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Native liver survival in bile salt export pump deficiency: results of a retrospective cohort study

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Abstract

Background: Bile salt export pump (*ABCB11*) deficiency [Progressive familial intrahepatic cholestasis (PFIC2)] is the most common genetic cause of PFIC and is associated with pruritus and progressive liver disease. Surgical biliary diversion or pharmacological [ileal bile acid transporter inhibitor (IBATi)] approaches can be used to block the recirculation of bile acids to the liver. There is a paucity of detailed data on the natural history and, in particular, the longitudinal evolution of bile acid levels to predict treatment response. Cross-sectional data from large international consortia suggested a maximum cutoff value of bile acids after the intervention to predict a successful outcome.

Methods: This retrospective, single-center, cohort study included all patients with confirmed biallelic pathogenic *ABCB11* genotype PFIC2 treated at our institution with $\geq$2 years follow-up. The outcomes of interventions and predictors of long-term health were analyzed.

Results: Forty-eight cases were identified with PFIC2. Eighteen received partial external biliary diversion (PEBD) surgery, and 22 patients underwent liver transplantation. Two patients developed HCC and 2 died. Improved survival with native liver was closely associated with genotype, complete normalization of serum bile acids following PEBD, and alleviation of pruritus. Persistence of mild-to-moderate elevation of bile acids or a secondary rise following normalization was associated with liver disease progression and led to transplantation, suggesting that any prolonged elevation of bile acids worsens the chance of native liver survival. Higher-grade fibrosis at the time of PEBD was not associated with reduced long-term native liver survival. Patients with PFIC2 benefit from PEBD even at a stage of advanced fibrosis.

Abbreviations: ABCB11, ATP-binding cassette, subfamily B member 11; BA, bile acid; BSEP, Bile salt export pump deficiency; IBAT, Ileal bile acid transporter inhibitor; LTx, Liver transplantation; MARS, Molecular adsorbent recirculating system; n, sample size/number; PEBD, Partial external biliary diversion; PFIC, Progressive familial intrahepatic cholestasis; PFIC2, Progressive familial intrahepatic cholestasis type 2; sBA, Serum bile acid; SNL, Survival with native liver.

Conclusion: Serum bile acid levels are an early predictor of treatment response and might serve as the gold standard in the evaluation of novel therapies including IBATi.

INTRODUCTION

The bile salt export pump is an ATP-binding cassette transporter in the canalicular membrane of the apical domain of hepatocytes. This transporter is of critical importance for bile salt homeostasis; its deficiency leads to cholestasis and progressive liver disease, usually with the need for liver transplantation (LTx) in infancy.[1] Partial biliary diversion has been successfully used since 1988 with no conclusive explanation for its physiologic-beneficial effect.[2] A recent publication of the Natural course and Prognosis of PFIC and Effect of biliary Diversion consortium has stirred interest in this surgical intervention as it is thought to be the gold standard for comparison, with a group of novel compounds that inhibit ileal bile acid (BA) reuptake, and thus mimic the effect of a "medical" biliary diversion.[3] Since the effects of this type of surgery have not been fully understood, it is also not clear which biochemical markers might predict therapeutic short-term and long-term success.

Various therapeutic options, such as chaperones or modulators, have been developed to try to counteract the effects of pathogenic *ABCB11* variants.[4–6] While the new group of ileal bile acid transporter inhibitors (IBAT) has been shown to sufficiently reduce pruritus, the effect on long-term prognosis in liver cirrhosis and HCC is currently unknown due to the limited follow-up duration.[7] It is also still unclear whether the effectiveness of medical IBAT inhibition can be equated with that of partial external biliary diversion (PEBD). So far, there are only individual short-term case reports.[8] Therefore, data on the natural history and early parameters of treatment response are needed to assess and compare novel medical interventions. We aimed to document our long-term experience with patients who are BSEP deficient (PFIC2), with a focus on the outcome of interventions and predictors of prognosis.

METHODS

Patients and methods

We performed a retrospective single-center cohort study based on patient records. We included all patients treated at our institution by medical or surgical intervention since 1980 with a documented pathogenic *ABCB11* genotype, as determined by biallelic *ABCB11* variants classified as likely pathogenic (class 4) or pathogenic (class 5) according to the guidelines of the American College of Medical Genetics and Genomics.[9] Variants of unknown significance were also included if they could not (or only with allele frequency <0.01%) be detected in the population database gnomAD, and additionally, at least 5 out of 7 *in silico* tools [Meta Learning Rate (MetaLR); Polymorphism Phenotyping v2 (PolyPhen2); Functional Analysis through Hidden Markov Models (FATHMM); Combined Annotation Dependent Depletion (CADD); Align Grantham Variation Score-Grantham Difference Score (AlignGVGD); and Sorting Intolerant From Tolerant (SIFT), MutationTaster] predicted a pathogenic effect. For patients no longer being cared for by us, a structured telephone interview was conducted by a graduate pediatric nurse. Patients were followed up until October 2020. The data collection included some information on patients who were also included in previous analyses from our institution.[10,11] The study was approved by the local ethics committee (No 8558_BO_K_2019), and was performed in accordance with the ethical principles of the Declaration of Helsinki.

DNA sequencing was performed as targeted panel sequencing or whole-exome sequencing.[12] For targeted panel sequencing, Tru-Seq Custom Amplicon libraries containing 21 genes related to pediatric or juvenile hepatopathies were prepared according to the manufacturer's instructions and were sequenced on a MiSeq system (Illumina Inc., San Diego, California). Read alignment to the human reference genome build (GRCh37), and variant calling were performed using the Sequence Pilot 4.1.2 software (SeqNext module, JSI medical systems GmbH, Ettenheim, Germany). For whole-exome sequencing, DNA enrichment, and library preparation were performed using the xGen Exome Research Panel (Integrated DNA Technologies, Inc., Coralville) or TruSeq Exome (Illumina, San Diego, CA). Sequencing was performed on a NextSeq. 500/550 using the NextSeq. 500/550 High Output v2 kit (Illumina) or on an MGI DNB SEQ-G400RS using the DNBSEQ-G400RS High-throughput Sequencing Set (BGI, Shenzhen, China). Alignment to the human reference genome build (GRCh37) was performed using megSAP, version 0.1-710-g52d2b0c (https://github.com/imgag/megSAP). Variant prioritization and visualization were performed with GSvar, version 2018_04 (https://github.com/imgag/ngs-bits), IGV [13] version 2.5.3. Variant interpretation was conducted in all cases with the help of Alamut visual, version 2.7–2.12 (Interactive Biosoftware, Rouen, France).

TABLE 1 ID, genetic variants in *ABCB11* gene and age at PEBD, LTx, and death in our BSEP patients

ID	*ABCB11* variants	Age PEBD (y)	Age LTx (y)	Age death (y)
1	c.3169C > T p.(Arg1057*) het; c.2178+1G > A p.? het	ND	1.43	Alive
2	c.22c > T, p.Arg8* hom	0.45	0.86	Alive
3	c.1770C > A p.(Asp590Glu) hom	ND	9.94	Alive
4	c.890A > G p.(Glu297Gly) hom	ND	ND	Alive
5	c.1445A > G p.(Asp482Gly) het; 890A > G p.(Glu297Gly) het	ND	ND	Alive
6	c.1243C > T, p.Arg415* hom	ND	0.43	Alive
7	c.2629G > A p.(Gly877Arg) het; c.779G > A p.(Gly260Asp) het	5.68	ND	Alive
8	c.22C > T p.(Arg8*) het; c.1709C > T p.(Ala570Val) het; c.2005A > G p.(Ile1669Val) het	ND	1.53	Alive
9	c.1445A > G p.(Asp482Gly) hom	ND	ND	Alive
10	c.3459_3460delCTinsG p.(Ser1154Glnfs*17) het; c.3491delT p.(Val1164Glyfs*7) het	ND	1.68	Alive
11	c.2617G > A p.(Gly873Ser) hom	ND	ND	Alive
12	c.890A > G p.(Glu297Gly) hom	1.69	ND	Alive
13	c.1384A > C p.(Ser462Arg) het; c.2636T > G p.(Ile879Arg) het	ND	ND	Alive
14	c.3703C > T p.(Arg1235*) hom	ND	4.67	Alive
15	c.2162A > G p.(Tyr721Cys) het; c.3084A > G p.(Ala1028Ala) het	ND	ND	Alive
16	c.3767C > T, p.(Thr1256Met) hom	ND	ND	Alive
17	c.3517A > G p.(Asn1173Asp) hom	ND	ND	Alive
18	c.1160G > A p.(Arg387His) het; c.2703C > G p.(Ser901Arg) het	ND	ND	Alive
19	c.890A > G (p.Glu297Gly) het; c.1445A > G p.(Asp482Gly) het	ND	ND	Alive
20	c.2178+1G > A p.? het; c.2029A > G p.(Met677Val) het	28.96	ND	Alive
21	c.1966_1967delTT p.(Leu656Alafs*9) het; c.890A > G p.(Glu297Gly) het	ND	3.33	Alive
22	c.1384A > C p.(Ser462Arg) het; c.2636T > G p.(Ile879Arg) het	ND	ND	Alive
23	c.2782C > T,p.Arg928* hom	ND	ND	1.8
24	c.1445A > G p.(Asp482Gly) hom	1.03	2.99	Alive
25	c.3382C > T p.(Arg1128Cys) het; c.3495_3497del p.(Leu1165del) het	ND	ND	Alive
26	c.1150G > C p.(Ala384Pro) hom	ND	ND	0.98
27	c.890A > G p.(Glu297Gly) het; c.99-1G > T;r.spl p.? het	0.41	14.77	Alive
28	c.3352G > A p.(Gly1118Ser); c.3362C > T p.(Thr1121Ile)	5.39	ND	Alive
29	c.1712G > C p.(Arg571Thr) het; c.2782C > T,p.Arg928* het	12.5	ND	Alive
30	c.3495_3497del p.(Leu1165del) hom	0.96	10.5	Alive
31	c.890A > G p.(Glu297Gly) hom	0.95	3.07	Alive
32	c.1827_1828insCA p.(Ile610Glnfs*45) hom	ND	6.42	Alive
33	c.1712G > C p.(Arg571Thr) het; c.2782C > T,p.Arg928* het	6.09	19.94	Alive
34	c.890A > G p.(Glu297Gly) het; c.1445A > G p.(Asp482Gly) het	ND	4.08	Alive
35	c.3703C > T p.(Arg1235*) het; c.1160G > A p.(Arg378His) het	ND	ND	Alive
36	c.1460G > C p.(Arg487Pro) het; c.764G > A p.(Gly255Glu) het	ND	2.28	Alive
37	c.890A > G p.(Glu297Gly) hom	ND	7.2	Alive
38	c.1160G > A p.(Arg378His) het; c.-27-789_99-48del het p.?	ND	ND	Alive
39	c.1150G > C p.(Ala384Pro) hom	0.44	1.24	Alive
40	c.890A > G p.(Glu297Gly) hom	0.36	7.1	Alive
41	c.3495_3497del p.(Leu1165del) hom	2.5	23.75	Alive
42	c.1445A > G p.(Asp482Gly) het; c.890A > G p.(Glu297Gly) het	ND	ND	Alive
43	c.890A > G p.(Glu297Gly) hom + microdeletion (10.06 Mb) 2q24.3q31.2 het	ND	1.01	Alive
44	c.1445A > G (Asp482Gly) het; c.1259T > A het p.(Ile420Asn)	ND	1.58	Alive

TABLE 1. (*continued*)

ID	*ABCB11* variants	Age PEBD (y)	Age LTx (y)	Age death (y)
45	c.3517A > G p.(Asn1173Asp) het; c.1548T > G p.(Ile516Met) het	12.51	ND	Alive
46	c.1445A > G p.(Asp482Gly) hom	8.1	ND	Alive
47	c.1460G > A p.(Arg487His) het; c.1460G > C, p.(Arg487Pro) het	1.32	ND	Alive
48	c.279C > A p.Tyr93* het; c.3083_3084delinsAG p.(Ala1028Glu) het	0.94	ND	Alive

Abbreviations: BSEP, bile salt export pump deficiency; LTx, liver transplantation; ND, not determined; PEBD, partial external biliary diversion.

Individual cases were referred to us for further treatment with a previously external, genetically confirmed diagnosis. The main part of these external genetic examinations, as well as the measurement of BSEP antibodies, was performed by Prof. Verena Keitel-Anselmino in Düsseldorf.[14,15]

Patients were categorized as heterozygous, homozygous, or compound heterozygous for *ABCB11* variants classified as (likely) pathogenic or suspicious variants of unknown significance, as described above. Where available, the genotypes of parents were analyzed to determine the compound heterozygosity. Detected variants were categorized into missense variants resulting in an amino acid substitution or nonfunctional variants (nonsense, frameshift, canonical ± 1 or 2 splice sites, and single, or multi-exon deletions) potentially resulting in protein truncation or absent protein expression because of nonsense-mediated mRNA decay.

The Ishak score was used to grade the histopathologic stage of fibrosis.[16]

Statistical analyses

Data were recorded in a specially designed case record form in REDCap (Research electronic data capture).[17] R version 4.0.3 was used for all analyses. Descriptive analyses were carried out for all patients with BSEP deficiency [progressive familial intrahepatic cholestasis type 2 (PFIC2)], as well as for the subgroups of those who received a PEBD and those who received LTx. Continuous variables were summarized as median (interquartile range) and categoric variables as absolute and relative frequencies. Kaplan-Meier survival curves and Cox proportional-hazards models were computed for survival with the native liver among all patients with BSEP deficiency (PFIC2), as well as in the subgroup of those who received a PEBD and those who received LTx (R packages "survminer" version 0.4.8 and "survival" version 3.2-7).[18,19]

RESULTS

Clinical characteristics

From 104 patients with low gamma-glutamyl transferase PFIC treated in our center since 1980, all patients with pathogenic *FIC1, TJP2, NR1H4,* or *MYO5B* variants, as well as patients with only a monoallelic pathogenic variant or variants of unknown significance (class 3) in the *ABCB11* gene, were excluded. Thus, 48 patients with genetically confirmed BSEP deficiency (PFIC2) due to biallelic pathogenic variants in *ABCB11* with at least 2 years of follow-up were evaluated. Table 1 lists the individual patients with their genetic variants in the *ABCB11* gene. Of these, 28 (58%) were male, 10 were from consanguineous families, 13 had affected siblings, and 5 had a family history of early childhood death from cholestatic cirrhosis. Most patients (n = 39, 81%) were of European (including Turkish) descent; the families of 7 patients (19%) were originally from the Arab or African region. The median birth weight and the duration of pregnancy did not differ from the healthy population. In 56% of the patients, compound heterozygous pathogenic variants were present in the *ABCB11* gene; 27% of these children had variants resulting in a nonfunctional protein. The 2 most common pathogenic *ABCB11* variants NM 003742.2: c.1445A > G p.(D482G) and c.890A > G p.(E297G) were identified on one or both alleles of 16 (33%) patients with BSEP deficiency (PFIC2). Three children presented with an additional syndromic phenotype. In 2 children, these additional phenotypes could be explained by *FGFR3*-associated achondroplasia and *SOX11*-associated Coffrin-Siris syndrome. In a third patient, a reciprocal translocation between chromosomes 5 and 11 was detected. However, its phenotypic consequences are unknown.

The median age at first clinical manifestation was 1.9 months. Jaundice was the most common initial symptom, followed by pruritus, vitamin K deficiency with coagulopathy, or symptoms of impaired liver function. On average, the final confirmation of BSEP deficiency (PFIC2) was made 10.8 months after the onset of symptoms, with differences ranging from a few days (with a positive family history) to decades (max. 23.3 years). Confirmed genetic diagnosis has increased rapidly in recent years due to improvements in genetic technology and a higher vigilance among health care professionals.

Three children developed 1 or more episodes of pancreatitis in a case associated with obstructive cholelithiasis. The other 2 had no evidence of choledocholithiasis. Three patients showed hearing impairment in adulthood and received hearing aids. Two of

these patients underwent LTx in early infancy, but the additive or causative role of multiple general anesthesia and therapies with potentially ototoxic drugs remains unknown. During the follow-up period of 2.3 to 40.8 years (median 19.3), 2 children (4.1%) developed HCC at the age of 2.2 and 3 years, respectively. Both had missense variants in the *ABCB11* gene and were successfully transplanted. Two children died due to liver cirrhosis and sepsis in chronic liver failure while on the waiting list for transplantation at the age of 12 months and 1.8 years, respectively. The final height reached in adulthood was below the expected target range. Even when excluding the 3 patients with additional syndromic diseases, the mean final height of 156 cm in women and 175 cm in men is below the mean in the Caucasian population (Tables 2 and 3).

Biliary diversion

A total of 18 (37.5%) children received biliary diversion surgery; 17 of those 18 primarily as PEBD. One child received primary partial internal biliary diversion surgery at 13 months of age, which was converted to external diversion (PEBD) after 6 months due to persistent pruritus. Exactly 50% of all biliary diversions led to excellent long-term results with normal liver function, no cholestasis, and no residual pruritus. In 2 adolescents, clinical relapse occurred in the long term due to stoma-associated complications with stricture-respective prolapse, which immediately resolved following stoma revision surgery. In contrast to the experience with patients with FIC1 or complete cirrhosis, we did not observe excessive cholorrhea with severe dehydration and electrolyte imbalances in any of the BSEP-deficient (PFIC2) cohort patients.[20,21]

TABLE 2 Baseline characteristics of the study population (N = 48)

	Median (range)
Year of birth	2002 (1976–2018)
Weight at birth (g)[a]	3,300 (2080–4400)
Pregnancy week[a]	39 (28–43)
Age at manifestation (mo)	1.9 (0–189)
Age at final diagnosis (mo)	14.3 (1.1–285)
Interval between first symptoms and diagnosis (mo)	10.8 (0.1–280)
Interval between first symptoms and first visit to the liver center (tertiary care center) (mo)	4.5 (0.6–292)
Age at LTx (mo)	38.4 (5.1–285)
Age at PEBD (mo)	20 (4–336)
Interval between PEBD and LTx (y)	6.7 (0.4–21.3)
Final height in males (cm)[b]	175 (153–192)
Final height in females (cm)[b]	156 (113–179)

[a]n = 2 missing.
[b]only patients > 15 years.
Abbreviations: LTx, liver transplantation; PEBD, partial external biliary diversion.

During follow-up, 9 patients (50%) underwent LTx after PEBD. Out of these, 5 patients were transplanted within the first 25 postoperative months with persistent uncontrolled pruritus, and elevated serum bile acid (sBAs). Two patients were transplanted after 7 and 14 years, respectively, 1 with intermittent severe itching episodes and 1 with a marked reduction in quality of life. sBAs in these patients was 40–817 μmol/l (median 273) 3 months or later after PEBD. Two patients had normalized sBAs following PEBD in childhood and maintained normal liver function well into adulthood; however, they became intolerant of the persistent stoma for social reasons. Their acceptance problems persisted despite recurrent medical counselling, surgical revision, and a switch to a button cholecystostomy. Both patients were temporarily noncompliant in adolescence or young adulthood, did not care for their stoma, and were not under hepatologic care for years. Because of this psychosocial burden of PEBD, they received LTx 14 and 21 years following PEBD.

Based on our initial experience with the unfavorable prognosis of PEBD in already cirrhotic patients,[10] we discontinued biliary diversion surgery in patients with complete cirrhosis (Ishak 5 and 6). In the cohort of patients with BSEP deficiency (PFIC2), we did not find evidence that the presence and grade of fibrosis (Ishak 0-1 versus Ishak 2-4) at the time of surgery are a prognostic parameter of native liver survival (HR 0.74; 95% CI, 0.27–2.01; $p = 0.56$). Figure 1 shows the degrees of fibrosis in detail according to the Ishak score. Only 1 patient had F4 fibrosis at the time of PEBD, and he lived with his own liver for more than 15 years. However, despite biochemical and clinical remission, he increasingly declined the biliary stoma for cosmetic reasons, was temporarily noncompliant, and underwent liver transplantation in young adulthood. On the other hand, there was a patient who, despite the absence of fibrosis (Ishak F0) at the time of PEBD, still needed liver transplantation at preschool age.

Liver transplantation

A total of 22 (46%) patients underwent LTx between the age of 5 months and 23.8 years (median 3.2 years). In more than 80% (n = 18) of these patients, therapy-refractory pruritus alone was the indication for admission to the transplant waiting list. Signs of portal hypertension with ascites (n = 10) or gastrointestinal bleeding (n = 6) developed in up to 23% of these children at a median age of 2 to 3 years. Four children with dysfunctional PEBD suffered at least 1 pathologic fracture as a result of chronic cholestasis and osteopenia. Complications of biliary cirrhosis, such as hepatic

TABLE 3 Clinical information of the study population (N = 48)

		n (%)
Sex	Female	20 (42)
	Male	28 (58)
Genetics	Compound heterozygous	27 (56)
	Homozygous	21 (44)
Type of genetic variants	Missense	35 (73)
	Nonfunctional	13 (27)
Consanguinity[a]	Yes	10 (22)
Affected siblings	Yes	13 (27)
Healthy siblings	Yes	36 (75)
Death	Yes	2 (4)
HCC	Yes	2 (4)
PEBD	Yes	18 (37.5)
LTx	Yes	22 (46)
MARS	Yes	3 (6)
Nasobiliary tubes	Yes	5 (10)
Upper endoscopy	Yes	22 (46)

[a]n = 3 missing.
Abbreviations: LTx, liver transplantation; MARS, Molecular adsorbent recirculating system; PEBD, partial external biliary diversion.

encephalopathy, hepatopulmonary syndrome, hepatorenal syndrome, and spontaneous bacterial peritonitis, developed only incidentally (Table 4). All transplanted patients suffered from moderate to severe itching pre-LTx, and had elevated sBAs (mean 227 μmol/l).

Five of the transplantations were performed by living donation from a first-degree relative, usually a heterozygous parent. The posttransplant course in these patients did not differ from those who received a donation following brain death organ. Two patients required re-LTx for transplant-related complications 2 and 4 years after primary LTx. One patient with a nonsense and a splice site variant in *ABCB11* developed a recurrence of the underlying disease 32 months after maternal living donation, with elevated anti-BSEP-antibodies 1:5000 in serum and histologic evidence of canalicular cholestasis. Complete remission was achieved after intensive combination therapy with plasmapheresis, rituximab, and bortezomib. Seven years later, this child showed unrestricted liver synthesis parameters under BSEP-antibody level-guided combined immunosuppression with mycophenolate mofetil and tacrolimus, and had no further episodes of cholestasis.

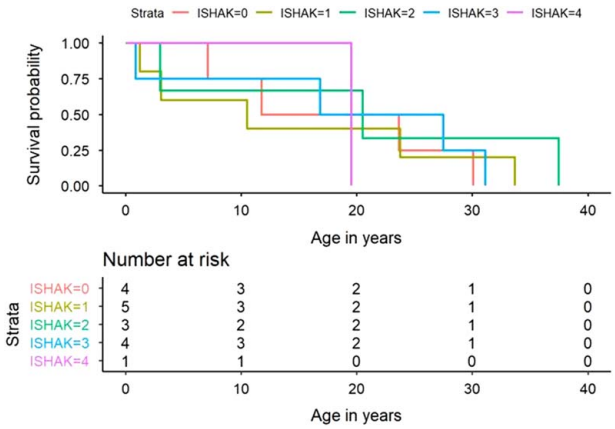

FIGURE 1 Kaplan-Meier plot of native liver survival of patients and Ishak score at the time of PEBD. Abbreviations: PEBD, partial external biliary diversion.

The probability of death or LTx was strongly associated with the genotype. Patients with variants leading to a nonfunctional protein had significantly reduced the probability of survival with native liver (SNL; HR, 3.94; 95% CI, 1.74–8.89; $p = 0.001$; Fig. 2). However, we identified 2 patients with truncating BSEP variants who are still surviving with their native livers and without surgical intervention. One is a 16-year-old girl with moderate pruritus, portal hypertension, a history of variceal ligations, and with compensated biliary cirrhosis. The other is a 30-year-old man who had first cholestatic symptoms aged 5 and underwent several molecular adsorbent recirculating system cycles due to intermittent severe pruritus in adolescence. Although chronically affected by pruritus, he has a normal liver function, stable partnership, and is fully employed.

Neither week of gestation (HR, 1.02; 95% CI, 0.86–1.22), age in years at first manifestation (HR, 0.84; 95% CI, 0.64–1.10), nor interval between first symptoms and diagnosis in years (HR, 0.85; 95% CI, 0.69–1.04), had a significant effect on patient survival with their own native liver. However, birth weight (HR, 0.4; 95% CI, 0.20–0.82) did have a significant effect on patient survival with their own native liver.

Serum bile acids

The level of sBAs in different patient groups showed characteristic patterns associated with the clinical course. After PEBD, sBA decreased immediately postoperatively. In patients surviving long term with their native liver, sBA remained within the normal range. In 2 patients with stoma-associated complications, sBAs spiked to >300 μmol/l in parallel with the recurrence of pruritus and returned to normal after surgical revision (Fig. 3). The group of liver-transplanted patients showed a decrease in the level of bile acids, but an unsteady course with fluctuations between 40–820 μmol/l. The 2 partially noncompliant patients with LTx, 20 and 21 years after PEBD, initially normalized their sBA to <25 μmol/l in a stable pattern for many years (Fig. 4).

Other parameters recorded in the study were glutamic oxaloacetic transaminase/aspartate transaminase, glutamic

TABLE 4 Frequency of liver decompensation events in the study population (N = 48)

		All N = 48	LTx n = 22	PEBD + LTx n = 9	PEBD only n = 9
Ascites	Yes	10	8	1	0
	No	33	12	6	9
	Missing	5	2	2	0
	Age at first onset (years)[a]	0.4–9.9 (2.5)	0.4–9.9 (2.5)	0.7	-
Variceal bleeding	yes	6	4	0	1
	no	37	16	8	8
	missing	5	2	1	0
	Age at first onset (years)[a]	1.5–10.3 (2.8)	1.5–9.6 (2.8)	-	10.3
HE	Yes	3	3	0	0
	No	40	18	8	9
	Missing	5	1	1	0
	Age at first onset (years)[a]	0.4–4.5 (1.5)	0.4–4.5 (1.5)	-	-
Hepatopulmonary syndrome	Yes	1	1	1	0
	No	43	20	7	9
	Missing	4	1	1	0
	Age at first onset (years)	23.3	23.3	23.3	-
Hepatorenal syndrome	Yes	1	1	0	0
	No	43	21	8	9
	Missing	4	0	1	0
	Age at first onset (years)	8.0	8.0	-	-
Spontaneous bacterial peritonitis	Yes	2	2	1	0
	No	41	18	6	9
	Missing	5	2	2	0
	Age at first onset (years)	0.6 resp 1.5	0.6 resp 1.5	0.6	-
Bone fracture	Yes	4	3	0	1
	No	39	17	7	8
	Missing	5	2	2	0
	Age at first onset (years)[a]	1.3–6.4 (3.1)	2.4–6.4 (3.8)	-	1.3

aMin-max median.
Abbreviations: LTx, liver transplantation; PEBD, partial external biliary diversion.

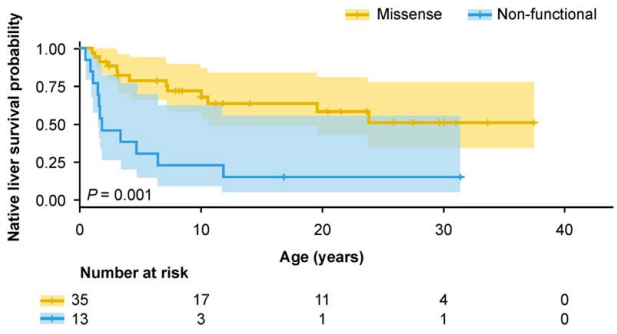

FIGURE 2 Kaplan-Meier plot of the native liver survival of patients with missense versus nonfunctional variants in *ABCB11*.

pyruvic transaminase/alanine transaminase, bilirubin, gamma-glutamyl transferase, quick/international normalized ratio, cholinesterase, albumin, protein, alkaline phosphatase, and thrombocyte level. For none of these parameters could we demonstrate a prognostically significant effect comparable to the bile acids between the different prognosis groups.

Special pruritus therapy

Due to severe pruritus, 3 patients each received a nasobiliary tube or molecular adsorbent recirculating system therapy once or more. Typically, these itching episodes were triggered by mild infections, and patients

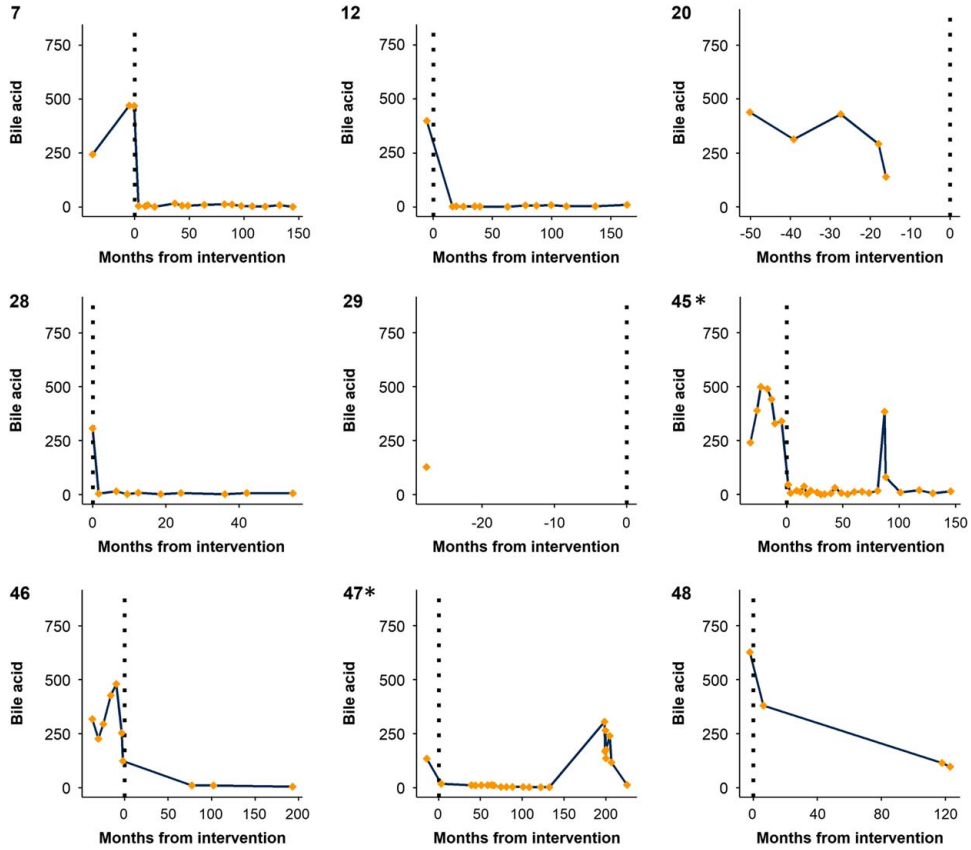

FIGURE 3 Individual longitudinal bile acid levels (μmol/l) in PEBD patients without LTx (n = 9). *n = 2 Relapse due to stoma-associated complication, resolved after stoma revision surgery. Patient ID = number in the top left of each panel. Dotted line = PEBD. Abbreviations: LTx, liver transplantation; PEBD, partial external biliary diversion.

responded immediately with a decrease in bile acids and resolution of pruritus. Naturally, the therapeutic effect of this intervention was limited over time and led to only partial remission. A 14-year-old girl and an adult man had recurrent episodes of pruritus but still lived with their native livers. They each underwent interventions for acute episodes of pruritus, such as 2 nasobiliary tubes and 3 cycles of the molecular adsorbent recirculating system, respectively. Fig. 5 gives an overview of the patient outcomes.

DISCUSSION

The aim of this study was to describe our long-term experience with patients who are BSEP-deficient (PFIC2), with a focus on interventions and predictors of long-term prognosis. Our single-center natural history review analyzes fewer patients than previous multicenter cohorts but has more granular data to investigate predictors of long-term response to surgical partial biliary diversion. Our data confirm that genotype is strongly associated with the natural history of BSEP deficiency (PFIC2).[3,22]

What is new in the current discussion on the gold standard for long-term SNL is the role of sBAs. It is well documented in various case series that partial biliary diversion may enable long-term SNL.[10,23,24] Especially with regard to the future use of IBAT inhibitors, it is important to understand which factors can be used to predict therapeutic response. The previous multicenter analysis identified a cutoff for postinterventional reduction in sBA levels below 102 μmol/l as a parameter to predict the therapeutic efficacy of diversion surgery.[3] In contrast, in our cohort, we found that any increase in sBA following biliary diversion is associated with a poorer prognosis, and long-term SNL is only possible with normal sBA. Accordingly, since this research did not have a predefined aim to identify endpoints for a medication intervention, we could not derive a cutoff level for sBA levels following surgical diversion.

Another important novel finding of our analysis is the missing impact of liver fibrosis on the outcome of partial biliary diversion and long-term SNL. In contrast to our expectations and previous reports[25] we could not demonstrate a worse outcome in patients with advanced fibrosis as compared with patients with lower-grade fibrosis. The risk of surgery and postoperative complications must be compared with the chance of avoiding transplantation with its lifelong consequences and/or the benefit of transplant-free years. Biliary diversion may allow long-term SNL, even

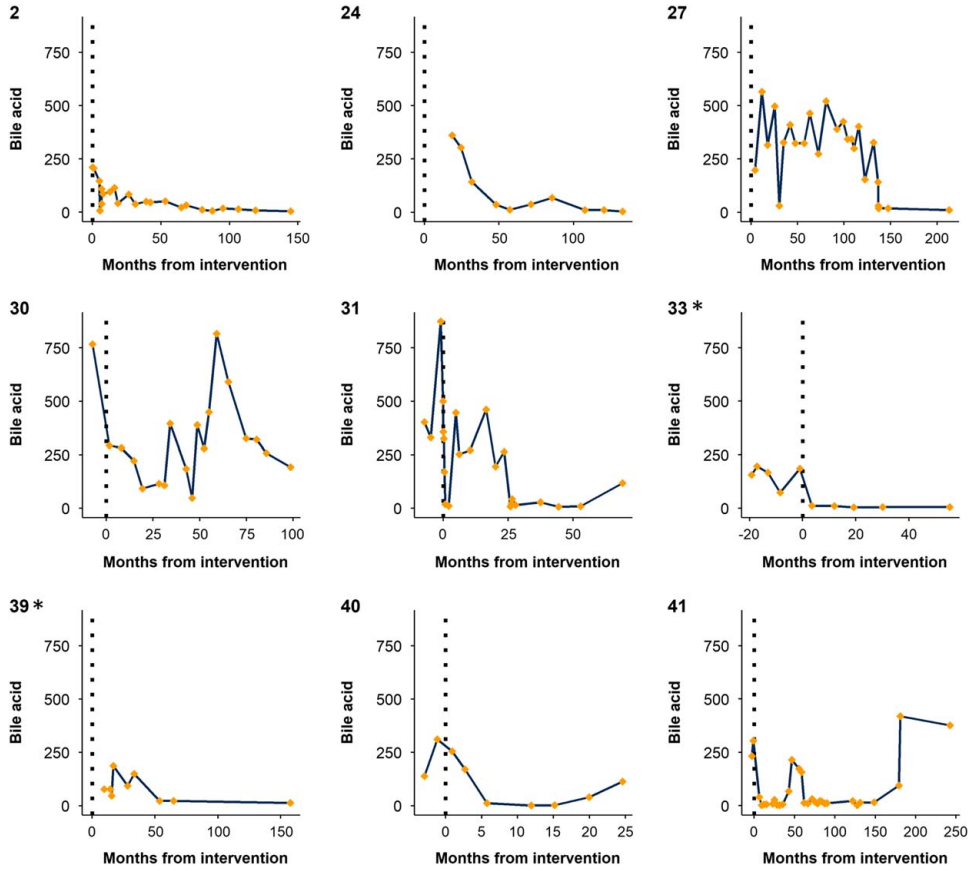

FIGURE 4 Individual longitudinal bile acid levels (μmol/l) in PEBD patients with LTx (n = 9). *n = 2 long-term responders with decompensation due to noncompliance 14 respectively 21 years after PEBD. Dotted line = PEBD. Patient ID = number in the top left of each panel. Abbreviations: LTx, liver transplantation; PEBD, partial external biliary diversion.

in patients with advanced fibrosis. Therefore, surgical (PEBD), or hopefully in the future, medical-biliary diversion (IBAT) should be evaluated in every patient before LTx evaluation. However, it requires further prospective studies if medical diversion with IBAT inhibition can live up to these expectations. Our report provides essential and novel information on the benchmarking of treatment response of sBA for further prospective studies.

Also, in our cohort, we found a significant association between the type of genetic variant in the *ABCB11* gene and the probability of SNL. Patients with missense variants showed a significantly better prognosis than children and adolescents with variants leading to a nonfunctional BSEP protein, which is in line with the data reported by the Natural course and Prognosis of PFIC and Effect of biliary Diversion consortium.[3] However, the risk of HCC development also exists in patients with *ABCB11* missense variants. In addition, we also report 2 patients older than 15 years of age who live with their own liver, despite nonsense variants and conservatively treatable severe itching episodes with good liver function. Therefore, besides the *ABCB11* genotype, additional factors impact long-term prognosis in PFIC2. We did not find evidence for any influence of duration of pregnancy, age at manifestation, or interval until diagnosis on long-term outcome in this retrospective study. By expanding genetic diagnostics with whole-exome and genome sequencing, we are now looking specifically for modifier genes, and in the context of epigenetics, we hope to learn more about the mechanisms of gene regulation in the future.

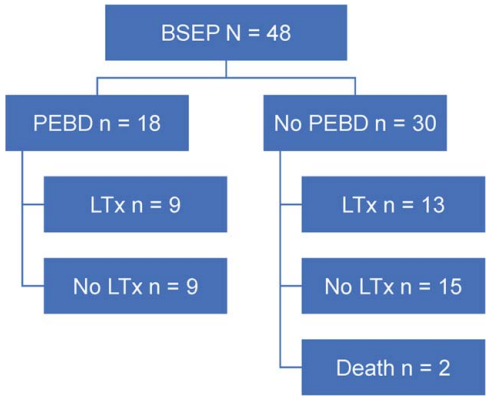

FIGURE 5 Cohort overview. Abbreviations: BSEP, bile salt export pump deficiency; LTx, liver transplantation; PEBD, partial external biliary diversion.

Alongside the medical aspects, a patient's quality of life, with acceptance of a permanent external stoma, has to be considered. While avoiding transplantation is a success for the attending physicians and the patient's family, a biliary stoma poses additional challenges for the patient.[11] Despite an initially excellent response with complete clinical remission, we saw 2 male adult patients who could no longer accept their stoma following puberty for cosmetic reasons and in the context of partnerships. Both decided to undergo LTx at the age of >20 years despite all the risks.

In our cohort study, therapy-refractory pruritus was the most frequent indication for LTx. The classic indications for pediatric LTx, complications of portal hypertension or impaired liver function, were less frequent in patients with BSEP deficiency. None of those patients with hepatic decompensation had normal sBA or were free of pruritus. Monitoring of patients with persistent itching and nonresponders to conservative therapy using clinical scratch scores and sBA might allow for early prognostic assessment.

In summary, we found that any long-term increase in sBAs was correlated with long-term SNL, providing a new benchmark for evaluating future medical interventions. Every patient with gamma-glutamyl transferase-negative cholestasis should be classified genetically based on exome or genome sequencing, and therapy with the interruption of the enterohepatic circulation should be considered regardless of the degree of fibrosis. If inhibitors prove to be as effective as a surgical diversion in the long term, this noninvasive form of therapy would be a major improvement in the quality of life of these patients. However, potential side effects, especially of higher bile acid exposure to the colonic mucosa and microbiome, will need to be in focus and warrant further evaluation.

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CONFLICTS OF INTEREST

Ulrich Baumann is a consultant for, advises for, is on the speakers' bureau, and received grants from Mirum Pharmaceuticals and Albireo Pharmaceuticals. He consults for, advises, and received grants from Alexion. He consults for and advises Vivet Pharma and Nestle. Eva-Doreen Pfister is on the speakers' bureau and received grants from Mirum Pharma. She advises, is on the speakers' bureau, and received grants from Albireo and Alexion Pharma. She advises Univar Pharma. Amelie Stalke received grants from BMBF. Verena Keitel-Anselmino advises Astra Zeneca; she is on the speakers' bureau of Albireo, Falk, Abbvie, Gilead, CSL Behring, and Sanofi. The remaining authors have no conflicts to report.

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