Emerging Patterns of Metabolic Disturbance in Autism Spectrum Disorders

Robert K. Naviaux, MD, PhD
Professor of Genetics, Medicine, Pediatrics, and Pathology
Co-Director, The Mitochondrial and Metabolic Disease Center
University of California, San Diego School of Medicine
September 23, 2014
Summary

- All ASD subjects examined to date have metabolic abnormalities
- Most of the mitochondrial dysfunction found in ASD is secondary, and is not the result of single-gene Mendelian or mtDNA defects
- Redox, glutathione, and methylation disturbances are common (>50%)
  - **Special Request:** present some of Dr. Jill James work on ASD biochemistry and treatment (4 slides)
- The Cell Danger Hypothesis
- Autism-like behaviors, metabolism, and synaptic defects were corrected by APT in mouse models of ASD
- NextGen metabolomics identifies the disturbances
  - Mouse models and humans have the same core pathway abnormalities
  - Previously identified as the effector pathways of the CDR
Message from Dr. Jill James

• “Please do not place my work in the category of the ‘oxidative stress school’ (ROS cause disease)”
• We found oxidative changes in glutathione and the methionine cycle in a majority of children with ASD (2004)
• Treatment of underlying redox disturbances with methyl-B12 and folinic acid restored extracellular glutathione balance in some (2013)
• Extracellular glutathione redox improvements were correlated with behavioral benefits in our open label study (2013)
Design: Open label treatment, no placebo
65 Screened, 48 Enrolled, 37 completed
75 µg/kg methyl-B12 sq 2/wk
400 µg folinic acid PO BID x 3 months

**Clinical Study**
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

Plasma, not cells


<table>
<thead>
<tr>
<th>Vineland subscale</th>
<th>Baseline age equivalent (months) (mean ± SE)</th>
<th>Postintervention age equivalent (months) (mean ± SE)</th>
<th>Change in age equivalent (months) (mean ± 95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<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>3.2 (–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>
<tr>
<td>Overall skills</td>
<td>26.6 ± 2.3</td>
<td>34.3 ± 3.6</td>
<td>7.7 (3.4, 12.0)</td>
</tr>
</tbody>
</table>

**TABLE 1**
Comparison of methionine cycle and transsulphuration metabolites between autistic children and control children

<table>
<thead>
<tr>
<th>Control children</th>
<th>Autistic children</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n = 33)</td>
<td>(n = 20)</td>
</tr>
<tr>
<td>Methionine (µmol/L)</td>
<td>Methionine (µmol/L)</td>
</tr>
<tr>
<td>31.5 ± 5.7 (23–48)</td>
<td>19.3 ± 9.7 (15–25)</td>
</tr>
<tr>
<td>SAM (µmol/L)</td>
<td>SAM (µmol/L)</td>
</tr>
<tr>
<td>96.9 ± 12 (77–127)</td>
<td>75.8 ± 16.2 (68–100)</td>
</tr>
<tr>
<td>SAH (mmol/L)</td>
<td>SAH (mmol/L)</td>
</tr>
<tr>
<td>19.4 ± 3.4 (16–27)</td>
<td>28.9 ± 7.2 (14–41)</td>
</tr>
<tr>
<td>SAM:SAH</td>
<td>SAM:SAH</td>
</tr>
<tr>
<td>5.2 ± 1.3 (4–8)</td>
<td>2.9 ± 0.8 (2–4)</td>
</tr>
<tr>
<td>Adenosine (µmol/L)</td>
<td>Adenosine (µmol/L)</td>
</tr>
<tr>
<td>0.27 ± 0.1 (0.1–0.4)</td>
<td>0.39 ± 0.2 (0.17–0.83)</td>
</tr>
<tr>
<td>Homocysteine (µmol/L)</td>
<td>Homocysteine (µmol/L)</td>
</tr>
<tr>
<td>6.4 ± 1.3 (4.3–9.0)</td>
<td>5.8 ± 1.0 (4.0–5.8)</td>
</tr>
<tr>
<td>Cystathionine (µmol/L)</td>
<td>Cystathionine (µmol/L)</td>
</tr>
<tr>
<td>0.17 ± 0.05 (0.1–0.27)</td>
<td>0.14 ± 0.06 (0.04–0.2)</td>
</tr>
<tr>
<td>Cysteine (µmol/L)</td>
<td>Cysteine (µmol/L)</td>
</tr>
<tr>
<td>202 ± 17 (172–252)</td>
<td>163 ± 15 (133–189)</td>
</tr>
<tr>
<td>tGSH (µmol/L)</td>
<td>tGSH (µmol/L)</td>
</tr>
<tr>
<td>7.6 ± 1.4 (3.8–9.2)</td>
<td>4.1 ± 0.5 (3.3–5.2)</td>
</tr>
<tr>
<td>Oxidized glutathione (nmol/L)</td>
<td>Oxidized glutathione (nmol/L)</td>
</tr>
<tr>
<td>0.32 ± 0.1 (0.11–0.43)</td>
<td>0.55 ± 0.2 (0.29–0.97)</td>
</tr>
<tr>
<td>tGSH:GSSG</td>
<td>tGSH:GSSG</td>
</tr>
<tr>
<td>25.3 ± 8.9 (13–49)</td>
<td>8.6 ± 3.5 (4–11)</td>
</tr>
</tbody>
</table>

F(1,33) = 9.66; p <0.01

Plasma, not cells
Clinical Trials in Complex Disease—A Cautionary Tale from Mitochondrial Medicine

35 of 1,039 Clinical Trials were described in enough detail to generate a Jadad Score

How do cells “smell” safety and danger in the world? (Hint: It’s all about metabolism.)

Vertebrate Chemosensory Receptor Evolution

7 Transmembrane GPCRs

<table>
<thead>
<tr>
<th></th>
<th>Sight</th>
<th>Smell</th>
<th>Pheremones</th>
<th>Taste</th>
</tr>
</thead>
<tbody>
<tr>
<td>Opsin</td>
<td>3</td>
<td>1,037(354)</td>
<td>165(165)</td>
<td>61(148)</td>
</tr>
<tr>
<td>OR</td>
<td>4</td>
<td>388(414)</td>
<td>2(115)</td>
<td>0</td>
</tr>
</tbody>
</table>


Shi/Zhang. Results Probl Cell Diff, 2009. PMID 19145414
“Mitokine Receptors” are Like Extranasal Cellular Odorant Receptors—7 Transmembrane GPCRs

The Ligands are Traceable to Mitochondria and adapt Rapidly to Environmental Change, eg, Infection, Injury, or Toxins

Short Chain Fatty Acids

Neuropeptides

Lysophosphatidylserine

Sphingosine-1-P and LPA

Purinergic Receptors are the most widely expressed class
Metabolic Features of the CDR and Its Evolutionary Origins in the Seasons

Suramin is a Competitive Antagonist of Purinergic Signaling

(a) Suramin

(b) ATP
APT Prevented Loss of Cerebellar Purkinje Cells When Started by 2 Months of Age

Synaptosomal Ultrastructural Abnormalities in the MIA Model—Corrected by Antipurinergic Therapy

Normal Post-Synaptic Densities

Hypomorphic Post-Synaptic Densities; Electron Dense Matrix Material

Normalized Post-Synaptic Densities And Matrix

Saline-Saline Control

Poly(IC)-Saline ASD

Poly(IC)-Suramin Treated
Synaptosomal Ultrastructural Abnormalities in the Fragile X Model—Corrected by Antipurinergic Therapy

Hypomorphic Post-Synaptic Densities; Electron Dense Matrix

ASD-Like

Fragile X/Saline

Treated

Fragile X/Suramin
Social Approach Abnormalities in the MIA Model Were Corrected by Antipurinergic Therapy (APT)
Social Approach Abnormalities in the Fragile X Model Were Corrected by Antipurinergic Therapy (APT)

![Graph showing social preference (percent time with stranger) for WT-Sal, WT-Sur, KO-Sal, and KO-Sur groups. The graph indicates a decrease in social preference for KO-Sal compared to WT-Sal and an increase in social preference for KO-Sur compared to KO-Sal. The p-values for comparisons are p < 0.01 and p < 0.05.]
UCSD Metabolomics

Sample Extraction
(to remove proteins, DNA, and RNA)

Polar + Nonpolar Metabolites

Biochemical Pathway Visualization

PLS-DA and PC Analysis

Volcano Plots

Quantitation of About 700 Metabolites, 300 Drugs, Toxins, and Xenobiotics

LC-MS/MS

UHPLC

Internal Standards

20-100 µl samples of urine and blood

10-50 mg of Liquid Nitrogen Powdered Tissue
Metabolic Abnormalities in the MIA Model were Improved by Antipurinergic Therapy
Metabolic Abnormalities in the Fragile X Model Were Improved by Antipurinergic therapy
20 Different Metabolic Pathways Were Improved by APT in the Fragile X Model

Purine Metabolism Was the Top Pathway Associated With Restoration of Social Behavior

Also found in a gene expression study of human ASD by Ginsberg/Natowicz PMID 22984548—Thank you Sophia Colamarino
Metabolomics of Autism Spectrum Disorders—Pathway Abnormalities Known in Humans Were Also Seen and Corrected by Suramin in Two Animal Models

Metabolic Features of the Cell Danger Response

- Stereotyped (ciston-like) coordination of biochemical responses to environmental and genetic departures from homeostasis that threaten cell function
  - Triggered by a redistribution in electron flow, oxygen, and substrate concentrations unmatched to collective enzymatic & effector $K_m$s
  - Maintained by hyperpurinergia
Take Home Messages

• The brain controls metabolism
  – Corollary: All brain disorders have metabolic disturbances
• Cells “smell” the world through conserved chemosensory receptors that continuously monitor metabolism
• Purinergic signaling and mitokines control the cellular response to safety and danger
  – “Safety” and “Danger” are not anthropomorphic constructs
  – “Danger” is the graded mismatch between the instantaneous concentrations of substrates and effectors, and the collective Kms and Kds of the enzymes and receptors evolved by natural selection in past environments and passed on to us by our ancestors
• About a dozen core metabolic pathway disturbances were shared by the environmental MIA, the genetic Fragile X mouse model, and human ASD
Thank You

- Jane Botsford Johnson Foundation
- The UCSD Christini Fund
- Autism Speaks Trailblazer Award
- Wright Family Foundation
- Lennox Foundation
- MRSII Demonstration Grant Program

[PII redacted]
1996-1998
Species and Cellular Survival and Persistence States, Hypometabolism, and the Cell Danger Response (CDR)

**Stress Conditions**
- Tardigrade tun state
- Nematode dauer & diapause
- Memory T-cells
- Mammalian embryo diapause
- Oocyte and egg cell metabolism
- Plant seed metabolism
- Hummingbird torpor
- Hibernation
- Estivation
- ?Autism

**Shared Metabolic Features**
- Decreased basal oxygen consumption
- Increased glycolysis
- Oxidize glutathione
- Decreased heat production
- Decreased fatty acid oxidation
- Intracellular lipid accumulation
- Increased mitochondrial coupling
- Increased mitochondrial reserve capacity
- Increased vitamin-independent methionine synthesis
  - Increased Betaine-Homocysteine Methyltransferase (BHMT) expression
- Increased capacity for ROS production
  - Increased SOD, GSH peroxidase
- Increased ATP turnover
  - **Hypothesis**: Hyperpurinergia maintains the abnormal metabolism and behavior
Understanding the Cell Danger Response: Follow the Electrons.....

An Archetypal Stressor: a Virus

Electron Steal Drives Rapid Mitochondrial Redox Change

0. Decrease oxygen consumption → increase dissolved O₂ concentration
1. Shift from polymer to monomer synthesis (ΔG)
2. Stiffen cell membranes
3. Release anti-viral and anti-microbial chemicals
4. Increase mitochondrial fission and autophagy
5. Change DNA methylation
6. Mobilize endogenous retroviruses and LINEs
7. Warn neighboring cells and call in effector cells—the “purinergic halo”
8. Alter host behavior to prevent spread of disease to kin

The Long Road to Purinergic Signaling in Autism Spectrum Disorders—1929-Present

Mitochondrial Disease ≠ Autism Spectrum Disorders
Cell Persistence Strategies Across Species—Thinking “Analogically”

**Stress Response**
- Stop eating
- Mitochondrial oxphos declines
- Oxygen consumption declines
- Heat production declines
- Lipid droplets accumulate

**Tardigrades**
("Water Bears")

Desiccation, heat stress, etc

Add water + carbs

Tun State

Resistant to: Drying, heating, Freezing, radiation, Toxins
Reproductive Cycle = 2.5 days
Menopause After 6 days
Normal life span = 13 days

C. elegans

Dauer Shifts
- Stop eating
- Mitochondrial oxphos declines
- Oxygen consumption declines
- Lipid droplets accumulate
- Glycolysis increases
- Glyoxylate shunt increases to increase OAA and gluconeogenesis

Lipid Droplets
In this review I report evidence that the mainstream field of oxidative damage biology has been running fast in the wrong direction for more than 50 years.

"Oxidative stress is not the prime cause of chronic disease. The prime cause of chronic disease may be the pathological persistence of the cell danger response—the evolutionarily conserved process that generates the metabolic features (biochemical symptoms) that protect the cell acutely from viral attack and homeostatic threats, but can persist chronically, causing disease."
Antipurinergic Therapy Corrects the Autism-Like Features in the Poly(IC) Mouse Model

Robert K. Naviaux¹,²,³,⁴*, Zarazuela Zolkipli¹,⁵, Lin Wang¹,², Tomohiro Nakayama¹,⁵, Jane C. Naviaux¹,⁶, Thuy P. Le¹,³, Michael A. Schuchbauer⁶, Mihael Rogac¹,²*, Qingbo Tang², Laura L. Dugan², Susan B. Powell⁶

ORIGINAL ARTICLE
Reversal of autism-like behaviors and metabolism in adult mice with single-dose antipurinergic therapy

JC Naviaux¹, MA Schuchbauer¹, K Li²,³, L Wang²,³, VB Risbrough¹,⁴, SB Powell¹ and RK Naviaux²,³,⁴,⁵,⁶
The MIA Mouse Model of ASD has Relative Hypothermia—Corrected by Antipurinergic Therapy

![Graph showing temperature changes over age with different treatments.](image)
The Fragile X Mouse Model of ASD has Relative Hypothermia—Corrected by Antipurinergic Therapy

Decreased Normalized (0.7°C)
Modified from Will Alaynick, 2014
<table>
<thead>
<tr>
<th>Feature</th>
<th>Dauer</th>
<th>Autism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decreased oxygen consumption</td>
<td>•</td>
<td>•</td>
</tr>
<tr>
<td>Decreased heat production</td>
<td>•</td>
<td>•</td>
</tr>
<tr>
<td>Decreased Fatty acid oxidation</td>
<td>•</td>
<td>•</td>
</tr>
<tr>
<td>Intracellular lipid accumulation</td>
<td>•</td>
<td>•</td>
</tr>
<tr>
<td>Increased mitochondrial coupling</td>
<td>•</td>
<td>•</td>
</tr>
<tr>
<td>Increase mitochondrial reserve capacity</td>
<td>•</td>
<td>•</td>
</tr>
<tr>
<td>Increased ATP turnover</td>
<td>•</td>
<td>•</td>
</tr>
</tbody>
</table>
2000—First Hints of a Mitochondrial DNA Connection to Autism—Compensatory Overfunction

**Autism Associated With the Mitochondrial DNA G8363A Transfer RNA<sup>Lys</sup> Mutation**

William D. Graf, MD; Jose Marin-Garcia, MD; H.G. Gao, MD; Senia Pizzo, PhD; Robert K. Naviaux, MD, PhD; David Markusic, BS; Bruce A. Barshop, MD, PhD; Eric Courchesne, PhD; Richard H. Haas, MB, BChir

**Table 1. Summary of Clinical, Morphologic, Biochemical, and Genetic Findings in the Family Described**

<table>
<thead>
<tr>
<th></th>
<th>II-1</th>
<th>II-2</th>
<th>I-1</th>
<th>II-3</th>
<th>II-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>II</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Irritable bowel</td>
<td>-</td>
<td>++</td>
<td>-</td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td>Epilepsy</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Learning disability</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cognitive regression</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Leig syndrome</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Autism</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Brain Magnetic Resonance Imaging</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Abnormal</td>
<td>Normal</td>
</tr>
<tr>
<td>Muscle analysis</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>Histology</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>Histochemistry</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>Biochemistry</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>G3863A mitochondrial DNA mutation</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>PCR analysis in blood</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Percent in blood</td>
<td>ND</td>
<td>ND</td>
<td>28%</td>
<td>62%</td>
<td>60%</td>
</tr>
<tr>
<td>Percent in muscle</td>
<td>ND</td>
<td>ND</td>
<td>86%</td>
<td>61%</td>
<td>61%</td>
</tr>
</tbody>
</table>

- = absent; + = mild; ++ = moderate; +++ = severe; ND = not determined; ↑ = increased, ↓ = decreased; C = citrate synthase corrected respiratory chain complex activity; COX- = absence of cytochrome c oxidase staining; PCR = polymerase chain reaction.

**Proband**
Leigh Syndrome

"Definite" Mitochondrial Disease

"Unlikely" Mitochondrial Disease (Without mtDNA)

Regression: 18-24 mos
ASD Dx at 4.3 yrs
ADOS = 20
CARS = 51.5

CSF Lactate 31 mg/dl 12 mg/dl

250%

24 mos
Elevated Brain Lactate

8 of 41 Adults (19-60 yrs) with ASD = 20% (95% CI = 9-35%)

2 of 34 Children (5-18 yrs) with ASD = 6% (95% CI = 0.7-20%)

Affected Voxels: Variable between subjects; Cingulate gyrus most commonly.
Pertinent Negative: Rarely in the basal ganglia

Unifying Observation: Brain lactate elevation can be either genetic or environmental
1969—The First Reported Case of Purine-Associated Autism

William Nyhan
UCSD 2012

  - Disorder of \textit{de novo} purine synthesis
- 3 year old boy with autism, hyperuricemia, hypospadius, and hearing loss
- Parasympathetic defect/Dysautonomia
  - Congenitally absent tear glands & ducts
  - Methacholine-insensitive pupillary response
- Phosphoribosyl Pyrophosphate Synthase (PRPPS) Superactivity
  - G547C/D182H—resistant to feedback inhibition by ADP and ATP
  - Increased ca 600%
- Resulted in excess ATP, ADP, GTP, and IMP synthesis by \textit{de novo} purine synthesis
- Treated with allopurinol and hearing aids with reduction in autistic symptoms

Fig. 1. S. M. at 3 years of age. His general appearance and characteristic odd grin are illustrated.
Ho: Poor dietary choline consumption $\rightarrow$ low choline and betaine (TMG) in ASD $\rightarrow$ worsened oxidative stress.

H1: The Cell Danger Response lowers choline and betaine to prevent DNA and RNA synthesis, increase the ratio of sphingo/phospholipid synthesis to stiffen membranes, and alters behavior and gut absorption to decrease dietary choline intake.

Treatment Implications
Ho: choline supplementation
H1: choline returns spontaneously after turning off the CDR
The Maternal Immune Activation (MIA) Model—The Autism-Schizophrenia Spectrum

Pregnant C57BL/6J Mice

Poly(I:C) E12.5

8-Week Old Offspring

Purinergic Antagonist (eg, Suramin)

Saline

Behavioral Testing, Molecular Studies, Metabolomics
What About the Microbiome?
Why Does Metabolomics Work for Autism and Neurologic Disease?

“The brain controls metabolism.”
(Through the autonomic nervous and endocrine systems.)

--All brain disorders produce a signature of abnormalities that can be detected in the blood and other biofluids.
The Work Capacity of the Cell is Set by the Thermodynamic Gradients Created by Mitochondrial Oxygen Consumption.
Figure 1. The Connection Between Mitochondrial Folate Metabolism and Nuclear DNA Methylation. In embryonic cells and cancer, MBE is expressed and one-carbon units are efficiently converted to Formyl-THF and formate for cytosolic nucleotide synthesis. Under these conditions, fewer one-carbon units are available for SAM synthesis and DNA methylation. When MBE is turned off in differentiated cells, less mitochondrial formate is produced and one-carbon units are directed through Methylenetetrahydrofolate reductase and MTHF. 1—NAD+ Dependent Methylenetetrahydrofolate Reductase, 2—Methylenetetrahydrofolate Cyclohydrolase, 3—Folic Acid Hydrolase, FTS can reverse directions in differentiated cells when MBE is turned off. 4—Mitochondrial Serine Hydroxymethyltransferase (mSHMT). 5—Dimethylglycine Dehydrogenase, ETF—Electron Transfer Flavoprotein, 6—Sarcosine Dehydrogenase, 7—Glycine Cleavage System, 8—Methylenetetrahydrofolate Reductase (MFR), 9—Thymidylate Synthase, 10—Dihydrofolate Reductase (DHFR). 11—Cytosolic Serine Hydroxymethyltransferase (cSHMT), 11’—cSHMT reverse reaction, 12/13/14—Cytosolic Tri-functional Enzyme, 12—Folic Acid Hydrolase, 12’—Methylenetetrahydrofolate Cyclohydrolase, 12’—NADPH-dependent Methylenetetrahydrofolate Dehydrogenase, 15—Formyltetrahydrofolate Dehydrogenase, 16—Homocysteine Methyltransferase (Methionine Synthase), 17—Methionine Adenosyltransferase (MAT). 18—Multiple DNA, RNA, Protein, and other Methyltransferase reactions in the nucleus, cytosol, and mitochondria. 19—S-Adenosyl Homocysteine Hydrolase (SAHH), 20—Cystathionine (l-Synthase (CBS), 21—Cystathionase, 22—. Glutamylcysteine Synthetase (GCS), 23—Glutathione Synthase, 24—Nucleoside Diphosphate Kinase, 25—ATP Synthase (Complex V), 26—Propionyl CoA Carboxylase, 27—Methylmalonyl CoA Mutase, GAR—Glycaminidase Ribonucleotide, AICAR—Aminomimidazole Carboxamidine Ribonucleotide, FAICAR—Formiminimidazole Carboxamidine Ribonucleotide, Addo—Adenosine.

Naviaux RK. Cancer Biol Ther, 2008. PMID 18719362