METHYLCOBALAMIN

Pharmacy Compounding Advisory Committee Meeting
June 9, 2021

A.J. Day, PharmD
Vice President of Clinical Services
PCCA
Methylcobalamin is a vitamer of vitamin B₁₂ and based upon the use of other cobalamins would be expected to be effective in treating vitamin B₁₂ deficiency. However, it is not clear that treatment of vitamin B₁₂ deficiency is currently the primary use of compounded methylcobalamin in the U.S. or that methylcobalamin provides a unique benefit over other vitamers of vitamin B₁₂ that are available in FDA approved drug products. It appears that the primary use of compounded methylcobalamin is to treat patients with conditions, in some cases serious, for which there is little evidence to support the effectiveness. We do not have information on the range of doses or the frequency of administration and cannot make a judgement on the safety of the current use of injectable products in patients.
Use of cyanocobalamin or hydroxocobalamin in ASD

- There are no clinical trials to assess the safety or efficacy of either cyanocobalamin or hydroxocobalamin for patients with Autism Spectrum Disorders (ASD)

- Clinicians would be starting from scratch
  - No dosing information
  - No safety data for this population
  - No anecdotal data
Pharmacokinetics

- FDA notes that exogenously administered methylcobalamin is not biologically active (Figure 4)
  - Absorption requires functional Intrinsic Factor
  - Transport to cells requires binding to specific transport proteins
  - Intracellular reduction of methylcobalamin to inactive cobalamin and methyl ligand

- This typical absorption pathway relies on fully functional gastrointestinal lining, transport proteins, and Intrinsic Factor
Pharmacokinetics

- A hallmark feature for many patients with ASD is compromised gut/intestinal health, sensitivity to proteins and foods, therefore oral absorption of cobalamins may not be the same in ASD patients.
- Data assessed by Paul and Brady 2017, and their references, does not explore altered gastrointestinal health or function in patients with ASD.
- Paul and Brady 2017, page 44

All conditions that involve impaired production of IF, such as autoimmune pernicious anemia or atrophic gastritis, and/or compromising intestinal absorptive function, as in celiac disease, ulcerative colitis, Crohn's disease, or tropical sprue, may greatly impair $B_{12}$ absorption by endocytosis.
Johns Hopkins University – Frequency of Use

Safety Assessment

• Reviews by FDA and Johns Hopkins University researchers did not identify significant safety concerns
• There are many trials which report safety measures and the safety profile is consistent

Source: FDA Briefing Document; Pharmacy Compounding Advisory Committee (PCAC) Meeting; June 9, 2021, Tab 2c page 14

Among the United States (U.S.) reports, there were no deaths reported and no events considered by OSE to be probably or certainly related to methylcobalamin. There were four reports that

Source: FDA Briefing Document; Pharmacy Compounding Advisory Committee (PCAC) Meeting; June 9, 2021, Tab 2c page 16

No published clinical trials were found that were conducted to specifically assess the safety of methylcobalamin in humans. In some studies investigating the use of oral, intramuscular, intrathecal and intravenous methylcobalamin at different doses as a treatment for various disorders, safety data is reported. Adverse events reported with methylcobalamin use are infrequent and non-serious. Adverse events in studies that reported safety outcomes are summarized below.
Safety Assessment

• Package Insert - Japan

• Package Insert - Australia

Australian Product Information
Biological Therapies Methylcobalamin 10 mg in 2 mL Injection
(Mecobalamin (co-methylcobalamin))

AUSTR: 22435

1. Name of the Medicine
Mecobalamin (co-methylcobalamin)

2. Qualitative and Quantitative Composition
Each vial contains a minimum of 2 mL of solution and has mecobalamin (co-methylcobalamin) 10 mg as an active ingredient.

3. Pharmaceutical Form
Biological Therapies Methylcobalamin 10 mg in 2 mL Injection is a clear bright red coloured solution for injection supplied in amber glass vials. The pH of the solution is 6.0-8.0.

For the full list of excipients, see Section 6.1 List of Excipients.

4. Clinical Particulars

4.1 Therapeutic Indications
Cobalamin deficiency may exist that is unable to be corrected by oral intake and in these cases parenteral administration may be preferable. Oral administration of vitamin B12 may be insufficient in pernicious anaemia, malabsorption disorders, gastrectomy and gastrointestinal pathologies.
Safety Assessment

- Package Insert - Japan

PRECAUTIONS

1. Adverse Reactions
   Adverse reactions were reported in 13 of 2,872 patients (0.45%). (At the end of the reexamination period)

(1) Clinically significant adverse reactions (incidence unknown)
   Anaphylactoid reaction
   Anaphylactoid reaction such as decrease in blood pressure or dyspnea, may occur. Patients should be carefully observed. In the event of such symptoms, treatment should be discontinued immediately and appropriate measures taken.

(2) Other adverse reactions

<table>
<thead>
<tr>
<th></th>
<th>&lt;0.1%</th>
<th>Incidence unknown</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypersensitivity (note)</td>
<td>Rash</td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td>Headache and hot sensation</td>
<td>Diaphoresis and pain/induration at the site of intramuscular injection</td>
</tr>
</tbody>
</table>

Note: In the event of such symptoms, treatment should be discontinued.
## Safety Assessment

### Database of Adverse Event Notifications - medicines

**Medicine summary**

You searched for the following 3 medicines between 01/01/1991 – 01/03/2021:

- Blackmores B12 Rapi-Melt 1000mcg AUST L 269572 (mecobalamin (co-methylcobalamin))
- Herbs of Gold Activated Sublingual B12 - AUST L 286757 (mecobalamin (co-methylcobalamin))
- Methyl B12 Chewable - AUSTL 250090 (mecobalamin (co-methylcobalamin))

### Results

<table>
<thead>
<tr>
<th>MedDRA system organ class¹</th>
<th>MedDRA reaction term²</th>
<th>Number of cases³</th>
<th>Number of cases with a single suspected medicine⁴</th>
<th>Number of cases where death was a reported outcome⁵</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiac disorders</td>
<td>Tachycardia</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Psychiatric disorders</td>
<td>Anxiety</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Product issues</td>
<td>Product complaint</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>General disorders and administration site conditions</td>
<td>Pain</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Investigations</td>
<td>Blood pressure increased</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Nervous system disorders</td>
<td>Burning sensation</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>General disorders and administration site conditions</td>
<td>Fatigue</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Psychiatric disorders</td>
<td>Insomnia</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>General disorders and administration site conditions</td>
<td>Malaise</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Musculoskeletal and connective tissue disorders</td>
<td>Muscle spasms</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

¹ MedDRA system organ class
² MedDRA reaction term
³ Number of cases
⁴ Number of cases with a single suspected medicine
⁵ Number of cases where death was a reported outcome
<table>
<thead>
<tr>
<th>Author (year of pub.)</th>
<th>Duration of Trial</th>
<th>N</th>
<th>Dose and Route</th>
<th>Adverse event (report)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hendren (2015) (ASD)</td>
<td>8 weeks</td>
<td>57</td>
<td>Methylcobalamin 75 µg/kg every 3 days. Subcutaneous</td>
<td>Treatment: 28; Control 29 3/27 (Cold); 2/27 (Fever); 1/27 (flu); 1/27 (growing pain); (2/27) Increasing hyperactivity; 1/27 (Increasing irritability); 1/27 (Lack of focus); 5/27 (mouthing); 2/27 (Nose bleeds); 1/27 (Rash); 1/27 (stomach flu); 1/27 (trouble sleeping);</td>
</tr>
<tr>
<td>Bertoglio (2010) (ASD)</td>
<td>12-weeks</td>
<td>30</td>
<td>All subjects received 6 weeks of placebo and 6 weeks of Methylcobalamin 64.5 µg/kg every 3 days Subcutaneously</td>
<td>Side effects were limited to increased hyperactivity and increased mouthing of objects. No serious adverse events were reported.</td>
</tr>
<tr>
<td>Frye (2013) (ASD)</td>
<td>3 months</td>
<td>48</td>
<td>Methylcobalamin 75 µg/kg twice weekly 400 µg folinic acid PO twice daily Subcutaneous</td>
<td>6/44 (Hyperactivity); 3/44 (reduced sleep); 2/42 (Discomfort with injections); 1/42 (insomnia); 1/44 (Impulsivity); 1/44 (Irritability). Four patients (2 had hyperactivity) dropped out of trial.</td>
</tr>
<tr>
<td>James (2004) (ASD)</td>
<td>4 months</td>
<td>53</td>
<td>Folinic acid 800 µg PO BID and 1000 mg betaine PO BID (intervention 1) administered after baseline blood draw for 3 months. Another baseline blood draw was compared with initial blood draw. Methylcobalamin 75 µg/kg twice a week in addition to the oral folinic acid and betaine for an additional month (2nd intervention) Subcutaneous</td>
<td>Treatment: 20; Control 33 Not reported</td>
</tr>
<tr>
<td>James (2009) (ASD)</td>
<td>3 months</td>
<td>82</td>
<td>Methylcobalamin 75 µg/kg 2 times per week and 400 µg folinic acid PO twice a day for 3 months Subcutaneous</td>
<td>Treatment: 40; Control 42 4/44 (moderate hyperactivity that was reduced with decreased dose of Folinic acid to 400 µg/d) 1/44 (sleep disruption) 1/44 (getting to sleep) 1/44 (Increased impulsiveness) 1/44 (Irritability).</td>
</tr>
<tr>
<td>Author (year of pub.)</td>
<td>Duration of Trial</td>
<td>N</td>
<td>Dose and Route</td>
<td>Adverse event (report)</td>
</tr>
<tr>
<td>----------------------</td>
<td>-------------------</td>
<td>----</td>
<td>---------------</td>
<td>-----------------------</td>
</tr>
<tr>
<td><strong>Zhang (2017)</strong> (BIPN) Bortezomib (Velcade) induced peripheral Neuropathy</td>
<td>5 months (5 cycles of Velcade therapy*)</td>
<td>65</td>
<td>Methylcobalamin, 2000 µg per dose in 100ml saline one hour before chemotherapy for &gt;1h, and continuously for 28 days to prevent BIPN Intravenous</td>
<td>Treatment: 27; Control 38 1/28 (Rash)</td>
</tr>
<tr>
<td><strong>Shibuya (2014)</strong> (Peripheral neuropathy)</td>
<td>12 months</td>
<td>14</td>
<td>Methylcobalamin 25 mg/day for 10 days followed by monthly 25 mg for 5 months Intravenous</td>
<td>2/14 (Pneumonia related to AIDP seborrheic dermatitis due to CMT) AIDP = acute inflammatory demyelinating polyneuropathy, CMT = Charcot-Marie-Tooth disease</td>
</tr>
<tr>
<td><strong>Chiu (2011)</strong> (Chronic back pain)</td>
<td>9 months</td>
<td>60</td>
<td>Methylcobalamin 500 µg three times a week for 2 weeks Intramuscular</td>
<td>Treatment:33; Control 27 1/33 (pain, hematoma and lack of improvement in symptoms).</td>
</tr>
<tr>
<td><strong>Kaji (2019)</strong> (Amyotrophic lateral sclerosis)</td>
<td>&lt; 36 months</td>
<td>373</td>
<td>Methylcobalamin 25 mg or 50 mg twice per week starting from the end of the observation period (12 weeks) and continued for 182 weeks Intramuscular</td>
<td>Treatment: 124 (25 mg), 123 (50 mg); Placebo 123 Placebo (5/123), methylcobalamin: 9/124 (25 mg) and 7/123 (50 mg) Serious adverse events was similar 64.2%, 62.1% and 65.0%, 6 died of causes other than ALS progression. 1 death in the 50 mg methylcobalamin group was due to cardiac arrest following MI or arrhythmia considered unrelated to medication based on patient’s history</td>
</tr>
</tbody>
</table>

* Not clear or not specified
<table>
<thead>
<tr>
<th>Author (year of pub.)</th>
<th>Duration of Trial</th>
<th>N</th>
<th>Dose and Route</th>
<th>Adverse event (report)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kuwabara (1999)</td>
<td>6 months</td>
<td>10</td>
<td>Methylcobalamin 500 µg three times a week for 6 months after each hemodialysis Intravenous</td>
<td>2/10 (pain scale unchanged) No adverse effect reported.</td>
</tr>
<tr>
<td>Tamura (1999)</td>
<td>2 years</td>
<td>19</td>
<td>Methylcobalamin 500 µg/day every other day for 2 weeks and 1000 µg every 3 months as outpatients Intramuscular</td>
<td>Treatment: 11; Control 8 0/11 (No adverse effects)</td>
</tr>
<tr>
<td>Nakano (2005)</td>
<td>6-25 months</td>
<td>13</td>
<td>Methylcobalamin 25-30 µg/kg/day to max of 1500 µg/day max Parenteral (not specified)</td>
<td>0/11 (No adverse effects)</td>
</tr>
<tr>
<td>Dave (2021)</td>
<td>3 weeks</td>
<td>18</td>
<td>Methylcobalamin nasal spray 500 µg, or methylcobalamin 100 µg IM injection (15 day washout)</td>
<td>Nasal spray (1/18 dizziness) IM (0/18 no adverse effect)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>531 weeks</strong></td>
<td><strong>842</strong></td>
<td><strong>Treated Patients: 531 Adverse events: 63 (12%) Serious Adverse Event: &lt; 1%</strong></td>
<td></td>
</tr>
</tbody>
</table>
Patients with ASD receive high-frequency in-depth medical supervision.
Patients require a multi-modal approach, involving behavioral therapy, pharmacotherapy, and more.
Core symptoms of autism include impairments in social interaction and communication.

Pharmacotherapy for the Core Symptoms in Autistic Disorder: Current Status of the Research
Cristan Farmer, PhD, Audrey Thurm, PhD, and Paul Grant, MD
Pediatrics and Developmental Neuroscience Branch, National Institute of Mental Health, Bethesda, MD, USA

There is no gold-standard for the measurement of change in autism symptoms in clinical trials. The gold-standard diagnostic instruments, the Autism Diagnostic Interview-Revised (ADI-R) and the Autism Diagnostic Observation Schedule (ADOS), were not created to measure severity or improvement of the disorder. None of the measures used in the reviewed studies holistically and comprehensively measure change in core symptom domains, with adequately established reliability and validity across all subgroups of individuals with ASD (e.g., developmental and chronological age levels). While we recognize this as a limitation...
There are no drugs approved to treat the core symptoms of ASD. Two drugs—risperidone and aripiprazole—are approved for the treatment of irritability associated with autism. Labeled warnings associated with both of these drugs include metabolic changes, neuroleptic malignant syndrome, and tardive dyskinesia, among others. Common adverse reactions include akathisia, extrapyramidal symptoms, and weight gain.
Frequency of Use – Industry Survey

• 24 pharmacies in the US
• 27,565 patients in last 12 months
• 662,000 mL of compounded methylcobalamin dispensed in last 12 months
  • Typical dose is 0.1mL
  • Thousands of patients will be impacted by this decision

• Docket has received 1,167 comments as of June 2

• Medicaid data from JHU research is not informative. Prescription claims for compounded methylcobalamin are unlikely to be submitted to CMS.
May 4, 2017 FDA public meeting: Patient-focused drug development for Autism

- https://www.fda.gov/media/105399/download
- Parents testified directly to FDA about the impact of methylcobalamin injections on their children’s autism symptoms
- Testimony addressed the various FDA-approved products and therapies they have attempted, and currently use
- Discussion included the inadequacies of current clinical trial process to measure certain outcomes in patients with ASD
May 23, 2021

Re: FDA-2021-N-0357

I am a licensed physician in the State of Michigan dually board certified in emergency and integrative medicine with over 30 years of experience caring for emotionally ill children and adults. I have had the honor of caring for children on the autism spectrum both through my ABA center and the therapies they provide (ABA, OT, SLP) as well as the bio-medical treatments I provide to address their health issues. Many of these children suffer from an impairment in their ability to digest resulting from a decreased ability to make reduced glutathione. This process is intimately linked with the methylion cycle for which both methylfolate and methylcobalamin (MB12) are essential. I have been using injectable MB12 for the children that are demonstrating these issues successfully since 2007, when I discovered its use. This is one of the interventions that has been a backbone of my biomedical treatment of these children. Unlike methylfolate, MB12 is much more difficult to absorb making the injectable form critical. In a survey of over 5000 parents done by the Autism Research Institute (ARD), MB12 was one of the top four interventions in terms of its positive response rate (over 75%) versus its negative response rate. I have personally witnessed recovery of language, eye contact, and extinction of tittering as a result of its administration. I, myself, have used it to manage some of my own symptoms and found it as useful as stimulants to manage my ADHD symptoms. MB12 has also lowered my homocysteine (a cardiac risk factor) from over 18 to around 7 (when injected or oral cyanocobalamin, oral MB12, and folate were ineffective). Other than the possibility of increasing hyperactivity and local redness at the injection site, there are no dangerous side effects, something that cannot be said for the two drugs approved for kids on the spectrum (Abilify and Risperdal). Removing this valuable asset from the bulk compounding list would be a disaster for three kids that can benefit from its effects. I would beg this committee to leave MB12 on the compounding list so that its benefits can continue to be enjoyed by the children that need it the most.

I thank you for your consideration in this matter.

Yours in health,

[Signature]

BIO ENERGY MEDICAL CENTER
TREATING CLINICALLY APPRAISED

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WWW.BIOENERGYMEDICALCENTER.COM

Source: https://www.regulations.gov/comment/FDA-2021-N-0357-0822
provide to address their health issues. Many of these children suffer from an impairment in their ability to detoxify resulting from a decreased ability to make reduced glutathione. This process is intimately linked with the methylation cycle for which both methylfolate and methylcobalamin (MB12) are essential. I have been using injectable MB12 for the children that are demonstrating these issues successfully since 2007, when I discovered its use. This is one of the interventions that has been a backbone of my biomedical treatment of these children. Unlike methylfolate, MB12 is much more difficult to absorb making the injectable form critical. In a survey of over 5000 parents done by the Autism Research Institute (ARI), MB12 was one of the top four interventions in terms of its positive response rate (over 70%) versus its negative response rate. I have personally witnessed recovery of language, eye contact, and extinction of stimming as a
May 25, 2021

Re: Docket Number FDA-2021-N-0357

To Whom It May Concern:

I have more than 15 years of clinical experience treating patients with autism, inborn errors of metabolism, vitamin B12 deficiency, and/or hyperhomocysteinemia.

I am writing with utmost concern over FDA’s position leading into the Agency’s scheduled review of methylcobalamin by the Pharmacy Compounding Advisory Committee (PCAC) on June 9, 2021. This compounded drug is essential for my patient’s medical treatment, wellbeing and health. In fact, in the years of my practice of medicine, I have witnessed profound beneficial outcomes, such as dramatic measurable improvement in speech, resolution of anxiety, reduction in homocysteine levels, and improvement in multiple measurable outcomes in terms of health for those with inborn errors of metabolism or B-12 deficiency. It is also worth noting that I have never had, in the same 15 years, had no significant sustained side effects.

It is my professional position that methylcobalamin, in forms outside of a nutritional supplement, is a vital and key treatment for my patients.

Sincerely,

[Signature]

Julie A. Buckley, MD
June 1, 2021

To whom it may concern:

The Medical Academy of Pediatrics Special Needs (MAPS) is a group of physicians and other medical providers who treat children with autism and other neurodevelopmental disorders using treatments that target biochemical abnormalities in an attempt to obtain optimal clinical outcomes. Through MAPS, over 1000 physicians and other providers have received training and can be considered a substantial minority. Part of the MAPS treatment protocol involves using Methylcobalamin injections which often lead to quick and obvious clinical improvements. These are an important treatment which is considered standard of care for our group and should continue to be available for compounding as the benefits are high and risks are low.

Sincerely,

Daniel Rossignol MD, FAAFP
President of MAPS
rossignolfm@gmail.com
www.medmaps.org

James Heyenschwander, MD
Co-Chair and Board Member MAPS
Conclusions

• Approved as injection in Japan and Australia
• No significant adverse event reports from all available literature, worldwide
  • “Adverse events reported with methylcobalamin use are infrequent and non-serious.” – FDA
• Has been compounded since at least 2005
• No alternative options for patients

• Dr. Frye to address clinical and biochemical assessment
THANK YOU
References

References for Safety Chart


Methylcobalamin Treatment
For Autism Spectrum Disorder

Richard E. Frye, M.D., Ph.D.
Chief, Neurodevelopmental Disorders
Director of Autism and Fragile X Programs,
Phoenix Children's Hospital, Phoenix AZ
Professor of Child Health
University of Arizona College of Medicine - Phoenix, Phoenix AZ

Presenting on Behalf of PCCA
Disclosures

Research Funding: National Institutes of Health; Autism Speaks; Department of Defense; The BRAIN Foundation; Turnabout for Autism; Gupta Family Foundation; Jonty Foundation; N of 1 Foundation; Finch Therapeutics; National Fragile X Foundation

Industry Clinical Trials: Finch Therapeutics; Zynerba Pharma

Advisory Board: Iliad Neurosciences, NeuroNeeds Inc
Disclaimer

Every attempt has been made to make this presentation as accurate as possible. The information is provided without any expressed or implied warranty. This presentation should not be substituted for medical advice. Treatments discussed are considered off-label and are not FDA-approved.
### Key Points

**Biochemical Pathways Involving Cobalamin Are Disrupted in Autism**
- Methylation
- Redox Metabolism

**Methylcobalamin Supports Disrupted Biochemical Pathways in Autism**
- Not Necessarily a Treatment of a Deficiency
- Treatment of an Insufficiency in Cofactors for Methylation and Redox Metabolism

**Evidence for Effectiveness of mB12 SQ Therapy**
- Improves Redox and Methylation Biochemical Abnormalities
- Improves Autism Symptoms
- Improvement in Symptoms is Related to Improvement in Biochemical Abnormalities

**Potential Biological Mechanisms in Autism**
- Production of Glutathione Can Rebalance Glutamate Metabolism
- Polymorphism in Cobalamin Carrier Protein Could Reduce Cobalamin Transportation
- Cobalamin Concentrations Are Reduced in the Autism Brain
- Methionine Synthase mRNA Expression is Reduced in the Autism Brain
- Cobalamin Intake is Lower in Children with Autism

Subcutaneous Methylcobalamin is Standard of Care for Physicians Treating Autism
Methycobalamin for Autism

**FOLATE CYCLE**
- Leucovorin Calcium
- DHFR
- DHFR Dihydrofolate Reductase
- MTHFR Methylenetetrahydrofolate Reductase
- THF TetraHydroFolate
- Purines and Thymidylate
- DNA / RNA
- GTP
- 5,10-CH$_2$THF
- 5,10-Methylenetetrahydrofolate
- THF TetraHydroFolate
- 5,10-CH$_2$THF
- MTHFR Methylenetetrahydrofolate Reductase
- Methylcobalamin for Autism

**METHYLATION**
- Methionine
- SAM S-Adenosyl methionine
- SAH S-Adenosyl homocysteine
- Reduced Glutathione
- Oxidized Glutathione
- Nitric Oxide
- Tetrahydrobiopterin
- Cellular Methylation Reactions
- Adenosine
- Homocysteine
- B12
- NAC
- Cystathionine
- GSH Reduced Glutathione
- GSSG Oxidized Glutathione
- Tyrosine
- L-DOPA
- BH4 Tetrahydrobiopterin
- Tryptophan
- L-arginine
- L-citrulline + NO
- TETRAHYDROBIOPTERIN METABOLISM
- GLUTATHIONE METABOLISM

**Metabolites**
- THF TetraHydroFolate
- SAM S-Adenosyl methionine
- SAH S-Adenosyl homocysteine
- GSH Reduced Glutathione
- GSSG Oxidized Glutathione
- NO Nitric Oxide
- BH4 Tetrahydrobiopterin

**Enzymes**
- DHFR Dihydrofolate Reductase
- MS Methionine Synthase
- MTHFR Methylenetetrahydrofolate Reductase

**Figure Key**
- Blue text outlines one of the four pathways related to folate metabolism
- Ovals represent enzymes
- Boxes represent metabolites
- Red indicates metabolites and enzymes repeatedly noted to be abnormal in autism
- Green highlights reduced folates
- Yellow highlights oxidized folates

Metabolic Endophenotype and Related Genotypes are Associated With Oxidative Stress in Children With Autism

S. Jill James, Stepan Melnyk, Stefanie Jernigan, Mario A. Cleves, Charles H. Halsted

1Department of Pediatrics, University of Arkansas for Medical Sciences, Arkansas Children’s Hospital Research Institute, Little Rock, Arkansas

**TABLE II. Transmethylation and Transsulfuration Metabolites in Autistic Cases and Controls**

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Control (n = 73)</th>
<th>Autistic (n = 80)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methionine (μmol/L)</td>
<td>28.0 ± 6.5</td>
<td>20.6 ± 5.2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>SAM (nmol/L)</td>
<td>93.8 ± 18</td>
<td>84.3 ± 11</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>SAH (nmol/L)</td>
<td>18.8 ± 4.5</td>
<td>23.3 ± 7.9</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>SAM/SAH ratio</td>
<td>5.5 ± 2.8</td>
<td>4.0 ± 1.7</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Adenosine (μmol/L)</td>
<td>0.19 ± 0.13</td>
<td>0.28 ± 0.13</td>
<td>0.001</td>
</tr>
<tr>
<td>Homocysteine (μmol/L)</td>
<td>6.0 ± 1.3</td>
<td>5.7 ± 1.2</td>
<td>0.03v</td>
</tr>
<tr>
<td>Cystathionine (μmol/L)</td>
<td>0.19 ± 0.1</td>
<td>0.24 ± 0.1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Cysteine (μmol/L)</td>
<td>207 ± 22</td>
<td>165 ± 14</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Cysteinylglycine (μmol/L)</td>
<td>39.4 ± 7.3</td>
<td>38.9 ± 11</td>
<td>0.78</td>
</tr>
<tr>
<td>Total GSH (μmol/L)</td>
<td>7.53 ± 1.7</td>
<td>5.1 ± 1.2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Free GSH (μmol/L)</td>
<td>2.2 ± 0.9</td>
<td>1.4 ± 0.5</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>GSSG (μmol/L)</td>
<td>0.24 ± 0.1</td>
<td>0.40 ± 0.2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Total GSH/GSSG ratio</td>
<td>28.2 ± 7.0</td>
<td>14.7 ± 6.2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Free GSH/GSSG ratio</td>
<td>7.9 ± 3.5</td>
<td>4.9 ± 2.2</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

SAM, S-adenosylmethionine; SAH, S-adenosylhomocysteine; GSH, glutathione; GSSG, glutathione disulfide.

*aMeans ± SD.*
### Oxidative stress-related biomarkers in autism: Systematic review and meta-analyses


- Significant reduction in blood GSH, Methionine and Cysteine
- Significant elevation in blood GSSG
- Significant reduction in blood glutathione peroxidase
- Significant association of MTHFR homozygous C677T polymorphism with ASD

### Glutathione Abnormalities are found in several tissues in children with ASD


### Redox Abnormalities can lead to DNA, protein and lipid oxidative damage in ASD

Classification and adaptive behavior prediction of children with autism spectrum disorder based upon multivariate data analysis of markers of oxidative stress and DNA methylation

Daniel P. Howsmon¹,², Uwe Kruger³, Stepan Melnyk⁴, S. Jill James⁴, Juergen Hahn¹,²,³,*

¹ Department of Chemical and Biological Engineering, Rensselaer Polytechnic Institute, Troy, New York, United States of America, ² Center for Biotechnology and Interdisciplinary Studies, Rensselaer Polytechnic Institute, Troy, New York, United States of America, ³ Department of Biomedical Engineering, Rensselaer Polytechnic Institute, Troy, New York, United States of America, ⁴ Department of Pediatrics, University of Arkansas for Medical Sciences, Little Rock, Arkansas, United States of America
Methylcobalamin for Autism

Metabolites Examined
(Highlighted Metabolites In Discriminant Fx)

<table>
<thead>
<tr>
<th>Metabolites</th>
<th>Actual</th>
<th>Predicted</th>
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</thead>
<tbody>
<tr>
<td>SAM</td>
<td>ASD</td>
<td>NEU</td>
</tr>
<tr>
<td>S-Adenosyl methionine</td>
<td>TP 81</td>
<td>FP 2</td>
</tr>
<tr>
<td>S-Adenosyl homocysteine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>fGSH</td>
<td>NEU</td>
<td>ASD</td>
</tr>
<tr>
<td>Free Reduced Glutathione</td>
<td>FN 3</td>
<td>TN 73</td>
</tr>
<tr>
<td>tGSH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Reduced Glutathione</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GSSG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oxidized Glutathione</td>
<td></td>
<td></td>
</tr>
<tr>
<td>fGSH/GSSG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glutathione Redox Ratio</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8-OHG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8-hydroxy-2'-deoxyguanosine</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Efficacy of methylcobalamin and folinic acid treatment on glutathione redox status in children with autism\textsuperscript{1–3}

S Jill James, Stepan Melnyk, George Fuchs, Tyra Reid, Stefanie Jernigan, Oleksandra Pavliv, Amanda Hubanks, and David W Gaylor

<table>
<thead>
<tr>
<th>Plasma metabolite concentration</th>
<th>Control children ((n = 42))</th>
<th>Pretreatment ((n = 40))</th>
<th>Posttreatment ((n = 40))</th>
<th>(P) value\textsuperscript{2}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methionine</td>
<td>24 ± 3</td>
<td>21 ± 4\textsuperscript{3}</td>
<td>22 ± 3\textsuperscript{4}</td>
<td>NS</td>
</tr>
<tr>
<td>SAM (nmol/L)</td>
<td>78 ± 22</td>
<td>66 ± 13\textsuperscript{3}</td>
<td>69 ± 12\textsuperscript{4}</td>
<td>NS</td>
</tr>
<tr>
<td>SAH (nmol/L)</td>
<td>14.3 ± 4.3</td>
<td>15.2 ± 5</td>
<td>14.8 ± 4</td>
<td>NS</td>
</tr>
<tr>
<td>SAM:SAH ((\mu)mol/L)</td>
<td>5.6 ± 2.0</td>
<td>4.7 ± 1.5\textsuperscript{3}</td>
<td>5.0 ± 2.0</td>
<td>NS</td>
</tr>
<tr>
<td>Homocysteine ((\mu)mol/L)</td>
<td>5.0 ± 1.2</td>
<td>4.8 ± 1.8</td>
<td>5.3 ± 1.1</td>
<td>0.04</td>
</tr>
<tr>
<td>Cysteine ((\mu)mol/L)</td>
<td>210 ± 18</td>
<td>191 ± 24\textsuperscript{3}</td>
<td>215 ± 19</td>
<td>0.001</td>
</tr>
<tr>
<td>Cysteinylglycine ((\mu)mol/L)</td>
<td>45 ± 6</td>
<td>40 ± 9\textsuperscript{3}</td>
<td>46 ± 9</td>
<td>0.002</td>
</tr>
<tr>
<td>tGSH ((\mu)mol/L)</td>
<td>7.5 ± 1.8</td>
<td>5.4 ± 1.3\textsuperscript{3}</td>
<td>6.2 ± 1.2\textsuperscript{4}</td>
<td>0.001</td>
</tr>
<tr>
<td>fGSH ((\mu)mol/L)</td>
<td>2.8 ± 0.8</td>
<td>1.5 ± 0.4\textsuperscript{3}</td>
<td>1.8 ± 0.4\textsuperscript{4}</td>
<td>0.008</td>
</tr>
<tr>
<td>GSSG ((\mu)mol/L)</td>
<td>0.18 ± 0.07</td>
<td>0.28 ± 0.08\textsuperscript{3}</td>
<td>0.22 ± 0.06\textsuperscript{4}</td>
<td>0.001</td>
</tr>
<tr>
<td>tGSH:GSSG</td>
<td>47 ± 18</td>
<td>21 ± 6\textsuperscript{3}</td>
<td>30 ± 9\textsuperscript{4}</td>
<td>0.001</td>
</tr>
<tr>
<td>fGSH:GSSG</td>
<td>17 ± 6.8</td>
<td>6 ± 2\textsuperscript{3}</td>
<td>9 ± 3\textsuperscript{4}</td>
<td>0.001</td>
</tr>
</tbody>
</table>

\textsuperscript{1} tGSH, free glutathione; SAH, S-adenosylhomocysteine; SAM, S-adenosylmethionine; tGSH, total glutathione; GSSG, oxidized glutathione disulfide. NS, \(P > 0.05\).

\textsuperscript{2} Pre- and posttreatment comparison.

\textsuperscript{3} Significantly different from control children, \(P < 0.005\).

\textsuperscript{4} Significantly different from control children, \(P < 0.01\).
Efficacy of methylcobalamin and folic acid treatment on glutathione redox status in children with autism\textsuperscript{1–3}

\begin{itemize}
\item S Jill James, Stepan Melnyk, George Fuchs, Tyra Reid, Stefanie Jernigan, Oleksandra Pavliv, Amanda Hubanks, and David W Gaylor
\end{itemize}
## Effectiveness of Methylcobalamin and Folinic Acid Treatment on Adaptive Behavior in Children with Autistic Disorder Is Related to Glutathione Redox Status

Richard E. Frye,¹ Stepan Melnyk,¹ George Fuchs,¹ Tyra Reid,¹ Stefanie Jernigan,¹ Oleksandra Pavliv,¹ Amanda Hubanks,¹ David W. Gaylor,² Laura Walters,¹ and S. Jill James¹

<table>
<thead>
<tr>
<th>Vineland Subscale</th>
<th>Baseline Age Equivalent (mean ± SE)</th>
<th>Post-Intervention Age Equivalent (mean ± SE)</th>
<th>Change (months) (mean; 95% C I)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Receptive Language</td>
<td>23.1 ± 1.8</td>
<td>31.4 ± 3.4</td>
<td>8.3 (2.9, 13.7)</td>
</tr>
<tr>
<td>Expressive Language</td>
<td>20.6 ± 1.9</td>
<td>27.5 ± 2.9</td>
<td>6.0 (3.3,9.4)</td>
</tr>
<tr>
<td>Written Language</td>
<td>40.5 ± 3.8</td>
<td>46.7 ± 4.0</td>
<td>6.2 (3.4, 9.0)</td>
</tr>
<tr>
<td>Personal Skills</td>
<td>30.5 ± 2.3</td>
<td>40.5 ± 3.8</td>
<td>10.0 (3.8, 16.2)</td>
</tr>
<tr>
<td>Domestic Skills</td>
<td>30.3 ± 4.1</td>
<td>39.3 ± 5.9</td>
<td>9.0 (-1.4, 19.4)</td>
</tr>
<tr>
<td>Community Skills</td>
<td>32.9 ± 2.9</td>
<td>36.1 ± 3.8</td>
<td>2.0 (-3.0, 6.9)</td>
</tr>
<tr>
<td>Interpersonal Skills</td>
<td>18.7 ± 2.7</td>
<td>24.1 ± 3.9</td>
<td>5.4 (0.0, 10.9)</td>
</tr>
<tr>
<td>Play/Leisure Skills</td>
<td>22.0 ± 4.5</td>
<td>34.0 ± 4.1</td>
<td>12.0 (4.1, 19.6)</td>
</tr>
<tr>
<td>Coping Skills</td>
<td>25.8 ± 2.5</td>
<td>34.3 ± 4.0</td>
<td>11.5 (4.9, 18.0)</td>
</tr>
</tbody>
</table>
Effectiveness of Methylcobalamin and Folinic Acid Treatment on Adaptive Behavior in Children with Autistic Disorder Is Related to Glutathione Redox Status

Richard E. Frye,¹ Stepan Melnyk,¹ George Fuchs,¹ Tyra Reid,¹ Stefanie Jernigan,¹ Oleksandra Pavliv,¹ Amanda Hubanks,¹ David W. Gaylor,² Laura Walters,¹ and S. Jill James¹

(A) Expressive Communication
(B) Personal
(C) Domestic
(D) Interpersonal
(E) Play-Leisure
(F) Coping

Change in free GSH/GSSG vs. Change in Expressive Communication Raw Score
Change in free GSH/GSSG vs. Change in Personal Daily Living Skills Raw Score
Change in free GSH/GSSG vs. Change in Domestic Daily Living Skills Raw Score
Change in free GSH/GSSG vs. Change in Interpersonal Social Skills Raw Score
Change in free GSH/GSSG vs. Change in Play-Leisure Social Skills Raw Score
Change in free GSH/GSSG vs. Change in Coping Social Skills Raw Score
Randomized, Placebo-Controlled Trial of Methyl B12 for Children with Autism

Robert L. Hendren, DO, S. Jill James, PhD, Felicia Widjaja, MPH, Brittany Lawton, BS, Abram Rosenblatt, PhD, and Stephen Bent, MD

1Department of Psychiatry, University of California, San Francisco, California.
2Arkansas Children’s Hospital Institute, Department of Pediatrics,
Methylcobalamin for Autism

Children screened (n=61)

- Screen failure (n=3)
- Not willing to take injections (n=1)

Randomized (n=57)

Allocated to B12 (n=28)
- Received B12 (n=28)
  - Lost to follow-up (n=0)
  - Discontinued intervention (n=0)
  - Did not complete HIPAA form (n=1)
  - Analyzed (n=27)

Allocated to placebo (n=29)
- Received placebo (n=29)
  - Lost to follow-up (n=3)
  - Discontinued intervention (n=0)
  - Did not complete HIPAA form (n=3)
  - Analyzed (n=23)
Randomized, Placebo-Controlled Trial of Methyl B12 for Children with Autism

Robert L. Hendren, DO, S. Jill James, PhD, Felicia Widjaja, MPH, Brittany Lawton, BS, Abram Rosenblatt, PhD, and Stephen Bent, MD

JOURNAL OF CHILD AND ADOLESCENT PSYCHOPHARMACOLOGY

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean change placebo (SD)</th>
<th>Mean change methyl B12 (SD)</th>
<th>Effect size</th>
<th>Difference in mean change</th>
<th>95% CI</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CGI-Improvement</td>
<td>3.1 (0.8)</td>
<td>2.4 (0.8)</td>
<td>0.84</td>
<td>0.7 (0.8)</td>
<td>-1.2 to -0.2</td>
<td>0.005*</td>
</tr>
<tr>
<td>ABC Hyperactivity</td>
<td>-3.9 (7.1)</td>
<td>-0.9 (4.8)</td>
<td>-0.48</td>
<td>-3.0 (6.2)</td>
<td>-7.0 to 1.1</td>
<td>0.11</td>
</tr>
<tr>
<td>ABC Inappropriate Speech</td>
<td>-0.3 (1.6)</td>
<td>0.3 (1.4)</td>
<td>-0.43</td>
<td>-0.6 (1.5)</td>
<td>-1.6 to 0.3</td>
<td>0.17</td>
</tr>
<tr>
<td>ABC Irritability</td>
<td>-2.6 (4.3)</td>
<td>-0.1 (3.7)</td>
<td>-0.61</td>
<td>-2.5 (4.0)</td>
<td>-5.1 to 0.2</td>
<td>0.08</td>
</tr>
<tr>
<td>ABC Lethargy</td>
<td>-1.2 (7.1)</td>
<td>-1.9 (5.8)</td>
<td>0.12</td>
<td>0.8 (6.6)</td>
<td>-3.5 to 5.0</td>
<td>1.00</td>
</tr>
<tr>
<td>ABC Stereotypy</td>
<td>0.3 (3.2)</td>
<td>-0.3 (2.2)</td>
<td>0.23</td>
<td>0.6 (2.8)</td>
<td>-1.2 to 2.5</td>
<td>0.58</td>
</tr>
<tr>
<td>ABC Total Score</td>
<td>-7.6 (17.4)</td>
<td>-2.9 (12.1)</td>
<td>-0.30</td>
<td>-4.7 (15.4)</td>
<td>-14.6 to 5.3</td>
<td>0.23</td>
</tr>
<tr>
<td>SRS Social Awareness</td>
<td>0.0 (18.3)</td>
<td>-3.9 (10.0)</td>
<td>0.26</td>
<td>3.9 (15.0)</td>
<td>-5.9 to 13.7</td>
<td>0.18</td>
</tr>
<tr>
<td>SRS Social Cognition</td>
<td>-3.6 (10.5)</td>
<td>-2.7 (6.9)</td>
<td>-0.10</td>
<td>-0.9 (9.0)</td>
<td>-6.8 to 5.0</td>
<td>0.87</td>
</tr>
<tr>
<td>SRS Social Communication</td>
<td>-3.6 (8.8)</td>
<td>0.1 (15.0)</td>
<td>-0.30</td>
<td>-3.6 (12.1)</td>
<td>-11.5 to 4.2</td>
<td>0.22</td>
</tr>
<tr>
<td>SRS Social Manerisms</td>
<td>-1.6 (14.3)</td>
<td>-0.8 (10.7)</td>
<td>-0.06</td>
<td>-0.8 (12.8)</td>
<td>-9.1 to 7.5</td>
<td>0.53</td>
</tr>
<tr>
<td>SRS Social Motivation</td>
<td>-6.1 (10.0)</td>
<td>0.2 (6.6)</td>
<td>-0.73</td>
<td>-6.3 (8.6)</td>
<td>-11.9 to -0.7</td>
<td>0.02*</td>
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<tr>
<td>SRS Total Score</td>
<td>-4.1 (7.7)</td>
<td>-1.6 (7.7)</td>
<td>-0.32</td>
<td>-2.5 (7.7)</td>
<td>-7.5 to 2.5</td>
<td>0.21</td>
</tr>
</tbody>
</table>

- Hendren Study did not use folate with mB12 Treatment
- The Clinical Global Impression (CGI) Scale is a Clinician Rated Instrument
- Both the Social Responsiveness Scale (SRS) and Aberrant Behavior Checklist (ABC) are Parent Reported Measures which are Notoriously Sensitive to Placebo Effects
Pharmacotherapy for the Core Symptoms in Autistic Disorder:
Current Status of the Research

Cristan Farmer, PhD, Audrey Thurm, PhD, and Paul Grant, MD
Pediatrics and Developmental Neuroscience Branch, National Institute of Mental Health,
Bethesda, MD, USA

There is no gold-standard for the measurement of change in autism symptoms in clinical trials (85). The gold-standard diagnostic instruments, the Autism Diagnostic Interview-Revised (86) and the Autism Diagnostic Observation Schedule (87) were not created to measure severity or improvement of the disorder. None of the measures used in the reviewed studies holistically and comprehensively measures change in core symptom domains, with adequately established reliability and validity across all subgroups of individuals with ASD (e.g., developmental and chronological age levels). While we recognize this as a limitation...
Methylcobalamin for Autism

CGI-I at Week 8 vs Change in SAM/SAH

Interaction P = 0.091

- Placebo
- MB 12

Change in SAM/SAH
### Clinical Studies on Injected Methylcobalamin

<table>
<thead>
<tr>
<th>Study Type</th>
<th># of Studies</th>
<th>Total N</th>
<th>Dose</th>
<th>Outcome</th>
<th>Adverse Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Double Blind Placebo Controlled</td>
<td>2</td>
<td>87</td>
<td>MB12 64.5-75 µg/kg SQ q 3 days</td>
<td>• CGI Improved</td>
<td>• Few, Mild</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• Improvement Associated with Methylation Biomarkers</td>
<td>• Hyperactivity</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• Mouthing Objects</td>
<td>• No Serious</td>
</tr>
<tr>
<td>Open Label</td>
<td>3 (2 same group)</td>
<td>48</td>
<td>mB12 SQ 75 µg/kg 2-3 TIW</td>
<td>• Improved VABS</td>
<td>• Few, Mild</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• Improved Redox Metabolism</td>
<td>• Hyperactivity</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• VABS Improvements Associated with Redox Biomarker</td>
<td>• Insomnia</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• Impulsiveness</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• No Serious</td>
</tr>
<tr>
<td>Case Report/ Series</td>
<td>4</td>
<td>7</td>
<td>Various Doses SQ and IM</td>
<td>• Improved Vision</td>
<td>None Reported</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• Improved ASD symptoms</td>
<td></td>
</tr>
</tbody>
</table>

VABS = Vineland Adaptive Behavior Scale
Metabolic Endophenotype and Related Genotypes are Associated With Oxidative Stress in Children With Autism

S. Jill James, Stepan Melnyk, Stefanie Jernigan, Mario A. Cleves, Charles H. Halsted, Donna H. Wong, Paul Cutler, Kenneth Bock, Marvin Boris, J. Jeffrey Bradstreet, Sidney M. Baker, and David W. Gaylor

Enzymes with Significant Polymorphisms

Yellow Highlight

RFC       Reduced folate carrier
TCN2       Transcobalamin II (B12 binding protein)
MTHFR      Methylene tetrahydrofolate reductase
GST        Glutathione S-Transferase

Other Important Enzymes

GCPII      Glutamate Carboxypeptidase II
CBS        Cystathionine beta-synthase
MSM        Methionine synthase
MSR        Methionine synthase reductase
MTase      Methyltransferase

Metabolic Endophenotype and Related Genotypes are Associated With Oxidative Stress in Children With Autism

S. Jill James,1* Stepan Melnyk,1 Stefanie Jernigan,1 Mario A. Cleves,1 Charles H. Halsted,2 Donna H. Wong,2 Paul Cutler,3 Kenneth Bock,4 Marvin Boris,5 J. Jeffrey Bradstreet,6 Sidney M. Baker,7 and David W. Gaylor8

<table>
<thead>
<tr>
<th>SNP</th>
<th>Genotype</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCN2 776C &gt; G/COMT 472G &gt; A</td>
<td>CC/CC</td>
<td>Reference</td>
</tr>
<tr>
<td></td>
<td>GG/GG</td>
<td>7.0 (2.32, 21.2)</td>
</tr>
<tr>
<td>RFC-1 80A &gt; G/MTHFR677C &gt; T</td>
<td>AA/CC</td>
<td>Reference</td>
</tr>
<tr>
<td></td>
<td>GA/CT</td>
<td>3.24 (1.55, 6.78)</td>
</tr>
<tr>
<td></td>
<td>GA/TT</td>
<td>4.40 (1.45, 14.0)</td>
</tr>
<tr>
<td></td>
<td>GG/CT</td>
<td>3.10 (1.39, 6.84)</td>
</tr>
<tr>
<td>RFC-1 80A &gt; G/GSTM1 Null</td>
<td>AA/+</td>
<td>Reference</td>
</tr>
<tr>
<td></td>
<td>GA/null</td>
<td>3.78 (1.80, 7.95)</td>
</tr>
<tr>
<td></td>
<td>GG/null</td>
<td>2.67 (1.22, 5.89)</td>
</tr>
<tr>
<td>MTHFR 677 CT/MTHFR 1298AC</td>
<td>CT/AC</td>
<td>1.78 (0.97, 3.26)</td>
</tr>
<tr>
<td>MTHFR 677CT/1298AC/RFC 80G</td>
<td>(CT/AC)/GA</td>
<td>1.33 (1.33, 15.81)</td>
</tr>
<tr>
<td></td>
<td>(CT/AC)/GG</td>
<td>3.57 (0.97, 13.49)</td>
</tr>
</tbody>
</table>

RFC = Reduced folate carrier  
TCN2 = Transcobalamin II (B12 binding protein)  
MTHFR = Methylenetetrahydrofolate reductase  
GST = Gluthathione S-Transferase  
COMT = Catechol-O-methyltransferase

Long-term Outcome of 4 Patients With Transcobalamin Deficiency Caused by 2 Novel TCN2 Mutations

Marwan Nashabat, MCR,* Gustavo Maegawa, MD, PhD, †
Peter H. Nissen, PhD, ‡ Ebba Nexo, MD, PhD, ‡ Hussain Al-Shamrani, MD, §
Mohammed Al-Owain, MD, FACMG, ¶ and Majid Alfadhel, MD, FCCMG*##

<table>
<thead>
<tr>
<th>TABLE 1. Summary of the Patient’s Clinical Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Patient 1</strong></td>
</tr>
<tr>
<td><strong>Molecular findings</strong></td>
</tr>
<tr>
<td><em>(TCN2 gene mutation)</em></td>
</tr>
<tr>
<td><strong>Laboratory findings on presentation</strong></td>
</tr>
<tr>
<td><em>(reference intervals are indicated in parenthesis)</em></td>
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<tr>
<td></td>
</tr>
<tr>
<td><strong>Follow-up (clinical)</strong></td>
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<td></td>
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</tbody>
</table>

**Autism Cases**

<table>
<thead>
<tr>
<th><strong>Patient 3</strong></th>
<th><strong>Patient 4</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonsense mutation:</td>
<td>Nonsense mutation:</td>
</tr>
<tr>
<td>exon 5 c.679C &gt;</td>
<td>exon 5 c.679C &gt;</td>
</tr>
<tr>
<td>T(p.R227X)</td>
<td>T(p.R227X)</td>
</tr>
<tr>
<td>Severe pancytopenia and macrocytic anemia</td>
<td>P-MMA: 29 µmol/L (GC-MS)</td>
</tr>
<tr>
<td>Intractable metabolic acidosis</td>
<td></td>
</tr>
<tr>
<td>P-MMA: 30.1 µmol/L (GC-MS)</td>
<td></td>
</tr>
<tr>
<td>P-Hcy: 7 µmol/L</td>
<td></td>
</tr>
<tr>
<td>One deterioration at the age of 4y</td>
<td>One deterioration at the age of 2y</td>
</tr>
<tr>
<td>Speech delay, social and communication impairment improving with behavioral therapy</td>
<td>ASD, language delay, speech and responsivity deficit</td>
</tr>
</tbody>
</table>
Long-term Outcome of 4 Patients With Transcobalamin Deficiency Caused by 2 Novel TCN2 Mutations

Marwan Nashabat, MCR,* Gustavo Maegawa, MD, PhD,†
Peter H. Nissen, PhD,‡ Ebba Nexo, MD, PhD,‡ Hussain Al-Shamrani, MD,§
Mohammed Al-Owain, MD, FACMG,¶ and Majid Alfadhel, MD, FCCMG*#

The management of TC deficiency is pharmacological doses of Cbl. Trakadis et al27 conducted the largest retrospective observational cohort on TC deficiency. Their recommendation supported the aggressive treatment proposed previously11 with parenteral hydroxocobalamin 1 mg or cyano-cobalamin 1 mg IM injection weekly. Patients 3 and 4 developed acute anemia while on cyano-cobalamin injection every 4 weeks. The 2 patients were started on methyl-cobalamin 1 mg injections weekly, and their condition was stabilized immediately until the date of this report. Obviously, we do not know whether this effect was related to change in treatment frequency or change in the form of cobalamin administered. As methyl-cobalamin is the active coenzyme, it could be considered to use this form of the vitamin. Follow-up and regular monitoring for these patients is an essential part to optimize the treatment regimen.11,13,27
Methylcobalamin was 3.1 times lower in the ASD Brain
Decreased Brain Levels of Vitamin B12 in Aging, Autism and Schizophrenia

Yiting Zhang¹, Nathaniel W. Hodgson¹, ², Malav S. Trivedi¹, ³, Hamid M. Abdolmaleky⁴, Margot Fournier⁵, Michel Cuenod⁵, Kim Quang Do⁵, Richard C. Deth¹, ³ *

C

Thiol status (%)

Control
Autism

HCY Methionine SAM SAH Cysteine GSH GSSG Cystathionine

GSH/GSSG ratio

SAM/SAH ratio

Control Autism Control Autism

* ** ***
Age-Dependent Decrease and Alternative Splicing of Methionine Synthase mRNA in Human Cerebral Cortex and an Accelerated Decrease in Autism

Christina R. Muratore¹, Nathaniel W. Hodgson¹, Malav S. Trivedi¹, Hamid M. Abdolmaleky², Antonio M. Persico³, Carla Lintas³, Suzanne De La Monte⁴, Richard C. Deth¹*

¹ Department of Pharmaceutical Sciences, School of Pharmacy, Northeastern University, Boston, Massachusetts, United States of America, ² Genetics Program, School of Medicine, Boston University, Boston, Massachusetts, United States of America, ³ Laboratory of Molecular Psychiatry and Neurogenetics, University Campus Bio-Medico, Rome, Italy, ⁴ Department of Medicine and Pathology, Rhode Island Hospital and Warren Alpert School of Medicine at Brown University, Providence, Rhode Island, United States of America
Methylcobalamin for Autism

B12 Intake is Lower in Children with Autism Spectrum Disorder

Differences in food consumption and nutritional intake between children with autism spectrum disorders and typically developing children: A meta-analysis

Patricia Esteban-Figuerola, Josefa Canals, José Cándido Fernández-Cao, and Victoria Arija Val

Figure 9. Forest plot of overall standardized mean difference in vitamin B12 intake between TD and ASD children.
June 1, 2021

To whom it may concern:

The Medical Academy of Pediatrics Special Needs (MAPS) is a group of physicians and other medical providers who treat children with autism and other neurodevelopmental disorders using treatments that target biochemical abnormalities in an attempt to obtain optimal clinical outcomes. Through MAPS, over 1000 physicians and other providers have received training and can be considered a substantial minority. Part of the MAPS treatment protocol involves using Methylcobalamin injections which often lead to quick and obvious clinical improvements. These are an important treatment which is considered standard of care for our group and should continue to be available for compounding as the benefits are high and risks are low.

Sincerely,

Daniel Rossignol MD, FAAFP
President of MAPS
rossignolmd@gmail.com
www.medmaps.org

James Neuschwander, MD
Co-Chair and Board Member MAPS

MAPS considers Subcutaneous Methylcobalamin as Standard of Care For Children with Autism Spectrum Disorder
Grants to Study Methylcobalamin in Autism are Not Easy to Get. Methylcobalamin was seen as Standard of Care and Widely Available So No Novelty or Need Was Seen for Further Clinical Research
Choline Chloride

Nomination for section 503A of the Federal Food, Drug, and Cosmetic Act, to the Pharmacy Compounding Advisory Committee (PCAC)

Paul S. Anderson, ND
for the Nominator,
American Association of Naturopathic Physicians (AANP)
Dietary Requirements:

“Current dietary recommendations for choline are likely too low for some men. In addition, these recommendations did not consider genetic variation as a modulator of dietary requirement because it was assumed that functional polymorphisms would be too rare (<5% of population) to be considered. It is clear that this assumption is not true for SNPs in folate metabolism (where 63% of subjects had at least one allele for the MTHFD1 SNP) or for SNPs in choline metabolism (where 74% of subjects had at least one allele for the PEMT promoter SNP). As discussed above, women with low dietary choline intake have a markedly increased risk of having a baby with neural tube defects (112). There is solid science in animal models that suggests choline is critical for normal brain development (31). Choline deficiency has other health consequences—it is associated with liver and muscle damage and with an exaggerated plasma homocysteine rise after a methionine load (34). Elevated plasma homocysteine is an independent risk factor for cardiovascular disease and stroke in humans (44,45,77)”

Quote from reference-11
Preterm Birth Nutritional Requirement:

According to the CDC website, there are approximately 374,754 babies born preterm and approximately 1% of all births of babies in the USA have fetal alcohol syndrome. This represents a large amount of new births in the USA that have special nutritional requirements.

There are no FDA approved Choline drugs available for physicians to prescribe. (One exception being parenteral egg-based lipid product)

These infants with special nutritional requirements can go under served in the medical community. Studies are providing valuable insight into the need for available treatment options.
Preterm Infant Need

One study done in preterm infants found that choline deficiency can be present in preterm infants.

Breast feeding does not supply enough choline to correct deficiency.1 Preterm babies will have higher levels of phosphatidyl choline leading to rapid turnover of choline levels. Decreased choline levels can result in hepatic damage and lung damage over time.1

Choline supplementation can be vital for normal development of preterm births.2,3
Preterm Infant Need

In another study in infants with preterm births Choline was seen increase docosahexaenoic acid (DHA) in preterm infants.4

Levels of these two essential nutrients can fall rapidly after birth in preterm infants and can lead to impaired lean body mass growth of the preterm infants.4

**Choline deficiency** results in the death of cells. This can lead to poor cellular repair and regeneration.5

Eventually the body can develop fragmented hepatocytes.
Fetal Alcohol Syndrome

In studies with babies with fetal alcohol syndrome, Choline supplementation is well tolerated and showed improvement in cognitive characteristics.6,7,8 Choline levels have been shown to be decreased by alcohol exposure to adults. The net result of high demand from infant and mother’s exposure to alcohol during the pregnancy, can result in deficiency for the child.9
Fetal Alcohol Syndrome

Choline supplementation can be beneficial when given during early developmental stages in preterm and fetal alcohol syndrome babies.\textsuperscript{8}

It is needed because of lack of any FDA approved drug treatments currently available.

Choline should be considered for addition to the FDA positive list.
Prenatal Requirements:

“Choline is critical for a number of physiological processes during the prenatal period with roles in membrane biosynthesis and tissue expansion, neurotransmission and brain development, and methyl group donation and gene expression. Studies in animals and humans have shown that supplementing the maternal diet with additional choline improves several pregnancy outcomes and protects against certain neural and metabolic insults. Most pregnant women in the U.S. are not achieving choline intake recommendations of 450 mg/day and would likely benefit from boosting their choline intakes through dietary and/or supplemental approaches.” 10
Prenatal Continued:

“Choline may be especially important during pregnancy when it modulates proliferation of stem cells needed to form a normal fetus. Furthermore, it may influence brain development throughout gestation, and these influences may continue throughout the life span. Choline deficiency has been associated with liver and muscle damage and increases in homocysteine” 12

“Shaw and colleagues [Am J Epidemiol 2004;160:102–109. PubMed: 15234930] found an increased incidence of neural tube defects in women consuming less than 300 mg choline per day during pregnancy compared with women consuming more than 500 mg/d” 12
Meeting Requirements?

Choline was officially recognized as an essential nutrient by the Institute of Medicine (IOM) in 1998. There is a significant variation in the dietary requirement for choline that can be explained by common genetic polymorphisms.

Because of its wide-ranging roles in human metabolism, from cell structure to neurotransmitter synthesis, **choline-deficiency is now thought to have an impact on diseases such as liver disease, atherosclerosis and possibly neurological disorders.**
Meeting Requirements?

Choline is found in a wide variety of foods. Egg yolks are the most concentrated source of choline in the American diet, providing 680 milligrams per 100 grams.

Mean choline intakes for older children, men, women and pregnant women are far below the Adequate Intake established by the IOM. 13

“the recommendation remains the same—to increase choline intake by diet or supplementation to attenuate de novo synthesis and reduce homocysteine levels. [Am J Clin Nutr 2005;82:836–842. PubMed: 16210714] These findings may indicate a need for intervention to ensure optimal choline intakes during pregnancy.” 13
ASPEN Call for Parenteral Choline Availability

“Although choline and carnitine are not technically vitamins or trace elements, choline is an essential nutrient in all age groups, and carnitine is an essential nutrient in infants, according to the Food and Nutrition Board of the Institute of Medicine. A parenteral choline product needs to be developed and available.”

Current FDA Approved Product:

“Current parenteral nutrition sources of choline are available as egg phospholipid in approved lipid products.” [06-09-PCAC-20210609-FDA_Backgrounder.pdf]

Compounding need:

Egg allergy: Adjusted mean incidence of HEA was 1.23% (95% CI 0.98-1.51) considering possible cases among eligible children who were not challenged. Centre-specific incidence ranged from United Kingdom (2.18%, 95% CI 1.27-3.47) 14
References:


