



## Critical Periods in the Neurodevelopment of Autism

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### Abstract:

Autism Spectrum Disorder (ASD) is a developmental disability that can create significant behavioral and communication challenges. The prevalence of ASD among children at 8 years of age is over 2%, and the prevalence is similar across ethnic groups and countries. Studies have shown that the majority of ASD children make an autoantibody to the high-affinity folate receptor in response to a dietary component. This Folate Receptor Antibody (FRA) blocks transport of folate across the Blood-Brain Barrier (BBB), resulting in a Cerebral Folate Deficiency (CFD). Parents of autistic children also have FRA at substantially higher rates than the general public, which may play a critical role during neurodevelopmental critical periods in

the fetus. In clinical trials, ASD children with the FRA had improvement in their communication when placed on a daily supplement of folate in its reduced form, which can enter the brain via a low-affinity transport. We reason that supplementing folate earlier in development, including *in utero* development, may be most effective in reducing the severity of ASD symptoms by facilitating typical passage through critical neurodevelopmental periods.

**Keywords:** *ASD, folate, autism, clinical, prenatal development, critical period.*

## Folate in Nervous System Development

### Folate Role in Development

Folate (Vitamin B-9) is a key component in normal nervous system development (Alam et al., 2020; Reynolds, 2006). Bio-available folate is present in many foods, including legumes, leafy greens, and citrus. The naturally occurring form is methylated, while the synthetic version in many vitamin supplements is the oxidized form, folic acid, which is stable for a much longer time than the reduced form of folate. Folate is necessary for neural tube formation and closure

in the human embryo and plays an essential role in fetal brain development (Pitkin, 2007; Blom et al., 2006; Bobrowski-Khoury et al., 2021). Low cerebral folate levels is causative of many developmental conditions, including spina bifida in the newborn (Imbard et al., 2013; Shapira et al., 2015). To reduce complications, prenatal vitamins contain Vitamin B-9, typically in its stable, oxidized form, rather the reduced form. Most individuals convert sufficient folic acid to folate. However, 5-10% of the population is limited in absorbing folate into the brain due to the presence of an autoantibody for the folate receptor (Frye et al., 2020).



## Folate Receptor Antibody

Folate Receptor Antibody (FRA) blocks the high affinity folate receptor, preventing folate from crossing the blood brain barrier (Frye et al., 2020; Ramaekers et al., 2005). Clinical studies have shown that blood levels of FRA can be reduced by changes in diet, particularly elimination of dairy (Ramaekers et al., 2008). If FRA levels are reduced or eliminated, this may allow sufficient folate to enter the brain. While FRA is present in a small subsection of the population, clinical tests show that 70% of ASD children have FRA (Frye et al., 2013). Meta-analysis from multiple studies show that ASD children are 20 times more likely to have FRA (Rossignol & Frye, 2021), indicating a likely genetic aspect that restricts brain absorption of Vitamin B-9. While FRA can be reduced with significant change in diet (Ramaekers et al., 2008), a symptom of ASD is resistance to dietary change. Thus, the dietary factors contributing to FRA are self-perpetuating due to this change resistance, as FRA reduces folate in the brain, creating Cerebral Folate Deficiency (CFD). This CFD can be countered by supplementation with elevated levels of the natural version of folate, in the form of methyl-folate or folinic acid (but not with folic acid) (Frye et al., 2020; Ramaekers et al., 2005, Ramaekers & Blau, 2004). These reduced forms of folate cross the blood brain barrier via a low-affinity transport, necessitating larger blood levels to obtain sufficient cerebral levels of folate.

## Immunological Impacts in Development

Antibodies are essential proteins produced by the adaptive immune system to limit the destruction of host tissue by foreign agents. Antibodies bind to target antigens, which are regions of infectious agents, and neutralize their harmful effects, such as blocking the spread of infection. Usually, B cells that produce antibodies that bind host proteins are eliminated to avoid an attack on host cells by the immune system. However, in some instances, misdirected attacks occur in which antibodies are generated against host tissue. These autoantibodies typically do not cause harm to the adult brain as the blood-brain barrier (BBB) prevents their

entry in the healthy state. Nonetheless, autoantibodies from the mother can cross the placenta during development and have been shown to be reactive against fetal brain proteins (Bruce et al., 2023).

The clinical finding of ASD diagnosis linked to maternal autoantibodies is known as maternal autoantibody-related (MAR)-ASD. Previous research has identified autoantibody targets that are common in mothers whose children were subsequently diagnosed with ASD. Specifically, one study found that maternal autoantibodies against LDHA, LDHB, CRMP1, and STIP1 were 23 times higher in mothers who went on to have children diagnosed with ASD compared to mothers who had neurotypical children. Within the cohort of ASD children studied, the presence of these circulating maternal autoantibodies was also associated with a greater incidence of stereotypical behaviors (Bruce et al., 2023).

Maternal stress, such as infection or exposure to environmental toxins, has been shown to trigger the immune response that results in the production of autoantibodies that can cross the placenta and react with fetal brain proteins. The resulting neurodevelopmental changes have been implicated in the pathogenesis of disorders such as autism spectrum disorder (ASD). The link between maternal stress and the development of ASD has been observed in both human and animal studies. For instance, maternal immune activation induced by viral or bacterial infection during pregnancy has been associated with an increased risk of ASD in the offspring. Similarly, exposure to environmental toxins such as polychlorinated biphenyls (PCBs) and organophosphate pesticides during pregnancy has been linked to increased autoantibody production and the development of ASD in the offspring. Overall, maternal stress appears to play a critical role in triggering the immune response that may result in neurodevelopmental changes leading toward a neuropsychiatric disorder such as ASD. (Goodrich et al., 2022; Ramirez-Celis et al., 2023; Ames et al., 2022).

## Clinical Trial Evidence

In clinical trials with ASD children, those who have FRA have improvement in their communication when given daily folate supplements (in the form of folic acid) for three months (Renard et al., 2020; Frye et al., 2018). It is clear that supplementation with reduced folate can help overcome CFD. Once CFD is reduced or eliminated, it may be possible to revise the diet of ASD children to reduce production of FRA, which we propose may provide a means to reduce ASD symptoms. The key elements in such a dietary treatment is to reduce or remove foods that can stimulate FRA production and to provide food sources of natural folate. These two things, elimination of autoantibody stimulating foods and consumption of the natural form of folate (the reduced form), are key conditions for reducing severity of ASD symptoms. Diets that are richer in natural folate include the Mediterranean diet that focuses on olive oil, fresh vegetables and fruits, nuts, legumes and fish.

A challenging fact of FRA contributing to CFD is the observation that this CFD likely starts early in development. Ramaekers et al. (2005; 2007) documented that when either or both parents have FRA, their child has higher odds of being ASD. It seems that elevated risk of childhood ASD is related to elevated prenatal FRA, more so when the FRA is present in the mother, but also when it is present in the father. One study found FRA was present in 75.6% of autistic children, while the FRA prevalence was 34% in their mothers and 29% in their fathers, as compared to 3% FRA positivity in healthy controls (Ramaekers, 2021). Another study found FRA prevalence of 76% in autistic children, 75% in unaffected siblings, 69% in fathers and 59% in mothers, while the prevalence of FRA in unrelated normal controls was 29% (Quadros et al., 2018).

## Critical Periods in Neurological Development

These findings are consistent with the presence of critical periods in development (Frye et al., 2018). The presence of critical periods, first shown for the visual system, is the

developmental time when sensory stimulation is necessary for visual perception to become established. If an animal is deprived of the sensory input during the critical period, the functional perception of visual images is impaired throughout its life (Wiesel, 1981; Wiesel, 1974). Similarly, depletion of cerebral folate during an infant's fetal development may be similar to a critical period that could lead to increased severity of autism. We postulate that ASD may result from a modified critical period of some type, where CFD impairs neurological development in some manner, increasing the probability of a later diagnosis of ASD.

In pregnancy, the presence of FRA in the mother blocks folate delivery to the developing fetus. These FRAs are common in pregnancies that have births with spina bifida and ASD. Identifying pregnancies where FRA is present and treating the pregnant mother with folic acid or methyl-folate may permit sufficient folate to reach the fetus, in turn lowering the risk of developing ASD and other developmental disorders (Levine et al., 2018; Cirillo et al., 2021).

There may be an additional advantage to diagnose FRA in prospective parents. A recent report documented that Vitamins B-9 and B-12 supplementation increase pregnancy and live birth in women who have experienced difficulty in conceiving, indicating an essential role of these vitamins in pregnancy and healthy fetal development.

Clinical studies have shown that when FRA is present, supplementation with folate and B-12 can overcome the FRA to permit sufficient folate for a child's brain development to continue its normal course (Frye et al., 2013; Frye et al., 2018).

Folate is a key vitamin in neural health. Evidence shows that most autistic people produce the autoantibody FRA, that blocks folate absorption into the brain, resulting in CFD. Stress may exacerbate this deficiency, worsening communication difficulties in ASD (Ahmavaara, & Ayoub, 2022). Diets that are rich in natural forms of folate, such as the Mediterranean diet may help alleviate the CFD, and when coupled

with reduction in stress may improve communication in autistic people.

We suggest there are critical periods in the development of ASD, where the first period is in utero and a later one is in the first five years of life. These periods can predispose a child to be more likely to develop ASD (in utero), and begin to set the conditions for ASD development (ages 2-5). Nutritional supplementation along with psychological counseling was effective for children under five in one study (Lam et al., 2022), suggesting therapy and nutrition should be provided in the first years of life to maximize positive outcomes. Additionally, reports indicating that the presence of FRA can predispose a child toward ASD (Bobrowski-Khoury et al., 2021; Rossignol & Frye, 2021) leads us to recommend that women presenting with FRA who may become pregnant be advised to take a prenatal supplement that includes a reduced form of folate (such as methyl-folate or folic acid), and that children born to parents with FRA or children who have FRA would be advised to have nutritional supplementation to ensure sufficient levels of bioavailable Vitamin B-9 for brain development.

## Diagnosis and Nutritional Intervention

### Early Diagnosis

Thus, for optimal treatment, early diagnosis may be essential, including making a prognostic diagnosis early in pregnancy. There is support for using the presence of FRA in either biological parent as a predictor for this antibody in the newborn and an increased probability of ASD development in childhood. The FRA requires a blood sample, so if a non-invasive test were available for widespread screening, the FRA test could be used as confirmation and point to the therapeutic method that would be successful.

Several types of screening tests are under development for ASD. Lai et al. (2020) are using retinal images to rapidly screen children for autism. Duan et al. (2023) are using computer algorithms to assess children based on

behavioral phenotypes. Elbattah et al. (2023) have a machine learning method to make more rapid diagnoses of autism in young children. We have proposed a fast assessment based on the presence of ASD, depression or spina bifida in any immediate family of the birth parents, followed by a FRA test. Any of these systems that allow a quick screening that can identify potential future ASD cases should be effective.

We thus hypothesize that testing for a biomarker in newborns or in parents at the onset of pregnancy is the optimal time to assess elevated potential for neurodevelopmental disorders, such as spina bifida and ASD.

### Treatment with Folate

In the case of ASD, testing for FRA in parents (testing needs to be of both maternal and paternal biological parents), and treating the mother with prenatal vitamins including reduce folate (methyl-folate or L-folinic acid) is optimal to reduce neurodevelopmental disorder severity and possibly reduce the fraction of children born with neurodevelopmental disorders such as spina bifida or ASD.

Treatment of the infant would continue post-partum, with the mother continuing to take the folate-containing prenatal vitamin while nursing, then the baby will receive folate directly in a liquid form (liquid preparation of L-folinic acid is available in a measured dosing bottle from Aprofol AG, Switzerland). Regular testing for FRA can be used to inform continuance of folate treatment. As the child becomes a toddler, vitamin treatment may continue if FRA remains present, and the family will be advised as to preferred diets that will foster folate absorption. Currently, a diet with plant sources of folate, such as legumes, leafy greens, is recommended, along with reduced exposure to bovine milk products.

### Therapy

At the toddler age and beyond, cognitive-behavioral therapy (CBT) will commence, in addition to any needed physical therapy. Along with CBT, other therapeutic methods may prove advantageous.



PISTA is a non-pharmacological intervention that is complementary to CBT and aims to improve attention and reduce impulsivity in children with Attention Deficit Hyperactivity Disorder (ADHD). It involves physical exercise, cognitive training, and behavioral therapy. While PISTA was originally developed for children with ADHD, some studies suggest it may improve social interaction, motor coordination, and adaptive behavior in autistic children (Pan et al., 2017; Sowa & Meulenbroek, 2012).

Use of electrophysiological methods such as electroencephalograms (EEG) and Transcranial Magnetic Stimulation (TMS) are being deployed in treatment of certain neurological disorders, primarily in treating depression. Recent work that measures the EEG and uses this data to inform the TMS creates an individualized TMS (iTMS) that is beginning to be tested in treatment of autism. The Brain Restoration Center, based in Hong Kong, has initial data indicating a positive impact of iTMS with autistic children (personal communication).

The development of assistive technologies is beginning to blossom, with promise of supportive technologies to aid families with an autistic child. A robot designed to be a constant companion to an autistic child has recently been developed by Lam Hanson Robotics (Hong Kong). This robot is designed to work with ASD children, provide safety and rapid communication with parents or caregivers when the adults are at work or otherwise away from home. The robot is able to engage and communicate with the child, and provide some therapeutic activities.

## Conclusion

Recent studies reveal that autism likely has origins *in utero* where, during certain critical periods, the fetus is pre-conditioned toward the development of particular neurological disorders. Early identification and intervention is essential to reducing the severity of ASD in a child. The interventions showing potential begin with nutritional supplementation to restore folate levels in the developing brain and foster

typical passage through these critical periods. Additional therapies are rapidly becoming available that hold promise of enhancing family life for those with autistic children.

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