



HAL
open science

Influence of early identification and therapy on long-term outcomes in early-onset MTHFR deficiency

Mathilde Yverneau, Stephanie Leroux, Apolline Imbard, Florian Gleich, Alina Arion, Caroline Moreau, Marie-Cecile Nassogne, Marie Szymanowski, Marine Tardieu, Guy Touati, et al.

► To cite this version:

Mathilde Yverneau, Stephanie Leroux, Apolline Imbard, Florian Gleich, Alina Arion, et al.. Influence of early identification and therapy on long-term outcomes in early-onset MTHFR deficiency. *Journal of Inherited Metabolic Disease*, 2022, 45 (4), pp.848-861. 10.1002/jimd.12504 . hal-03711581

HAL Id: hal-03711581

<https://hal.science/hal-03711581>

Submitted on 20 Jul 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Influence of early identification and therapy on long-term outcomes in early-onset MTHFR deficiency

Mathilde Yverneau¹, Stéphanie Leroux^{1*}, Apolline Imbard^{2,3,4*}, Florian Gleich⁵, Alina Arion⁶, Caroline Moreau⁷, Marie-Cécile Nassogne⁸, Marie Szymanowski⁹, Marine Tardieu¹⁰, Guy Touati¹¹, María Bueno¹², Kimberly A. Chapman¹³, Yin-Hsiu Chien¹⁴, Martina Huemer^{15,16}, Pavel Ješina¹⁷, Mirian CH Janssen¹⁸, Stefan Kölker⁵, Viktor Kožich¹⁷, Christian Lavigne¹⁹, Allan Meldgaard Lund²⁰, Fanny Moche²¹, Andrew Morris^{22,23}, Mónica Ruiz Pons²⁴, Gloria Liliana Porras-Hurtado²⁵, Jean-François Benoit^{2,3,4}, Léna Damaj^{26**}, Manuel Schiff^{3,27**}, E-HOD consortium

¹ Department of Child and Adolescent Medicine, Rennes Hospital, Rennes, France

² Biochemistry Laboratory, Robert Debré Hospital, APHP, Paris, France

³ Reference Center for Inborn Error of Metabolism, Department of Pediatrics, Necker and Robert-Debré Hospital, APHP, Université de Paris, Paris, France

⁴ LYPSSIS, Université Paris-Saclay, Châtenay-Malabry, France

⁵ Division of Child Neurology and Metabolic Medicine, Center for Child and Adolescent Medicine, University Hospital Heidelberg, Heidelberg, Germany

⁶ Department of Pediatrics, Caen Hospital, Caen, France

⁷ Biochemistry Laboratory, Rennes Hospital, Rennes, France

⁸ Pediatric Neurology Unit, Cliniques Universitaires Saint-Luc, UCLouvain, Brussels, Belgium

⁹ Department of Pediatrics, Estaing Hospital, Clermont-Ferrand, France

¹⁰ Department of Pediatrics, Tours Hospital, Tours, France

¹¹ Reference Center for Inborn Error of Metabolism, Department of Pediatrics, Toulouse Hospital, Toulouse, France

¹² Hospital Universitario Virgen del Rocío, Sevilla, Spain

¹³ Section of Genetics and Metabolism, Children's National Health System, Washington DC, USA

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the [Version of Record](#). Please cite this article as doi: [10.1002/jimd.12504](https://doi.org/10.1002/jimd.12504)

¹⁴ Department of Pediatrics, National Taiwan University Hospital and National Taiwan University College of Medicine, Taipei, Taiwan

¹⁵ Division of Metabolism and Children's Research Center, University Children's Hospital, Zürich, Switzerland

¹⁶ Department of Paediatrics, Landeskrankenhaus Bregenz, Bregenz, Austria

¹⁷ Department of Pediatrics and Inherited Metabolic Disorders, Charles University-First Faculty of Medicine and General University Hospital, Prague 12808, Czech Republic

¹⁸ Department of Internal Medicine, Radboud University Medical Centre, Nijmegen, The Netherlands

¹⁹ Department of Internal Medicine, Angers University Hospital, 4 Rue Larrey, 49000 Angers, France

²⁰ Centre for Inherited Metabolic Diseases, Departments of Paediatrics and Clinical Genetics, Copenhagen University Hospital, Rigshospitalet, 2100 Copenhagen, Denmark

²¹ AP-HP, Pitié-Salpêtrière University Hospital, Department of Genetics, Paris, France

²² Willink Metabolic Unit, Manchester Centre for Genomic Medicine, Manchester University Hospitals NHS Foundation Trust, Manchester

²³ Alder Hey Children's Hospital, Liverpool, UK

²⁴ H.U. Ntra. Sra. de la Candelaria, Santa Cruz de Tenerife, Spain

²⁵ Clínica Comfamiliar, Colombia, Comfamiliar Health Research Group, Colombia

²⁶ Competence Center of Inherited Metabolic Disorders, Department of Pediatrics, Rennes Hospital, Rennes, France

²⁷ Inserm UMR_S1163, Institut Imagine, Paris, France

*, **: equally contributing

Abstract

MTHFR deficiency is a severe inborn error of metabolism leading to impairment of the remethylation of homocysteine to methionine. Neonatal and early-onset patients mostly exhibit a life-threatening acute neurologic deterioration. Furthermore, data on early-onset patients' long-term outcomes are scarce. The aims of this study were 1) to study and describe the clinical and laboratory parameters of early-onset MTHFR-deficient patients (i.e. ≤ 3 months of age) and 2) to identify predictive factors for severe neurodevelopmental outcomes in a cohort with early and late onset MTHFR-deficient patients.

To this end, we conducted a retrospective, multicentric, international cohort study on 72 patients with MTHFR deficiency from 32 international metabolic centres. Characteristics of the 32 patients with early-onset MTHFR deficiency were described at time of diagnosis and at the last follow-up visit. Logistic regression analysis was used to identify predictive factors of severe neurodevelopmental outcome in a broader set of patients with early and non-early-onset MTHFR deficiency.

The majority of early-onset MTHFR-deficient patients (n=32) exhibited neurologic symptoms (76%) and feeding difficulties (70%) at time of diagnosis. At the last follow-up visit (median follow-up time of 8.1 years), 76% of treated early-onset patients (n=29) exhibited a severe neurodevelopmental outcome. Among the whole study population of 64 patients, pre-symptomatic diagnosis was independently associated with a significantly better neurodevelopmental outcome (adjusted OR 0.004, [0.002-0.232]; p=0.003).

This study provides evidence for benefits of pre-symptomatic diagnosis and appropriate therapeutic management, highlighting the need for systematic newborn screening for MTHFR deficiency and pre-symptomatic treatment that may improve outcome.

Synopsis

Pre-symptomatic diagnosis and prompt therapeutic management of early-onset MTHFR deficiency is associated with better neurodevelopmental outcomes. This highlights the need for systematic newborn screening for this inborn error of folate metabolism.

Details of the contributions of individual authors

Mathilde Yverneau participated in the study concept and design, acquisition of data, analysis and interpretation of the data, drafting and critical revision of the manuscript. Stéphanie Leroux participated in the study design, analysis and interpretation of the data, drafting and critical revision of the manuscript, and supervision of the study. Apolline Imbard participated in analysis and interpretation of the data, and critical revision of the manuscript. Florian Gleich and Stefan Kölker conceptualized, established, and managed the E-HOD registry. Alina Arion, Caroline Moreau, Marie-Cécile Nassogne, Marie Szymanowski, Marine Tardieu, Guy Touati, María Bueno, Kimberly A. Chapman, Yin-Hsiu Chien, Martina Huemer, Mirian Janssen, Pavel Ješina, Viktor Kožich, Christian Lavigne, Allan Meldgaard Lund, Fanny Mochel, Andrew Morris, Mónica Ruiz Pons, Gloria Liliana Porrás-Hurtado, Ana Maria Martins, Javier Blasco Alonso, Brigitte Chabrol, Ellen Crushell, Carlo Dionisi-Vici, Stephanie Grünewald, Karine Mention, Helen Mundy, Elaine Murphy, Pilar Quijada Fraile, Carlos José Ruiz, María Ángeles Ruiz Gómez, Saikat Santra, Thomas Scherer, Collette Stainforth, Karolina M. Stepień, Gere Sunder-Plassmann and Johan L.K. Van Hove participated in the acquisition of data. Manuel Schiff, Jean-François Benoist and Léna Damaj participated in the study concept and design, critical revision of the manuscript and supervision of the study.

Guarantor for the article

Prof. Manuel Schiff

Competing interest statement

All of the authors declare that they have no conflict of interest.

Details of funding

The European network and registry for homocystinurias and methylation defects (E-HOD) project has been established with funding from the European Union in the framework of the Health Program (Chafea grant N°. 2012 12 02). From the end of the EU project phase, the E-HOD project has received ongoing support from SOBI, Recordati Rare Disease Foundation, Vitaflo, Aeglea Bio Therapeutics, and Nutricia Metabolics Germany. The authors confirm independence from the sponsors; the content of the article has not been influenced by the sponsors.

Details of ethics approval

This study has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki). The Ethics Committee of the University Hospital in Heidelberg (N ° S-525/2010; 14.3.2013) first approved the E-HOD registry. All participating centres received approval from their local ethics committees before enrolling patients and all patients provided written informed consent for entering pseudonymized data into the registry and subsequent publication.

A patient consent statement

Not applicable.

Keywords

Homocystinuria; remethylation defects; MTHFR deficiency; neurodevelopmental outcome; newborn screening; patient registry

General Rules

This study has not been published previously neither has it been submitted to any other journal. All authors contributed substantially to the study, inspected the manuscript and agreed with the submission. The author(s) confirm(s) independence from the sponsors; the content of the article has not been influenced by the sponsors.

An availability of data and material statement

Only aggregated data are being published in this study to comply with the GDPR rules.

Introduction

5,10 - Methylene tetrahydrofolate reductase (MTHFR) (MIM #607093) deficiency is a severe autosomal recessive inborn error of metabolism. Though rare, MTHFR deficiency is the most prevalent inborn error of folate metabolism.

The enzyme MTHFR catalyses the reduction of 5,10 - methylene tetrahydrofolate (5,10-MTHF) to 5 - methyl tetrahydrofolate (5-MTHF), that acts as a methyl donor for the remethylation of homocysteine into methionine ¹⁻³. Consequently, MTHFR deficiency leads to hyperhomocysteinemia and low methionine due to the dysfunction of the remethylation pathway.

Pathogenesis of MTHFR deficiency is complex. On the one hand, the accumulating homocysteine is a well-known multisystem toxic agent ⁴. Homocysteine toxicity induces central nervous system (CNS) damages, either directly through endothelial toxicity ⁴ and neuronal cell death ⁵⁻¹⁰, or indirectly *via* its conversion to S - adenosylhomocysteine (SAH), a potent inhibitor of methyltransferases ^{1,11}. On the other hand, MTHFR deficiency causes a secondary impairment of methionine synthase with subsequent methionine and S-adenosylmethionine (SAM) depletion. The latter causes an additional reduction of cellular methylation capacity in the CNS ^{1,12}. CNS methylation reactions are critical for myelination and brain development ^{6,13}.

Remethylation of homocysteine into methionine can also proceed *via* an alternate pathway ¹ using betaine as methyl donor and catalysed by betaine-homocysteine methyltransferase (BHMT), mainly expressed in liver and kidney. BHMT converts a significant part of tHcy (total homocysteine) formed in the body, however not in the brain due to its limited expression in this tissue. Betaine is thus a central drug and the cornerstone of the treatment for MTHFR deficiency, which general therapeutic goal is to bypass the remethylation defect, in

order to normalize plasma methionine and SAM levels ⁸ and reduce plasma tHcy accumulation ^{1,14–16}. Other, less validated therapeutic means include folate supplementation either as methylfolate¹⁷ or folic acid and L-methionine.

MTHFR deficiency can present at any age. The clinical course varies according to the age of onset: the early-onset presentations (neonatal period and early infancy [≤ 3 months]) and the late-onset presentations (late infancy or early childhood [from 3 months to 10 years] and adolescence or adulthood [> 10 years]) ¹⁵. Early-onset patients mostly exhibit a life-threatening acute neurologic deterioration frequently with apnoea following a free interval after birth that can be short (a few weeks). Data on initial phenotype and long-term outcomes of these early-onset patients are scarce, predominantly derived from case reports or small case series ^{14,18–22}. Several authors suggested that early initiation of treatment prevents mortality and allows better neurodevelopmental outcomes ^{1,19,23}. More specifically, a pivotal meta-analysis demonstrated that early betaine treatment prevented mortality and allowed normal psychomotor development in 36 patients with severe MTHFR deficiency ¹⁹. The majority of patients are diagnosed during late infancy and early childhood, with progressive neurologic deterioration ¹, delayed or arrested development and acquired microcephaly ^{1,24}. Adolescent or adult patients exhibit, after a period of normal development or mild developmental delay, a neurologic and/or psychiatric deterioration ¹.

The aims of this study were 1) to retrospectively study clinical and laboratory parameters of patients with early-onset MTHFR deficiency (≤ 3 months of age) at time of diagnosis and at last follow-up visit and 2) to identify predictive factors of severe neurodevelopmental outcomes in a broader set of patients with MTHFR deficiency without restriction of age at disease presentation (early and non-early-onset forms).

Patients and Methods

Study design and setting

A retrospective multicentric cohort study was conducted on 62 patients from the E-HOD registry and 10 separately recruited French patients with MTHFR deficiency.

Subjects, ethical issues and E-HOD registry

The E-HOD (European Network and Registry for Homocystinurias and Methylation Defects) registry was first approved by the Ethics Committee of University Hospital in Heidelberg (N° S-525/2010; 14.3.2013). All participating centres received approval from their local ethics committees before enrolling patients and all patients provided written informed consent before pseudonymized data were entered into the registry. The E-HOD registry documents natural history (symptoms by organs), biochemical and treatment data. It records intelligence and developmental scores (IQ, DQ) from age- appropriate standardized instruments. Standardized self- and proxy report questionnaires inform about neuropsychological development and behaviour. In 2019, participating centres were asked for permission to use the data of the MTHFR-deficient patients for analysis and publication, and the project was approved by the E-HOD Steering Committee. Analysis of data and publication of results was approved by the Ethics Committee of all E-HOD centres and for patients not included in the E-HOD registry (n=7). Regarding the additional survey, informed written consent was obtained from parents for all of the patients regarding reporting patient data. Each of the local ethics committees provided approval for patient data collection.

Data collection

Data were extracted from two sources: the web-based E-HOD registry database URL: <https://www.ehod-registry.org/about-ehod>) and *via* an additional French survey especially designed for this study. Physicians entered the data in this multinational registry at enrolment

and at regular follow-up visits using standardized forms. The additional French survey aimed at identifying a French cohort of children with MTHFR deficiency. For this purpose, a data collection sheet was developed on the basis of relevant literature on the disease and expert discussions. Physicians shared the totality of anonymized medical reports. These reports were used to fill in the questionnaire for each patient of the French cohort. Three of the French patients were already included in E-HOD and data were not duplicated. Of note, forty-three of previously reported patients by Huemer et al. were included into our study. As a pre-requisite for data analysis, we ensured that there was full interoperability of the data models between the E-HOD registry and this French survey.

Data collection included patients' socio-demographic characteristics, medical history, clinical and laboratory data at disease onset and at the last follow-up visit, brain magnetic resonance imaging (MRI), electroencephalogram (EEG) results and treatment modalities.

“Early-onset” and “non-early-onset” disease were defined as “ ≤ 3 months of age” and “ > 3 months of age” at time of diagnosis, respectively, in accordance with literature¹⁵ data. Outcomes were categorized as mild or severe. Severe neurodevelopmental outcome was defined in patient with motor disability ranging from need of assistance to walk to impossibility to walk or to sit alone and/or behavioral problems and/or special educational needs at the time of the last follow-up visit or who died during their follow-up, in accordance with previous studies evaluating neurodevelopmental outcomes²⁵. Mild patients were those whose phenotype did not fall into the “severe” category.

Among the whole cohort of 72 patients with MTHFR deficiency, eight were excluded because of incomplete or missing outcome information.

Statistical analysis

First, early-onset MTHFR patients were described at time of diagnosis and at the last follow-up visit. Continuous variables were presented as median and range (minimum, maximum). Categorical variables were presented as number and/or percentage of the total number of patients with available data. Results were presented as “n/N (%)” where n and N are the concerned patients and the total number of patients with available data respectively. Continuous variables were compared using the Student’s t-test. Normality of the distribution and homogeneity of variances were previously verified.

Second, all MTHFR-deficient patients (early- and late-onset) were analyzed in terms of clinical outcomes. A logistic regression analysis was performed to assess the relation between patients’ characteristics at the time of disease diagnosis and the risk of severe neurodevelopmental outcomes evaluated at the last follow-up visit. Only variables with less than 10% of missing data were included in this analysis. The variables that were not included in the logistic regression model due to missing data >10% were the following: microcephaly at birth, disturbance of consciousness, age at first symptoms, delay of diagnosis, EEG and MRI parameters, plasma total homocysteine concentration at first and last visit, plasma methionine concentration at last visit. Continuous variables were examined for their linearity in relation to the logit of the outcome. In case of non-linearity, a categorization was applied (based on graphical inspection). Factors associated with a risk of severe neurodevelopmental outcome were first identified by univariate analysis. This was followed by a manual backward multivariate logistic regression analysis of variables with p value less than 0.25 in the univariate analysis²⁶. These variables were examined in multiple clinically relevant 2×2 analyses to assess first-order interactions. The odds ratio, with its 95% confidence interval (CI), was computed for each identified factor. The model’s goodness of fit was assessed using the Hosmer-Lemeshow test: the p value of 1 indicated that there was no significant difference

between observed and predicted values. Statistical tests were performed using R software (version 3.6.1).

Results

MTHFR deficiency was suspected based on clinical signs and symptoms except for the patients diagnosed at the pre-symptomatic stage (either prenatally or at birth) associated with equivocal biochemical findings and later on confirmed by the presence of two pathogenic/likely pathogenic variants in the *MTHFR* gene and/or MTHFR enzyme activity in fibroblasts (data not shown). The study cohort consisted of 32 and 40 patients with early and late-onset MTHFR deficiency, respectively: 62 from the E-HOD registry and 10 from the French survey. Patients were followed by 32 European and North American metabolic centers (25 E-HOD partner sites and 7 additional French centers).

Presentation of the 32 patients with early-onset MTHFR deficiency

The population of patients with early-onset MTHFR deficiency included 17 females and 15 males, born between 1985 and 2019.

Pregnancy and birth data

Initial patients' characteristics are detailed in **Table 1 and Supplemental Table 1**. Pregnancy was uneventful in most cases. Nonspecific complications were reported during 5 out of 27 pregnancies (gestational diabetes, folate deficiency, gastroenteritis). The adaptation to extra-uterine life was optimal for all patients (N=10/10). Among patients with early-onset MTHFR deficiency, 28/29 (97%) were term newborns (one individual was born at 36 weeks gestation), 26/27 (96%) had appropriate birth weight for gestational age (GA) and 5/19 (26%) had neonatal microcephaly (*i.e.* head circumference below the third percentile matched for gestational age and sex). Consanguinity was reported in 7/10 (70%) of patients.

Presentation at time of diagnosis

Four of the 32 patients were pre-symptomatically diagnosed thanks to positive family history. The median age at diagnosis was 32 days (range: 6-90 days) for the 28 clinically diagnosed patients. The median diagnosis delay, defined as the time between first symptoms and diagnosis, was 4 days (range: 0-65 days). Major symptoms at diagnosis were feeding difficulties (21/28, 75%) and neurologic symptoms (23/28, 82%) including hypotonia (17/28, 61%), microcephaly (9/28, 32%), and seizures (6/28, 21%). Respiratory failure (including respiratory distress and/or apnoea) was reported in 30% (9/28) of patients. Poor or absent eye contact was reported in 38% (10/26) of patients. Other main symptoms included hypothermia (5/10, 50%), drowsiness and impaired level of consciousness (3/10, 30%), haemodynamic failure (3/10, 30%). Cardiac, kidney, liver diseases and thromboembolic events were rare (2/28 (7%), 0/28 (0%), 2/28 (7%) and 2/28 (7%) of patients respectively.

Initial brain MRI reports were available for nine patients: all were abnormal. Main abnormalities included ventricle dilatation (6/9, 67%), cortical atrophy (5/9, 56%), external CSF spaces dilatation (5/9, 56%) and myelination delay (4/9, 44%) (**Table 1, Supplemental Table 2**).

Seven out of the eight available initial EEGs were abnormal (**Table 1, Supplemental Table 2**).

Among the 22 patients with available data, the median initial plasma tHcy concentration (laboratory reference levels: <10 $\mu\text{mol/L}$) was 202.8 $\mu\text{mol/L}$ (range: 45-353 $\mu\text{mol/L}$) (**Table 1**). Median initial methionine concentration (laboratory reference levels: 17-43 $\mu\text{mol/L}$) was 6 $\mu\text{mol/L}$ (range: 4-18 $\mu\text{mol/L}$). Additional metabolic data are provided in **Figure 1**.

Treatment modalities and outcome at time of the last follow-up visit

Table 2 presents patients' characteristics at the last follow-up visit. One out of the 32 patients died during the follow-up period. This patient was diagnosed at 13 days of life with seizures and feeding problems. He died at 39 months of age from cardiomyopathy that had not been identified at time of diagnosis. The median follow-up time of the 31 other patients was 8.1 years (range: 7.2 months-34.1 years). Median age of patients at the last follow-up visit was 8.6 years (range: 7.5 months-34.3 years) (**Table 2**).

At the last follow-up visit, all patients received betaine with a median daily dose of 157 mg/kg/day (range: 52-350 mg/kg/day). Vitamin B9 (folic or folinic acid) and vitamin B12 (hydroxocobalamin - by intramuscular or oral route - or cyanocobalamin) were administered to 24/29 (83%) and 23/29 (79%) of the patients respectively. Other treatments were inconsistently given: pyridoxine (vitamin B6), riboflavin (vitamin B2), L-methionine, L-carnitine, 5-MTHF were administered to 11/29 (38%), 10/29 (34%), 7/29 (24%), 4/29 (14%) and 2/29 (7%) of the patients respectively. The combinations of (betaine+B12+B9) and (betaine+B12+B9+B6) were the two most frequent combinations used (31% of patients for both – N=29) (**Table 2**).

At the last follow-up visit, 7/23 (30%) of patients had microcephaly. Among them, 17% had had a normal head circumference at birth (acquired microcephaly). Neurologic examination was abnormal in 19/29 (65%) of the patients. The most frequent abnormality was pyramidal syndrome (14/28, 50%) mainly characterized by hyperactive patellar reflexes (10/27, 37%), hypertonia (9/27, 33%), spasticity (9/27, 32%), and abnormal motion range (6/26, 23%). Gait abnormalities (ataxia and/or abnormal balance) were observed in 9/27 (33%) of patients and hypotonia (abnormal head control and/or hypotonia muscle tone) in 9/28 (32%) of patients.

Global abnormal muscle strength concerned 7/24 (29%) of patients. One patient (early-onset) exhibited hydrocephalus.

Seventy-six percent (22/29) of early-onset patients exhibited severe neurodevelopmental outcomes at the last follow-up visit. Gross motor was abnormal in 14/28 (50%) of the patients, with 21% of mild disability, 18% of moderate disability and 11% of severe disability. Fine motor evaluation was abnormal in 14/26 (54%) of patients. Behavioural problems were reported in 13/27 (48%) of patients. Language difficulties (absence of speech development or speech delay) were reported in 7/9 (78%) of patients. Special educational needs were retrieved for 12/20 (60%) of patients. Feeding problems were reported in only two patients; both had a G-tube (2/23, 9%). Forty-eight percent of patients (14/29) exhibited eye issues (mainly nystagmus and strabismus; and more rarely uncoordinated eye movements, optic nerve atrophy, cataract). Treatment significantly reduced the frequency of feeding problems ($p < 0.001$). There were no other affected organ systems. Between time of diagnosis and last follow-up visit, “general state and overall well-being” were considered stable over time for 14/21 (67%) and were improved for 4/21 (19%) of patients (**Table 2**).

Among the patients with abnormal initial brain MRI, 6/9 (67%) had severe neurodevelopmental outcomes at the last follow-up visit. EEG was abnormal for 14/17 (82%) of patients. Clinical epilepsy was reported in 3/10 (30%) of patients and was easily managed with one antiepileptic drug.

As shown in **Figure 1**, plasma tHcy concentrations significantly decreased under treatment for all patients (on average decreased by 1.8 times $p < 0.001$), but remained higher than the reference levels for 26/28 (93%) of them. Plasma tHcy decreased below 70 $\mu\text{mol/L}$ for 2/28 (7%) of patients. Methionine plasma concentrations significantly increased under treatment (on average increased by more than 3 times $p < 0.001$) and were in the laboratory reference

range for all patients with available data (N=24) at the last follow-up visit (**Figure 1**). Plasma tHcy concentrations were not significantly different in patients with severe *vs* non-severe outcome at the initial evaluation (p=0.35) and at the last follow-up visit (p=0.93).

Predictors of severe neurodevelopmental outcomes in 64 patients with early and non-early-onset MTHFR deficiency

Among the whole cohort of 72 patients with MTHFR deficiency, eight were excluded because of incomplete or missing outcome information. Among the remaining 64 patients, 30 and 34 patients had early-onset and late-onset disease manifestation, respectively. The median follow-up time was 8.2 years (range: 94 days-37.4 years). Factor(s) associated with a risk of severe neurodevelopmental outcome are shown in **Table 3** (univariate analysis) and **Table 4** (multivariate analysis). Four factors were introduced in the multivariate regression analysis because of p-values less than 0.25 in the univariate analysis: age at diagnosis (with 53% of patients with early-onset disease in the severe outcome group *vs* 33% in the other group), Caucasian ethnic background (with 77% in the severe outcome group *vs* 62%), respiratory failure at time of diagnosis (with 28% of patients presenting respiratory failure in the severe outcome group *vs* 14%), and pre-symptomatic disease diagnosis (with 2% of pre-symptomatically diagnosed patients in the severe outcome group *vs* 38% in the non-severe group). Follow-up time was also added to the multivariate analysis because of a potential clinically confounding effect. Pre-symptomatic disease diagnosis was the only independent factor associated with outcome in multivariate analysis. Pre-symptomatically diagnosed patients (see next paragraph) were significantly less likely to exhibit severe neurodevelopmental outcomes (aOR 0.04, [95% CI, 0.002-0.232], p=0.003). We did not identify any significant interaction between pre-symptomatic diagnosis and early-/late-onset disease. The goodness of fit of the model was assessed using the Hosmer-Lemeshow test: the

p value of 1 indicated that there was no significant difference between observed and predicted values.

Focus on the pre-symptomatically diagnosed patients (n=9)

As pre-symptomatic diagnosis was identified as an independent protective factor for severe neurodevelopmental outcome, the following information focused on this subset of patients (**Supplemental Tables 1 and 3**). These nine patients were pre-symptomatically diagnosed and treated because of a symptomatic sibling. Nine out of nine patients exhibited normal cognitive outcome. Eight of nine patients did not exhibit any symptom during the follow-up period. One patient required special educational needs due to severe visual problems and therefore potentially arbitrarily fell into the severe group. Regardless, he exhibited a normal neurologic examination and a normal neurodevelopment. The neurologic examination was normal for 9/9 patients at the last follow-up visit (range: 3 months old – 52 years old). For two pre-symptomatic patients, information were available on the outcomes of their late treated sibling showing unequivocally a much poorer outcome in the late treated than in the pre-symptomatic ones. Initial plasma tHcy concentrations did not significantly differ from clinically diagnosed patients ($p=0.17$) (**Supplemental Figure 1**). Plasma tHcy decreased from diagnosis to the last follow-up visit in all cases. Plasma tHcy at the last follow-up visit did not significantly differ from clinically diagnosed patients ($p=0.89$). In keeping with this, we analysed treatment data in these nine pre-symptomatic patients. In terms of treatment either at initial management or for long-term therapy, no major differences was observed among patients (**Supplemental Table 3**): all of the patients for whom data were available (5/5) were treated with betaine at a median dose of 174.5 mg/kg/d (range 44-284). Remaining therapies were variable and included, folic or folinic acid, L-Methionine and vitamin B12.

Discussion

Though previous studies already reported clinical data on MTHFR-deficient patients, these studies either included late-onset MTHFR deficiency or did not focus on neurodevelopmental outcomes^{23,27}. Huemer *et al* analysed the clinical presentation of 50 MTHFR-deficient patients²⁸. Forty-three of these previously reported patients (including two with pre-symptomatic diagnosis) were included into our study, which, in contrast to the earlier publication focused on early-onset presentation and neurodevelopmental outcomes of MTHFR-deficient patients.²⁸

We reported in detail clinical and laboratory parameters of 32 patients with an age of less than 3 months at time of diagnosis. Seventy six % of these patients exhibited severe neurodevelopmental outcomes at the last follow-up visit. The median follow-up period was 8 years for these early-onset patients. Using multivariate analysis, factors associated with severe neurodevelopmental outcomes were assessed in 64 patients with early and late-onset MTHFR deficiency. Our data emphasizes that pre-symptomatic diagnosis and subsequent treatment are associated with a significantly better outcome.

Similar to literature data^{15,18}, our patients with early-onset MTHFR deficiency were born after uneventful pregnancy, delivery and perinatal adaptation. Among the 32 patients with early-onset MTHFR deficiency, more than 70% exhibited feeding difficulties and neurologic symptoms (mainly muscular hypotonia) at time of diagnosis. In brain, remethylation of homocysteine into methionine is exclusively dependent on 5-MTHF¹⁸ since BHMT is not expressed in this tissue²⁹. This could explain the particular vulnerability of the CNS in MTHFR deficiency.

Six out of 32 patients exhibited central apnoea at time of diagnosis as reported elsewhere^{1,8,18,30}.

Detailed analysis of initial brain MRI showed abnormalities in all patients with available data. Ventricle dilatation, cortex atrophy and myelination delay were among the most frequent MRI abnormalities, similar to previous case series ^{15,31}. Low methionine levels and concomitant SAM deficiency characterizing the disease were shown to be associated with demyelination ^{8,32}, as well as accumulation of SAH that would also contribute to hypomyelination ¹¹. Of note, one patient of the early-onset disease cohort needed a ventriculoperitoneal shunt during follow-up. This complication was previously reported in a few case reports and points to a malformation of the neural tube in mid-gestation at the origin of hydrocephalus ^{16,33}.

Deficiency of methionine and SAM, affecting protein synthesis and all methylation dependent activities, possibly provides the basis for epileptogenesis ²⁴. A wide variation in seizure phenotypes was documented in previous case reports ^{8,16,33}. Unfortunately, it was not possible to precisely describe epilepsy in the present retrospective study apart from its occurrence in 30% of the patients. Similarly, data on psychometric tests were missing.

Ophthalmologic manifestations were frequent (38%) in the present cohort, with a wide heterogeneity of clinical damages. Patients mainly exhibited strabismus and nystagmus, but a variety of symptoms were also inconsistently observed such as uncoordinated eye movements, optic nerve atrophy, and secondary cataract. Frequency of ophthalmologic issues is variable in the literature ^{18,23,34}. The precise pathomechanism has not yet been elucidated, and multiple factors have been considered to be involved ²³. Studies on human and mouse models mainly suggest the role of hyper-homocysteinemia in inducing retinal degeneration and/or impairing retinal function ³⁵⁻³⁷. MTHFR deficiency in mice was suggested to induce eye disease ³⁷. More importantly, impaired vision might be due to visual cortex damage, even though no related MRI lesion was identified ³⁴. Given our results, we recommend a frequent eye follow-up for early-onset MTHFR-deficient patients. As compromised remethylation processes play an important role in the poor removal of retinal homocysteine, decrease of

plasma tHcy levels might improve retinal damage ³⁸. Frequency of eye issues was not significantly improved under treatment in line with recent literature data ²⁸.

In our cohort, “general state and overall well-being” were considered stable in 14/21 patients and improved in only 4/21 patients with early-onset MTHFR deficiency. Despite the short diagnosis delay (median of 4 days), a total of 76% of treated patients exhibited severe neurodevelopmental outcomes as reported in the literature ²³. Irreversible brain damage caused by severe demyelination may explain this finding ³⁴. Plasma tHcy levels significantly decreased under treatment but were not normalized at the last follow-up visit, in line with previous findings ¹. There is no clear explanation for that.

It was previously argued that decreasing plasma tHcy to 50 to 70 $\mu\text{mol/L}$ was a reasonable goal of treatment in patients with remethylation disorders ¹ but it could be achieved for a small number of patients only. In contrast to plasma tHcy concentrations, plasma methionine concentrations were normalized at the time of last follow-up in all of the patients with early-onset MTHFR deficiency as previously reported ^{7,8,32}. Of note, normalization of CSF SAM level was reported to be associated with improved remyelination under betaine treatment ⁸. Therefore, one could hypothesize that pre-symptomatic treatment would be beneficial because of normalization of methionine and SAM with correction of the remethylation defect in spite of persisting high tHcy concentrations.

The multivariate analysis on 64 patients with early and late-onset MTHFR deficiency highlighted that pre-symptomatic diagnosis was an independent protective factor for severe neurodevelopmental outcome. This significantly better neurodevelopmental outcome of pre-symptomatically diagnosed patients highlights the need for timely diagnosis through newborn screening (NBS). Indeed, NBS is indicated if early intervention is effective with a better outcome in a severe disease generally not detectable early in life on a clinical basis, with

sensitive and specific biochemical markers ³⁹. A prospective study of pre-symptomatically diagnosed and treated patients preferably identified through NBS would be warranted to further confirm this benefit. MTHFR deficiency is currently detected by very few NBS programs using methionine concentration and methionine-to-phenylalanine ratio in dried blood spots (DBS) and DBS tHcy concentrations as a second tier test ⁴⁰⁻⁴³. The yield of methionine or methionine/phenylalanine ratios seem however imperfect and the cost of tHcy determination on DBS high therefore questioning the practical feasibility of NBS for MTHFR deficiency. Regardless, an argument for NBS is the availability of a generally effective therapy for MTHFR deficiency *i.e.* betaine. This would however require confirmation by a prospective study.

Despite the previously suggested different clinical patterns between early and non-early-onset forms of the disease ^{1,23}, our results did not show any significant impact of age at diagnosis on the risk of severe neurodevelopmental outcomes among clinically-diagnosed patients ($p=0.13$). The limited number of patients can explain this result.

The limitations of this study are inherent with the retrospective methodology. The E-HOD registry includes data from several countries with different health care systems and standards of care. Treatment regimens varied by site, and there was a high inter-patient variability regarding schedules of follow-up visits. Thus, the main result regarding neurodevelopmental outcomes should be adjusted on this potentially confounding variable. Second, the problem of a relatively high proportion of missing data needs to be highlighted. For the same reason, it was not possible to integrate microcephaly, median diagnosis delay, initial plasma tHcy concentrations and initial brain MRI abnormalities, in the prognosis model. Importantly microcephaly at birth was not associated with a more severe outcome (not shown). Due to the high proportion of missing data regarding speech difficulties, it was not possible to include

this item in our composite criteria of severe neurodevelopmental outcome. All these criteria should be assessed in further larger studies.

Conclusions

Paediatricians need to be aware of MTHFR deficiency as a rare and severe but potentially treatable inborn error of metabolism. Treatment should be initiated as early as possible, prior to irreversible brain damage. As previously alluded by a seminal meta-analysis¹⁹, this study provides clear evidence for a benefit of pre-symptomatic diagnosis and subsequent therapeutic management, highlighting the need for newborn screening for MTHFR deficiency and pre-symptomatic treatment that may improve outcomes.

Acknowledgements

The authors would like to thank patients and data managers for providing data for the registry. Several authors of this publication are members of the European Reference Network for Rare Hereditary Metabolic Disorders (MetabERN) - Project ID No 739543. The European network and registry for homocystinurias and methylation defects (EHOD) project has been established with funding from the European Union in the framework of the Health Program (Chafea grant N°. 2012 12 02). From the end of the EU-project phase, the E-HOD project has received ongoing support from SOBI, Recordati Rare Disease Foundation, Vitaflo, Aeglea, and Nutricia Metabolics, Germany. E-HOD was initially funded by the European Union in the framework of the Health Program, Chafea grant no. 2012 12 02; registry coordination and management: F. Gleich and S. Kölker, Heidelberg.

E-HOD consortium members

Ana Maria Martins²⁸, Javier Blasco Alonso²⁹, Brigitte Chabro³⁰, Ellen Crushell³¹, Carlo Dionisi-Vici³², Stephanie Grünewald³³, Karine Mention³⁴, Helen Mundy³⁵, Elaine Murphy³⁶,

Pilar Quijada Fraile³⁷, Carlos José Ruiz³⁸, Maria Ángeles Ruiz Gómez³⁹, Saikat Santra⁴⁰,
Thomas Scherer⁴¹, Collette Stainforth⁴², Karolina M. Stepień⁴³, Gere Sunder-Plassmann⁴⁴,
Johan L.K. Van Hove⁴⁵

Accepted Article

Affiliations of E-HOD consortium members

²⁸ Centro de Referência em Erros Inatos do Metabolismo, Universidade Federal de São Paulo (UNIFESP), Sao Paulo, Brazil

²⁹ Pediatric Gastroenterology and Nutrition Unit, Hospital Regional Universitario de Málaga, Malaga, Spain

³⁰ Service de neurologie pédiatrique, hôpital d'Enfants, CHU de la Timone, 13385 Marseille Cedex 05, France

³¹ National Centre for Inherited Metabolic Disorders, Children's Health Ireland, Temple St, Dublin Ireland and University College, Dublin, Ireland

³² Division of Metabolism, Bambino Gesù Children's Research Hospital, Rome, Italy

³³ Metabolic Unit, Great Ormond Street Hospital, Institute of Child Health, Great Ormond Street, London

³⁴ Centre de référence des Maladies Héritaires du métabolisme, Hôpital Jeanne de Flandre, Lille, France

³⁵ Evelina London Children's Hospital, London, United Kingdom

³⁶ Charles Dent Metabolic Unit, National Hospital for Neurology and Neurosurgery, London, United Kingdom

³⁷ Pediatrics, Hospital Universitario 12 de Octubre, Madrid, Spain

³⁸ Paediatric Gastroenterology, Hepatology and Nutrition, Hospital Sant Joan de Déu, Barcelona, Spain

³⁹ Metabolic Neuropediatric Unit, University Hospital Son Espases, Palma de Mallorca, Spain

⁴⁰ Department of Clinical Inherited Metabolic Disorders, Birmingham Women's and Children's NHS Foundation Trust, Birmingham, United Kingdom

⁴¹ Division of Endocrinology and Metabolism, Department of Medicine III, Medical University of Vienna, Vienna, Austria

⁴² Bradford Teaching Hospitals NHS Foundation Trust, Bradford, United Kingdom

⁴³ Adult Inherited Metabolic Diseases, Salford Royal NHS Foundation Trust, Salford, United Kingdom

⁴⁴ Medical University Vienna, Department of Medicine III, Division of Nephrology and Dialysis, Vienna, Austria

⁴⁴ Department of Pediatrics, Clinical Genetics and Metabolism, Children's Hospital Colorado, Denver, Colorado, USA

Figures legends

Figure 1. Evolution of plasma total homocysteine and methionine concentrations in patients with early-onset MTHFR deficiency

Plasma total homocysteine (A) and methionine (B) concentrations in early onset MTHFR patients at the time of diagnosis (circle), at the first visit after treatment initiation (square) and at the last follow-up visit (triangle). Upper reference range (total homocysteine, 10 μ M) and low and upper reference range (methionine, 17-43 μ M) are represented by grey dashed line(s).

Table 1. Initial presentation of 32 patients with early-onset MTHFR deficiency

Table 2. Presentation of 32 patients with early-onset MTHFR deficiency, at their last follow-up visit

Table 3. Factors predictive of severe neurodevelopmental outcomes

Table 4. Independent factors predictive of severe neurodevelopmental outcomes in multivariate analysis in univariate analysis

References

1. Schiff M, Blom H. Treatment of Inherited Homocystinurias. *Neuropediatrics*. 2012;43(06):295-304.
2. Matthews RG, Sheppard C, Goulding C. Methylene tetrahydrofolate reductase and methionine synthase: biochemistry and molecular biology. *Eur J Pediatr*. 24 mars 1998;157(S2):S54-9.
3. Huemer M, Baumgartner MR. The clinical presentation of cobalamin-related disorders: From acquired deficiencies to inborn errors of absorption and intracellular pathways. *J Inherit Metab Dis*. juill 2019;42(4):686-705.
4. Castro R, Rivera I, Blom HJ, Jakobs C, de Almeida IT. Homocysteine metabolism, hyperhomocysteinaemia and vascular disease: An overview. *J Inherit Metab Dis*. 2006;29(1):3-20.
5. Mattson MP, Shea TB. Folate and homocysteine metabolism in neural plasticity and neurodegenerative disorders. *Trends Neurosci*. 2003;26(3):137-46.
6. Kishi T, Kawamura I, Harada Y, Eguchi T, Sakura N, Ueda K, et al. Effect of betaine on S-adenosylmethionine levels in the cerebrospinal fluid in a patient with methylene tetrahydrofolate reductase deficiency and peripheral neuropathy. *J Inherit Metab Dis*. 1994;17(5):560-5.
7. Strauss KA, Morton DH, Puffenberger EG, Hendrickson C, Robinson DL, Wagner C, et al. Prevention of brain disease from severe 5,10-methylene tetrahydrofolate reductase deficiency. *Mol Genet Metab*. 2007;91(2):165-75.
8. Surtees R, Leonard J, Austin S. Association of demyelination with deficiency of cerebrospinal-fluid S-adenosylmethionine in inborn errors of methyl-transfer pathway. *The Lancet*. 1991;338(8782-8783):1550-4.
9. D'Aco KE, Bearden D, Watkins D, Hyland K, Rosenblatt DS, Ficicioglu C. Severe 5,10-Methylene tetrahydrofolate Reductase Deficiency and Two MTHFR Variants in an Adolescent With Progressive Myoclonic Epilepsy. *Pediatr Neurol*. 2014;51(2):266-70.
10. Wang X, Chen W, Fu X, Ma J, Wang M, Hou Y, et al. Reversal of homocysteine-induced neurotoxicity in rat hippocampal neurons by astaxanthin: evidences for mitochondrial dysfunction and signaling crosstalk. *Cell Death Discov*. 2018;4(1):50.
11. Schatz RA, Wilens TE, Sellinger OZ. Decreased Transmethylation of Biogenic Amines After In Vivo Elevation of Brain S-Adenosyl-L-Homocysteine. *J Neurochem*. 1981;36(5):1739-48.
12. Surtees R. Demyelination and inborn errors of the single carbon transfer pathway. *Eur J Pediatr*. 1998;157(S2):S118-21.

- Accepted Article
13. Chiang PK, Gordon RK, Tal J, Zeng GC, Doctor BP, Pardhasaradhi K, et al. S-Adenosylmethionine and methylation. *FASEB J.* 1996;10(4):471-80.
 14. Schiff M, Benoist J-F, Tilea B, Royer N, Giraudier S, Ogier de Baulny H. Isolated remethylation disorders: do our treatments benefit patients? *J Inherit Metab Dis.* 2011;34(1):137-45.
 15. Ogier de Baulny H, Gérard M, Saudubray JM, Zittoun J. Remethylation defects: guidelines for clinical diagnosis and treatment. *Eur J Pediatr.* 1998;157(S2):S77-83.
 16. Holme E, Kjellman B, Ronge E. Betaine for treatment of homocystinuria caused by methylenetetrahydrofolate reductase deficiency. *Arch Dis Child.* 1989;64(7):1061-4.
 17. Knowles L, Morris A a. M, Walter JH. Erratum to: Treatment with Mefolate (5-Methyltetrahydrofolate), but Not Folic Acid or Folinic Acid, Leads to Measurable 5-Methyltetrahydrofolate in Cerebrospinal Fluid in Methylenetetrahydrofolate Reductase Deficiency. *JIMD Rep.* 2016;29:117.
 18. Huemer M, Mulder-Bleile R, Burda P, Froese DS, Suormala T, Zeev BB, et al. Clinical pattern, mutations and in vitro residual activity in 33 patients with severe 5, 10 methylenetetrahydrofolate reductase (MTHFR) deficiency. *J Inherit Metab Dis.* 2016;39(1):115-24.
 19. Diekman EF, de Koning TJ, Verhoeven-Duif NM, Rovers MM, van Hasselt PM. Survival and Psychomotor Development With Early Betaine Treatment in Patients With Severe Methylenetetrahydrofolate Reductase Deficiency. *JAMA Neurol.* 2014;71(2):188.
 20. Al Tawari AA, Ramadan DG, Neubauer D, Heberle LC, Al Awadi F. An early onset form of methylenetetrahydrofolate reductase deficiency: a report of a family from Kuwait. *Brain Dev.* 2002;24(5):304-9.
 21. Balasubramaniam S, Salomons G, Blom H. A case of severe methylenetetrahydrofolate reductase deficiency presenting as neonatal encephalopathy, seizures, microcephaly and central hypoventilation. *J Pediatr Neurol.* 2015;11(02):135-40.
 22. Watkins D, Rosenblatt DS. Update and new concepts in vitamin responsive disorders of folate transport and metabolism. *J Inherit Metab Dis.* 2012;35(4):665-70.
 23. Huemer M, Diodato D, Martinelli D, Olivieri G, Blom H, Gleich F, 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):333-52.
 24. Prasad AN, Rupa CA, Prasad C. Methylenetetrahydrofolate reductase (MTHFR) deficiency and infantile epilepsy. *Brain Dev.* 2011;33(9):758-69.
 25. Duncan AF, Matthews MA. Neurodevelopmental Outcomes in Early Childhood. *Clin Perinatol.* 2018;45(3):377-92.
 26. Zhang Z. Model building strategy for logistic regression: purposeful selection. *Ann Transl Med.* 2016;4(6):7.

- Accepted Article
27. Marelli C, Lavigne C, Stepien KM, Janssen MCH, Feillet F, Kožich V, et al. Clinical and molecular characterization of adult patients with late-onset MTHFR deficiency. *J Inherit Metab Dis.* 2021;44(3):777-86.
 28. Huemer M, Diodato D, Martinelli D, Olivieri G, Blom H, Gleich F, 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):333-52.
 29. McKeever MP, Weir DG, Molloy A, Scott JM. Betaine-homocysteine methyltransferase: organ distribution in man, pig and rat and subcellular distribution in the rat. *Clin Sci.* 1991;81(s25):551-6.
 30. Fattal-Valevski A, Bassan H, Korman SH, Lerman-Sagie T, Gutman A, Harel S. Methylene tetrahydrofolate Reductase Deficiency: Importance of Early Diagnosis. *J Child Neurol.* 2000;15(8):539-43.
 31. Engelbrecht V, Rassek M, Huismann J, Wendel U. MR and Proton MR Spectroscopy of the Brain in Hyperhomocysteinemia Caused by Methylene tetrahydrofolate Reductase Deficiency. 1997;4.
 32. Hyland K, Smith I, Bottiglieri T, Perry J, Wendel U, Clayton PT, et al. Demyelination and decreased S-adenosylmethionine in 5,10-methylene tetrahydrofolate reductase deficiency. *Neurology.* 1988;38(3):459-62.
 33. Baethmann M, Wendel U, Hoffmann GF, Göhlich-Ratmann G, Kleinlein B, Seiffert P, et al. Hydrocephalus Internus in Two Patients with 5,10-Methylene tetrahydrofolate Reductase Deficiency. *Neuropediatrics.* 2000;31(6):314-7.
 34. Ronge E, Kjellman B. Long term treatment with betaine in methylene tetrahydrofolate reductase deficiency. *Arch Dis Child.* 1996;74(3):239-41.
 35. Tsina EK, Marsden DL, Hansen RM, Fulton AB. Maculopathy and retinal degeneration in cobalamin C methylmalonic aciduria and homocystinuria. *Arch Ophthalmol Chic Ill* 1960. 2005;123(8):1143-6.
 36. Lahey JM, Kearney JJ, Tunc M. Hypercoagulable states and central retinal vein occlusion. *Curr Opin Pulm Med.* 2003;9(5):385-92.
 37. Lawrance AK, Racine J, Deng L, Wang X, Lachapelle P, Rozen R. Complete deficiency of methylene tetrahydrofolate reductase in mice is associated with impaired retinal function and variable mortality, hematological profiles, and reproductive outcomes. *J Inherit Metab Dis.* 2011;34(1):147-57.
 38. Kowluru RA, Mohammad G, Sahajpal N. Faulty homocysteine recycling in diabetic retinopathy. *Eye Vis.* 2020;7(1):4.
 39. Wilson J, Jungner F. Principles and Practice of Screening for Disease. *Public Health Pap.* 1968;34.

40. Huemer M, Kožich V, Rinaldo P, Baumgartner MR, Merinero B, Pasquini E, et al. Newborn screening for homocystinurias and methylation disorders: systematic review and proposed guidelines. *J Inherit Metab Dis.* 2015;38(6):1007-19.
41. Gramer G, Abdoh G, Ben-Omran T, Shahbeck N, Ali R, Mahmoud L, et al. Newborn screening for remethylation disorders and vitamin B12 deficiency-evaluation of new strategies in cohorts from Qatar and Germany. *World J Pediatr.* 2017;13(2):136-43.
42. Tortorelli S, Turgeon CT, Lim JS, Baumgart S, Day-Salvatore D-L, Abdenur J, et al. Two-Tier Approach to the Newborn Screening of Methylenetetrahydrofolate Reductase Deficiency and Other Remethylation Disorders with Tandem Mass Spectrometry. *J Pediatr.* 2010;157(2):271-5.
43. Keller R, Chrastina P, Pavlíková M, Gouveia S, Ribes A, Kölker S, et al. Newborn screening for homocystinurias: Recent recommendations versus current practice. *J Inherit Metab Dis.* 2019;42(1):128-39.

Table 1. Initial presentation of 32 patients with early-onset MTHFR deficiency

	Total number of patients with available data (N)	Number	Median (range)
Demographic data			
Gender (Female/Male)	32	17/15	
Ethnic background	30		
Caucasian		17	
Asian		6	
Black		4	
Arab		3	
Pregnancy and birth data			
Normal pregnancy	27	22	
Gestational age (weeks)	29		39 (36 – 41)
Birth weight (grams)	27		3423 (1830 – 4300)
Head circumference (cm)	19		34 (32 – 36)
Diagnosis data			
Pre-symptomatically diagnosed	32	4	
Age at diagnosis ^a (days)	28		32 (6 – 90)
Diagnosis delay ^a (days)	25		4 (0 – 65)
Symptoms at time of diagnosis	28		
Feeding problems	28	21	
Neurologic symptoms	28	23	
Hypotonia	28	17	
Microcephaly	28	9	
Developmental delay	28	8	
Seizure	28	6	
Myelopathy	21	0	
Respiratory failure	28	9	
Eye issues	26	10	
Other symptoms	10	6	
Brain MRI ^b at time of diagnosis			
Normal	9	0	
Dilated ventricles	9	6	
External cerebrospinal fluid (CSF) spaces dilated	9	5	
Cortex atrophy	9	5	
Myelination delay	9	4	
Deep gray matter structure abnormality	9	2	
White matter T2/Flair hyperintensity	7	3	
Pontine or cerebellar hypoplasia	9	5	
Corpus callosum agenesis	9	2	
Brainstem abnormality	9	1	
Thrombosis or stroke	9	1	
EEG ^c at time of diagnosis	8		
Abnormal basal rhythm		7	
Paroxysmal abnormalities		6	
Laboratory parameters at time of diagnosis			
Plasma total homocysteine concentration (μmol/L) ^d	22		202.8 (45-353) [154.25; 233.25] ^f
Plasma methionine concentration (μmol/L) ^e	10		6 (4-18) [5; 7] ^f

^a among clinically diagnosed patients; ^b Magnetic Resonance Imaging; ^c Electroencephalogram;

^d reference laboratory level <10 μmol/L; ^e reference laboratory level 17-43 μmol/L; ^f [25th percentile; 75th percentile]

Table 2. Presentation of 32 patients with early-onset MTHFR deficiency, at their last follow-up visit

	Total number of patients with available data (N)	Number	Median (range)
<u>Treatment modalities</u>	29		
Betaine		29	157 (52-350) ^a
B9 (folinic acid)		17	0.8 (0.1-14) ^a
B9 (folic acid)		7	0.05 (0.04-0.37) ^a
B12 (hydroxocobalamin)		19	0.03 (0.0003-0.11) ^b
B12 (cyanocobalamin)		4	0.03 (0.006-0.05) ^a
B6 (pyridoxine)		11	2.7 (0.39-22) ^a
Riboflavine		10	0.46 (0.09-8.9) ^a
L-Methionine		7	8 (4-12.5) ^a
L-Carnitine		4	8.8 (7.4-31.5) ^a
5-Methyltetrahydrofolate		2	0.3 (0.07-0.5) ^a
<u>Clinical data</u>			
Age at the last follow-up visit	32		8.6 y (7.5 m-34.3 y)
Microcephaly	23	7	
Analytic neurologic examination data			
Pyramidal syndrome	28	14	
Hypotonia	28	9	
Extrapyramidal syndrome	28	2	
Gait abnormalities	27	9	
Muscle strength abnormalities	24	7	
Clinical epilepsy	10	3	
Neurodevelopmental outcome data			
Gross motor abnormalities	28	14	
Interactions abnormalities	27	13	
Fine motor abnormalities	26	14	
Language abnormalities	9	7	
Special educational needs	20	12	
Visual problems	29	14	
Feeding problems	23	2	
General state and overall well-being	21		
Stable		14	
Improved		4	
Deteriorated		3	
Other affected organ systems			
Thromboembolic events	30	0	
Abnormal liver size	26	0	
Cardiac disease	26	0	
Psychiatric disease	10	4	
<u>Laboratory data</u>			
Plasma total homocysteine concentration (μmol/L) ^c	28		111 (16-228)
Plasma methionine concentration (μmol/L) ^d	24		21 (10-45)
<u>Electroencephalographic examination</u>	17		
Abnormal result		14	

^a daily oral dose in mg/kg/day; ^b intramuscular dose in mg/kg/day; ^c reference laboratory level <10 μmol/L;

^d reference laboratory level 17-43 μmol/L

Table 3. Factors predictive of severe neurodevelopmental outcomes^a in univariate analysis

	Patients, n (%)		OR (95% CI) ^b	P value
	Severe outcome (N=43)	Non-severe outcome (N=21)		
Characteristics at time of diagnosis				
Age ≤ 3 months (early-onset disease)	23 (53.4)	7 (33.3)	2.3 (0.8-7.2)	0.13
Sex male	21 (48.9)	12 (57.1)	0.7 (0.2-2.0)	0.53
Caucasian ethnic background	33 (76.7)	13 (61.9)	2.0 (0.6-6.3)	0.22
Pre-symptomatic diagnosis	1 (2.3)	8 (38.1)	0.039 (0.002-0.238)	0.003
Seizures	13 (30.2)	4 (19.0)	1.8 (0.6-7.3)	0.34
Respiratory failure	12 (27.9)	3 (14.3)	2.3 (0.6-11.2)	0.23
Follow-up time > 11 years	22 (51.1)	13 (61.9)	0.6 (0.2-1.8)	0.42

^a severe neurodevelopmental outcome is defined as patients with moderate or severe gross motor disability and/or behavioral problems and/or special educational needs at time of the last follow-up visit or who died during their follow-up; ^b CI, confidence interval; OR, odds ratio

Table 4. Independent factors predictive of severe neurodevelopmental outcomes^a in multivariate analysis

	aOR (95% CI) ^b	P value
Pre-symptomatic diagnosis	0.04 (0.002-0.232)	0.003
Follow-up time > 11 years	0.57 (0.15-1.91)	ns

^a severe neurodevelopmental outcome is defined as patients with moderate or severe gross motor disability and/or behavioral problems and/or special educational needs at time of the last follow-up visit or who died during their follow-up; CI, confidence interval; ^b aOR, adjusted odds ratio; ns, not significant

