OXIDATIVE STRESS IN AUTISM SPECTRUM DISORDERS

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Walsh Research Institute

- **Nonprofit public charity**
- **Experimental research**
- **Expertise in biochemical therapy**
- **International physician training**

Clinical Experience

William J. Walsh, Ph.D.

- 10,000 Behavior & ADHD
- 6,500 Autism-Spectrum Disorder
- 6,500 Mental Illness

AUTISM

- Developmental disorder,
- Onset: Prior to age 4,
- Deficits in cognition, speech, socialization – Many autistics institutionalized,
- Worldwide epidemic – More than 1 in every 100 USA births (2009 data),
- About 80% involve regression – Normalcy followed by shocking decline at 16-24 months,

Autism Spectrum Database

- About 90 to 150 assays of chemical factors for each of 6,500 patients,
- More than 800,000 chemical test results.

-- *Compared with reference levels* --

Autism Database Analysis

- Major biochemical abnormalities observed in the autism population.
- Autism biochemical imbalances are more severe than those for violent behavior, depression, and schizophrenia.
- Female autistics have more disordered chemistry than male autistics.

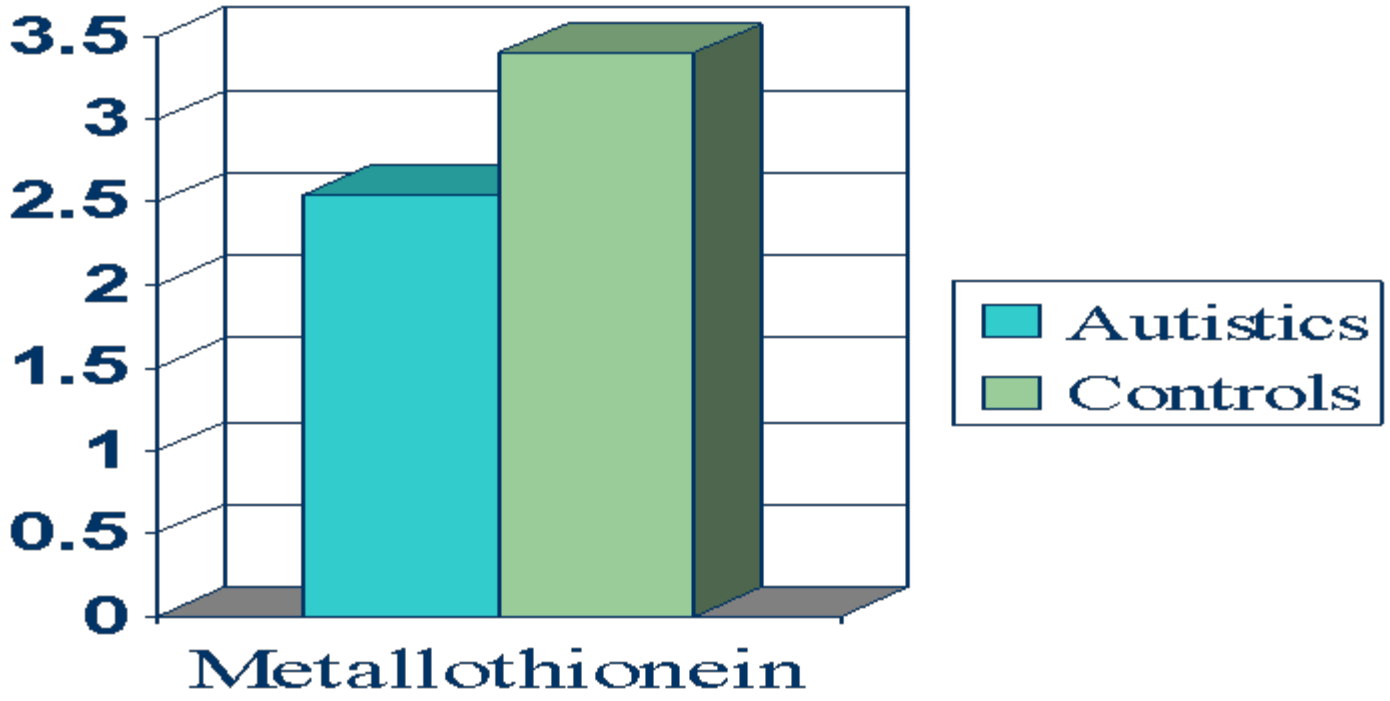
High Incidence Biochemical Abnormalities in Autism

- **Depressed Glutathione & Cysteine**
- **Elevated toxic metals**
- **Hypomethylation**
- **High Copper & low Ceruloplasmin**
- **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.

Low Metallothionein Levels in Autism

$p < 0.0092$



Oxidative Stress and Autism

1. Excessive oxidative stress is evident throughout the autism spectrum,
2. An oxidative stress model can explain most symptoms of autism,
3. Most autism therapies have antioxidant properties,
4. Oxidative stress has become a leading focus of autism research.

Consequences of Oxidative Stress Mirror Classic Symptoms of Autism

- Hypersensitivity to Hg and other toxic metals
- Hypersensitivity to certain proteins (casein, gluten, etc)
- Poor immune function
- Disruption of the methylation cycle
- Inflammation of the brain & G.I. tract
- Depletion of glutathione & metallothionein
- Excessive amounts of “unbound” copper

Most Popular Autism Therapies Enhance Antioxidant Protection

- 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

Distinctive Features of Autism

- Strong genetic predisposition
- Onset after environmental insult
- High oxidative stress
- Altered brain development

Autism Brains Are Different

- Incomplete maturation – Excessive number of short, undeveloped brain cells in cerebellum, amygdala, pineal gland and hippocampus,
- Poverty of brain dendrites and synapses,
- Narrowed minicolumns in brain cortex,
- Brain inflammation and increased head size,
- High oxidative stress and damaged PUFA's,
- Abnormal levels of calcium and iron.

Oxidative Stress Can Impair Brain Development

- High oxidative stress depletes glutathione,
- Ample glutathione is required for proper functioning of metallothionein,
- Metallothionein is a key factor in early brain development.

Why is Metallothionein Important?

- Required for pruning, growth and growth-inhibition of brain cells in early development,
- Prevents Hg, and other metal toxics from passing intestinal and blood/brain barriers,
- Required for homeostasis of Cu and Zn,
- Supports immune function.

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

Consequences of Oxidative Stress 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.

Oxidative Stress and Methylation
The Chicken or the Egg?

- Excess oxidative stress can deplete GSH, impair the one-carbon cycle, and produce undermethylation.
- Undermethylation can reduce production of GSH, cysteine, and MT, and cause excess oxidative stress.
- A genetic weakness in either factor can produce the other.
- Both factors are distinctive features of autism.

Autism Rates
A Continuing Medical Mystery

- Strong genetic predisposition: Greater than 60% concordance in identical twins; Less than10% concordance in fraternal twins,
- Dramatic increase in autism cases over the past 50 years.
- Autism rates continue to escalate – October, 2009 data indicates one case per 100 births.

How can there be an epidemic of a genetic condition?

The Role of Environment

- Concordance of only 60-80% in identical twins indicates that environment plays a significant role.

- Since DNA mutations can take centuries to develop, the autism epidemic has been attributed to changes in environment.

The Recipe for Autism

1. Genetic Predisposition

2. Environmental Insult

Environmental Insults: A Multitude of Possibilities

1. Attention has been focused on direct insults to the child from conception to age three.

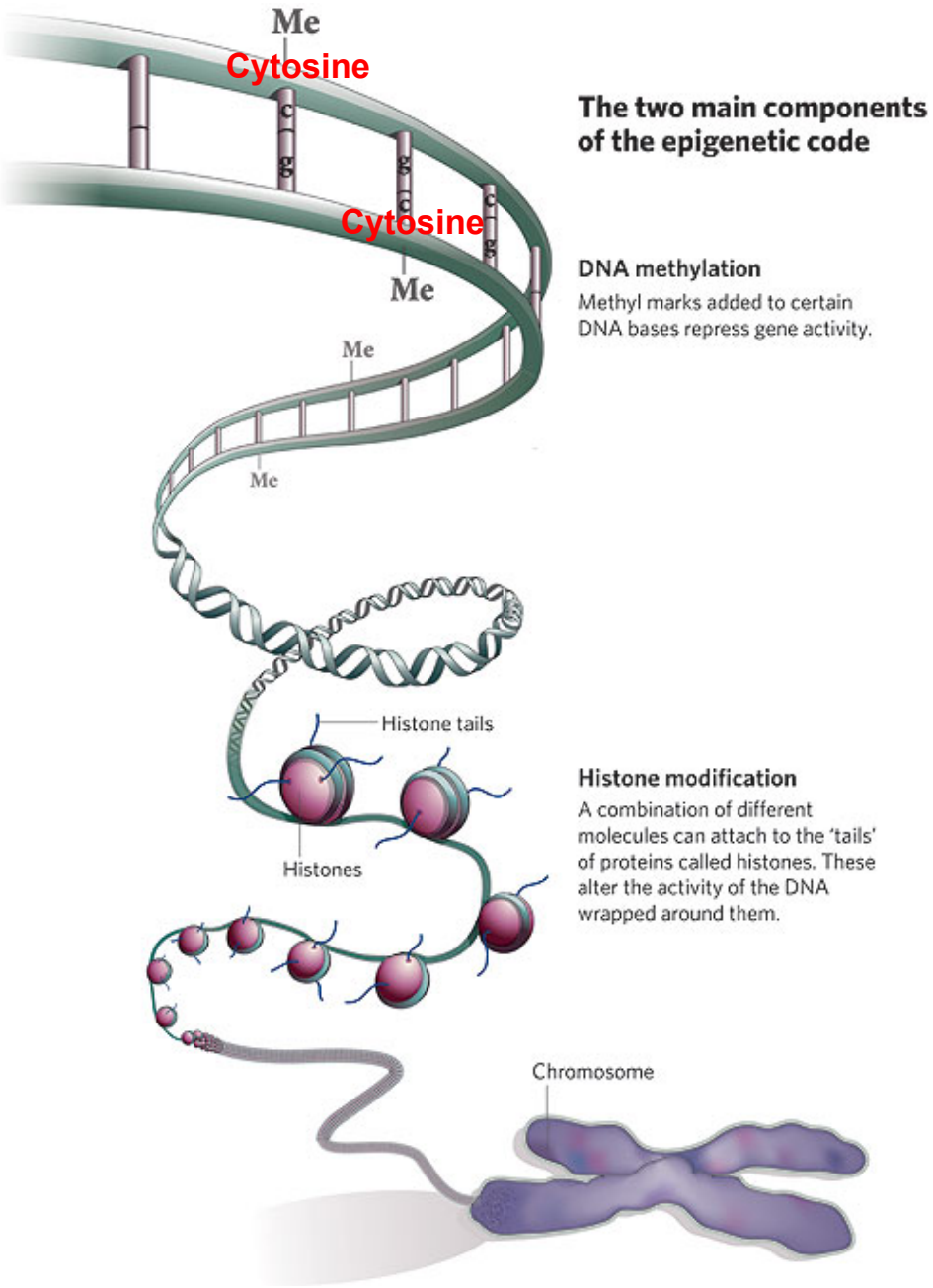
2. More than 25 environmental insults have been proposed, including mercury exposures, vaccines, changes in diet, viruses, increased Cu in the water supply, etc, etc.

Another Possibility - Epigenetics

- Chemical insults during the first month of gestation can produce abnormalities in gene expression that may persist throughout life.
- In some cases, these abnormalities can be transferred to future generations.
- This could result in a geometric increase in the number of autism-prone families.

Epigenetic Processes During Early Fetal Development

- Every cell has the potential for expressing any of the >20,000 genes in DNA,
- In utero chemical environment determines which genes will be expressed or inhibited throughout life (bookmarking),
- Gene expression tendencies can be transmitted to future generations by a process called transgenerational epigenetic inheritance (TEI),
- Methylation is a primary factor in TEI, and is abnormal in autistic children.



The two main components of the epigenetic code

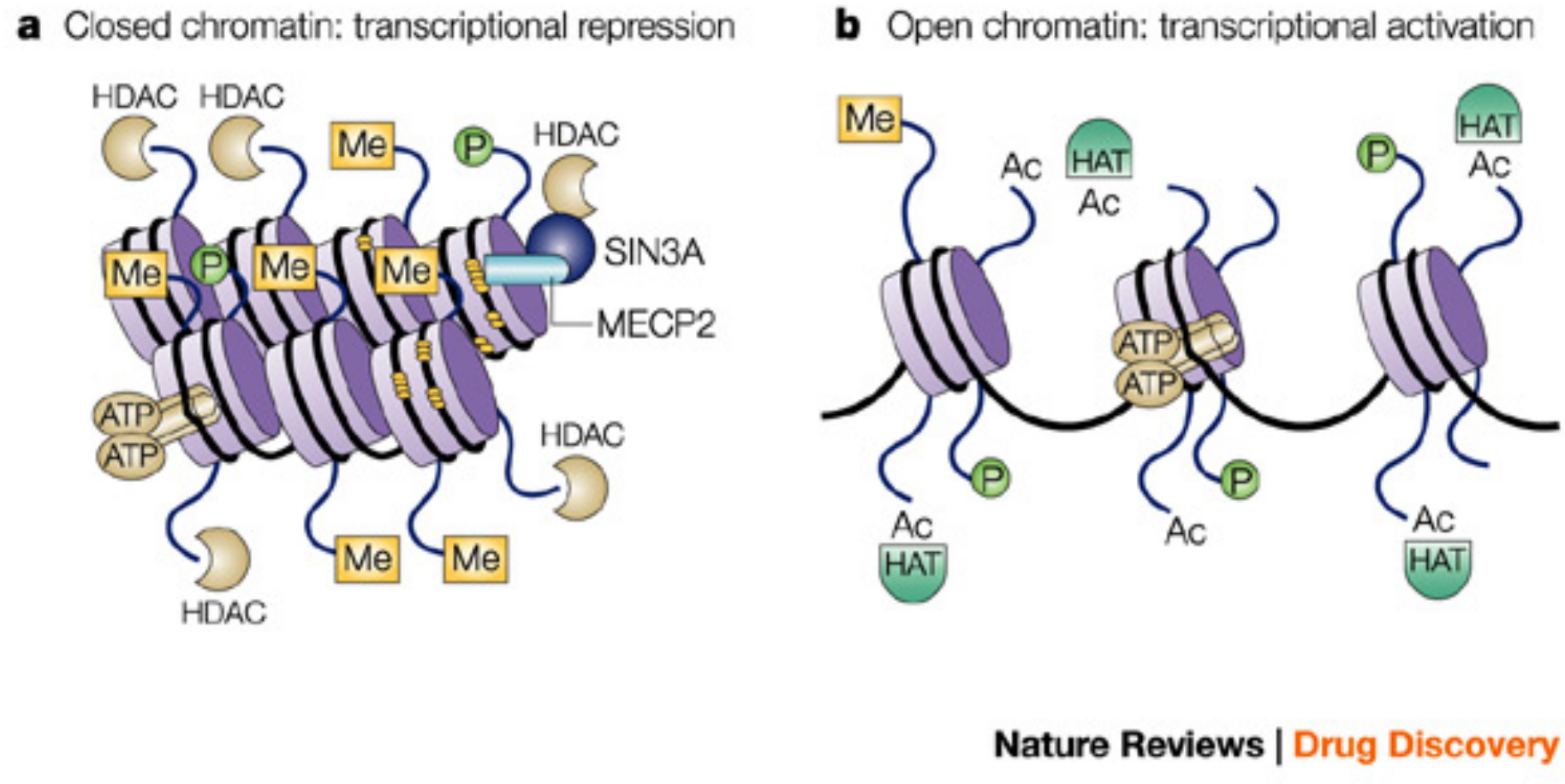
DNA methylation

Methyl marks added to certain DNA bases repress gene activity.

Histone modification

A combination of different molecules can attach to the 'tails' of proteins called histones. These alter the activity of the DNA wrapped around them.

Methylation Inhibits Gene Expression



Nature Reviews | Drug Discovery

A Clue From the Past -- Thalidomide Babies

- Deformed thalidomide babies of the 1960's had a high incidence of autism,
- Autism occurred only if the anti-nausea pill was taken between days 20-24 of gestation,
- This is the period when most epigenetic decisions regarding gene expression or inhibition are established,
- This may be the time of greatest sensitivity to environmental insults.

Recent Research

- Dominant importance of oxidative stress,
- Evidence of neurodegeneration,
- Hypomethylation is a feature of autism,
- Poverty of brain dendrites & synapses
- Male/Female differences in brain chemistry,
- Evidence that Hg brain levels are at normal levels several years after significant exposure.

Autism and Neurodegeneration

- Increased evidence of neurodegeneration in autism…. attributed to severe oxidative stress,
- Gradual loss of brain cells and IQ may occur in the absence of antioxidant therapy,
- Young autistics appear very bright despite behavioral, speech, and socialization deficits,
- Most adult autistics exhibit mental retardation (exception: Aspergers patients).

Note: Antioxidant therapy may be needed throughout life.

The Final Battlefield – The Brain

- Autism involves a brain that has not completed the maturation process,
- Brain cells and organelles may have been damaged in early development,
- In either case, development of immature brain cells, and production of new dendrites and synapses is a high priority in autism therapy.

Behavioral Therapies and Brain Plasticity

- ABA stimulates organization of synaptic connections & cortex minicolumns.
- ABA promotes brain maturation, but is greatly slowed by oxidative overload and inflammation.
- ABA is especially promising when coupled with antioxidant therapy.

Hebb's Rule:

Brain cells that fire together, wire together.

Unique Advantages of Metallothionein-Promotion Therapy

- Directly aimed at development of brain cells,
- Potential for permanently correcting the intestinal and blood/brain barriers,
- Restores a key antioxidant system.

Limitation: **Does not directly enhance development of dendrites and synapses.**

Important Questions

- Why do most autism regressions occur during months 16-22? Environmental insults are present throughout development.
- Why do many autism regressions result in radical changes in speech, socialization, food sensitivities, etc., in just a few days?
- Why do autism symptoms persist after onset?

Conclusion: A dramatic EVENT has occurred!!

Oxidative Stress Theory of Autism

(Bill Walsh, October, 2009)

- Genetic/Epigenetic predisposition involves weakened defense against oxidative stress,
- Cumulative oxidative insults gradually deplete GSH, MT, SOD and other protective factors,
- A threshold is reached in which antioxidant protection collapses, causing (a) sudden brain & G.I. tract inflammation, (b) leaky intestinal & brain barriers, (c) interruption of normal brain development.

A Strategy for Enhanced Cognition, Speech, and Socialization

- Elimination of excess oxidative stress and inflammation,
- Normalization of blood/brain & intestinal barriers,
- Therapies that enhance brain maturation.

The Bottom Line

OXIDATIVE STRESS MAY BE
THE PRIMARY CAUSE OF AUTISM

1. The genetic predisposition in autism may be weakness in coping with oxidative stress,
2. The environmental component may involve a variety of oxidative insults.

Welcome to the Advanced Photon Source

Photon Beam Nanoanalysis of Brain Tissues

Advanced Photon Source in Illinois

- Simultaneous assays for 10-15 elements,
- High-brilliance beams may be concentrated to less than 0.1 micron diameter,
- Typical protocol: Automated raster scanning using 1 sec. pulses,
- Early experiments involved 10 & 15 micron thick tissues,
- Non-destructive: Chemically-characterized tissues may be used in later experiments.

Comparison of Elemental Levels in Autism & Control Brains

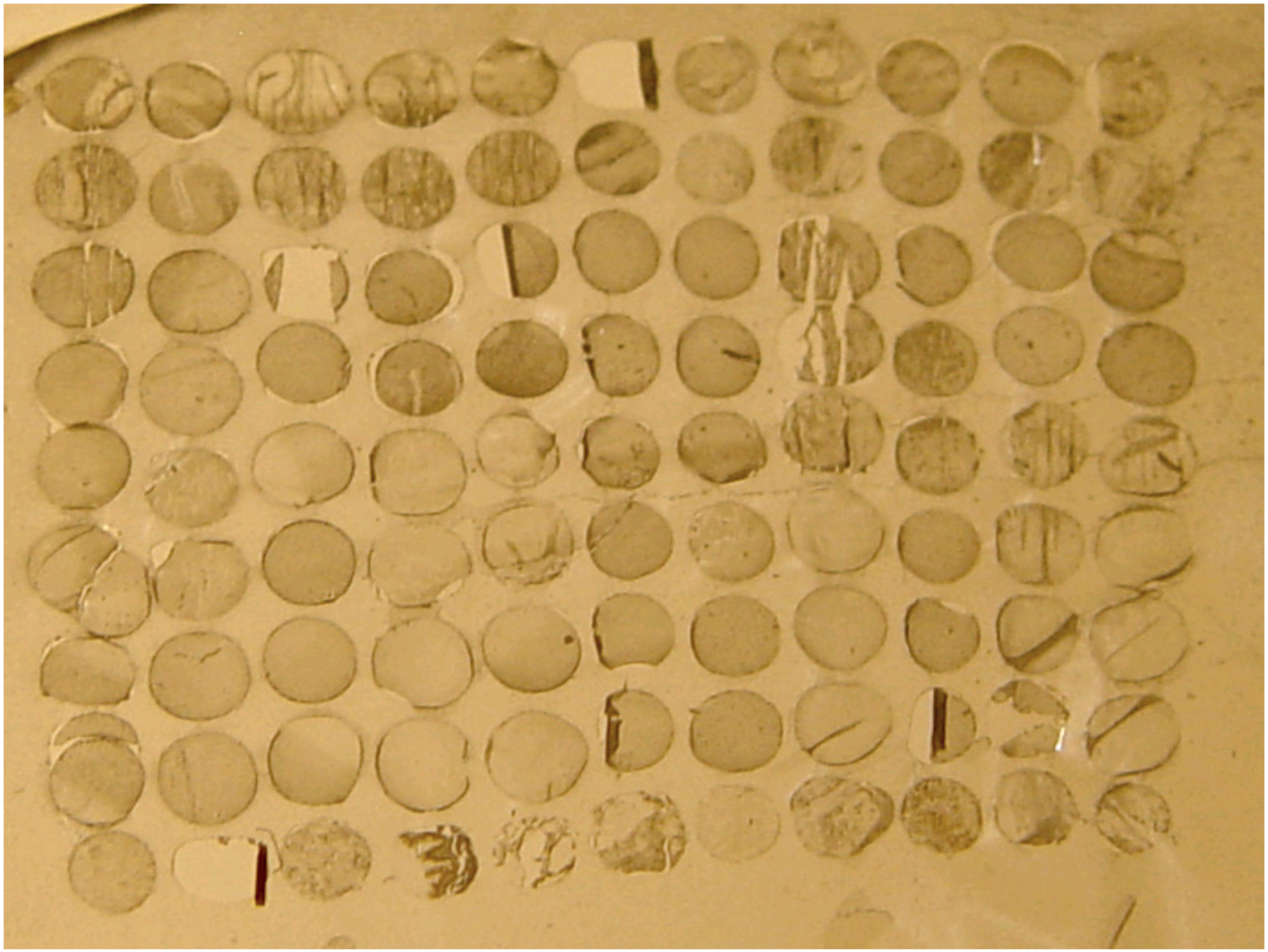
- Double blind, controlled study,
- 176 brain tissues & 22 peripheral samples from U. of Maryland's Autism Brain Bank,
- Elemental analysis for 16 elements, including Hg, Pb, Cu, Zn, and Se using high-brilliance photons,
- First elemental assays ever attempted for autism & control brain tissues.

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



Status of Brain Tissue Study

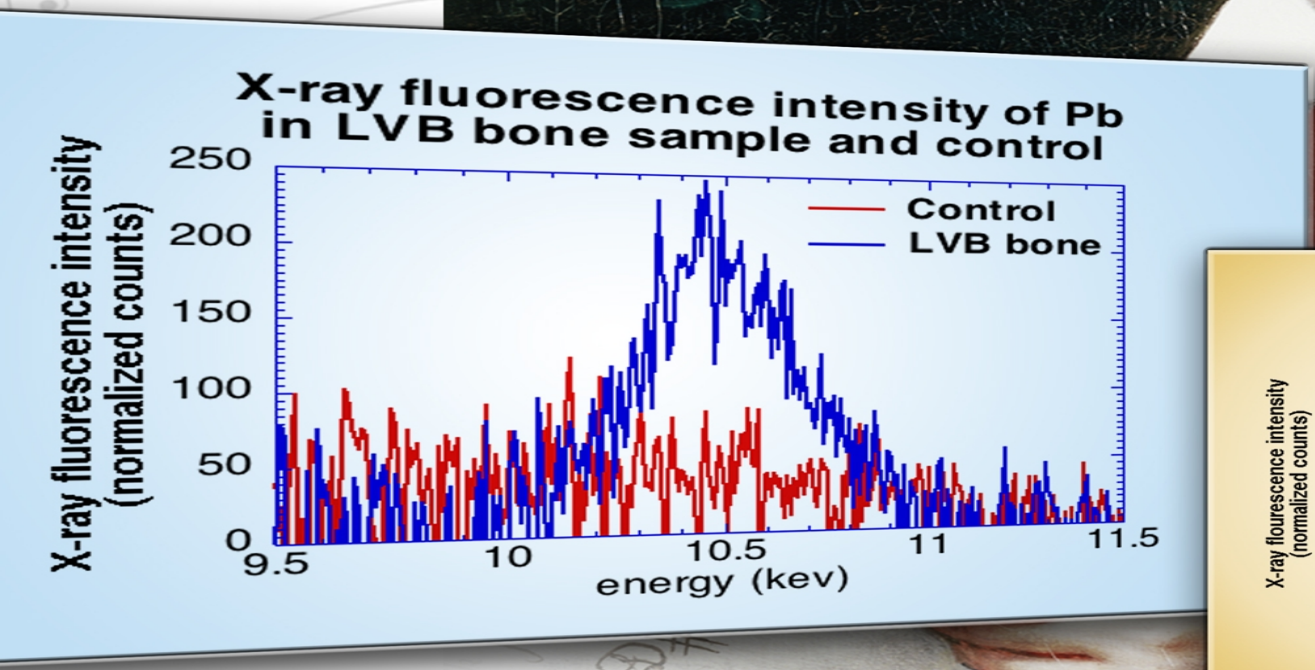
- Testing of 198 coded samples completed,
- Abnormal overloads of specific elements found throughout autism brains and not in the controls,
- The abnormalities are different for male and female autistic subjects, suggesting that male and female autism may have different genetic origins.

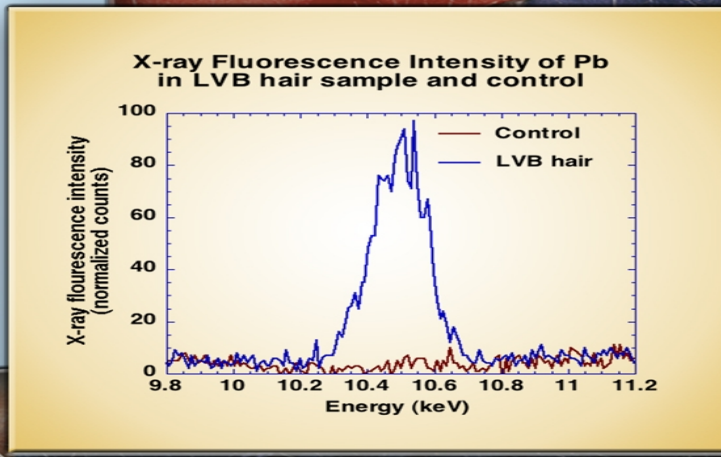
Alzheimer Brain Tissue Assays

- Collaboration with Argonne and LSU,
- Chemical analysis of 10-micron-thick tissues from AD subjects and controls,
- Large circular Ca-rich regions found in AD samples (15 times Ca levels in adjacent areas); Controls had Ca-rich circles that were much smaller in diameter,
- Elevated Cu/Zn regions observed only in the AD samples.

ARGONNE NATIONAL LABORATORY

LEAD LEVELS IN BEETHOVEN SAMPLES





THE UNIVERSITY OF CHICAGO

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Research Goal

- Elemental analyses at hundreds of locations within individual brain cells.
- Identification of intra-cell chemical abnormalities for brain disorders.

THANK YOU!

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