Autism and Epigenetics
What’s the connection?

AUTISM

Rett Syndrome
*MECP2* mutation

Angelman, Prader-Willi, 15q syndromes
15q11-13 deficiency or duplication
Autism

- Complex developmental disorder that usually appears in first three years of life

- Not a single disorder but a spectrum of neurodevelopmental disorders characterized by:
  - Impairments in social interactions and communication
  - Impairments in language
  - Restrictive and repetitive interests and behaviors
Autism

- **Regressive autism**: apparently normal infancy followed loss of reciprocal social interactions, loss of language, gain of stereotypical behaviors around 18 mo to 4 years of age
- **Early onset autism**: no apparent loss of language or social interactions
- Male bias for autism 4:1; Asperger’s 10:1
Autism most likely results from alterations in brain development and maturation due to a combination of genetic and environmental factors.
Genetics of Autism

• Strong genetic component to risk for autism:
  - Family studies: 50x greater risk for sibs of children with autism compared to the general population.
  - Identical twin studies
    - MZ concordance = 60-90%
    - DZ concordance = 0-10%
  - $H^2 > 90\%$

But genetic basis is likely complex; multiple approaches are needed
Loci identified by genome scans that might increase risk of autism

Folstein and Rosen-Sheidley, 2001, Nature Reviews Genetics
• The majority of autism cases are a result of *de novo* mutations, occurring first in the parental germ line.

• For reasons yet to be determined, female offspring are considerably more resistant to displaying the effects of such mutations than are males.

• Resistant individuals, but females in particular, carrying a mutation may marry and, with a probability of 50%, pass the mutation to their offspring, who will display the symptoms with high probability if male.
Is autism prevalence on the rise?

California’s Developmental Services System
*Schechter and Grether, 2008*

Is this an increase in diagnosis, prevalence, or both?
Rett Syndrome

• Rett syndrome is the only one of the pervasive developmental disorders with a single known genetic cause
  – DSM IV Pervasive Developmental Disorders:
    • Autism
    • Asperger syndrome
    • Childhood disintegrative disorder
    • Rett syndrome
    • PDD-NOS
Rett Syndrome

- X-linked dominant, ~80% MECP2 mutation
- ~1/10,000 in US population
- Neurodevelopmental regression around 6 to 18 months of age
- MECP2 encodes a known epigenetic factor, methyl CpG binding protein 2

Rett syndrome involves epigenetics at 2 levels
Clinical Progression of Rett syndrome

Chahrou and Zoghbi, Neuron, 2007
MeCP2 is a marker for mature neurons in the post-natal mammalian brain.
MeCP2 appears to have multiple roles in regulating gene expression in neurons

Activity dependent gene regulation

Regulation of alternative splicing

Chahrou and Zoghbi, Neuron, 2007
### Genetic and environmental interactions in regressive autism

**What Rett syndrome reveals**

<table>
<thead>
<tr>
<th>Rett syndrome</th>
<th>MECP2 mt</th>
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<tbody>
<tr>
<td>In utero</td>
<td>Normal infancy</td>
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<tr>
<th>Regression</th>
<th>Autism/MR</th>
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<tr>
<td>In utero</td>
<td>Normal infancy</td>
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**Environmental exposures**

Etiologic environmental exposures in autism could be causitive (thalidamide, valproate, Rubella), or additive to genetic susceptibility (likely to be more common).

Long-term effects from in utero exposures could alter epigenetic mechanisms, leading to behavior and cognitive dysfunction in the child and adult.
Epigenetics

DNA methylation

Histone modifications

Chromatin structure

Spatial organization of chromosomes

Inherited and reversible modifications to nucleotides or chromosomes that do not change the sequence but can alter gene expression

Kosak and Groudine, 2004

Horn and Peterson, 2002

Cook, 1999

Kosak and Groudine, 2004
Epigenetics

Examples of epigenetic mechanisms

X chromosome inactivation

Calico cats are females and are mosaics of cells expressing black and orange coat colors.

Rett girls are mosaics of cells expressing mutant MECP2.
Epigenetics

Examples of epigenetic mechanisms

Parental Imprinting

Angelman syndrome

Prader-Willi syndrome

Chromosome 15

15q11-13
Epigenetics

Examples of epigenetic mechanisms
Tissue-specific and developmental differences in gene expression
Epigenetics

Examples of epigenetic mechanisms
Environmental effects on gene expression

Bisphenol A (BPA)
Bisphenol A (BPA) + folic acid

Dolinooy et al, PNAS, 2007
Epigenetic disorders on the autism spectrum

• The imprinted disorders Prader-willi and Angelman syndromes are on the autism spectrum.
  – 2-42% of AS and PWS cases have comorbid autism, depending on study
  – Uniparental disomy cases of PWS may be more frequently autistic

• Maternal 15q11-13 duplications are the most common cytogenetic cause of autism (1-3%)
Angelman and Prader-Willi syndromes

Imprinted disorders caused by 15q11-13 deletions or deficiency (~1/20,000)

AS: Maternal 15q11-13 deletion, paternal disomy, maternal UBE3A mutation, imprinting defects

PWS: Paternal 15q11-13 deletion, maternal disomy, imprinting defects
Parental Imprinting and Mammalian Reproductive Technologies

- Many cloned livestock exhibit “large offspring syndrome” due to dysregulated expression of Igf2.
- Cloned mice and embryonic stem cells have many epigenetic defects in imprinted genes.
- Human ES cell lines exhibit altered methylation patterns compared to normal human tissue.
- Human children from in vitro fertilization (IVF) have increased rates of Angelman and Beckwith-Wiedemann syndromes.
The Rosetta Stone approach to “decoding” the complex genetics and epigenetics in autism

Autism

Angelman

Rett

Autism Tissue Program
The Gift of Hope
Evidence for epigenetic overlap between autism, RTT, and AS

• MeCP2 expression is significantly reduced in 79% of autism post-mortem brain samples
• Methylation of the MECP2 promoter correlates with reduced expression in male autism brain samples
• GABRB3 expression (15q11-13) is significantly reduced in 56% of autism post-mortem brain samples
• Biallelic expression levels of GABRB3 are epigenetically dysregulated in Rett and autism postmortem brain
• Homologous pairing of 15q11-13 in mature neurons is deficient in RTT, autism, and AS
MeCP2 binds to the imprinting control region of 15q11-13 and regulates *UBE3A* and *GABRB3* expression.

ChIP-chip analysis of MeCP2 binding at *SNRPN* and 62 additional sites within 13 MB of 15q11-13

*MECP2* mutation or deficiency does not alter imprinted expression, but reduces levels of *UBE3A* and *GABRB3*

*Samaco et al., 2005*

*Yasui et al., 2007*
Reduced MeCP2 in autism frontal cortex correlates with aberrant methylation

Nagarajan et al, Epigenetics, 2006
Identification of a methylation boundary element upstream of MECP2 bound by CTCF

Nagarajan et al, Autism Research, in revision
GABRB3 expression positively correlates with MeCP2

Significant correlation between MeCP2 and GABRB3 protein levels suggests that MeCP2 positively regulates GABRB3 expression.

R² = 0.5772
Nonimprinted GABRB3 is epigenetically dysregulated in a subset of autism and Rett syndrome brains

Hogart et al, Hum. Mol. Genet., 2007
What is the future for epigenetics and autism?

Defining precise genetic and environmental risk factors and develop tests for precise epigenetic alterations.
Future directions examining environmental pollutants on epigenetics in neurodevelopment

Animal model component

MeCP2

GABRB3

UBE3A

BDE-47

PCB-95

MeCP2$^{308/+}$

MeCP2$^{308/y}$

Social behavior
Cognition
Seizures

Human subject component

MeCP2

GABRB3

UBE3A

MeCP2$^{308/+}$

MeCP2$^{308/y}$

Future directions examining environmental pollutants on epigenetics in neurodevelopment
Epigenetic interaction of MECP2 and organic pollutants in neurodevelopment

Perinatal exposure
BDE-47
4 w prior/ 3 w in utero/ 3 w lactation

0.03 mg/kg/day
1 mg/kg/day
vehicle control

0.03 mg/kg/day
1 mg/kg/day
vehicle control

Test perinatally exposed mice for social and cognitive behavior
Test mouse brains for epigenetic changes in MeCP2, UBE3A, global DNA methylation and histone modifications, etc

12 different treatment x genotype categories

$Mecp2^{308/+}$  $C57Bl6/J$

$Mecp2^{+/+}$  $Mecp2^{308/+}$  $Mecp2^{+/y}$  $Mecp2^{308/y}$
Behavioral Testing

Growth & Reflex Assessment

**Ultrasonic Vocalization Measurement**

Sociability Test

Social Dyadic Interaction

**Acoustic Startle and Pre-Pulse Inhibition test**

Social Transmission of Food Preference

Elevated Plus Maze

Locomotor Activity Integra

Spatial Memory and Learning in the Water Maze
Preliminary evidence of epigenetic changes with perinatal BDE-47 exposure
Irva Hertz-Picciotto, PI

Comprehensive, collaborative evaluation of autism
- Medical evaluations
- Environmental exposures/epidemiology
- Behavior and neuropsychology
- Genomics
- Brain structure/imaging
- Immune function
- Epigenetics

DNA samples from four diagnostic categories
- Early onset autism
- Regressive autism
- Developmental delay
- Typically developing controls

Parental DNA also available
Epigenetic analyses on human samples

**CHARGE blood DNA samples**
- X chromosome inactivation
- DNA methylation at chromosome 15 imprinting control regions
- *MECP2* promoter methylation

**Human postmortem brain samples**
- X chromosome inactivation
- DNA methylation at chromosome 15 imprinting control regions
- *MECP2* promoter methylation
- MeCP2 and GABRB3 expression

Correlate epigenetic changes with PBDE tissue levels
No evidence for X chromosome inactivation skewing differences between mothers of males with autism

<table>
<thead>
<tr>
<th></th>
<th>number of uninformative samples (%)</th>
<th>avg age (y)</th>
<th>avg small allele size (bp)</th>
<th>avg large allele size (bp)</th>
<th>avg % skewing&lt;sup&gt;a,b&lt;/sup&gt;</th>
<th>avg inactive allele size (bp)&lt;sup&gt;a,c&lt;/sup&gt;</th>
<th>% mothers with &lt;5% or &gt;95% skewing&lt;sup&gt;a&lt;/sup&gt;</th>
<th>% mothers with &lt;15% or &gt;85% skewing&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Typical Development</td>
<td>23 (5)</td>
<td>33</td>
<td>274</td>
<td>286</td>
<td>53</td>
<td>280</td>
<td>11</td>
<td>17</td>
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<tr>
<td>Delayed Development</td>
<td>24 (3)</td>
<td>32</td>
<td>275</td>
<td>287</td>
<td>45</td>
<td>282</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>Autism</td>
<td>27 (2)</td>
<td>35</td>
<td>276</td>
<td>288</td>
<td>46</td>
<td>284</td>
<td>8</td>
<td>16</td>
</tr>
<tr>
<td>ASD</td>
<td>25 (3)</td>
<td>36</td>
<td>277</td>
<td>286</td>
<td>60</td>
<td>280</td>
<td>9</td>
<td>18</td>
</tr>
</tbody>
</table>

Notes:
- <sup>a</sup> - for informative samples
- <sup>b</sup> - percent of cells with small allele inactive
- <sup>c</sup> - average of allele sizes (bp) of the alleles that are inactivated > 50%

PBDEs protective for XCI skewing?
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Human tissue samples
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