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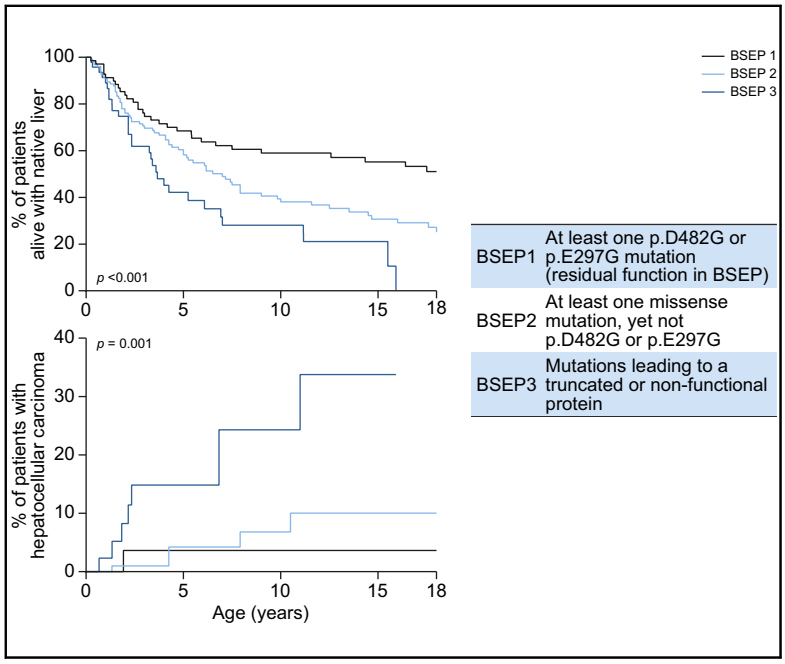
Genotype correlates with the natural history of severe bile salt export pump deficiency

Graphical abstract



Highlights

- NAPPED is the largest global database of genotyped patients with BSEP deficiency.
- The genotype of patients with BSEP deficiency predicts survival with native liver.
- Genotype predicts long-term benefit of interruption of enterohepatic circulation.
- Serum bile acids can be a surrogate marker for long-term outcome.
- Treatment of patients with BSEP deficiency should be based on genotype.

Authors

Daan B.E. van Wessel, Richard J. Thompson, Emmanuel Gonzales, …, Ton M.G. Bunt, Bettina E. Hansen, Henkjan J. Verkade

Correspondence

h.j.verkade@umcg.nl (H.J. Verkade).

Lay summary

This study presents data from the largest genetically defined cohort of patients with severe bile salt export pump deficiency to date. The genotype of patients with severe bile salt export pump deficiency is associated with clinical outcomes and the success of therapeutic interventions. Therefore, genotypic data should be used to guide personalized clinical care throughout childhood and adulthood in patients with this disease.

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Genotype correlates with the natural history of severe bile salt export pump deficiency

Daan B.E. van Wessel[1], Richard J. Thompson[2], Emmanuel Gonzales[3,4], Irena Jankowska[4,5], Etienne Sokal[4,6], Tassos Grammatikopoulos[2], Agustina Kadaristiana[2], Emmanuel Jacquemin[3], Anne Spraul[3b], Patryk Lipiński[4,5], Piotr Czubkowski[4,5], Nathalie Rock[6], Mohammad Shagrani[7,7b], Dieter Broering[7], Talal Algoufi[7], Nejat Mazhar[7], Emanuele Nicastro[8], Deirdre A. Kelly[4,9], Gabriella Nebbia[10], Henrik Arnell[4,11], Björn Fischler[4,11], Jan B.F. Hulscher[4,12], Daniele Serranti[13], Cigdem Arikan[14], Esra Polat[15], Dominique Debray[16], Florence Lacaille[16], Cristina Goncalves[4,17], Loreto Hierro[4,18], Gema Muñoz Bartolo[4,18], Yael Mozer-Glassberg[19], Amer Azaz[20], Jernej Brecelj[21], Antal Dezsőfi[22], Pier Luigi Calvo[23], Enke Grabhorn[24], Ekkehard Sturm[4,25], Wendy J. van der Woerd[26], Binita M. Kamath[27], Jian-She Wang[28], Liting Li[28], Özlem Durmaz[29], Zerrin Onal[29], Ton M.G. Bunt[1], Bettina E. Hansen[30,30b], Henkjan J. Verkade [1,4,*], and the NAtural course and Prognosis of PFIC and Effect of biliary Diversion (NAPPED) consortium

[1]Pediatric Gastroenterology and Hepatology, University Medical Centre Groningen, University of Groningen, The Netherlands; [2]King's College London, United Kingdom; [3]Service d'Hépatologie et de Transplantation Hépatique Pédiatriques, Bicêtre Hôspital, AP-HP, Université Paris-Sud, Paris Saclay, Inserm UMR-S 1174, France; [3b]Service de Biochemie, Bicêtre Hôspital, AP-HP, Université Paris-Sud, Paris Saclay, Inserm UMR-S 1174, France; [4]European Reference Network on Hepatological Diseases (ERN RARE-LIVER); [5]Gastroenterology, Hepatology, Nutritional Disorders and Paediatrics, The Children's Memorial Health Institute, Warsaw, Poland; [6]Université Catholique de Louvain, Cliniques St Luc, Brussels, Belgium; [7]Liver & SB Transplant & Hepatobiliary-Pancreatic Surgery, King Faisal Specialist Hospital & Research Centre, Riyadh, Saudi Arabia; [7b]Alfaisal University, College of Medicine, Riyadh, Saudi Arabia; [8]Pediatric Hepatology, Gastroenterology and Transplantation, Ospedale Papa Giovanni XXIII, Bergamo, Italy; [9]Liver Unit, Birmingham Women's and Children's Hospital, Birmingham, United Kingdom; [10]Servizio Di Epatologia e Nutrizione Pediatrica, Fondazione Irccs Ca' Granda Ospedale Maggiore Policlinico, Milano, Italy; [11]Pediatric Digestive Diseases, Astrid Lindgren Children's Hospital, Karolinska University Hospital, Karolinska Institutet, Stockholm, Sweden; [12]Paediatric Surgery, University Medical Centre Groningen, Groningen, The Netherlands; [13]Paediatric and Liver Unit, Meyer Children's University Hospital of Florence; [14]Koc University School of Medicine, Paediatric GI and Hepatology Liver Transplantation Centre, Kuttam System in Liver Medicine, Istanbul; Turkey; [15]Hospital Umraniye Training and Research Hospital, Istanbul; [16]Unité d'hépatologie Pédiatrique et Transplantation, Hôpital Necker, Paris, France; [17]Coimbra University Hospital Center, Coimbra, Portugal; [18]Pediatric Liver Service, La Paz University Hospital, Madrid, Spain; [19]Institute of Gastroenterology, Nutrition and Liver Diseases, Schneider Children's Medical Centre of Israel; [20]Sheikh Khalifa Medical City, Abu Dhabi, United Arab Emirates; [21]Department of Gastroenterology, Hepatology and Nutrition, University Children's Hospital Ljubljana, and Department of Paediatrics, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia; [22]1st Department of Paediatrics, Semmelweis University, Budapest, Hungary; [23]Pediatic Gastroenterology Unit, Regina Margherita Children's Hospital, Azienda Ospedaliera Città Della Salute e Della Scienza University Hospital, Torino, Italy; [24]Klinik Für Kinder- Und Jugendmedizin, Universitätsklinikum Hamburg Eppendorf, Hamburg, Germany; [25]University Children's Hospital Tübingen, Tübingen, Germany; [26]Wilhelmina Children's Hospital, University Medical Centre Utrecht, Paediatric Gastroenterology, Hepatology and Nutrition, Utrecht, The Netherlands; [27]The Hospital for Sick Children and the University of Toronto, Toronto, Canada; [28]Children's Hospital of Fudan University, Shanghai, China; [29]Istanbul University, Istanbul Faculty of Medicine, Istanbul, Turkey; [30]Toronto Centre for Liver Disease, University Health Network, Canada; [30b]IHPME, University of Toronto, Canada

Background & Aims: Mutations in *ABCB11* can cause deficiency of the bile salt export pump (BSEP), leading to cholestasis and end-stage liver disease. Owing to the rarity of the disease, the associations between genotype and natural history, or outcomes following surgical biliary diversion (SBD), remain elusive. We aimed to determine these associations by assembling the largest genetically defined cohort of patients with severe BSEP deficiency to date.

Methods: This multicentre, retrospective cohort study included 264 patients with homozygous or compound heterozygous pathological *ABCB11* mutations. 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 20.4 years; BSEP2, 7.0 years; BSEP3, 3.5 years; p <0.001). At 15 years of age, the proportion of patients with hepatocellular carcinoma was 4% in BSEP1, 7% in

Keywords: Severe BSEP deficiency; PFIC2; ABCB11; Natural history; Surgical biliary diversion.
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* Corresponding author. Address: Paediatric Gastroenterology & Hepatology, Department of Paediatrics, Beatrix Children's Hospital, University Medical Centre Groningen, University of Groningen, PO Box 30.001, 9700 RB Groningen, The Netherlands.
E-mail address: h.j.verkade@umcg.nl (H.J. Verkade).
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BSEP2 and 34% in BSEP3 (p = 0.001). SBD was associated with significantly increased NLS (hazard ratio 0.50; 95% CI 0.27–0.94: p = 0.03) in BSEP1 and BSEP2. A serum bile acid concentration below 102 μmol/L or a decrease of at least 75%, each shortly after SBD, reliably predicted NLS of ≥15 years following SBD (each p <0.001).

Conclusions: The genotype of severe BSEP deficiency strongly predicts long-term NLS, the risk of developing hepatocellular carcinoma, and the chance that SBD will increase NLS. Serum bile acid parameters shortly after SBD can predict long-term NLS.

Lay summary: This study presents data from the largest genetically defined cohort of patients with severe bile salt export pump deficiency to date. The genotype of patients with severe bile salt export pump deficiency is associated with clinical outcomes and the success of therapeutic interventions. Therefore, genotypic data should be used to guide personalized clinical care throughout childhood and adulthood in patients with this disease.

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 transferase (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 with ursodeoxycholic acid (UDCA), however, most patients progress to 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.[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 (LT) 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 LT.[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 1:7,200[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 from this disease.

We aimed to obtain more detailed insights into 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), LT and mortality.

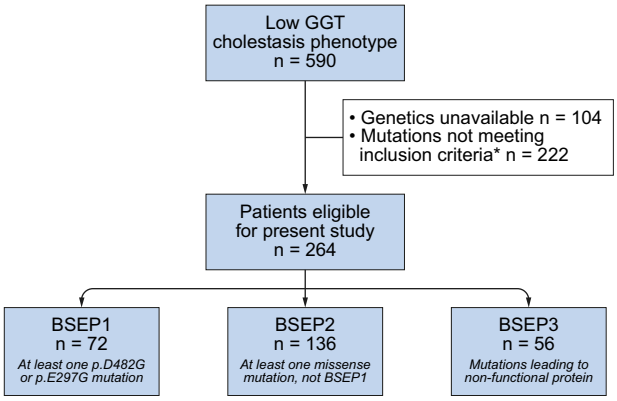

Fig. 1. Flowchart of patient inclusion from NAPPED database. *Heterozygous mutations, *ABCB11* mutations of no (known) clinical consequence or mutations in *ATP8B1/TJP2*. ABCB11, ATP-binding cassette, sub-family B member 11; ATP8B1, ATPase class I type 8B member 1; NAPPED, NAtural course and Prognosis of PFIC and Effect of biliary Diversion.

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 in accordance with 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* (Fig. 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 1 p.D482G (c.1445A>G) or p.E297G (c.890A>G) mutation; 2 common European mutations associated with residual BSEP functionality,[26] BSEP2 (at least 1 missense mutation, not p.D482G or p.E297G) or BSEP3 (mutations leading to a predicted, non-functional protein). Biochemistry data were collected at presentation in the tertiary centre, before SBD (pre-SBD) and at least 2 months after SBD (post-SBD, ≤1 year after SBD). Parameters were converted to standardized units. Pruritus was scored as 'absent', 'mild to moderate' or 'severe', at the discretion of the centre and was dichotomized into 'absent' or 'present' for statistical purposes. The 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 (years between birth and SBD, last visit, LT or death) and native liver survival (NLS, years between birth and either LT, death, or last visit, whichever occurred first). Follow-up ended at last known visit, LT or death.

Statistical evaluation

Continuous variables are expressed as medians (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 (UDCA, rifampicin, phenobarbital, cholestyramine, antihistamines), serum bile acid (sBA), total serum bilirubin (TSB), alanine aminotransferase (ALT), aspartate aminotransferase (AST), GGT and platelet count (PLT). Unadjusted differences in diversion-free survival, NLS and HCC between subgroups were assessed using Kaplan-Meier estimates and compared using the log-rank test. Time-dependent Cox regression was used to study the association between SBD and NLS in patients with BSEP1 or BSEP2 genotypes (hazard ratios [HRs] and 95% CIs). The genotype (BSEP1 or BSEP2), sex, birth year and SBD were included as time-dependent factors for NLS in the multivariate model. Patients with a BSEP3 genotype were excluded due to low numbers. The model was extended one by one to assess the association of each factor 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 best cut-off level of sBAs for the prediction of NLS after SBD. Imputation of missing data was not attempted.

A 2-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 were lacking (n = 104) (Fig. 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, 2 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, 50%). 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 LT, 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 LT. In total, 16 patients (BSEP1 n = 3/72 [4%], BSEP2 n = 8/136 [6%], BSEP3 n = 5/56 [9%]) died prior to LT (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), 5 patients underwent LT (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 <0.001). Biochemistry at presentation did not differ significantly. ALT and AST, however, tended to be higher in BSEP3. Table S3 shows baseline data of patients with BSEP1 and homozygous p.E297G or p.D482G mutations. p.D482G patients presented later (2.9 years old *vs.* 0.2 years old, p <0.001), with lower ALT levels (66 *vs.* 231 U/L, p = 0.01), 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 homozygous p.E297G mutations presented at a younger age than patients with compound heterozygous p.E297G mutations (0.2 [0.2–0.6] years old *vs.* 0.6 [0.3–3.8] years old, respectively, p = 0.02, Tables 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

Patients with BSEP1 had better long-term outcomes than those with BSEP2 or BSEP3, with a median NLS of 20.4 years, *vs.* 7.0 years and 3.5 years, respectively (BSEP1 *vs.* BSEP2 p = 0.009; BSEP1 *vs.* BSEP3 p <0.001; BSEP2 *vs.* BSEP3 p = 0.02; Fig. 2). SBD was more often performed in BSEP1, as opposed to BSEP2 and BSEP3 (p <0.001, % of patients with SBD at 15 years: 74%, 38% and 28% respectively; BSEP1 *vs.* BSEP2 p <0.001, BSEP1 *vs.* BSEP3 p = 0.004, BSEP2 *vs.* BSEP3 p = 0.90, Fig. 2). NLS was similar in patients with homozygous p.D482G or homozygous p.E297G mutations (NLS at 15 years: 73% *vs.* 69%, respectively; p = 0.41, Fig. S1), despite patients with homozygous p.D482G undergoing SBD less often (% with SBD at 15 years: 26% *vs.* 90%; p = 0.006, Fig. S1). Patients with compound heterozygous p.D482G mutations underwent SBD more often than those with homozygous p.D482G mutations (% with SBD at 15 years: 91% *vs.* 26%, respectively; p = 0.01, Fig. S2). Each of the 3 patients with compound heterozygous p.D482G/p.E297G mutations underwent SBD (at 1.4, 8.9 and 9.4 years of age). None had undergone LT at a median age at last follow-up of 17.8 years.

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, Fig. 3, p = 0.001). HCC occurred in 2/61 (3%) patients

Table 1. Baseline characteristics in all patients, and BSEP1, BSEP2 and BSEP3 genotypes separately.

		Genetic severity category BSEP deficiency			
Parameter	**Overall (n = 264)**	**BSEP 1 (n = 72)**	**BSEP 2 (n = 136)**	**BSEP 3 (n = 56)**	***p* value***
Year of birth	2004 [1995-2012]	1995 [1991-2004]	2007 [1999-2013]	2008 [2001-2012]	**<0.001**
Available n (%)	263 (99)	72 (100)	135 (99)	56 (100)	
Year of birth timeframe	1964-2018	1979-2017	1964-2018	1991-2018	–
Males, n (%)	125 (50)	33 (46)	69 (59)	23 (47)	0.77
Available n (%)	252 (95)	72 (100)	117 (86)	49(88)	
Age first presentation in referral center, year	0.7 [0.2-1.9]	0.8 [0.2-1.8]	0.7 [0.3-3.0]	0.5 [0.2-1.3]	0.49
Available n (%)	251 (95)	69 (96)	128 (94)	53 (95)	
Year first presentation in referral center	2007 [1997-2013]	1997 [1992-2007]	2010 [2003-2013]	2009 [2003-2014]	**<0.001**
Available n (%)	251 (95)	69 (96)	128 (94)	53 (95)	
Timeframe year first presentation in referral centre	1977-2018	1982-2018	1977-2018	1992-2018	–
Treated before first presentation in referral centre with:					
UDCA, n (%)	122/264 (46)	36/72 (50)	60/136 (44)	25/56 (45)	0.52
Rifampicin, n (%)	52/264 (20)	11/72 (15)	26/136(19)	14/56(25)	0.17
Phenobarbital, n (%)	16/264 (6)	6/72 (8)	7/136(5)	3/56(5)	0.45
Cholestyramine, n (%)	40/264 (15)	11/72 (15)	22/136(16)	7/56(13)	0.70
Antihistamines, n (%)	21/264 (8)	7/72 (10)	9/136(7)	5/56(9)	0.81
Laboratory data *at presentation in referral center*					
Serum bile acids, μmol/L	252 [161-363]	281 [183-448]	240 [157-339]	254 [158-341]	
Available n (%)	141 (53)	40 (55)	68 (50)	33 (59)	0.17
Total serum bilirubin, μmol/L	107 [43-162]	96 [31-156]	115 [53-187]	106 [59-145]	
Available n (%)	200 (75)	56 (77)	104 (76)	39 (70)	0.49
Alanine aminotransferase, IU/L	199 [83-386]	175 [71-366]	186 [65-351]	243 [135-466]	
Available n (%)	189 (71)	55 (76)	99 (73)	34 (61)	0.11
Aspartate aminotransferase, IU/L	242 [97-422]	243 [100-426]	222 [75-398]	314 [160-542]	
Available n (%)	169 (64)	44 (61)	92 (68)	33 (59)	0.06
Gamma-glutamyltransferase, IU/L	24 (16–36)	22 (12–36)	24 (17–37)	24 (17–35)	
Available n (%)	182 (69)	54 (75)	93 (68)	35 (63)	0.26
Platelet count, 10^9/L	384 [275-517]	412 [310-562]	351 [258-499]	374 [257-529]	
Available n (%)	176 (67)	55 (76)	84 (62)	37 (66)	0.11

A p-value <0.05 was considered statistically significant.
*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-glutamyltransferase; UDCA, ursodeoxycholic acid.

that underwent SBD and in 13/180 (7%) that did not ($p = 0.32$). In patients with homozygous p.E297G and p.D482G mutations, HCC was observed in 6% (1 patient) and 0%, respectively ($p = 0.41$). HCC was not observed in the 3 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 ileal exclusion and 1 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. LT followed closure in 5/6 patients, 6.2 (0.8–10.2) years after initial SBD. LT 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 <0.001). The improvement of pruritus post-SBD was semi-quantified: 12/41 patients (29%) had no improvement of pruritus, 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. Patients with BSEP1 achieved sustained partial or complete relief of pruritus (18/27, 66%) more often than those with BSEP2 (4/11, 36%) and BSEP3 (0/3, 0%) ($p = 0.002$, Table S6).

SBD was associated with a decrease in sBAs (363 [254–452] to 48 [4–258] μmol/L; median 90% decrease; p <0.001), TSB (59 [21–129] to 15 [9–51] μmol/L; median 56% decrease; p <0.001), ALT (117 [64–247] to 63 [22–111] U/L; median 54% decrease; p <0.001) and AST (165 [91–358] to 83 [92–176] U/L; median 45% decrease; $p = 0.002$) (Fig. 4). Post-SBD sBAs were significantly lower in patients with 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; $p = 0.03$, Fig. 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% CI 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 on outcomes (HR 0.32–0.62). Including patients with BSEP3 in the original model yielded comparable results (n = 3 added, HR 0.51; 95% CI 0.29–0.91; $p = 0.02$).

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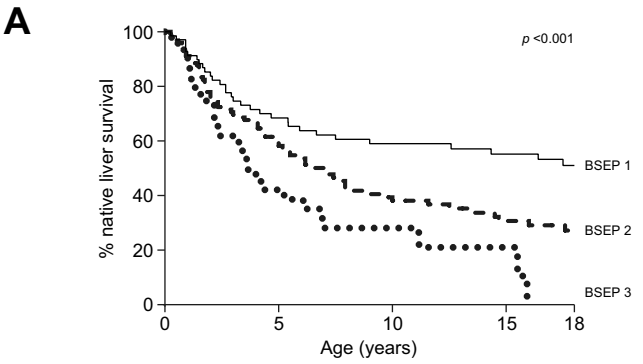
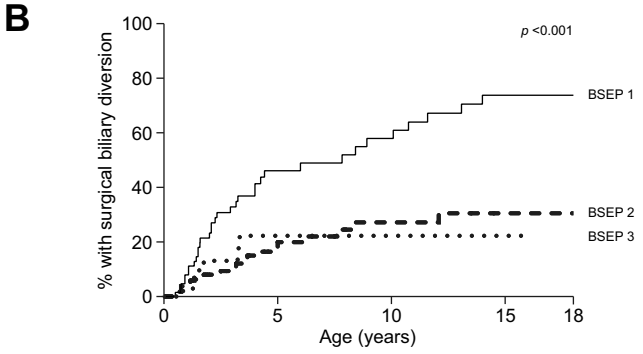
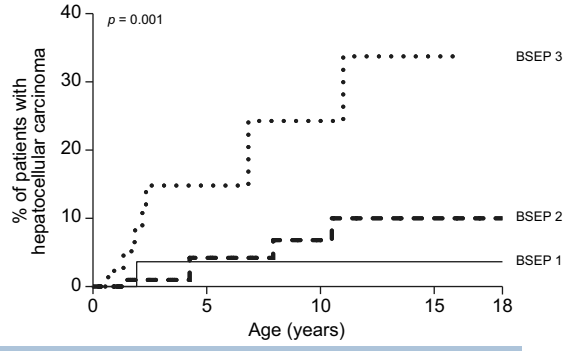


N° at risk					
BSEP 1	72	43	36	29	18
BSEP 2	136	53	31	20	12
BSEP 3	56	13	8	2	0



N° at risk					
BSEP 1	72	20	14	8	5
BSEP 2	136	43	22	16	11
BSEP 3	56	12	9	3	0

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.



N° at risk					
BSEP 1	72	43	36	29	18
BSEP 2	136	52	30	19	12
BSEP 3	56	12	8	2	0

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

Follow-up after diversion

NLS after SBD significantly decreased with genotype severity (p = 0.002–0.03, Fig. 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 (Fig. 6; p <0.001, AUC sBAs: 0.778; cut-off 102 μmol/L: sensitivity 80%, specificity 75%). Additionally, a decrease of at least 75% in sBAs was associated with improved NLS after SBD (Fig. 6; p <0.001; AUC % change sBAs 0.774; cut-off 75%: sensitivity 73%; specificity 78%).

Fig. 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 more often had BSEP1 mutations compared to patients with NLS <2.5 years after SBD (proportion BSEP1: 71% *vs.* 31%, respectively; p = 0.001, Table S7), providing further evidence that SBD improves NLS in patients with BSEP1 (and BSEP2). Pre-SBD ALT levels were significantly higher in patients with short NLS (160 *vs.* 101 IU/L, p = 0.005). Post-SBD sBAs were significantly lower in patients with prolonged NLS than in patients with shorter NLS (sBA 29 [3–214] *vs.* 259 [39–297], respectively; p = 0.03), as were TSB levels (9 [8–19] μmol/L *vs.* 41 [12–73] μmol/L, respectively; p = 0.02).

Discussion

Our aim was to characterize the natural history of severe BSEP deficiency, to determine genotype/phenotype associations and to assess the effect of genetic, clinical and therapeutic parameters on clinical outcome. We succeeded in assembling the largest genetically defined cohort of patients with severe BSEP deficiency to date. Our data indicate that the majority of patients with severe BSEP deficiency undergo an LT 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 1 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. Patients with BSEP2 harboured at least 1 missense mutation (not p.D482G or p.E297G). While these groups showed distinct courses of disease, we suggest that BSEP2 needs further sub-characterization; many different mutations existed among the 136 patients in this group. 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

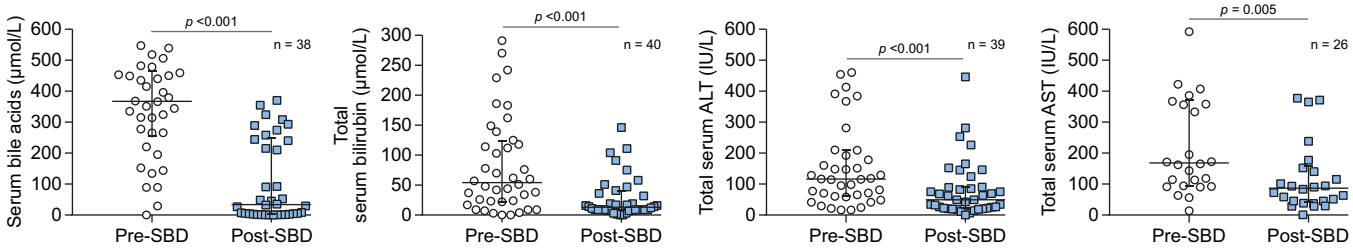


Fig. 4. Paired pre- and post-surgical biochemical parameters in all patients undergoing surgical biliary diversion. Wilcoxon signed-rank test. ALT, alanine aminotransferase; AST, aspartate aminotransferase; SBD, surgical biliary diversion.

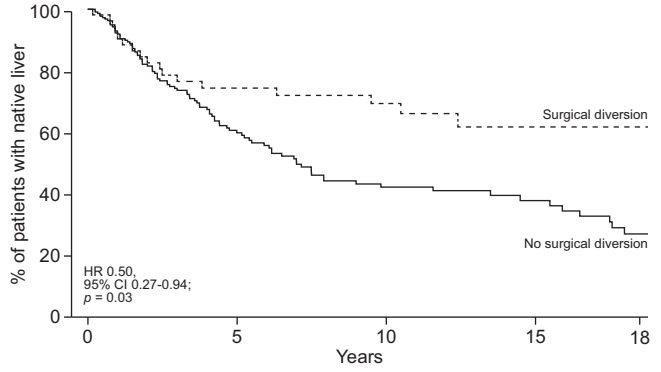
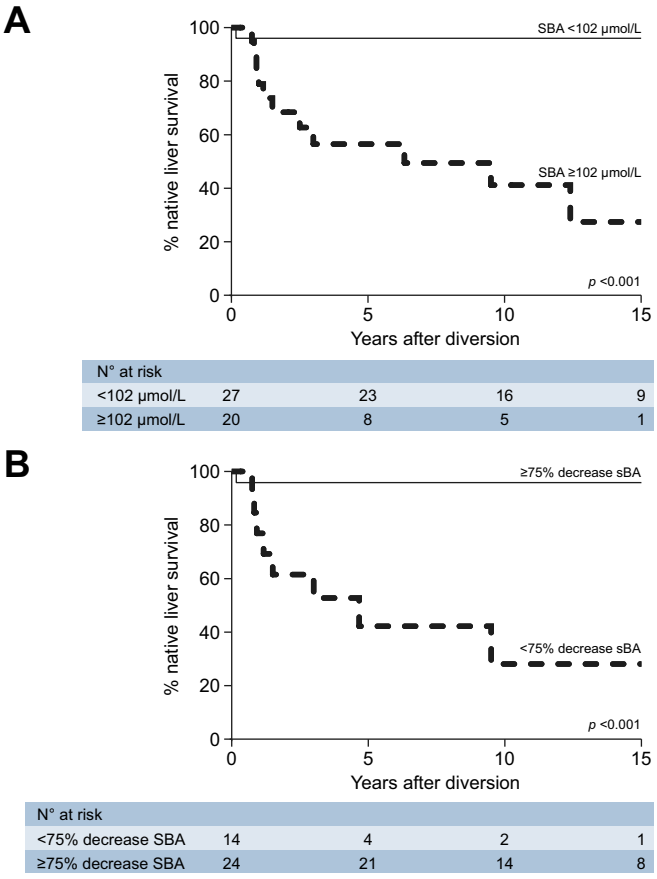


Fig. 5. Observed native liver survival in BSEP1 or BSEP2 patients undergoing SBD or not. The clock-reset approach allows visualization of native liver survival up to SBD (solid line, all patients) and after SBD (dotted line, only patients that underwent SBD). The estimated HR is achieved by Cox regression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



N° at risk				
<102 μmol/L	27	23	16	9
≥102 μmol/L	20	8	5	1

N° at risk				
<75% decrease SBA	14	4	2	1
≥75% decrease SBA	24	21	14	8

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

reports.[20,32] However, it should be noted that initial presentation may occur later, even at adulthood, *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 patients with BSEP3. These observations may indicate that biochemical parameters are not sensitive enough to discriminate between patients according to genotype. The median year of birth was highest for BSEP3. It might be that these patients were rarely reported previously, either due to early mortality or early LT 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 herein 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 patients with severe BSEP deficiency, especially in the BSEP3 category.[18]

By adulthood, only a third of patients with severe BSEP deficiency 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 reporting LT in up to 50% of patients.[20,32] While patients with a BSEP1, BSEP2 and BSEP3 genotype are apparently clinically and biochemically indistinguishable at baseline, their natural history differed significantly. To date, research has not been able to adequately characterize large groups of patients based on genotype, or solely by distinguishing between patients with or without copies of p.D482G/p.E297G.[18,20,32] Besides categorizing patients into 3 genetic severity groups, we were 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 p.D482G might be

associated with a milder phenotype. Patients who are homozygous for p.D482G 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, regarding baseline biochemistry, the proportion undergoing SBD, and NLS. A note of caution is warranted as 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 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 patients with BSEP1/2. Our data do not support performing SBD in BSEP3 because of apparent lack of a beneficial effect on long-term outcomes. In BSEP1 and BSEP2, however, the interruption of the enterohepatic circulation (EHC) seemed to postpone or even remove the need for LT. Also, patients with BSEP1 were most likely to achieve long-term NLS after SBD. Similarly, a recent, in-depth study by Bull *et al.* indicated that patients harbouring at least 1 copy of p.D482G or p.E297G were less likely to undergo LT after SBD.[8]

Apart from postponing or eliminating the need for LT, SBD aims to relieve patients of pruritus. Pruritus is regarded as 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 patients with BSEP3 seemed to benefit from SBD. Therefore, our present data 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 guiding expectations towards the need for alternative treatments after SBD (*e.g.* LT) and for counselling patients and their families. Furthermore, we propose that sBA parameters could function as markers of long-term outcomes after interruption of the EHC. It would be interesting to explore whether biliary bile acid concentrations could also provide (more) prognostic information regarding long-term outcomes after SBD.[20,38]

Whilst SBD is helpful in a fraction of patients with severe BSEP deficiency, 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 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 the residual function of BSEP. Patients with BSEP1 or BSEP2 might particularly benefit from these medical strategies, based on the current observations.

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 a young age, it is likely that the first symptomatic presentation of disease had been at an even younger age. The present study included 11 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 not include currently 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 LT remains the most definitive treatment for severe BSEP deficiency, the disease might reoccur after transplant.[42,43] Our study design, with follow-up ending at LT, 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 patients with BSEP1 or BSEP2. Based on the data provided, we propose that sBA levels after interruption of the EHC 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 the care for patients with severe BSEP deficiency can and should, at least partially, be based on the severity of their genotype.

Abbreviations

ABCB11, ATP-binding cassette, sub-family B member 11; ATP8B1, ATPase class I type 8B member 1; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BSEP, bile salt export pump; GGT, gamma-glutamyltransferase; HCC, hepatocellular carcinoma; HR, hazard ratio; LT, 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; sBAs, serum bile acids; SBD, surgical biliary diversion; TJP2, tight junction protein 2; TSB, total serum bilirubin; UDCA, ursodeoxycholic acid.

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Conflicts of interest

Daan B.E. van Wessel: [nothing to disclose], Richard J. Thompson [consultancy for Shire, Albireo, Mirum, Horizon Pharmaceuticals, Sana Biotechnology, GenerationBio, Retrophin and Qing Bile Therapeutics], Emmanuel M. Gonzales [Consultant for CTRS and Mirum Pharmaceuticals], Irena Jankowska [nothing to disclose], Tassos Grammatikopoulos [nothing to disclose], Agustina Kadaristiana [nothing to disclose], Patryk Lipiński [nothing to disclose], Piotr Czubkowski [nothing to disclose], Nathalie Rock [nothing to disclose] Emmanuel Jacquemin [nothing to disclose], Anne Spraul [nothing to disclose], Etienne M. Sokal [founder and CSMO of Promethera Biosciences], Mohammad Shagrani [nothing to disclose], Dieter Broering [nothing to disclose], Talal Algoufi [nothing to disclose], Nejat Mazhar [nothing to disclose], Emanuele Nicastro [nothing to disclose] Deirdre Kelly [Consultant for Albireo], Gabriela Nebbia [nothing to disclose], Henrik Arnell [consultant for Albireo and Mirum Pharmaceuticals], Björn Fischler [has attended one advisory board meeting with Albireo in 2016], Jan Hulscher [nothing to disclose], Daniele Serranti [nothing to disclose], Cigdem Arikan [nothing to disclose], Esra Polat [nothing to disclose], Dominique Debray [consultant for Alexion pharmaceuticals], Florence Lacaille [nothing to disclose], Cristina Goncalves [nothing to disclose], Loreto Hierro [nothing to disclose], Gema Muñoz Bartolo [nothing to disclose], Yael Mozer-Glassberg [nothing to disclose], Amer Azaz [nothing to disclose], Jernej Brecelj [nothing to disclose], Antal Dezsőfi [nothing to disclose], Pier Luigi Calvo [nothing to disclose], Enke Grabhorn [nothing to disclose], Ekkehard Sturm [nothing to disclose] Wendy van der Woerd [nothing to disclose], Binita Kamath [consultant for Mirum Pharmaceuticals, Shire and DCI], Jian-She Wang [nothing to disclose], Liting Li [nothing to disclose], Özlem Durmaz [nothing to disclose], Zerrin Onal [nothing to disclose], Ton Bunt [nothing to disclose], Bettina Hansen [consultant for Mirum Pharmaceuticals, Albireo AB, Chemomab, Calliditas, Intercept, Cyma Bay, unrestricted grants from Cyma bay, Intercept, Mirum and Albireo], Henkjan J. Verkade [Consultant for Danone/Nutricia Research, Ausnutria BV, Albireo AB, GMP+Orphan, Mirum Pharmaceuticals, Intercept and Vivet].

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

Daan van Wessel: study concept and design, acquisition of data, analysis and interpretation of data, statistical analysis, drafting of the manuscript, obtained funding; Richard Thompson: study concept and design, acquisition of data, critical revision of the manuscript for important intellectual content; Emmanuel Gonzales: study concept and design, acquisition of data, critical revision of the manuscript for important intellectual content; Irena Jankowska: study concept and design, acquisition of data, critical revision of the manuscript for important intellectual content; Etienne Sokal: study concept and design, acquisition of data, critical revision of the manuscript for important intellectual content; Tassos Grammatikopoulos: acquisition of data, critical revision of the manuscript for important intellectual content; Agustina Kadaristiana: acquisition of data, critical revision of the manuscript for important intellectual content; Emmanuel Jacquemin: critical revision of the manuscript for important intellectual content; Anne Spraul: acquisition of data, critical revision of the manuscript for important intellectual content; Patryk Lipiński: acquisition of data, critical revision of the manuscript for important intellectual content; Piotr Czubkowski: acquisition of data, critical revision of the manuscript for important intellectual content; Nathalie Rock: acquisition of data, critical revision of the manuscript for important intellectual content; Mohammad Shagrani: acquisition of data, critical revision of the manuscript for important intellectual content; Dieter Broering: acquisition of data, critical revision of the manuscript for important intellectual content; Talal Algoufi: acquisition of data, critical revision of the manuscript for important intellectual content; Nejat Mazhar: acquisition of data, critical revision of the manuscript for important intellectual content; Emanuele Nicastro: acquisition of data, critical revision of the manuscript for important intellectual content; Deirdre Kelly: acquisition of data, critical revision of the manuscript for important intellectual content; 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Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhep.2020.02.007.

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Author names in bold designate shared co-first authorship

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