Folate Receptor Autoimmunity in Autism Spectrum Disorders

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While Dr. Rossignol has attempted to make the information in this presentation as accurate as possible, the information is provided without any express or implied warranty. The purpose of this lecture is to provide information about different conditions or treatments that affect individuals with autism and other conditions. Please be advised that Dr. Rossignol is not giving medical advice and that circumstances may dictate different treatments. If the issues that are discussed in this lecture affect you or your loved ones, seek professional advice. All of the reviewed treatments in this lecture are considered off-label and not FDA-approved. Before beginning any treatment, please consult with your or your child’s physician.

Autism Spectrum

Asperger Syndrome PDD-NOS Autistic Disorder

Psychologically / Behaviorally defined

Communication Stereotypical behaviors Social interaction

Underlying pathophysiology (biomedical problems): ???

Autisms

- There are many types of autism and thus multiple subgroups
- There are probably many causes of autism
- Biomarkers will help subgroup children and identify metabolic abnormalities that may be treatable

Biomarker-Guided Interventions of Clinically Relevant Conditions Associated with Autism Spectrum Disorders and Attention Deficit Hyperactivity Disorder

This article reviews the medical literature and discusses the authors’ clinical experience using various biomarkers for measuring oxidative stress, methylation capacity and transsulfuration, immune function, gastrointestinal problems, and toxic metal burden. These biomarkers provide useful guides for selection, efficacy, and sufficiency of biomedical interventions. The use of these biomarkers is of great importance in young children with ADHD or individuals of any age with ASD, because typically they cannot adequately communicate regarding their symptoms.

Genetics

- Genetic syndromes only account for an estimated 6-15% of autism cases
- Genetics do not account for epigenetics – e.g., DNA methylation
- Genetics also do not account for environmental factors

**Metabolism**

- **Definition:** set of chemical reactions that occur in living organisms to maintain life
- **Catabolism:** breaks down organic matter, for example to harvest energy in cellular respiration
- **Anabolism:** uses energy to construct components of cells such as proteins and nucleic acids

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**Autism as a Metabolic Disorder**

- If metabolic abnormalities cause or contribute to autistic symptoms, then this implies that some of the symptoms of autism may be treatable or reversible
- **Shades of gray: not an “all or none” phenomenon**
  - Mitochondrial dysfunction vs. disorder
  - Epileptiform vs. epileptic activity
  - Gluten intolerance vs. celiac disease

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**Examples: Metabolic Problems**

- Inhibitory substances
  - Toxins
  - Propionic acid
  - Abnormal antibodies (e.g., folate receptor)
- **Deficiencies**
  - Glutathione (GSH)
  - Antioxidants
  - Antioxidant enzymes
  - Iron

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**Metabolic Disorders Associated with ASD**

- Phenylketonuria
- Disorders of purine metabolism
- Creatine deficiency
- Biotinidase deficiency
- Cerebral folate deficiency
- SSADH deficiency
- Smith-Lemli-Opitz syndrome
- Infantile ceroid lipofuscinosisis

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**Metabolic Abnormalities: ASD**

- Cerebral Folate Deficiency
- Mitochondrial Dysfunction
- Oxidative stress
- Impaired methylation / sulphation
- Inflammation
- Seizures
- Hypothyroidism: ASD and ADHD
- Deficiencies: iron (ASD and ADHD)
**Folate Transport into Brain**

- Folate = folic acid, inactive and oxidized form
- 5-methyltetrahydrofolate (5MTHF): form of folate that crosses the blood brain barrier and is concentrated 2-4 times higher in cerebral spinal fluid (CSF) than in the blood
- 5MTHF binds to a Folate Receptor (FR1) in the choroid plexus and then undergoes endocytosis, storage and then delivery into the CSF. It is then transported into neurons by the Reduced Folate Carrier (RFC)
- Folate transport into the CSF is ATP-dependent

**Folate Receptors (FR)**

- FR1: found at the luminal surface of intestinal cells, choroid plexus epithelium, pulmonary alveolar cells, thyroid cells (may play a role in hypothyroidism), and renal tubular cells (does not affect kidney function as antibodies cannot cross there)
- FR2: found in mesenchymal cells such as red blood cells
- Proper FR function requires normal levels of homocysteine, sphingolipid and cholesterol content in membranes

**Reduced Folate Carrier (RFC)**

- The Reduced Folate Carrier (RFC) carries folate across the intestinal barrier and also from the CSF into neuronal cells
- Is located at the blood brain barrier as well, but has low folate affinity and does not play much of a role in transporting folate into the brain
- Jill James reported significant genetic variants in the RFC in children with ASD

**FR Autoantibodies**

- Associated with Cerebral Folate Deficiency (CFD) syndrome, low-functioning autism, and Rett syndrome
- Block the folate binding site on the plasma surface of choroid plexus epithelial cells, impairing folate transport into the CNS
- An antibody mediated inflammatory response could also contribute to CFD
Types of FR Autoantibodies

- Blocking antibodies: blocks the binding of folic acid to the folate receptor. As in the case of all autoimmune diseases, the presence of autoantibody should be considered as abnormal. However, a low titer in some may not be pathological. Typically, lower CSF folate levels have been associated with higher titer but sometimes, low CSF folate values have been observed in patients with low titer.
- Binding antibody: may also be pathological by a yet unidentified mechanism.

FR Autoantibodies

- Implicated in neural tube defect pregnancy and in subfertility (in one study 12-fold increased risk of subfertility)
- FR autoantibodies might block transplacental transport of folate by neural crest cells in the developing embryo.
- High doses of folic acid can circumvent this blockade.
- 5MTHF, in theory, should work, but has not been studied (yet) in CFD.

Prevalence of Folate Receptor Autoantibodies

- Not formally studied or published.
- Appears underrecognized.
- Informal data: autoantibodies are present in ~10-15% of the U.S. population; prevalence is 3 fold higher in the Irish population; incidence of neural tube defects is 5-8 fold higher in Irish population.
- Without treatment, the concentration of autoantibodies increases over time.
Cerebral Folate Deficiency (CFD)

- **Definition:** any neurological or psychiatric condition associated with lowered CSF 5MTHF in the presence of normal folate levels outside the CSF (a neurometabolic syndrome)
- **Most common cause** is autoimmune mechanism (antibodies to the folate receptor)
- **First described in 1994; in autism in 2004**
- **Most studies report** a male-to-female ratio of 2.5-3:1 (in general, females are usually more likely to have autoimmune disorders)
- **Classified as primary or secondary CFD**

Clinical Features: CFD

- **Usually there is normal development** in the first 4-6 months of age
- **Symptoms typically begin** with marked unrest, irritability, and insomnia
- **Improvements can occur** if cow’s milk is eliminated
- **By 1-2 years of age,** there is usually onset of hypotonia and ataxia

Clinical Features: CFD

- **Often there is moderate to severe deceleration of head growth** from age 6 months, but not always observed (especially in autism)
- **Onset of spasticity in lower limbs,** and seizures develop in 1/3 children; seizures can become intractable
- **Most children develop moderate to severe mental disability,** but some have regression in mental and motor function later in life

Clinical Features: CFD

- Many children with CFD have speech delay
- Some develop hearing or vision loss
- 20% of children with CFD develop autistic features; CFD is a known cause of regression in some children with autism
- Some children develop normally in first year of life, followed by spasticity and ataxia; cognitive functions are mildly impaired or normal (late-onset spastic-ataxia CFD syndrome)
- There is a spectrum of CFD

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Ramaekers et al., 2008  Dev Med Child Neurol 50(5):346-52

Ramaekers and Quadros, 2010  Wiley Publishing
### Diagnosis: CFD Syndrome

- Low CSF 5MTHF, plus 3 of 7 features:
  - Irritability, marked unrest and insomnia
  - Deceleration of head growth after 6 months
  - Psychomotor retardation, sometimes with regression
  - Hypotonia and cerebellar ataxia
  - Spasticity in lower limbs
  - Dyskinesias (involuntary movements)
  - Epilepsy


### Primary CFD

- Infantile-onset CFD: autoantibodies of the blocking type
- Low-IQ autism with neurological deficits: autoantibodies of the blocking type
- Late-onset spastic-ataxia CFD: autoantibodies of the blocking type

### Secondary CFD

- Rett syndrome: MECP2 mechanism
- Mitochondrial encephalopathies: disrupted active folate transport at choroid plexus
- Kearns-Sayre syndrome: disrupted active folate transport at choroid plexus
- 3-Phosphoglycerate dehydrogenase deficiency
- Dihydropteridine reductase deficiency
- Aromatic L-amino acid decarboxylase deficiency

### Rett Syndrome and CFD

- Studies in Europe and Israel report low 5MTHF in the CSF in 50% of children with Rett syndrome; these children often have epilepsy that is resistant to treatment
- A much smaller percentage in the U.S. have low 5MTHF in CSF
- Prevalence differences may be due to folate fortification in the diet in the U.S.

### Reduced folate transport to the CNS in female Rett patients

CONCLUSION: Irrespective of the MECP2 genotype, 5MTHF transfer to the CNS is reduced in Rett syndrome. Folinic acid supplementation restores 5MTHF levels and serotonergic turnover. The lowered folate binding capacity of FBP is not explained by a defect of the FBP1 or FBP2 gene, but most likely occurs as a secondary phenomenon in Rett syndrome.


### Mitochondrial Dysfunction and CFD

- In mitochondrial dysfunction, the choroid plexus epithelial cells may not produce enough ATP to actively transport 5MTHF into the CSF
- Interestingly, folic acid is used by some physicians in “mitochondrial cocktails”
**Mitochondrial Complex I Encephalomyopathy and Cerebral 5-Methyltetrahydrofolate Deficiency**

Folate transport to the brain depends on ATP-driven folate receptor-mediated transport across choroid plexus epithelial cells. Failure of ATP production in Kearns-Sayre syndrome provides one explanation for the finding of low spinal fluid (CSF) 5-MTHF levels in this condition. In the present patient with mitochondrial complex I encephalomyopathy a low 5-MTHF level was found in the CSF. Serum folate receptor autoantibodies were negative and could not explain the low spinal fluid folate levels. Addition of folic acid led to partial clinical improvement including full control of epilepsy, followed by marked recovery from demyelination of the brainstem, thalamus, basal ganglia and white matter.

Ramaekers et al., 2007 Neuropediatrics 38(4):184-7

**ORIGINAL ARTICLE**

Mitochondrial dysfunction in autism spectrum disorders: a systematic review and meta-analysis

Dr. Ruslan and RC Frye

The prevalence of MD in the general population of ASD was 5.0% (95% confidence interval 3.2, 6.9%), much higher than found in the general population (approximately 0.01%). The prevalence of abnormal biomarker values of mitochondrial dysfunction was high in ASD, much higher than the prevalence of MD. Taken together, these findings suggest children with ASD have a spectrum of mitochondrial dysfunction of differing severity. Eighteen publications representing a total of 112 children with ASD and MD (ASD/MD) were identified. The prevalence of developmental regression (52%), seizures (41%), motor delay (51%), gastrointestinal abnormalities (74%), female gender (39%), and elevated lactate (78%) and pyruvate (45%) was significantly higher in ASD/MD compared with the general ASD population. 53% of ASD/MD had a complex I deficiency.


**MITOCHONDRIAL DISEASES ASSOCIATED WITH CEREBRAL FOLATE DEFICIENCY**

Twenty-eight patients with different mitochondrial disorders and fulfilling the previously defined diagnostic criteria were recruited from the Hospital Sant Joan de Deu, Barcelona, Spain (21 patients), the University Clinic Aachen, Germany (5 patients), and Hospital 12 de Octubre, Madrid, Spain (2 patients). Despite normal serum folate levels, 14 patients (mean age: 9.5 years; range: 3 months–34 years; Patients 1–14, table) had low CSF 5-MTHF concentrations (mean: 22.1 nmol/L; range: 0.6–48.8 nmol/L).

Garcia-Cazorla et al., 2008 Neurology 70(16):1360-1362

**MTHFR**

- Methylenetetrahydrofolate reductase (MTHFR) converts folic acid (folate) into 5MTHF
- Defects in MTHFR can lead to decreased production of 5MTHF
- 3 studies in autism report this; 2 do not
- Can be associated with low serine, glycine, and histidine—these are the most important one-carbon donors to replenish 5MTHF
- Defects in the dihydrofolate reductase enzyme (involved in making 5MTHF) have also been reported in some children with ASD

**Systemic Folate Deficiency**

- Congenital folate malabsorption: transporter gene defect
- Malnutrition
- Malabsorption: celiac disease, Crohn’s disease
- Antifolate agents: methotrexate, isoniazide, sulphonamides, anti-convulsants drugs, carbidopa
Drugs Depleting Folate

- Sodium bicarbonate
- Sulfasalazine
- Steroids, prednisone
- NSAIDS: motrin, naprosyn
- Aspirin
- Bactrim/Septa
- Cephalosporins
- Zithromax
- Amoxicillin
- Augmentin
- Quinolones (Cipro)
- Minocycline
- Phenobarbital
- Dilantin
- Tegretol
- Valproic acid
- Metformin
- Estrogen
- Spironolactone
- Famotidine
- Ranitidine

Milk and Folate Receptor Autoantibodies

- Soluble FR antigen from human, bovine and goat’s milk are structurally homologous; Human and bovine milk FR antigen are 91% similar
- Serum folate receptor autoantibodies cross-react with milk-derived soluble folate receptor antigens
- Elimination of cow’s milk significantly lowers FR autoantibody production

Folate Receptor Autoimmunity in Autism Spectrum Disorders

Described 20 children with CFD, 7 had autism (ADOS). 18 of 20 children had normal development during the first 4 months of life, followed by deceleration of head growth from 4 to 6 months, marked unrest, irritability and sleep disturbances. 9 of 20 children had reduced CSF 5-hydroxy-indoleacetic acid (5HIAA) and normal homovanillic acid (HVA) levels. Almost all children had normalization of 5HIAA levels after folic acid treatment.
The authors describe a 6-year-old girl with developmental delay, psychomotor regression, seizures, mental retardation, and autistic features associated with low CSF levels of 5MTHF, the biologically active form of folates in CSF and blood. Folate and B12 levels were normal in peripheral tissues, suggesting cerebral folate deficiency. Treatment with folinic acid corrected CSF abnormalities and improved motor skills. Parents described mild increases in verbalizations and social interaction.

Folate Receptor Autoimmunity and Cerebral Folate Deficiency in Low-Functioning Autism with Neurological Deficits

Twenty-five patients with early-onset low-functioning autism with or without neurological deficits, were evaluated for serum folate, cerebrospinal fluid (CSF) 5MTHF, and serum FR autoantibodies of the blocking type to determine the significance of folate receptor (FR) autoantibodies with respect to folate transport across the blood-CSF barrier. In spite of normal serum folate, CSF 5MTHF was low in 23 of 25 patients. The reduced CSF folate in 19 of these 23 patients could be explained by serum FR autoantibodies blocking the folate binding site of the membrane-attached FR on the choroid epithelial cells. Oral folinic acid supplements led to normal CSF 5MTHF and partial or complete clinical recovery after 12 months.

In infantile-onset cerebral folate deficiency, 5MTHF levels in the cerebrospinal fluid are low, but folate levels in the serum and erythrocytes are normal. We examined serum specimens from 28 children with cerebral folate deficiency, 5 of their mothers, 28 age-matched control subjects, and 41 patients with an unrelated neurologic disorder. Serum from 25 of the 28 patients and 0 of 28 control subjects contained high-affinity blocking autoantibodies against membrane-bound folate receptors that are present on the choroid plexus. Oral folinic acid normalized 5MTHF levels in the cerebrospinal fluid and led to clinical improvement.

“The four children with mental retardation associated with autism had very high titers of blocking autoantibodies (i.e., 1.27, 1.20, 0.65, and 1.27 pmol of folate receptor blocked per milliliter of serum). Treatment with folate acid or folinic acid improved the communication skills and neurologic abnormalities in the two younger autistic children, who received the diagnosis of cerebral folate deficiency at the ages of two and three years. The two older children with this diagnosis, who were treated beginning at the ages of 5 and 12 years, had a poorer outcome and remained autistic.”

“Two patients (patients 2, 4) who were diagnosed early and received treatment were cured with full recovery from autism and neurological deficits. In the whole group these two patients were among the youngest and were detected at 2 years 8 months and at 3 years and 2 months. Three other patients (patients 11, 23, 25), diagnosed and treated from the age of 4.9, 8, and 11.9 years, did not recover from autism but showed improvement of their neurological deficits. The remaining thirteen patients in the age range of three and seven years showed a good response after treatment with improvement of most neurological deficits, but only partial recovery from their autism. The partial recovery in the latter group of 13 patients consisted of amelioration of social impairment in 4 of 13 patients, reversal of impaired communication in 9 of 13 patients and disappearance of perseverative behaviour and restricted interests in 6 of 13 patients.”
We studied seven children with CNS folate deficiency (CFD). 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. This study did not see deceleration of head growth. Four of 7 (57%) children demonstrated some improvements in cognition, motor skills, social interaction, communication and a reduction in the frequency of seizures with folic acid treatment.

10 of 24 children had autism: “Folic acid therapy had an overall positive effect on irritability and insomnia, arrested further head growth deceleration, decreased ataxia, spasticity, and seizure frequency, and had a variable effect on mental retardation and dyskinesias. Among the 10 patients with low IQ autism associated with neurological deficits (patients 1, 6, 8, 9, 10, 11, 15, 16, 20, and 23), a marked improvement was observed in two (patients 1 and 6) and a partial response in four (patients 8, 9, 10, and 11) with regard to improved attention, communication, and less stereotypies.”

Testing: Folate Receptor Autoimmunity

Initial Workup: Labs
- CBC (? Macrocytosis related to FR antibody)
- Serum homocysteine
- RBC folate and serum folate (serum may be elevated due to inadequate transport of folate into cells)
- Serum B12
- Mitochondrial dysfunction markers
- MTHFR
- Folate receptor autoantibodies
- CSF: metabolites of dopamine, serotonin, pterins and 5MTHF
- Folate receptor genes (FR1 and FR2) are normal
Lumbar Puncture

- In children with CFD who are not treated, CSF 5MTHF levels typically drop with age
- May need to repeat test over time to measure levels and effect of treatment
- In 50% of patients with CFD, 5-hydroxyindoleacetic acid (the main metabolite of serotonin) CSF levels are low but return to normal after folinic acid supplementation
- Child needs to be off folate supplementation (including multivitamin containing folate) for 3 months for CSF testing to be completely accurate

Other Investigations

- EEG: Common findings are generalized epileptic discharges and intermittent slowing of background activity
- Some children demonstrate optic atrophy
- CT and MRI are completely normal in 50% of children with CFD; some children have cerebral atrophy of fronto-temporal regions and delayed myelination

Types of Folate

- Folate (folic acid) is an oxidized, inactive form and some investigators suggest that folic acid should not be used because it has a higher binding affinity to FR1 than 5MTHF, and thus might further deplete CNS 5MTHF
- 5MTHF
- Folinic acid

Treatments: Folate Receptor Autoimmunity

Treatments

- Folinic acid at 0.5 to 1 mg/kg divided twice per day for at least one year
- 5MTHF should work, but has not been studied in CFD
- Cow's milk free diet
- Predisone and IVIG might be helpful

Side Effects of Folates

- Gastrointestinal upset
- Sleep disturbances
- Irritability, agitation
- Hyperactivity
- Tics
- Increased risk of seizures in a small number
- The above occur in up to 10% of children
- If side effects occur, some investigations suggest stopping treatment for a short period of time and restarting at 1/2 of the initial dose
Folate Supplementation and Seizures

- Supplementation of folate and folate derivatives can increase seizure activity
- Folate (folic acid) is an oxidized form and more likely to increase seizures
- 5MTHF and folinic acid can increase seizures as well, but folinic acid has been shown to decrease the incidence of seizures in individuals with CFD over time

Pregnancy, birth, early development were normal. At 3.5 years she presented with spasticity, gait problems, and speech difficulties. She progressively had stagnation of mental development. At age 9.5 years she had increase leg spasticity and drooling. At age 12, she started having ataxia, difficulty walking, and bladder spasticity. At age 12 years/10 months, she had a very low 5MTHF on CSF testing. She was started on 15 mg/day of folinic acid and had “an amazing effect after less than one week.” She could take a seat without falling, could negotiate stairs, was more alert, and more interested in school. By age 14 years/7 months, her gait and stability improved and her speech was near normal.

<table>
<thead>
<tr>
<th>Age</th>
<th>Folinic acid (mg/day)</th>
<th>5MTHF (mg/day)</th>
<th>CSF (nmol/L)</th>
<th>Non-CSF (nmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 years/11 months</td>
<td>15.4</td>
<td>19.4</td>
<td>174</td>
<td>148</td>
</tr>
<tr>
<td>21 months/university</td>
<td>17.2</td>
<td>21.8</td>
<td>195</td>
<td>159</td>
</tr>
<tr>
<td>45 years/15 months (university CSF)</td>
<td>25.6</td>
<td>29.4</td>
<td>205</td>
<td>178</td>
</tr>
</tbody>
</table>


Prognosis: CFD

- The earlier CFD is treated, the more reversible the disorder
- Treatment after 1 year of age is associated with variable or no improvement in mental disability, autistic features and dyskinesias
- However, almost all children improve in irritability, ataxia, spasticity and epilepsy, with complete reversibility of these problems in many children

FR Autoantibodies in ASD

- May explain why many parents have reported that a cow’s milk free diet helps some children with autism: this is much worse than a “milk allergy”
- In many cases, CFD appears to be an autoimmune disease
- Some symptoms overlap with PANDAS
- May be a potential cause or contributor to seizures, mitochondrial dysfunction, autism, ataxia and other neurological problems

FR Autoantibodies in ASD

- The presence of the folate blocking or binding antibody does not mean the child has Cerebral Folate Deficiency Syndrome or low levels of 5MTHF in the CSF; a lumbar puncture is needed to test for this
- In two studies, 4/23 (17%) and 1/5 (20%) children with ASD and CFD had normal FR autoantibodies
- It may be reasonable in some cases to empirically begin folinic acid treatment, but consult with your physician first
Synergistic Effects?

- Things that can go wrong:
  - Inadequate folate intake (poor nutrition)
  - Poor GI absorption of folate due to celiac disease or inflammatory bowel disease
  - Drugs that deplete folate
  - Autoantibodies to the FR
  - Cow’s milk intake
  - Genetic defects in RFC and other rare genetic defects
  - MTHFR defects
  - Dihydrofolate reductase defects
  - Too much folate (folic acid): inactive form

Testing Implications

- Some investigators suggest that all infants who have early signs and symptoms of infantile-onset CFD should be tested for FR auto-antibodies:
  - Irritability, marked unrest, insomnia
  - Delay of motor development with hypotonia
  - Early signs of ataxia
  - Early indicators of autism
  - Seizures
  - Regression

Current Data