**METAL IMBALANCES IN AUTISM BRAIN TISSUES**

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### Walsh Research Institute
- Nonprofit organization
- Expertise in autism, ADHD, depression, behavior disorders, schizophrenia, bipolar disorder, and Alzheimers
- International physician training
- Research

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### Massive Autism Database
- 6,500 ASD Patients,
- More than 1.5 million chemical assays of blood and urine,
- Striking biochemical differences between ASD children and non-affected children.

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### Autism Database Highlights
- Autism imbalances more severe than in violent behavior and mental illness,
- Discovery of undermethylation in more than 95% of ASD patients (1999),
- Clear evidence of oxidative stress and metallothionein depletion (2000).

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### Pervasive Biochemical Abnormalities in Autism
- Depressed Glutathione & Cysteine
- Elevated toxic metals
- Hypomethylation
- Copper/Ceruloplasmin dysregulation
- Depleted Zinc & Metallothionein
- Elevated Pyrroles
- Low B-6, C, and Selenium
- Elevated Urine Isoprostanes

*Note: Each of these imbalances is associated with elevated OXIDATIVE STRESS.*

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### Some Consequences of Excess Oxidative Stress
1. Hypersensitivity to Hg & other toxic metals,
2. Hypersensitivity to casein, and gluten,
3. Poor immune function,
4. Inflammation of the brain & G.I. tract,
5. Depletion of glutathione & metallothionein.
Consequences of Oxidative Overload in the G.I. Tract

- Destroys digestive enzymes needed to break down casein & gluten,
- Increases candida/yeast levels,
- Diminishes Zn levels and production of stomach acid,
- Produces inflammation,
- Results in a “leaky intestinal barrier, allowing toxics to enter the bloodstream.

Many Popular Autism Therapies Have Antioxidant Properties

- Methyl B-12
- Metallothionein Promotion
- Transdermal or Injected Glutathione
- Zn, Se, CoQ-10, Vitamins A,C,D,E
- Chelation with DMSA, DMPS, EDTA.
- Alpha Lipoic Acid
- Risperdal

The Three Musketeers of Antioxidant Protection

Glutathione: First line of defense,

Metallothionein: Nature’s back-up system,

Selenium: Speeds up the process.

A Clue From Cancer Research

Severe oxidative stress can alter established epigenetic bookmarks,

Certain deviant marks can turn on a cancer gene or silence a cancer-protective gene, initiating a cancer condition,

Regressive autism may arise from a similar mechanism – severe oxidative stress that permanently alters gene regulation.

Methylation and Oxidative Stress

- Undermethylation is a distinctive feature of autism,
- Undermethylation results in reduced synthesis of glutathione and cysteine – and weakened ability to cope with toxic metals and other sources of oxidative stress,
- An undermethylation environment during pregnancy may alter gene regulation of antioxidant protectors.

The Bermuda Triangle of Autism

Undermethylation

Oxidative Stress

Epigenetics
An Epigenetic Model of Autism

- Undermethylation from genetic inheritance and/or folate deficiency results in life-long vulnerability to oxidative stresses,
- Environmental insults overwhelm antioxidant protectors and produce deviant epigenetic bookmarks resulting in autism,
- Since deviant marks survive cell divisions, the autism condition can persist a lifetime.

Mounting Evidence that Autism is an Epigenetic Disorder


Autism Brains Are Different

- Narrowed minicolumns in brain cortex,
- Incomplete maturation in cerebellum, amygdala, pineal gland and hippocampus,
- Poverty of brain dendrites and synapses,
- Brain inflammation and increased head size,
- Damaged fats in autism brains,
- Abnormal levels of calcium and iron,
- Reduced structural connectivity between brain regions.

Low Metallothionein Levels in Autism p < 0.0092

Why is Metallothionein Important?

- Required for brain cell development,
- Prevents Hg, and other metal toxics from passing intestinal and blood/brain barriers,
- Can safely bind to Hg that enters the brain.

Note: MT functioning can be disabled by severe oxidative stress.

Brain Tissue Studies

- Very limited amount of autism brain tissue available for research,
- Conventional chemical analysis for zinc, copper, mercury, lead, calcium, etc requires significant sample size,
- Until now, little or no data for levels of most elements in ASD brains.
**Advanced Photon Source**

1.4 billion dollar facility at Argonne National Laboratory in Illinois,

The APS produces photon beams 100 times brighter than at the surface of the sun,

World’s greatest capability for accurate elemental analysis of tiny samples.

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**Photon Beam Nanoanalysis of Autism Brain Tissues**

- Double blind, controlled study of 176 brain tissues from U. of Maryland’s Autism Brain Bank,
- Elemental analysis for Hg, Pb, Cu, Zn, Ca, and other elements using high-brilliance photons,
- More than 35,000 individual assays obtained for autism & control brain tissues.

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**Study Researchers**

- Bill Walsh PhD, Principal Investigator
- Aditi Gulibani MD, Research Associate
- Woody McGinnis MD, Consultant
- Stephon Vogt PhD, Argonne Scientist
- Barry Lai PhD, Argonne Scientist

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**Test Subjects**

- **Five autistics:** Age range 5-9; 3/2 M/F ratio; All children exposed to Thimerosal,
- **Five controls:** Age range 5-11, 2/3 M/F ratio.

Brain tissues samples prepared at Johns Hopkins
Brain Regions Studied

- Cerebellum
- Superior Cortex
- Deep Cortex
- White Matter

Note: 20 autistic & 20 control tissue samples from each brain region.

Autism/Control Tissue Array

Experimental Procedure

- Samples prepared using special plastic substrate,
- Simultaneous assays for 10-14 elements,
- High-brilliance beam of 0.3 mm diameter,
- Typical protocol: Automated raster scanning using 1 sec. pulses,
- 15 micron thick tissues,
- Non-destructive analysis: Tissue samples available for future experiments.

Proficiency Testing

- Modified Gaussian’s fitted to elemental peaks,
- NITS/NBS standards 1832 and 1833 used,
- Control ppb levels exhibited close agreement with published elemental levels, including expected variations between brain regions.

Conclusion: Assay results appear highly reliable.

Results of Brain Tissue Study

1. Testing of 153 intact samples,

2. Abnormal overloads of specific elements found throughout autism brains and not in the controls,

3. Major chemical differences between male and female ASD brains, suggesting that male and female autism may represent distinctly different conditions.
What About Mercury?

- All ASD brain tissues tested were from children exposed to Thimerosal,
- Strong evidence that mercury insults can initiate autism,
- Are brain mercury levels high in ASD brains several years after exposure to Thimerosal?

“Normal” Hg Levels in Brain

- Hg present in brains of all humans,
- Some Hg enters and departs the brain daily,
- Hg concentrations of 5 to 25 ppb considered typical for healthy persons,
- Hg levels exceeding 75 ppb considered a serious health risk.
Mercury Results

- Mercury not detected in any of the autism or control samples, in any brain region,
- Detection limit in this experiment believed to be 40-50 ppb.

Study Limitation

- Small number of autism subjects (five),
- Testing of additional subjects needed to determine if the chemical imbalances and gender differences reported in this study are characteristic features of autism.

Summary of Results

1. World's first extensive measurements of metal levels in autism brains (35,000 assays compared to dozens previously),
2. Abnormal metal levels and gender differences found in autism brains,
3. Testing of additional ASD subjects needed to verify results.

Thank You!

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