THE COLLISION OF UNDERMETHYLATION, EPIGENETICS, AND OXIDATIVE STRESS IN AUTISM SPECTRUM DISORDERS

William J. Walsh, Ph.D.
Walsh Research Institute
Naperville, IL

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 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 imbalances more severe than in violent behavior, depression, and schizophrenia.
- Discovery of hypomethylation in >95% of persons in the autism spectrum (1999),
- Evidence of oxidative stress & metallothionein depletion (2000).

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.

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

Autism Brains Are Different

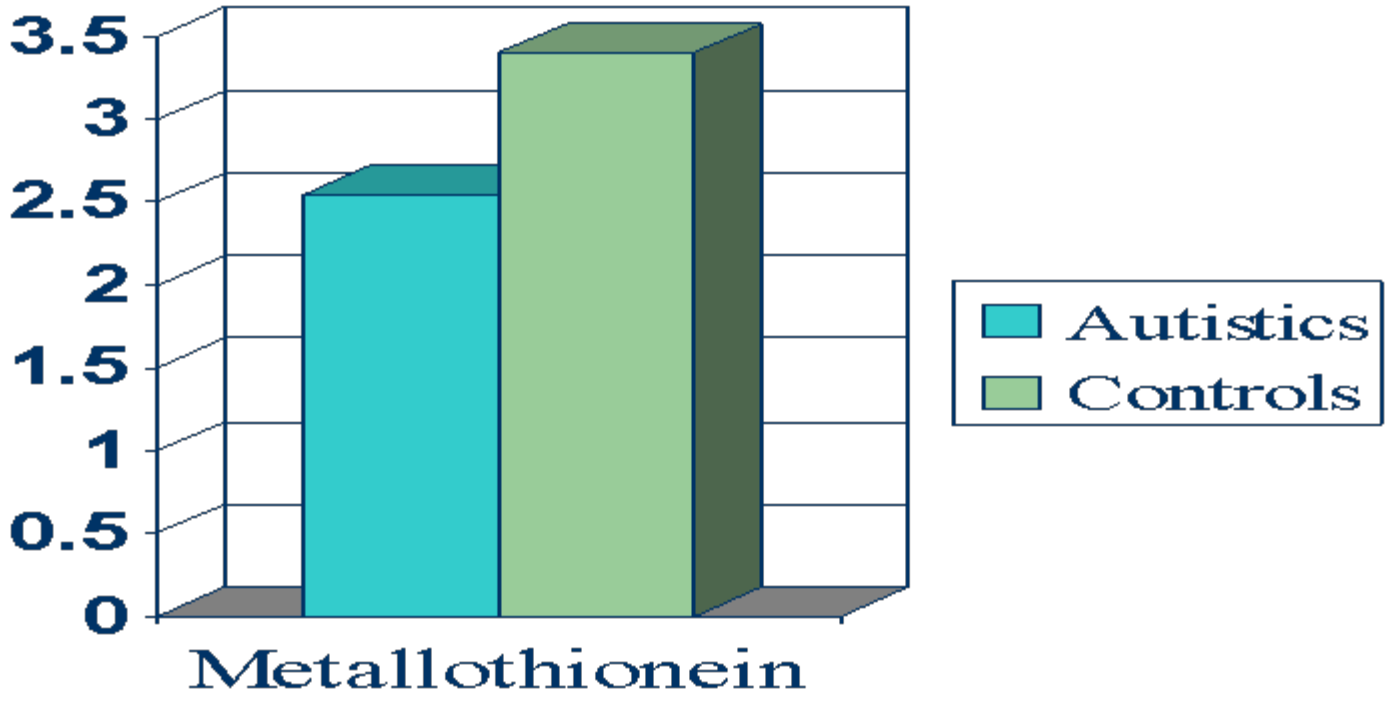
- Narrowed minicolumns in brain cortex,
- Incomplete maturation in cerebellum, amygdala, pineal gland and hippocampus,
- Poverty of brain dendrites and synapses,
- Brain inflammation and increased head size,
- Damaged fats in autism brains,
- Abnormal levels of calcium and iron,
- Reduced structural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

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.

Low Metallothionein Levels in Autism

$p < 0.0092$



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.

The McGinnis Hypothesis

- The brain-stem area receives little or no protection from the blood-brain barrier,

- This provides an avenue for oxidative damage to developing autism brains, caused by toxic metals, viruses, etc.

Note: This may explain immaturity in the limbic system & cerebellum not observed in other brain regions.

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

- Clear inborn 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 110 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. Inborn 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 28 environmental insults have been proposed, including mercury exposures, vaccines, changes in diet, viruses, increased Cu in the water supply, etc, etc.

A New Explanation - Epigenetics

- Environmental 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.

Epigenetics

- Altered gene expression without changes in DNA sequence,
- Abnormal chemical environment during in-utero bookmarking of genes,
- Post-natal gene expression changes resulting from toxics or chemical imbalances,
- Two major epigenetic mechanisms:
  - -- Direct DNA methylation
  - -- Histone modification

Epigenetic Processes During Early Fetal Development

- In utero chemical environment determines which genes will be expressed or inhibited throughout life (bookmarking),
- Gene expression errors can be transmitted to future generations by a process called transgenerational epigenetic inheritance (TEI),
- Methylation is a dominant factor in TEI, and is abnormally low in autistic children.

“Epigenetics”

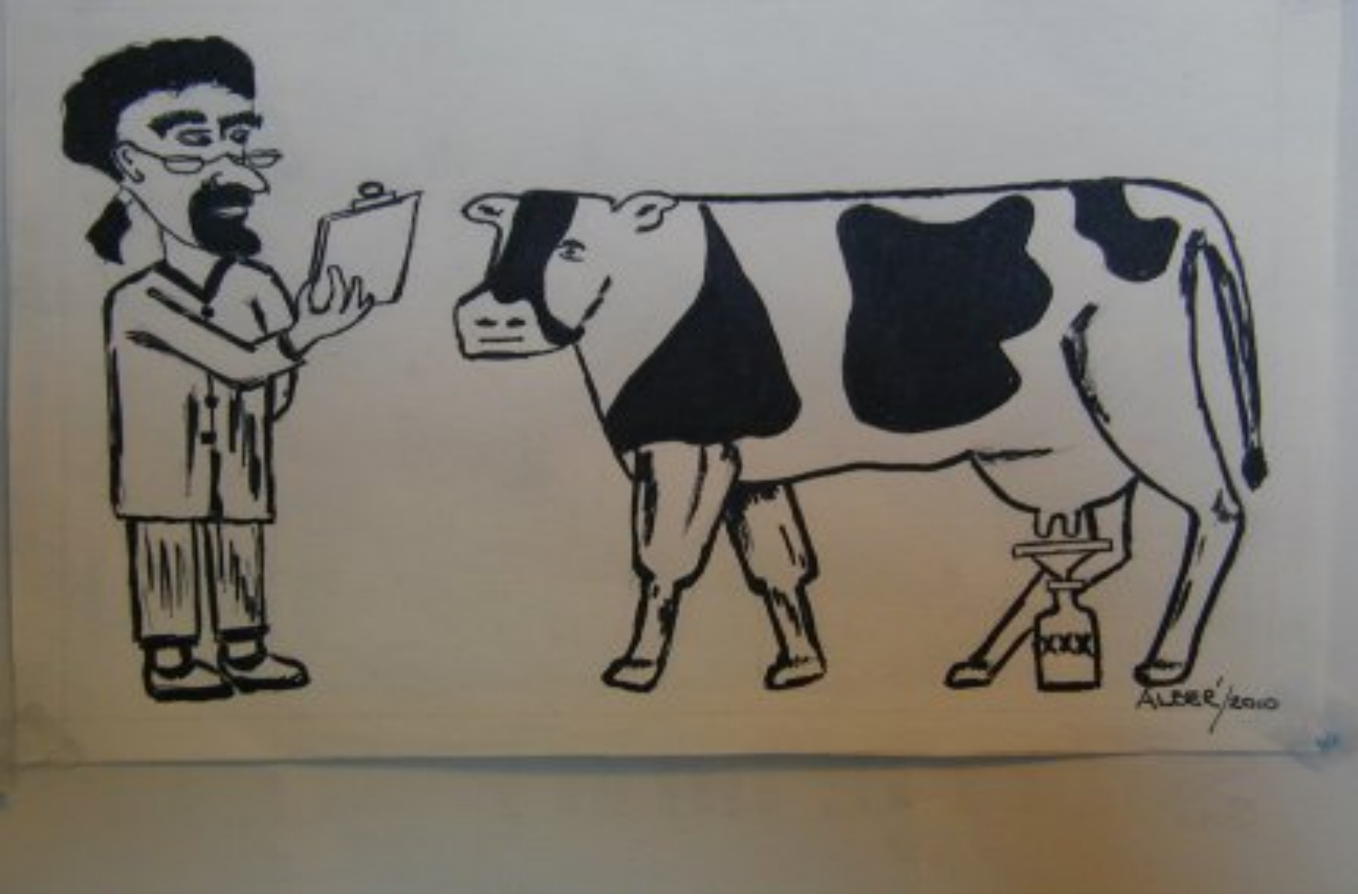
A Limerick by George Marino

Said a scientist once feeling frisky

I know altering genes can be risky

but I want to learn how

to develop a cow….



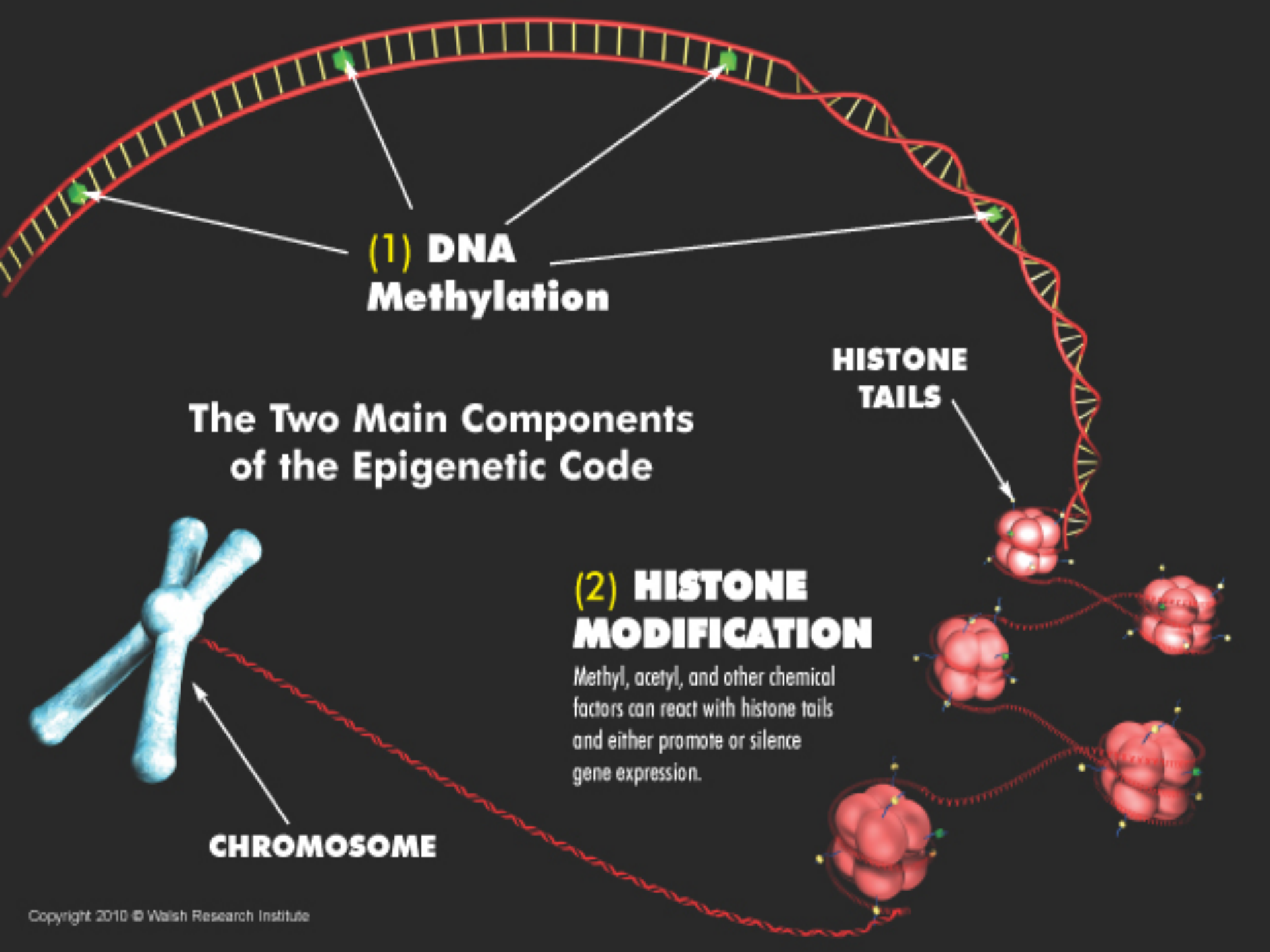
That will stop giving milk and give whiskey.

Undermethylation Enclaves and Increasing Autism Rates

- Undermethylation is associated with OCD, perfectionism & high career accomplishment,
- High frequency for doctors, lawyers, CEO's, scientists, great athletes; also in affluent neighborhoods and universities,
- Increased social mobility in the past 50 years has resulted in increasing numbers of low-methyl persons who marry each other,
- Undermethylated parents are more vulnerable to epigenetic insults that can cause autism.

Histones

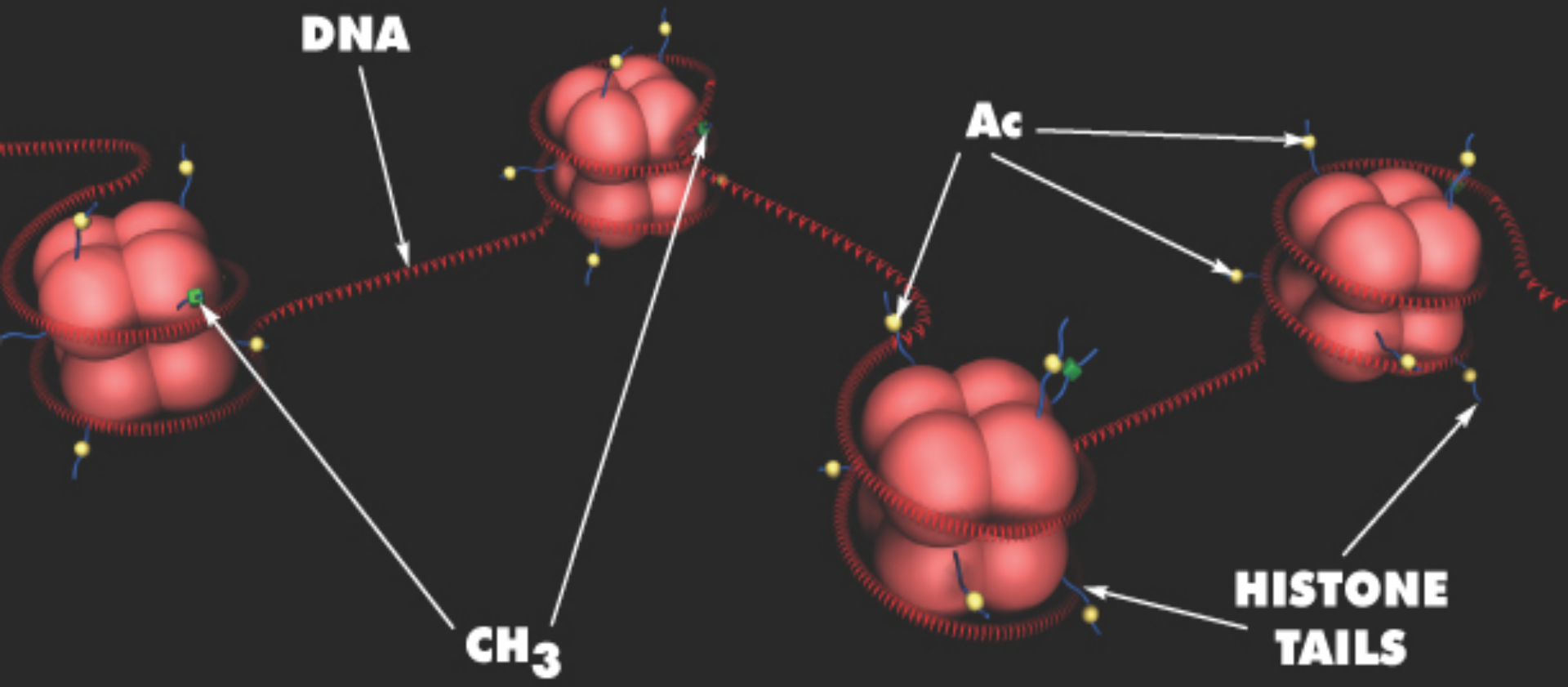
- Composed of 8 linear proteins twisted together like a ball of yarn,
- Originally believed to serve only as structural support for DNA packaging,
- Later found to inhibit/promote gene expression depending on chemical reactions at histone tails, that alter electrostatic attraction to DNA's double helix,
- Complex histone code under development.



Methyl-Acetyl Competition

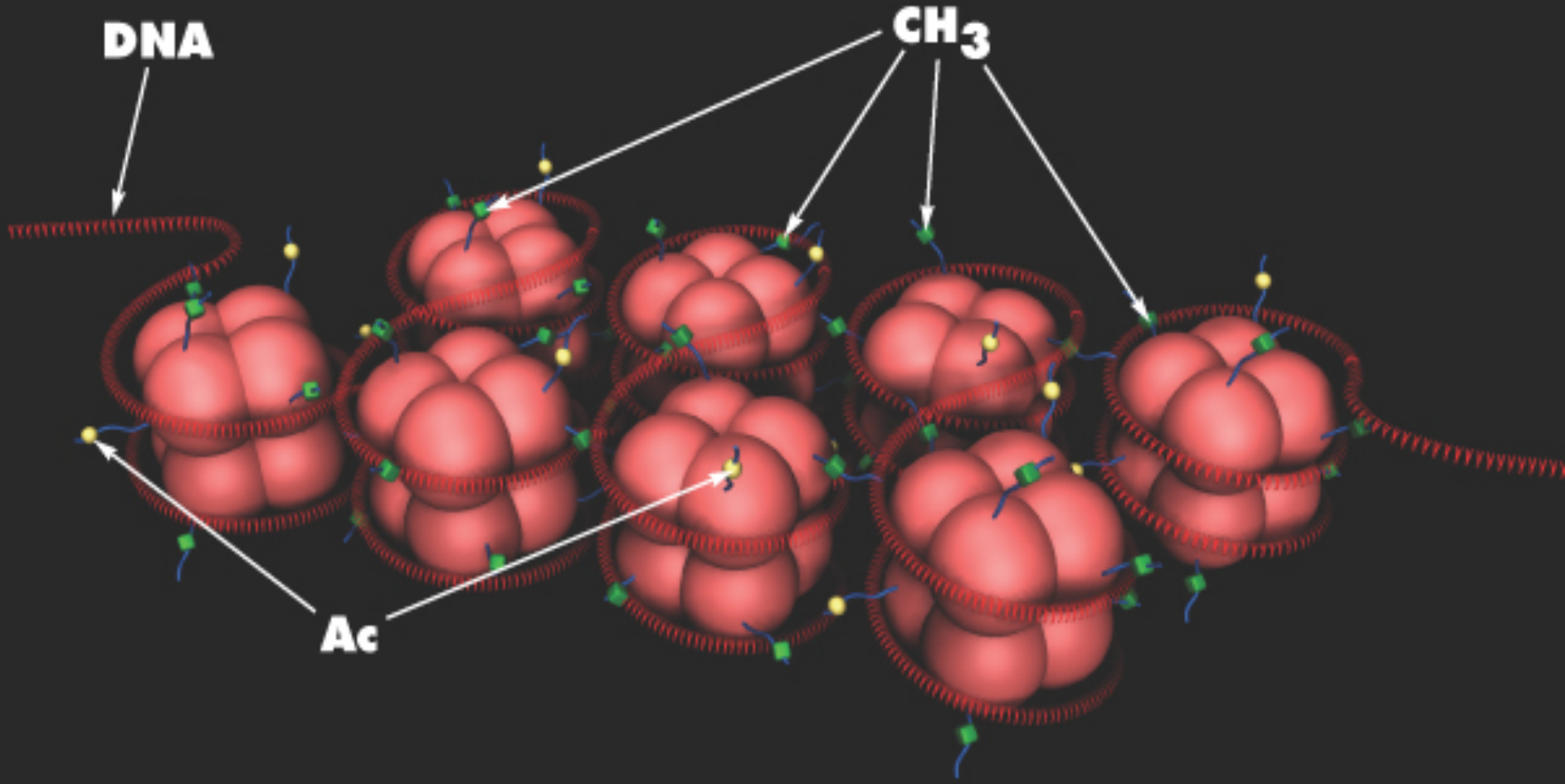
- Competition between acetyl and methyl groups at histone tails often determines whether genes are expressed or silenced,
- Acetylation tends to promote gene expression,
- Methylation generally inhibits expression.

LOW METHYLATION PROMOTES GENE EXPRESSION



OPEN CHROMATIN

HIGH METHYLATION INHIBITS GENE EXPRESSION



CLOSED CHROMATIN

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,
- Most epigenetic decisions regarding gene expression or inhibition are established at this time,
- This suggests the greatest vulnerability to autism-causing environmental insults may be during in-utero epigenetic bookmarking.

Epigenetic versus Genetic

1. Epigenetic processes are far more vulnerable to toxic metals, viruses, etc., compared to genetic processes,

2. Epigenetic errors are enhanced by abnormal methylation,

3. Nearly all autism spectrum persons are undermethylated,

4. Epigenetic errors may be passed on to future generations.

Epigenetic Errors in Autism

1. Nguyen et al; Identical twin study (FASEB Journal, April 2010): Finding: Abnormal methylation at DNA CpG sites.
2. Beaudet (2007), Nat. Med. 13, 534-536.
3. Schanen (2006), Hum. Mol. Genet. 15(2), R138-R150.
4. Nakayama, et al (2006), NSSYZ Journal (Japanese).

CONCLUSIONS

1. EPIGENETIC ERRORS MAY BE THE PRIMARY CAUSE OF AUTISM.

2. EPIGENETIC ERRORS CAN BE IDENTIFIED IN INFANCY.

3. NEW THERAPIES TO REVERSE EPIGENETIC ERRORS ARE VERY PROMISING.

Other 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

- Recent evidence of neurodegeneration in autism…. attributed to severe oxidative stress,
- Gradual loss of brain cells and IQ may occur if antioxidant therapy is not provided,
- Young autistics appear very bright despite behavioral, speech, and socialization deficits,
- Most adult autistics exhibit mental retardation (exception: Aspergers patients).

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.

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!!

An Epigenetic Theory of Autism

1. Fetal undermethylation promotes epigenetic errors,
2. In-utero environmental insults alter epigenetic bookmarking producing weakened defenses against oxidative stress,
3. Oxidative insults gradually deplete GSH, MT, SOD, catalase, and other protective factors,
4. 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 (the regression event),

Result = Autism

The Bermuda Triangle of Autism

- Hypomethylation
- Epigenetic errors, triggered by environmental insults,
- Oxidative Stress

A Strategy for Enhanced Cognition, Speech, and Socialization

- Powerful antioxidant therapy,
- Methylation protocols,
- Biochemical therapies aimed at reversing epigenetic errors,
- Therapies that enhance brain maturation.

Potential Epigenetic Therapies

- Methylation normalization (SAMe),
- Deacetylase promotion,
- Acetyl-CoA adjustments,
- Therapies to stimulate or suppress DNA promoter regions,

We must never forget….

Autism is Treatable

Recovery is Possible

THANK YOU!

Bill Walsh, PhD

Walsh Research Institute

Naperville, Illinois

www.walshinstitute.org