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Genotype Correlates to the Natural History of Severe Bile Salt Export Pump Deficiency - Results from the NAPPED Consortium

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Abbreviations: ABCB11 (ATP-binding cassette, sub-family B member 11), ATP8B1 (ATPase class I type 8B member 1), ALT (alanine-aminotransferase), AST (aspartate-aminotransferase), ATP (Adenosine triphosphate), BSEP (bile salt export pump), DFS (diversion-free survival), GGT (gamma-glutamyltranspeptidase), HCC (hepatocellular carcinoma), IE (ileal exclusion), LTx (liver transplantation), NAPPED (NAtural course and Prognosis of PFIC and Effect of biliary Diversion), NLS (native liver survival), PEBD (partial external biliary diversion), PFIC (Progressive Familial Intrahepatic Cholestasis), PLT (platelet count), REDCap (Research Electronic Data Capture), sBA (serum bile acids), SBD (surgical biliary diversion), TJP2 (tight junction protein 2), TSB (total serum bilirubin), UDCA (ursodeoxycholic acid).

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ABSTRACT

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BACKGROUND & AIMS

Mutations in *ABCB11* can cause deficiency of the bile salt export pump (BSEP), leading to cholestasis and end-stage liver disease. The rarity of the disease has prevented determination of associations between genotype and either natural history or the effect of surgical biliary diversion (SBD) on long-term outcome (liver transplantation (LTx), hepatocellular carcinoma (HCC), death). We aimed to determine these associations by assembling the largest genetically defined cohort of severe BSEP deficiency patients to date.

METHODS

This multicentre, retrospective cohort study of patients with homozygous or compound heterozygous pathological *ABCB11* mutations included 264 patients. Patients were categorized according to genotypic severity (BSEP1, BSEP2, BSEP3). The predicted residual BSEP transport function decreased with each category.

RESULTS

Genotype severity was strongly associated with native liver survival (NLS, BSEP1 median 20.4y; BSEP2, 7.0y; BSEP3, 3.5y; *P*<.001). At age 15y, the proportion of patients with HCC was 4% in BSEP1, 7% in BSEP2 and 34% in BSEP3 (*P*=0.001). SBD was associated with significantly increased NLS (HR:0.50; 95%CI 0.27-0.94, *P*=.03) in BSEP1 and BSEP2. A serum bile acid (sBA) concentration below 102 µmol/L and a decrease in sBA by at least 75%, each shortly after SBD, reliably predicted NLS of at least 15 years after SBD (each *P*<.001).

CONCLUSIONS

The genotype of severe BSEP deficiency strongly predicts the long-term native liver survival, the risk to develop hepatocellular carcinoma, and the chance that surgical biliary diversion increases native liver survival. Serum bile acid parameters shortly after surgical biliary diversion allow the reliable identification of patients with long-term native liver survival.

Introduction

Deficiency of the bile salt export pump (BSEP) can result from mutations in the *ABCB11* gene and may lead to intrahepatic cholestasis. The most severe form of BSEP deficiency has been labelled progressive familial intrahepatic cholestasis (PFIC) type 2[1,2]. This autosomal disorder, which is (mostly) recessive in nature, belongs to the group of low gamma-glutamyl transpeptidase (GGT) cholestatic diseases[3]. BSEP is a canalicular ATP binding cassette transporter which transports conjugated bile acids into bile[4,5]. Patients with severe BSEP deficiency (i.e. PFIC due to mutations in *ABCB11)* typically present in early childhood with jaundice, pruritus, elevated serum bile acids, malabsorption and failure to thrive. Some patients respond to medical therapy such as ursodeoxycholic acid (UDCA), however, most patients progress into end-stage liver disease[6,7]. It has been observed that patients may benefit from surgical biliary diversion (SBD) procedures, such as partial external biliary diversion (PEBD) or ileal exclusion (IE)[8-17]. SBD aims to decrease the size of the bile acid pool by interrupting the enterohepatic circulation. Unfortunately, not all patients benefit from SBD and, at some point, many require a liver transplantation (LTx) for refractory pruritus or end-stage liver disease. Severe BSEP deficiency has also been associated with the development of hepatocellular carcinoma (HCC) at an early age, which by itself may necessitate LTx[8,18-20].

Severe BSEP deficiency is a rare disease (incidence estimated between 1:50.000 and 1:100.000 births[18,21]), yet relatively high incidences are reported in Saudi-Arabia (approximately 1:7200 [22]). Due to its rarity, the natural course of severe BSEP deficiency and the genotype-phenotype relationships have remained poorly

characterized, precluding clinicians from providing optimal care and counselling for patients suffering this disease.

We aimed to obtain more detailed insights in the natural history of the disease and to determine associations between genotype and phenotype. We set up a global consortium; *NAPPED* (NAtural course and Prognosis of PFIC and Effect of biliary Diversion). NAPPED aims to characterize the natural history of severe BSEP deficiency, to assess the effect of genetic, clinical and therapeutic parameters (including SBD) on major surgical and clinical events, such as native liver survival (NLS), LTx and mortality.

Patients and Methods

Data collection, patient selection and genetic categorization

Since its start in 2017, NAPPED set out to collect retrospective individual data of patients with a clinical phenotype of progressive low GGT cholestasis. The consortium currently comprises 48 tertiary referral centres from all over the globe. Data were collected conforming to the 1975 Declaration of Helsinki. Data collection used a pre-specified case-record form and was performed using REDCap[23]. Demographic, clinical, and outcome data were collected by investigators within each centre, who identified all consecutive patients who had ever been under paediatric care (age 0-18 years) since 1977. Data reported in the present manuscript were exported from REDCap on March 1, 2019. Patients with compound heterozygous or homozygous pathological *ABCB11* mutations were selected. Where available, functional studies were used in the determination of the pathogenicity of the observed mutations[24-28]. ACMG criteria were applied. Variants of unknown significance were evaluated with the Combined Annotation Dependent Depletion score; a score >25 was sufficient for inclusion in the study. Patients were excluded if genetic reports were unavailable, if they had *ABCB11* mutations of no or unknown pathogenicity, or mutations in *ATP8B1* or *TJP2* (Figure 1). Included patients were further categorized based on their predicted mildest mutation, as estimated from functional *in vitro* and/or genetic *in silico* data, if available[2,18,20,24,26,29]. Patients were categorized as BSEP1 (at least one p.D482G (c.1445A>G) or p.E297G (c.890A>G) mutation; two common European mutations associated with residual BSEP functionality[26], BSEP2 (at least one missense mutation, not p.D482G or p.E297G) or BSEP3 (mutations leading to a predicted, non-functional protein). Biochemistry at presentation in the tertiary centre, as well as most recent before SBD

(pre-SBD) and at least two months after SBD (post-SBD, maximally one year after SBD) were collected. Parameters were converted to standardized units. Pruritus was scored as ‘absent’, ‘mild to moderate’ or ‘severe’, at the discretion of the centre, what, for statistical purposes, was dichotomized into ‘absent’ or ‘present’. Effect of SBD on pruritus was noted as ‘no improvement in pruritus’, ‘transient (partial or complete) relief of pruritus’ and ‘sustained (partial or complete) relief of pruritus’. Outcome parameters were diversion-free survival (DFS, years between birth and SBD, last visit, LTx or death) and native liver survival (NLS, years between birth and either LTx or death, whichever occurred first, or last visit). Follow-up ended at last known visit, LTx or death.

Statistical evaluation

Continuous variables are expressed as medians [interquartile range] (IQR). Data were analysed using appropriate methods, including Mann-Whitney and Kruskal-Wallis tests. Categorical variables were analysed using Chi-square, McNemar or Mantel’s trend-test. We included sex, birth year, age at presentation in the tertiary centre, use of medical therapy prior to presentation (ursodeoxycholic acid (UDCA), rifampicin, phenobarbital, cholestyramine, antihistamines), sBA, total serum bilirubin (TSB), alanine aminotransferase (ALT), aspartate aminotransferase (AST), GGT and platelet count (PLT). Unadjusted differences in DFS, NLS and hepatocellular carcinoma (HCC) between subgroups were assessed using Kaplan-Meier estimates and compared using the log-rank test. Time-dependent Cox-regression was applied for studying the association between SBD and NLS in patients with BSEP1 or BSEP2 genotypes (hazard ratios [HR] and 95% confidence intervals [CI]). The genotype (BSEP1 or BSEP2), sex, birth year and SBD as a time-dependent factor for NLS

were included in the multivariate model. Patients with a BSEP3 genotype were excluded due to low numbers. The model was extended with additional factors one by one to assess the association with the endpoint. Sensitivity analyses (including geographic region and caseload) were performed to control for heterogeneity between sites. A clock-reset approach was used to visualize the association of the time-dependent risk of SBD with NLS: all patients start without SBD. Then, patients that underwent SBD during follow-up are censored at the age of SBD and restart with a new risk in the SBD curve. Receiver Operating Characteristic (ROC) curve analysis was used to determine the cut-off for post-SBD sBAs in relation to predicting NLS after SBD. Imputation of missing data was not attempted.

A two-sided P-value <0.05 was considered statistically significant. All analyses were performed using IBM SPSS Statistics 23.0 (Armonk, NY). Figures were constructed using Prism 7.02, GraphPad Software, La Jolla, CA, USA.

Results

Baseline data

At time of data export, NAPPED had 590 patients in its database. This study included 264 patients with compound heterozygous or homozygous disease associated mutations in *ABCB11* with a phenotype of low-GGT cholestasis. Of these patients, 84 had been described in previously published literature (Table S1). Three hundred twenty-six patients were excluded since mutations did not meet inclusion criteria (n=222) or genetic analysis results lacked (n=104) (Figure 1). Eleven patients previously presented with a BRIC2 phenotype (i.e. episodic cholestasis and/or pruritus and transient hepatocellular damage). These patients later presented with severe BSEP deficiency phenotypes (i.e. continuous cholestasis and/or pruritus and continuous hepatocellular damage) and had pathological mutations. Within BSEP1, two homozygous subgroups were identified: 17 patients with p.E297G mutations and 11 patients with p.D482G mutations. The final 264 included patients were followed at European (number of patients, n=202), Middle Eastern (n=25) and Asian (n=37) centres. Table S1 provides genetic profiles and corresponding genotype severity allocations. Table S2 provides the predicted effects of all mutations observed on a single allele (n=194).

The median birth year was 2004 [1995–2012]. Half of the patients were male (125/252, 47%). Age at presentation in the tertiary referral centre was 0.7 [0.2-1.9] years. Prior to presentation, 46% patients used or had ever used UDCA (Table 1). Follow-up ended at LTx, death or last visit. The median follow-up was 4.1 [1.5-12.3] years. During follow-up, 61 patients had undergone SBD and 120 patients had undergone LTx. In total, sixteen patients (6%, BSEP1 n=3/72 (4%), BSEP2 n=8/136

(6%), BSEP3 n=5/56 (9%) died prior to LTx (age 1.6 [1.1-3.5] years). Deaths were all related to liver disease. At 18 years of age, 32% of patients were alive with native liver. During adulthood (age ≥18 years), five patients underwent LTx (aged 19.6-27.5 years).

Associations between genetic severity category and baseline characteristics

Table 1 depicts patient characteristics and biochemistry at presentation in the referral centre for BSEP1 (n=72), BSEP2 (n=136) and BSEP3 (n=56). The birth year and year of first visit were earlier in BSEP1 than in BSEP2 or BSEP3 (BSEP1 vs. BSEP2, BSEP1 vs. BSEP3, *P*<.001). Biochemistry at presentation did not differ significantly. ALT and AST, however, tended to be higher in BSEP3. Table S3 shows baseline data of BSEP1 patients with homozygous p.E297G or p.D482G mutations. p.D482G patients presented later in hospital (2.9y vs. 0.2y, *P*<.001), with lower ALT levels (66 vs. 231 U/L, *P*=.01), compared to p.E297G. Patients with homozygous p.E297G or p.D482G mutations presented with lower sBA and ALT levels compared to patients with compound heterozygous mutations, although statistical significance was not reached in either of these variables. Patients with compound heterozygous p.E297G mutations presented at younger age than patients with homozygous p.E297G mutations (0.2 [0.2-0.6]y vs. 0.6 [0.3-3.8]y, respectively, *P*=.02, Table S4, S5). Patients with compound heterozygous p.D482G/p.E297G mutations (n=3) presented at a median age of 1.2 years.

Genetic severity category is associated with diversion-free survival and with survival with native liver

BSEP1 patients had better long-term outcomes than BSEP2 or BSEP3 patients: a median NLS of 20.4 years, versus 7.0 and 3.5 years, respectively (BSEP1 vs. BSEP2 *P*=0.009; BSEP1 vs. BSEP3 *P*<0.001; BSEP2 vs. BSEP3 *P*=0.02; Figure 2). SBD was more often performed in BSEP1, as opposed to BSEP2 and BSEP3 (*P*<.001, % of patients with SBD at 15y: 74%, 38% and 28% respectively; BSEP1 vs. BSEP2 *P*<0.001, BSEP1 vs. BSEP3 *P*=0.004, BSEP2 vs. BSEP3 *P*=0.90, Figure 2). Patients with homozygous p.D482G mutations had comparable NLS to that of the homozygous p.E297G patients (NLS at 15y: 73% vs. 69% respectively; *P*=.41, Figure S1), despite undergoing SBD less often (% with SBD at 15y: 26% vs. 90%; *P*=0.006, Figure S1). Compound heterozygous p.D482G patients underwent SBD more often compared to homozygous p.D482G patients (% with SBD at 15y: 91% vs. 26% respectively; *P*=.01, Figure S2). Each of the three patients with compound heterozygous p.D482G/p.E297G mutations underwent SBD (at 1.4, 8.9 and 9.4 years of age). None underwent LTx at a median age at last follow-up of 17.8y.

Association between the severity of genotype and occurrence of hepatocellular carcinoma

Severe BSEP deficiency is associated with a significant risk of HCC[19], which was confirmed in our cohort. The incidence of HCC increased with the genotype severity: at 15 years of age, the observed incidence increased from 4% in BSEP1, to 7% and even to 34% in BSEP2 and BSEP3, respectively (derived from the survival curve, Figure 3, *P*=.001). HCC occurred in 2/61 (3%) patients that underwent SBD and in 13/180 (7%) that did not (*P*=.32). In patients with homozygous p.E297G and

p.D482G mutations, HCC was observed in 6% (one patient) and 0%, respectively (*P*=.41). HCC was not observed in the three patients with compound heterozygous p.D482G/p.E297G mutations

Surgical biliary diversion: baseline data

Median age at time of SBD was 2.3 [1.2-4.7] years (n=61). Of these patients, 47 underwent PEBD, 13 underwent IE and one underwent gallbladder-colic diversion. The observed proportion of patients with an SBD at 5, 10 and 18 years of age was 29%, 37% and 47%, respectively (derived from the diversion-free survival curve). One patient received an SBD during adulthood (age 25.6 years). Follow-up after SBD was 8.4 [1.6-12.0] years. The diversion was surgically closed in 6 patients (BSEP1 n=2, BSEP2 n=3, BSEP3 n=1) at 2.0 [0.1-4.0] years after SBD. LTx followed closure in 5/6 patients, 6.2 [0.8-10.2] years after initial SBD. LTx was performed in 18 (30%) of the 61 patients at 2.4 [1.3-10.0] years after SBD.

Prior to SBD, pruritus was present in 36 (97%) of the 37 patients for whom paired data was available pre- and post-SBD. After SBD, 17 patients (46%) experienced pruritus (*P*<.001). The improvement of pruritus post-SBD was semi-quantified: in 12/41 patients (29%), no improvement of pruritus was reported, whereas 7/41 (17%) had transient partial or complete relief of pruritus and 22/41 patients (54%) had sustained partial or complete relief of pruritus. BSEP1 patients achieved sustained partial or complete relief of pruritus (18/27, 66%) more often compared to BSEP2 (4/11, 36%) and BSEP3 (0/3, 0%) (*P*=.002, Table S6).

SBD was associated with a decrease in sBAs (363 [254-452] to 48 [4-258] µmol/L,

median 90% decrease, *P*<.001), TSB (59 [21-129] to 15 [9-51] µmol/L; median 56% decrease, *P*<.001), ALT (117 [64-247] to 63 [22-111] U/L; median 54% decrease, *P*<.001) and AST (165 [91-358] to 83 [92-176] U/L, median 45% decrease, *P*=.002) (Figure 4). Post-SBD sBAs were significantly lower in BSEP1, compared to BSEP2 (27 [3-210] vs. 249 [43-332] µmol/L, *P*=0.02).

Relationship between surgical biliary diversion and native liver survival

Time-dependent Cox regression analysis (corrected for genotype severity, sex and birth year) showed that SBD was associated with significantly higher NLS (HR 0.50; 95%CI 0.27-0.94, *P*=.03, Figure 5) in BSEP1 and BSEP2. Time-dependent Cox-regression for BSEP1 and BSEP2 separately yielded HRs of 0.42 (95%CI 0.16-1.07) and 0.64 (95%CIs 0.22-1.82), respectively (difference between HRs: *P*=0.57). To assess the model's robustness and the impact of centre heterogeneity on outcome, we performed sensitivity analyses, stratifying for geographical region and caseload of the centres. Neither region, nor the caseload significantly impacted outcomes (HRs 0.32-0.62). Including BSEP3 patients in the original model yielded comparable results (n=3 added, HR 0.51; 95% CI 0.29-0.91, *P*=0.02).

Follow-up after diversion

NLS after SBD significantly decreased with genotype severity (*P*=.002-0.03, Figure S3). Since sBAs likely play an important role in hepatocellular damage, we performed ROC analyses on post-surgical sBA levels in relation to NLS. A post-SBD sBA level <102 µmol/L was associated with prolonged NLS after SBD (Figure 6; *P*<.001, AUC sBAs: 0.778; cut-off 102µmol/L: sensitivity 80%, specificity 75%). Additionally, a decrease of at least 75% in sBAs was associated with improved NLS after SBD

(Figure 6; *P*<.001; AUC %change sBAs: 0.774; cut-off 75%: sensitivity 73%, specificity 78%).

Figure 5, as stated before, shows NLS up to and after SBD. The NLS after SBD (dotted line) declines rapidly until 2.5 years. Patients with NLS ≥2.5 years after SBD harboured more often BSEP1 mutations compared with patients with NLS <2.5 years after SBD (proportion BSEP1: 71% vs. 31% respectively, *P*=.001, Table S7), providing further support for SBD being able to improve NLS in BSEP1 (and BSEP2) patients. Pre-SBD ALT levels were significantly higher in patients with short NLS (160 vs. 101 IU/L, *P*=.005). Post-SBD sBAs in patients with prolonged NLS were significantly lower than in patients with shorter NLS (sBA 29 [3-214] vs. 259 [39-297] respectively (*P*=.03), as were TSB levels (9 [8-19] µmol/L vs. 41 [12-73] µmol/L respectively, *P*=.02).

Discussion

Our aim was to characterize the natural history in severe BSEP deficiency, to determine genotype/phenotype associations and to assess the effect of genetic, clinical and therapeutic parameters on clinical outcome. We succeeded to assemble the largest genetically defined cohort of severe BSEP deficiency patients to date. Our data indicate that the majority of severe BSEP deficiency patients undergo an LTx before reaching adulthood. SBD, however, can postpone or obviate the need for transplantation in selected patients. The probability of undergoing SBD, the NLS, the incidence of HCC and follow-up after SBD were all associated with predicted genotype severity.

To study genotype-phenotype relations we categorized patients based on their predicted mildest mutation. We categorized patients harbouring at least one copy of p.D482G or p.E297G as the mildest genotype (i.e. BSEP1); these mutations are associated with less severe disease[8,20,26,29]. Patients within BSEP3 (i.e. the most severe category) harboured mutations known or predicted to lead to a non-functional protein or to absent BSEP expression. BSEP2 patients harboured at least one missense mutation (yet not p.D482G or p.E297G). While these groups showed distinct courses of disease, we suggest that BSEP2 needs further sub-characterization. A large number of different mutations in this category with 136 patients exists. Studying patients with mutations leading to residual BSEP transport activity[30] other than p.D482G and p.E297G could be one of these initiatives. As stated in the introduction, severe BSEP deficiency is an autosomal recessive disorder, belonging to low-GGT cholestatic diseases (including familial intrahepatic cholestasis protein type 1 and the tight junction protein 2 deficiencies[3]). The

recessive nature of *ABCB11* mutations can be somewhat questioned, based on heterozygous *ABCB11* mutations in patients with, for example, intrahepatic cholestasis of pregnancy[31]. This may suggest that the actual phenotype can be determined by the residual transport capacity in relation to environmental challenges.

Our data indicate that patients generally presented before their first birthday, which is consistent with previous reports[20,32]. However, it should be realized that initial presentation may occur later, even at adult age, e.g. in patients with BRIC phenotypes[29,33,34]. Interestingly, the age and biochemistry at presentation did not differ significantly between the genetic severity groups. Yet, ALT and AST levels tended to be higher in BSEP3 patients. These observations may indicate that biochemical parameters are not sensitive enough to discriminate patients according to genotype. The median year of birth was highest in BSEP3. It might be that these patients were rarely reported before, either due to early mortality or early LTx without full diagnostic work up.

HCC is rare in young children, yet severe BSEP deficiency is associated with relatively high incidences of HCC[19,20]. HCC was indeed encountered in a significant proportion of our patients; in up to 34% in patients with a BSEP3 genotype. Although already high, the here reported incidences may actually be an underestimation of the incidence during childhood or lifetime, since the present values were based on a median follow-up of 4.1 years, and data regarding the diagnosis of HCC at explant were not consistently collected. Our study, in addition to earlier reports, underlines the need to screen for HCC in all severe BSEP deficiency

patients, especially in the BSEP3 category [18].

By adulthood, only a third of severe BSEP deficiency patients were alive with their native liver, among these patients over half had undergone SBD. The relatively low NLS highlights the severity of the disease, illustrated by smaller scale studies describing that up to 50% of patients undergo LTx [20,32]. While patients with a BSEP1, BSEP2 and BSEP3 genotype are at baseline apparently clinically and biochemically indistinguishable, their natural history differed significantly. Research so far has not been able to adequately characterize large groups of patients based on genotype at all, or solely by distinguishing between patients with either one or two copies of p.D482G/p.E297G on the one hand, and patients that did not have one of these mutations on the other hand[18,20,32]. The present study was, besides categorizing patient in three genetic severity groups, able to analyse patients with homozygous p.D482G or p.E297G mutations. Our data indicate that these patients (i.e. harbouring the mildest, BSEP1 genotype) have a milder phenotype than patients not carrying either p.D482G or p.E297G. Apart from confirming earlier findings[8,20,26,29], we add that of these two, p.D482G might be the milder. Homozygous p.D482G patients present in hospital 2.8 years later, with lower ALT levels. To our knowledge, this study is the first to describe the natural history of these mutations in homozygous cohorts. Moreover, patients with homozygous mutations had a slightly favourable prognosis compared to patients with compound heterozygous p.D482G or p.E297G mutations, in regard to baseline biochemistry, proportion undergoing SBD, and NLS. A note of caution is due here since statistical significance was not reached in all variables and a centre bias cannot be ruled out.

Future studies scrutinizing the natural history in these patient groups are therefore recommended.

One of the mechanisms of action of surgical biliary diversion (SBD) involves re-targeting of BSEP to the canalicular membrane, thereby improving bile acid excretion[35]. We show that SBD is associated with increased NLS in BSEP1 and BSEP2 patients. Our data do not support to perform SBD in BSEP3 because of apparent lack of beneficial effect on long-term outcome. In BSEP1 and BSEP2, however, the interruption of the enterohepatic circulation (EHC) seemed to postpone or even remove the need for LTx. Also, BSEP1 patients were most likely to achieve long-term NLS after SBD as opposed to BSEP2 or BSEP3. Similarly, a recent, in-depth study by Bull et al. indicated that patients harbouring at least one p.D482G or p.E297G copy were less likely to undergo LTx after SBD[8].

Apart from postponing or eliminating the need for LTx, SBD aims to relieve patients of pruritus. Pruritus is regarded one of the most burdensome symptoms of severe BSEP deficiency, negatively affecting health related quality of life within the PFIC spectrum[36,37]. The efficacy of current medical antipruritogens is limited. Yet, SBD has been reported to decrease pruritus in some patients [8-17]. In agreement with earlier observations, our data show that SBD was associated with a sustained or complete improvement in pruritus, especially in BSEP1, even though a standardized itch score was absent in this retrospective cohort. None of the analysed BSEP3 patients seemed to benefit from SBD. Our present data therefore also do not support surgical interruption of the EHC as a viable and successful short-term treatment strategy for pruritus in patients with this genotype.

In our study, post-surgical sBA levels were highly prognostic of NLS. This information provides valuable information for expectations towards the need for alternative treatments after SBD (e.g. LTx) and for counselling patients and their families. Furthermore, we propose that sBA parameters could function as markers for long-term outcome after interruption of the EHC. It is interesting to explore whether also biliary bile acid concentrations could provide (more) prognostic information for long-term outcome after SBD[20,38].

Whilst SBD is helpful in a fraction of severe BSEP deficiency patients, it remains an invasive procedure with unwanted cosmetic consequences. Alternative treatments for severe BSEP deficiency are currently being developed and studied, such as medical interruption of the EHC by means of apical sodium-dependent bile acid transporter inhibitors[39,40], and chaperone drugs[30,41]. The present data do provide further support for a personalized strategy to change the natural history of the disease for the better by interrupting the EHC and/or by increasing residual function of BSEP. Especially BSEP1 or BSEP2 patients might benefit from these medical strategies, based on the current observations of SBD with respect to long-term outcome.

We are aware of the limitations of our retrospective study. Inevitably, we encountered missing data. Although this might have influenced our outcomes to some extent, we believe that our numbers are sufficient to nevertheless draw adequate and relevant conclusions. In this study, the age at first presentation was defined as the first visit in the tertiary centre. Although the first presentation was at young age, it is likely that the first symptomatic presentation of disease had been at even younger age. The

present study included eleven patients who initially presented with a BRIC-phenotype. This did not impact our main conclusions; excluding these patients from major analyses yielded comparable results. Our database does currently not include data regarding liver histology. Therefore, we could not assess if histological features at either presentation or at the time of SBD were associated with long-term outcome. The global nature of NAPPED might have resulted in a degree of selection-bias. Therefore, we performed sensitivity analyses regarding the effect of the centre (caseload, geographical region) on outcome. Our main conclusions were not affected by these potential confounders. Although LTx remains presently the most definitive treatment for severe BSEP deficiency, the disease might reoccur after transplant[42,43]. Our study design, with follow-up ending at LTx, precluded analysis of this phenomenon. Despite these limitations NAPPED offers a solution to obtain relevant clinical and follow-up data in a rare disease over an extended period of time.

In conclusion, our study shows that only a third of severe BSEP deficiency patients reach adulthood with their native liver. Moreover, the genotype severity of severe BSEP deficiency strongly predicts long-term NLS. Our results indicate that SBD is associated with significantly prolonged NLS in BSEP1 or BSEP2 patients. Based on the data provided, we propose that serum bile acids after interruption of the enterohepatic circulation can be used as a marker for long-term outcome. The present results increase the understanding and characterization of severe BSEP deficiency, allowing for improved personalized clinical care for these patients throughout childhood and into adulthood, for better targeting of novel therapeutic strategies and, finally, for an improved means to assess their effect via candidate surrogate markers. Our data indicate that that the care for patients with severe BSEP

deficiency can and should, at least partially, be based on the severity of their genotype.

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[1] Elferink RO, Groen AK. Genetic defects in hepatobiliary transport. Biochim Biophys Acta 2002;1586:129-145.

[2] Strautnieks SS, Bull LN, Knisely AS, Kocoshis SA, Dahl N, Arnell H et al. A gene encoding a liver-specific ABC transporter is mutated in progressive familial intrahepatic cholestasis. Nat Genet 1998;20:233-238.

[3] Hadzic N, Verkade HJ. The Changing Spectrum of Neonatal Hepatitis. JPediatr Gastroenterol Nutr 2016;63:316-319.

[4] Noe J, Stieger B, Meier PJ. Functional expression of the canalicular bile salt export pump of human liver. Gastroenterology 2002;123:1659-1666.

[5] Thompson R, Strautnieks S. BSEP: function and role in progressive familial intrahepatic cholestasis. Semin Liver Dis 2001;21:545-550.

[6] Shneider BL. Progressive intrahepatic cholestasis: mechanisms, diagnosis and therapy. Pediatr Transplant 2004;8:609-612.

[7] Whitington PF, Freese DK, Alonso EM, Schwarzenberg SJ, Sharp HL. Clinical and biochemical findings in progressive familial intrahepatic cholestasis. JPediatr Gastroenterol Nutr 1994;18:134-141.

[8] Bull LN, Pawlikowska L, Strautnieks S, Jankowska I, Czubkowski P, Dodge JL et al. Outcomes of surgical management of familial intrahepatic cholestasis 1 and bile salt export protein deficiencies. Hepatol Commun 2018;2:515-528.

[9] Ellinger P, Stindt J, Droge C, Sattler K, Stross C, Kluge S et al. Partial external biliary diversion in bile salt export pump deficiency: Association between outcome and mutation. World J Gastroenterol 2017;23:5295-5303.

[10] Lemoine C, Bhardwaj T, Bass LM, Superina RA. Outcomes following partial external biliary diversion in patients with progressive familial intrahepatic cholestasis. JPediatr Surg 2017;52:268-272.

[11] Ng VL, Ryckman FC, Porta G, Miura IK, de Carvalho E, Servidoni MF et al. Long-term outcome after partial external biliary diversion for intractable pruritus in patients with intrahepatic cholestasis. JPediatr Gastroenterol Nutr 2000;30:152-156.

[12] Yang H, Porte RJ, Verkade HJ, De Langen ZJ, Hulscher JB. Partial external biliary diversion in children with progressive familial intrahepatic cholestasis and Alagille disease. JPediatr Gastroenterol Nutr 2009;49:216-221.

[13] Arnell H, Bergdahl S, Papadogiannakis N, Nemeth A, Fischler B. Preoperative observations and short-term outcome after partial external biliary diversion in 13 patients with progressive familial intrahepatic cholestasis. JPediatr Surg 2008;43:1312-1320.

[14] Kurbegov AC, Setchell KD, Haas JE, Mierau GW, Narkewicz M, Bancroft JD et al. Biliary diversion for progressive familial intrahepatic cholestasis: improved liver morphology and bile acid profile. Gastroenterology 2003;125:1227-1234.

[15] Melter M, Rodeck B, Kardorff R, Hoyer PF, Petersen C, Ballauff A et al. Progressive familial intrahepatic cholestasis: partial biliary diversion normalizes serum lipids and improves growth in noncirrhotic patients. Am J Gastroenterol 2000;95:3522-3528.

[16] Emond JC, Whitington PF. Selective surgical management of progressive familial intrahepatic cholestasis (Byler's disease). J Pediatr Surg 1995;30:1635-1641.

[17] Wang KS, Tiao G, Bass LM, Hertel PM, Mogul D, Kerkar N et al. Analysis of surgical interruption of the enterohepatic circulation as a treatment for pediatric cholestasis. Hepatology 2017;65:1645-1654.

[18] Strautnieks SS, Byrne JA, Pawlikowska L, Cebecauerova D, Rayner A, Dutton L et al. Severe bile salt export pump deficiency: 82 different ABCB11 mutations in 109 families. Gastroenterology 2008;134:1203-1214.

[19] Knisely AS, Strautnieks SS, Meier Y, Stieger B, Byrne JA, Portmann BC et al. Hepatocellular carcinoma in ten children under five years of age with bile salt export pump deficiency. Hepatology 2006;44:478-486.

[20] Davit-Spraul A, Fabre M, Branchereau S, Baussan C, Gonzales E, Stieger B et al. ATP8B1 and ABCB11 analysis in 62 children with normal gamma-glutamyl transferase progressive familial intrahepatic cholestasis (PFIC): phenotypic differences between PFIC1 and PFIC2 and natural history. Hepatology 2010;51:1645-1655.

[21] Davit-Spraul A, Gonzales E, Baussan C, Jacquemin E. Progressive familial intrahepatic cholestasis. Orphanet JRare Dis 2009;4:1-1.

[22] Shagrani M, Burkholder J, Broering D, Abouelhoda M, Faquih T, El-Kalioby M et al. Genetic profiling of children with advanced cholestatic liver disease. Clin Genet 2017;92:52-61.

[23] Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research electronic data capture (REDCap)--a metadata-driven methodology and workflow process for providing translational research informatics support. JBiomed Inform 2009;42:377-381.

[24] Byrne JA, Strautnieks SS, Ihrke G, Pagani F, Knisely AS, Linton KJ et al. Missense mutations and single nucleotide polymorphisms in ABCB11 impair bile salt export pump processing and function or disrupt pre-messenger RNA splicing. Hepatology 2009;49:553-567.

[25] Noe J, Kullak-Ublick GA, Jochum W, Stieger B, Kerb R, Haberl M et al. Impaired expression and function of the bile salt export pump due to three novel ABCB11 mutations in intrahepatic cholestasis. JHepatol 2005;43:536-543.

[26] Hayashi H, Takada T, Suzuki H, Akita H, Sugiyama Y. Two common PFIC2 mutations are associated with the impaired membrane trafficking of BSEP/ABCB11. Hepatology 2005;41:916-924.

[27] Plass JR, Mol O, Heegsma J, Geuken M, de Bruin J, Elling G et al. A progressive familial intrahepatic cholestasis type 2 mutation causes an unstable, temperature-sensitive bile salt export pump. JHepatol 2004;40:24-30.

[28] Wang L, Soroka CJ, Boyer JL. The role of bile salt export pump mutations in progressive familial intrahepatic cholestasis type II. JClin Invest 2002;110:965-972.

[29] Droge C, Bonus M, Baumann U, Klindt C, Lainka E, Kathemann S et al. Sequencing of FIC1, BSEP and MDR3 in a large cohort of patients with cholestasis revealed a high number of different genetic variants. JHepatol 2017;67:1253-1264.

[30] Gonzales E, Grosse B, Schuller B, Davit-Spraul A, Conti F, Guettier C et al. Targeted pharmacotherapy in progressive familial intrahepatic cholestasis type 2: Evidence for improvement of cholestasis with 4-phenylbutyrate. Hepatology 2015;62:558-566.

[31] Dixon PH, Sambrotta M, Chambers J, Taylor-Harris P, Syngelaki A, Nicolaides K et al. An expanded role for heterozygous mutations of ABCB4, ABCB11, ATP8B1, ABCC2 and TJP2 in intrahepatic cholestasis of pregnancy. Sci Rep 2017;7:11823-x.

[32] Pawlikowska L, Strautnieks S, Jankowska I, Czubkowski P, Emerick K, Antoniou A et al. Differences in presentation and progression between severe FIC1 and BSEP deficiencies. JHepatol 2010;53:170-178.

[33] van Ooteghem NA, Klomp LW, van Berge-Henegouwen GP, Houwen RH. Benign recurrent intrahepatic cholestasis progressing to progressive familial intrahepatic cholestasis: low GGT cholestasis is a clinical continuum. JHepatol 2002;36:439-443.

[34] van Mil SW, van der Woerd, WL, van der Brugge G, Sturm E, Jansen PL, Bull LN et al. Benign recurrent intrahepatic cholestasis type 2 is caused by mutations in ABCB11. Gastroenterology 2004;127:379-384.

[35] Varma S, Revencu N, Stephenne X, Scheers I, Smets F, Beleza-Meireles A et al. Retargeting of bile salt export pump and favorable outcome in children with progressive familial intrahepatic cholestasis type 2. Hepatology 2015;62:198-206.

[36] Kamath BM, Chen Z, Romero R, Fredericks EM, Alonso EM, Arnon R et al. Quality of Life and Its Determinants in a Multicenter Cohort of Children with Alagille Syndrome. JPediatr 2015;167:390-6.e3.

[37] Baker A, Kerkar N, Todorova L, Kamath BM, Houwen RHJ. Systematic review of progressive familial intrahepatic cholestasis. Clin Res Hepatol Gastroenterol 2019;43:20-36.

[38] Emerick KM, Elias MS, Melin-Aldana H, Strautnieks S, Thompson RJ, Bull LN et al. Bile composition in Alagille Syndrome and PFIC patients having Partial External Biliary Diversion. BMC Gastroenterol 2008;8:47-47.

[39] Baghdasaryan A, Fuchs CD, Osterreicher CH, Lemberger UJ, Halilbasic E, Pahlman I et al. Inhibition of intestinal bile acid absorption improves cholestatic liver and bile duct injury in a mouse model of sclerosing cholangitis. JHepatol 2016;64:674-681.

[40] Miethke AG, Zhang W, Simmons J, Taylor AE, Shi T, Shanmukhappa SK et al. Pharmacological inhibition of apical sodium-dependent bile acid transporter changes bile composition and blocks progression of sclerosing cholangitis in multidrug resistance 2 knockout mice. Hepatology 2016;63:512-523.

[41] Gonzales E, Jacquemin E. Mutation specific drug therapy for progressive familial or benign recurrent intrahepatic cholestasis: a new tool in a near future?. JHepatol 2010;53:385-387.

[42] Stindt J, Kluge S, Droge C, Keitel V, Stross C, Baumann U et al. Bile salt export pump-reactive antibodies form a polyclonal, multi-inhibitory response in antibody-induced bile salt export pump deficiency. Hepatology 2016;63:524-537.

[43] Maggiore G, Gonzales E, Sciveres M, Redon MJ, Grosse B, Stieger B et al. Relapsing features of bile salt export pump deficiency after liver transplantation in two patients with progressive familial intrahepatic cholestasis type 2. JHepatol 2010;53:981-986.

Fig. 1. Flowchart of patient inclusion from NAtural course and Prognosis of PFIC and Effect of biliary Diversion (NAPPED) database. *:heterozygous mutations, *ABCB11* mutations of no (known) clinical consequence or mutations in *ATP8B1/TJP2*.

Table 1. Baseline characteristics in all patients, and BSEP1, BSEP2 and BSEP3 genotypes separately. *Mantel-Haenszel or Kruskal-Wallis test, as appropriate, to test differences between BSEP1, BSEP2 and BSEP3. Genotypic categorization clarified in Methods. *BSEP (bile salt export pump); GGT (gamma-glutamyl transpeptidase); UDCA (ursodeoxycholic acid).*

Fig. 2. Observed native liver survival and diversion-free survival per genotypic severity category. (A) Proportion of patients alive with native liver over time with a BSEP1 (solid line), BSEP2 (dashed line) or BSEP3 (dotted line) genotype. Log-rank test. (B) Proportion of patients with a surgical biliary diversion over time with BSEP1 (solid line), BSEP2 (dashed line) or BSEP3 (dotted line) genotypes. Genotypic categorization clarified in Methods. Log-rank test. *BSEP (bile salt export pump).*

Fig. 3. Observed proportion of patients with a hepatocellular carcinoma per genotypic severity category. Genotypic categorization clarified in Methods. Log-rank test. *BSEP (bile salt export pump).*

Fig. 4. Paired pre- and post-surgical biochemical parameters in all patients undergoing surgical biliary diversion. Kruskal-Wallis test. *ALT (alanine aminotransferase); AST (aspartate aminotransferase); SBD (surgical biliary diversion).*

Fig. 5. **Observed native liver survival in BSEP1 or BSEP2 patients undergoing surgical biliary diversion (SBD) or not.** The clock-reset approach allows visualization of native liver survival up to SBD (solid line, all patients) and after SBD (dotted line, only patients that underwent SBD). The estimated hazard ratio (HR) is achieved by Cox-regression with SBD as a time-dependent risk-factor, adjusted for genotype, sex and birth year. Patients in analysis: n=173.

Fig. 6. **Observed native liver survival after surgical biliary diversion, stratified for post-surgical serum bile acids cut-offs** (A) In patients with a post-surgical serum bile acid concentration < or ≥ 102µmol/L. (B) In patients with a relative decrease in serum bile acids of < or ≥ 75%. Log-rank test. *SBA (serum bile acids)*

Lay summary

By using the largest genetically defined cohort of severe BSEP deficiency (i.e. PFIC due to *ABCB11* mutations) patients known to date, this study increases the understanding and characterization of severe BSEP deficiency. The present data allow for improved personalized clinical care throughout childhood and into adulthood, for better targeting of novel therapeutic strategies and, for an improved means to assess their effect via candidate surrogate markers.