

# Cerebral folate deficiency with developmental delay, autism, and response to folinic acid

**Abstract**—The authors describe a 6-year-old girl with developmental delay, psychomotor regression, seizures, mental retardation, and autistic features associated with low CSF levels of 5-methyltetrahydrofolate, the biologically active form of folates in CSF and blood. Folate and B12 levels were normal in peripheral tissues, suggesting cerebral folate deficiency. Treatment with folinic acid corrected CSF abnormalities and improved motor skills.

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Intracellular folates participate in essential one-carbon transfer reactions (see figure E-1 on the *Neurology* Web site).<sup>1</sup> Slowly progressive neurologic dysfunction has been described in adults and children with an isolated deficiency of folate in CSF.<sup>2-5</sup> Phenotypic manifestations associated with CSF folate deficiency remain to be fully characterized. Some patients demonstrate clinical improvement with folinic acid, but the molecular etiology of folate deficits in the brain remains unknown.

**Methods.** The patient was followed at Texas Children's Hospital between the immediate postnatal period (day of life 2) and 6 years of age. Written permission was obtained from the patient's parents. CSF levels of 5-methyltetrahydrofolate (5-MTHF), pterins, and neurotransmitter metabolites were measured by high-performance liquid chromatography (HPLC).<sup>6</sup> 5-MTHF was analyzed using a coulometric electrochemical detection method. Briefly, CSF (30  $\mu$ L) was injected directly onto a reversed phase Spherclone 5- $\mu$ m C18 ODS2 column (25 cm  $\times$  4.6 mm inner diameter; Phenomenex, Torrance, CA), and the 5-MTHF was separated isocratically using 0.05 mol/L  $\text{KH}_2\text{PO}_4$  (pH 4.0) containing 14% methanol. The 5-MTHF was detected after oxidation using the second electrode of a model 5010 dual-cell analytical electrode set at +0.05 V. The first electrode was set at -0.2 V. The flow rate was 1.3 mL/min, and the HPLC column was maintained at 35  $^\circ$ C using a column oven. The system was calibrated against a 100-

nmol/L external standard (Sigma, St. Louis, MO), with the lower limit of detection being 0.15 pmol on column.

**Results.** The child was born to a 34-year-old  $G_4P_4A_{b_0}$  mother. Prenatal, birth, and family history were normal. There was a neonatal history of seizures and gastroesophageal reflux. A neonatal EEG showed multifocal seizure discharges. Mild spasticity was noted at 2 to 3 months, and signs of developmental delay were observed at 9 months. She sat unsupported at 13 months. At 18 months, she was able to use some signs (including simple "phrase" speech in the form of signs), eat on her own, pull to a stand, and walk with support. According to the mother, the infant imitated some actions and was interactive with others. Her development began to regress around age 3.5 years. There was chronic progressive loss of motor skills and language, loss of purposeful hand use, cerebellar ataxia, dyskinesia, pyramidal signs, and occasional seizures. By age 5 years, the child was wheelchair bound, had no purposeful hand use, was unable to communicate, and was fed via a gastrostomy tube. Her growth measurements were normal. Her facial features were reminiscent of Angelman syndrome (AS) with midface retrusion, relative prognathism, and wide mouth. Neurologic examination showed reduced eye contact, decreased arm strength, and increased tone and deep tendon reflexes in the lower extremities. Ophthalmologic evaluation was normal. Routine blood chemistry, CSF analysis, brain MRI, karyotype, plasma amino acids, urine organic acids,  $\alpha$ -fetoprotein, very long chain fatty acid levels, and isoelectrofocusing of transferrin were normal. Urine orotic acid was increased at 31 mmol/mol creatinine (normal range, 0.3 to 2.82). EMG, skeletal muscle histochemistry, and mitochondrial respiratory chain analysis were unremarkable. Angelman DNA methylation studies, *UBE3A* mutation analysis, telomere fluorescence in situ hybridization, and fragile X DNA testing gave negative results. There were no point mutations or deletions in the *MECP2* coding regions and no expansion mutations in *SCA-1*, *SCA-2*, *SCA-3*, *SCA-6*, *SCA-7*, and *SCA-10*. A heterozygous GAA expansion was found in *Frataxin* with no mutations in the second allele. There was no evidence of serum or red blood cell folate deficiency. Vitamin B12 levels and metabolism were normal in peripheral tissues (table 1). Although methylenetetrahydrofolate reductase (*MTHFR*) activity was slightly lower than the control reference, it was not consistent with severe enzyme deficiency. A heterozygote 1298A $\rightarrow$ C polymorphism was identified in *MTHFR* (see figure E-2 on the *Neurology* Web

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**Table 1** Analysis of folate and vitamin B12 in peripheral tissues

Assay	Result	Normal range
Plasma homocysteine, $\mu\text{mol/L}$	6	4–14
Plasma methionine, $\mu\text{mol/L}$	32	11–50
Serum B12, $\text{pg/mL}$	2,000	200–1,100
Uptake in skin fibroblasts, $\text{nmol/mg protein/18 h}$		
Propionate (–OHCbl)	13.0	7.1–14.5
Propionate (+OHCbl)	13.5	7.4–14.4
5-MTHF (–OHCbl)	340	60–390
5-MTHF (+OHCbl)	380	180–430
Cobalamin uptake in skin fibroblasts, $\text{pg/mL}$	17.8	8.4–18.0
Cobalamin distribution in skin fibroblasts, %		
Aq-cobalamin	9.1	4.45–12.25
CN-cobalamin	8.6	4.37–18.17
Ado-cobalamin	12.4	11.09–19.49
Me-cobalamin	58.0	51.25–64.65
Other	11.9	4.48–9.88
MTHFR assay, $\text{nmol CHO/mg protein/h}$	5.2	8.7–17.9

5-MTHF = 5-methyltetrahydrofolate; MTHFR = methylenetetrahydrofolate reductase.

site), but sequencing showed no coding sequence mutations.

CSF analysis revealed low 5-MTHF levels with normal pterins and neurotransmitter metabolites (table 2). There was a significant elevation of CSF total homocysteine and S-adenosylhomocysteine and a decrease of S-adenosylmethionine. No mutations were found in the coding regions of *RFC1* and *FBP1*, two genes encoding components of the choroid plexus folate transport system (see figure E-3 on the *Neurology* Web site). Oral treatment with folinic acid (5-formyl-THF) was initiated at 0.5 mg/kg/d, and

**Table 2** Analysis of CSF before and after treatment with folinic acid for 3 months

Metabolite	Before treatment, $\text{nmol}$	After treatment, $\text{nmol}$	Normal range, $\text{nmol}$
5-Methyltetrahydrofolate	34	113	40–128
Total homocysteine	424	83.9	32.3–113.7
S-adenosylhomocysteine	88.8	28	8.9–14.1
S-adenosylmethionine	106	308	137–385
Methionine	3240	NT	900–3,500
5-Hydroxyindoleacetic acid	103	156	66–338
Homovanillic acid	350	449	218–852
3-O-methyldopa	<10	<10	0–100
Neopterin	9	14	7–65
Tetrahydrobiopterin	36	35	9–40
Lactate	1.2	1.5	1–2.4

**Table 3** Developmental evaluation results after folinic acid treatment

Bayley Scales of Infant Development, Second Edition			
Mental Scale			
Developmental Age			9 months
Motor Scale			
Developmental Age			15 months
Vineland Adaptive Behavior Scales, Interview Edition			
	Standard score	Percentile	Age equivalent
Communication	37	<0.1	1–1
Daily Living Skills	20	<0.1	1–6
Socialization	43	<0.1	0–4
Composite	34	<0.1	

the dose was doubled after 2 weeks. This dose was selected based on published reports.<sup>3–5</sup> Clinical follow-up evaluation once a month and repeat CSF analysis after 3 months showed correction of the biochemical CSF abnormalities (see table 2) and remarkable motor improvement, including independent gait, and restoration of hand use and oral feeding, leading to removal of the gastrostomy tube (see table E-1 on the *Neurology* Web site). A follow-up EEG showed no epileptiform discharges; however, the background activity continued to be slow. The mother reported a mild increase of verbal output and social reactivity. A brief attempt at dose escalation was associated with the development of facial dyskinesias, and treatment was then continued at 1 mg/kg/d. During treatment, the child showed neurologic features evocative of AS and autistic spectrum manifestations. The child's development was formally evaluated at age 6 years, 7 months, while still receiving 1 mg/kg/d of folinic acid. Using the Bayley Scales of Infant Development, cognitive/developmental skills and motor skills were estimated at 9 and 15 months (table 3). Using the Vineland Adaptive Behavior Scales, her adaptive behavior was within the severe deficits range of the low adaptive level (see table 3). Her self-help and communication skills were rated at the 18- and 13-month-old levels. However, her socialization skills were the most impaired, at the 4-month-old level. Using the Autism Diagnostic Observation Schedule (ADOS) and the Autism Diagnostic Interview–Revised (ADI-R), the child exceeded the cutoff criteria for autism in all areas (nonverbal communication, reciprocal social interaction, play, and repetitive behaviors). She did not spontaneously use nonverbal gestures; she used other people's hands as tools to gesture "for" her; and her vocalizations were only on occasion socially directed. She also played with toys in a repetitive manner, fixated on objects of interest to her, and was more focused on interactions with objects than she was on interactions with people. Furthermore, the child did not demonstrate "joint-attention" skills (the ability to look where someone else is looking), responsive social smiles to unfamiliar others, shared enjoyment in interactions, or imitative/social play.

**Discussion.** We report a child with a progressive neurologic syndrome consisting of seizures, mental

retardation, and autistic features in association with reduced 5-MTHF in CSF. The age at onset, clinical features, biochemical abnormalities, and striking response to treatment differentiate our patient from previous cases. Isolated CSF deficiency of folates was originally reported in an 18-year-old man with progressive sensorineural hearing loss, cerebellar ataxia, distal spinal muscular atrophy, and pyramidal tract dysfunction.<sup>2</sup> Low CSF levels of 5-MTHF have also been documented in pediatric cases with various manifestations. Five children with cerebral folate deficiency (CFD) presented at ~6 months of age with deceleration of head growth, irritability, disturbed sleep, psychomotor retardation, cerebellar ataxia, dyskinesia, pyramidal signs, seizures, and cerebral atrophy<sup>3</sup> ([www.folates.com](http://www.folates.com)). Four girls met established diagnostic criteria for Rett syndrome (RTT).<sup>5</sup> Three patients with Aicardi-Goutieres syndrome showed microcephaly, severe developmental delay, dyskinesia or spasticity, seizures, and CNS calcifications.<sup>4</sup> In contrast to these reports, the child described here presented in the neonatal period with seizures and was subsequently diagnosed with developmental delay. Folinic acid treatment resulted in objective neurologic improvements that allowed for a more accurate cognitive and behavioral assessment. The evaluation showed that despite improvement of motor skills and parental reports of increased responsiveness, her cognitive, language, and socialization skills remained delayed. She met criteria for mental retardation, and even after taking into consideration her cognitive and language delays, she demonstrated deficits in communication and socialization that mirrored those observed in children with idiopathic autism. Her socialization deficits were further corroborated by her mother's reports of her adaptive behavior. Thus, even considering the profile of her strengths and her cognitive delays, her socialization skills were significantly lower than expected. In summary, after 1 year of continuous treatment with folinic acid resulted in considerable neurologic improvements, this child now demonstrated features of "classic" autism. Although restricted to a single case, this report suggests that patients with mental retardation and autism may benefit from analysis of CSF folate abnormalities when basic workup is noncontributory. The clinical overlap between autism, RTT, and AS<sup>7,8</sup> and published data on CNS folate deficiency in RTT<sup>5</sup> suggest the possibility of an association between reduced CNS folates and a spectrum of neurodevelopmental disorders. It is tempting to speculate that deficits of CNS folate may change the methylation of cellular substrates affecting similar brain functions in these disorders.

From a biochemical standpoint, CSF levels of pterins and neurotransmitter metabolites were normal in the case reported here, in contrast with pub-

lished pediatric cases.<sup>3-5</sup> Orotic aciduria has not previously been reported in CFD. Although this is consistent with an impairment of folate metabolism as observed in hereditary folate malabsorption<sup>1,9</sup> or formiminoglutamate transferase deficiency,<sup>10</sup> our patient had neither common features of hereditary folate malabsorption nor urinary excretion of formiminoglutamic acid. Abnormally high urinary levels of orotic acid have also been found in other metabolic conditions. Laboratory abnormalities diagnostic of urea cycle disorders or hereditary orotic acidurias were absent in our patient. Finally, this case appears to exceed the degree of improvement obtained with folinic acid treatment in other patients. The individual we describe gained the ability to ambulate and feed herself independently.

The CNS folate deficit associated with sparing of peripheral tissues suggests the presence of defective folate transfer across the blood-brain barrier. Two distinct transport systems for folates across mammalian cell membranes have been described: the reduced folate carrier 1 and a family of membrane-associated folate binding proteins (see figure E-3 on the *Neurology* Web site).<sup>1</sup> We found no mutations in the coding regions of *RFC1* and *FBP1*. Sequencing of *MTHFR* showed no evidence of pathologic mutations. Future studies are required to elucidate the mechanism of reduced CSF folate transport in CFD.

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