Epigenetics and multiple risk factors in development: Perspectives from autism research

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Autism spectrum disorders

- 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

Challenge for research: Changing diagnoses and lack of molecular tests

DSM IV to DSM V: one heading termed “Autism Spectrum Disorders”
Genetics of autism spectrum disorders and the ongoing nature vs. nurture debate

**Heritability estimates of Autism**

**Twins**
- 90% based on a small monozygotic versus dizygotic twin study in 1960s (Steffenberg et al, 1989)
- 70-90% from a variety of larger twin studies pre-2010

- 38% based on 2011 study that separated calculated effect of shared in utero environment (58%) (Hallmayer et al, 2011)

**Siblings**
- 50-100x greater risk for sibs of children with autism compared to the general population

Most recent and largest family analysis from a population of 2 million in Sweden put estimate of 50% heritability, suggesting a 50% “environmental” or non-genetic component

**ASDs are complex genetic disorders**
- Multiple known genetic causes of ASD, but each contribute to at most 1% of ASDs
Is autism incidence on the rise?

California’s Developmental Services System
Hertz-Picciotto and Delwiche, *Epidemiology, 2009*

Only 200% of the 600% increase can be explained by increased diagnosis
Gene x environment interactions in autism risk
Epigenetic mechanisms act at the interface of genes and the environment.

Genetic
- Single gene mutations
- Copy number variations
- Single gene polymorphisms
- XY male

Environmental
- In utero Rubella
- In utero thalidomide
- Maternal age
- Paternal age
- Grandparental age
- Maternal obesity
- Maternal inflammation
- Proximity to freeways
- Pesticide exposure

Risk
- XX female (Female Protective Effect)

Protection
- Periconceptional folic acid supplements
Prenatal Vitamin Use by Perinatal Month

Months Before or During Pregnancy

Percent Reporting Prenatal Vitamin

OR = 0.6 (95%CI: 0.4 to 0.9)

Schmidt et al, 2011 *Epidemiology*
Absence of folic acid at conception modifies autism risk with multiple environmental exposures

data from the CHARGE case-control study

<table>
<thead>
<tr>
<th>Environmental Exposure During Pregnancy</th>
<th>800+ mcg FA Preg Month 1 aOR (95% CI)</th>
<th>&lt;800 mcg FA Preg Month 1 aOR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Traffic-Related Air Pollution (Total Nox)</td>
<td>No</td>
<td>Reference</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>1.25 (0.79-1.98)</td>
</tr>
<tr>
<td>Any Exposure to Sprays/Foggers</td>
<td>No</td>
<td>Reference</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>1.65 (1.06-2.55)</td>
</tr>
<tr>
<td>Regular Use of Sprays/Foggers (6+ months)</td>
<td>No</td>
<td>Reference</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>2.27 (1.29-4.00)</td>
</tr>
<tr>
<td>Pregnancy Chlorpyrifos</td>
<td>No</td>
<td>Reference</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>0.75 (0.36-1.55)</td>
</tr>
<tr>
<td>Pregnancy Organophosphates</td>
<td>No</td>
<td>Reference</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>0.87 (0.51-1.50)</td>
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<tr>
<td>Pre-conception Pyrethroids</td>
<td>No</td>
<td>Reference</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>0.95 (0.45-2.00)</td>
</tr>
</tbody>
</table>
400 Mothers of a child with autism planning pregnancy or pregnant with another child

Recurrence rate is ~ 1 in 5 (Ozonoff et al 2011)

EEQ, FFQs  Biologic Samples  Parent Forms & Clinical Assessments
CCEH Project 2- Center interactions

Birgit Puschner

Toxicant Exposure: Core C

Project 1. Epidemiology, Environment, and Bio-Informatics
Irva Hertz-Picciotto

Project 2. Perinatal epigenetic signatures of environmental exposures

Project 3. Immune Environment Interactions and Neurodevelopment
Judy Van de Water
Paul Ashwood

Maternal Exposure immune profile- MARBLES:

Child Exposure immune profile-CHARGE and MARBLES

Project 4. Mechanism of changes in calcium signaling
Isaac Pessah
Pam Lein
MARBLES analysis of folic acid protection and global DNA methylation

Folic Acid Intake (mcg): By Month

\[ P = 0.02 \]

LINE1 DNA methylation by Folic Acid

\[ N=41 \]
(17 TD, 8 ASD, 16 Other Dx)

\[ P=.02 \]
Environmental impacts on the brain methylome
An integrative genome point-of-view

LaSalle, Epigenetics, 2011
Apply genomic and bioinformatic tools to investigate MARBLES placenta and cord blood samples

- Assess the global impact on DNA methylation over partially methylated domains and repeats
- Find specific differentially methylated regions associated with environmental exposure and/or ASD diagnosis

Methyl-C Sequencing
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Brain Tissues
Jane Pickett
Autism Speaks ATP
NICHD BTB
Mapping the methylome in brain and placenta
The landscape feature of partially methylated domains in early life
## Prenatal vitamin protection in MARBLES

<table>
<thead>
<tr>
<th>Prenatal Vitamin ≤ P1</th>
<th>ASD N (%)</th>
<th>Typical N (%)</th>
<th>RR (95% CI)</th>
<th>( P )</th>
<th>( \text{adj}_{\text{RR}^*} (95% \text{ CI}) )</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>5 (24)</td>
<td>42 (62)</td>
<td>Reference</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>No</td>
<td>16 (76)</td>
<td>26 (38)</td>
<td>3.7 (1.5, 9.1)</td>
<td>.005</td>
<td>4.0 (1.5, 10.0)</td>
<td>.003</td>
</tr>
</tbody>
</table>
MARBLES

Plasma: 422 Homocysteine
127 mothers, 19 children

Homocysteine

Nutrient Status
Folate
B–Complex Vitamins

Food Frequency Questionnaires:
184 Analyzed
Folate, B12, B6, choline, Hcy

Serum/Plasma:
432 serum, 422 plasma
127 mothers, 19 children
Folate, B12, B6, Choline, Hcy

DNA Methylation

Child Neurodevelopment
Autism, ASD
Other Neurodevelopmental Outcomes

Whole Blood/Plasma:
LINE-1 344 samples, 599 more selected

Placentas:
Pyrosequencing

Cord Blood:
Pyrosequencing

Completed Visits
130 36-month
MARBLES

Markers of Autism Risk in Babies: Learning Early Signs
PI: Irva Hertz-Picciotto

400 Mothers of a child with autism planning pregnancy or pregnant with another child

Nutrient Status

Folate, B12, B6, Choline

Biologic Mechanisms

DNA Methylation

Child Neurodevelopment

ASD

Other Outcomes

Parent Forms and Clinical Assessments

Maternal & Child Serum

FFQs