Journal of Pediatric Biochemistry 2 (2012) 263–271
DOI 10.3233/JPB-120067
IOS Press

Review Article

Folate receptor alpha autoimmunity and cerebral folate deficiency in autism spectrum disorders

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Abstract. Cerebral folate deficiency (CFD) is a neurometabolic syndrome characterized by low levels of 5-methyltetrahydrofolate (5MTHF) in the brain despite normal systemic folate levels. Notably, CFD represents one of a few progressive neurological disorders that is treatable and potentially reversible. One common cause of CFD is an autoantibody that binds to the folate receptor-α (FRα) making it non-functional and blocking the transportation of 5MTHF from the blood into the central nervous system. Cow's milk contains soluble FRα antigen, which is 91% similar to human FRα. Autoantibodies to the FRα cross-react with the soluble FRα antigen in cow's milk, increasing the concentration of autoantibodies and resulting in worsen of CFD, while elimination of cow's milk lowers the autoantibody concentration and improves CFD symptoms. Notably, some cases of CFD are due to mitochondrial disease (MD). To date, three studies have reported an association between CFD and Rett syndrome, seven studies have reported that CFD is associated with autism spectrum disorders (ASD) in some children, and five studies have reported FRα autoantibodies in children with ASD, some of whom also had CFD. One study of 93 children with ASD reported that FRα autoantibodies were found in 75.3%. From these studies of children with concomitant ASD and CFD, treatment with oral folinic acid (leucovorin, 0.5 to 2 mg/kg/day) resulted in various improvements ranging from partial improvements in communication, social interaction, attention and stereotypical behavior to complete recovery of both neurological and ASD symptoms. Notably, an overlap between ASD, MD and CFD is found in some children with ASD, and therefore we recommend testing for MD and CFD/FRα autoantibodies in all individuals with ASD. Further studies examining FRα autoantibodies and CFD in children with ASD are warranted.

Keywords: Autism, cerebral folate deficiency, folate, methylfolate, folinic acid

1. Introduction

Autism spectrum disorders (ASD) are a heterogeneous group of neurodevelopmental disorders that are behaviorally defined and characterized by impairments in communication and social interaction along with restrictive and repetitive behaviors [1]. ASD includes autistic disorder, Asperger syndrome, and pervasive developmental disorder-not otherwise specified (PDD-NOS). An estimated 1 out of 88 individuals in the United States (U.S.) is currently affected with an ASD [2]. ASD affects approximately four times as many males as females [3]. The etiology of ASD is unclear at this time. Although several genetic syndromes, such as Fragile X, have been associated with ASD, empirical studies have estimated that single gene and chromosomal defects only account for approximately 6–15% of ASD cases [4]. In fact, one recent study of dizygotic twins

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reported that environmental factors were estimated to account for 55% of autism risk compared to 37% for genetic factors, with risks for developing the broader diagnosis of ASD nearly identical [5]. Therefore, the majority of ASD cases are not due to a simple single gene or chromosomal disorder. Although many of the cognitive and behavioral features of ASD are thought to arise from dysfunction of the central nervous system (CNS), evidence from many fields of medicine has documented multiple non-CNS physiological abnormalities associated with ASD [6–9], suggesting that ASD arises from systemic, rather than organ specific, abnormalities. This article reviews cerebral folate deficiency (CFD) and folate receptor (FRα) autoimmunity in individuals with ASD.

1.1. Folate metabolism

Folate is an essential B vitamin (B9) required for normal neurodevelopment [10,11]. Defects in folate metabolism can cause secondary physiological abnormalities, some of which have been associated with ASD. Since folate is essential for the production of purines and pyrimidines, the nucleotide precursors of RNA and DNA, low folate levels can result in abnormalities in cell proliferation, transcription, and translation, thus contributing to DNA instability [12] and chromosomal breakage [13]. Folate depletion can cause DNA methylation alterations in the brain [14] and deficits in folate metabolism have been shown to be associated with methylation deficits and oxidative stress in some children with ASD [8,15,16].

Deficits in folate metabolism could explain several CNS abnormalities documented in ASD. For example, examination of postmortem ASD brains has shown alterations in DNA methylation in the frontal cortex [17] and increased oxidative stress in cortical regions associated with speech, emotion, and social behavior [18]. Since chronic oxidative stress can result in mitochondrial dysfunction [19], the increased oxidative stress noted in postmortem ASD brain samples [18] could account for the mitochondrial dysfunction found in similar cortical regions in the postmortem ASD brain [20, 21].

ASD has also been associated with defects in folate metabolism. Polymorphisms in the genes coding for methylenetetrahydrofolate reductase (MTHFR) [8, 22–29] and dihydrofolate reductase [30] enzymes have been reported in some children with ASD. Both of these enzymes are involved in the production of 5-methyltetrahydrofolate (5MTHF), a metabolically important and reduced form of folate which is the active metabolite of folate in the CNS. MTHFR is the enzyme that converts folate into 5MTHF. Moreover, polymorphisms in MTHFR have been associated with more severe ASD behaviors, including unusual body movements, hyperactivity and self-injury [28].

1.2. Cerebral folate deficiency

Idiopathic CFD syndrome is a neurometabolic syndrome characterized by low levels of 5MTHF in the CNS despite normal systemic folate levels. This condition is a recently described disorder, being first reported by Ramaekers et al. in 2002 with a patient presenting with psychomotor retardation, spastic paraplegia, cerebellar ataxia, and dyskinesia who had low cerebrospinal fluid (CSF) 5-MTHF levels with normal red blood cell (RBC) and serum folate levels, implying a disturbance of transport of folates across the blood-brain barrier [31]. The authors described two different folate transport mechanisms into the CNS: the reduced folate carrier 1 (RFC1) and the FRα proteins. Normally, 5MTHF is transported across the blood-brain barrier by the FRα, but it can also be transported by RFC1. In the aforementioned patient with idiopathic CFD syndrome, the FRα proteins were suggested to be defective and folinic acid was utilized as a treatment because it can enter the CNS via the alternate RFC1 at the choroid plexus [31]. Additional studies have now reported that treatment of CFD with folinic acid can, in some cases, dramatically improve motor skills, even in as little as one week, as well as improve speech impairments [32]. In fact, CFD represents one of a few progressive neurological disorders that is treatable and potentially reversible.

Idiopathic CFD syndrome was further defined in 2004 by Ramaekers and colleagues who reported that individuals with CFD generally have normal early development until the typical onset at four to six months of age. Symptoms include marked irritability, unrest, slow head growth, psychomotor retardation, cerebellar ataxia, spastic paraplegia, pyramidal tract signs in the legs, dyskinesias (choreoathetosis and ballismus) and, in some cases, seizures. Central visual disturbances (optic atrophy and blindness) and hearing loss occurred after age 3 and 6 years old, respectively, in some patients [33].

In 2005, Ramaekers and colleagues then identified an autoantibody which attached to the FRα, making it non-functional [34]. Normally, 5MTHF binds to the FRα Normally, 5MTHF binds to the FRa through a gly-

cosylphosphatidylinositol (GPI) moiety anchored to the basolateral endothelial surface of the choroid plexus. Through receptor-mediated endocytosis, 5MTHF is then transported across the cell in an ATP-dependent process, where it is normally concentrated two-fold higher in the CNS compared to the blood. The FRα has a high affinity for both folate and 5MTHF derivatives. RFC1 has a lower affinity for folates and lies on both the basolateral as well as the apical surface of the choroid plexus, and in other locations like neuronal axons. RFC1 transports 5MTHF into neurons.

The FRα autoantibodies have a high affinity for the FRα and block the transport of the folates across this carrier on the basolateral surface. These autoantibodies bind to the FRα located in the choroid plexus; however these autoantibodies could potentially impede the folate receptors located in the thyroid, placenta and lung as well. These autoantibodies do not bind significantly to folate receptor protein-2, another folate receptor found in the prostate, liver, testicular, ovarian tissues and blood cells [34]. In healthy adult women, the prevalence of blocking FRα autoantibodies has been estimated to be 10–15% in the United States [35], 4–7% in Spain [36], and 9–13% in Ireland [37]. These autoantibodies have previously been described to be associated with neural tube defects, although this has not been found in every study [37]. One study reported a 12-fold increased risk of subfertility in women with the presence of these autoantibodies [36]. However, in adult populations, a low titer of this autoantibody may not necessarily be pathogenic [38]. Notable, folic acid (an inactive, oxidized form of folate used to fortify food and found in some nutritional supplements) can also attach to the FRα and block this receptor as well [39]. Therefore, in patients with CFD, the use of folic acid should be avoided, when possible.

Notably, reactive oxygen species (ROS) have been reported to inhibit FRα mediated 5MTHF uptake [40]. This finding may be particularly significant in individuals with ASD because they have been shown, as a group, to be under higher oxidative stress and have reduced levels of antioxidants as compared to controls [8, 15,19,41–47].

1.3. The genetics of cerebral folate deficiency

In children with CFD, some studies have reported several mutations in the folate receptor 1 gene (*FOLR1*) which codes for the FRα [48–50]. In one study, mutations in this gene did not correlate with clinical severity, suggesting other factors contributed to CFD [48]. In addition, in one study, all 10 patients with mutations in *FOLR1* had an extremely low CSF 5MTHF (< 5 nmol/l) [48]. This suggests that screening for *FOLR1* defects should be performed in individuals with CFD who have extremely low CSF 5MTHF. However, even in this specific phenotype of very severe CFD, mutations were only identified in a fraction (14%) of the cases studied. To date, studies in individuals with ASD have not reported any mutations in *FOLR1* [51,52].

1.4. The influence of dietary factors in cerebral folate deficiency

Cow's milk contains soluble FRα antigen, which is 91% similar to the human FRα. Autoantibodies to the FRα cross-react with the soluble FRα antigen in cow's milk, which can lead to an increase in the circulating serum FRα autoantibody concentration. Exposure to cow's milk has been shown to increase the concentration of the FRα autoantibody and lead to worsening of CFD symptoms, while elimination of cow's milk has been reported to lower the autoantibody concentration and improve CFD symptoms [53]. Moreover, re-exposure to cow's milk after a period of being cow's milk-free substantially worsens the condition and increases the autoantibody concentration [53]. These findings may help explain why some parents of children with ASD report improvements in their child on a cow's milk-free diet [54,55]. Notably, exposure to cow's milk has also been associated with constipation and megarectum in some children with ASD [56] and a recent study of 199 children with ASD reported that 58% had lactase deficiency [57]. Recently, some parents have been using camel's milk as a treatment in some children with ASD because camel's milk appears to help food allergies in some individuals [58,59]. However, the concentration of FRα antigen in camel's milk is similar to that found in cow's milk and its immunoreactivity with FRα is also similar to the FRα antigen in cow's milk and is 2–3 fold higher than with human milk (Dr. Quadros, personal communication, 12/21/11). Therefore, the use of camel's milk in children with FRα autoantibodies may be problematic.

1.5. Mitochondrial disease as a cause of cerebral folate deficiency

In 1983, low CSF folate was linked to mitochondrial disease (MD) in a woman with Kearns-Sayre syndrome [60]. In 2006, CFD was linked to MD in a case report of a child with an incomplete form of Kearns-

Sayre syndrome [61]. Further case reports and case series later expanded the association between CFD and MD to include complex I deficiency [62], Alpers' disease [63] and complex IV overactivity [64], as well as a wide variety of mitochondrial disorders in both children and adults [65]. One study reported CFD in three individuals with Kearns-Sayre syndrome and in four patients with MD [66]. A larger study [65] of 28 patients with MD, diagnosed by standard criteria [67], reported that 50% had CFD. Notably, one study reported a child with ASD who also had MD and CFD [68]. In many of these cases, autoantibodies to FRα were not found, suggesting that it was the lack of ATP availability secondary to mitochondrial dysfunction that resulted in the impaired transportation of 5-MTHF into the CNS.

1.6. Autism spectrum disorder is associated with cerebral folate deficiency and the folate receptor alpha autoantibody

To date, three studies have reported a connection between CFD and Rett syndrome [66,69,70], seven studies have reported that CFD is associated with ASD in some children [33,34,51–53,68,71] and five studies have reported FRα autoantibodies in children with ASD, some of whom also had CFD [34,38,52,53,72]. In CFD, the male-to-female ratio is approximately 2.5–3:1 [33,52,53], approaching the prevalence reported in ASD [3].

CFD was first described in ASD in 2004 in a study of 20 children with CFD, of whom seven children met the criteria for autism on the Autism Diagnostic Observation Schedule (ADOS). In this study, 18 of the 20 (90%) children had normal development during the first 4 months of life, followed by a deceleration of head growth from four to six months of age, as well as sleep disturbances, marked unrest and irritability. Interestingly, 9 of 20 (45%) children had reduced CSF 5-hydroxy-indolacetic acid (5-HIAA, a metabolite of serotonin) levels in the face of normal homovanillic levels. Seven of these nine (78%) children had 5-HIAA levels return to normal after folinic acid supplementation [33]. Notably, two other studies in individuals without autism also reported a normalization in CSF serotonin levels from folinic acid administration, including one child with CFD [32] and four children with Rett syndrome who also had CFD [69].

In 2005, another group of investigators described a 6 year old girl with CFD who met the criteria for autism as measured on the ADOS and the Autism Diagnostic Interview-Revised (ADI-R). Treatment of this child with folinic acid corrected the low 5-MTHF levels in the CSF and led to improved motor skills as well as parentally reported mild improvements in verbalizations and social interaction [71].

A larger study in 2005 reported that out of 28 children with CFD, five met the criteria for autism on the ADOS. These children had "low functioning" autism along with neurological deficits. One child "recovered completely" after taking 400 μg of folic acid daily and was reported to be attending regular school; this child did not produce autoantibodies to the FRα. The other four children with autism had mental retardation and high titers of blocking autoantibodies (ranging from 0.65 to 1.27 pmol of FRα blocked per ml of serum) and treatment with folinic acid or folic acid led to improved communication in the two youngest children, while the two older children had poorer outcomes. Notably, in this study, four out of the five (80%) children with autism produced blocking autoantibodies that accounted for the CFD [34].

In another study from 2007 of 25 children with regressive autism (diagnosed on ADOS and ADI-R) who were "low functioning" with or without neurological defects, 23 (92%) children had low CSF 5MTHF levels consistent with CFD. Of these 23, 19 (83%) had measurable blocking autoantibodies to the FRα which could account for the low CSF 5MTHF. In one of the children with CFD and autism, the FRα autoantibody concentration measured weekly over 6 weeks strongly correlated with increasing aggressive behavior. These children were treated with oral folinic acid, and two younger children (ages two years and eight months, and three years and two months) were "cured with full recovery from autism and neurological deficits." Three older children had improvements in neurological deficits but not in autism symptoms. The remaining 13 children had improvement in neurological deficits, and partial improvements in autistic symptoms, including social impairment (four children, 31%), communication impairments (nine children, 69%), and restricted interests (six children, 46%) [52].

In another study from 2008 of 7 children with CFD, five children were examined for possible autism (two children displayed symptoms considered to be too severe to be tested for autism) and all five of these children met the criteria for autism based on the ADOS and the ADI-R. Notably, none of these five children had a history of deceleration of head growth, a common finding in CFD. Four of the seven (57%) children demonstrated various improvements in cognition, mo-

tor skills, social interaction, communication and a reduction in the frequency of seizures with folinic acid treatment [51].

In one study from 2008 of 24 children with CFD, 10 met criteria for autism as measured by ADOS and ADI-R. Folinic acid was given to all 24 children, which led to improvements in irritability, insomnia, ataxia, seizure frequency and spasticity as well as ceasing the deceleration in head growth. In the 10 children with autism, folinic acid led to marked improvements in two children and partial improvements in four children in communication, attention and stereotypies. In addition, elimination of cow's milk in some of the children led to a significant reduction in FRα autoantibody concentration, with a significant increase when cow's milk was reintroduced. Elimination of cow's milk also led to improvements in CFD symptoms. In other children, the continuation of cow's milk over a two year period led to a significant rise in FRα autoantibody concentration. Notably, children who had a blocking autoantibody titer of approximately 0.5 pmol of FRα blocked per ml of serum or higher were very likely to have below normal levels of CSF 5MTHF. However, some children with a very low blocking autoantibody titer (e.g., 0.1 pmol of FRα blocked per ml of serum) still had low levels of CSF 5MTHF and subsequent CFD [53].

Recently, Frye et al. [72] reported that autoantibodies to the FRα were present in approximately 75% of children with ASD, and administration of folinic acid (2 mg/kg/day; max 50 mg/day) in children with ASD and FRα autoantibodies resulted in significant improvements in parental ratings of receptive and expressive language, verbal communication, stereotypic behavior, and attention compared to parental ratings for children who did not undergo did not undergo any intervention (wait-list control group) over a similar time period. In this study, FRα autoantibody titers were collected in 93 children with ASD as part of a medical workup. Concentrations of both the blocking and binding FRα autoantibodies were measured and categorized as negative, low, medium, or high [34,37]. The sample included 84 male and nine female children with ASD (mean age = 7y 3 m, SD = 3y 1 m; range = 2y 11 m – 17y 5 m). Overall, 60% and 44% were positive for the blocking and binding FRα autoantibody, respectively. For children who were positive for the blocking FRα autoantibody, a low, medium, or high titer was found in 33%, 17%, and 10% of the sample, respectively. For children with the binding FRα autoantibody, a low, medium, and high titer was found in 40%, 4% and 0% of the sample, respectively. Overall, 29% of children were positive for both blocking and binding FRα autoantibodies, 46% were positive for only one FRα autoantibody and 75% were positive for at least one FRα autoantibody. Review of the clinical and medical characteristics between children positive for at least one FRα autoantibody as compared to those negative for both FRα autoantibodies demonstrated no significant differences. Notably, 27 parents underwent FRα autoantibody testing and 10 (37%) were found to be low positive for the blocking FRα autoantibody, 2 (7%) were low positive for the binding FRα autoantibody, and none were positive for both autoantibodies. Six siblings without ASD were also tested for FRα autoantibodies and 1 (17%) was low positive for the blocking FRα autoantibody and 1 (17%) was low positive for the binding FRα autoantibody, with none being positive for both autoantibodies.

In addition, 44 children (age mean = 6y 10 m; SD = 2y 8 m) of the 70 children positive for at least one FRα autoantibody were given 2 mg/kg/day of folinic acid in two divided doses (maximum 50 mg daily). The dose was escalated over a two-week period with half of the final dose given during the first two weeks. Intervention response and adverse events were assessed during a follow-up after at least one-month of intervention (mean follow-up time = 4.0 months; SD = 2.6 m). No significant changes were made in other interventions during the follow-up period. Parents were asked to rate intervention responses using a modified Clinical Global Impression (CGI) Improvement subscale on cognition and behavior dimensions: verbal communication, receptive language, expressive language, nonverbal communication, stereotypical behavior, hyperactivity, mood, attention, and aggression.

Twenty-six FRα autoantibody positive children were not treated with folinic acid because they were awaiting FRα autoantibody or CSF results. During follow-up, nine children were found to have not made any changes in interventions since the blood draw for the FRα autoantibodies (age mean = 6y 11 m, SD = 2y 8 m); therefore the wait-list control group was composed of these nine patients. Parents were asked to rate changes in their child's behavior since the blood draw on the CGI scale. The mean time between blood draw and rating was 3.1 months (SD = 1.3 months) for the wait-list control group, which was not significantly different than the treatment length for the intervention group.

To determine if parental ratings demonstrated greater improvement in the intervention group compared to the control group, one-tailed Mann-Whitney U nonparametric tests were used. The one-tailed test was used

since it was hypothesized that an improvement, not a decrement, in cognitive-behavioral function would occur with the intervention. Parametric t-tests were also computed for comparison. Significantly higher improvement ratings were found for treated compared to untreated children on ratings of verbal communication, receptive and expressive language, attention, and stereotypical behavior. These significant differences were confirmed using the Student's t-test. The Mann-Whitney U test did not find significant differences between groups for the ratings of non-verbal communication, mood, hyperactivity, or aggression although the t-test did demonstrate significantly higher improvement ratings for treated compared to untreated children on ratings of non-verbal communication, mood, and aggression. Approximately two-thirds (66%) of the children treated were rated as manifesting improvement in receptive and expressive language, verbal communication, attention, and stereotyped behavior, with one-third (~33%) of children rated as demonstrating moderate or much improvement. Thus, folinic acid treatment appears to result in improvement in core (i.e., communication, stereotyped behavior) and associated (i.e., attention) symptoms of ASD in children with ASD who are positive for FRα autoantibodies.

In this study, adverse effects of folinic acid were minimal for children positive for the FRα autoantibody who received the intervention for at least one month. Four (9%) of the 44 treated children discontinued the intervention due to an adverse effect. Three boys, all taking risperidone, discontinued within two weeks due to worsening aggression. One other patient was taking risperidone but did not demonstrate adverse effects. The fourth boy developed insomnia and gastroesophageal reflux six weeks after the intervention started. Thus, overall, there was a very low rate of intervention discontinuation and a low rate of reported adverse effects. As a comparison, for children who underwent the folinic acid and methylcobalamin intervention in the James et al. [73] study, four children discontinued the study (9%); two due to the fact that parents were uncomfortable giving methylcobalamin injections and two due to sleep disruption and increased impulsivity and irritability.

Recently, Ramaekers et al. examined the prevalence of FRα autoantibodies in 75 children with autism and their parents, compared to 30 non-autistic controls who had developmental delay [38]. Age and serum folate levels were similar in both groups of children. In the children with ASD, 47% (35 out of 75) were positive for the blocking autoantibody while only 3% (1 out of 30) of control children were positive ($p < 0.001$). In the mothers, 19 out of 74 (26%) were positive, while 9 out of 50 (18%) fathers were positive. Fluctuations in autoantibody titers were observed in some children, and two children fluctuated between 0 and 1.14 pmol FRα blocked per ml serum. Based on these findings, the investigators suggested that children with ASD should be tested on multiple occasions for autoantibodies to the FRα to avoid missing those who might be intermittently negative. In addition, in 40 children who were negative for the FR autoantibody, 10 mothers and 4 fathers tested positive, suggesting that parental autoantibodies to the FRα might have played some role in the ASD.

1.7. The clinical implications of cerebral folate deficiency and the folate receptor alpha autoantibody in autism spectrum disorder

In the reviewed studies, most children with ASD who had CFD possessed FRα autoantibodies. However, in two of these studies, 17% [52] to 20% [34] of children with CFD and concomitant ASD did not have these autoantibodies, indicating another cause for CFD was present. Since the transport of 5MTHF into the CSF is ATP-dependent, one potential reason for this finding is mitochondrial dysfunction [65], which is a relatively common finding in ASD [6,74]. In one study, children with ASD who had a high FRα autoantibody concentration were likely to have a below normal level of 5MTHF in the CSF. However, even some children with very low FRα autoantibody concentrations may have CFD [53]. Therefore, it is noteworthy that some children with ASD who have either a very low concentration of FRα autoantibodies or no autoantibodies may still have CFD. Treatment of these children with oral folinic acid may lead to beneficial effects.

From the reviewed studies of children with concomitant ASD and CFD, treatment with oral folinic acid (leucovorin, 0.5 to 2 mg/kg/day) resulted in various improvements ranging from partial improvements in communication, social interaction, attention and stereotypical behavior [34,51,53,71] to complete recovery of both neurological and ASD symptoms [34,52]. In one study, treatment with folinic acid corrected low serotonin related findings in a majority of children with CFD and concomitant ASD who also had low levels of CSF 5-HIAA [33].

Only two of the reviewed studies reported the prevalence of FRα autoantibodies in control children. One study reported that 34% of siblings without ASD were positive for one of the FRα autoantibodies, but the

sample size was quite small (six children) [72]. The other study reported 3% of control children were positive for the blocking FRα autoantibody, but these control children had developmental delay [38]. Previous studies have demonstrated the prevalence of blocking FRα autoantibodies in the general population to range from 4% [36] to 15% [35]. Therefore, the reviewed studies strongly support the notion that there is a higher prevalence of FRα autoantibodies in children with ASD compared to the general population.

Because FRα autoantibodies appear to be highly prevalent in children with ASD, we recommend that FRα autoantibody testing should be considered in all patients with ASD. Testing may need to be performed on multiple occasions since some children may be intermittently positive for the FRα autoantibody [38]. Early identification and treatment is paramount as younger children generally respond more robustly than older children, with "cure" reported in some of the youngest children [52]. It may also be prudent to test parents for the presence of FRα autoantibodies since two studies reported that 18–44% of the parents are positive for at least one of the autoantibodies [38,72]. Furthermore, since one study [72] reported that 34% of typically developing siblings are positive for at least one of the FRα autoantibodies, it may be prudent to test siblings.

Notably, an overlap between ASD, MD and CFD is found in some children with ASD [68], and therefore we also recommend testing for MD in individuals with ASD [6,74]. In children with ASD who have FRα autoantibodies or who have CFD, treatment with oral folinic acid can lead to improvements in receptive and expressive language, attention, and stereotypical behavior [52,72]. Interestingly, one study reported an improvement in seizure activity with folinic acid treatment [51]. Elimination of cow's milk is also essential [53]. Further studies examining FRα autoantibodies and CFD in children with ASD are warranted.

References

[1] APA, Diagnostic and statistical manual of mental disorders. American Psychiatric Association (4th ed.), Washington, DC, 1999.

[2] Autism and Developmental Disabilities Monitoring Network Surveillance Year 2008 Principal Investigators, Centers for Disease Control and Prevention. Prevalence of autism spectrum disorders – autism and developmental disabilities monitoring network, 14 sites, United States, 2008. MMWR Surveill Summ 2012; 61: 1-19.

[3] Autism and Developmental Disabilities Monitoring Network Surveillance Year 2008 Principal Investigators, Centers for Disease Control and Prevention. Prevalence of autism spectrum disorders–autism and developmental disabilities monitoring network, 14 sites, United States, 2002. MMWR Surveill Summ 2007; 56: 12-28.

[4] Schaefer GB, Mendelsohn NJ. Genetics evaluation for the etiologic diagnosis of autism spectrum disorders. Genet Med 2008; 10: 4-12.

[5] Hallmayer J, Cleveland S, Torres A, et al. Genetic Heritability and Shared Environmental Factors Among Twin Pairs With Autism. Arch Gen Psychiatry 2011; 68: 1095-1102.

[6] Rossignol DA, Frye RE. Mitochondrial dysfunction in autism spectrum disorders: a systematic review and meta-analysis. Mol Psychiatry 2012; 17: 290-314.

[7] Buie T, Campbell DB, Fuchs GJ, 3rd, et al. Evaluation, diagnosis, and treatment of gastrointestinal disorders in individuals with ASDs: a consensus report. Pediatrics 2010; 125: 1-18.

[8] James SJ, Melnyk S, Jernigan S, et al. Metabolic endophenotype and related genotypes are associated with oxidative stress in children with autism. Am J Med Genet B Neuropsychiatr Genet 2006; 141: 947-956.

[9] Ashwood P, Krakowiak P, Hertz-Picciotto I, Hansen R, Pessah I, Van de Water J. Elevated plasma cytokines in autism spectrum disorders provide evidence of immune dysfunction and are associated with impaired behavioral outcome. Brain Behav Immun 2011; 25: 40-45.

[10] Greenblatt JM, Huffman LC, Reiss AL. Folic acid in neurodevelopment and child psychiatry. Prog Neuropsychopharmacol Biol Psychiatry 1994; 18: 647-680.

[11] Black MM. Effects of vitamin B12 and folate deficiency on brain development in children. Food Nutr Bull 2008; 29: 126-131.

[12] Duthi SJ, Hawdon A. DNA instability (strand breakage, uracil misincorporation, and defective repair) is increased by folic acid depletion in human lymphocytes *in vitro*. FASEB J 1998; 12: 1491-1497.

[13] Crott JW, Mashiyama ST, Ames BN, Fenech M. The effect of folic acid deficiency and MTHFR C677T polymorphism on chromosome damage in human lymphocytes *in vitro*. Cancer Epidemiol Biomarkers Prev 2001; 10: 1089-1096.

[14] Pogribny IP, Karpf AR, James SR, Melnyk S, Han T, Tryndyak VP. Epigenetic alterations in the brains of Fisher 344 rats induced by long-term administration of folate/methyl-deficient diet. Brain Res 2008; 1237: 25-34.

[15] James SJ, Cutler P, Melnyk S, et al. Metabolic biomarkers of increased oxidative stress and impaired methylation capacity in children with autism. Am J Clin Nutr 2004; 80: 1611-1617.

[16] Melnyk S, Fuchs GJ, Schulz E, et al. Metabolic Imbalance Associated with Methylation Dysregulation and Oxidative Damage in Children with Autism. J Autism Dev Disord 2012; 42: 367-377.

[17] Nagarajan RP, Hogart AR, Gwye Y, Martin MR, LaSalle JM. Reduced MeCP2 expression is frequent in autism frontal cortex and correlates with aberrant MECP2 promoter methylation. Epigenetics 2006; 1: e1-11.

[18] Sajdel-Sulkowska EM, Xu M, McGinnis W, Koibuchi N. Brain Region-Specific Changes in Oxidative Stress and Neurotrophin Levels in Autism Spectrum Disorders (ASD). Cerebellum 2011; 10: 43-48.

[19] Chauhan A, Chauhan V. Oxidative stress in autism. Pathophysiology 2006; 13: 171-181.

[20] Rose S, Melnyk S, Pavliv O, et al. Evidence of oxidative damage and inflammation associated with low glutathione redox status in the autism brain. Transl Psychiatry 2012; 2: e134.
[21] Chauhan A, Gu F, Essa MM, et al. Brain region-specific deficit in mitochondrial electron transport chain complexes in children with autism. J Neurochem 2011; 117: 209-220.
[22] Frustaci A, Neri M, Cesario A, et al. Oxidative stress-related biomarkers in autism: Systematic review and meta-analyses. Free Radic Biol Med 2012; 52: 2128-2141.
[23] Boris M, Goldblatt A, Galanko J, James SJ, et al. Association of MTHFR gene variants with autism. Journal of American Physicians and Surgeons 2004; 9: 106-108.
[24] Mohammad NS, Jain JM, Chintakindi KP, Singh RP, Naik U, Akella RR. Aberrations in folate metabolic pathway and altered susceptibility to autism. Psychiatr Genet 2009; 19: 171-176.
[25] Guo T, Chen H, Liu B, Ji W, Yang C. Methylenetetrahydrofolate Reductase Polymorphisms C677T and Risk of Autism in the Chinese Han Population. Genet Test Mol Biomarkers 2012; 16: 968-973.
[26] Schmidt RJ, Tancredi DJ, Ozonoff S, et al. Maternal periconceptional folic acid intake and risk of autism spectrum disorders and developmental delay in the CHARGE (CHildhood Autism Risks from Genetics and Environment) case-control study. Am J Clin Nutr 2012; 96: 80-89.
[27] Liu X, Solehdin F, Cohen IL, et al. Population- and family-based studies associate the MTHFR gene with idiopathic autism in simplex families. J Autism Dev Disord 2011; 41: 938-944.
[28] Goin-Kochel RP, Porter AE, Peters SU, Shinawi M, Sahoo T, Beaudet AL. The MTHFR 677C–>T polymorphism and behaviors in children with autism: exploratory genotype-phenotype correlations. Autism Res 2009; 2: 98-108.
[29] Pas¸ca SP, Dronca E, Kaucsa´r T, et al. One Carbon Metabolism Disturbances and the C677T MTHFR Gene Polymorphism in Children with Autism Spectrum Disorders. J Cell Mol Med 2009; 13: 4229-4238.
[30] Adams M, Lucock M, Stuart J, Fardell S, Baker K, Ng X. Preliminary evidence for involvement of the folate gene polymorphism 19bp deletion-DHFR in occurrence of autism. Neurosci Lett 2007; 422: 24-29.
[31] Ramaekers VT, Ha¨usler M, Opladen T, Heimann G, Blau N. 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 2002; 33: 301-308.
[32] Hansen FJ, Blau N. Cerebral folate deficiency: life-changing supplementation with folinic acid. Mol Genet Metab 2005; 84: 371-373.
[33] Ramaekers VT, Blau N. Cerebral folate deficiency. Dev Med Child Neurol 2004; 46: 843-851.
[34] Ramaekers VT, Rothenberg SP, Sequeira JM, et al. Autoantibodies to folate receptors in the cerebral folate deficiency syndrome. N Engl J Med 2005; 352: 1985-1991.
[35] Rothenberg SP, da Costa MP, Sequeira JM, et al. Autoantibodies against folate receptors in women with a pregnancy complicated by a neural-tube defect. N Engl J Med 2004; 350: 134-142.
[36] Berrocal-Zaragoza MI, Fernandez-Ballart JD, Murphy MM, Cavalle´-Busquets P, Sequeira JM, Quadros EV. Association between blocking folate receptor autoantibodies and subfertility. Fertil Steril 2009; 91: 1518-1521.
[37] Molloy AM, Quadros EV, Sequeira JM, et al. Lack of association between folate-receptor autoantibodies and neural-tube defects. N Engl J Med 2009; 361: 152-160.
[38] Ramaekers VT, Quadros EV, Sequeira JM. Role of folate receptor autoantibodies in infantile autism. Mol Psychiatry, 2012, in press.
[39] Hyland K, Shoffner J, Heales SJ. Cerebral folate deficiency. J Inherit Metab Dis 2010; 33: 563-570.
[40] Opladen T, Blau N, Ramaekers VT. Effect of antiepileptic drugs and reactive oxygen species on folate receptor 1 (FOLR1)-dependent 5-methyltetrahydrofolate transport. Mol Genet Metab 2010; 101: 48-54.
[41] Pastural E, Ritchie S, Lu Y, et al. Novel plasma phospholipid biomarkers of autism: mitochondrial dysfunction as a putative causative mechanism. Prostaglandins Leukot Essent Fatty Acids 2009; 81: 253-264.
[42] Kurup RK, Kurup PA. A hypothalamic digoxin-mediated model for autism. Int J Neurosci 2003; 113: 1537-1559.
[43] James SJ, Melnyk S, Fuchs G, et al., Efficacy of methylcobalamin and folinic acid treatment on glutathione redox status in children with autism. Am J Clin Nutr 2009; 89: 425-430.
[44] Geier DA, Kern JK, Garver CR, Adams JB, Audhya T, Geier MR. A prospective study of transsulfuration biomarkers in autistic disorders. Neurochem Res 2009; 34: 386-393.
[45] Geier DA, Kern JK, Garver CR, et al. Biomarkers of environmental toxicity and susceptibility in autism. J Neurol Sci 2009; 280: 101-108.
[46] James SJ, Rose S, Melnyk S, et al. Cellular and mitochondrial glutathione redox imbalance in lymphoblastoid cells derived from children with autism. FASEB J 2009; 23: 2374-2383.
[47] Rose S, Melnyk S, Trusty TA, et al. Intracellular and extracellular redox status and free radical generation in primary immune cells from children with autism. Autism Res Treat 2012; 2012: 986519.
[48] Grapp M, Just IA, Linnankivi T, et al. Molecular characterization of folate receptor 1 mutations delineates cerebral folate transport deficiency. Brain 2012; 135: 2022-2031.
[49] Steinfeld R, Grapp M, Kraetzner R, et al. Folate receptor alpha defect causes cerebral folate transport deficiency: a treafig neurodegenerative disorder associated with disturbed myelin metabolism. Am J Hum Genet 2009; 85: 354-363.
[50] Cario H, Bode H, Debatin KM, Opladen T, Schwarz K. Congenital null mutations of the FOLR1 gene: A progressive neurologic disease and its treatment. Neurology 2009; 73: 2127-2129.
[51] Moretti P, Peters SU, Del Gaudio D, et al. Brief report: autistic symptoms, developmental regression, mental retardation, epilepsy, and dyskinesias in CNS folate deficiency. J Autism Dev Disord 2008; 38: 1170-1177.
[52] Ramaekers VT, Blau N, Sequeira JM, Nassogne MC, Quadros EV. Folate receptor autoimmunity and cerebral folate deficiency in low-functioning autism with neurological deficits. Neuropediatrics 2007; 38: 276-281.
[53] Ramaekers VT, Sequeira JM, Blau N, Quadros EV. A milk-free diet downregulates folate receptor autoimmunity in cerebral folate deficiency syndrome. Dev Med Child Neurol 2008; 50: 346-352.
[54] Whiteley P, Haracopos D, Knivsberg AM, et al. The ScanBrit randomised, controlled, single-blind study of a gluten- and casein-free dietary intervention for children with autism spectrum disorders. Nutr Neurosci 2010; 13: 87-100.
[55] Pennesi CM, Klein LC. Effectiveness of the gluten-free, casein-free diet for children diagnosed with autism spectrum

disorder: Based on parental report. Nutr Neurosci 2012; 15: 85-91.
[56] Afzal N, Murch S, Thirrupathy K, Berger L, Fagbemi A, Heuschkel R. Constipation with acquired megarectum in children with autism. Pediatrics 2003; 112: 939-942.
[57] Kushak RI, Lauwers GY, Winter HS, Buie TM, et al. Intestinal disaccharidase activity in patients with autism: Effect of age, gender, and intestinal inflammation. Autism 2011; 15: 285-294.
[58] Shabo Y, Barzel R, Margoulis M, Yagil R. Camel milk for food allergies in children. Isr Med Assoc J 2005; 7: 796-798.
[59] Shabo Y, Yagil, R. Etiology of autism and camel milk as therapy. Int J Disab Hum Develop, 2005; 4(2): 67-70.
[60] Allen RJ, DiMauro S, Coulter DL, Papadimitriou A, Rothenberg SP. Kearns-Sayre syndrome with reduced plasma and cerebrospinal fluid folate. Ann Neurol 1983; 13: 679-682.
[61] Pineda M, Ormazabal A, Lo´pez-Gallardo E, et al. Cerebral folate deficiency and leukoencephalopathy caused by a mitochondrial DNA deletion. Ann Neurol 2006; 59: 394-398.
[62] Ramaekers VT, Weis J, Sequeira JM, Quadros EV, Blau N. Mitochondrial complex Iencephalomyopathy and cerebral 5-methyltetrahydrofolate deficiency. Neuropediatrics 2007; 38: 184-187.
[63] Hasselmann O, Blau N, Ramaekers VT, Quadros EV, Sequeira JM, Weissert M. Cerebral folate deficiency and CNS inflammatory markers in Alpers disease. Mol Genet Metab 2010; 99: 58-61.
[64] Frye RE, Naviaux RK. Autistic Disorder with complex IV overactivity: A new mitochondrial syndrom. J Ped Neurol, 2011; 9: 427-34.
[65] Garcia-Cazorla A, Quadros EV, Nascimento A, et al. Mitochondrial diseases associated with cerebral folate deficiency. Neurology 2008; 70: 1360-1362.
[66] Pe´rez-Duen˜as B, Ormaza´bal A, Toma C, et al. Cerebral folate deficiency syndromes in childhood: clinical, analytical, and etiologic aspects. Arch Neurol 2011; 68: 615-621.
[67] Bernier FP, Boneh A, Dennett X, Chow CW, Cleary MA, Thorburn DR. Diagnostic criteria for respiratory chain disorders in adults and children. Neurology 2002; 59: 1406-1411.
[68] Shoffner J, Hyams L, Langley GN, et al. Fever plus mitochondrial disease could be risk factors for autistic regression. J Child Neurol 2010; 25: 429-434.
[69] Ramaekers VT, Hansen SI, Holm J, et al. Reduced folate transport to the CNS in female Rett patients. Neurology 2003; 61: 506-515.
[70] Ramaekers VT, Sequeira JM, Artuch R, et al. Folate receptor autoantibodies and spinal fluid 5-methyltetrahydrofolate deficiency in Rett syndrome. Neuropediatrics 2007; 38: 179-183.
[71] Moretti P, Sahoo T, Hyland K, et al. Cerebral folate deficiency with developmental delay, autism, and response to folinic acid. Neurology 2005; 64: 1088-1090.
[72] Frye RE, Sequeira JM, Quadros EV, James SJ, Rossignol DA. Cerebral folate receptor autoantibodies in autism spectrum disorder. Mol Psychiatry 2012, in press.
[73] James SJ, Melnyk S, Fuchs G, et al. Efficacy of methylcobalamin and folinic acid treatment on glutathione redox status in children with autism. Am J Clin Nutr 2009; 89: 425-430.
[74] Frye RE, Rossignol DA. Mitochondrial dysfunction can connect the diverse medical symptoms associated with autism spectrum disorders. Pediatr Res 2011; 69: 41R-47R.