Received: 14 January 2022 | Revised: 25 March 2022 | Accepted: 29 March 2022

DOI: 10.1002/jimd.12499

ORIGINAL ARTICLE

JIMD SSIEM WILEY

Postauthorization safety study of betaine anhydrous

Ulrike Mütze[1] | Florian Gleich[1] | Sven F. Garbade[1] | Céline Plisson[2] | Luis Aldámiz-Echevarría[3] | Francisco Arrieta[4] | Diana Ballhausen[5] | Matthias Zielonka[1] | Danijela Petković Ramadža[6] | Matthias R. Baumgartner[7] | Aline Cano[8] | María Concepción García Jiménez[9] | Carlo Dionisi-Vici[10] | Pavel Ješina[11] | Henk J. Blom[12] | Maria Luz Couce[13] | Silvia Meavilla Olivas[14] | Karine Mention[15] | Fanny Mochel[16] | Andrew A. M. Morris[17,18] | Helen Mundy[19] | Isabelle Redonnet-Vernhet[20] | Saikat Santra[21] | Manuel Schiff[22,23] | Aude Servais[24] | Isidro Vitoria[25] | Martina Huemer[7,26] | Viktor Kožich[11] | Stefan Kölker[1]

[1]Division of Child Neurology and Metabolic Medicine, Centre for Child and Adolescent Medicine, University Hospital, Heidelberg, Germany

[2]Recordati Rare Diseases, Puteaux, France

[3]Instituto de Investigación Santiago de Compostela (IDIS), Spain

[4]Endocrinology & Nutrition, Metabolic Congenital Disease, H.U. Ramon y Cajal, Madrid, Spain

[5]Pediatric Unit for Metabolic Diseases, Woman-Mother-Child Department, Lausanne University Hospital, Lausanne, Switzerland

[6]Department of Pediatrics, University Hospital Centre Zagreb and University of Zagreb, School of Medicine, Zagreb, Croatia

[7]Division of Metabolism and Children's Research Center, University Children's Hospital, University of Zurich, Zurich, Switzerland

[8]Centre de Référence des Maladies Héréditaires du Métabolisme, CHU La Timone Enfants, Marseille, France

[9]Metabolic Department, University Children Miguel Servet Hospital, Aragon, Spain

[10]Division of Metabolism, Bambino Gesù Children's Hospital IRCCS, Rome, Italy

[11]Department of Pediatrics and Inherited Metabolic Disorders, Charles University-First Faculty of Medicine and General University Hospital, Prague, Czech Republic

[12]Department of Clinical Genetics, Center for Lysosomal and Metabolic Diseases, Erasmus Medical Center, Rotterdam, Netherlands

[13]Unit of Diagnosis and Treatment of Congenital Metabolic Diseases, Service of Neonatology, Department of Pediatrics, Hospital Clínico Universitario de Santiago, CIBERER, Health Research Institute of Santiago de Compostela (IDIS), Santiago de Compostela, Spain

[14]Pediatrics, Gastroenterology, Hepatology and Nutrition, Hospital Sant Joan de Déu, Barcelona, Spain

[15]Centre de Référence des Maladies Héréditaires du Métabolisme, Hôpital Jeanne de Flandre, Lille, France

[16]Ap.HP, Sorbonne University, Reference Center for Adult Neurometabolic Diseases, La Pitié-Salpêtrière University Hospital, Paris, France

[17]Alder Hey Children's NHS Foundation Trust, Liverpool, UK

[18]Manchester Centre for Genomic Medicine, Manchester University Hospitals NHS Trust, Manchester, UK

[19]Evelina London Children's Hospital, London, UK

[20]Endocrinology, Nutrition and Metabolic Diseases, Haut-Lévêque Hospital, Bordeaux University, Bordeaux, France

[21]Department of Clinical Inherited Metabolic Disorders, Birmingham Women's and Children's NHS Foundation Trust, Birmingham, UK

[22]Necker Hospital, APHP, Reference Center for Inborn Error of Metabolism and Filière G2M, Pediatrics Department, University of Paris, Paris, France

[23]Inserm UMR_S1163, Institut Imagine, Paris, France

[24]Nephrology and Transplantation, MAMEA Reference Center, Necker hospital, APHP, Paris, France
[25]Unit of Metabolic Disorders, Universitary Hospital La Fe, Valencia, Spain
[26]Department of Pediatrics, Landeskrankenhaus Bregenz, Bregenz, Austria

Correspondence
Ulrike Mütze, Division of Child Neurology and Metabolic Medicine, University Hospital Heidelberg, Im Neuenheimer Feld 430, 69120 Heidelberg, Germany.
Email: ulrike.muetze@med.uni-heidelberg.de

Funding information
Czech Health Research Council, Grant/Award Number: NV19-01-00307; Czech Ministry of Health, Grant/Award Number: RVO VFN 64165; European Union, Grant/Award Number: CHAFEA agreement no. 2012 12 02; Medizinischen Fakultat Heidelberg, Universitat Heidelberg, Grant/Award Number: F.206852; Orphan Europe SARL

Communicating Editor: Sander M Houten

Abstract

Patient registries for rare diseases enable systematic data collection and can also be used to facilitate postauthorization safety studies (PASS) for orphan drugs. This study evaluates the PASS for betaine anhydrous (Cystadane), conducted as public private partnership (PPP) between the European network and registry for homocystinurias and methylation defects and the marketing authorization holder (MAH). Data were prospectively collected, 2013–2016, in a noninterventional, international, multicenter, registry study. Putative adverse and severe adverse events were reported to the MAH's pharmacovigilance. In total, 130 individuals with vitamin B_6 nonresponsive ($N = 54$) and partially responsive ($N = 7$) cystathionine beta-synthase (CBS) deficiency, as well as 5,10-methylenetetrahydrofolate reductase (MTHFR; $N = 21$) deficiency and cobalamin C ($N = 48$) disease were included. Median (range) duration of treatment with betaine anhydrous was 6.8 (0–9.8) years. The prescribed betaine dose exceeded the recommended maximum (6 g/day) in 49% of individuals older than 10 years because of continued dose adaptation to weight; however, with disease-specific differences (minimum: 31% in B_6 nonresponsive CBS deficiency, maximum: 67% in MTHFR deficiency). Despite dose escalation no new or potential risk was identified. Combined disease-specific treatment decreased mean ± SD total plasma homocysteine concentrations from 203 ± 116 to 81 ± 51 μmol/L ($p < 0.0001$), except in MTHFR deficiency. Recommendations for betaine anhydrous dosage were revised for individuals ≥ 10 years. PPPs between MAH and international scientific consortia can be considered a reliable model for implementing a PASS, reutilizing well-established structures and avoiding data duplication and fragmentation.

KEYWORDS
betaine anhydrous, E-HOD, homocystinuria, orphan drug, postauthorization safety study, public private partnership, rare disease

1 | INTRODUCTION

Reliable data on natural history, therapy, and long-term health outcomes of individuals with rare diseases, especially inherited metabolic diseases (IMDs), are often scarce. With more than 1600 distinct genetic diseases (IEMbase, http://www.iembase.org/), IMDs form the largest group of rare diseases. Data fragmentation has significantly hampered the advancement of epidemiological, clinical, and pharmacological knowledge in this field.[1,2] Specialists on IMDs address this challenge through utilization of uniform accepted standards to achieve syntactic and semantic interoperability.[3] These efforts resulted amongst others in the foundation of the EU-funded European network and registry for homocystinurias and methylation defects (E-HOD).

The challenges for research-oriented studies in the field of IMDs also translate into clinical trials or the structural assessment of safety data for drugs. This is of special significance since medicines with an orphan drug status are common for IMDs and generally require the marketing authorization holder (MAH) to perform postauthorization safety studies (PASS) as specified by the Pharmacovigilance Risk Assessment Committee (PRAC) of the European Medicines Agency (EMA). Data protection compliant reutilization of data collected by observational patient registries is feasible and efficient, helping to fulfill a

postauthorization measure in the framework of a public private partnership (PPP) and to avoid data fragmentation. This paper presents the "Cystadane® (betaine anhydrous) Surveillance Protocol in cooperation with the E-HOD" (CSP) as an example for orphan drug surveillance as PPP between the MAH and an international scientific consortium focusing on rare diseases.

2 | MATERIAL AND METHODS

2.1 | The E-HOD

E-HOD, initiated in 2013 as an EU-funded activity (CHAFEA agreement no. 2012 12 02), manages a web-based registry (https://www.ehod-registry.org/; German Clinical Trials register: DRKS00013085) which reutilizes the IT solution and modular design of the European registry and network for intoxication type metabolic diseases (E-IMD: https://www.eimd-registry.org/; initiated in 2011, CHAFEA agreement no. 2010 12 01).[4] The E-HOD registry gathers comprehensive information on individuals with cystathionine beta-synthase (CBS) deficiency (synonym, classic homocystinuria; OMIM #23600), 5,10-methylenetetrahydrofolate reductase (MTHFR) deficiency (OMIM #236250), cobalamin C disease (CblC; OMIM #277400), cobalamin D disease (CblD; OMIM #277410), methionine synthase reductase deficiency (CblE; OMIM #236270), methionine synthase deficiency (CblG; OMIM #250940), cobalamin F disease (OMIM #277380), cobalamin J disease (OMIM #614857), methionine adenosyl transferase I/III deficiency (OMIM #250850), glycine *N*-methyltransferase deficiency (OMIM #606664); *S*-adenosylhomocysteine hydrolase deficiency (OMIM #613752), adenosine kinase deficiency (OMIM #102750), methylenetetrahydrofolate dehydrogenase deficiency (OMIM #617780); glutamate formiminotransferase deficiency (OMIM #229100), and intestinal folate carrier 1 deficiency (OMIM #600424). The most common diagnoses are CBS deficiency, CblC disease, and MTHFR deficiency.[5–8] CBS deficiency is for all patients further subdivided by vitamin B_6 responsiveness using the classification system recently introduced by Kožich et al.[7]

E-HOD is implemented as an observational, non-interventional, multicenter registry study (November 2021: 71 participating sites). Upon enrollment patients receive a baseline visit, including detailed information on initial disease manifestation, mode of diagnosis, case confirmation, as well as family and individual medical history. Subsequent regular annual visits map the disease course, with unscheduled admissions at the hospital and fatal disease outcomes being covered by specific emergency and fatal disease course visits tailored to the context. At every visit the clinical and neurological phenotype is systematically recorded, as well as results from technical investigations, neuropsychological tests, quality of life assessments, dietary and drug treatment, and biochemical markers. At present (November 2021), the E-HOD registry contains comprehensive patient health data on 713 baseline visits, 1353 regular (annual) visits, as well as 62 emergency and 3 fatal disease course visits, averaging 3.0 visits per patient.

2.2 | Betaine anhydrous

Cystadane® (active substance: betaine anhydrous) was granted an orphan drug designation on July 9, 2001, subsequently receiving market authorization on February 15, 2007 for the adjunctive treatment of homocystinurias, including CBS deficiency, MTHFR deficiency, and diseases of cobalamin cofactor metabolism.[9] As methyl group donor betaine activates betaine-homocysteine-methyltransferase, converting homocysteine to methionine as a bypass to the cobalamin-dependent methionine synthase, and is used in combinations with vitamin B_6 (pyridoxine), vitamin B_{12} (cobalamin), folic acid, folinic acid, and for some diseases a low methionine diet.

2.3 | Public private partnership

The company Orphan Europe SARL (acquired by the Recordati Group in 2007 and continued as Recordati Rare Diseases since 2019) was granted the EU marketing authorization for the drug Cystadane® on February 15, 2007 by the EMA. To guarantee the safe use of Cystadane®, with special consideration of cerebral edema due to extreme hypermethioninemia as potential adverse drug reaction the company conducted the Registry for Cystadane-Homocystinuria (RoCH[10]) as part of its Risk Management Plan. RoCH collected retrospective and prospective health data of 125 patients with homocystinuria enrolled at 29 centers in France and Spain (2009–2013). RoCH was terminated in 2013 and replaced by CSP, a PPP between the newly established E-HOD consortium and Orphan Europe SARL in coordination with the EMA, to continue the postauthorization measure (http://www.encepp.eu/encepp/viewResource.htm?id=40022) and to act along recommendations of the EMA and European Union Committee of Experts on Rare Diseases.

2.4 | Study organization

The study protocol for CSP was developed by the sponsor of the study in collaboration with the principal

investigator of the E-HOD study center coordinating the registry (Heidelberg University Hospital [UKHD]) and under oversight of the E-HOD consortium and the PRAC of the EMA.

Putative (severe) adverse events [(S)AEs] were primarily reported directly to the pharmacovigilance of the sponsor to honor legal timelines, but were additionally also entered into the registry. The coordinating investigator at UKHD was responsible for administrative management and communication with the local investigators, providing assistance to participating clinical sites in study management and record keeping, and acted as primary liaison with the sponsor providing the agreed descriptive and analytical reports.

2.5 | Ethical and legal aspects

E-HOD was first approved by the local ethics committee of UKHD (Application no. S-525/2010, as the first amendment to E-IMD), followed by approvals of the respective ethics committees of further associated and collaborating partners contributing to the registry. CSP was first approved by the local ethics committee of UKHD (Application no. S-525/2010, as the second amendment to E-IMD), followed by approvals of the respective ethics committees of further associated and collaborating partners contributing to the PASS. All participating E-HOD partners had to obtain an additional ethics vote for the CSP study protocol. The patients' participation in the study was voluntary. Prospective written informed consent of participants and/or their parents and/or legal representatives, respectively, for both E-HOD and CSP were requested before inclusion of all eligible patients.

2.6 | Data protection and data management

CSP augmented the E-HOD data model by inclusion of additional variables for the start of Cystadane® treatment, previous participation in Cystadane® PASS studies, current pregnancy status, management of Cystadane® treatment, and putative (S)AE. The data collection for CSP started in June 2013 with the acceptance of the CSP study protocol by the first local ethics committee and ended in December 2016 with the update of the Cystadane Summary of Product Characteristics (SmPC).[11] Data collection was performed using standardized paper-based case report forms which were subsequently entered into the E-HOD registry. E-HOD uses a custom-made registry based on a MySQL database and a Typo3 backend for user administration. Data transfer between webserver and clients is protected by hypertext transfer protocol secure which enables encrypted communication and secure identification. Access to the patient registry is protected by secure individualized passwords. All entered data is pseudonymized, and users receive access limited to their institutional list of patients. Access to the complete dataset is only granted to the administrators (coordinator, data manager, biostatistical manager) for regular data plausibility checks and statistical analyses agreed by the consortium. Third parties, especially the MAH, do not have access to the primary data. Confidential personal information is handled in accordance to medical confidentiality, and the commitments of the federal and local data protection laws. Participants give written informed consent before data entry.

2.7 | Inclusion and exclusion criteria

Inclusion criteria for E-HOD are (1) confirmed diagnosis of a homocystinuria, methylation defect or folate disorder and (2) written informed consent for the E-HOD registry. Exclusion criteria for E-HOD are: (1) Metabolic derangement induced by any IMD not included in this study, congenital or acquired cobalamin (vitamin B_{12}) deficiency due to malnutrition or failure of absorption, or hyperhomocystinemia due to *MTHFR* gene polymorphism, (2) extreme low birth weight (<1500 g). Inclusion criteria for CSP are (1) participation in E-HOD, (2) being treated with Cystadane® and (3) giving written informed consent for CSP. CSP does not have additional exclusion criteria to E-HOD.

2.8 | Statistical analysis

For the present study data analysis was limited to individuals with vitamin B_6 nonresponsive and partially responsive CBS deficiency, MTHFR deficiency, and CblC disease. CSP data sets of individuals with cobalamin metabolism disorders in small sample sizes [CblD ($N = 3$), CblE ($N = 7$), CblG ($N = 5$), as well as unclassified remethylation defects ($N = 3$) and CBS patients with unclassified vitamin B_6 responsiveness according to Kožich et al.[7] ($N = 3$)] and incomplete data sets regarding betaine therapy ($N = 2$) were excluded from the analysis.

Age in years, diagnostic delay, and duration of treatment were compared with Kruskal–Wallis and Mann–Whitney U test; when appropriate, the Holm method was used to adjust p values. Pearson-product moment coefficient was used to correlate two continuous variables. Multiple regression models were used to analyze the response of betaine anhydrous dose and homocysteine

and methionine levels with respect to predictors sex, age group (< and ≥ 10 years) and disease. Variable selection was done with forward and backward step-wise variable selection with AIC. Tukey method was used for contrast analysis in multiple regression. When initial and last betaine and methionine levels were available, these were analyzed with linear mixed-effect (LME) models with the predictors sex and disease. Contrast analyses for LME were computed by estimated marginal means with Bonferroni adjustment, or paired *t*-test. Due to data protection, only month and year of birth were recorded and the day of birth imputed as the first day of the month. No other data was imputed. Missing data was handled by pairwise deletion. For all statistical analyses and figures R version 4.0.2 was used.

3 | RESULTS

3.1 | Model for PASSs in PPP with a rare disease registry

The organization structure established to perform a PASS (CSP) as PPP with a rare disease registry (E-HOD) is shown in detail in Figure 1. CSP was conducted between July 2013 and December 2016, recruiting patients in 25 metabolic centers in 9 European countries (Austria 1, Croatia 1, Czech Republic 1, France 6, Germany 1, Italy 1, Spain 6, Switzerland 2, United Kingdom 6). Data were collected during baseline visits ($N = 153$), scheduled follow-up visits ($N = 233$), and emergency visits ($N = 2$). No fatal disease course was recorded during the observation period. Three CSP participants were recorded to also having been participants of the preceding PASS RoCH.[10]

3.2 | Study sample

In total, data sets of 130 (58 female, 72 male) CSP participants with vitamin B_6 nonresponsive ($N = 54$) and partially responsive CBS deficiency ($N = 7$), MTHFR deficiency ($N = 21$), and CblC disease ($N = 48$) were analyzed.

Cohort characteristics are summarized in Table 1. Median age at last study visit (interquartile range [IQR]; range) was 16.2 (7.0–29.9; 0.3–65.3) years; however, it showed disease-specific variations ($p = 0.0001$; Kruskal–Wallis test), with a minimum in CblC disease [10.8 (4.7–16.9; 0.3–51.7) years] and a maximum in vitamin B_6 nonresponsive CBS deficiency [20.1 (11.3–32.1; 0.5–65.2) years; $p = 0.0074$, Mann–Whitney *U* test; Table 1]. Age at onset of first symptoms and age at diagnosis also differed (Table 1; $p < 0.0001$; Kruskal–Wallis test): in individuals with vitamin B_6 nonresponsive CBS deficiency, first symptoms [median (IQR, range) 4.2 (1.5–6.2; 0–27) years] were observed much later than in those with MTHFR deficiency [0.2 (0.0–0.7; 0–18) years; $p = 0.0049$; Mann–Whitney *U* test] and CblC disease [0.1 (0.0–0.3; 0–42) years; $p < 0.0001$; Mann–Whitney *U* test]. In analogy, individuals with vitamin B_6 nonresponsive CBS deficiency were diagnosed later [median age (IQR, range) 6.0 (1.8–13.0; 0–46) years] than individuals with CblC disease [0.2 (0.1–4.0; 0–45) years; $p = 0.0006$; Mann–Whitney *U* test] and in tendency later than those with MTHFR deficiency [0.7 (0.1–8.2; 0–24) years; $p = 0.0979$; Mann–Whitney *U* test]. Overall the diagnostic delay (time elapsed between first symptoms and diagnosis), median 0.1 years, did not show any difference between the diagnoses ($p = 0.7077$; Kruskal–Wallis test, Table 1).

3.3 | Safety findings

Apart from supporting direct reporting of putative (S)AEs to the MAH's pharmacovigilance, the CSP also gathered information on drug safety in the registry that was used as an additional data source for the obligatory periodic safety update report compiled by the MAH during the reporting period of CSP. One new case of diarrhea and two follow-up visits to this case, with the events diarrhea and vision blurred were identified. Causality was reported as noncompliance by the patient. Events resolved without sequelae when the patient followed the diet and the dosage prescribed by the caring physician. One event of extreme hypermethioninemia (defined as plasma methionine levels equal to or exceeding 1000 μmol/L) was identified in a 14-year-old adolescent with vitamin B_6 nonresponsive CBS deficiency on 6 g betaine anhydrous (105 mg/kg/day) who showed behavioral problems and a diet noncompliance (total plasma homocysteine 242 μmol/L, plasma methionine 1056 μmol/L). No potential severe complications like cerebral edema were observed and thus the episode was not registered as (S)AE. In total, no new or potential risks were identified after review of safety data from the CSP registry.

3.4 | Betaine anhydrous dosage: Disease-specific variations and nonadherence to recommendations

Median (IQR, range) duration of treatment with betaine anhydrous was 6.8 (3.2–8.3; 0–9.8) years and did not differ between the four disease groups ($p = 0.4284$; Kruskal–Wallis test; Table 1). Betaine anhydrous treatment was recommended to be adapted to body weight (maximum 150 mg/kg/day) for children below 10 years

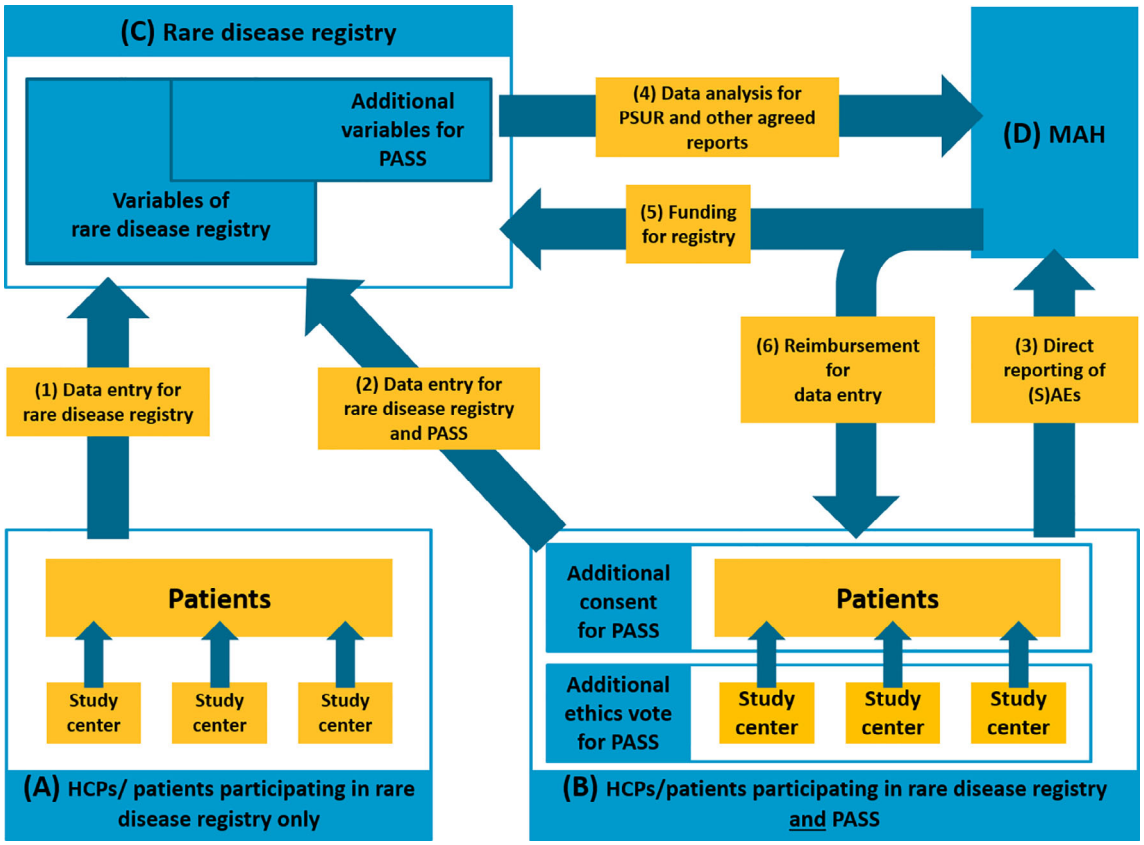

FIGURE 1 Organization structure for PASS as PPP with a rare disease registry. Model of organizing a PASS in cooperation with a rare disease registry: Healthcare providers (HCPs) (A) are free to only participate in the rare disease registry and consequently only enter data for the associated variables (1) or to additionally also participate in the PASS (B) and consequently enter data for an expanded set of variables (2) and directly report (S)AEs (3) to the pharmacovigilance of the MAH (D) according to legal timelines. HCPs participating in the PASS (B) need to obtain an additional ethics vote and informed consent for the PASS. The rare disease registry (C) provides data analysis (4) to the MAH (D) to facilitate PSURs and other reports. The MAH (D) provides funding (5) for the rare disease registry (C) and reimbursement (6) for entered data to the HCPs participating in the PASS (B). MAH, marketing authorization holder; PASS, postauthorization safety studies; PPP, public private partnership; PSUR, periodic safety update report

of age, and from then onwards to be prescribed using fixed doses (maximum 6 g/day). Since in individuals older than 10 years of age the dosage of betaine anhydrous continuously increased with age in parallel to increasing weight (Pearson; $R = 0.296$; $p = 0.0065$), we unraveled that age-dependent treatment recommendations were disregarded by participating metabolic centers in a significant number of individuals receiving betaine anhydrous treatment. Furthermore, the absolute betaine dose (g/day) increased by age (Pearson; $R = 0.237$; $p = 0.0265$) and exceeded the maximum recommended dose in 49% of participants ≥10 years, indicating that treating physicians continued to adapt doses relative to body weight according to the recommendations for individuals younger than 10 years of age. Noteworthy, for all ages prescribed amounts of betaine anhydrous depended on the diagnosis (Table 1, Figure 2; $p = 0.0074$; ANOVA): Compared to individuals with vitamin B_6 nonresponsive CBS deficiency doses were higher in MTHFR deficiency ($p = 0.0057$, Tukey HSD). In analogy, an excess of the betaine anhydrous dose above the maximum recommended dose (44%) also differed between diagnoses (likelihood ratio test; $p = 0.0391$), ranging from 31% of participants in B_6 nonresponsive CBS deficiency to 67% in MTHFR deficiency (Figure 3).

3.5 | Disease-specific biochemical response to specific therapy including betaine anhydrous

Betaine anhydrous was one component of the specific treatment in our study participants. Further therapy components, depended on the diagnosis, were: low protein diet, amino acid supplements, supplementation of micronutrition, and drugs. Therefore, the biochemical response could not be selectively analyzed for betaine anhydrous, but for the combined specific therapy including betaine

TABLE 1 Cohort characteristics, betaine anhydrous treatment and metabolic laboratory results

	Total	B_6 nonresponsive CBS def.	B_6 partially responsive CBS def.	MTHFR def.	CblC disease
N (male/female)	130 (72/58)	54 (31/23)	7 (4/3)	21 (10/11)	48 (27/21)
	Median (IQR; range)				
Age at last study visit (years)	16.2 (7.0–29.9; 0.3–65.3)	20.1 (11.3–32.1; 0.5–65.2)	39.8 (27.8–57.5; 18.6–65.3)	17.3 (7.5–25.4; 3.5–39.4)	10.8 (4.7–16.9; 0.3–51.7)
Age at onset of first symptoms (years)	$N = 106$ 1.0 (0.1–5.9; 0–55)	$N = 46$ 4.2 (1.5–6.3; 0–27)	$N = 6$ 8.4 (5.4–22.7; 2–55)	$N = 18$ 0.2 (0.0–0.7; 0–18)	$N = 36$ 0.1 (0.1–0.3; 0–42)
Age at diagnosis (years)	$N = 126$ 3.0 (0.1–10.3; 0–55)	$N = 54$ 6.0 (1.8–13.0; 0–46)	$N = 7$ 10.0 (6.5–23.5; 1–55)	$N = 20$ 0.7 (0.1–8.2; 0–24)	$N = 45$ 0.2 (0.1–4.0; 0–45)
Diagnostic delay (years)	$N = 105$ 0.1 (0–2; 0–14.7)	$N = 46$ 0.5 (0–3; 0–12.0)	$N = 6$ 1.5 (0.3–6.5; 0–8.1)	$N = 18$ 0.1 (0–0.4; 0–14.7)	$N = 35$ 0.0 (0–0.1; 0–3.1)
Betaine treatment	Median (IQR; range) Mean (SD)				
Duration of treatment (years)	$N = 128$ 6.8 (3.2–8.3; 0–9.8) 5.7 (3.1)	$N = 54$ 7.4 (3.2–7.4; 0–9.8) 6.0 (3.1)	$N = 7$ 7.4 (5.5–7.5; 3.7–8.2) 6.5 (1.9)	$N = 20$ 7.3 (5.5–8.3; 0.5–9.2) 6.3 (2.8)	$N = 47$ 4.9 (1.6–8.2; 0.1–9.8) 5.0 (3.3)
Daily dose at last visit (mg/kg/day) *All*	$N = 123$ 113 (71–167; 5–484) 122 (74)	$N = 53$ 98 (68–120; 5–316) 102 (61)	$N = 7$ 101 (67–124; 39–195) 102 (52)	$N = 19$ 148 (118–183; 23–484) 166 (103)	$N = 44$ 139 (59–173; 18–293) 130 (68)
Daily dose at last visit (mg/kg/day) *Individuals < 10 years*	$N = 40$ 120 (91–162; 25–484) 134 (82)	$N = 12$ 99 (87–118; 27–168) 101 (37)	$N = 0$	$N = 8$ 159 (133–194: 89–484) 196 (124)	$N = 20$ 130 (58–172; 25–293) 128 (70)
Daily dose at last visit (mg/kg/day) *Individuals ≥ 10 years*	$N = 83$ 105 (64–168; 5–316) 116 (70)	$N = 41$ 90 (56–123; 5–316) 101 (67)	$N = 7$ 101 (67–124; 39–195) 102 (52)	$N = 11$ 131 (95–190; 23–283) 143 (85)	$N = 24$ 149 (70–179; 18–288) 131 (67)
Daily dose at last visit (g/day) *Individuals ≥ 10 years*	$N = 88$ 6 (4.8–10.1; 0.3–18) 7.6 (4.5)	$N = 42$ 6 (5–9; 0.3–18) 7.1 (4.8)	$N = 7$ 9 (6–9; 3–12) 7.0 (2.9)	$N = 13$ 10 (4–12; 2–18) 9.2 (5.1)	$N = 26$ 7 (4.5–11.6; 0.9–15) 7.6 (4.0)
Metabolic laboratory results[a]	Median (IQR; range) Mean (SD)				
Homocysteine in plasma (μmol/L) *At diagnosis (initial)* *Last measurement under treatment*	$N = 79$ 211 (114–264; 18–650) 203 (116) 71 (45–104; 14–294) 81 (51)	$N = 39$ 249 (213–307; 70–650) 260 (112) 67 (46–106; 17–281) 83 (55)	$N = 6$ 272 (239–292; 74–400) 257 (107) 95 (52–123; 19–294) 112 (98)	$N = 7$ 163 (106–193; 18–233) 145 (77) 106 (93–126; 70–137) 107 (24)	$N = 27$ 112 (62–183; 23–282) 123 (73) 69 (41–82; 14–135) 66 (28)

(Continues)

TABLE 1 (Continued)

	Total	B_6 nonresponsive CBS def.	B_6 partially responsive CBS def.	MTHFR def.	CblC disease
p Value, initial vs. last visit	<0.0001	<0.0001	0.0038	0.9999	0.0163
Methionine in plasma (μmol/L)	$N = 46$	$N = 33$	$N = 5$	$N = 2$	$N = 6$
At diagnosis (initial)	367 (103–551; 4–1280)	442 (200–579; 61–1280)	360 (130–464; 76–650)	87 (47–127; 7–167)	9 (8–13; 4–20)
Last measurement under treatment	354 (290)	436 (278)	336 (237)	87 (113)	10 (6)
	104 (44–533; 14–960)	412 (75–584; 14–960)	69 (44–105; 30–358)	32 (28–35; 24–39)	21 (17–43; 14–55)
	283 (303)	370 (315)	121 (135)	32 (11)	30 (18)
p Value, initial vs. last visit	0.3418	0.2804	0.3804	N.a.	0.0690

Abbreviations: Cblc, cobalamin C; CBS, cystathionine beta-synthase; Def, deficiency; IQR, interquartile range; MTHFR, 5,10-methylenetetrahydrofolate reductase; N.a, not applicable.
[a]Patients with reported plasma concentrations for total homocysteine and methionine before and during treatment with betaine anhydrous.

anhydrous. To analyze the treatment effect on total homocysteine and methionine concentrations before initiation of treatment (initial) and at last measurement (last) were recorded and analyzed with a LME model with the predictor variables treatment (initial, last), sex, and disease and the interaction treatment with disease. For total plasma homocysteine, we found significant main effects of disease and treatment (initial to last) as well as the interaction disease with treatment (Table 1). Overall, total plasma homocysteine concentration (mean ± SD; $N = 79$) before the start of specialized therapy (203 ± 116 μmol/L) was higher than at last visit (81 ± 51 μmol/L; $p < 0.0001$); however, differed between disease groups ($p < 0.0001$) but not between females and males ($p = 0.2246$). Noteworthy, total plasma homocysteine concentrations decreased in treated individuals with vitamin B_6 nonresponsive CBS deficiency ($N = 39$; $p < 0.0001$), vitamin B_6 partially responsive CBS deficiency ($N = 6$; $p = 0.0038$), and CblC disease ($N = 27$; $p = 0.0163$), while it remained virtually unchanged in individuals with MTHFR deficiency ($N = 7$; $p = 0.9999$, all p values were adjusted with Bonferroni correction; Figure 4).

Overall, plasma methionine concentrations (mean ± SD; $N = 46$) did not reveal significant changes before (354 ± 290 μmol) and after start of treatment (283 ± 303 μmol/L; $p = 0.3418$); however, they differed between disease groups ($p < 0.0004$). As expected, the highest initial methionine concentrations (mean ± SD) were found in individuals with vitamin B_6 nonresponsive (436 ± 278 μmol/L) and vitamin B_6 partially responsive CBS deficiency (336 ± 237 μmol/L), while they were low in CblC disease (10 ± 6 μmol/L; Table 1; Figure 4). Although treatment with betaine anhydrous is known to bear the risk of further increasing plasma methionine concentrations to toxic levels in individuals with CBS deficiency, this was not observed during the study interval. Plasma methionine and total homocysteine concentrations of the last study visit did not correlate with the prescribed betaine anhydrous dose, neither for all diseases (Pearson; $R = -0.186$, $p = 0.0864$; $R = 0.135$, $p = 0.1684$, respectively) nor for single diseases.

3.6 | Comparison of RoCH and CSP studies

The RoCH study[10] and, in succession, the CSP study were designed to evaluate betaine anhydrous following market launch. Therefore, the same set of key variables were recorded, mainly retrospectively in RoCH and prospectively in CSP (Table 2). Although only three patients participated in both studies, comparable study cohorts

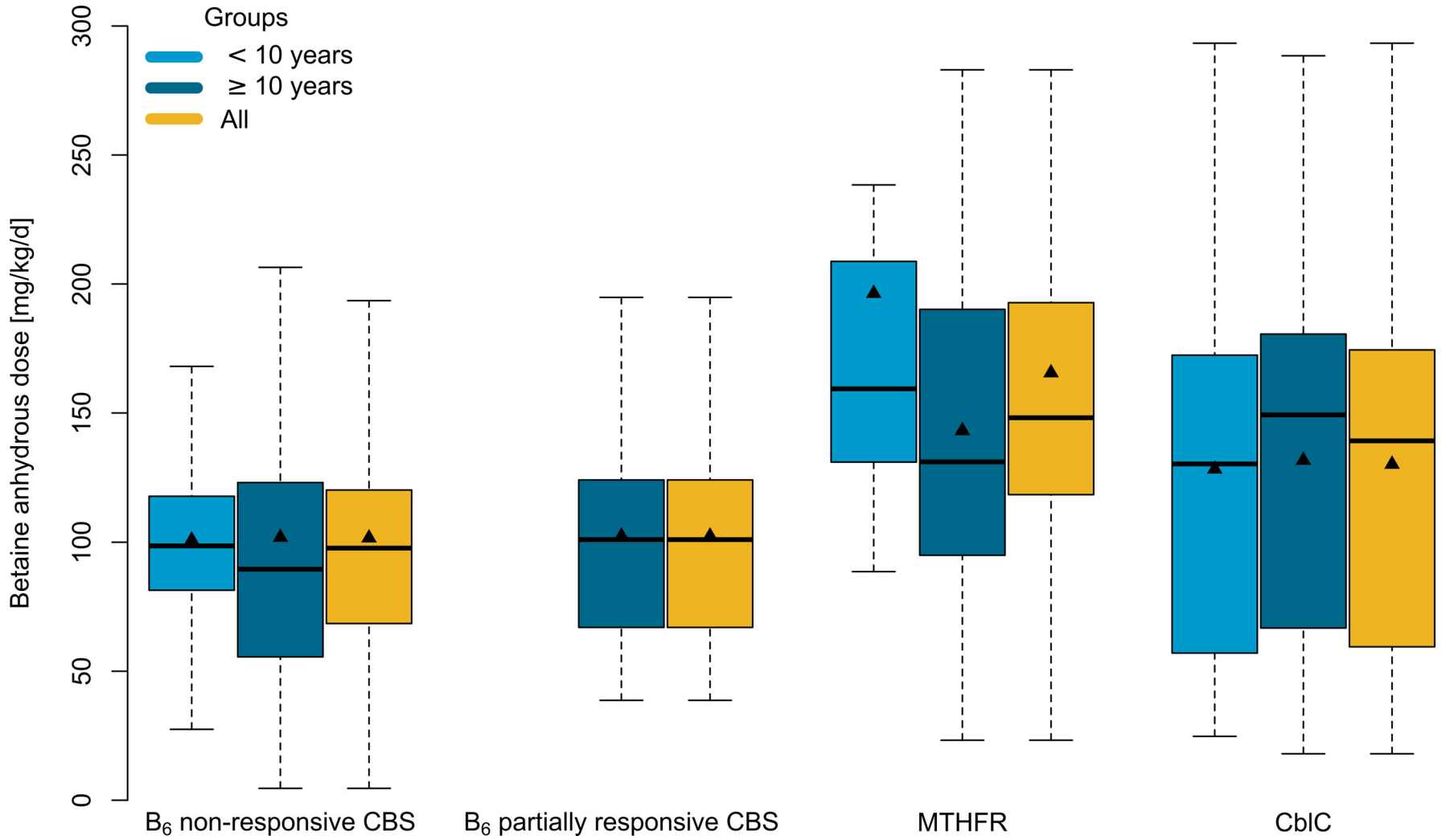

FIGURE 2 Dosing of betaine anhydrous is disease-dependent. Prescribed amounts of betaine anhydrous for all groups (<10 years; ≥10 years, all) depended on the diagnosis (Table 1; $p = 0.0074$; ANOVA): Doses were higher in MTHFR deficiency ($p = 0.0057$, Tukey HSD) compared to individuals with vitamin B_6 nonresponsive CBS deficiency. Boxplots show median (bold horizontal lines), IQR (boxes), and mean (black triangles). Outliers are not shown. CBS, cystathionine beta-synthase; IQR, interquartile range; MTHFR, 5,10-methylenetetrahydrofolate reductase

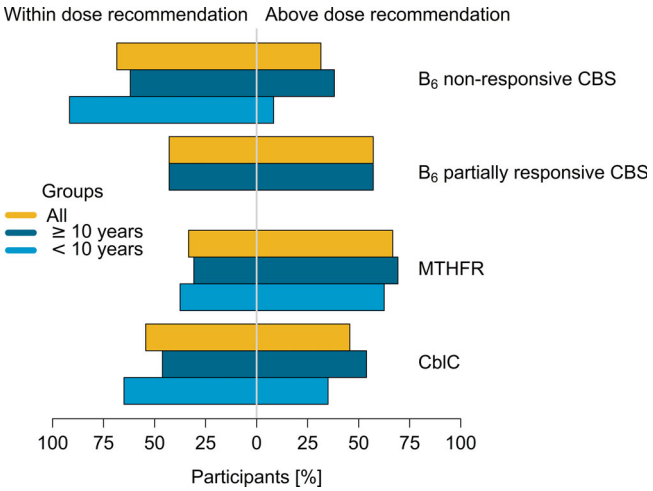

FIGURE 3 Adherence to dosing recommendations for betaine anhydrous. In 44% of individuals the betaine anhydrous dose exceeded the maximum recommended dose, with wide differences differing between the diagnoses ranging from 31% of participants in B_6 nonresponsive CBS deficiency up to 67% in MTHFR deficiency (likelihood ratio test; $p = 0.0391$). CBS, cystathionine beta-synthase; MTHFR, 5,10-methylenetetrahydrofolate reductase

(number of patients and diagnoses, sex, age, onset of symptoms) were enrolled. CSP expanded the geographical coverage from France and Spain (RoCH; $N = 29$ study sites) to a wider European survey (9 countries; $N = 25$ study sites), confirming most results of RoCH. However, CSP revealed the following different results and additions to RoCH: patients with CblC disease were not evaluated separately in RoCH, but within a combined entity of cobalamin disorders ("Cbl"; Table 2), consisting of CblC (88.2%), CblE/G (9.5%), and transcobalamin deficiency (2.4%) in the RoCH study. Therefore, comparison is impaired due to case mix differences. In individuals with vitamin B_6 partially responsive CBS and MTHFR deficiency, median ages at first symptoms and diagnosis were lower in CSP (Table 2). Although overall dosages of betaine anhydrous were similar in both studies, only the prospectively recorded data in CSP enabled a detailed evaluation of betaine treatment for the different diagnoses and age groups, unraveling the frequent dose excess depending on diagnoses and age groups (<10 years; ≥10 years; Table 2).

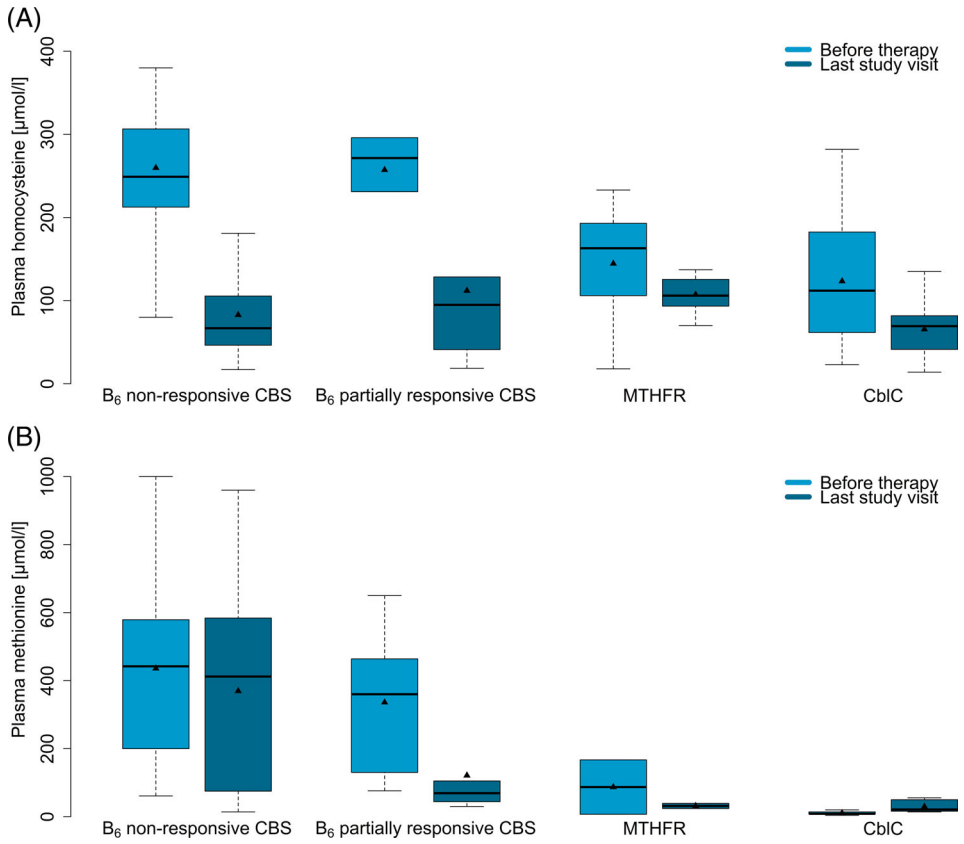

FIGURE 4 Disease-specific biochemical response to disease specific treatment including betaine anhydrous. Boxplots show median (bold horizontal lines), IQR (boxes), and mean (black triangles) total plasma concentrations of homocysteine (A) and methionine (B) before start of therapy and at the last documented visit under specific therapy including betaine anhydrous. Outliers are not shown. Total homocysteine (A) and methionine (B) plasma concentrations differed between the diagnoses ($p < 0.0001$; $p < 0.0004$, respectively). Total homocysteine levels decreased under specific therapy for all diagnoses but MTHFR deficiency (Table 1). In contrast, methionine plasma concentrations changes before therapy to last study visit did not reach statistical significance ($p = 0.3418$). IQR, interquartile range; MTHFR, 5,10-methylenetetrahydrofolate reductase

TABLE 2 Cohort data for CSP and RoCH (according to Valayannopoulos et al.[10])

	Total	B_6 nonresponsive CBS def.	B_6 partially responsive CBS def.	MTHFR def.	Cbl disease
CSP—*N* (male/female)	130 (72/58)	54 (31/23)	7 (4/3)	21 (10/11)	48 (27/21)
RoCH—*N* (male/female)	125 (71/54)	49 (31/18)	9 (7/2)	21 (10/11)	45 (22/23)
	Years (median, range)				
CSP—Age at last study visit	16.2 (0.3–65.3)	20.1 (0.5–65.2)	39.8 (18.6–65.3)	17.3 (3.5–39.4)	10.8 (0.3–51.7)
RoCH—Age at last study visit	14 (0–56.9)	18 (0–55.5)	39 (17.6–56.9)	20 (0–56.5)	6 (0–44.3)
CSP—Age at onset of first symptoms	$N = 106$ 1.0 (0–55)	$N = 46$ 4.2 (0–27)	$N = 6$ 8.4 (2–55)	$N = 18$ 0.2 (0–18)	$N = 36$ 0.1 (0–42)
RoCH—Age at onset of first symptoms	$N = 114$ 2 (0–50.2)	$N = 41$ 4 (0–28.7)	$N = 9$ 31 (0.8–48.0)	$N = 20$ 2 (0.0–50.2)	$N = 44$ 0 (0.0–41.2)
CSP—Age at diagnosis	$N = 126$ 3.0 (0–55)	$N = 54$ 6.0 (0–46)	$N = 7$ 10.0 (1–55)	$N = 20$ 0.7 (0–24.0)	$N = 45$ 0.2 (0.0–45.0)
RoCH—Age at diagnosis	$N = 124$ 4 (0–56.6)	$N = 49$ 6 (0–28.7)	$N = 9$ 31 (0.8–56.6)	$N = 20$ 5 (0.0–56.5)	$N = 45$ 0 (0.0–42.2)
CSP—Diagnostic delay	$N = 105$ 0.1 (0–14.7)	$N = 46$ 0.5 (0–12)	$N = 6$ 1.5 (0–8.1)	$N = 18$ 0.1 (0–14.7)	$N = 35$ 0.0 (0–3.1)
RoCH—Diagnostic delay	$N = 125$ 0 (0–17)	$N = 41$ 0.1 (0–15)	$N = 9$ 0 (0–17)	$N = 20$ 0 (0.0–6.3)	$N = 44$ 0 (0.0–7.6)
CSP—Duration of betaine treatment	$N = 128$ 6.8 (0–9.8)	$N = 54$ 7.4 (0–9.8)	$N = 7$ 7.4 (3.7–8.2)	$N = 20$ 7.3 (0.5–9.2)	$N = 47$ 4.9 (0.1–9.8)
RoCH—Duration of betaine treatment	$N = 125$ 7 (0–22.8)	N/A	N/A	N/A	N/A
Dosage	Median				
CSP—Daily dose at last visit (mg/kg/day) All	$N = 123$ 113	$N = 53$ 98	$N = 7$ 101	$N = 19$ 148	$N = 44$ 139
RoCH—Daily dose at last visit (mg/kg/day) All	108–167	N/A	N/A	N/A	N/A
CSP—Daily dose at last visit (mg/kg/day) Individuals <10 years	$N = 40$ 120	$N = 12$ 99	N/A	$N = 8$ 159	$N = 20$ 130
RoCH—Daily dose at last visit (mg/kg/day) Pediatric group <18 years	$N = 75$ 107–181	N/A	N/A	N/A	N/A
CSP—Daily dose at last visit (mg/kg/day) Individuals ≥10 years	$N = 83$ 105	$N = 41$ 90	$N = 7$ 101	$N = 11$ 131	$N = 24$ 149

(Continues)

TABLE 2 (Continued)

	Total	B_6 nonresponsive CBS def.	B_6 partially responsive CBS def.	MTHFR def.	Cbl disease
RoCH—Daily dose at last visit (mg/kg/day)	$N = 50$	N/A	N/A	N/A	N/A
Adult group ≥18 years	92–184				
CSP—Daily dose at last visit (g/day)	$N = 88$	$N = 42$	$N = 7$	$N = 13$	$N = 26$
Individuals ≥10 years	6	6	9	10	7
RoCH—Daily dose at last visit (g/day)	NA	9	6	9	6

Note: Data for CSP was acquired in 25 study centers in 9 European countries and for RoCH in 29 centers in France and Spain.[10] Three individuals participated the RoCH study prior to enrollment to the CSP study. The Column Cbl consists exclusively CblC disease patients for CSP, whereas in the RoCH it clusters individuals with CblC, CblE/G, and transcobalamin deficiency.
Abbreviations: CblC, cobalamin C; CBS, cystathionine beta-synthase; CSP, Cystadane® (betaine anhydrous) Surveillance Protocol; Def, deficiency; MTHFR, 5,10-methylenetetrahydrofolate reductase; N/A, not available; RoCH, Registry for Cystadane-Homocystinuria.

The RoCH evaluation[10] demonstrated an overall reduction of total plasma homocysteine levels of 29% following betaine anhydrous introduction and a methionine increase in CBS deficiency without exceeding the safety threshold of 1000 μmol/L.[10] Prospective follow-up of patients treated with betaine anhydrous did not allow to evaluate this effect selectively, but from the perspective of clinical everyday life of a combined and continuously adapted specific therapy.

4 | DISCUSSION

The presented CSP study serves as a successful model for PPPs between MAH and international scientific consortia focusing on rare diseases for implementing a PASS. Such collaborations allow recruitment of well-established patient cohorts and efficient data collection using already existing structures, thus avoiding data duplication and low enrollment turnouts due to burdening patients with multiple study protocols.

E-HOD highlighted that networking activities and the establishment of patient registries have a relevant impact for improving the health of patients with IMDs and for harmonizing diagnostic algorithms, therapy, long-term follow-up, and care on a European and international level. It described in detail the natural history including disease variants of more than six different IMDs, evaluated the impact of interventional and noninterventional parameters (including evaluation of newborn screening programs), and allowed the development of evidence-based care protocols being the basis for national guidelines and being translated into information brochures for patients, families, and healthcare professionals.[5–8,12,13]

For rare diseases treated with orphan drugs requiring a PASS, the model of implementing a PPP between MAH and international patient registries was demonstrated to be successful in generating the needed cohort size and data quality. Apart from helping to enable the market introduction of critically needed drugs, the reuse of existing data also lowers the impact on patients and health infrastructure posed by data duplication and provides funding for the sustainability of research registries after the end of the initial funding period. Thereby, research registries will help to improve treatment and care in rare diseases like IMDs.

The CSP study presented has a similar cohort size and structure compared to the previously reported RoCH data[10] enrolled in France and Spain. It is representative for the selected diagnoses, but features a greater geographical coverage in Europe. As a valuable extension of the former RoCH study[10] which also reported relative betaine anhydrous dosage regimes exceeding treatment

recommendation, our evaluation could now analyze this practice in greater detail.

We demonstrated continuous dose adaptation of betaine anhydrous to weight in most patients aged 10 years and older, exceeding the former maximum dosage recommendations by a factor of up to three. This was especially true for individuals with MTHFR deficiency and CblC disease (two diseases with *hypo*-methioninemia), but also for 30%–50% of the CBS-deficient patients (with *hyper*-methioninemia). Noteworthy, this prescription practice in CBS deficiency did not result in reports of severe adverse effects associated with toxic methionine concentrations such as cerebral edema or signs of cerebral pressure. Plasma methionine concentrations in vitamin B_6 nonresponsive CBS-deficient patients remained stable in all but one patient under betaine anhydrous therapy due to further concomitant treatment, including restricted dietary intake of natural methionine containing protein.[8] Adding this information to the RoCH evaluation and further pharmacovigilance data collected by the sponsor, the CSP study data resulted in an update of the Cystadane SmPC in 2016,[14] introducing the observed clinical practice of weight-adapted dose titration of 100–200 mg/kg/day depending on the therapeutic goal as new dosage recommendation.[11]

Total homocysteine levels in plasma at last visit were lower than pretreatment in all diagnoses; in contrast, methionine levels remained stable in vitamin B_6 nonresponsive CBS-deficient patients under betaine anhydrous-containing specific treatment, but did not increase to toxic levels. Presumably this was supported by regular controls and adaption of dietary treatment if necessary.

In individuals with MTHFR deficiency and CblC disease, observed therapy did not normalize plasma methionine concentrations, supporting the notion of necessary regular laboratory surveillance and continuous adaptation of medication in these patients to maintain methionine in the normal range.[6] Patients with CblC disease having the highest betaine anhydrous dosages ≥10 years, usually as additional treatment to the high effective parenteral hydroxocobalamin,[5,6] might surprise and points on the necessity of regular dosage reevaluation of all treatment components in consideration of the metabolic laboratory to avoid under- and overdosages.

5 | LIMITATIONS

Not all E-HOD study sites participated in the CSP and as participation is voluntary and requires informed consent not all eligible patients participated in E-HOD and CSP. This might be a source of bias for the presented study. However, the implementation of the study as an observational study with none or very low impact on patients, makes refusal to participate low. Due to the different incidences and the observational design of the study the enrolled disease groups differ in size, with the MTHFR group being especially small. This could hamper the robustness of our results.

Observational studies collect real world data on heterogenous disease groups regarding disease severity, age, sex, and treatment components. Furthermore, often only data on the prescribed treatment is recorded without detailed information on individual treatment compliance. This might be a source of bias for the study. However, PASSs are designed and done to assess the medication (severe) adverse and desired effects in a real-world setting.

Analysis of metabolic laboratory parameters as paired data (initial and last value) was only possible for small cohorts, due to the retrospective nature of data collection of the initial level and the resulting fragmentation. This reduced the power of the analysis. Furthermore, data collection for CSP as extension of RoCH was not long enough to generate a meaningful sequence of longitudinal metabolic laboratory data. However, results from metabolic laboratory tests at last visit were comparable to the follow-up data collected in RoCH.

6 | CONCLUSION

The presented CSP study serves as a successful model for PPPs between MAH and international scientific consortia focusing on rare diseases for implementing a PASS using well-established structures and avoiding data duplication. The results of this study which observed a continuous dose titration relative to body weight without reported occurrence of severe side effects resulted in adaption of Cystadane dosage recommendations and thereby warrants a safe drug administration in patients with homocystinurias.

ACKNOWLEDGMENTS

The authors are indebted to all patients and their parents who participated in this study by sharing their experience of daily practice and life with homocystinurias for their motivation and trust. The authors thank Collette Stainforth, Bradford Teaching Hospitals NHS Foundation Trust, Bradford, UK, for providing data sets from her site. The Cystadane Surveillance Protocol (CSP) was funded by Orphan Europe SARL (currently Recordati Rare Diseases SARL being part of Recordati S.P.A.). The European Network and Registry for Homocystinurias and Methylation Defects (E-HOD) was funded by the European Union, in the framework of the Health Programme 2008–2013

(CHAFEA agreement no. 2012 12 02). Ulrike Mütze was supported by Olympia Morata research fellowship of the Medical Faculty of the Ruprecht Karl University of Heidelberg (grant number F.206852). Viktor Kožich and Pavel Ješina received institutional support from the Ministry of Health (General University Hospital in Prague- RVO VFN 64165) and from the Czech Health Research Council (grant NV19-01-00307). The authors confirm independence from the sponsor; the content of the article has not been influenced by the sponsor.

CONFLICTS OF INTEREST

The CSP was funded by Orphan Europe SARL (currently Recordati Rare Diseases SARL being part of Recordati S.P.A.) and data entry of the study sites was reimbursed. Celine Plisson is a Recordati Rare Diseases employee. Carlo Dionisi-Vici received honoraria for educational activity by Orphan Europe/Recordati Rare Diseases. Luis Aldámiz-Echevarría declares payment for lectures from Mead-Johnson Nutrition, Takeda Pharma, Nutricia Metabolics, and Sanofi Genzyme, payment for expert testimonies on urea cycle defects and support for attending meetings by Nutricia Metabolics and Amicus Therapeutics. Matthias R. Baumgartner declares research funds from Nutricia Metabolics and participation on advisory boards for Hemoshear Therapeutics and Moderna and participation on a data safety monitoring board for Lysogene. Saikat Santra declares benefitting from travel and educational grants payed by Recordati Rare Diseases to his institutional charity fund. Karine Mention declares payments for lectures by Sanofi Genzyme and Chiesi. Andrew Morris declares having received an honorarium from the Recordati Rare Disease Foundation for lecturing on their Inherited disorders of glucose homeostasis course. Manuel Schiff declares having received an honorarium from the Recordati Rare Disease Foundation for lecturing on their webinar on homocystinurias and lecturing for the Recordati Rare Disease Foundation on mitochondrial diseases and metabolic cardiomyopathies free of charge. Helen Mundy declares having received fees for lectures from the British Dietetic Association, consulting fees form Ultragenyx for GSD gene therapy safety monitoring and support for attending the SSIEM from Vitaflo. Silvia Meavilla Olivas declares support for attending meetings by Nutricia Metabolics, Nestlé and Mead-Johnson Nutrition. Isidro Vitoria Miñana declares having received honoraria for lectures from Vitaflo, PIAM Farmaceutici, Nutricia Metabolics and Recordati Rare Diseases. The other authors declare no conflicts of interest.

ETHICS STATEMENT

E-HOD was first approved by the local ethics committee of UKHD (application no. S-525/2010, as the first amendment to E-IMD), followed by approvals of the respective ethics committees of further associated and collaborating partners contributing to the registry. CSP was first approved by the local ethics committee of UKHD (application no. S-525/2010, as the second amendment to E-IMD), followed by approvals of the respective ethics committees of further associated and collaborating partners contributing to the PASS. All participating E-HOD partners had to obtain an additional ethics vote for the CSP study protocol.

ORCID

Ulrike Mütze https://orcid.org/0000-0002-4251-7257
Florian Gleich https://orcid.org/0000-0001-7397-5640
Sven F. Garbade https://orcid.org/0000-0002-7420-4757
Luis Aldámiz-Echevarría https://orcid.org/0000-0003-0687-6604
Diana Ballhausen https://orcid.org/0000-0003-2940-3439
Matthias Zielonka https://orcid.org/0000-0001-9653-2914
Matthias R. Baumgartner https://orcid.org/0000-0002-9270-0826
María Concepción García Jiménez https://orcid.org/0000-0002-5563-3177
Carlo Dionisi-Vici https://orcid.org/0000-0002-0007-3379
Henk J. Blom https://orcid.org/0000-0001-5202-9241
Isabelle Redonnet-Vernhet https://orcid.org/0000-0002-5102-3814
Aude Servais https://orcid.org/0000-0002-8165-8212
Martina Huemer https://orcid.org/0000-0002-0590-678X
Viktor Kožich https://orcid.org/0000-0001-5820-5277

REFERENCES

1. Opladen T, Gleich F, Kozich V, et al. U-IMD: the first unified European registry for inherited metabolic diseases. *Orphanet J Rare Dis*. 2021;16(1):95. doi:10.1186/s13023-021-01726-3
2. Rodwell C, Aymé S. 2014 Report on the State of the Art of Rare Disease Activities in Europe.European Union; 2014. https://ec.europa.eu/health/sites/health/files/rare_diseases/docs/2014report_rare_disease_activitieseu_1_en.pdf. Accessed March 24, 2021.
3. Wilkinson MD, Dumontier M, Aalbersberg IJ, et al. The FAIR guiding principles for scientific data management and stewardship. *Sci Data*. 2016;3:160018. doi:10.1038/sdata.2016.18
4. Kölker S, Dobbelaere D, Häberle J, et al. Networking across borders for individuals with organic acidurias and urea cycle disorders: the E-IMD consortium. *JIMD Rep*. 2015;22:29-38. doi:10.1007/8904_2015_408
5. Huemer M, Diodato D, Martinelli D, et al. Phenotype, treatment practice and outcome in the cobalamin-dependent remethylation disorders and MTHFR deficiency: data from the E-HOD registry. *J Inherit Metab Dis*. 2019;42(2):333-352. doi:10.1002/jimd.12041

6. Huemer M, Diodato D, Schwahn B, et al. Guidelines for diagnosis and management of the cobalamin-related remethylation disorders cblC, cblD, cblE, cblF, cblG, cblJ and MTHFR deficiency. *J Inherit Metab Dis*. 2017;40(1):21-48. doi:10.1007/s10545-016-9991-4
7. Kožich V, Sokolová J, Morris AAM, et al. Cystathionine β-synthase deficiency in the E-HOD registry-part I: pyridoxine responsiveness as a determinant of biochemical and clinical phenotype at diagnosis. *J Inherit Metab Dis*. 2021;44(3):677-692. doi:10.1002/jimd.12338
8. Morris AA, Kožich V, Santra S, et al. Guidelines for the diagnosis and management of cystathionine beta-synthase deficiency. *J Inherit Metab Dis*. 2017;40(1):49-74. doi:10.1007/s10545-016-9979-0
9. EMA Cystadane: EPAR—Sientific Discussion. 2007. https://www.ema.europa.eu/en/documents/scientific-discussion/cystadane-epar-scientific-discussion_en.pdf. Accessed March 25, 2021.
10. Valayannopoulos V, Schiff M, Guffon N, et al. Betaine anhydrous in homocystinuria: results from the RoCH registry. *Orphanet J Rare Dis*. 2019;14(1):66. doi:10.1186/s13023-019-1036-2
11. EMA Cystadane: EPAR—Product information. 2019. https://www.ema.europa.eu/en/documents/product-information/cystadane-epar-product-information_en.pdf. Accessed March 25, 2021.
12. Huemer M, Kožich V, Rinaldo P, et al. Newborn screening for homocystinurias and methylation disorders: systematic review and proposed guidelines. *J Inherit Metab Dis*. 2015;38(6):1007-1019. doi:10.1007/s10545-015-9830-z
13. Keller R, Chrastina P, Pavlíková M, et al. Newborn screening for homocystinurias: recent recommendations versus current practice. *J Inherit Metab Dis*. 2019;42(1):128-139. doi:10.1002/jimd.12034
14. EMA Cystadane: EPAR—procedural steps taken sientific information after authorisation. 2019. https://www.ema.europa.eu/en/documents/procedural-steps-after/cystadane-epar-procedural-steps-taken-scientific-information-after-authorisation_en.pdf. Accessed March 25, 2021.

How to cite this article: Mütze U, Gleich F, Garbade SF, et al. Postauthorization safety study of betaine anhydrous. *J Inherit Metab Dis*. 2022;45(4):719-733. doi:10.1002/jimd.12499