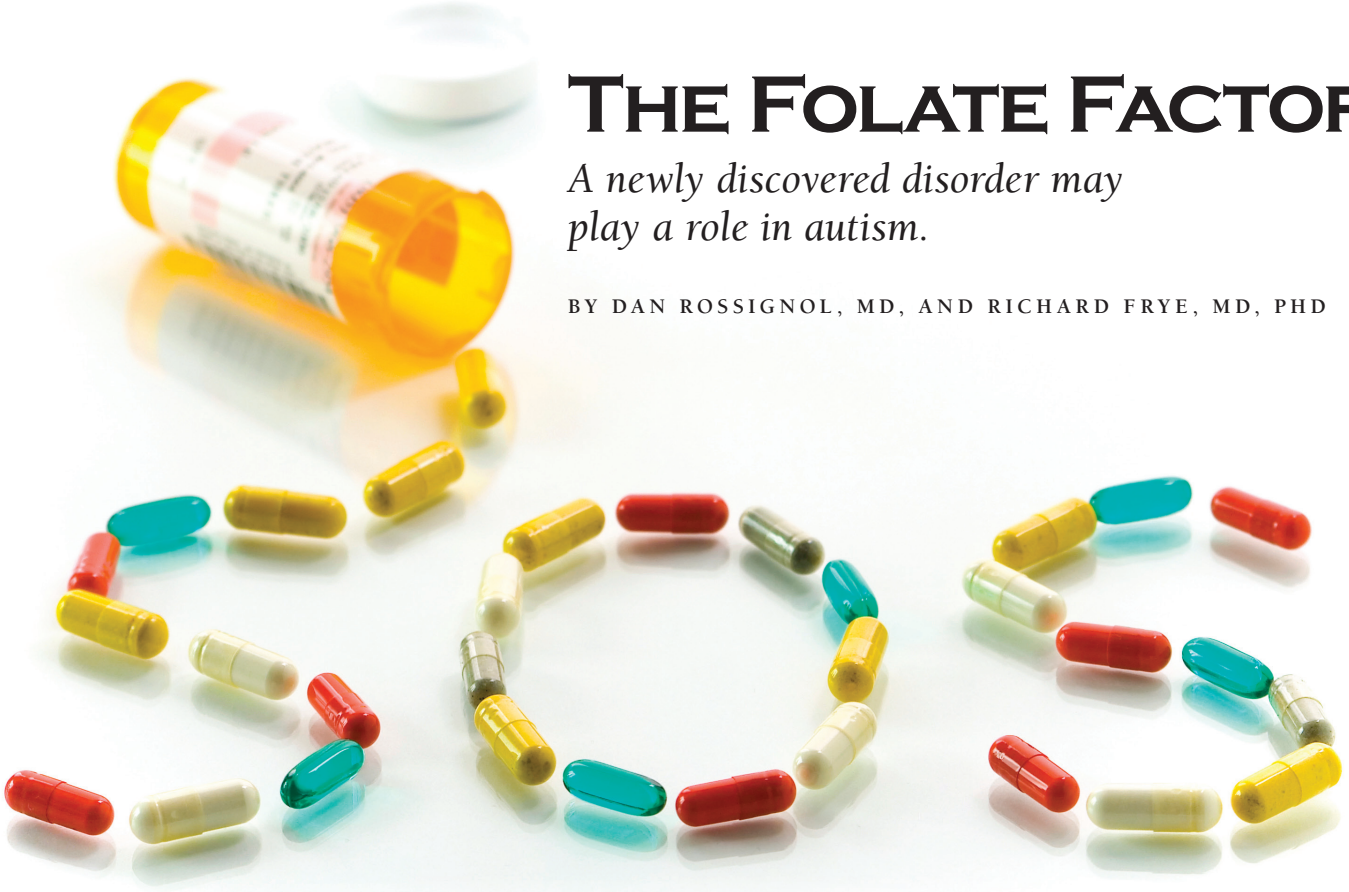

The Folate Factor

A newly discovered disorder may play a role in autism.

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Autism spectrum disorders (ASDs)—which include autistic disorder, Asperger's Syndrome, and pervasive developmental disorder-not otherwise specified (PDD-NOS)—are defined by behavioral observations and characterized by impairments in communication and social interaction, along with restrictive and repetitive behaviors [1].

An estimated one out of 110 individuals in the U.S. is currently affected with an ASD [2], but the cause of ASD is not known at this time. Several genetic syndromes—such as Fragile X and Rett syndromes—have been associated with ASD, but scientific studies have found that genetic syndromes are only observed in a small percentage (6-15 percent) of children with ASD [3]. Therefore, the cause of ASD in most children with these disorders is not due to a simple abnormality of the DNA code, or a missing or extra piece of a chromosome.

The association of ASD with a number of physiological abnormalities, including immune dysfunction and inflammation, mitochondrial dysfunction, oxidative stress and environmental toxicant exposures, has gained increased attention in the last five years [4]. Findings from these areas of research suggest that there are many physiological abnormalities that could contribute to the development of ASD in some children.

An example of a physiological abnormality that might cause ASD is mitochondrial disease. Recently we reviewed the evidence for children with ASD having abnormal functioning of mitochondria [5]. The mitochondrion is an essential part of each cell, and is responsible for producing energy for the cellular metabolic processes. When this energy engine of the cell does not work correctly, many organs in the body—especially those that require a high amount of energy (like the brain, gastrointestinal tract, and immune system)—may not function correctly, resulting in symptoms seen in mitochondrial disorders. Recently we also pointed out that symptoms of mitochondrial disorders overlap the symptoms commonly seen in ASD [5, 6].

As we discover more about ASD and its underlying physiological abnormalities, we learn about new disorders that can also result in ASD. In this article, we describe a newly discovered disorder called cerebral folate deficiency (CFD) that has recently been closely linked with the general population of children with ASD [7].

WHAT IS CFD?

CFD is characterized by below normal levels of the active metabolite of folate known as 5-methyltetrahydrofolate (5MTHF) in the central nervous system (CNS), despite normal levels of folate metabolites in the blood. 5MTHF is normally transported into the CNS through one of two pathways. The CNS folate receptor protein alpha (FRA) transports 5MTHF directly into the CNS in a process that is dependent on mitochondrial function (ATP production). 5MTHF is also transported into the CNS through the reduced folate carrier (RFC). Impaired transport of 5MTHF into the CNS can lead to reduced levels of 5MTHF in the brain and cause CFD.

Children with CFD can have severe symptoms such as difficulty walking, abnormal balance, speech problems and

autistic symptoms [8]. Treatment of CFD with folinic acid (leucovorin calcium), which can enter the brain through the RFC, has been shown in some cases to dramatically improve motor skills, even in as little as one week, as well as improve speech impairments [9].

In 2005, an autoantibody was discovered which attaches to the FRA, making it dysfunctional [10]. Normally, 5MTHF binds to the FRA on the blood side of the brain and is then transported across in an ATP-dependent process. 5MTHF is normally concentrated two-fold higher in the CNS compared to the blood. The FRA has a high affinity for both folate (folic acid) and 5MTHF derivatives. The RFC has a lower affinity for folate metabolites, and it lies on both the blood and brain sides as well as in other locations including brain cells.

The RFC transports 5MTHF from the cerebrospinal fluid (CSF) into neurons. The FRA has a high affinity for the FRA autoantibodies, which block the transport of folate metabolites across this carrier on the blood side. These FRA autoantibodies have been described to be associated with neural tube defects, although this has not been found in every study [11]. One study reported a 12-fold increased risk of subfertility in women with the presence of these autoantibodies [12].

CFD AND DIETARY FACTORS

Cows' milk contains soluble FRA antigen, which is 91 percent similar to the human FRA. Autoantibodies to the FRA cross-react with the soluble FRA antigen in cows' milk, which causes an increase in the circulating serum FRA autoantibody concentration. Exposure to cows' milk has been shown to increase the concentration of the FRA autoantibody and lead to worsening of CFD symptoms, while elimination of cows' milk has been reported to lower the autoantibody concentration and improve CFD symptoms [13]. Moreover, re-exposure to cows' milk after a period of being cows' milk-free substantially worsens the condition and increases the autoantibody concentration [13]. These findings may help explain why some parents of children with ASD report improvements in their child on a cows' milk-free diet [14].

Exposure to cows' milk has also been associated with constipation and megarectum in some children with ASD [15] and a recent study of 199 children with ASD reported that 58 percent had lactase deficiency [16]. Recently, some parents have been using camels' milk as a treatment in some children with ASD because camels' milk appears to help food allergies in some individuals [17, 18]. However, the concentration of FRA antigen in camels' milk is similar to that found in cows' milk, and its immunoreactivity with FRA is also similar to the FRA antigen in cows' milk and is two to three-fold higher than with human milk (Dr. Quadros, personal communication, 12/21/11). Thus, the use of camels' milk in children with FRA autoantibodies may be problematic.

CFD AND MITOCHONDRIAL DISEASE

In 2006, CFD was linked to mitochondrial disease in a case report of a child with an incomplete form of Kearns-Sayre syndrome [19]. Further case reports and case series later expanded the association between CFD and mitochondrial disorders to include complex I deficiency [20], Alpers' disease [21] and complex IV hyperfunctioning [22], as well as a wide variety of mitochondrial disorders in both children and adults [23]. One study reported a child with ASD who also had mitochondrial disease and CFD [24]. In most of these cases, autoantibodies to FRA 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.

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CFD AND ASD

To date, three studies have reported a connection between CFD and Rett syndrome [25-27], and seven studies have reported an association with ASD [10, 13, 24, 28-31].

To date, three studies have reported a connection between CFD and Rett syndrome [25-27] and seven studies have reported an association with ASD [10, 13, 24, 28-31]. CFD was first described in ASD in a study of 20 children with CFD, of whom seven (35 percent) met the criteria for autism on the Autism Diagnostic Observation Schedule (ADOS). In this study, 18 of the 20 (90 percent) children had normal development during the first four 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, nine of the 20 (45 percent) children had a reduced CSF 5-hydroxy-indolacetic acid (5-HIAA) level, which is a metabolite of serotonin. Seven of these nine (78 percent) children had 5-HIAA levels return to normal after folinic acid supplementation. Treatment with folinic acid also increased 5MTHF levels in the CSF in these children [30].

Another group of investigators described a six-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 mild improvements in verbalizations and social interaction [31]. A larger study reported that out of 28 children with CFD, five met the criteria for

In several small studies, children treated with folinic acid have demonstrated improvements in neurological deficits and in social and communication impairments.

autism on the ADOS. These children had "low functional" 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 FRA.

The other four children with autism had mental retardation as well as high concentrations of FRA autoantibodies (ranging from 0.65 to 1.27 pmol of FRA blocked per ml of serum), and treatment with oral folinic acid led to improved communication in the two youngest children (ages two and five years), while the older two children (ages five and 12 years) had minimal changes. Interestingly, in this study, four out of the five children with autism produced autoantibodies to the FRA that accounted for the CFD [10].

In another study of 25 children with regressive autism who were "low functioning" with or without neurological defects, 23 (92 percent) had low CSF 5MTHF levels consistent with CFD. Of these 23, 19 (83 percent) had measurable autoantibodies to the FRA which could account for the low CSF 5MTHF. In one of the children with CFD and autism, the FRA autoantibody concentration significantly correlated with aggressive behavior. These children were treated with oral folinic acid, and two of the younger children (ages two and a half and three years) were "cured with full recovery from autism and neurological deficits." Three older patients (ages four and a half, eight, and 11 years) had improvements in neurological deficits but not in autism symptoms. The remaining 13 children (age range three to seven years) had improvements in neurological deficits, and partial improvements in autistic symptoms, including social impairment (four children, 31 percent), communication impairments (nine children, 69 percent), and restricted interests (six children, 46 percent) [29].

In another study of seven children with CFD, five were examined for possible autism (two displayed symptoms too severe to be tested for autism) and all five met the criteria for autism based on the ADOS and ADI-R. Notably, none of the five had a history of deceleration of head growth, a common feature in CFD. Four of the seven (57 percent) demonstrated improvements in cognition, motor skills, social interaction, communication and a reduction in the frequency of seizures with folinic acid treatment [28].

Finally, in another study of 24 children with CFD, 10 met the criteria for autism and oral folinic acid was given to all 24, 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 improvement in two and partial improvement in four, in communication, attention and stereotypical behaviors. Elimination of cows' milk also dramatically lowered the FRA autoantibody concentration [13]. From these studies of children with concomitant ASD and CFD, treatment with oral leucovorin (0.5 to 2 mg/kg/day) resulted in improvements ranging from partial improvements in communication, social interaction, attention and stereotypical behavior [10, 13, 28, 31] to complete recovery of both neurological and ASD symptoms [10, 29].

ASD, CFD AND FRA AUTOANTIBODIES

In the aforementioned studies, most children with ASD who had CFD typically possessed FRA autoantibodies. However, in two of these studies, 17 percent [29] to 20 percent [10] of these children did not have 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 [23], which is a relatively common finding in ASD [5]. In one study, children with ASD who had a high FRA autoantibody concentration were very likely to have a below normal level of 5MTHF in the CSF [13]. However, some children had low levels of CSF 5MTHF, even when the FRA autoantibody concentration was very low. Therefore, it is apparent that some children with ASD who have either a very low concentration of FRA autoantibodies or no autoantibodies may still have CFD or below normal levels of 5MTHF in the CSF. Treatment of these children with oral folinic acid may lead to beneficial effects.

Recently, we reported a study that measured serum FRA autoantibody concentrations in 93 children with ASD [7]. A high prevalence (75.3 percent) of FRA autoantibodies was found. Unlike previous studies of FRA autoantibodies in children with ASD, none of these children had CFD or significant neurological deficits. In 16 children, the concentration of blocking FRA autoantibody significantly correlated with the CSF 5MTHF concentration, which, in each case, was below the mean level found in typically developing children. Children who possessed FRA autoantibodies were treated with oral leucovorin calcium (2 mg/kg/day; maximum 50 mg/day). Treatment response was measured and compared to a wait-list control group who also possessed FRA autoantibodies but was not being treated with folinic acid.

Compared to controls, significantly higher improvement ratings were observed in treated children over a mean period of four months in verbal communication, receptive and expressive language, attention and stereotypical behavior. Approximately one-third of treated children demonstrated moderate to much improvement, and the incidence of adverse effects was low. Given the results of that study, empirical treatment with leucovorin calcium without performing a lumbar puncture appears to be a reasonable and non-invasive approach in FRA autoantibody positive children with ASD.

FRA AUTOANTIBODY TESTING ADVISABLE?

Because FRA autoantibodies appear to be highly prevalent in children with ASD, we recommend that FRA

autoantibody testing should be considered in all patients with ASD. Early identification and treatment is paramount, as younger children generally respond more robustly than older children, with "cure" reported in some children. An overlap between ASD, mitochondrial disease and CFD is found in some children with ASD, and therefore we also recommend testing for mitochondrial disease. In children with ASD who have FRA autoantibodies or who have CFD, treatment with oral folinic acid can lead to improvements in receptive and expressive language, attention, stereotypical behavior and social interaction [7, 29]. Interestingly, one study reported an improvement in seizure activity with folinic acid treatment [28]. Elimination of cows' milk is also essential [13]. Further studies examining FRA autoantibodies and CFD in children with ASD are warranted. ◂

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