What is Autism?

• Neurodevelopmental disorder with three defining domains of deficit:
  – Social reciprocity
  – Communication
  – Repetitive behaviors or restricted interests

• Symptoms present by 36 months of age

• Considered to be lifelong, no cure
Facts about Autism

- **Prevalence in U.S.**: 1 in 110
- **Sex ratio**: 4:1

**Neurobiologic basis**: aberrant brain development
- Post-mortem autopsy
- Electrophysiologic
- Magnetic Resonance Imaging and fMRI

**Highly heritable**
- Twin studies
- Family recurrence
- Candidate gene
- GWAS (Genome-wide association studies)
What Causes Autism?

- Genes
- Advanced Parental Age (clues rather than causes)
- Obstetric/Perinatal factors
- Prenatal Infections (Reubella, Chess et al 1979)
- Medications (Thalidomide, SSRIs Croen et al 2011)
- Pesticides (Roberts et al 2007, Eskenazi et al 2007)
- Closely Spaced Pregnancies (Cheslack-Postava et al 2010)
- Month of Conception (Zerbo et al 2011)
The CHARGE* Study

*Childhood Autism Risks from Genetics and the Environment

PI: Irva Hertz-Picciotto

First comprehensive study of environmental factors in autism
The CHARGE Study Design: Case-Control - 3 Groups

1. Children with autism
2. Children with developmental delay
3. Children drawn from general population of births, frequency matched to projected distributions, in cases, of age, gender & geography
Eligibility for All 3 Groups

- 24-60 months
- Born in California
- English / Español
- Living in study catchment area
Diagnoses Confirmed

- Clinic visit:
  - Autism Diagnostic Observation Schedule (ADOS)
  - Autism Diagnostic Inventory – Revised (ADI-R)

- Medical examination and history

- Broad assessment of behavior, development, co-morbidities
Data Collection

- Interview: lifestyle, demographics, medical conditions, reproductive hx, residential hx, etc.
- Permission to obtain medical charts:
  - prenatal
  - labor and delivery
  - pediatrician
  - fertility clinics
- Collect specimens from family members:
  - blood
  - urine
  - hair
1. Traffic-Related Air Pollution
Residential Proximity to Freeways

• Proxy for Traffic-Related Air Pollution
• Calculated distance to nearest freeway from maternal address at time of delivery
• ArcGIS software

Volk et al, Environ Health Persp 2010
## Residential Proximity to Freeways
(a proxy for air pollution exposure)

<table>
<thead>
<tr>
<th>Distance to Freeway at Delivery</th>
<th># Cases / # Controls</th>
<th>Adjusted OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;309m (closest 10%)</td>
<td>38 / 19</td>
<td>1.86</td>
<td>1.03, 3.45</td>
</tr>
<tr>
<td>309-647m (10th to 25th %)</td>
<td>43 / 41</td>
<td>0.96</td>
<td>0.58, 1.56</td>
</tr>
<tr>
<td>647-1419m (25th to 50th %)</td>
<td>77 / 63</td>
<td>1.11</td>
<td>0.73, 1.67</td>
</tr>
<tr>
<td>&gt;1419m (farthest 50%)</td>
<td>146 / 136</td>
<td>reference</td>
<td>--</td>
</tr>
</tbody>
</table>

*Adjusted for child’s sex and ethnicity, parental education, and maternal age and smoking

Volk et al, Environ Health Persp 2010
Comments

- Components of traffic-related air pollution (benzo(a)pyrene; diesel, O$_3$) induce neurodevelopmental deficits in rodents.

- Polycyclic Aromatic Hydrocarbons (PAHs) → oxidative stress, inflammation, endocrine disruption, immunologic alterations.

Volk et al, Environ Health Persp 2010
1. Air Pollution
2. The Immune System
Immunology Findings

• Elevated leptin levels in children with autism. 

• Lower overall IgG in children with autism 
  (Heuer et al, Autism Res 2008)

• Higher concentrations of IgG4 subclass in peripheral blood 
  (Enstrom et al, Brain, Behav Immun 2008)

• Reduced TGF-beta (regulates immune responses) 
  (Ashwood et al, J. Neuroimmunol 2008)

• Abnormal NK (natural killer) cell functioning: over-
  stimulated, but less able to respond to specific challenge 
  (Enstrom et al, Brain, Behav Immun 2008)

• Autoantibodies to cerebellum in children with ASD 
  (Goines et al, Brain, Behav Immun 2011)

Immune dysregulation in children with autism
Maternal Antibodies to Fetal Brain Tissue

About 7% of mothers of children with autism produce specific antibodies to fetal brain tissue compared to 0% of mothers of children with TD.

Braunschweig et al., JADD 2011
• Direction of connection to autism unclear:
  – immune aberrations could be downstream or upstream of neuropathology or neither

• Environmental factors could work through immune pathways (rather than direct effects)

• Not solely a disorder of the CNS
1. Air Pollution
2. The Immune System
3. Maternal Metabolic Conditions
## Maternal Metabolic Conditions

<table>
<thead>
<tr>
<th>Conditions in index pregnancy</th>
<th>ASD vs. TD OR† 95% CI</th>
<th>DD vs. TD OR† 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetes (type 2 or gestational)</td>
<td>1.5 (0.8, 2.9)</td>
<td>2.5 (1.1, 5.5)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>3.1 (1.1, 8.8)</td>
<td>5.3 (1.5, 18.6)</td>
</tr>
<tr>
<td>Obesity</td>
<td>1.7 (1.1, 2.6)</td>
<td>2.1 (1.2, 3.7)</td>
</tr>
<tr>
<td>Diabetes, hypertension, and/or obesity</td>
<td>1.6 (1.1, 2.4)</td>
<td>2.5 (1.5, 4.1)</td>
</tr>
</tbody>
</table>

†Multinomial logistic regression models were adjusted for mother’s age at delivery, race/ethnicity, and education, delivery payer, calendar time, and frequency-matching variables.

Krakowiak et al, in press Pediatrics
Insulin Resistance and Fetal Brain Damage

Maternal Metabolic Condition

- ↑ Pro-inflammatory cytokines (e.g. IL-6)
- Maternal IL-6 crosses placenta
- Fetal immune cells in brain stimulated
- ↑ Pro-inflammatory cytokines in fetal brain

Maternal glucose ↑

- Fetal insulin ↑
- ↑ Fetal growth and oxygen consumption
- Fetal hypoxia and iron deficiency

Neuronal damage
CHARGE STUDY RESULTS

1. Air Pollution
2. The Immune System
3. Maternal Metabolic Conditions
4. Maternal Vitamin Intake
Prenatal Vitamin Use by Perinatal Month

Percent Reporting Prenatal Vitamin

Months Before or During Pregnancy

Schmidt et al, Epidemiology 2011

OR = 0.6 (95% CI: 0.4 to 0.9)
Folate, Methionine, and Transmethylation Pathways

Gene x Environment (Vitamin) Interaction

Maternal environment combined with child’s genetic susceptibility could determine child’s risk for autism

Schmidt et al, Epidemiology 2011

<table>
<thead>
<tr>
<th>Child COMT 472 Genotype</th>
<th>Prenatal Vitamin B3-P1</th>
</tr>
</thead>
<tbody>
<tr>
<td>GG+GA</td>
<td>REF</td>
</tr>
<tr>
<td>AA</td>
<td></td>
</tr>
</tbody>
</table>

- YES: 1.3
- NO: 7.2

Synergistic effect of child’s COMT genotype with peri-conceptional supplementation
## Interaction with Maternal Genes

<table>
<thead>
<tr>
<th>Maternal Gene</th>
<th>Genotype</th>
<th>Prenatal Vitamin B3-P1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td><strong>MTHFR 667</strong></td>
<td>CC+CT</td>
<td>REF 1.2</td>
</tr>
<tr>
<td></td>
<td>TT</td>
<td>0.7 4.5</td>
</tr>
<tr>
<td><strong>CBS</strong></td>
<td>GG</td>
<td>REF 1.0</td>
</tr>
<tr>
<td></td>
<td>GT+TT</td>
<td>1.0 2.6</td>
</tr>
<tr>
<td><strong>MTRR 66</strong></td>
<td>AA</td>
<td>REF 0.9</td>
</tr>
<tr>
<td></td>
<td>AG+GG</td>
<td>0.9 1.6</td>
</tr>
<tr>
<td><strong>BHMT 716</strong></td>
<td>GG</td>
<td>REF 1.1</td>
</tr>
<tr>
<td></td>
<td>GA+AA</td>
<td>0.9 1.7</td>
</tr>
<tr>
<td><strong>FOLR2</strong></td>
<td>GG+GA</td>
<td>REF 1.1</td>
</tr>
<tr>
<td></td>
<td>AA</td>
<td>1.0 1.9</td>
</tr>
</tbody>
</table>
Comments

• First to demonstrate gene/environment interactions in autism

• First to suggest a concrete step that may reduce the risk of having a child with autism
Significance of CHARGE:
First comprehensive study of environmental factors in autism

Our focus is on modifiable risk and protective factors - goal is to intervene and prevent autism!

Limitation is retrospective data collection: recall can be faulty, or influenced by child’s condition.
MARBLES

Markers of Autism Risk in Babies—Learning Early Signs

EARLI

Early Autism Risk Longitudinal Investigation

Studies of pregnant moms, who already have a child with autism, following their pregnancies and new child
Investigators & Collaborators

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  Bruce German
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  Virginia Chaidez
  Kristen Lyall

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  Flora Tassone
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- USC (Southern Calif)
  Pat Levitt
  Dan Campbell
  Rob McConnell
  Heather Volk

- Johns Hopkins
  Li-Ching Lee
  Rebecca Harrington
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Special Appreciation To:

• >1600 families who have generously given their time to participate in the CHARGE Study

• Over 250 families who invited us into their homes & lives (repeatedly!) for the MARBLES Study

• Families participating in EARLI
And Thanks to Our Funders:

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- NIEHS 1R01 ES015359
- NIEHS 1R01 ES 015359–S1, S2, S3, S4
- NIEHS 1R01 ES 015171
- NIEHS 1R01-ES020392
- NIH T32-MH073124
- Autism Speaks
- Cure Autism Now
- The Allen Foundation
- National Institute for Occupational Safety and Health
End

Thank you!
Multifactorial Causation

- Genetics: ~38-90% (twin studies)
- Environment: 10-62% = minimum
  …Sum not 100%

Assumes *no interaction with environment*

- across populations *and* within each individual
Seasonality in Autism

Zerbo et al, Epidemiology 2011
Seasonal Variation in Month of Conception

- Implies seasonality of causes
- Time of conception not necessarily critical window
- Potential seasonal causes include:
  - Infectious diseases such as influenza
  - Pesticide exposures
  - Deficiencies: vitamin D, other nutrients

Zerbo et al, Epidemiology 2011
Time Trends in Autism

Quarterly Incidence Rates

Quarterly number of new cases per 10,000 Children

Hertz-Picciotto and Delwiche, Epidemiology 2009
Cumulative Incidence by Birth Cohort

~7-fold increase 1990-2001

Hertz-Picciotto & Delwiche, Epidemiology 2009
The effect of maternal age across strata of paternal age
The effect of paternal age across strata of maternal age

[Diagram showing the effect of paternal age across different maternal age groups with odds ratios (OR) and 95% confidence intervals (CI)].
Goal

• To determine whether current blood Hg levels differ in children with autism or ASD as compared with typically developing (TD) children
## Proportions exposed by case status

<table>
<thead>
<tr>
<th></th>
<th>Autism/ ASD N=249</th>
<th>Dev Delay (DD) N=60</th>
<th>Typical Dev (TD) N=143</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ate any fish</td>
<td>43%</td>
<td>68%</td>
<td>76%</td>
</tr>
<tr>
<td>Dental amalgams and chew or grind teeth</td>
<td>1.5%</td>
<td>2.4%</td>
<td>1.9%</td>
</tr>
<tr>
<td>Thimerosal-containing vaccine in last 90 days</td>
<td>3%</td>
<td>8%</td>
<td>5%</td>
</tr>
<tr>
<td>Nasal spray or earwax removal</td>
<td>19%</td>
<td>23%</td>
<td>13%</td>
</tr>
</tbody>
</table>

*Hertz-Picciotto et al, Environ Health Persp 2009*