

Cerebral Folate Deficiency Syndromes in Childhood

Clinical, Analytical, and Etiologic Aspects

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Background: Cerebral folate deficiency may be amenable to therapeutic supplementation. Diverse metabolic pathways and unrelated processes can lead to cerebrospinal fluid 5-methyltetrahydrofolate (5-MTHF) depletion, the hallmark of cerebral folate deficiency.

Objective: To analyze cerebral folate abundance in a large prospective series of children diagnosed with any neurologic disorder for which a diagnostic lumbar puncture was indicated.

Design: We studied the spectrum and frequency of disorders associated with cerebral folate deficiency by measuring cerebrospinal fluid 5-MTHF, biogenic amines, and pterins. Direct sequencing of the *FOLR1* transporter gene was also performed in some patients.

Setting: Academic pediatric medical center.

Participants: We studied 134 individuals free of neurometabolic disease and 584 patients with any of several diseases of the central nervous system.

Results: Of 584 patients, 71 (12%) exhibited 5-MTHF deficiency. Mild to moderate deficiency ($n=63$; range,

19–63 nmol/L) was associated with perinatal asphyxia, central nervous system infection, or diseases of probable genetic origin (inborn errors of metabolism, white matter disorders, Rett syndrome, or epileptic encephalopathies). Severe 5-MTHF depletion ($n=8$; range, 0.6–13 nmol/L) was detected in severe MTHF reductase deficiency, Kearns-Sayre syndrome, biotin-responsive striatal necrosis, acute necrotizing encephalitis of Hurst, and *FOLR1* defect. A strong correlation was observed between cerebrospinal fluid and plasma folate levels in cerebral folate deficiency.

Conclusions: Of the 2 main forms of cerebral folate deficiency identified, mild to moderate 5-MTHF deficiency was most commonly associated with disorders bearing no primary relation to folate metabolism, whereas profound 5-MTHF depletion was associated with specific mitochondrial disorders, metabolic and transporter defects, or cerebral degenerations. The results suggest that 5-MTHF can serve either as the hallmark of inborn disorders of folate transport and metabolism or, more frequently, as an indicator of neurologic dysfunction.

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FOlate is a B vitamin essential for brain metabolism. It participates in the de novo synthesis of purines and thymidine and, together with cobalamin, represents an important cofactor for homocysteine remethylation and S-adenosylmethionine production by the brain. As with other vitamin deficiency states, folate deficiency may be the consequence of acquired or inherited disorders. Several inborn errors of metabolism can lead to defective folate transport or impaired metabolism, resulting in systemic folate deficiency and, obligatorily, 5-methyltetrahydrofolate (5-MTHF) depletion in the nervous system detectable by assay of cerebrospinal fluid (CSF) levels.¹ Cerebral folate deficiency (CFD), however, is characterized by decreased concentrations

of 5-MTHF in the CSF in the context of normal systemic folate metabolism.² The principal mechanistic hypothesis put forth for this condition implies defective transport of folate across the blood-CSF or blood-brain barriers.^{3,4} Indeed, mutations in the *FOLR1* gene (OMIM 136430), encoding the folate receptor α (FR α), can cause brain-specific folate transport deficiency leading to neurodegeneration in early childhood.⁵ In other patients with CFD, blocking autoantibodies against FRs may also play a pathogenic role.⁶

In contrast with these observations, CFD caused by less understood mechanisms has also been identified in a variety of neurologic disorders, mostly as part of anecdotal reports or small case series.^{6–10} However, to our knowledge, no systematic analysis of 5-MTHF in central ner-

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nervous system (CNS) disorders has yet been performed, so both the prevalence and the significance of low 5-MTHF concentrations in diseases of the nervous system in children remain unknown.

We set out to analyze cerebral folate abundance in a large prospective series of children diagnosed with any neurologic disorder for which a diagnostic lumbar puncture was indicated. As a prerequisite to this approach, we first established control values of CSF 5-MTHF in a pediatric population. Subsequently, we determined 5-MTHF, biogenic amine, and pterin concentrations in CSF as well as total serum folate levels in a cohort of children diagnosed with neurologic disorders. Finally, after stratification of 5-MTHF deficiency, we further investigated select individuals characterized by a profound reduction of 5-MTHF in CSF and decreased CSF to plasma folate ratio not explained by other known causes of CFD, and we detected mutations in the *FOLR1* transporter gene associated with severe manifestations of disease.

METHODS

Samples from patients and controls were obtained in accordance with the Helsinki Declaration of 1964, revised in 2000. Informed consent was obtained from parents, and the Ethics Committee of the Hospital Sant Joan de Déu approved the study.

PATIENTS

We analyzed CSF samples obtained from 625 patients with encephalopathies investigated at the Department of Paediatric Neurology of Hospital Sant Joan de Déu from 2005 to 2010. These CSF samples were analyzed as part of the diagnostic investigation of conditions that alter the cellular or biochemical profile of the CSF, including CFD, primary and secondary disorders of biogenic amines and pterin metabolism, amino acid and organic acid defects, glucose transporter type 1 deficiency, mitochondrial disorders, and other acquired (nonmetabolic) conditions. An initial analysis (including cell count, protein, lactate, and glucose abundance) was performed in all CSF samples. After excluding traumatic punctures contaminated by blood (41 CSF samples), a final sample of 584 patients was considered for this study (age range, 1 day to 34 years; mean, 4.8 years). The concentration of total plasma folate was measured in 318 of 584 patients. In the rest of the patients, either blood samples were unavailable or hemolysis prevented accurate determination of folate concentrations.

CONTROL SUBJECTS

To establish control values, we analyzed CSF samples from 134 children (age range, 1 day to 18 years; mean, 3.8 years). In this group, lumbar puncture was performed to rule out CNS infection and other acute neurologic conditions typically encountered in a large general pediatric academic medical center. Exclusion criteria were CNS infection, hemorrhage, traumatic or neoplastic disorders of the CNS, clinical or analytical suspicion of neurometabolic disorders, and traumatic lumbar puncture containing blood.

BIOCHEMICAL ANALYSIS

Plasma and CSF samples were collected on the same day in accordance with a protocol that we established to ensure assay

reliability.¹¹ Following lumbar puncture, the CSF was immediately stored at -80°C until analysis. We analyzed 5-MTHF, pterins (neopterin, biopterin), and biogenic amine metabolites (5-hydroxyindoleacetic acid and homovanillic acid) by reverse-phase high-performance liquid chromatography with electrochemical and fluorescence detection, as previously reported by our laboratory.^{11,12} Plasma total folate concentrations were analyzed by automated chemiluminescent immunoassays (ADVIA Centaur; Bayer, Tarrytown, New York). We defined low plasma folate values as those below the lower limit of our reference value (for ages 0-5 years: 13 nmol/L, and ages ≥ 6 years: 10 nmol/L [to convert to nanomoles per liter, multiply by 2.266]). Cerebrospinal fluid 5-MTHF deficiency was arbitrarily classified from an analytical point of view as mild (<30% decrease below the lower limit of reference values), moderate (30%-60%), and severe (>60%).

SEQUENCE ANALYSIS OF THE *FOLR1* GENE

Genomic DNA was prepared from peripheral blood leukocytes using the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany). The entire coding sequence and splice junctions of the *FOLR1* gene were amplified by polymerase chain reaction assay and sequenced using standard methods. Polymerase chain reaction conditions and primers are available upon request.

ADDITIONAL DIAGNOSTIC STUDIES

Systematic studies were performed to evaluate neurologic disorders in the patient cohort, including karyotype, specific genetic tests for several neurogenetic diseases according to syndromic manifestations, magnetic resonance imaging, and neurophysiologic studies (electroencephalogram and evoked potentials). Biochemical tests in urine and plasma were also performed for the diagnosis of inborn errors of metabolism, including lactate, pyruvate, ammonia, uric acid, amino acids, organic acids, glycosaminoglycans, creatine and guanidinoacetate, purines and pyrimidines, and sialotransferrin.

STATISTICAL METHODS

For control value calculation, the Pearson product moment correlation test was used to search for correlations between patient age and CSF 5-MTHF values. Because a strong negative correlation was found, the control values were stratified according to patient age. Analysis of variance with the Bonferroni correction test was used for comparisons of different age groups. The nonparametric Mann-Whitney test was applied to compare CSF 5-MTHF and serum folate concentrations between children with and without epilepsy. To uncover further potential statistical correlations among select variables involved in the study (age, total serum folate, and CSF concentrations of 5-MTHF and proteins) in the group of 584 patients, we used a multiple linear regression analysis.

RESULTS

DETERMINATION OF CSF 5-MTHF REFERENCE VALUES

The 5-MTHF control values are summarized in the Figure. A strong negative correlation was observed between 5-MTHF values and the age of controls ($r=-0.515$; $P<.001$). Control values were stratified into 3 age groups: 0 to 10 months ($n=40$): mean (SD), 119 (37) nmol/L (range,

66–203 nmol/L); 11 months to 4 years (n=54): mean (SD), 80 (18) nmol/L (range, 48–127 nmol/L); and 5 to 18 years (n=40): mean (SD), 66 (18) nmol/L (range, 35–124). These reference ranges were significantly different (analysis of variance with Bonferroni; $P < .05$ for all groups).

PATIENTS WITH DECREASED CSF 5-MTHF

According to our control values, 71 of 584 patients (12%) (mean [range] age, 6.5 years [3 days to 34 years]) had decreased 5-MTHF values (Figure). They were classified into mild (n=43; mean [range], 39 [24–63] nmol/L), moderate (n=20; mean [range], 29 [19–41] nmol/L), and severe (n=8; mean [range], 6 [0.6–13] nmol/L) biochemical 5-MTHF deficiency. In patients with low 5-MTHF, the mean (SD) CSF to plasma folate ratio was 2.49 (1.5) (range, 0.02–4.57).

Forty-seven patients with a definitive etiologic diagnosis were affected by either an acquired neurologic condition (most often asphyxia or CNS infections) or a genetic or otherwise congenital disease (Table 1). Patients with severe 5-MTHF deficiency were diagnosed with Kearns-Sayre syndrome (KSS) (n=2); severe 5,10-methylenetetrahydrofolate reductase (MTHFR) deficiency (n=2); striatal necrosis responsive to biotin (n=1); or postinfectious acute necrotizing encephalitis of Hurst (n=1).

Despite extensive diagnostic investigations, an etiologic diagnosis remained elusive in 24 patients. Among them, 2 individuals exhibited both profound deficiency of CSF 5-MTHF (patient 1 [age, 2 years]: 2 nmol/L with control values ranging from 48–127 nmol/L, and patient 2 [age, 13 years]: 12 nmol/L with control values ranging from 35–124 nmol/L) and reduced CSF to plasma folate ratios (patient 1: 0.4, patient 2: 1.3, and control values ≥ 1.5), suggestive of defective folate transport into the brain. Molecular analysis of the coding exons of the *FOLR1* gene and genomic splice sites was performed in these patients.

A therapeutic trial with folic acid was attempted in 19 of 71 patients with CFD (Table 2). Overall, a good clinical response was observed in patients with profound CFD deficiency due to KSS and *FOLR1* defect, whereas inconsistent responses were observed in children with partial CFD.

Of note, 40 of 71 children with decreased 5-MTHF values had seizures and were being treated with antiepileptic drugs at the time of the lumbar puncture. Antiepileptic drugs were administered in monotherapy (19 patients), double therapy (14 patients), or a combination of 3 or more drugs (3 patients). The most frequent administered drugs were valproic acid (17 patients), phenobarbital (12 patients), benzodiazepine hydrochloride (8 patients), carbamazepine (4 patients), topiramate (3 patients), and vigabatrin (3 patients). Children with and without epilepsy did not show significant differences in CSF 5-MTHF values (mean, 35.0 and 30.9 nmol/L, respectively) or total serum folate (mean, 18.1 and 17.8 nmol/L, respectively).

CEREBRAL FOLATE TRANSPORT DEFICIENCY CAUSED BY MUTATIONS IN *FOLR1*

Mutational screening of *FOLR1* allowed the identification of a homozygous missense mutation, p.Cys105Arg

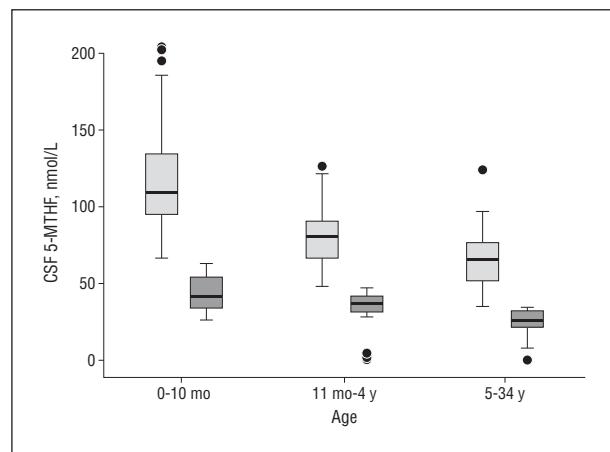


Figure. Normative cerebrospinal fluid (CSF) 5-methyltetrahydrofolate (5-MTHF) concentrations (light gray bars) and 5-MTHF values in patients with 5-MTHF deficiency (dark gray bars). The length of the boxes indicates the interquartile space (25th–75th percentiles). The horizontal line in each box represents the median (50th percentile), and the error bars indicate the adjacent values, ie, the maximum and minimum values of the distribution. The black dots indicate the outlier values.

(c.313T>C), in patient 1, which previously has not been described.^{5,15} The asymptomatic parents, first cousins, were heterozygous for the same transition. The substitution was not found in a screening of 210 chromosomes from healthy individuals, suggesting pathogenicity. *FOLR1* mutations in patient 2 are still under investigation because of limitations in the quantity and quality of the DNA sample.

DECREASED 5-MTHF AND SECONDARY ABNORMALITIES OF PTERINS AND BIOGENIC AMINES

Thirty-five of 71 patients (49%) with decreased 5-MTHF displayed other abnormalities of biogenic amines and pterins in CSF compared with age-matched reference values.¹¹ These abnormalities involved pterin (n=22), homovanillic acid (n=21), and 5-hydroxyindoleacetic acid (n=17) concentrations. Only 1 patient had primary deficiency of biogenic amines and pterin metabolism (autosomal recessive guanosine triphosphate cyclohydrolase 1 deficiency), whereas the other abnormal biochemical profiles may be considered secondary to other disorders. High neopterin concentrations were detected in the following neurologic conditions: perinatal asphyxia (4 of 7 patients), CNS infections (4 of 6 patients), KSS (3 of 3 patients), and CNS disorders associated with seizures (4 of 16 patients). High protein concentrations in CSF were also present in most of these patients (data not shown), suggesting the intercurrent association of an inflammatory process and/or impaired blood-brain barrier function with these disorders.

DECREASED 5-MTHF AND TOTAL SERUM FOLATE CONCENTRATIONS

Twenty-five of 46 patients (54%) with decreased 5-MTHF values in CSF exhibited systemic folate deficiency (mean [range], 8.6 [5.3–12.5] nmol/L). With the exception of

Table 1. Etiologic Diagnosis and Biochemical Data in 47 Patients With Secondary 5-MTHF Deficiency^a

Age, Disorder	No. of Patients	CSF 5-MTHF, nmol/L ^b	Serum Folate, nmol/L	CSF to Plasma Folate Ratio
0 to 10 mo				
Perinatal asphyxia	7	48 (35-61)	c	c
Central nervous system infections	4	45 (28-61)	c; c; c; 15	c; c; c; 2.2
Posthemorrhagic hydrocephalus	1	51	c	c
Glutaric aciduria type 1	1	26	c	c
Serine deficiency	1	34	54	0.6
Benign idiopathic neonatal seizures	1	27	43	0.6
Neonatal seizures (cortical dysplasia)	1	63	24	2.6
Infantile spasms (stroke)	1	39	38	1
Nonketotic hyperglycinemia	1	50	11 ^b	4.5
11 mo to 4 y				
Central nervous system infections	2	31; 32	c	c
Mitochondrial disorders	3	34; 42; 35	28; 55	1.19; 0.67
5,10-Methylenetetrahydrofolate reductase deficiency	2	1; 4	41; 12 ^b	0.02; 0.3
Rett syndrome	2	34; 47	9 ^b ; c	3.7; c
Williams syndrome	1	40	16	2.5
H-ABC syndrome	1	34	18	1.9
5 to 18 y				
Kearns-Sayre syndrome	3	0.6; 8; 24	10 ^b ; 14; 5 ^b	0.06; 0.6; 4.8
Other mitochondrial disorders	1	32	7 ^b	4.3
Biotin-responsive striatal necrosis	1	13	45	0.3
Glucose transporter type 1 deficiency	2	32; 26	9 ^b ; 7 ^b	3.5; 3.7
Recessive GTP cyclohydrolase type 1 deficiency	1	33	8 ^b	4.1
Niemann-Pick type C disease	1	32	7 ^b	4.6
Infantile spasms (cortical dysplasia)	1	34	c	c
Drug-refractory epilepsy after viral encephalitis	1	27	6 ^b	4.5
Rett syndrome	5	29 (19-34)	18 (7 ^b -45)	2.6 (0.4-3.6)
Multiple sclerosis	1	32	10	3.2
Postinfectious acute necrotizing encephalitis of Hurst	1	10	17	0.59

Abbreviations: CSF, cerebrospinal fluid; GTP, guanosine triphosphate; H-ABC, hypomyelination with atrophy of the basal ganglia and cerebellum; 5-MTHF, 5-methyltetrahydrofolate.

^aSevere 5-MTHF deficiencies are in bold. More than 3 values are expressed as mean (range).

^bSerum folate concentrations below the lower limit of the reference value.

^cNo serum folate value is available for the corresponding CSF 5-MTHF value in the same patient.

3 patients (with KSS, severe MTHFR deficiency, and cerebral folate transport deficiency), all patients had normal folate CSF to plasma ratios (mean [range], 3.7 [1.5-4.51]), indicating preserved transport of 5-MTHF across the blood-CSF barrier.

CORRELATION BETWEEN 5-MTHF VALUES AND OTHER VARIABLES

To investigate variables that may influence 5-MTHF values, we performed multiple linear regression analyses including CSF 5-MTHF values as a dependent variable and patient age, plasma folate values, or CSF protein concentrations as independent variables. The CSF 5-MTHF values were negatively associated with patient age (slope = -0.397; $P < .001$) and positively with serum folate (slope = 0.229; $P < .001$). No correlation was observed between CSF 5-MTHF levels and protein concentration.

COMMENT

Our results indicate that deficit of CSF 5-MTHF may be a relatively common—although underrecognized—biochemical abnormality found in children diagnosed with various neurologic disorders. This conclusion is based

on an estimation of the control values more accurate than that previously reported by our group.¹² As a result, the lower limit of the reference range used was established at 35 nmol/L in patients older than 5 years. This modification accounted for the lower prevalence of 5-MTHF deficiency detected in the current series (71 of 584 patients [12%]) compared with the prevalence in our previous study (26%).¹² These results are in line with the control values reported by other groups.¹⁶

We were able to identify the etiology underlying secondary CSF 5-MTHF deficiency in 47 of 71 patients (66%). Children younger than 1 year were mostly affected by epilepsy (neonatal seizures and infantile spasms) or acquired CNS conditions (perinatal asphyxia and CNS infections). Older children experienced more heterogeneous disorders, predominantly inherited diseases. Among these, conditions such as Rett syndrome,^{6,17,18} mitochondrial disorders,^{7,13,19} and hypomyelination with atrophy of the basal ganglia and cerebellum syndrome⁹ were previously associated with CFD. In addition, to our knowledge, the following disorders have been recognized for the first time in this study: cortical dysplasia, Williams syndrome, multiple sclerosis, atypical nonketotic hyperglycinemia, biotin-responsive striatal necrosis, and other specific inborn errors of metabolism (glutaric aciduria

Table 2. Summary of 19 Patients Included in the Present Study Who Received Folate Supplementation^a

Etiology of CSF 5-MTHF Deficiency	No. of Patients	CSF 5-MTHF Values at Diagnosis	Treatment With Folinic Acid	CSF 5-MTHF Values After Folinic Acid	Clinical Response
Unknown	11	30 (12-54) nmol/L	15-60 mg/d	NA	Only mild and transitory improvement in seizure control and behavior in 2 of 11 patients
Rett syndrome ⁶	6	35 (19-47) nmol/L	0.5-1.5 mg/kg/d	NA	Improvement of seizures in 3 patients
Kearns-Sayre syndrome ¹³	1	8 nmol/L	2.5 mg/kg/d	48 nmol/L	Recovered ambulation, improvement in CNS myelination
<i>FOLR1</i> defect ¹⁴	1	2 nmol/L	4 mg/kg/d	NA	Seizure free without antiepileptic drugs, improvement in psychomotor development

Abbreviations: 5-MTHF, 5-methyltetrahydrofolate; CNS, central nervous system; CSF, cerebrospinal fluid; NA, not available.

^aData are given as mean value or mean (range) value.

type 1, glucose transporter type 1 deficiency, and Niemann-Pick type C disease). Most secondary deficiencies in our series manifested mild to moderate reductions of 5-MTHF, whereas severe deficiencies were ascribed to children with impaired transport or metabolism of folate (ie, KSS or MTHFR deficiency) and cerebral degeneration (ie, biotin-responsive striatal necrosis or acute necrotizing encephalopathy).

In 1 patient with severe 5-MTHF deficiency in CSF and decreased CSF to plasma folate ratio not explained by other known causes of CFD, mutational analysis of *FOLR1* revealed a novel homozygous missense mutation, p.Cys105Arg (c.313T>C). His healthy consanguineous parents were analyzed and shown to be heterozygous for the same mutation.¹⁴ Recently, pathogenic mutations in the *FOLR1* gene coding for the FR α have been identified in 3 patients with cerebral folate transport deficiency.^{5,15} Loss-of-function mutations in *FOLR1* cause a loss of FR-specific folate binding to patients' fibroblasts. Among all human folate transporters, FR α messenger RNA expression is the most abundant folate carrier in the choroid plexus, confirming the notion that FR α is the principal molecule that regulates folate transport across the blood-CSF barrier.

In 23 of 71 children (32%) with decreased 5-MTHF, no etiologic diagnosis was identified after extensive investigation. Most of these patients exhibited mild to moderate reductions of 5-MTHF and normal CSF to plasma folate ratios, making it unlikely that there was a transport defect across the blood-CSF barrier. These patients manifested diverse clinical phenotypes (mostly infantile spasms, drug-refractory epilepsies of infancy or early childhood, or progressive motor deterioration syndromes), disease courses, and neuroimaging findings, suggesting broad disease heterogeneity. Because CFD is, in principle, amenable to therapeutic supplementation, a trial with folic acid was attempted in 11 patients with decreased 5-MTHF of unknown cause, to investigate whether decreased CNS folate availability might play a causative or significant role in their encephalopathies. Clinical benefits were limited to 2 patients who experienced only mild transitory improvement in communicative skills and seizure control. These observations agree with poor treatment response observed in patients with mild CSF 5-MTHF deficiencies associated with Rett syndrome.^{6,17} In contrast, favorable clinical responses have been reported in patients with profound CSF 5-MTHF deficiency due to KSS¹³ and *FOLR1* de-

fect.^{5,14,15} A study (including validated clinical scales for the evaluation of communicative skills, seizures, and other neurologic signs; biochemical monitoring; and assessment of concomitant antiepileptic therapy) is under way to evaluate the efficacy and safety of folic acid supplementation in CFD syndrome, particularly in mild and moderate deficiencies.

There is great variability in the range of 5-MTHF concentrations reported in children with suspected primary CFD. A few case series or case reports described reduced 5-MTHF concentrations in CSF that are in the range of mild or moderate 5-MTHF deficiencies observed in our patients and may therefore represent secondary or coincidental forms of CFD without major pathophysiologic implications.^{4,10,20,21} Our results illustrate that it is also possible that mild or moderate reductions of CSF 5-MTHF concentration indicate transient biochemical derangement in acquired conditions such as asphyxia or CNS infections. In fact, even children with genetically determined diseases can manifest transient CSF 5-MTHF deficiency. In our experience, 1 patient with benign idiopathic neonatal seizures and reduced 5-MTHF values showed spontaneous normalization of 5-MTHF values 6 months later, whereas 4 patients with initial normal values of 5-MTHF (mean, 58 nmol/L) exhibited a significant drop in 5-MTHF concentrations (mean, 24 nmol/L) after a mean period of 12 months. During this time, progressive neurologic deterioration was evident in 2 of them that was due to postinfectious acute necrotizing encephalitis and biotin-responsive striatal necrosis.

Children with epilepsy are at risk of manifesting low serum folate and increased homocysteine concentrations, especially while receiving long-term and/or multiple antiepileptic drug treatments.^{22,23} It is conceivable that pharmacologic agents contribute to decreased 5-MTHF concentrations in CSF. In this respect, a recent study by Opladen et al²⁴ suggested that antiepileptic drugs might disturb the *FOLR1*-mediated 5-MTHF cellular uptake, thus playing a hypothetical role in the pathogenesis of secondary CFD syndrome. In our sample, 56% of children with decreased 5-MTHF values manifested seizures and were treated with antiepileptic drugs at the time of the lumbar puncture. However, no differences were detected in the concentrations of folate in serum and CSF when comparing children with and without epilepsy.

Cerebral folate is involved in nearly 100 metabolic reactions; hence, reduced CSF 5-MTHF could represent a secondary biochemical manifestation of other disor-

ders. In agreement with this hypothesis, we found that approximately 50% of children with reduced 5-MTHF concentrations showed secondary abnormalities of pterin and biogenic amine metabolites. High neopterin concentration, commonly associated with high CSF protein levels, was identified in children with perinatal asphyxia, CNS infections, KSS, and epilepsy. High pterin levels have been previously reported in most of these conditions,^{17,25,26} whereas decreased 5-MTHF constitutes a novel biochemical finding for these disorders. Neopterin is released from activated macrophages, and its production is inducible by different cytokines implicated in inflammatory and hypoxic-ischemic damage.^{27,28} Under these conditions, the combination of several factors, such as increased oxidative stress, inflammatory cell activation, and blood-brain barrier dysfunction, might lead to defective cerebral folate transport and/or increased folate consumption within the CNS.

One of the major findings in our study was the strong positive correlation observed between CSF 5-MTHF and plasma folate values. Moreover, one-half of the patients with reduced 5-MTHF in our series exhibited associated low serum folate concentrations, highlighting the importance of performing simultaneous analyses of folate in plasma and CSF. These results are in agreement with the strong correlation observed by others between serum and CSF folate at the lowest range of serum folate levels (5.4–15.6 nmol/L).²⁹

In conclusion, we have surveyed 5-MTHF deficiency across a broad spectrum of diseases of the nervous system in childhood. Partial deficiency of 5-MTHF is a common secondary abnormality found in acquired injuries to the CNS and in genetic and congenital encephalopathies. The mechanisms that account for cerebral folate depletion within the CNS have not been completely elucidated, although nutritional determinants such as total plasma folate values strongly influence 5-MTHF concentration in CSF. Profound deficiencies of 5-MTHF are caused by inborn errors of systemic folate metabolism or cerebral transport across the blood-CSF barrier, including mutation of *FOLR1*.

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