“Prenatal and Postnatal Epigenetic Programming (PreP and PEP):
Implications for GI, Immune and Neuronal Function in Autism”

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Boston, MA

Overview
- Oxidation and antioxidant metabolism
- Autism: An epigenetic disorder
  Prenatal epigenetic programming (PREP)
  Postnatal epigenetic programming (PEP)
- Brain-specific redox features
- Redox signaling in the immune response
- Gluten and casein-derived opiate peptides

Viewing Life Through Redox Glasses

Oxidation: Loss of an electron
Reduction: Gain of an electron

Life:
The evolved ability to resist oxidation

Death:
The inevitable outcome of oxidation

Evolution:
Gradual acquisition and manifestation of novel adaptive strategies to survive oxidation

Development:
Programmed and progressive changes in gene expression, driven by changes in oxidation status, via epigenetic mechanisms

Earliest life appears arose at hydrothermal vents emitting hydrogen sulfide.

From Paul G. Falkowski; Science 311 1724 (2006)

The ability to control oxidation is at the core of evolution
Each addition is strengthened because it builds on the solid core already in place.

EVOLUTION = LAYER UPON LAYER OF USEFUL ADAPTIVE RESPONSES TO OFFSET THE THREAT OF OXIDATION
Ergo:
1. Life, from beginning to end, depends upon sufficient levels of antioxidant.
2. Disorders and diseases commonly reflect an interference with the capacity to resist oxidation.
3. Agents or organisms which interfere with antioxidant capacity will disrupt normal development and cause diseases.
4. Metabolic treatments that restore and sustain antioxidant capacity will be effective in reversing many disorders and diseases.

Eleven studies showing significantly lower GSH in autism

<table>
<thead>
<tr>
<th>Authors</th>
<th>Control n</th>
<th>Autistic n</th>
<th>GSH</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>James et al. (2004)</td>
<td>33</td>
<td>20</td>
<td>46%↓</td>
<td>0.0001</td>
</tr>
<tr>
<td>James et al. (2006)</td>
<td>73</td>
<td>80</td>
<td>32%↓</td>
<td>0.0001</td>
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<tr>
<td>Geier and Geier (2006)</td>
<td>10</td>
<td>36%↓</td>
<td>0.0001</td>
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<tr>
<td>Adams et al. (2011)</td>
<td>55</td>
<td>43</td>
<td>21%↓</td>
<td>0.0001</td>
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<tr>
<td>Pasca et al. (2009)</td>
<td>13</td>
<td>15</td>
<td>33%↓</td>
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<tr>
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<td>12</td>
<td>15</td>
<td>35%↓</td>
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<td>Al-Gadani et al. (2009)</td>
<td>30</td>
<td>30</td>
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<td>Melnyk et al. (2011)</td>
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<td>40</td>
<td>29%↓</td>
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<tr>
<td>James et al. (2009)</td>
<td>42</td>
<td>40</td>
<td>28%↓</td>
<td>0.0001</td>
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<tr>
<td>Geier et al. (2009)</td>
<td>120</td>
<td>28</td>
<td>24%↓</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

The available level of antioxidant (GSH) controls the level of aerobic metabolism and ATP formation = Redox Signaling

$\text{O}_2 + 4\text{H}^+ \rightarrow \text{2H}_2\text{O} + \text{ATP}$

**Autism is associated with inadequate levels of glutathione and impaired methylation**

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

**TABLE II: Transmethylation and Transsulfuration Metabolism in Autistic Cases and Controls**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Control (n = 75)</th>
<th>Autistic (n = 80)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methionine (µmol/L)</td>
<td>25.0 ± 0.5</td>
<td>20.6 ± 1.5</td>
<td>0.0001</td>
</tr>
<tr>
<td>SAM (µmol/L)</td>
<td>93.6 ± 16</td>
<td>94.1 ± 11</td>
<td>0.0001</td>
</tr>
<tr>
<td>SAH (µmol/L)</td>
<td>18.6 ± 6.5</td>
<td>23.2 ± 7.6</td>
<td>0.0001</td>
</tr>
<tr>
<td>NADPH (µmol/L)</td>
<td>0.73 ± 0.1</td>
<td>0.87 ± 0.3</td>
<td>0.0001</td>
</tr>
<tr>
<td>Homocysteine (µmol/L)</td>
<td>16.9 ± 5.1</td>
<td>23.2 ± 7.6</td>
<td>0.0001</td>
</tr>
<tr>
<td>Cysteine (µmol/L)</td>
<td>37.0 ± 13</td>
<td>14.0 ± 11</td>
<td>0.0001</td>
</tr>
<tr>
<td>Cystidine (µmol/L)</td>
<td>246 ± 73</td>
<td>380 ± 33</td>
<td>0.75</td>
</tr>
<tr>
<td>Total GSH (µmol/L)</td>
<td>5.5 ± 1.7</td>
<td>5.1 ± 1.2</td>
<td>0.0001</td>
</tr>
<tr>
<td>Free GSH (µmol/L)</td>
<td>3.5 ± 0.9</td>
<td>1.4 ± 0.0</td>
<td>0.0001</td>
</tr>
<tr>
<td>GSH/GSSG ratio</td>
<td>182.2 ± 51.4</td>
<td>141.7 ± 32.5</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

**The available level of intracellular cysteine is rate-limiting for GSH synthesis**

GLYCATOMY...
Cysteine for glutathione synthesis can be provided by either transsulfuration of homocysteine or by uptake from outside the cell.

\[
\text{HCY} \xrightarrow{\text{Methionine Synthase}} \text{MET} \xrightarrow{\text{SAH}} \text{SAM}
\]

\[
\text{SAM} \xrightarrow{\text{Methylation Reactions}} \text{SAH} \xrightarrow{\text{Adenosine}} \text{MethylTHF} \xrightarrow{\text{THF}} \text{Cystathionine} \xrightarrow{\gamma-\text{Glutamylcysteine Synthase}} \text{Cysteine}
\]

\[
\text{Cysteine} \xrightarrow{\gamma-\text{Glutamylcysteine Synthase}} \text{GSH}
\]

Absorption and distribution of cysteine/cystine

The GI tract is a critical site for immune cell activation, especially during postnatal development. Decreased cysteine uptake can promote oxidative stress and inflammation, resulting in epigenetic effects which can last throughout life.

Postnatal Epigenetic Programming (PEP)

Prenatal Epigenetic Programming (PREP)

Methylation is inhibited by oxidative stress

\[
\text{Oxidative Stress} \xrightarrow{(-)} \text{Adenosine} \xrightarrow{A \text{ reversible reaction?!}} \text{Methionine Synthase} \xrightarrow{(+) \text{MethylTHF}} \text{SAM}
\]

\[
\text{DIETARY PROTEIN} \xrightarrow{\text{Vitamin B12 (Cobalamin)}} \text{Methionine Synthase} \xrightarrow{(+)} \text{SAM}
\]

Regulation of gene expression during development

Transcription Factor Regulation:

Epigenetic Regulation:

DNA + histone + chromatin; genes are silenced and transcription is blocked.
Epigenetic changes in gene expression are the primary mechanism underlying development.

Epigenetic marks change in response to the environment and contribute to adaptive capabilities gained through evolution.

Essentially:

Epigenetics = A memory system
- linked to gene expression
- responsive to redox status
- driver of development
- active in all cell types
- active at all ages
- capable of transgenerational effects

Agents which interrupt antioxidant and/or methylation pathways will interfere with the many roles of epigenetic regulation.

GI tract absorption of cysteine is critical for postnatal epigenetic programming (PEP).

PrEP
- Prenatal Epigenetic Programming
  - Maternal Metabolism
  - Maternal Nutrition
  - Maternal Toxic Exposures
  - Placental Function
  - Genetic Factors

PEP
- Postnatal Epigenetic Programming
  - Breast Milk (Formula)
  - Toxic Exposures
  - Physical Experiences
  - Emotional Experiences

BIRTH
Odds of autism decrease with increasing duration of breastfeeding

Folate and B12 Levels are Markedly Lower

Thus malnutrition-based autism in Oman shares a similar metabolic profile to autism in the U.S.
i.e. Oxidative stress and impaired methylation are fundamental features of autism

Effect of breast milk and weaning on epithelial growth of the small intestine in humans

<table>
<thead>
<tr>
<th>Growth factor</th>
<th>Growth factor activity</th>
<th>Pharmacological action</th>
<th>In vivo effect</th>
<th>Bioactive</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGF</td>
<td>Present in milk Yes</td>
<td>Yes</td>
<td>Yes, KO</td>
<td>Yes, KO</td>
</tr>
<tr>
<td>TGF</td>
<td>Present in milk Yes</td>
<td>Yes</td>
<td>Yes, KO</td>
<td>Yes, KO</td>
</tr>
<tr>
<td>SHH</td>
<td>Present in milk Yes</td>
<td>Yes</td>
<td>Yes, KO</td>
<td>Yes, KO</td>
</tr>
<tr>
<td>EGF</td>
<td>Present in serum Yes</td>
<td>Yes</td>
<td>Yes, KO</td>
<td>Yes, KO</td>
</tr>
<tr>
<td>TGF</td>
<td>Present in serum Yes</td>
<td>Yes</td>
<td>Yes, KO</td>
<td>Yes, KO</td>
</tr>
<tr>
<td>SHH</td>
<td>Present in serum Yes</td>
<td>Yes</td>
<td>Yes, KO</td>
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</tr>
<tr>
<td>Growth factors</td>
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<td>Yes, KO</td>
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<tr>
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<td>Present in serum Yes</td>
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<td>Yes, KO</td>
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<tr>
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<td>Growth factors</td>
<td>Present in serum Yes</td>
<td>Yes</td>
<td>Yes, KO</td>
<td>Yes, KO</td>
</tr>
</tbody>
</table>

Growth factors stimulate cysteine uptake, providing a powerful mechanism to regulate redox and methylation
FOOD-DERIVED OPIATE PEPTIDES

A1 casein contains HIS at position 67 and is readily hydrolyzed to BCM7
A2 casein contains PRO at position 67 and is resistant to hydrolysis

Cysteine uptake in CaCo2 cells in presence of various drugs. 04/04/2010.

Inhibition of cysteine uptake by SH-SY5Y human neuronal cells by bovine vs. human beta casomorphin-7

Bovine casomorphin 7 decreases cysteine, glutathione, and methionine levels in neuronal cells.

Opiate peptides can inhibit cysteine uptake and promote oxidative stress
Effects of morphine and bovine and human casomorphin-7 peptides on redox/methylation gene expression in human neuronal cells

Breast Milk Constituents Can Exert Epigenetic Effects

Terminal ileum is critical site for absorption of cysteine and selenocysteine via the EAAT3 transporter

Terminal ileum is also critical for vitamin B12 and folic acid absorption

Breast Milk

[Diagram showing Breast Milk Constituents and Epigenetic Effects]

Effects of morphine and bovine and human casomorphin-7 peptides on redox/methylation gene expression in human neuronal cells

Breast Milk Constituents Can Exert Epigenetic Effects

- Casein
- Growth Factors
- DHA
- Modulation of GI cysteine uptake
- Systemic availability of cysteine
- Δ Cellular Redox Status
- Δ Methylation Status
- Δ Gene Expression
- GI Tract
- Brain Immune System

Terminal ileum is critical site for absorption of cysteine and selenocysteine via the EAAT3 transporter

Terminal ileum is also critical for vitamin B12 and folic acid absorption


Neonatal lymphoid nodular hyperplasia, non-specific colitis, and pervasive developmental disorder in children

Antioxidant Availability (i.e. cysteine and GSH) → Homeostatic Equilibrium

Antioxidant Availability (Higher antioxidant demand) → Oxidative Stress → Adaptive Epigenetic Changes

Metabolic Activity (Antioxidant demand)
Autism-associated changes in gene expression in terminal ileum
Data from Drs. Steve Walker and Arthur Krigsman

<table>
<thead>
<tr>
<th>Gene</th>
<th>Fold Change</th>
<th>Direction</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBS</td>
<td>2.37</td>
<td>Down</td>
</tr>
<tr>
<td>GSS</td>
<td>1.52</td>
<td>Down</td>
</tr>
<tr>
<td>TNFA</td>
<td>1.63</td>
<td>Up</td>
</tr>
<tr>
<td>GSR</td>
<td>1.72</td>
<td>Down</td>
</tr>
<tr>
<td>DRD4</td>
<td>2.02</td>
<td>Down</td>
</tr>
<tr>
<td>GCLC</td>
<td>1.58</td>
<td>Down</td>
</tr>
<tr>
<td>CTH</td>
<td>2.63</td>
<td>Down</td>
</tr>
<tr>
<td>GCLM</td>
<td>1.7</td>
<td>Down</td>
</tr>
<tr>
<td>EAAT3</td>
<td>2.19</td>
<td>Down</td>
</tr>
<tr>
<td>MTHFR</td>
<td>1.55</td>
<td>Down</td>
</tr>
<tr>
<td>NRF2</td>
<td>3.12</td>
<td>Down</td>
</tr>
</tbody>
</table>

A preliminary qRT-PCR evaluation of redox/methylation-related gene expression in blood-derived RNA of autistic vs. control subjects

The brain extracellular environment (CSF) has more than 10-fold less cysteine than plasma!!


Redox and Methylation Pathways in Neurons

Methionine synthase mRNA in cortex progressively decreases with increasing age in normal subjects

The level of methionine synthase mRNA is reduced by 50% in postmortem brain of autistic subjects vs. age-matched controls.

Redox signaling plays a central role in the immune response.

Dendritic cells release GSH, similar to astrocytes in brain.

Effector T-cells take up cysteine, similar to neurons in brain.

Regulatory T-cells compete with T$_{eff}$ for cysteine and restrict the immune response.

EAAT3 provides Cysteine uptake.

Regulatory T Cells Interfere with Glutathione Metabolism in Dendritic Cells and T Cells

Zhanghua Yan, Sairaj R. Garg, and Noise Fawaz

EAAT3 is expressed in T cell lymphocytes and highest expression is in Treg cells.

Autoimmune-prone SJL/J mice have lower EAAT3 expression in mixed T cell lymphocytes.

EAAT3-mediated cysteine/selenocysteine uptake in the terminal ileum.

A milk-free diet downregulates folate receptor autoimmunity in cerebral folate deficiency syndrome.
Autoimmune-prone SJL/J mice have lower levels of GSH in brain, unaffected by postnatal thimerosal treatment.

Autoimmune-prone SJL/J mice have lower methionine synthase activity in brain, unaffected by postnatal thimerosal treatment.

A1 Beta Casein Consumption is correlated with increased risk of juvenile-onset diabetes. 

Using A1 cow milk formula with hydrolyzed casein lowered autoimmunity and incidence of type I diabetes by ~ 50%.

GI uptake of cysteine is critical throughout life, but especially during postnatal epigenetic programming (PEP).
ACKNOWLEDGEMENTS

Brain Samples:
Autism Tissue Program
Harvard Brain Tissue Resource Center
Tissue Resource Center (Australia)
Stanley Medical Research Foundation
and donor families.

Collaborators:
Sultan Qaboos University
Mostafa Waly and Yahya Al-Farsi

Grant Support:
Autism Research Institute
SafeMinds
National Autism Association
Autism Speaks