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ORIGINAL ARTICLE

Disease burden and management of Crigler-Najjar syndrome: Report of a world registry

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Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; CB, conjugated bilirubin; CNS, Crigler-Najjar syndrome; GGT, gamma glutamyl transpeptidase; PB, phenobarbital; PT, phototherapy; TB, total bilirubin; UCB, unconjugated bilirubin; UGT1A1, uridine 5′-diphosphate glucuronosyltransferase; ULN, upper limit of normal.

Sem J. Aronson and Norman Junge contributed equally

[Correction added on 26 May 2022, after first online publication: Sem J. Aronson and Norman Junge contributed equally - first authorship statement has been added.]

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Abstract

Background and Aims: Crigler-Najjar syndrome (CNS) is a disorder of bilirubin conjugation leading to brain damage and death without treatment. Although cohort studies of limited size have been published, uncertainty about outcome, co-morbidities, occurence of liver fibrosis and treatment outcome remains. With this worldwide cohort study, we aim to add substantial knowledge to the previously published data.

Methods: Anonymized retrospective data of CNS patients were collected in a web-based registry platform.

Results: Clinical data of 221 CNS patients (46% female, severe phenotype $n = 209$) were collected. At the time of analysis, 59 CNS patients were deceased. Mean serum total bilirubin (TB) was 300 μmoL/L, mean conjugated bilirubin (CB) 14.4 μmoL/L. The incidence of neurologic symptoms was 10.7%. Elevated alanine aminotransferase (ALT, >70 U/L) was present in 43/221 (19.5%) and the mean gamma-glutamyl transpeptidase (GGT) was 54 U/L. CB and TB did not correlate with aspartate aminotransferase (AST), ALT or GGT. TB was higher in males than in females (316 vs. 287 μmol/L; $p < .001$). Cholelithiasis was detected in 14 of 91 ultrasound examinations. In 5 of 31 CNS patients with liver fibrosis evaluation advanced fibrosis and significant higher AST were detected. Liver transplantation (LT) was performed in 26 CNS patients at the median age of 9 years (0–32 years). A subgroup of 75 CNS patients showed high TB (444 ± 141 μmoL/L) and high mortality/morbidity because of inadequate access to treatment.

Conclusions: This largest cohort of CNS patients to date shows the urgent need to globally expand access to therapy and gives insight into the clinical course and outcome of different treatment strategies. However, some questions need ongoing examination, especially regarding liver fibrosis and the timing of LT.

KEYWORDS
encephalopathy, liver transplantation, phototherapy, UGT1A1, unconjugated hyperbilirubinemia

1 | INTRODUCTION

Crigler-Najjar syndrome (CNS; OMIM 218800) is an ultra-rare (<1/1 million newborns) autosomal recessive monogenic disorder of bilirubin conjugation that results in the accumulation of neurotoxic unconjugated bilirubin (UCB).[1] Accumulation of UCB in CNS is caused by uridine 5′-diphosphate glucuronyltransferase (UGT1A1) deficiency, a non-secreted intracellular enzyme, mainly located in hepatocytes, which catalyses the conjugation of UCB with sugar moieties.[2] Elevations in levels of UCB up to ~300 μmol/L can lead to bilirubin-induced encephalopathy (kernicterus) with a wide range of irreversible neurological consequences.[3]

Current management aims to prevent bilirubin-induced encephalopathy by reducing serum UCB. Until the discovery of phototherapy (PT),[4] which consists of blue light with a wavelength of 475–478 nm and produces water-soluble photo isomers of UCB, all severely affected patients without residual UGT1A1 activity died soon after birth.[1] Optimization of the PT units made it possible to prevent brain damage and to keep patients alive, until liver transplantation (LT). A registry published 25 years ago revealed orthotopic LT and daily PT as the two main treatment methods.[5] Patients with some degree of residual UGT1A1 activity can be treated with phenobarbital (PB) to induce the expression of the residual UGT1A1.[6]

Epidemiological evidence revealed that patients with CNS carry a substantial burden, in spite of the advancement in the standard treatments and care.[7]

Although earlier cohort studies have been published[5,8] relevant questions remain about CNS associated comorbidities such as cholecystolithiasis and liver fibrosis, regional differences in treatment outcome and optimal timing of LT. Therefore, we aimed with this worldwide cohort study to add substantial knowledge about these aspects.

Lay Summary

Crigler-Najjar syndrome implies a high burden for affected patients because of the necessity of lifelong phototherapy and/or liver transplantation. Additionally, our worldwide registry revealed large regional differences in morbidity and mortality rates because of inequal availability of treatment, emphasizing a high unmet medical need to improve access to therapy. Analysis of our registry containing data of 221 patients futher showed that liver fibrosis developed in some patients and that total bilirubin in males was higher than in females.

2 | METHODS

To collect retrospective data of CNS patients worldwide a Good Clinical Practice (GCP) and General Data Protection Regulation (GDPR) compliant web-based registry platform were developed (commercial platform Castor EDC®; https://castoredc.com). For data management, we used a pseudonymization procedure by which personally identifiable information fields within the data record are replaced by a pseudonym under the control of the local PI. The ethical committee of the Amsterdam University Medical Center (AUMC) reviewed and approved the study and the registry platform. Physicians from 16 centres (13 countries) contributed data after obtaining approval from local ethical committees and obtaining informed consent of patients. The study was performed in accordance with the principles of the Declaration of Helsinki. Data collection took place from September 2016 up to the final analysis in January 2020, inclusion of additional sites and patients is still ongoing.

At the time of inclusion, serial lifetime retrospective data of each patient were captured in the registry. Data on patient characteristics (including medical history, genetic diagnosis, clinical features, blood values, fibroscan, imaging), treatment and outcome were included. An overview of the registry structure is included in the Table S1. Included data of blood values were measured during treatment, as far as treatment was available for the individual patient. In the manuscript text, the number of patients of which the data were available for analysis was specified. The registry was developed for sequential updates of the cohort to facilitate a 5-yearly evaluation. The registry will not be used to collect prospective data. For liver-transplanted patients, data after LT were analysed separately for LT-outcome.

Serum liver enzymes are expressed in U/L and bilirubin in μmol/L. Upper limits of normal (ULN) are based on average ULN for standard techniques in European laboratories. Liver fibrosis was evaluated either from liver biopsies and explanted livers by local liver pathologists or by transient elastography (Fibroscan®), which was performed according to common guidelines.[9] We interpreted a Fibroscan result ≥8 kPa as a sign of significant fibrosis (ISHAK Fibrosis score ≥3; Metavir Fibrosis Score ≥2).[10] In case of differences between Fibroscan and liver histology results, liver histology was leading.

Sufficient PT is defined as daily PT with special blue light fluorescent light tubes or high-intensity light-emitting diodes (LED) (emission 400–525 [peak 450–460] nm) for 6–12 h with an irradiance at the skin surface of at least 40 μW/cm^2 ·nm, up to 100 or more and a body exposition of at least 35%–50% (skin lamp distance should be 30–60 cm) resulting in total bilirubin (TB) ≤20 mg/dl or 340 μmoL/L. White bed sheets and/or reflective material around the bed should be used. Fluorescent light tubes have to be tested and changed regularly (every 4 weeks to 4 months). In case of increased TB, it is more effective to add a PT-Slot during daytime than further expanded nighttime-slot because PT efficiency becomes weaker over treatment time (UCB needs to recirculate into the capillaries).

The registry is still open and patient inclusion is ongoing and follow-up data of included patients are continuously updated if available.

2.1 | Statistical analysis

Data are presented as frequency (% of total cohort), median (interquartile range, IQR), or mean (±standard deviation, SD). After checking for normal distribution analysis, the independent *t*-test for the comparison of parametric variables between two groups was used, unless stated otherwise. For nonparametric variables (normal distribution not given) we performed a Mann–Whitney test. $p < .05$ was considered significant. For statistical analyses, we used SPSS Statistics v25 software (IBM).

3 | RESULTS

3.1 | Cohort description

At the time of analysis, the CNS World Registry contained retrospective data of 221 CNS patients (46% females; Cohort description, Table 1). Clinical data were mainly collected from North Africa $n = 96$ (43.4%), Europe $n = 85$ (38.5%), North America $n = 21$ (9.5%), and Middle-East/Asia $n = 19$ (8.6%) (Figure S1 "World Map"). All patients were diagnosed based on persistent high levels of UCB in serum and 83.7% by genetic analysis. The median age of the patients was 8 (IQR 4–19, maximum 68) years. At the time of analysis, 59 patients (26.7%) were deceased.

3.2 | Clinical characteristics

The clinical characteristics of all patients are shown in Table 2. The average serum TB level in this cohort was 299.5 ± 121.2 μmol/L (ULN 17 μmol/L) and conjugated bilirubin (CB) level 14.4 ± 13.3 μmol/L (ULN 5.1 μmol/L). TB and CB were significantly correlated ($p < .001$). From 198/221 patients (90.0%), serum bilirubin levels

TABLE 1 Cohort description

Patients included	$n = 221$
North Africa	96 (43.4%)
Europe	85 (38.5%)
North America	21 (9.5%)
Middle-East/Asia	19 (8.6%)
Genetic diagnosis	185 (83.7%)
Allele frequency (most common ones)	
c.1070A>G (p.Gln357Arg)[a]	39.9%
c.222C>A (p.Tyr74Ter)[b]	7.1%
c.1220delA (p.Lys407Argfs*5)[c]	4.9%
c.1489delG (p.Ala497Profs*4)[c]	3.4%
c.1381T>C (p.Trp461Arg)[a]	2.2%
Consanguinity	96 (43.3%)
First-degree	47 (21.3%)
Second-degree	38 (17.2%)
Third-degree	11 (5.0%)
Male	120 (54.3%)
Age [years]	8 (4–19)
Deceased	59 (26.7%)

Note: Data presented as frequency (% of the total cohort) or median (IQR).

Abbreviation: IQR, interquartile range.

[a]Missense variant.

[b]Nonsense variant.

[c]Deletion variant.

TABLE 2 Clinical and biochemical characteristics

Serum total bilirubin [μmol/L]	299.5 ± 121.2 (17.6 × ULN)
Direct bilirubin [μmol/L]	14.4 ± 13.3 (2.8 × ULN)
ALT [U/L]	62.5 ± 41.8 (~1.8 × ULN)
AST [U/L]	51.9 ± 33.1 (~1.2 × ULN)
GGT [U/L]	54.3 ± 99.3 (~1.4 × ULN)
ALP [U/L]	214.8 ± 141.9 (~1.8 ULN)
Co-morbidity	33 (14.9%)
Gallstone disease	14 (6.3%)
Malignancy	2 (0.9%)
Abdominal ultrasound	91 (41.2%)
Gallstones or cholecystic sludge	14/91 (15.4%)
Portal hypertension	9/91 (9.9%)
Bile duct dilatation	3/91 (3.3%)
Fibroscan available	18/221 (8.1%)
>8 kPa	5/18 (27.8%)
Liver histology available	18/221 (8.1%)
Fibrosis/cirrhosis (ISHAK ≥3)	3/18 (16.6%)
Total amount of fibrosis	5/31 (16.1%)

Note: Data presented as frequency (% of the total cohort), median (IQR) or mean ± SD (range).

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma glutamyl transpeptidase; IQR, interquartile range; SD, standard deviation; ULN, upper limit normal.

were available. The amount of data points per patient strongly varies from 1 to 90. In 111/198 patients (66%), we collected at least three data points. The serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were increased with 62.5 ± 41.8 U/L for ALT and 51.9 ± 33.1 U/L for AST, ULN is age- and gender-dependent around 35 and 45 U/L respectively.[11] From 113/221 patients (51.1%), liver enzymes (ALT/AST) and cholestatic markers (alkaline phosphatase [ALP]/gamma-glutamyl transpeptidase [GGT]) were available. Elevated ALT >2 × ULN or >70 U/L was present in 43/113 (38.1%). Serum levels of GGT 54.3 ± 99.3 U/L (ULN 38–55 U/L) and ALP 214.8 ± 141.9 U/L (ULN 120 U/L) were both elevated, although the high standard deviation confirms a large variability within this cohort. ALT (Figure 1; $p = .543$, R^2 0.003), AST and GGT (data not shown) did not correlate with TB levels. Furthermore, also CB did not correlate with AST, ALT or GGT. The TB level in males, 316.0 ± 125.3 μmol/L, was significantly higher than in females 286.6 ± 116.6 μmol/L (Table 3, $p = .0003$). Phenobarbital treatment was not associated with higher AST or ALT but with higher GGT ($p = .013$).

The most frequently reported comorbidity for longtime survivors was cholelithiasis, defined as symptomatic or asymptomatic gallstones or gallbladder sludge, which was identified in 6.3% of all patients, and seen in 15.4% ($n = 14$) of patients with documented abdominal ultrasound ($n = 91$) (Table 2).

In some of the older patients skin problems were reported as a result of the intensive PT such as skin xerosis, lichenification or hyperpigmentation ($n = 8$) or even erythematous skin rash ($n = 3$). One patient developed skin melanoma, but the relation with the use of PT remains uncertain.

3.3 | Liver fibrosis

In 31 patients, transient elastography (Fibroscan) data ($n = 18$) and/or liver histology ($n = 18$) from liver biopsies ($n = 16$) or liver explant ($n = 2$) were available (Table 2). In 5 of 18 patients Fibroscan result was >8 kPa (widely accepted as cut off for advanced fibrosis [ISHAK Fibrosis Score ≥3]). Median Fibroscan result was 7.1 kPa with IQR 1.0. In two patients with a Fibroscan result >8 kPa, liver biopsy was performed and confirmed fibrosis, in one it did not. In 3 of 18 patients advanced fibrosis or cirrhosis was detected based on liver histology (ISHAK Fibrosis Score ≥3). The two liver explant samples were from LTs in CNS patients below 2 years and do not show any fibrosis. Overall, five CNS patients out of 31 (16%) showed signs of advanced liver fibrosis (two by histology + fibroscan, one by histology, two by fibroscan). In sub analyses including patients dependent on PT and with an age of ≥7 years at evaluation 5 out of 15 patients had liver fibrosis (33%). That means all patients with fibrosis had severe phenotypes and were at least 7 years of age at analysis. The age

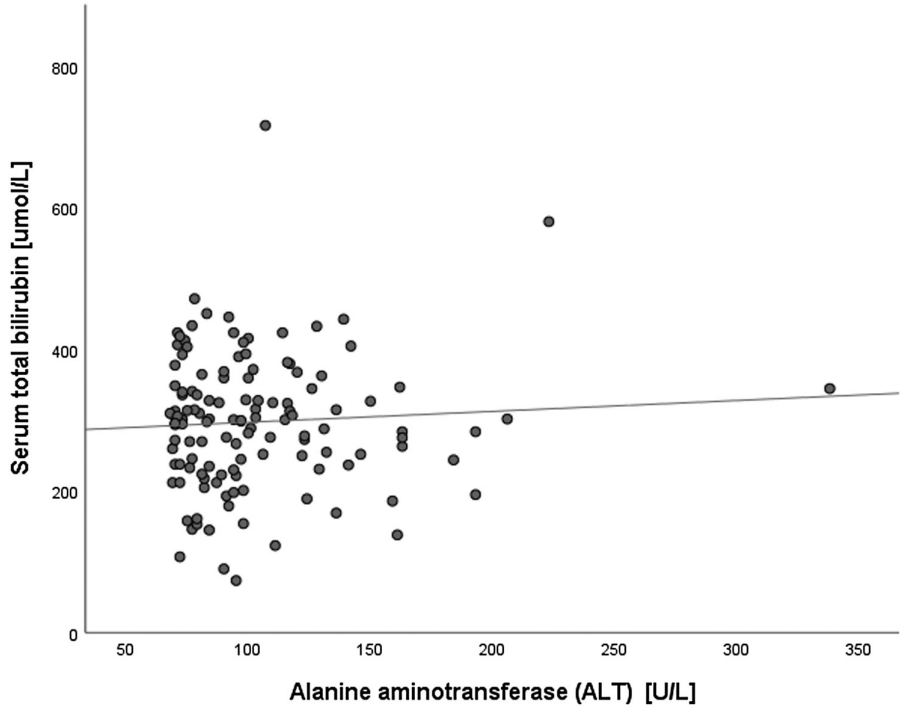

FIGURE 1 Correlation ALT and TB. Elevated alanine aminotransferase (ALT) (>2 × ULN) does not correlate with serum total bilirubin levels ($p = .543$, R^2 0.003). ALT, alanine aminotransferase; TB, total bilirubin

TABLE 3 Gender differences for bilirubin, AST, ALT, GGT, ALP

	Total	Male	Female	M vs. F *p*
Serum total bilirubin [μmol/L]	299.5 ± 121.2 (17.6 × ULN)	316.0 ± 125.3 (18.6 × ULN)	286.6 ± 116.6 (16.9 × ULN)	<.001
Direct bilirubin [μmol/L]	14.4 ± 13.3 (2.8 × ULN)	16.4 ± 14.1 (3.2 × ULN)	12.9 ± 12.6 (2.5 × ULN)	.001
ALT [U/L]	62.5 ± 41.8 (~1.8 × ULN)	64.3 ± 45.6 (~1.4 × ULN)	60.7 ± 37.6 (~1.8 × ULN)	.379
AST [U/L]	51.9 ± 33.1 (~1.2 × ULN)	54.7 ± 40.8 (~1.6 × ULN)	49.0 ± 22.4 (~1.6 × ULN)	.078
GGT [U/L]	54.3 ± 99.3 (~1.4 × ULN)	63.5 ± 129.2 (~1.2 × ULN)	45.1 ± 53.9 (~1.2 × ULN)	.085
ALP [U/L]	214.8 ± 141.9 (~1.8 × LN)	222.7 ± 153.2 (~1.7 × ULN)	207.3 ± 130.6 (~2.0 × ULN)	.354

Note: Data presented as mean ± SD.

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma glutamyl transpeptidase; SD, standard deviation; ULN, upper limit normal.

of the patients at fibrosis evaluation did not differ between those with or without, however, AST was significantly higher in the fibrosis group ($p = .048$). Further results are shown in Table 4. Three of the five patients with fibrosis had ultrasound-proven gallstones.

3.4 | Treatment strategies and outcomes

The vast majority of patients was reported to receive treatment (95.9%) aiming to reduce UCB and prevent bilirubin-induced encephalopathy (Table 5; Figure 2). A small fraction of patients remained untreated (3.2%) or it is unknown if they were treated (<1%).

Phototherapy was the most abundantly used treatment (Table 5), either as a monotherapy ($n = 132$, 59.7%) or in combination with PB ($n = 37$, 16.7%). Amongst patients treated with PT alone, two subgroups were identified with distinctly different treatment outcomes. A subgroup of patients ($n = 75$) requiring PT, but with inadequate access to equipment of sufficient quality, maintained high TB levels (443.6 ± 141.4 μmol/L) and showed high morbidity with 42% of patients with neurologic symptoms (generalized muscle hypertonia and dystonia, hypotonia, cerebellar dysfunctions, dysphasia) and high mortality rates (62% deceased at a median age of 4 years). These patients were identified first by the fact that they had TB levels above the targeted limit and second by living in a similar region. On the contrary, within the group of patients ($n = 57$) that received lifelong high standard PT based on existing recommendations[12] (daily exposure 9.3 ± 2.3 h), TB was adequately reduced to 298.3 ± 90.3 μmol/L and the majority survived (92.9%), most of them reaching adulthood

TABLE 4 Thirty-one patients with available liver fibrosis analysis

	(A) Patients with liver fibrosis[a] (all patients are >7 years and PT dependent)	(B) Patients without fibrosis	(C) Patients without fibrosis and PT dependent	(D) Patients without fibrosis, PT dependent and age >7	Mann–Whitney-*U*-Test A vs. B/A vs. C/A vs. D *p*
Number of patients	*n* = 5	*n* = 26	*n* = 16	*n* = 10	
Age (mean/median/95% CI)	21.40/25.00/11.25–31.55	24.00/20.50/15.71–32.29	15.06/16.00/7.87–22.26	23.20/23.00/15.88–30.52	.648/.172/.854
Sex	3 female (60%)	11 female (42%)	8 female (47%)	7 female (70%)	
AST	51.20/04.00/26.85–75.55 (*n* = 5)	33.10/25.50/25.41–40.79 (*n* = 20)	39.50/41.0/26.66–52.34 (*n* = 10)	39.11/39.00/24.51–53.71 (*n* = 9)	.048/.269/.256
ALT	65.80/70.00/35.93–95.67 (*n* = 5)	47.05/41.00/36.63–60.47 (*n* = 20)	53.90/46.50/34.09–73.71 (*n* = 10)	50.22/46.00/29.74–70.71 (*n* = 9)	.153/.391/.257
GGT	81.60/35.00/−24.95 to 188.15 (*n* = 5)	48.15/27.00/26.39–69.91 (*n* = 20)	57.00/44.50/23.15–90.85 (*n* = 10)	57.89/40.00/19.38–96.40 (*n* = 9)	.377/.806/.739
Total bilirubin	322.60/305.00/203.82–441.38 (*n* = 5)	268.77/244.00/211.97–325.58 (*n* = 25)	341.22/328/271.56–410.99 (*n* = 15)	335.70/345.50/266.26–405.14 (*n* = 10)	.254/.827/.806
Conjugated bilirubin	12.67/13.00/1.47–23.87 (*n* = 3)	18.49/14.75/13.15–43.81 (*n* = 22)	19.63/16.00/14.00–25.25 (*n* = 14)	21.99/15.00/13.76–30.02 (*n* = 9)	.558/.230/.164
Body-mass-index (kg/m^2)	21.20/19.82/16.48–25.91 (*n* = 5)	21.66/20.69/18.57–24.75 (*n* = 16)	21.97/20.28/18.13–25.81 (*n* = 13)	23.96/25.06/18.86–29.07 (*n* = 9)	.804/.882/.641

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma glutamyl transpeptidase; PT, phototherapy.

[a]Liver fibrosis diagnosed by liver histology ISHAK Fibrosis Score ≥3 or Fibroscan ≥8 kPa.

TABLE 5 Treatment and outcome

	Number (% of cohort)	Serum total bilirubin [μmol/L]	Neurologic symptoms	Deceased	Age at death
Phenobarbital (PB) (monotherapy)	15 (6.8%)	146 ± 77.6 (8.6 × ULN)	0/15	0	n.a.
Phototherapy (PT) (monotherapy)	132 (59.7%)	n.a.	n.a.	n.a.	n.a.
Indicated but insufficient access	75 (34%)	443.6 ± 141.4 (26.1 × ULN)	32/75 (42.7%)	47 (62.7%)	4.0 (0–11)
Sufficient access with average daily exposure to PT of 9.3 ± 2.3 [h]	57 (26%)	298.3 ± 90.3 (17.6 × ULN)	5/56 (8.9%)	4 (7.1%)	14.0 (1–31)
Combination therapy PB and PT with daily exposure to PT of 9.6 ± 2.9 [h]	37 (16.7%)	289.4 ± 119.5 (17.0 × ULN)	5/37 (13.5%)	0	n.a.
Liver transplantation	26 (11.8%)	20.3 ± 26.0 (1.2 ULN)	3/26 (11.5%)	6 (23.0%)	10.0 (0–23)

Note: Data presented as frequency (%), mean ± SD or median (spread).

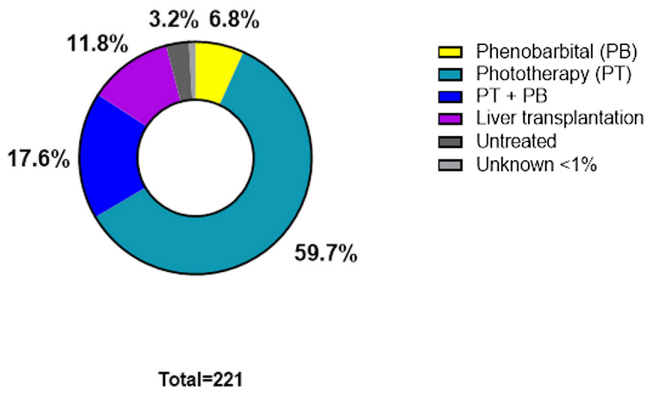

FIGURE 2 Treatment strategies. Data presented as percentage of the complete cohort (%). Unknown <1%

without signs of bilirubin encephalopathy. Of the four patients in this group who passed away, only in one case the cause of death could be directly attributed to CNS (neurotoxicity).

A subgroup of patients was treated with a combination of PT and PB (n = 37, 16.7%). When comparing the outcome with the group that received only PT, the daily exposure time and serum TB levels were similar (Table 5). The incidence of neurologic symptoms (13.5 vs. 8.9%) tended to be higher for PT + PB than PT alone (Table 5).

PB monotherapy was used to treat a small group of patients in the described cohort (n = 15, 6.8%). The vast majority of this group comprises patients with a less severe phenotype (n = 12, 80%), formerly called CNS type 2, with some residual UGT1A1 activity, explaining their lower average serum TB levels (146 ± 77.6 μmol/L).

The most frequently reported co-medication in the whole cohort was ursodeoxycholic acid in 32 patients (14.5%).

Twenty-six patients (11.8% of the cohort, 12.4% of patients with severe phenotype) underwent LT, which resulted in a marked reduction of serum TB (20.3 ± 26.0 μmol/L). The median age at LT was 9 years, ranging from 0 to 32 years of age. Procedure-related complications (n = 13) were reported in 6/11 patients (unknown in 15 patients). The most common complications were stenosis of the hepatic artery (n = 4) and stenosis of the biliary anastomosis (n = 3), leading to a total of three re-LTs. Six of the 26 LT patients are deceased at the time of this analysis, only two of them are related to CNS (severe encephalopathy). A detailed description of the LT patients is given in Table S2.

4 | DISCUSSION

Crigler-Najjar syndrome requires lifelong treatment to prevent lethal brain damage. Even though several cohort studies have been published, additional insight into the natural course of the disease, incidence of complications and outcome of different treatment strategies remains valuable. We aimed to address some of the remaining questions by reporting data from the largest multicenter cohort study of CNS patients (n = 221) to date. The worldwide nature of our study made it possible to show regional differences in disease occurrence, genetic background and treatment outcome, all relevant to improve the care for CNS patients.

4.1 | Genetic analyses

Sequencing of the *UGT1A1* gene in 185 patients (83.7% of the cohort) revealed several frequently occurring pathogenic variants (Table 1) pointing towards a significant founder effect. The most striking being the p.Gln357Arg (c.1070A>G) reported in Tunisia, a variant that appeared 32 generations ago.[13] This founder effect and the frequent consanguineous marriages that occur in Tunisia explain the higher prevalence of eight CNS patients per million inhabitants in this country compared to the frequency of <1 per million in Western Europe. Indeed, consanguineous unions range between 25% in Central Tunisia[14] to 60% in rural areas.[15] Additional frequently found variants that have been previously reported were identified, such as the p.Tyr74* (c.222C>A) variant seen in the Amish population[8,16] and the p.Lys407Argfs*5 (c.1220delA) variant seen in the South-Western region of the Netherlands.[17]

4.2 | Blood values

Mildly elevated liver tests (ALT, AST, GGT, AP) are frequently identified in CNS patients. More than a third of CNS patients were detected to have elevated ALT >70 U/L or >2 × ULN. We could show that serum levels of AST, ALT and GGT were neither correlated with TB nor with CB. But AST, and in a subgroup also ALT, were associated with advanced fibrosis. We found significant higher TB (and CB) blood levels in male CNS patients compared to female CNS patients. However, higher UCB levels in males are reported for a disease with slightly reduced UGT1A1 activity (Gilbert syndrome) for a long time already. First, this phenomenon was described in rats[18] and thereafter in a U.S. population study[19] and a large Italian cohort of patients with Gilbert syndrome.[20] For CNS patients, we describe this sex difference for the first time. In Gilbert syndrome this difference is explained by the influence of sex steroids on UGT1A1 activity.[18,21,22] In CNS, the difference cannot fully be explained by this mechanism since in most patients, it is impossible to increase UGT1A1 activity, which is shown by failed treatment with PB. However, gender differences for TB in healthy adults are described and higher total heme content in males compared to females may partially contribute to this since bilirubin is the major metabolic product of heme.[23,24] Though the gender difference which is described in healthy adults[23,24] is smaller than in our cohort.

4.3 | Cholelithiasis

A high prevalence of cholelithiasis has been reported in patients with severe CNS.[8] UCB in bile can promote gallstone formation, for instance as a nucleating factor for cholesterol gallstones as indicated by their central pigment-protein nidus.[25] In normal bile, the amount of UCB is very low but in the bile of patients treated by PT, water-soluble UCB photo isomers are excreted into the bile. These can partially revert back into UCB that can form crystals inducing gallstone formation. Whilst the prevalence of gallstone disease reported in our cohort (6.3% overall; 15.4% in patients with documented ultrasound) is significantly higher compared to the estimated prevalence in the normal population (0.2%–1.9%),[26–28] it is not nearly as high as reported previously for CNS (43%).[12] Reasons for this relatively lower prevalence in our cohort could be under-reporting or the limited use of ultrasound screening, but also the preemptive use of ursodeoxycholic acid (14.5%) in some regions. The use of PB as an activator of the constitutive androstane receptor could also prevent gallstones and could have a protective effect since it leads to a shift in the bile acid synthesis.[29]

4.4 | Liver fibrosis

Liver fibrosis in CNS patients is an increasingly observed issue[30] even though in relevant rodent models liver fibrosis has not been observed.[31] Our analyses on fibrosis is limited by the small number of patients with fibrosis evaluation (n = 31 overall and n = 15 severe phenotypes and age ≥7). We found in 31 patients with liver fibrosis evaluation (liver histology or Fibroscan) five patients (16%) with signs of advanced liver fibrosis. Only higher AST was associated with fibrosis. Except differences for body-mass-index (as an indicator for steatohepatitis), we cannot exclude all other causes of fibrosis in these five patients. Since liver fibrosis is only described in patients with the severe phenotype and development of fibrosis is unlikely in early childhood we further focused on patients with these characteristics (PT dependent and ≥7 years of age). This led to the prevalence of fibrosis in 33% (5/15) of this sub-analysis. This is still less than described by Mitchell et al.[30] However, this study is based on a cohort with low genetic diversity compared to our cohort with profound genetic diversity. The causes for liver fibrosis in CNS patients are still unclear. Even though CNS is not a cholestatic disease based on primary pathophysiology and textbook knowledge it is increasingly recognized, that liver histology shows cholestatic-like aspects and that prevalence of cholecystolithiasis and sludge is higher than in the general population. Furthermore, elevated GGT and even CB elevation is observed in a substantial amount of CNS patients. Therefore, one cause for liver fibrosis could be subclinical cholestasis. But neither CB nor GGT was associated with fibrosis or with each other. Additionally, the increased CB levels could be caused by interference with the high levels of UCB (falsely high) and not by cholestasis. UCB covalently bound to albumin (delta-bilirubin) or bilirubin photo isomers, or a combination of both may result in an overestimation of CB levels.[32] This is supported by the observation that CB did not correlate with AST, ALT or GGT in our cohort and that CB was significantly correlated with TB and showed the same sex difference (significant higher levels in male patients).

Another cause for liver fibrosis could be a direct hepatotoxic effect of UCB mediated by photo isomers or by PT products. This has not been reported so far,[33,34] although in vitro studies have shown that high levels of UCB impair mitochondrial membranes and these could play a role in both brain and liver damage.[35] If this hypothesis holds true, higher UCB or TB levels should correlate with AST/ALT or fibrosis, but we did not find such a correlation (Figure 1) and thus, our data cannot support this mechanism.

A recent study[36] showed in a mouse model with reduced UGT1A1 activity that the liver is more vulnerable and that UCB leads to an increase in inflammatory markers and upregulates the activity of Kupffer and stellate cells. This is an important point that should be verified in the CNS mouse or rat model. However, the results of this study are contrary to a large amount of studies that showed that UCB has a substantial anti-inflammatory and antioxidative effect.[37–39] One could argue that not the bilirubin itself is protective but the enzyme heme oxygenase-1, which is responsible for bilirubin formation, and which has been shown to have an antifibrogenic effect, harbours this function.[40–42] However, Tang et al.[43] showed that cultured hepatic stellate cells (HSC), if exposed to bilirubin, have reduced inflammatory activation. Activated HSC is known to be involved in liver fibrosis. Lanone et al.[44] could even

show in Gunn rats (the primary CNS animal model) that they are more resistant to endotoxin-induced hypotension and death than non-hyperbilirubinemia rats.

That in our small subcohort of fibrosis evaluated patients only patient with PT showed fibrosis is most likely caused because of the more severe phenotype in these patients but an association between long-term PT use and development of fibrosis cannot be excluded based on our data.

All these controversial and unclear aspects display the importance of liver fibrosis evaluation in the standard care of CNS patients. Better detection and characterizing of liver fibrosis in CNS patients is necessary to gain an understanding of aetiology, to identify patients at risk, to develop individual treatment plans (e.g. LT vs. gene therapy in the future) and to probably find a way to avoid the development of fibrosis. It may be necessary to also evaluate patients with a milder phenotype who are not PT dependent for liver fibrosis. Furthermore, it could be helpful to test CNS patients for additional genetic modulators (e.g. heterozygote pathogenic variants in genes for CFTR [Cystic Fibrosis Transmembrane Conductance Regulator] or MDR3 [multidrug resistance protein 3]) by whole genome or exome sequencing more frequently in future.

4.5 | Natural course of the disease

Of the seven CNS patients firstly described in 1952, six died in early childhood from bilirubin-induced encephalopathy (kernicterus).[1] Since the introduction of PT and the progress in the field of LT, CNS patients can now reach adulthood without brain damage. But still, a significant subset of patients ($n = 75$) requiring PT had inadequate access to treatment, because of a multitude of factors (geographic factors, such as great distance between home and hospital in rural areas; economic factors; system of health care and health insurance). This resulted in high serum TB levels and in extremely high mortality and morbidity rates, which could be underestimated because of missing data on neurologic symptoms in at least 33 cases and loss of follow up in 10 cases. The likely sad reality is that the subset of patients with no access to therapy represents the natural history of untreated CNS, which is associated with a life expectancy of 4 years (median age at death). This study is the first study, which can clearly show the extraordinary regional differences of treatment quality in CNS and its devastating consequences. The differences are not explained by delayed diagnosis (the symptom icterus is obvious and the indication for PT is not dependent on the diagnosis of CNS) or general knowledge gaps, but instead by mundane factors like poverty, poor access to electricity (for PT), limited availability of home PT systems and inadequate medical infrastructure to support LT. This underlines the need to eliminate these socioeconomic barriers or get high-quality PT units into resource-limited settings to improve the overall outcome for CNS patients worldwide. Furthermore, new treatment approaches should be aware of these problems and should target to overcome these barriers.

4.6 | Treatment and outcome, still much to improve

CNS patients are dependent on treatment, whether this is oral PB, daily PT or LT. The clinical management of CNS patients in the United States is well described[8] and has been adopted (and adapted) in most countries. Recent detailed studies evaluating the efficacy of PT provided valuable guidelines for optimal treatment.[12]

4.6.1 | Lifelong PT has a favourable outcome

A group of patients ($n = 57$) in our cohort received life-long high standard PT, which adequately reduced TB levels to <300 μmol/L. With a survival rate of >92% and a significant number of patients that currently live in their fourth decade without bilirubin encephalopathy, this treatment option has shown to be safe and effective. In five cases, neurologic symptoms of bilirubin encephalopathy did arise and four patients died with at least one death that can be directly attributed to CNS (bilirubin encephalopathy). Although there is a concern about the loss of efficacy of PT over time because of skin lichenification and decreasing body surface area-to-weight ratio,[5] the outcomes of long-term PT are favourable. However, daily PT with a mean exposure time of 9.3 h per day has a major impact on the quality of life of patients. This represents a heavy burden, requiring dedicated compliance of the patient and families. In some patients, the life-long PT was associated with skin damage (12.8%) and in one patient developed skin cancer (melanoma) that has not been described previously in CNS. A causal relationship between long-term PT and skin cancer cannot be established at this time, but in older units, UV light was not completely absent. The complete absence of UV light in the current PT light sources, such as LED, makes the increased risk for skin cancer unlikely, though LED eye protection is important.

4.6.2 | The combination of phototherapy with phenobarbital might not benefit patients without residual UGT1A1 activity

Phenobarbital increases the transcription of the *UGT1A1* gene which results in higher *UGT1A1* mRNA and protein levels in the liver and therefore lowers the serum bilirubin levels in patients with residual UGT1A1 activity. Patients that lack residual UGT1A1 activity have no benefit of PB treatment additionally to their PT. Therefore the use of PB is not recommended in these patients.

4.6.3 | Liver transplantation, high overall survival, but are we underestimating procedure-related complications?

Besides a uniform strong rationale to perform LT before the onset of irreversible bilirubin encephalopathy and when TB levels rise to

dangerous levels (>400 μmol/L) despite optimal PT, other factors such as availability of donor livers or country-specific local expertise and differences in management guidelines lead to the variability of LT timing. Within our cohort, 26 patients received LT that was curative in all patients with available follow-up. An overview of these 26 patients is given in Table S2. Procedure-related complications were reported in 6/11 patients (unknown in 15 patients). Six patients are deceased at the time of analysis, but none of them were related to LT or immune suppressive therapy. However, a complication rate of 54% is at the high end of the spectrum compared to previous reports and indeed 10-year post-transplant survival rates of up to 96% have been documented.[7] A limitation of the current study is the underrepresentation of the patients that received a liver transplant and the limited follow-up data after transplantation. So far, the data of the registry cannot answer the question, if an early (pre-emptive) or late LT is more favourable for the patient´s outcome.

4.7 | Disease burden and future perspective

In patients with severe phenotype, the lifetime dependency on PT or LT has a major impact on the quality of life. Even though different treatment options exist in most regions, 59 patients from the registry died, of which a substantial part before adulthood and the majority coming from regions where access to treatment is limited. Unfortunately, we have no detailed information on the causes of death for most of them. A recent publication confirms the general struggle with PT adherence and its emotional impact on children and their parents, who face limitations in social interactions and may feel stigmatized by their appearance.[7] The impact of CNS and the burden of the cumbersome treatment on the quality of life of patients has not been reported and deserves prompt recognition. Unfortunately, we also have no sufficient data on quality of life so far, but the quality of life analysis is planned as the next step within the cohort. Undisputed is the urgent need for novel treatment options that are safer and curative, especially in regions with limited access to the current standard of care. In vivo gene therapy especially adeno-associated virus (AAV) vector-mediated gene transfer is an approach that has already shown its value for the treatment of inherited monogenic disorders and long-term follow-up data reveal safe and persistent phenotype correction of haemophilia B.[45,46] CNS is an ideal disease for liver-directed gene therapy.[47] A vector encoding for the human *UGT1A1* gene (AAV8-h*UGT1A1*) showed complete and sustained correction in two relevant animal models after a single intravenous administration[48,49] and is currently under clinical evaluation (NCT03466463).[50] This gene therapy approach has the potential to dramatically change the natural course and future management of CNS patients. Unfortunately, immunologic challenges that arise for the formation of neutralizing antibodies to the viral vector capsid proteins may restrict part of the patients from enrollment in gene therapy trials,[51] although different solutions to that problem are under development.[52]

In conclusion, we report the data on the CNS World Registry that harbours the largest cohort of CNS patients to date and gives a unique insight into the natural course of the disease, its co-morbidities, including liver fibrosis, and regional differences in treatment outcomes. The study showed us that better characterization of patients including liver fibrosis evaluation is necessary to increase our understanding of aetiology. Liver fibrosis and quality of life questionnaires should be implemented in the standard care of CNS patients. The burden of this disease is high and there is an urgent need to globally expand the access to therapy and to develop novel treatment options that further improve complication-free survival and quality of life of affected patients.

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CONFLICT OF INTEREST

F.M. is an employee and equity holder of Spark Therapeutics. F.M. is an inventor in patents describing liver gene transfer approaches for metabolic diseases. L.D. has consultancy agreements with Alexion Biosciences and Vivet Therapeutics. U.B. and N.J. have consultancy agreements with Vivet Therapeutics. None of the other authors declares any conflicts of financial interest.

ETHICS STATEMENT

Approval by local ethical committees was obtained from all participating centres. From all patients, informed consent was obtained. The study was performed in accordance with the principles of the Declaration of Helsinki.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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