Brief Report: Autistic Symptoms, Developmental Regression, Mental Retardation, Epilepsy, and Dyskinesias in CNS Folate Deficiency

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Abstract We studied seven children with CNS folate deficiency (CFD). All cases exhibited psychomotor retardation, regression, cognitive delay, and dyskinesia; six had seizures; four demonstrated neurological abnormalities in the neonatal period. Two subjects had profound neurological abnormalities that precluded formal behavioral testing. Five subjects received ADOS and ADI-R testing and met diagnostic criteria for autism or autism spectrum disorders.

They exhibited difficulties with transitions, insistence on sameness, unusual sensory interests, and repetitive behaviors. Those with the best language skills largely used repetitive phrases. No mutations were found in folate transporter or folate enzyme genes. These findings demonstrate that autistic features are salient in CFD and suggest that a subset of children with developmental regression, mental retardation, seizures, dyskinesia, and autism may have CNS folate abnormalities.

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Introduction

Isolated deficiency of folate in the CSF has been described in rare individuals with progressive postnatal neurological dysfunction of varying presentations and manifestations. Isolated CSF deficiency of folates was originally reported in an 18-year-old male with progressive sensorineural hearing loss, cerebellar ataxia, distal spinal muscular atrophy, and pyramidal tract dysfunction (Wevers et al. 1994). Subsequently, other authors described several pediatric cases. A series of five children showed deceleration of head growth, psychomotor retardation, cerebellar ataxia, dyskinesia, pyramidal signs, and seizures (Ramaekers et al. 2002). These findings were largely confirmed by the same authors in a series of 20 subjects (Ramaekers and Blau 2004). Isolated Rett syndrome was described in four cases (Ramaekers et al. 2003). Three children demonstrated microcephaly, severe developmental delay, dyskinesia or spasticity, seizures, and CNS calcifications and were diagnosed with atypical Aicardi-Goutieres

syndrome (Blau et al. 2003). One female was diagnosed with developmental delay, spastic paraparesis, dystonia, and speech difficulties (Hansen and Blau 2005). One female was identified with developmental delay, mental retardation (MR), seizures, dyskinesias, and autism spectrum manifestations (Moretti et al. 2005). An incomplete form of Kearns-Sayre syndrome associated with a mitochondrial DNA deletion was described in one case (Pineda et al. 2006). Finally, one patient was identified with hypomyelination with atrophy of basal ganglia and cerebellum syndrome (Mercimek-Mahmutoglu and Stockler-Ipsiroglu 2007). Some cases demonstrated clinical improvement after treatment with folinic acid (Hansen and Blau 2005; Mercimek-Mahmutoglu and Stockler-Ipsiroglu 2007; Moretti et al. 2005; Ramaekers et al. 2002). This heterogeneity poses a challenge for the clinician and to our understanding of the association between neurological dysfunction and deficiency of folate metabolites in the CSF. Only recently clinicians have reported the presence of autism spectrum manifestations in some cases of CNS folate deficiency (CFD) (Moretti et al. 2005; Ramaekers et al. 2005). We described a 6-year-old girl with developmental delay, psychomotor regression, seizures, MR, and autistic features (Moretti et al. 2005). This child presented in the neonatal period with seizures and was subsequently diagnosed with developmental delay. After the identification of low folate levels in the CSF, the child was treated with folinic acid. This resulted in objective neurological improvements that allowed a standardized cognitive and behavioral assessment using the Bayley Scales of Infant Development, the Vineland Adaptive Behavior Scales, the Autism Diagnostic Observation Schedule (ADOS), and the Autism Diagnostic Interview—Revised (ADI-R). These tests showed that despite improvement of motor skills and parental reports of increased responsivity, her cognitive, language, and socialization skills remained delayed. The child met criteria for MR and exceeded the cutoff criteria for autism in all areas (nonverbal communication, reciprocal social interaction, play, and repetitive behaviors). Even after taking into consideration her cognitive and her language delays, she demonstrated deficits in communication and socialization that mirrored those observed in children with idiopathic autism. Very few clinical details were published concerning the diagnosis of MR and autism in 4 of 28 cases of CSF folate deficiency (Ramaekers et al. 2005). These authors noted improvement of communication skills in two of these subjects in contrast with results of Moretti et al.

Based on our observation (Moretti et al. 2005) and reports from other authors (James et al. 2004; Ramaekers et al. 2005), we hypothesized that autism is a manifestation of CFD. To investigate this association, we performed detailed clinical evaluations in six additional consecutive

subjects with CFD. Standardized neurobehavioral assessments were performed in four of these subjects whose cognitive function was adequate enough to allow this evaluation. The two remaining subjects were evaluated clinically, but were severely impaired and non-ambulatory, precluding any type of formal neurobehavioral assessment. In addition, genes encoding proteins that mediate folate transport or metabolism were sequenced in the whole cohort.

Methods

Patients were referred to Baylor College of Medicine because of an ongoing study regarding children with cerebral folate deficiency. The referrals originated from geneticists aware of the existence of this study. The seven subjects described in this report represent seven consecutive cases evaluated by the authors. Cerebrospinal fluid (CSF) analysis was performed by HPLC as previously described (Hyland and Surtees 1992). The normative range for CSF 5-MTHF was developed over a period of 15 years using the following methodology. Cerebrospinal fluid was collected from patients spanning 0 to 79 years of age in a standardized fashion from the first drop and the 7th 0.5 ml fraction was used for the analysis of 5-MTHF. Over 2,500 CSF samples were analyzed from males and females from a wide ethnic origin and with a wide variety of neurological disorders. Values from patients treated with drugs known to interfere with folate metabolism (anticonvulsants, methotrexate) or affected with infections, seizures, movement disorders, inborn errors of folate, cobalamin, serine or biogenic amine metabolism, and disorders leading to altered methylation were excluded. Four hundred and eighty-nine values remained from the total data set and were used to develop age related reference ranges. The reference range used in the current paper was extracted from this set and defines the highest and lowest values obtained from subjects 2 to 15 years of age.

DNA sequencing, chromosome analysis by karyotyping and array-comparative genomic hybridization (CGH) were performed at the Baylor College of Medicine Medical Genetics Laboratories. Analysis of the *PCFT* gene was performed as previously described (Qiu et al. 2006). Five of the seven patients also received a formal psychological evaluation. The two remaining subjects were too severely impaired neurologically to undergo neuropsychological assessments. The Bayley Scales of Infant Development, Second Edition (BSID-II) was used to assess cognitive and motor skills. Parents were interviewed using the standardized administration of the Vineland Adaptive Behavior Scales—Interview Edition (VABS). All patients were also given the Autism Diagnostic Observation Schedule

(ADOS) Module I. Module I was selected based on developmental level and expressive language abilities of the patients, and is appropriate for children who are non-verbal or have phrase speech, and who are functioning developmentally under the age of three. The parents of all patients were also given the ADI-R. Questions from the ADI-R were not only used to substantiate the diagnosis of autism, but were also utilized to document the presence of a true developmental regression. The ADOS and the ADI-R were administered by an expert clinician (SUP) with research reliability for both instruments. Additional research testing was performed after acquiring informed consent approved by the Institutional Review Board for Human Subject Research at Baylor College of Medicine and appropriate institutions.

Results

The clinical features and CSF analysis for the seven unrelated subjects are summarized in Table 1. All children demonstrated developmental delay, regression, dyskinesia and low CSF levels of 5-methyltetrahydrofolate (5-MTHF), the biologically active form of folate. Red blood cell folate, serum folate, total plasma homocysteine,

karyotype, chromosomal microarray analysis, fragile X DNA testing, plasma and CSF amino acids, urine organic acids, and evaluations for mitochondrial disease did not show any abnormalities. The results of psychological testing are depicted in Tables 2, 3, and 4. Of the seven consecutive cases, five children were able to undergo formal neuropsychological evaluation. The poor neurological status of the two remaining subjects precluded this type of evaluation. All five patients who received neuropsychological testing were diagnosed with MR and autism or autism spectrum disorders. These children had difficulty with transitions, were more interested in objects than interactions with people, did not compensate for language delays by using gestures, had limited shared enjoyment in interactions, and exhibited repetitive/sensory behaviors. Even after taking into consideration their cognitive and language impairments, they demonstrated deficits in communication, socialization, play and repetitive behaviors as observed in idiopathic autism.

Oral treatment with folinic acid (5-Formyl-tetrahydrofolate) was initiated at 0.5 mg/kg/day and the dose was doubled after approximately 2 weeks. Clinical follow-up and repeat CSF analysis showed variable treatment response and correction of 5-MTHF levels (Table 1). There were improvements of gait, dyskinesia, and hand use in

Table 1 Clinical features and results of CSF 5-MTHF analysis

	Subject 1	Subject 2	Subject 3	Subject 4	Subject 5	Subject 6	Subject 7
Sex	F	F	F	F	M	M	F
Age (years)	8	9	8	10	7	2	15
Normal early development	No	Until 24 months	Until 18 months	No	Until 8 months	No	No
Developmental delay	Severe delay	Severe delay	Severe delay	Profound delay	Profound delay	Severe delay	Severe delay
Regression	+	+	+	+	+	+	+
Seizures	+	+	+	+	+	+	—
Dyskinesia	+	+	+	+	+	+	+
Angelman-like facial features	+	—	+	—	—	—	—
Rett manifestations	—	—	—	+	—	—	—
CSF 5-MTHF levels at diagnosis ^a	34	30	35	20	33	37	37
CSF 5-MTHF levels during FA treatment ^a	113	175	76	81	73	90	120

^a Normal range 40–150 nM.

ND: not determined; FA: folinic acid

Table 2 Bayley Scales of infant development, second edition

	Subject 1	Subject 2	Subject 3	Subject 6	Subject 7
Age in months (years)	79 (6 7/12)	92 (7 8/12)	89 (7 5/12)	27 (2 3/12)	180 (15)
Mental scale (developmental age in months)	9	24	25	5	20
Motor scale (developmental age in months)	15	NT	27	6	19

NT: not tested

Table 3 Vineland adaptive behavior scales, interview edition

	Subject 1		Subject 2		Subject 3		Subject 6		Subject 7	
	Centile	Age equiv. (months)	Centile	Age equiv. (months)	Centile	Age equiv. (months)	Centile	Age equiv. (months)	Centile	Age equiv. (months)
Communication	<.1	13	<.1	22	<.1	30	1	11	<.1	29
Daily living skills	<.1	18	<.1	24	<.1	41	1	12	<.1	54
Socialization	<.1	4	<.1	15	.1	26	2	11	<.1	16
Motor skills ^a							.3	10		
Composite	<.1		<.1		<.1		.3		<.1	

^a The domain of motor skills is only assessed in children under the age of 6 years

Table 4 Autism diagnostic observation schedule Module 1 scores

	Subject 1	Subject 2	Subject 3	Subject 6	Subject 7
ADOS communication	7	3	5	2	5
ADOS social interaction	14	12	12	7	7
ADOS Communication + Socialization	21	15	17	9	12
Play skills	4	4	3	4	4
Stereotyped behaviors and restricted interests	3	4	6	4	3

Module 1 Cutoff scores for communication: Autism = 4, autism spectrum = 2. Cutoff scores for reciprocal social interaction: Autism = 7, autism spectrum = 7. Cutoff scores for communication + social interaction: Autism = 12, autism spectrum = 7. There are no cutoff scores for play skills or for stereotyped behavior and restricted interests

subjects 1–3 and 7. Subject 1 was also able to tolerate oral feeds.

No mutations were found in the exons and intron/exon boundaries of genes encoding proteins involved in folate transport (*RFC*, *FR α* , *FR β* , *PCFT*), methylenetetrahydrofolate reductase (*MTHFR*), dihydrofolate reductase (*DHFR*), and formiminotransferase cyclodeaminase (*FTCD*).

Subject 1

This previously reported 8-year-old girl (Moretti et al. 2005) had a history of neonatal seizures and hypotonia. Until the age of 3½ years, her development progressed slowly. She developed the ability to sit unsupported at 13 months, and at age 1½ years, she had acquired the use of some signs. She was eating on her own, was pulling herself to stand, and was walking with support. She had not yet been toilet trained at that time. She used to imitate some actions (waving, clapping, etc), and was interactive with other people. Her development then began to regress severely around the age of 3½ years. She lost the use of her hands, lost the use of sign language, and lost her feeding skills in addition to other motor skills. Her gait became ataxic and she developed spasticity and involuntary movements, eventually becoming wheelchair bound. She had to be fed through a gastrostomy tube and had several episodes of seizures on a daily basis. Different diagnostic

possibilities were entertained including Lennox-Gastaut syndrome, Angelman syndrome, Rett syndrome, cerebral palsy, and MR with epilepsy. Upon initial evaluation by Genetics at age 4 years and 7 months, she was found to have a weight of 18.1 kg (50th centile) and relatively short stature of 99 cm (5th centile). She was normocephalic with an FOC of 51.6 cm (75th centile). The patient exhibited an Angelman Syndrome-like phenotype with normal diagnostic studies (DNA methylation analysis, 15q11q13 deletion analysis by microarray-CGH, and *UBE3A* sequencing). CNS folate deficiency was identified at age 5 years. She showed cognitive and motor improvement with folinic acid treatment, as evidenced by her ability to walk independently, and she demonstrated some vocalizations but did not regain her prior level of functioning with regard to fluency in the use of sign language. The frequency of her seizures substantially decreased after treatment with folinic acid was initiated, although her seizures have not abated completely. Despite these improvements, she was diagnosed with severe MR and autism at age 6 years (see Tables 2–4). Her cognitive level of 9 months is below the mental age floor for the ADOS and ADI-R but she demonstrated scattered skills up to 18 months of age. The patterns of her behavior cannot be fully accounted for by her developmental delays since even children at her cognitive level have achieved some basic social-communication milestones that were not present in this child. Specifically, she used some single words, although her vocalizations were only occasionally socially

directed (typically when she was extremely upset or when she was highly motivated to acquire an object) and she had trouble modulating her voice tone. She routinely used other people's hands to communicate "for" her, had difficulties using eye contact for the purpose of modulating social interactions, was not responsive to her name being called, did not exhibit shared enjoyment in interactions, engaged in repetitive play and insisted upon her own structure and routine, had trouble with transitions, and had unusual sensory interests.

Subject 2

This 9-year-old girl presented with language delay at age 2 years, prior to the diagnosis of leukemia (see below). Diagnosed with acute lymphoblastic leukemia at 26 months, she was treated with intrathecal methotrexate and folinic acid. She exhibited regression (particularly in her language skills), seizures, ataxia, tremor, and chorea. She also stopped pretending to talk on the phone and stopped engaging in doll play. CNS folate deficiency was identified at age 6 years (2 years post-remission). On physical exam, this subject had a height of 119 cm (10th – 25th centile) and a weight of 23.4 kg (50th centile). She was relatively macrocephalic with a head circumference of 53 cm (95th centile). She presented with mild midfacial retrusion, but otherwise she had no other dysmorphic features. Folinic acid afforded some cognitive and motor improvement as evidenced by improvements on standardized testing. Her seizure control was significantly improved after initiation of folinic acid treatment. She was diagnosed with severe MR and autism spectrum disorder at age 7 years. Her cognitive level was above the mental age floor for the ADOS and ADI-R. She demonstrated some isolated higher-level cognitive skills in areas involving rote memorization and visual memory (e.g. reading sight words). Although she used single words and some phrases, much of her language was repetitive in nature and her vocalizations were not socially directed. She also had trouble modulating her voice tone (often speaking using a loud voice), had difficulties with eye gaze, was very insistent upon her own structure and routine in interactions and did not exhibit shared enjoyment. In addition, she exhibited repetitive play and repetitive interests, had unusual sensory interests, trouble with transitions, and tactile defensiveness.

Subject 3

This 8-year-old girl had normal development until age 18 months, after which she developed rapid regression and

dyskinesia in the absence of acute illness or exposure. She was eventually diagnosed with Landau-Kleffner syndrome and was noted to exhibit some autistic features because she did not acknowledge others within her environment. At 3 years she developed seizures and ataxia. She had features of Angelman syndrome, however molecular studies were normal. CNS folate deficiency was identified at age 6 years. On physical exam, her growth parameters revealed a weight of 95.9 kg (>95th percentile), a height of 127 cm (90th percentile), and an FOC of 54 cm (>98th centile). She had features of Angelman syndrome, however molecular studies were normal. On folinic acid supplementation, she had improved social interactions according to reports by her parents. However, her seizure disorder did not improve. She was diagnosed with severe MR and autism at age 7 years and her cognitive level was above the mental age floor for the ADOS and ADI-R. She exhibited some isolated higher-level skills related to auditory and visual memory. Her language consisted of single words and some phrases, and much of her language was rote and repetitive in nature. She exhibited immediate echolalia, was consistently loud when she spoke, and many of her vocalizations were not socially directed. She had difficulties with eye gaze, did not exhibit shared enjoyment in interactions, had difficulties with transitions and was highly insistent upon her own structure and routine, exhibited anxiety in response to certain toys, and displayed unusual sensory interests.

Subject 4

This 10-year-old girl presented with irritability, jitteriness, and feeding difficulties in the neonatal period. Developmental delay was diagnosed at 6 months and she presented with epilepsy at one year of age. The child showed regression at age 18 months. She lost her ability to walk, talk and feed herself. Moreover, she developed breathing dysrhythmias, breath holding spells, bruxism, and was found to have long QT interval on electrocardiogram. She was given the clinical diagnosis of Rett syndrome. *MECP2* sequencing, deletion–duplication analysis by Southern blot, and *CDKL5* sequencing were normal. CNS folate deficiency was identified at age 6 years. Upon her genetics evaluation at age 10 9/12 years she presented with a normal weight of 27 kg (5th centile) and a height of 52.6 cm (10th centile). Her head circumference was 51.4 cm (25th centile). Her facial features were significant for prognathism and an open mouth, but otherwise had no other dysmorphic features. Folinic acid treatment afforded no clinical improvement. Her neurological deficits precluded formal cognitive/behavioral assessment.

Subject 5

This 7-year-old boy presented with developmental delay. The child had normal development until 8 months. Subsequently he showed regression, hypotonia, and nystagmus. CNS folate deficiency was identified at age 4 years. His height was 102 cm (50th centile) and his weight was 16.8 kg (50th–75th centiles). His head circumference was normal at 51.5 cm (75th centile). He had no clinical improvement with folinic acid supplementation. Serial MRI revealed progressive atrophy of cerebellum and corpus callosum. More recently, he developed a seizure disorder, sleep apnea, and severe scoliosis secondary to hypotonia. His neurological impairments precluded formal cognitive/behavioral assessment.

Subject 6

This 2-year-old boy presented in the neonatal period with mild hypotonia and feeding difficulties followed by developmental delay, dyskinesia and seizures at 4 months of age. CNS folate deficiency was identified at 1 year of age. On physical exam he had normal growth parameters with a height of 77.8 cm (13th centile), a weight of 8.61 kg (2.8 SD below the mean), and a head circumference of 46 cm (10th centile). He had no dysmorphic features. Folinic acid treatment afforded no clinical improvement. The child currently exhibits cognitive, motor, and language delays. He also met criteria for an autism spectrum disorder. His cognitive level was below the mental age floor for the ADOS; however, several features of autism were noted for him that cannot be fully accounted for by his developmental delays. He does not consistently use single words and most of his language consists of jargon. He initially had a difficult time with forced interactions and would turn away from the examiner, exhibiting some high-pitched squeals and crying to leave the room. He was tactile defensive, and exhibited a strong preference for cause/effect toys. He exhibited limited shared enjoyment in interactions, had trouble with transitions, and exhibited several unusual sensory interests.

Subject 7

This 15-year-old girl presented with feeding difficulties, hypotonia and jitteriness in the neonatal period. She was diagnosed at 9 months of age with developmental delay. Her course was characterized by dyskinesia, MR, and regression. She was first diagnosed with a static encephalopathy of unknown etiology and her course was significant for hypotonia. A brain MRI revealed an enlarged cisterna

magna, delayed myelination, and possible cerebellar hypoplasia. She was subsequently found to have some cortical visual impairment. CNS folate deficiency was identified at 14 years of age. Weight was 39.9 kg (10th centile) and stature was 139 cm (<5th centile). She was normocephalic with a head circumference of 53.5 cm (25th–50th centiles) and no dysmorphic features were found on physical exam. A repeat brain MRI revealed progressive cerebellar atrophy. On folinic acid supplementation, she demonstrated motor and verbal improvement according to reports by her parents and her teachers, and through formal observation. She was diagnosed with severe MR and autism at 15 years of age. Her cognitive level was above the mental age floor for the ADOS and ADI-R. Her language consisted of single words and phrases and most of her phrase speech was rote and mechanical. She exhibited echolalia, and she often spoke using a loud voice tone. She had difficulties with direct eye gaze, had limited shared enjoyment in interactions, did not engage in pretend play, had several rituals and repetitive behaviors, was extremely prompt-dependent, and exhibited some symptoms of anxiety.

Discussion

We have demonstrated the presence of autism spectrum manifestations in five subjects with CFD. All five patients who were formally evaluated achieved cutoff scores that placed them within the autistic (patients 1, 3, and 7) or autism spectrum (patients 2 and 6) ranges on the ADOS-G and the ADI-R. All five patients exhibited features consistent with those observed in children with idiopathic autism. The overlap in symptomatology between patients with idiopathic autism and patients with CFD is more evident in those patients with higher cognitive and language functioning and seems related to the severity of developmental regression and the level of developmental functioning prior to the regression. In those patients with autistic features and lower functioning in language and cognition (patients 1 and 6), we found that their specific difficulties with social communication cannot be fully accounted for by their delays, given that typically developing children with similar functional levels have acquired these social communication milestones.

Limitations of our study include the small sample number and the fact that the neurobehavioral evaluations were not performed in a blinded fashion. However, these patients with CFD clearly differ in their neurological presentations and complexity from those with autism alone since they present with accompanying neurological features such as seizures, hypotonia, movement disorders, and ataxia. Some of these features such as the seizures and

hypotonia may become evident in the neonatal period. In addition, the regression observed in these patients with cerebral folate deficiency occurs at different points during development, whereas the regression in autism typically occurs prior to age 3 years.

Although the association of CFD and autism has been anecdotally reported by us and others (Moretti et al. 2005; Ramaekers et al. 2005), it has not previously been the subject of direct study. Our previous identification of one child with CFD and autism (Moretti et al. 2005) together with our current observations clearly establish an expanded phenotypic spectrum of CFD suggesting that children with psychomotor retardation, regression, seizures, dyskinesia and autism may benefit from analysis of CSF folate levels when the initial genetic and metabolic workup fails to identify an etiology. Clinical improvement with folinic acid treatment in a subset of our patients highlights the importance of considering this diagnosis. However, clinical improvement was observed only in cognitive, language, and motor domains with no amelioration of autistic features, which have remained consistent over time despite ongoing treatment with folinic acid. Moreover, it could be said that the autistic features became more prevalent as their level of developmental functioning improved. This suggests that autism is not simply the consequence of significant developmental delays—otherwise the autistic behaviors should decline as development improves.

Interestingly, in contrast to previous reports, four of the seven children described here manifested with neonatal abnormalities, while the remaining three subjects had a period of normal early development. This suggests that normal development in early infancy is not a necessary feature of CFD and that an abnormality of CSF folate may be present early in the neonatal period. Moreover, in our patients we did not observe the progressive deceleration of head growth that had been previously reported in children with CFD.

The variability in clinical history, neurological manifestations, and laboratory findings found in CFD suggests two considerations. First, the spectrum of neurological dysfunction associated with this metabolic abnormality remains to be fully characterized. Second, different pathways/disorders may lead to low CSF 5-MTHF. For instance, in some cases a severe impairment of CNS folate transport or metabolism may lead to early-onset of neurologic dysfunction, as occurs in hereditary folate malabsorption where CSF folate levels are very low (Geller et al. 2002). In contrast, a more subtle defect may allow some degree of compensation in the neonatal period and lead to clinically detectable impairments later in life. However, developmental delay/regression, dyskinesia, seizures, and autism seem to constitute core manifestations of this condition. The presence of isolated CSF folate

deficits led us to consider the possibility of defective folate transport into the brain as the pathophysiological basis for CFD. However, we found no mutations in genes regulating folate transport and metabolism (Rosenblatt and Fenton 2000) suggesting either the presence of genetic alterations in regulatory pathways or a potential defect in folate turnover as possible causes for this neurometabolic condition. Although autoantibodies are found in some children with CFD (Ramaekers et al. 2005), their causative role remains to be determined. However, in the author's opinion the presence of significant neurological deficits in the neonatal period in a subset of patients with CFD decreases the likelihood of an autoimmune etiology in these particular individuals. Regardless of the precise mechanism of CFD, we hypothesize that reduced 5-MTHF levels in the brain, reflected in the low CSF folate levels, lead to an impairment of methylation reactions in the brain (Rosenblatt and Fenton 2000) with potential consequences for protein function (protein methylation) and regulation of gene expression (DNA and/or histone methylation).

These observations also raise the question of the presence of CNS folate abnormalities in children with idiopathic autism and developmental regression. A recent study in 20 children with idiopathic autism identified biochemical abnormalities in the plasma consistent with impaired methylation capacity and increased oxidative stress (James et al. 2004). In a follow-up study, plasma methionine and the ratio of *S*-adenosylmethionine (SAM) to *S*-adenosylhomocysteine (SAH), an indicator of methylation capacity, were significantly decreased in 80 autistic children relative to 73 age-matched controls (James et al. 2006). Two of our patients (subjects one and two) exhibited elevated total homocysteine (424 and 138 nM, respectively, with a normal range of 32–113 nM) and increased *S*-adenosylhomocysteine (SAH) values (88.8 and 34.5 nM, respectively, with a normal range of 8.9–14.1) in CSF pointing toward a decreased capacity for methylation as a possible contributing factor in the development of autism. The elucidation of the role of methylation reactions in the pathogenesis of autism will require additional studies. However, the partial overlap of clinical manifestations between CFD and neurodevelopmental disorders including autism suggests the intriguing possibility that these conditions may share common molecular/cellular substrates or abnormalities of CNS methylation reactions.

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References

- Blau, N., Bonafe, L., Krageloh-Mann, I., Thony, B., Kierat, L., Hausler, M., & Ramaekers, V. (2003). Cerebrospinal fluid pterins and folates in Aicardi-Goutieres syndrome: A new phenotype. *Neurology*, 61(5), 642–647.
- Geller, J., Kronn, D., Jayabose, S., & Sandoval, C. (2002). Hereditary folate malabsorption: Family report and review of the literature. *Medicine (Baltimore)*, 81(1), 51–68.
- Hansen, F. J., & Blau, N. (2005). Cerebral folate deficiency: Life-changing supplementation with folinic acid. *Molecular Genetics and Metabolism*, 84(4), 371–373.
- Hyland, K., & Surtees, R. (1992). Measurement of 5-methyltetrahydrofolate in cerebrospinal fluid using HPLC with coulometric electrochemical detection. *Pteridines*, 3, 149–150.
- James, S. J., Cutler, P., Melnyk, S., Jernigan, S., Janak, L., Gaylor, D. W., Neubrandner, J. A. (2004). Metabolic biomarkers of increased oxidative stress and impaired methylation capacity in children with autism. *The American Journal of Clinical Nutrition*, 80(6), 1611–1617.
- James, S. J., Melnyk, S., Jernigan, S., Cleves, M. A., Halsted, C. H., Wong, D. H., Cutler, P., Bock, K., Boris, M., Bradstreet, J. J., Baker, S. M., & Gaylor, D. W. (2006). Metabolic endophenotype and related genotypes are associated with oxidative stress in children with autism. *American Journal of Medical Genetics. Part B, Neuropsychiatric Genetics*, 141(8), 947–956.
- Mercimek-Mahmutoglu, S., & Stockler-Ipsiroglu, S. (2007). Cerebral folate deficiency and folinic acid treatment in hypomyelination with atrophy of the basal ganglia and cerebellum (H-ABC) syndrome. *The Tohoku Journal of Experimental Medicine*, 211(1), 95–96; author reply 97.
- Moretti, P., Sahoo, T., Hyland, K., Bottiglieri, T., Peters, S., del Gaudio, D., Roa, B., Curry, S., Zhu, H., Finnell, R. H., Neul, J. L., Ramaekers, V. T., Blau, N., Bacino, C. A., Miller, G., & Scaglia, F. (2005). Cerebral folate deficiency with developmental delay, autism, and response to folinic acid. *Neurology*, 64(6), 1088–1090.
- Pineda, M., Ormazabal, A., Lopez-Gallardo, E., Nascimento, A., Solano, A., Herrero, M. D., Vilaseca, M. A., Briones, P., Ibanez, L., Montoya, J., & Artuch, R. (2006). Cerebral folate deficiency and leukoencephalopathy caused by a mitochondrial DNA deletion. *Annals of Neurology*, 59, 394–398.
- Qiu, A., Jansen, M., Sakaris, A., Min, S. H., Chattopadhyay, S., Tsai, E., Sandoval, C., Zhao, R., Akabas, M. H., & Goldman, I. D. (2006). Identification of an intestinal folate transporter and the molecular basis for hereditary folate malabsorption. *Cell*, 127(5), 917–928.
- Ramaekers, V. T., & Blau, N. (2004). Cerebral folate deficiency. *Developmental Medicine and Child Neurology*, 46(12), 843–851.
- Ramaekers, V. T., Hansen, S. I., Holm, J., Opladen, T., Senderek, J., Hausler, M., Heimann, G., Fowler, B., Maiwald, R., & Blau, N. (2003). Reduced folate transport to the CNS in female Rett patients. *Neurology*, 61(4), 506–515.
- Ramaekers, V. T., Hausler, M., Opladen, T., Heimann, G., & Blau, N. (2002). Psychomotor retardation, spastic paraplegia, cerebellar ataxia and dyskinesia associated with low 5-methyltetrahydrofolate in cerebrospinal fluid: a novel neurometabolic condition responding to folinic acid substitution. *Neuropediatrics*, 33(6), 301–308.
- Ramaekers, V. T., Rothenberg, S. P., Sequeira, J. M., Opladen, T., Blau, N., Quadros, E. V., & Selhub, J. (2005). Autoantibodies to folate receptors in the cerebral folate deficiency syndrome. *The New England Journal of Medicine*, 352(19), 1985–1991.
- Rosenblatt, D. S., & Fenton, W. A. (2000). Inherited disorders of folate and cobalamin transport and metabolism. In C. R. Scriver, et al. (Eds.), *The metabolic and molecular bases of inherited disease*. New York: McGraw-Hill.
- Wevers, R. A., Hansen, S. I., van Hellenberg Hubar, J. L., Holm, J., Hoier-Madsen, M., & Jongen, P. J. (1994). Folate deficiency in cerebrospinal fluid associated with a defect in folate binding protein in the central nervous system. *Journal of Neurology, Neurosurgery, and Psychiatry*, 57(2), 223–226.