Oxidative Stress Induced Mitochondrial Dysfunction in Children with Autism Spectrum Disorder

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Director of Autism Multispecialty Clinic
Arkansas Children’s Hospital
Associate Professor of Pediatrics
University of Arkansas for Medical Sciences
Acquired Mitochondrial Disorders in Children with Autism Spectrum Disorder
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Note: farther from the center is more impaired.

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Acquired Mitochondrial Disorders in Children with Autism Spectrum Disorder

Age of Onset

Autistic Disorder and Pervasive Development Disorder
- By Definition (DSM-IV/ICD-10) – Before 36 Months – Three patterns
  - 33% Regression from normal development
    - Usually Between 12 and 24 months
  - 33% Symptoms from Early Infancy
  - 33% Symptoms obvious after 1 year old – developmental plateau
- Onset after 36 months – other diagnosis
- Regression after 36 months Childhood Disintegrative Disorder

Asperger’s Syndrome
- No Age Criteria for diagnosis
- Typically not diagnosed until later childhood because less obvious when language development is normal.
Early Behavior in Children who Later Developed Autism

• Abnormalities the differentiate autism from both developmental delay and typically developing children are primarily considered social behaviors and include

  • Responding to Name
  • Looking at other people
  • Showing objects
  • Joint Attention (Pointing and Following a Point)
  • Decreased Social Interactions – Playing Peek-a-boo
  • Looking at others – looking for parents
Acquired Mitochondrial Disorders in Children with Autism Spectrum Disorder

**Pointing**

- Starts Around 8-10 Months
- Majority of Gestures at 12 Months

**Protoimperative**
- Desired Object
- Impaired in younger ASD
- May develop in older ASD

**Protodeclarative**
- Deficient in ASD
- Shared Experience
- Joint Attention
Protodeclarative Gestures

• Start Around 8-10 Months
• Pointing
• Showing: Extending arms holding object towards someone’s face to share interest
• Giving: Placing an object in someone’s hand to share object of interest with them (should not be confused with giving object in order for someone to do something necessary to fulfill child’s need)
Causes of Autism
The Etiology of Autism: More than Genetic Disorders

<table>
<thead>
<tr>
<th>Genetic Abnormality</th>
<th>Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytogenetic Abnormalities</td>
<td>5%</td>
</tr>
<tr>
<td>Fragile X</td>
<td>5%</td>
</tr>
<tr>
<td>Rett Syndrome (Females only)</td>
<td>5% (~1% overall)</td>
</tr>
<tr>
<td>Chromosomal Microarray</td>
<td>10%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>21%</strong></td>
</tr>
</tbody>
</table>

This leaves about 79%+ children with ASD without an identified genetic diagnosis.

(Schaefer and Mendelson, Genetics in Medicine, 2008)
Inherited Metabolic Disorders – Mostly Case Reports
Mitochondrial Disease Cases (~25%)
Pyrimidine and Purine metabolism: Dihydropyrimidinase deficiency, Phosphoribosylpyrophosphate synthetase superactivity, Adenylosuccinate lyase deficiency
Disorders of γ-aminobutyric acid metabolism: Succinic semialdehyde dehydrogenase deficiency
Carnitine Biosynthesis: 6-N-trimethyllysine dioxygenase deficiency
Disorders of amino acid metabolism: Phenylketonuria, Histidinemia Branched Chain Ketoacid Dehydrogenase Kinase Deficiency
Disorders of Cholesterol Metabolism: Smith–Lemli–Opitz Syndrome
Disorders of creatine metabolism
Sulfation defects
Biotinidase deficiency
Urea Cycle Defects: Ornithine transcarbamylase deficiency, Citrullinemia, Argininosuccinic aciduria, Carbamoyl phosphate synthetase deficiency
Lysosomal Storage Disease: Sanfilippo syndrome, Infantile ceroid lipofuscinosis

Schaefer and Mendelson, Genetics in Medicine, 2013
## Acquired Mitochondrial Disorders in Children with Autism Spectrum Disorder

### Non-inherited Metabolic Conditions Associated with Autism

<table>
<thead>
<tr>
<th>Mitochondrial Disorders</th>
<th>Redox Abnormalities</th>
<th>Folate Abnormalities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mitochondrial Disease with no genetic abnormalities (75%)</td>
<td>Decreased reduced Glutathione &amp; Cysteine</td>
<td>Cerebral Folate Insufficiency</td>
</tr>
<tr>
<td>Electron Transport Chain Deficiencies in Immune Cells and Brain Tissue ETC Complex I and IV overactivity</td>
<td>Reduced Glutathione Peroxidase function</td>
<td>Autoantibodies to Folate Receptor α</td>
</tr>
<tr>
<td>Acyl-carnitine Elevations</td>
<td>Increased oxidized Glutathione, DNA, Proteins and Lipids</td>
<td>Mitochondrial Disease/Dysfunction</td>
</tr>
</tbody>
</table>

### Genetics Disorders Associated with ASD & Metabolic Abnormalities

<table>
<thead>
<tr>
<th>Mitochondrial Disorders</th>
<th>Redox-Folate Metabolism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rett syndrome</td>
<td>Rett syndrome</td>
</tr>
<tr>
<td>Down syndrome</td>
<td>Down syndrome</td>
</tr>
<tr>
<td>PTEN mutations</td>
<td>Phenylketonuria</td>
</tr>
<tr>
<td>15q11-q13 duplication</td>
<td></td>
</tr>
<tr>
<td>Angelman syndrome</td>
<td></td>
</tr>
<tr>
<td>Septo-optic dysplasia</td>
<td></td>
</tr>
</tbody>
</table>
Acquired Mitochondrial Disorders in Children with Autism Spectrum Disorder

A review of research trends in physiological abnormalities in autism spectrum disorders: immune dysregulation, inflammation, oxidative stress, mitochondrial dysfunction and environmental toxicant exposures

DA Rossignol¹ and RE Frye²

¹International Child Development Resource Center, Melbourne, FL, USA and ²Arkansas Children’s Hospital Research Institute, University of Arkansas for Medical Sciences, Little Rock, AR, USA
Acquired Mitochondrial Disorders in Children with Autism Spectrum Disorder

ARCHIVES OF GENERAL PSYCHIATRY

ONLINE FIRST  July 2011

Genetic Heritability and Shared Environmental Factors Among Twin Pairs With Autism

Joachim Hallmayer, MD; Sue Cleveland, BS; Andrea Torres, MA; Jennifer Phillips, PhD; Brianne Cohen, BA; Tiffany Torigoe, BA; Janet Miller, PhD; Angie Fedele, BA; Jack Collins, MBA; Karen Smith, BS; Linda Lotspeich, MD; Lisa A. Croen, PhD; Sally Ozonoff, PhD; Clara Lajonchere, PhD; Judith K. Grether, PhD; Neil Risch, PhD

Objective: To provide rigorous quantitative estimates of genetic heritability and effects of shared environment

Conclusion: Susceptibility to ASD has moderate genetic heritability (38%) and a substantial shared twin environmental component (58%)
New Understanding of Autism

- Autism is defined as a collection of symptoms.
- Symptoms of autism are associated with underlying medical disorders in many cases.
- In many cases, autism is a multisystemic disorder with primary neurological manifestations.
- The rise in Autism cases is probably due to complex interactions between genetics, environment and the dynamics of physiological development.
The Mitochondria And Autism
Mitochondrial disease

- Relatively new field

- First disease described in 1988
  - Wallace, Leber’s hereditary optic neuropathy, published in Science
  - Holt, Mitochondrial Myopathy, published in Nature

- Usually defined by extremely clinical symptoms with a progressive course
  - High energy dependent tissues
  - Neurological Disease
  - Gastrointestinal Disease
  - Immune Dysfunction

- Not just powerhouse, also important in
  - programmed (apoptotic) cell death
  - Oxygen Radical Regulation
Acquired Mitochondrial Disorders in Children with Autism Spectrum Disorder
Acquired Mitochondrial Disorders in Children with Autism Spectrum Disorder
### Acquired Mitochondrial Disorders in Children with Autism Spectrum Disorder

<table>
<thead>
<tr>
<th>Respiratory chain complex</th>
<th>nDNA subunits</th>
<th>mtDNA subunits</th>
<th>Redox cofactors</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (EC 1.6.6.3)</td>
<td>38</td>
<td>7</td>
<td>FMN, [Fe–S] centres, ubiquinones</td>
</tr>
<tr>
<td>II (EC 1.3.5.1)</td>
<td>0</td>
<td>4</td>
<td>FAD, [Fe–S] centres, cytochrome $b_{560}$</td>
</tr>
<tr>
<td>III (EC 1.10.2.2)</td>
<td>10</td>
<td>1</td>
<td>Cytochromes b and c1, Rieske protein</td>
</tr>
<tr>
<td>IV (EC 1.9.3.1)</td>
<td>10</td>
<td>3</td>
<td>[Cu$_a$] centre, [Cu$_b$-haem a3] centre</td>
</tr>
<tr>
<td>V (EC 3.6.1.34)</td>
<td>14</td>
<td>2</td>
<td>None</td>
</tr>
</tbody>
</table>

[Diagram of mitochondrial genome with mutations and markers.](http://www.mitomap.org/MITOMAP/mitomapgenome.pdf)
Mitochondrial dysfunction in autism spectrum disorders: a systematic review and meta-analysis

DA Rossignol and RE Frye

Table 1 Pooled prevalence estimates for MD in ASD and for abnormal biomarkers of mitochondrial dysfunction in ASD

<table>
<thead>
<tr>
<th>General ASD population</th>
<th>Studies</th>
<th>Total N</th>
<th>Overall prevalence</th>
<th>Minimum (%)</th>
<th>Maximum (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mitochondrial disease in ASD</td>
<td>3</td>
<td>536</td>
<td>5.0% (3.2%, 6.9%)</td>
<td>3.6</td>
<td>9.1</td>
</tr>
<tr>
<td>Elevated lactate</td>
<td>6</td>
<td>479</td>
<td>31.1% (27.0%, 35.3%)</td>
<td>17</td>
<td>77</td>
</tr>
<tr>
<td>Elevated pyruvate</td>
<td>2</td>
<td>110</td>
<td>13.6% (7.2%, 20.1%)</td>
<td>8</td>
<td>30</td>
</tr>
<tr>
<td>Elevated lactate/pyruvate ratio</td>
<td>1</td>
<td>192</td>
<td>27.6% (21.2%, 33.9%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elevated alanine</td>
<td>1</td>
<td>36</td>
<td>8.3% (0.0%, 20.1%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low total carnitine</td>
<td>1</td>
<td>30</td>
<td>90.0% (81.0%, 99.0%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elevated creatine kinase</td>
<td>1</td>
<td>47</td>
<td>46.8% (32.4%, 61.2%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elevated ammonia</td>
<td>1</td>
<td>80</td>
<td>35.0% (24.5%, 45.5%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elevated AST</td>
<td>1</td>
<td>147</td>
<td>45.6% (37.5%, 53.7%)a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elevated ALT</td>
<td>1</td>
<td>87</td>
<td>7.0% (0.5%, 13.5%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Mitochondrial dysfunction in autism spectrum disorders: a systematic review and meta-analysis

DA Rossignol¹ and RE Frye²

Table 2  Pooled statistics and meta-analysis of group differences for mitochondrial biomarkers in ASD compared with controls

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Number of studies</th>
<th>ASD</th>
<th>Control</th>
<th>F-value</th>
<th>Hedge's g (CI)</th>
<th>Q for g</th>
<th>Glass's A (CI)</th>
<th>Q for A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactate (mM⁻¹)</td>
<td>5</td>
<td>114</td>
<td>0.91 (0.87, 0.96)</td>
<td>8.72*</td>
<td>1.42 (0.92, 1.92)*</td>
<td>3.64</td>
<td>3.22 (2.66, 3.78)*</td>
<td>45.89*</td>
</tr>
<tr>
<td>Pyruvate (mM⁻¹)</td>
<td>1</td>
<td>24</td>
<td>0.06 (0.06, 0.06)</td>
<td>20.25*</td>
<td>1.96 (0.85, 3.08)*</td>
<td>6.40</td>
<td>(5.04, 7.76)*</td>
<td></td>
</tr>
<tr>
<td>Carnitine (mg ml⁻¹)</td>
<td>1</td>
<td>30</td>
<td>6.40 (6.22, 6.62)</td>
<td>4.61*</td>
<td>2.51 (1.61, 3.42)*</td>
<td>4.21</td>
<td>(3.01, 5.41)*</td>
<td></td>
</tr>
<tr>
<td>Ubiquinone</td>
<td>1</td>
<td>15</td>
<td>144.2 (130.4, 161.1)</td>
<td>2.13</td>
<td>1.90 (0.79, 3.01)*</td>
<td>1.63</td>
<td>(0.62, 2.64)*</td>
<td></td>
</tr>
<tr>
<td>Creatine kinase</td>
<td>2</td>
<td>55</td>
<td>92.2 (89.9, 121.9)</td>
<td>6.93*</td>
<td>0.57 (–0.15, 1.30)</td>
<td>0.05</td>
<td>0.94 (0.19, 1.69)</td>
<td>0.69</td>
</tr>
<tr>
<td>AST</td>
<td>1</td>
<td>147</td>
<td>29.7 (28.1, 31.7)*</td>
<td>2.34*</td>
<td>0.49 (–0.22, 1.32)</td>
<td>0.67</td>
<td>(–0.07, 1.41)</td>
<td></td>
</tr>
<tr>
<td>ALT</td>
<td>1</td>
<td>87</td>
<td>20.6 (18.7, 23.1)</td>
<td>7.37*</td>
<td>0.18 (–0.61, 0.97)</td>
<td>0.38</td>
<td>(–0.42, 1.19)</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: ALT, alanine aminotransferase; ASD, autism spectrum disorder; AST, aspartate aminotransferase; CI, confidence interval.

*Standard error of AST was misstated in the publication as 3.0 but in actuality it is 1.0.

*P<0.0001.
A review of metabolic studies from 133 consecutive patients evaluated in a medically-based autism clinic

Examined a wide range of metabolic markers in children with autism including markers of fatty-acid oxidation disorders
Acquired Mitochondrial Disorders in Children with Autism Spectrum Disorder

6 Biomarkers Reviewed
3 Groups with high prevalence Identified
Lactate, Alanine-to-Lysine & Acyl-Carnitine  55.6%

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Total Tested</th>
<th>Abnormal at Least Once</th>
<th>Patients with Abnormalities Tested Twice</th>
<th>Abnormal Twice</th>
<th>Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactate</td>
<td>96</td>
<td>34 (35%)</td>
<td>20 (59%)</td>
<td>9 (45%)</td>
<td>15.9%</td>
</tr>
<tr>
<td>Alanine</td>
<td>94</td>
<td>8 (9%)</td>
<td>5 (63%)</td>
<td>1 (20%)</td>
<td>1.7%</td>
</tr>
<tr>
<td>AST</td>
<td>113</td>
<td>20 (18%)</td>
<td>14 (70%)</td>
<td>8 (57%)</td>
<td>10.1%</td>
</tr>
<tr>
<td>CK</td>
<td>81</td>
<td>11 (14%)</td>
<td>4 (36%)</td>
<td>2 (50%)</td>
<td>6.8%</td>
</tr>
<tr>
<td>Alanine-to-Lysine Ratio</td>
<td>98</td>
<td>39 (40%)</td>
<td>20 (51%)</td>
<td>8 (40%)</td>
<td>15.9%</td>
</tr>
<tr>
<td>Acyl-carnitine</td>
<td>58</td>
<td>23 (40%)</td>
<td>10 (44%)</td>
<td>6 (60%)</td>
<td>23.8%</td>
</tr>
</tbody>
</table>

Acyl-Carnitine Group Had Rate of Regression of 67%
VERY HIGH

<table>
<thead>
<tr>
<th></th>
<th>Regression</th>
<th>Epilepsy</th>
</tr>
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<tbody>
<tr>
<td>Lactate (n=9)</td>
<td>2 (22%)</td>
<td>3 (33%)</td>
</tr>
<tr>
<td>AST (n=8)</td>
<td>3 (38%)</td>
<td>1 (13%)</td>
</tr>
<tr>
<td>Alanine-to-Lysine Ratio (n=8)</td>
<td>2 (25%)</td>
<td>6 (75%)</td>
</tr>
<tr>
<td>Acyl-carnitine (n=6)</td>
<td>4 (67%)</td>
<td>1 (17%)</td>
</tr>
<tr>
<td>ASD Control (n=9)</td>
<td>5 (55%)</td>
<td>3 (33%)</td>
</tr>
</tbody>
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Mitochondrial dysfunction in autism spectrum disorders: a systematic review and meta-analysis

DA Rossignol\textsuperscript{1} and RE Frye\textsuperscript{2}

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</table>
The Oxidative Stress
And
Autism
Acquired Mitochondrial Disorders in Children with Autism Spectrum Disorder

- **Endogenous sources**
  - Mitochondria
  - Peroxisomes
  - Lipoxigenases
  - NADPH oxidase
  - Cytochrome P450

- **Antioxidant defences**
  - Enzymatic systems
    - CAT, SOD, GPx
  - Non-enzymatic systems
    - Glutathione
    - Vitamins (A, C, and E)

- **Exogenous sources**
  - Ultraviolet light
  - Ionizing radiation
  - Chemotherapeutics
  - Inflammatory cytokines
  - Environmental toxins

- **Impaired physiological function**
  - Decreased proliferative response
  - Defective host defences

- **Homeostasis**
  - Normal growth and metabolism

- **Impaired physiological function**
  - Random cellular damage
  - Specific signalling pathways

- **Ageing**
  - Disease
  - Cell death
Acquired Mitochondrial Disorders in Children with Autism Spectrum Disorder
Imbalance in the Equilibrium

- Equilibrium ($\text{AOX} = \text{ROS}$)
- Oxidative stress (Excess ROS)
- Oxidative stress (Depleted AOX)

Antioxidants
Oxidants
Acquired Mitochondrial Disorders in Children with Autism Spectrum Disorder
Oxidative Stress can weaken mitochondrial Function and cause programmed cell death.
Acquired Mitochondrial Disorders in Children with Autism Spectrum Disorder

Folic Acid

DHFR

5,10-CH₂THF

MTHFR

5-CH₃THF

Purines and Thymidylate

DNA SYNTHESIS

PROLIFERATION

Methionine

SAM

METHYLATION

Cellular Methylation Reactions

EPIGENETICS

B12

SAH

Homocysteine

Cystathionine

Cysteine

GSH ↔ GSSG

REDOX HOMEOSTASIS

OXIDATIVE STRESS

Use with permission of S. Jill James, Ph.D.
# Acquired Mitochondrial Disorders in Children with Autism Spectrum Disorder

## Metabolic Imbalance Associated with Methylation Dysregulation and Oxidative Damage in Children with Autism

**Stepan Melnyk • George J. Fuchs • Eldon Schulz • Maya Lopez • Stephen G. Kahler • Jill J. Fussell • Jayne Bellando • Oleksandra Pavliv • Shannon Rose • Lisa Seidel • David W. Gaylor • S. Jill James**

<table>
<thead>
<tr>
<th>Plasma metabolites</th>
<th>Cases (n = 40)</th>
<th>Paired sibling (n = 40)</th>
<th>p Value*</th>
<th>Controls (n = 54)</th>
<th>p Value**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methionine (μmol/L)</td>
<td>19.8 ± 2.6 a</td>
<td>22.3 ± 4.3</td>
<td>&lt;0.001</td>
<td>23.3 ± 3.9</td>
<td>ns</td>
</tr>
<tr>
<td>SAM (nmol/L)</td>
<td>61.6 ± 8.9 a</td>
<td>70.7 ± 19.4</td>
<td>&lt;0.006</td>
<td>71.0 ± 15.6</td>
<td>ns</td>
</tr>
<tr>
<td>SAH (nmol/L)</td>
<td>20.0 ± 4.6 a</td>
<td>16.9 ± 3.9</td>
<td>&lt;0.001</td>
<td>14.8 ± 4.1</td>
<td>0.05</td>
</tr>
<tr>
<td>Adenosine (μmol/L)</td>
<td>0.16 ± 0.07</td>
<td>0.11 ± 0.05</td>
<td>&lt;0.001</td>
<td>0.14 ± 0.07</td>
<td>ns</td>
</tr>
<tr>
<td>Homocysteine (μmol/L)</td>
<td>4.86 ± 1.5</td>
<td>4.69 ± 1.0</td>
<td>ns</td>
<td>4.68 ± 1.0</td>
<td>ns</td>
</tr>
<tr>
<td>Folate (ng/ml)</td>
<td>19.9 ± 5.1</td>
<td>21.6 ± 4.1</td>
<td>ns</td>
<td>19.4 ± 4.2</td>
<td>ns</td>
</tr>
<tr>
<td>B12 (pg/ml)</td>
<td>872 ± 528</td>
<td>719 ± 288</td>
<td>ns</td>
<td>864 ± 552</td>
<td>ns</td>
</tr>
<tr>
<td>SAM/SAH</td>
<td>3.29 ± 1.1 a</td>
<td>4.4 ± 1.7</td>
<td>&lt;0.001</td>
<td>5.08 ± 1.8</td>
<td>ns</td>
</tr>
<tr>
<td>DNA methylation (%5mC)</td>
<td>3.03 ± 0.8 a</td>
<td>3.9 ± 0.7</td>
<td>&lt;0.001</td>
<td>4.13 ± 1.0</td>
<td>ns</td>
</tr>
<tr>
<td>Total Cysteine (μmol/L)</td>
<td>189 ± 21 a</td>
<td>203 ± 26</td>
<td>&lt;0.002</td>
<td>212 ± 18</td>
<td>ns</td>
</tr>
<tr>
<td>Free Cysteine (μmol/L)</td>
<td>21.6 ± 6.45</td>
<td>22.5 ± 5.0</td>
<td>ns</td>
<td>23.6 ± 5.3</td>
<td>ns</td>
</tr>
<tr>
<td>Free Cystine (μmol/L)</td>
<td>34.1 ± 7.5 a</td>
<td>27.1 ± 8.7</td>
<td>&lt;0.001</td>
<td>26.4 ± 5.7</td>
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<td>Free Cysteine/Cystine</td>
<td>0.68 ± 0.25 a</td>
<td>0.89 ± 0.25</td>
<td>&lt;0.001</td>
<td>0.93 ± 0.27</td>
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<td>GSH (μmol/L)</td>
<td>1.84 ± 0.40 a</td>
<td>2.06 ± 0.41</td>
<td>&lt;0.001</td>
<td>2.58 ± 0.79</td>
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<td>GSSG (μmol/L)</td>
<td>0.23 ± 0.10 a</td>
<td>0.15 ± 0.08</td>
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<td>GSH/GSSG</td>
<td>9.45 ± 4.08 a</td>
<td>17.4 ± 10.3</td>
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<td>% Oxidized GSH (2GSSG/(GSH + 2GSSG))</td>
<td>22 ± 8.1 a</td>
<td>12.7 ± 5.9</td>
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<td>3-Nitrotyrosine (nmol/L)</td>
<td>143 ± 74 a</td>
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<td>3-Chlorotyrosine (nmol/L)</td>
<td>51 ± 18 a</td>
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<td>8-Oxo- deoxyguanosine (pmol/mg DNA)</td>
<td>95 ± 35 a</td>
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<td>63 ± 24</td>
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Acquired Mitochondrial Disorders in Children with Autism Spectrum Disorder

**INCREASED PRO-OXIDANTS**

- Endogenous
- Exogenous (Environmental factors)
  - NO
  - Xanthine oxidase
  - Homocysteine
  - Heavy metals (Hg, Pb)
  - Thalidomide, Valproic acid, Retinoic acid
  - Air pollutants
  - Chemicals and Toxins
  - Pathogenic bacteria
  - Viral infection

**DECREASED ANTI-OXIDANTS**

- Antioxidant enzymes (SOD, GPx, catalase)
- Glutathione
  - Ceruloplasmin
  - Transferrin
  - Abnormal Cu/Fe metabolism

↑ Production of free radicals

- OXIDATIVE STRESS IN AUTISM

- Genetic factors
  - ↑ Lipid peroxidation
  - ↑ Protein oxidation
  - ↑ DNA oxidation

- Mitochondrial damage
  - Impaired energy production
  - Increased excitotoxicity
Redox metabolism abnormalities in autistic children associated with mitochondrial disease

RE Frye\textsuperscript{1,2}, R DeLaTorre\textsuperscript{3}, H Taylor\textsuperscript{4}, J Slattery\textsuperscript{1,2}, S Melnyk\textsuperscript{1,2}, N Chowdhury\textsuperscript{1} and SJ James\textsuperscript{1,2}
Acquired Mitochondrial Dysfunction In Autism
Seahorse Bioscience XF96 Extracellular Flux Analyzer for 96-well microplate assays
Seahorse Extracellular Flux Analysis

• Simultaneously quantify mitochondrial respiration and glycolysis in real time

• Bioenergetic Profile
  – Measure the basal respiration rate of cells
  – Compounds modulating mitochondrial function are added sequentially
  – The effect on oxygen consumption rate (OCR) measured after each compound addition
  – Reveals the four fundamental parameters of mitochondrial function: basal respiration, ATP turnover, proton leak, and maximal respiratory capacity
• Oligomycin (ATP coupler)
  – Inhibits ATP synthesis by blocking Complex V
  – Reveals the % OCR devoted ATP synthesis vs the % OCR to overcome proton leak

• FCCP (ETC accelerator)
  – Uncoupler: collapses mito membrane potential
  – Results in maximal uncontrolled OCR
  – Allows calculation of spare respiratory capacity (Max-Basal)

• Rotenone: Complex I inhibitor and

• Antimycin A: Complex III inhibitor
  – Combo shuts down mito respiration and enables mitochondrial and non-mitochondrial factors contributing to respiration to be calculated
Acquired Mitochondrial Disorders in Children with Autism Spectrum Disorder
Acquired Mitochondrial Disorders in Children with Autism Spectrum Disorder
Oxidative Stress Induces Mitochondrial Dysfunction in a Subset of Autism Lymphoblastoid Cell Lines in a Well-Matched Case Control Cohort

Shannon Rose, Richard E. Frye*, John Slattery, Rebecca Wynne, Marie Tippett, Oleksandra Pavliv, Stepan Melnyk, S. Jill James

Department of Pediatrics, Arkansas Children’s Hospital Research Institute, Little Rock, Arkansas, United States of America
Acquired Mitochondrial Disorders in Children with Autism Spectrum Disorder

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24 hour pretreatment with 50 uM Genipin, a UCP2 inhibitory
Acquired Mitochondrial Disorders in Children with Autism Spectrum Disorder
Acquired Mitochondrial Disorders in Children with Autism Spectrum Disorder

A

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UCP2

Total Protein Stain (band approx. 45kDa)

B

![Graph showing relative UCP2 content](image)

- AD-N
- AD-A

* Significant difference
Acquired Mitochondrial Disorders in Children with Autism Spectrum Disorder
Acquired Mitochondrial Disorders in Children with Autism Spectrum Disorder

A. Intracellular GSH Redox Potential

B. Intracellular Cysteine and NAD Redox Potentials

C. 3-Nitrotyrosine

D. Intracellular ROS

E. Mitochondrial Superoxide

F. Mitochondrial Membrane Potential
Mitochondrial Function in the PMBCs of 35 ASD children
Acquired Mitochondrial Disorders in Children with Autism Spectrum Disorder

(A) Change in ATP Linked Respiration (pmol/min/uM DMNQ) with DMNQ Challenge

(B) Basal Resp (pmol/min)

(C) Proton (pmol/min)

(D) ATP Resp (pmol/min)

(E) Max Resp (pmol/min)

(F) Reserve (pmol/min)

(G) Scaled Score

- ASD-L / Low Functioning
- ASD-H / Higher Functioning
Acquired Mitochondrial Disorders in Children with Autism Spectrum Disorder
Acquired Mitochondrial Disorders in Children with Autism Spectrum Disorder

**AD-A Bioenergetic Response**
- \( \uparrow \) ATP turnover
- \( \uparrow \uparrow \) UCP2
- \( \uparrow \) Proton leak
- \( \uparrow \) Maximal capacity
- \( \uparrow \) Reserve capacity

**Maladaptive Response**
- Chronic
  - \( \uparrow \) ROS
  - \( \downarrow \) GSH/GSSG

**Normal Response**
- AD-N Bioenergetic Response
  - Small \( \uparrow \) Proton leak
  - Small \( \uparrow \) UCP2
  - Small \( \downarrow \) Reserve capacity

**Mild Acute Oxidative Insult**
- High ATP Demand
  - \( \uparrow \uparrow \) ATP turnover
  - \( \uparrow \uparrow \) Proton leak
  - \( \downarrow \downarrow \) Reserve capacity

**Severe Oxidative Insult**
- Reserve capacity depleted
- Damaged membranes
- Damaged ETC
- **Cell Death**

**Mild Acute Oxidative Insult**
- High ATP Demand
  - \( \uparrow \) ATP turnover
  - \( \uparrow \) Proton leak
  - \( \downarrow \) Reserve capacity
Oxidative stress induces mitochondrial dysfunction in a subset of autistic lymphoblastoid cell lines

S Rose, RE Frye, J Slattery, R Wynne, M Tippett, S Melnyk and SJ James
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Acquired Mitochondrial Disorders in Children with Autism Spectrum Disorder

![Graph showing GSH, GSSG, and GSH/GSSG levels](image-url)
• LCLs and PMBCs from children with autism demonstrate mitochondrial function abnormalities when challenged to increased level of oxidative stress.

• There are subgroups of autistic children with abnormal mitochondrial function and others with normal mitochondrial function.

• Mitochondrial function in PMBCs from children with autism spectrum disorder is related to development and behavior.

• N-acetyl-L-Cysteine normalizes mitochondrial function in those with abnormal mitochondrial function.
Pulling it Together
Acquired Mitochondrial Disorders in Children with Autism Spectrum Disorder

- Mitochondrial Dysfunction
- Redox Regulation & Oxidative Stress
- Immune Dysfunction & Inflammation
- Gene Expression
- Genetic Code
- Epigenetics
Studies in Our Center
Defining subgroups of mitochondrial disease and dysfunction in autism spectrum disorder

Aim: This research aims to better understand abnormalities in mitochondrial energy metabolism, and consequences of such abnormalities, in autism spectrum disorder (ASD).

Protocol: 1 to 5 visits to ACH with blood draws and cognitive and behavior evaluations. Primary measures are oxidative stress and mitochondrial function

Participants: Between the ages of 3-14 years.
Four Groups Matched on Age and Gender
• 50 Children with ASD who have mitochondrial disease (ASD/MD)
• 50 Children with ASD who do not have mitochondrial disease (ASD/NoMD)
• 50 Children with no ASD but have mitochondrial disease (NoASD/MD)
• 50 Children with developmental delays but no ASD or no MD (NoASD/NoMD)

150 children with general ASD
50 TD controls (ASD ruled out using SCQ)

Contact: John Slattery, jcslattery@uams.edu
Funding: Jane Johnson Foundation (partial)
A Folinic acid intervention for ASD

Specific Aim 1: To determine whether an intervention of folinic acid over a 12-week period is a safe and effective treatment for ASD and improves mitochondrial function.

Specific Aim 2: To determine whether the metabolic, immune and genetic biomarkers can predict individual participant response to folinic acid treatment.

1. Folate Receptor alpha autoantibody
2. Glutathione Metabolism
3. Mitochondrial Function
4. Genetic Polymorphisms:
   Methylenetetrahydrofolate Reductase (MTHFR): 677C>T & 1298A>C
   Reduced folate carrier: 80G>A

Inclusion: ASD, 3-14 years of age, Language Impairment, No major changes in therapy
Exclusion: Antipsychotic medication, Severe Irritability, Severe Prematurity, GERD

Contact: John Slattery, jcslattery@uams.edu
Funding: Lee Silsby Compounding Pharmacy / BHARE Foundation / Fraternal Order of Eagles
Acquired Mitochondrial Disorders in Children with Autism Spectrum Disorder

1st International Symposium on the Microbiome in Health and Disease with a Special Focus on Autism
June 26th, 2014  Arkansas Children’s Hospital

A collaborative effort between the Arkansas Autism Alliance and the N of One: Autism Research Foundation focusing on mechanisms of action in Autism Research.

The microbiome is the next frontier in medicine and research groups are investigating its contribution to certain diseases, along with its role in maintaining health. This unique cutting-edge conference will review the evidence for the role of the microbiome in health and disease with a special focus on how alterations in the microbiome may influence behavioral manifestations of autism.

Invited Speakers include:
  Dr. Susan Swedo, National Institute of Health
  Dr. William Parker, Duke University
  Dr. Tore Midtvedt, Karolinska Institute
  Dr. Jim Adams, Arizona State
  Dr. Carl Cerniglia, NCTR
  Dr. Derrick MacFabe, University of Western Ontario
  Dr. Rosa Krajmalnik-Brown, Arizona State
  Dr. Richard Frye, UAMS
  Dr. Emma Allen-Vercoe, University of Guelph

Register Here: http://www.microbiome-autism.com/
Lunch Provided to 1st 50 Registered
Free Admission
Media Contact: John Slattery jcslattery@uams.edu
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**Autism Seahorse Laboratory**
Shannon Rose  
Rebecca Wynne

**Autism Translational Research Center**
John Slattery  
Marie Tippet

**Autism Metabolic Laboratory**
Jill James  
Stepan Melnyk  
Teresa Evans  
Oleksandra Pavliv

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Jane Botsford Johnson Foundation  
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