,8] A next-generation sequencing-based multigene panel of

ALT	Alanine transaminase
BSEP	Bile salt export pump
ESLD	End-stage liver disease
GGT	Gamma-glutamyl transferase
HCC	Hepatocellular carcinoma
HR	Hazard ratio
INR	International normalized ratio
PFIC	Progressive familial intrahepatic cholestasis
TSB	Total serum bilirubin
UDCA	Ursodeoxycholic acid

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Supported financially by Prince Abdullah bin Khalid Celiac Disease Research Chair, Vice Deanship of Research Chairs, Department of Pediatrics, Faculty of Medicine, King Saud University. The authors declare no conflicts of interest.

https://doi.org/10.1016/j.jpeds.2021.04.040

98 pediatric patients with advanced cholestatic liver disease identified 29 patients with PFIC (PFIC 1 = 1, PFIC 2 = 16, PFIC 3 = 12), but the authors did not provide details on epidemiology, clinical, and natural course.[9] The objectives of this study were to estimate the frequency of gene-confirmed PFIC 1-3 among Saudi children presenting with cholestasis; characterize their epidemiologic, clinical, laboratory, molecular features, and outcome; and to determine whether a given genotype has highly predictable outcome, ie, "genotype–genotype" correlation.

Methods

We retrospectively reviewed our database of patients with cholestasis (defined clinically as the presence of jaundice, acholic stools, and/or itching with a conjugated bilirubin of >20 μmol/L, and/or total serum bile acids >10 [normal, 0-10 μmol/L]) who presented to our center, a tertiary care, nontransplant center for children with liver disorders in Saudi Arabia, during the period from 2008 until 2019. We included patients with at least 1 disease-causing mutation in the *ATP8B1*, *ABCB11*, or *ABCB4* genes.

Study Procedures

Hospital Protocol. All children with cholestasis underwent an extensive workup to exclude infectious, structural, metabolic, endocrine, infiltrative, and familial causes. After collection of serum for bile acid analysis, all cases were treated with UDCA (Dr Falk; 20 mg/kg/d, in divided doses twice a day) and supplied with fat-soluble vitamins. If itching persisted, we added an antihistamine (like hydroxyzine or cetirizine) and rifampicin (10 mg/kg/d). If itching was intractable and affected quality of life or end-stage liver disease (ESLD) developed, we referred the case to a liver transplant center.

Data Collection. Medical records were reviewed to collect demographics and clinical characteristics, laboratory investigations at presentation such as total serum bilirubin (TSB) and direct bilirubin, alanine transaminase (ALT), aspartate transaminase, international normalized ratio (INR), gamma-glutamyl transferase (GGT), and total serum bile acids levels, imaging findings, histopathologic findings, treatment provided, and outcomes, including resolution of cholestasis, response of pruritus to medical therapy, development, and age at onset of complications (eg, portal hypertension, bone fracture, hepatocellular carcinoma [HCC], liver failure, liver transplantation, and death). We analyzed findings at presentation and during the course of disease until last follow-up visit with native liver, death, or liver transplantation. Normal values of GGT were as follows: <1 month old, GGT <200 IUL; 1-2 months old, GGT <150 IU/L; 2-3 months old, GGT <100 IU/L; >6 months, GGT <60 IU/L.[10,11]

Histopathologic Studies. The liver specimens were fixed in 10% buffered formaldehyde, paraffin-embedded, and stained with hematoxylin and eosin, Masson's trichrome stain for fibrous tissue and Perls' method for iron, reticulin, and periodic acid–Schiff diastase. Immunohistochemistry for BSEP to diagnose PFIC2 and for multidrug resistance p-glycoprotein 3 protein to diagnose PFIC3 was not possible.

Molecular Genetic Investigations. Throughout the study period, samples were examined by targeted sequencing, next-generation sequencing (Jaundice chip or cholestasis panel), or whole-exome sequencing. Missense variations that are predicted by PolyPhen-2, Sorting Intolerant From Tolerant (SIFT), or Mutation Taster tools to have a deleterious effect on the function of the protein were considered pathologic and confirmed the genetic diagnosis. We collected blood DNA samples from parents of patients with novel mutations, which were analyzed for the novel allele variants by Sanger sequencing. All procedures were conducted with informed consent.

Study Outcomes

Primary outcomes included resolution of cholestasis (defined as TSB level <20 μmol/L); need for liver transplantation (because of liver failure, intractable pruritus, severe portal hypertension, or HCC); death due to complications of the disease; transplant-free survival rate, defined as starting at birth and ending at death; liver transplantation; or last follow-up in patients with native liver.

Secondary outcomes included response to medical therapy: complete (resolution of cholestasis and normalization of liver enzymes), partial (defined as a reduction of liver enzymes without reaching normal values and some improvement in itching according to patient and parent assessment), no response (worsening of cholestasis and intractable itching), and development of complications (portal hypertension, bone fracture, gallstones, liver failure).

Definitions

Clinically evident portal hypertension was diagnosed when there was either a history of a complication of portal hypertension (esophageal or gastric variceal bleed or ascites) or clinical findings consistent with portal hypertension (both splenomegaly [spleen palpable >2 cm below the costal margin] and thrombocytopenia [platelet count <150 000/mL]). Liver failure was defined as biochemical evidence of liver injury and coagulopathy (INR ≥2, normal 0.9-1.2) not corrected by vitamin K.

Clinical phenotype of the disease was based on the disease course and outcome at last follow-up visit: (1) mild (defined as resolution of cholestasis and pruritus with or without medical therapy associated with normalization of ALT/aspartate aminotransferase, and regression of liver and spleen to normal size, or a disease characterized by intermittent episodes of cholestasis and normal liver enzymes in-between); (2) severe (defined as worsening of cholestasis and intractable pruritus leading to development of portal hypertension, liver failure, or need for liver transplantation); and (3) intermediate disease phenotype (a status between mild and severe phenotypes characterized by partial improvement of liver tests under UDCA without development of portal hypertension,

liver failure, or need for liver transplantation). The severe disease phenotype has been subdivided into "rapid" and insidious, if patient's condition progressed to the worse outcome (severe portal hypertension, death, liver transplantation) within the first 5 years or after 5 years from disease onset, respectively.

Ethical Consideration

The local review board has approved the study (number 14-009).

Statistical Analyses

Mann–Whitney *U*/Kruskal–Wallis test was applied to find out median differences between gene classifications and titers ALT, GGT, and TSB. Kaplan–Meier survival curves were used to estimate the transplant-free survival with respect to disease type (PFIC1, PFIC2, and PFIC3) and *ABCB11* and *ABCB4* mutations to determine the median survival and transplant survival rate of the patients.

Results

We identified 65 children (40 male) with pathogenic mutations in *ATP8B1* (n = 5), *ABCB11* (n = 35), and *ABCB4* (n = 25), in 60 Saudi and 5 Yemeni families. During the study period, 533 cases of infantile cholestasis were referred to our center for evaluation; 52 of the 65 patients (80%) had disease onset during infantile period, representing 10% of the 533 cases of infantile cholestasis.

Clinical Phenotype of the 65 Patients

Patients' demographic, clinical, and laboratory characteristics are summarized in **Table I**. The onset of symptoms in patients with *ATP8B1* and *ABCB11* genes defect was in the first 7 months of life. All patients with *ATP8B1* and *ABCB11* gene defects presented to our center with a combination of jaundice, itching, hepatomegaly, and variable elevation of liver transaminases, except 1 patient in the *ABCB11* gene mutation group (patient AB1), who presented at age 4 months with seizure and hemiplegia due to intracranial bleeding secondary to severe vitamin K–responsive coagulopathy (INR = 4) and found to have cholestasis. Two of the 3 patients with PFIC1, with homozygous *ATP8B1* mutation, reported diarrhea (patients A1 and C1). In patients with *ABCB4* mutation, the onset of disease occurred at a median age of 10 months (range 1-72 months), and they presented to our hospital at median age 24 months (range 6-84 months). Overall, family history for a similar disease was positive in 30 cases (46%), and parents were consanguineous in 85% of the 65 cases.

Serum ALT activity was significantly greater in the *ABCB11* gene mutation group than in the *ATP8B1* gene mutation group (median 300 U/L, IQR 193-418 vs a median of 82 U/L, IQR 54-105 U/L); $P = .003$ [**Figure 1**, B; available at www.jpeds.com]). Serum GGT was low/normal in all patients with ATP8B1 and ABCB11 genes mutations except 3 patients: patient C1 with PFIC1 (GGT = 83 U/L at age 5 months), patient G1 with PFIC2 (GGT = 75 U/L at age 11 months), and patient I1 with transient infantile cholestasis due to heterozygous ABCB11 mutation (GGT = 104 U/L at age 3 months) (**Figure 1**, C; available at www.jpeds.com).

Patients with PFIC3 were notable for the absence of clinically apparent jaundice at presentation in 22 of 25 patients (**Figure 1**, A; available at www.jpeds.com). During the disease course, all the 22 patients with PFIC3 remained nonicteric, even those whose disease progressed to severe portal hypertension. GGT in the first blood sample was elevated in all except 3 patients (AH1 = 49 IU/L, AR1 = 19 IU/L, AT1 = 29 IU/L) in whom GGT rose up after 6-12 months (**Figure 1**, C; available at www.jpeds.com).

Histopathologic Findings

Twenty-seven of the 65 patients underwent percutaneous needle liver biopsy as part of the diagnostic work up of cholestasis; the histopathologic findings are shown in **Table II** (available at www.jpeds.com) and **Figure 2**, A-C (available at www.jpeds.com).

Molecular Analysis

Sequence analysis revealed 27 different mutations in *ATP8B1* (n = 5), *ABCB11* (n = 11), and *ABCB4* (n = 11) genes; 12 of the 27 mutations are novel (*ATP8B1* = 3, *ABCB11* = 2, and *ABCB4* = 7) (**Table I**). Three of the 11 *ABCB11* mutations (c.2494C>T, p.Arg832Cys; c.379delA, p.Thr127Hisfs*6; c.3457CT, p.Arg1153Cys) were the most common and have occurred in 3 tribes that included 21 unrelated families. One of the patients with p.Arg1153Cys mutation (AA1) also harbors a homozygous variant (c.1331T>C, p.Val444AIa) in *ABCB11* gene.

The 25 patients with PFIC3 (from 20 unrelated families) carry 10 homozygous and 1 compound heterozygous mutations; 11 families (AH to AL; AN to AP; AS to AU) from 6 different large tribes in Saudi Arabia harbor 3 missense mutations; one of them is novel (c.2692G>A, p.Glu898Lys). Sanger sequence confirmed the parental carriage of the novel monoallelic mutation variants.

Study Outcomes

Primary Outcomes. By the end of the study period, 33 of the 65 patients had worse outcome (51%): 25 underwent liver transplantation (38.5%) and 8 died (12.5%). The overall transplant free-survival rate was 48%. The details of outcome, indications for liver transplantation, and causes of death are shown in **Table I**. Seven patients with PFIC2 died due to ESLD (median age 2 years, range 1.5-5 years) either because parents refused liver transplantation or no availability of donor. Seventeen of the 25 patients with PFIC3 are still alive (68%) (Median age 10 years, range 1-15 years). Kaplan–Meier survival curve analysis in **Figure 3** (after excluding the heterozygous mutations) summarizes the cumulative survival rates without liver transplantation in the 3 PFIC groups and shows that patients with PFIC2 had significantly shorter survival time than patients with

Table I. Clinical, laboratory, and molecular characteristics and outcome of all the 65 patients

Family (no.)	Sex	Age at onset of symptoms, mo	Age at presentation (mo)	Gene	Gene mutation	Clinical presentation	Laboratory values at presentation: TSB/D, μmol/L	ALT/AST, IU/L	GGT, IU/L	s.bile acid	Response of itch to meds	Complication on follow-up, age	Outcome/age, mo	Clinical phenotype
A1	M	1	9	*ATP8B1*	Homozygous, splicing c.698+1 G>T (IVS8+1G>T)	Cholestasis, itching, and hepatomegaly	373/290	82/96	50	101	Partial	PHTN (6 y) Severe itch	LT (8 y)	Severe (insidious)
B1	M	4	8		Homozygous, missense **c.1234C>T (p.Arg412Cys)**	Cholestasis, itching,	149/128	129/114	44	252	Complete	None	Alive (9 y)	Mild
C1	M	1	5		Homozygous, frameshift **c.386_390delTCTTA (p.Ile129ThrfsX38)**	Cholestasis and hepatomegaly	139/118	82/141	83	47	Partial	Rickets (2 y) Severe itch PHTN (6 y)	Lost F/U at 1 y LT (6 y)	Severe (insidious)
D1	M	6	20		Heterozygous, missense **c.2053G>A (p.E685K)**	Cholestasis, itching, and hepatomegaly	28/22	55/69	16	187	Partial	None	Alive (5 y)	Mild (resolved)
E1	F	80	84		Heterozygous, missense c.208G>A	Recurrent cholestasis and itching	85/65	54/62	7	NA	Complete	None	Alive (10 y)	Mild (resolved)
F1	M	1	12		Homozygous, missense c.2494C>T (p.Arg832Cys)	Cholestasis, itching, and hepatomegaly	262/151	82/44	45	279	Complete	None	Alive (14 y)	Mild (resolved) developed BRIC
F2	M	5	7		Homozygous, missense c.2494C>T (p.Arg832Cys)	Cholestasis, itching, and hepatomegaly	121/104	87/83	8	329	Complete	None	Alive (13 y)	Mild (resolved) developed BRIC
F3	M	2	2		Homozygous, missense c.2494C>T (p.Arg832Cys)	Cholestasis and hepatomegaly	14/	40/29	11	NA	Complete	None	Alive (8 y)	Mild (resolved) developed BRIC
G1	M	7	11		Homozygous, missense c.2494C>T (p.Arg832Cys)	Cholestasis, itching, and hepatomegaly	190/172	568/488	75	169	Complete	None	Alive (11 y)	Intermediate
H1	F	2	4	*ABCB11*	Homozygous, missense c.2494C>T (p.Arg832Cys)	Cholestasis and HSM	259/221	830/1041	55	NA	No response	LF (7 mo)	LT (10 mo)	Severe (rapid)
I1	M	1	3		Heterozygous, missense c.2092C>T (p.Arg698Cys)	Cholestasis and hepatomegaly	75/71	300/223	104	96	Complete even without Rx	None	Alive (4.5 y)	Mild (resolved)
J1	M	1	2		Heterozygous, missense c.936G>T (p.Gln312His)	Cholestasis and hepatomegaly	80/57	79/88	37	47	Complete even without Rx	None	Alive (2 y)	Mild (resolved)
K1	M	1	5		Homozygous, frameshift c.379delA (p.Thr127Hisfs*6)	Cholestasis, itching, and hepatomegaly	103/86	253/212	40	572	No response	Rickets (1 y) PHTN (1 y) fracture (1 y) LF (1.5)	Died (2 y)	Severe (rapid)
K2	F	1	1		Homozygous, frameshift c.379delA/p.Thr127Hisfs*6	Cholestasis and hepatomegaly	185/135	281	23	238	No response	Rickets (1 y) PHTN (1.5 y)	LT (3 y)	Severe (rapid)
L1	F	2	2		Homozygous, frameshift c.379delA (p.Thr127Hisfs*6)	Cholestasis and HSM	128/96	363/357	26	158	No response	Rickets(1.5 y) PHTN (2.5 y) LF (3.5 y)	Died (5 y)	Severe (rapid)
M1	F	3	4		Homozygous, frameshift c.379delA (p.Thr127Hisfs*6)	Cholestasis and hepatomegaly	64/50	362/343	26	242	No response	PHTN (1 y) LF (1.5 y)	Died (2.5 y)	Severe (rapid)
N1	F	1	15		Homozygous, frameshift c.379delA (p.Thr127Hisfs*6)	Cholestasis, itching, and hepatomegaly	65/43	123/311	20	279	No response	Rickets (1 y) PHTN (2 y) LF (3.5 y)	Died (5.5 y)	Severe (rapid)
O1	F	2	2		Homozygous, frameshift c.379delA (p.Thr127Hisfs*6)	Cholestasis and hepatomegaly	116/90	418/469	48	135	No response	Rickets (9 mo) PHTN (1 y) LF (1.5 y)	Died (2 y)	Severe (rapid)
P1	F	2	4		Homozygous, frameshift c.379delA (p.Thr127Hisfs*6)	Cholestasis and hepatomegaly	150/122	1168/1221	19	351	No response	PHTN (10 mo) LF (1.5)	Died (1.5 y)	Severe (rapid)
Q1	M	3	13		Homozygous, frameshift c.379delA (p.Thr127Hisfs*6)	Cholestasis, itching HSM, FTT	94/88	773/959	37	>180	No response	PHTN (2.5 y) HCC (3 y) LF (3 y)	LT (3.5 y)	Severe (rapid) HCC

(*continued*)

Table I. Continued

Family (no.)	Sex	Age at onset of symptoms, mo	Age at presentation (mo)	Gene	Gene mutation	Clinical presentation	Laboratory values at presentation: TSB/D, μmol/L	ALT/AST, IU/L	GGT, IU/L	s.bile acid	Response of itch to meds	Complication on follow-up, age	Outcome/age, mo	Clinical phenotype
R1	F	1	1	*ABCB11*	Homozygous, frameshift c.379delA (p.Thr127Hisfs*6)	Cholestasis and hepatomegaly	52/44	223/210	38	132	No response	PHTN (1.5 y) Rickets (1 y) fracture (2 y) LF (2 y)	LT (2.5 y)	Severe (rapid)
R2	M	1	1		Homozygous, frameshift c.379delA (p.Thr127Hisfs*6)	Cholestasis and hepatomegaly	105/28	206/40	40	111	No response	Rickets (5 mo) PHTN (8 mo)	LT (1 y)	Severe (rapid)
S1	M	1	2		Heterozygous, frameshift c.379delA (p.Thr127Hisfs*6)	Cholestasis	200/149	373/454	45	135	Complete even without Rx	None	Alive (3 y)	Mild (resolved)
T1	M	1	2		Homozygous, frameshift c.379delA (p.Thr127Hisfs*6)	Cholestasis and hepatomegaly	119/92	193/227	24		No response	LF (8 mo)	LT (10 mo)	Severe (rapid)
T2	M	2	2		Homozygous, frameshift c.379delA (p.Thr127Hisfs*6)	Cholestasis and hepatomegaly	101/93	211	24	75	No response	LF (7 mo)	LT (10 mo)	Severe (rapid)
U1	M	2	2	*ABCB11*	Homozygous, frameshift c.379delA (p.Thr127Hisfs*6)	Cholestasis and hepatomegaly	80/59	294/352	26	114	No response	LF (1 y)	LT (1.2 y)	Severe (rapid)
V1	F	5	24		Homozygous, missense c.3457CT (p.Arg1153Cys)	Cholestasis, itching, and hepatomegaly	72/66	100/161	39	361	Partial	PHTN (10 y) GB stones (8y)	Alive (13 y)	Severe (insidious)
W1	M	2	2		Homozygous, missense c.3457CT (p.Arg1153Cys)	Cholestasis and hepatomegaly	232/187	324	49	225	Partial	PHTN (9 y)	Alive (12 y)	Severe (insidious)
X1	F	1	3		Homozygous, missense c.3457CT (p.Arg1153Cys)	Cholestasis and hepatomegaly	246/196	367/342	25	70	No response	PHTN (1 y) LF (2 y)	LT (2.5 y)	Severe (rapid)
Y1	F	1	6		Homozygous, missense c.3457CT (p.Arg1153Cys)	Cholestasis, itching, and hepatomegaly	114/102	567/709	20	>180	No response	PHTN (2 y) LF (3 y)	LT (4 y)	Severe (rapid)
Y2	F	1	5		Homozygous, missense c.3457CT (p.Arg1153Cys)	Cholestasis, itching, and hepatomegaly	123/98	892/1086	25	NA	No response	PHTN (1.5 y) LF (2.5 y)	LT (2.5 y)	Severe (rapid)
Z1	M	1	10	*ABCB11*	Homozygous, missense c.3457CT (p.Arg1153Cys)	Cholestasis, itching, and HSM	295/258	330/777	40	>180	No response	LF (11 mo)	LT (1 y)	Severe (rapid)
AA1	M	0.5	1		Homozygous, missense c.3457CT (p.Arg1153Cys) + Homozygous c.1331T>C (p.Val444Ala)	Cholestasis and hepatomegaly	112/166	473/882	46	244	No response	LF (1 y)	Died (2 y)	Severe (rapid)
AB1	F	1	4		Homozygous, missense c.3382CT (p.Arg1128Cys) + Homozygous, missense c.936 G>T (p.Q312H)	Cholestasis, hepatomegaly, and intracranial bleeding	90/74	360/663	75	NA	Partial	PHTN (4 y) LF (5.5 y)	LT (6 y)	Severe (insidious)
AB2	M	2	7		Homozygous, missense c.3382CT (p.Arg1128Cys) + Homozygous, missense c.936 G>T (p.Q312H)	Cholestasis, itching, and hepatomegaly	292/232	126/515	40	NA	No response	PHTN (1.2 y) LF (1.8 y)	LT (2 y)	Severe (rapid)
AC1	M	1	2		Heterozygous, missense **c.224C>A (p.Ala75Asp)**	Cholestasis and hepatomegaly	179/137	345/718	60	120	Complete even without Rx	None	Alive (9 y)	Mild (resolved)
AD1	M	1	3		Homozygous, missense c.3011G>A (p.Gly1004Asp)	Cholestasis and HSM	97/66	222/496	27	127	Partial	None	Alive (7 y)	Intermediate

(*continued*)

Table I. Continued

Family (no.)	Sex	Age at onset of symptoms, mo	Age at presentation (mo)	Gene	Gene mutation	Clinical presentation	Laboratory values at presentation: TSB/D, μmol/L	ALT/AST, IU/L	GGT, IU/L	s.bile acid	Response of itch to meds	Complication on follow-up, age	Outcome/age, mo	Clinical phenotype
AE1	M	96	96		Homozygous, missense c.1907A>G (p.Glu636Gly)	Cholestasis, itching, and hepatomegaly	243/204	17/31	17	>180	Complete	None	Alive (13 y)	Mild (resolved)
AF1	M	4	24		Homozygous, missense **c.3624T>A (p.Ty1208Ter)**	Cholestasis, itching HSM, and FTT	50/44	269/414	33	480	No response	Rickets (1.5 y) PHTN (2 y) LF (3 y)	LT (3.5 y)	Severe (rapid)
AG1	F	1	10		Homozygous, splicing c.611+5G>A	Cholestasis, itching, and hepatomegaly	155/145	500/688	40	>300	No response	HCC (2 y)	LT (3 y)	Severe (rapid) HCC
AH1	M	6	13	*ABCB4*	Homozygous, missense **c.2692G>A (p.Glu898Lys)**	Itching and hepatomegaly	5.8/1.17	260/169	49	21	Complete	None	Alive (10 y)	Mild (resolved)
AH2	F	11	11		Homozygous, missense **c.2692G>A (p.Glu898Lys)**	Itching and hepatomegaly	11.3	99	271		Complete	None	Alive (7 y)	Mild (resolved)
AH3	F	60	75		Homozygous, missense **c.2692G>A (p.Glu898Lys)**	Itching	10.8	83/72	234	104	Complete	None	Alive (11 y)	Mild (resolved)
AI1	M	30	36		Homozygous, missense **c.2692G>A (p.Glu898Lys)**	Itching and HSM	12/7.4	73/125	78	210	Partial	None	Alive (8 y)	Intermediate
AJ1	M	72	84		Homozygous, missense **c.2692G>A (p.Glu898Lys)**	Itching, recurrent epistaxis, hepatomegaly, and coagulopathy	21/14	303/473	200	90	Partial	None	Alive (9 y)	Severe (insidious)
AK1	F	6	6		Homozygous, missense **c.2692G>A (p.Glu898Lys)**	FTT and HSM	5/3	167/140	81	54.5	Partial	PHTN (6 y)	LT (9.5 y)	Severe (insidious)
AL1	M	48	84		Homozygous, missense **c.2692G>A (p.Glu898Lys)**	Jaundice, itching, epistaxis, and FTT	14	252	200	90	Partial	Portal hypertension	Alive (99)	Severe (insidious)
AL2	M	1	2		Homozygous, missense **c.2692G>A (p.Glu898Lys)**	Jaundice	30	54	495	–	Complete	No complications	Alive (10)	Mild
AM1	F	10	24		Homozygous, missense c.221T>G (p.Met74Arg)	Itching and HSM	20/16	68/160	149	245	Partial	PHTN (3 y)	LT (8 y)	Severe (insidious)
AN1	M	24	24		Homozygous, missense **c.2906G>A (p.R969H)**	Itching and hepatomegaly	13	106/123	151	169	Complete	None	Alive (10 y)	Mild
AO1	M	8	10		Homozygous, missense **c.2906G>A (p.R969H)**	Cholestasis, itching, hepatomegaly, and FTT	25/21	361	209		Complete	None	Alive (11 y)	Mild
AP1	F	17	18	*ABCB4*	Homozygous, missense **c.2906G>A (p.R969H)**	Cholestasis, itching, and HSM	9/2	84/97	207	45	Complete	Rickets	Alive (14 y)	Mild
AQ1	M	6	21		Heterozygous, splicing **c.456G>A (p.K152K)** + Heterozygous, missense c.1584G>C (p.E528D)	Cholestasis, itching, and HSM	65/56	106/386	107	20	Complete	None	Alive (9 y)	Mild
AR1	M	1	40		Homozygous, missense **c.2908T>C(p.Phe970Leu)**	Itching, hepatomegaly	8/1	250/153	157	43	Complete	Firm liver No PHTN	Alive (15 y)	Intermediate
AS1	F	12	53		Homozygous, missense c.526C>T (p.R176W)	Mild itching and hepatomegaly	5/2	96/48	19*	5.2	Complete	Firm liver No PHTN	Alive (15 y)	Intermediate
AT1	F	6	7		Homozygous, missense c.526C>T (p.R176W)	Itching and mild hepatomegaly	4/3	281	160		Complete	None	Alive (3 y)	Mild
AT2	F	6	36		Homozygous, missense c.526C>T (p.R176W)	HSM and itching	10/4	224/225			Partial	PHTN (5 y)	LT (9 y)	Severe (insidious)
AU1	M	6	16		Homozygous, missense c.526C>T (p.R176W)	Itching and hepatomegaly	4/2	102/65	29	NA	Complete	None	Aive (3.5 y)	Mild

(*continued*)

Table I. Continued

Family (no.)	Sex	Age at onset of symptoms, mo	Age at presentation (mo)	Gene	Gene mutation	Clinical presentation	Laboratory values at presentation: TSB/D, μmol/L	ALT/AST, IU/L	GGT, IU/L	s.bile acid	Response of itch to meds	Complication on follow-up, age	Outcome/age, mo	Clinical phenotype
AV1	M	11	27		Homozygous, missense c.296C>T (p.Ser99Phe) + Heterozygous, missense c.2495G>A (p.Arg832His) (*ABCB11*)	Itching	4/1	44/86	209	15	Partial	None	Alive (8.5 y)	Intermediate
AW1	F	18	18		Homozygous, missense c.296C>T (p.Ser99Phe)	Itching	6/1	144/79	122	10	Complete	None	Alive (3 y)	Mild
AX1	M	11	12		Homozygous, missense **c.1378A>T (p.Ile460Phe)**	Cholestasis, itching, and HSM	40/36	91/74	147	238	Partial	PHTN (4y) Fracture (10 y)	LT (11 y)	Severe (insidious)
AY1	M	1	72		Homozygous, splicing **c.2064+G>C (r.spl?)**	Itching, cholestasis Upper GI bleeding	33/29	21/42	350		Partial	PHTN (6 y)	Died (7 y)	Severe (insidious)
AZ1	M	2	50		Homozygous, frameshift **c.628_643del (p.Phe210Serfs*5)**	Failure to thrive, intracranial bleeding hepatomegaly	43/41	137/77	314		Partial	Fracture (4.5 y) PHTN (5y)	LT (9 y)	Severe (insidious)
AAA1	F	18	84		Homozygous, frameshift **c.628_643del (p.Phe210Serfs*5)**	Cholestasis, HSM, itch, Upper GI bleeding	298/194	153/283	206		No response	PHTN (7y)	LT (8 y)	Severe (insidious)
AAB1	M	18	66		Homozygous, frameshift **c.628_643del (p.Phe210Serfs*5)**	Cholestasis, HSM, itch, ascites	216/153	140/443	123		No response	PHTN (5y)	LT (6 y)	Severe (insidious)

AST, aspartate aminotransferase; *BRIC*, benign recurrent intrahepatic cholestasis; *F*, female; *F/U*, follow-up; *GI*, gastrointestinal; *HSM*, hepatosplenomegaly; *LF*, liver failure; *LT*, liver transplant; *M*, male; *NA*, not available; *PHTN*, portal hypertension; *Rx*, treatment; *s.bile acid*, serum bile acid.

Novel mutations described in this study are shown in bold.

Clinical phenotype was categorized into (1) mild: defined as resolution of cholestasis and pruritus with or without medical therapy associated with normalization of ALT/AST, regression of liver and spleen to normal size, or intermittent episodes of cholestasis and normal liver enzymes in-between; (2) severe:defined as worsening of cholestasis and intractable pruritus leading to development of portal hypertension, LF, or need for LT; and (3) intermediate disease phenotype: a status between mild and severe phenotypes characterized by an improvement of liver tests under UDCA without development of portal HTN, LF, or need for LT.

*GGT initially was normal, then later in the course of illness rose up to 100-150.

PFIC3 ($P < .009$, hazard ratio [HR] 4.6453; 95% CI 2.2-9.7). All the patients with heterozygous mutations in ATP8B1 and ABCB11 had transient cholestasis that never recurred during the follow-up time of median 4 years and ranged from 2 to 9 years.

Secondary Outcomes. The median follow-up time for patients with PFIC 1-3 was 8 years, range 5-10 years; 3 years, range 0.9-14 years; 9 years, range 1-15 years, respectively. Among the patients with PFIC3s, 13 responded completely with normalization of liver tests accompanied with disappearance of pruritus, hepatomegaly, or splenomegaly. The median age to start UDCA in this group was 18 months (range 7-75 months). The median age to start UDCA in patients with partial or no response to UDCA was 36 months (range 12-84 months).

Overall, 57% of all the 65 patients developed portal hypertension; development of portal hypertension in patients with PFIC 2 (n = 26) occurred more rapidly, at a median age of 1.5 years (range 0.8-10 years), in contrast to the 10 patients with PFIC3 who developed portal hypertension at median age of 5 years (3-7 year). Signs of liver failure developed in 22 of the 23 patients with PFIC2 who either died or underwent liver transplantation (median age at onset 1.65 years, range 0.7-5.5 years); 7 of the 22 developed liver failure before 1 year of age (32%). Two patients with PFIC2 (Q1 and AG1) (6%) developed HCC at 3 and 2 years of age, respectively; in patient AG1, HCC occurred without signs of liver failure and was the main indication for liver transplantation. The level of serum alpha-fetoprotein was 8459 ng/ml (normal <7 ng/mL) in patient Q1 and 447 ng/ml in patient AG1.

Genotype–Phenotype Correlations

On analysis of the homozygous mutations, we observed that homozygous splicing and frameshift mutations in *ATP8B1*, *BCB11*, and *ABCB4* genes always correlated with severe disease outcome and partial or no response to medical therapy. Indeed, the 2 patients with HCC harbored a frameshift and splicing mutations. In contrast, the homozygous missense mutations in the *ATP8B1*, *ABCB11*, and *ABCB4* genes in our study cohort were associated with better response to medical therapy and subsequently less severe disease outcome. However, different phenotypes could be observed between siblings or members from the same or different tribe sharing the same genotype. For example, 2 homozygous missense mutation in ABCB4 (c.2692G>A/p.Glu898Lys; c.526C>T/p.R176W) led to a wide range of disease severity

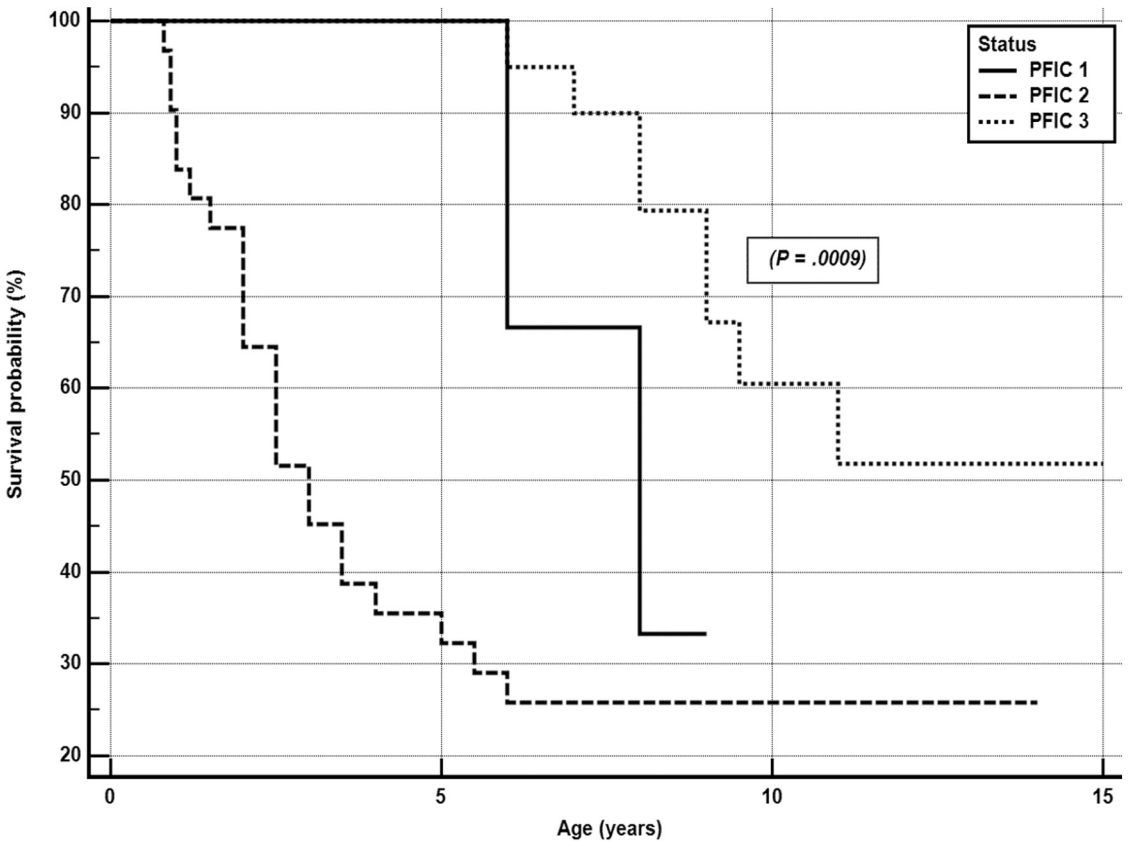

Figure 3. Kaplan–Meier transplant-free survival analysis of the 3 types of PFIC.

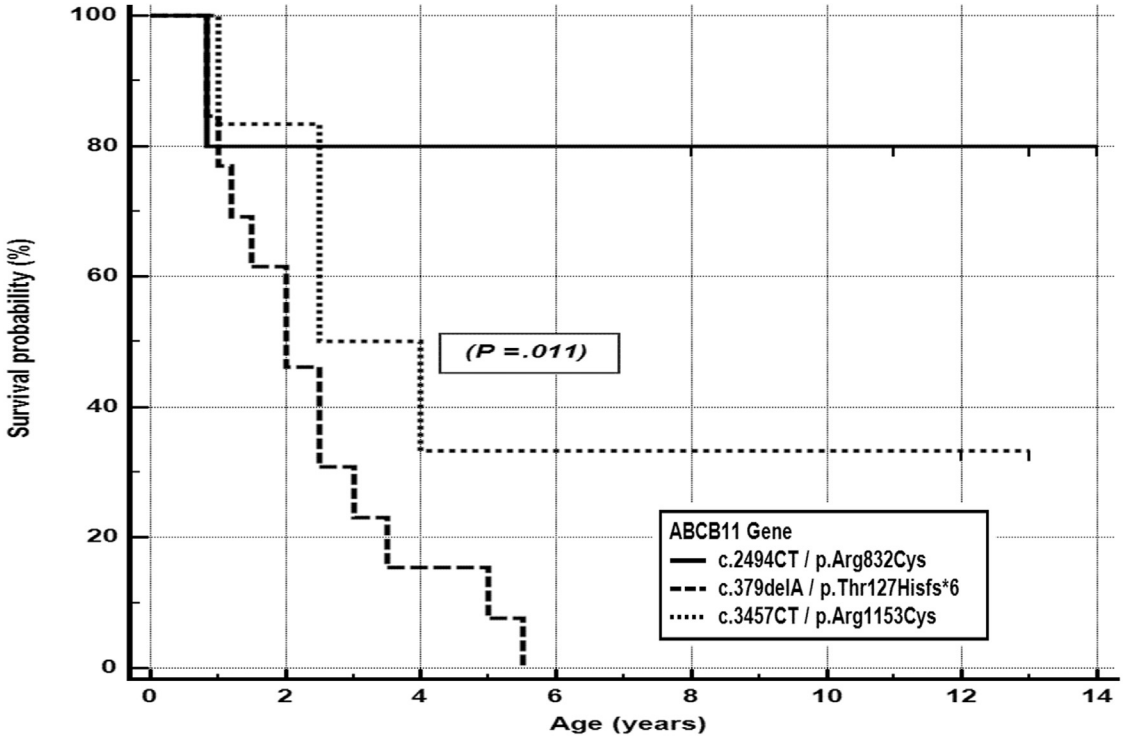

Figure 4. Kaplan–Meier transplant-free survival analysis of the 3 most common ABCB11 gene mutations.

in 12 patients (AH-AL; AS1-AU1, respectively). The same applies to the homozygous missense mutation in ABCB11 (c.2494C>T/p.Arg832Cys), which led to different clinical phenotypes in 5 patients from same tribe (F-H) with outcome ranging from mild to severe.

On subanalysis of genotypes of patients with PFIC2, the 3 most common ABCB11 gene mutations mentioned in the 3 large tribes in Saudi Arabia had variable disease severity and response to therapy (**Table I**). Kaplan–Meier analysis for survival (**Figure 4**) shows that cases harboring the p.Thr127Hisfs*6 [frameshift mutation] had significantly shorter survival time than cases harboring the 2 missense gene mutations (mean 2.4 years vs mean 6 years for the missense mutation p.Arg1153Cys, and mean 11.3 year for the other missense mutation p.Arg832Cys; P = .011; HR 2.2 [0.73-6.8] and HR 9 [CI 2.9-27.6], respectively). Similarly, the 3 most common missense mutations in ABCB4 gene in our patients with PFIC3 (c.2692G>A, p.Glu898Lys; c.2906G>A, p.R969H; c.526C>T, p.R176W) had better significantly better survival than the frameshift mutation c.628_643del (p.Phe210Serfs*5) (**Figure 5**).

Discussion

This study provides insight into the prevalence, wide-spectrum clinical presentation, molecular genetics, and outcomes of 65 patients with *ATP8B1*, *ABCB11*, and *ABCB4* gene mutations from the Middle East. Several novel disease-causing variants were identified, which add to the growing list of pathologic mutations. The results of our study provide further evidence of the broad allelic heterogeneity of the disease leading to a phenotypic continuum between mild, transient, nonprogressive disease and rapidly progressive, fatal, early-onset disease phenotypes. Mutations in *ATP8B1*, *ABCB11*, and *ABCB4* genes accounted for 10% of the causes of infantile cholestasis in our Saudi population, which is similar to 10%-15% rate reported in other countries.[1] The high percentage (29-33%) of PFIC1-3 reported from the liver transplant centers in Saudi Arabia likely over-represented PFIC1-3 as a cause of severe liver disease leading to ESLD and need for liver transplantation.[12,13] We were able to correlate certain gene mutations with disease outcomes. First, patients with PFIC2 had significantly shorter survival time and more rapid disease progression than patients with PFIC3. Second, in keeping with previous studies, splicing and frameshift mutations leading to truncating protein had the worse outcome as compared with the less severe missense mutations. Third, we observed a clear "genotype–phenotype correlation" among patients harboring the 3 most common *ABCB11* mutations in our cohort (p.Thr127Hisfs*6; p.Arg1153Cys; p.Arg832Cys) with patients with frameshift mutations having significantly shorter survival time than cases harboring the other 2 missense gene mutations. In our cohort, we identified the presence of 6 homozygous mutations (3 in *ABCB11* gene [p.Thr127Hisfs*6; p.Arg1153Cys; p.Arg832Cys] and 3 in *ABCB4* gene [p.Glu898Lys; p.R969H; p.R176W]) in apparently unrelated 31 families from 6 tribes in Saudi Arabia, that have not been reported in other

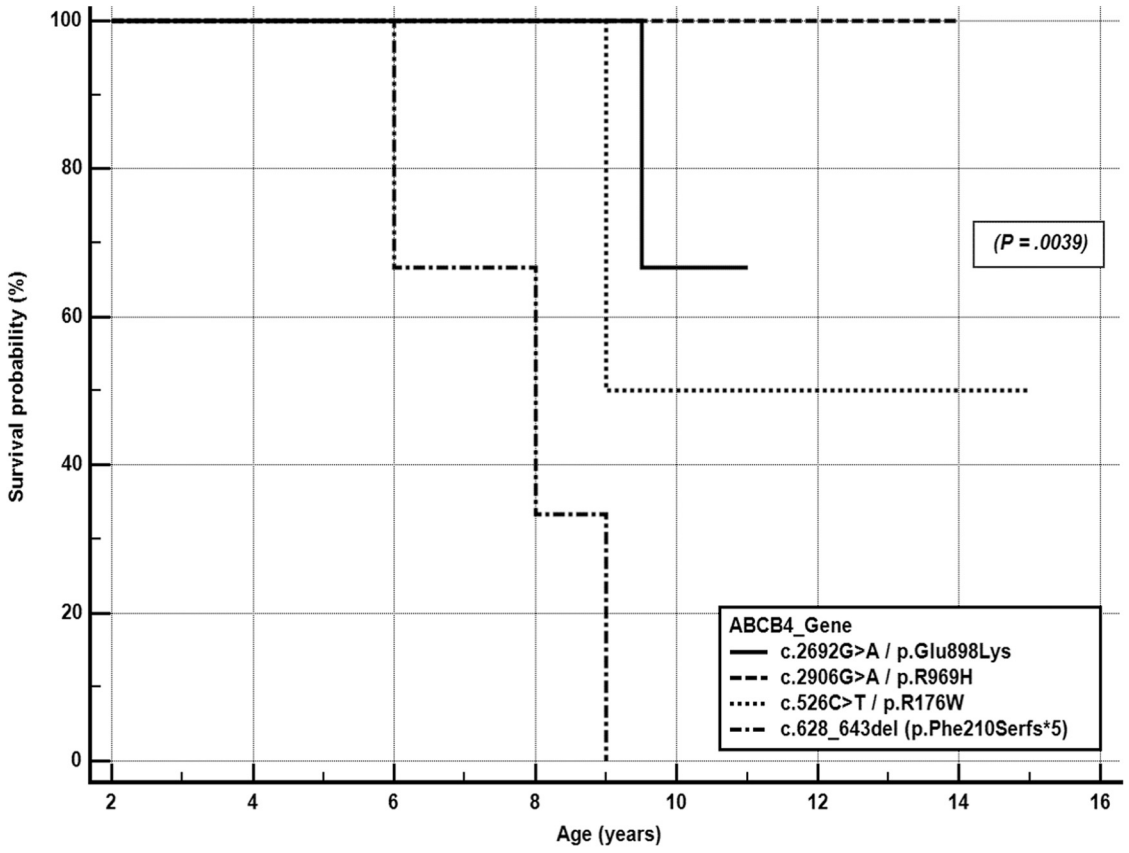

Figure 5. Kaplan–Meier transplant-free survival analysis of the 4 most common ABCB4 gene mutations.

populations, indicating that these mutations probably came from common origin ie, "founder gene mutations."

The clinical phenotype at presentation of the 65 patients in our cohort can be differentiated into 2 distinct patterns. The first pattern, represented by patients harboring *ABCB11* and *ATP8B1* mutations, was characterized by onset of normal or low GGT cholestatic jaundice in early infancy. The second pattern of presentation, represented by patients harboring *ABCB4*, was characterized by onset of pruritus and hepatomegaly without jaundice in late infancy or, more frequent, in early childhood, and typically elevated GGT. Although it has been reported that GGT is a reliable marker to differentiate between *ABCB11* and *ATP8B1* mutations at one end and *ABCB4* mutations at the other one, the normal GGT values found in 3 of our patients with *ABCB4* mutations indicates that normal GGT values at initial presentation do not exclude *ABCB4* mutations.[14] One patient with *ATP8B1* (C1) and 2 with *ABCB11* mutations (G1, I1) had mildly elevated GGT for age (84, 75, and 104 U/L at age 5, 11, and 3 months, respectively) at initial presentation that normalized after 6-12 months, indicating that GGT might fluctuate initially in patients with *ATP8B1* and *ABCB11* mutations.

After the first 6-12 months of presentation and compliance with medical therapy (UDCA ± rifampicin), the behavior of the disease and response to medical therapy depends to great extent on the type of mutation and the remaining function of the transporter protein.[4,13-16] *ABCB11* mutations resulting in severely dysfunctional or absent BSEP protein (splicing and frameshift mutations) did not respond to medical therapy and rapidly progressed to liver failure, whereas mutations that retain some residual BSEP protein function (missense mutations) responded to medical therapy and ultimately led to resolution of cholestasis or to slower progression of the disease. In a large series of 62 PFIC1 and 2 patients, only 30% cases responded to medical management.[6] A second important factor that influences the disease course and determines response to medical therapy, particularly in patients with missense *ABCB4* mutations with residual functioning protein, is initiation of UDCA early in the course of the disease. Two homozygous missense mutations in *ABCB4* gene (p.Glu898Lys and p.R176W) led to a wide range of disease severity in 12 patients (AH-AL; AS1-AU1, respectively).

The presence of modifiers, genetic, epigenetic, or environmental, might partly explain the variable expression of liver disease in family members with the same homozygous gene mutation. An example of a genetic modifier is monoallelic "heterozygous" mutation variant of other hepatocanalicular

transporter genes, which impairs expression and/or function of protein encoded by that gene but to a lesser degree than the homozygous "disease-causing" mutations.[15] Patients from unrelated families (F to H), from one tribe, harbor a missense homozygous *ABCB11* mutation (p.Arg832Cys), and received UDCA during infancy, but the clinical phenotype was mild in 3, intermediate in one, and severe in one patient. One of our patients with PFIC 2 (AA1), who developed rapid severe phenotype, harbors a homozygous variant (c.1331T>C, p.Val444AIa) in addition to the homozygous missense *ABCB11* mutation. This variant, considered to be a common polymorphism in Caucasians, could lead to reduced expression of BSEP protein in the canalicular membrane and as consequence reduce bile acid secretion.[15,17] Another example of gene modifier in our cohort is the presence of a heterozygous missense mutation c.2495G>A, p.Arg832His in *ABCB11* gene in a patient with PFIC3 (AV1). This patient developed intermediate disease phenotype, and another patient with PFIC3 from the same tribe harboring the same ABCB4 gene (c.296C>T/p.Ser99Phe), in absence of p.Arg832His variant in *ABCB11* gene, developed mild disease phenotype. All the 3 prediction programs, Mutation Taster, PolyPhen-2, and SIFT, predict the variant p.Arg832His in *ABCB11* gene to be "disease causing," "damaging," and "deleterious." The mere presence of monoallelic "heterozygous" mutation variant could confer genetic susceptibility to trigger onset of liver disease in specific circumstances (eg, pregnancy, viral infection, drugs).[15] In addition, because the neonatal liver is vulnerable to injury due to its limited capacity of bile acids synthesis and transport, these monoallelic mutations or polymorphism variants in *ATB8B1* and *ABCB11* genes could cause prolonged but transient neonatal jaundice; the cholestasis resolving after maturity of bile synthetic and excretory function of the liver.

Although our study had a retrospective design, its strength is the large number of patients with gene-confirmed PFIC included from a single center with limited variations in medical practice, and long-term follow-up. Our cohort represents the experience in a tertiary pediatric nonliver transplant center that gives us the advantage of receiving a wide spectrum of clinical presentation of patients with defects in *ATP8B1*, *ABCB11*, and *ABCB4* genes, unlike cohorts reported from liver transplant centers where severe forms of PFIC prevail.

In conclusion, we identified "genotype–phenotype" correlations among several mutations in *ABCB11* and *ABCB4* genes, which underscores the importance of early genetic confirmation of the diagnosis to provide additional guidance to physicians and patients about the likely disease course. The disease course in patients with PFIC3, particularly in those harboring missense mutations, could be favorably modified by UDCA therapy. ■

Submitted for publication Nov 22, 2020; last revision received Apr 17, 2021; accepted Apr 20, 2021.

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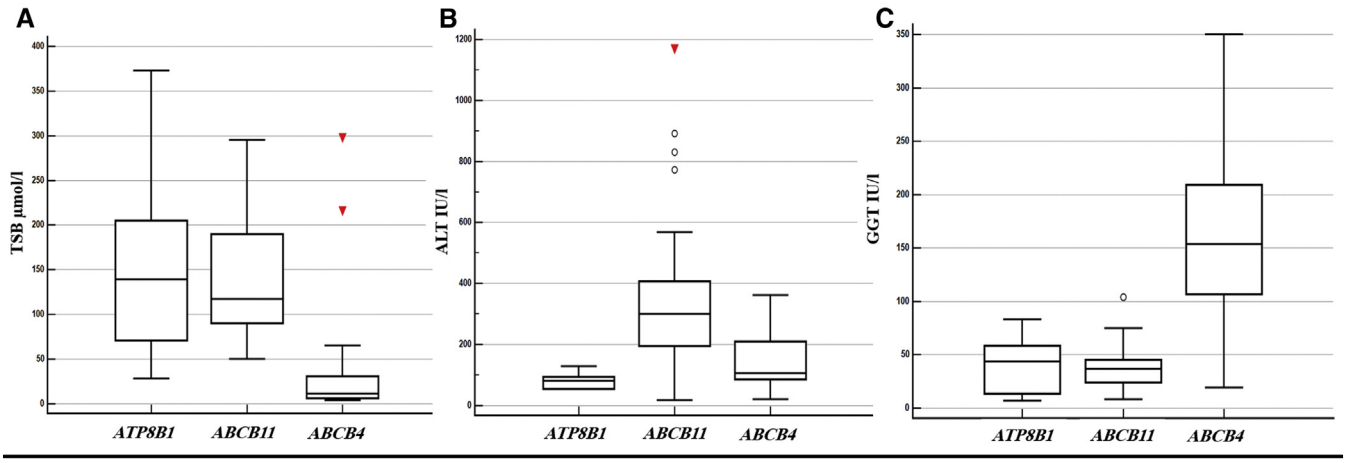

Figure 1. **A,** TSB; **B**, ALT; **C**, GGT levels among the 3 types of PFIC.

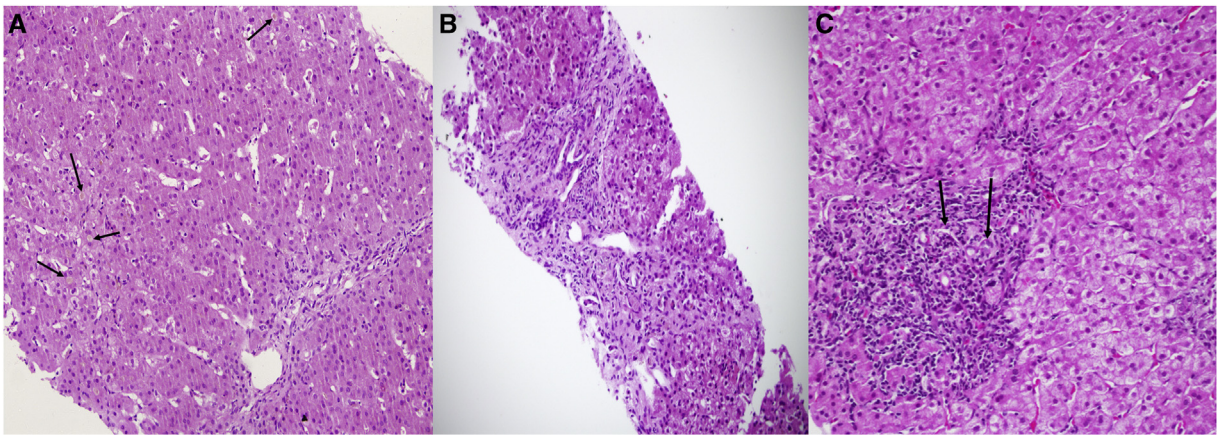

Figure 2. Histopathologic examination (hematoxylin and eosin stains) of liver biopsies depicts: **A**, Bland canalicular cholestasis (*arrows*) with no significant fibrosis or giant cell transformation or inflammation in PFIC1; **B**, Lobular disarray, giant cells formation, portal and lobular fibrosis with bridging fibrosis, and moderate-marked portal and lobular inflammatory cell infiltration in PFIC2; **C**, Bile ductular proliferation (*arrows*), and moderate fibrosis and mononuclear chronic inflammatory response within the portal tract and lack of canalicular cholestasis in PFIC3.

Table II. Histopathologic features of PFIC1-3

Patients	Gene defect	Family	Histopathologic findings			
			Portal fibrosis grade	Giant cell formation	Bile duct proliferation	Canalicular cholestasis
1	ATP8B1	A1	+	0	0	++
2		C1	+	0	0	++
3	ABCB11	F2	++	++	0	+
4		G1	++	++	+	++
6		K1	++	+++	0	+
7		K2	++	+++	0	+
8		L1	++	+++	0	+
9		N1	+++	++	0	+
10		P1	++	+++	+	+
11		T1	++	+++	+	+
12		V1	+++	++	0	+
13		W1	++	+++	0	+
14		X1	+++	++	0	+
15		Y1	+++	++	0	+
16		AB1	+++	++	0	+
17		AD1	+++	++	0	+
18	ABCB4	AH1	+++	0	++	0
19		AM1	+++	0	++	0
20		AN1	+++	0	++	0
21		AO1	+	0	++	0
22		AP1	++	0	++	0
23		AQ1	++	0	++	0
24		AR1	+	0	+	+
25		AS2	+++	0	+	0
26		AW1	+++	0	++	+
27		AX1	+++	0	++	0

0, absent; +, mild; ++, moderate; +++, severe.