Reducing Prenatal Risk for Autism

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UC Davis School of Medicine and the MIND Institute
Autism Spectrum Disorder

- Neurodevelopmental Disorder
  - Impairments in Social Reciprocity
  - Language / Communication Deficits
  - Repetitive Behaviors / Restricted Interests

- Neurobiologic Basis: Aberrant Brain Development

- Sex ratio 4-5 M : 1 F  (CDC 2014)

- Prevalence in US: 1 in 68  (CDC 2014)
Rising Autism Prevalence

7-Fold Increase

Adapted Figure from Autism Speaks
ASD Etiology

Causes Unknown

Highly Heritable
Sibling Risk ~1/5

Hallmayer et al, 2011 (US)
H=38% (14%-67%) for ASD

Sandin et al, 2014 – Largest study (Sweden)
H=50% (45%-56%) for ASD
Risk Factors for ASD

- Advance Parental Age (Shelton et al 2010)
- Prenatal Infections (Rubella) (Chess 1971; Arndt et al 2005)
- Closely Spaced Pregnancies (Cheslack-Postava et al 2014)
- Month of Conception (Zerbo et al 2011)
- Medications (Deykin & MacMahon 1980; Gillberg & Gillberg 1983; Piven et al 1993)
  - Thalidomide (for morning sickness) (Stromland et al 1994; Lotter 1966)
  - Valproic acid (anti-epileptic) (Moore et al 2000; Rasalam et al 2005)
  - SSRIs (antidepressant) (Croen et al 2011)
- Maternal Metabolic Conditions (Krakowiak et al 2012; Li et al 2016)
Risk Factors for ASD
Traffic-Related Air Pollution

Kalkbrenner, Schmidt & Penlesky 2014
Risk Factors for ASD

Pesticides

Kalkbrenner, Schmidt & Penlesky 2014
**Critical Periods of Susceptibility Indicated from Studies of ASD**

<table>
<thead>
<tr>
<th>Trimester</th>
<th>First</th>
<th>Second</th>
<th>Third</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational Weeks</td>
<td>1-20</td>
<td>16-20</td>
<td>28-38</td>
</tr>
</tbody>
</table>

**Brain Pathology**

- **Neurogenesis**
  - Weeks 1-20
- **Neuronal Migration**
  - Weeks 1-16
- **Neuronal Maturation**
  - Weeks 1-24
- **Cortical Layer Formation, Organization, and Neuronal Differentiation**
  - Weeks 1-30

**Exposure**

- **Freeway Proximity**
  - 3rd Trimester
- **Traffic-related Air Pollution**
  - 1st, 2nd, and 3rd Trimester
- **Pesticides**
  - Days 26-81
- **Prenatal Vitamins**
  - 1st Month and 3 Months Before
- **Folic Acid**
  - 1st Month
- **Rubella Infection**
  - Weeks 1-8
- **Fever**
  - 1st and 2nd Trimester
- **Thalidomide**
  - Days 20-24
- **Valproic Acid**
  - Days 22-28
- **SSRI**
  - 1st Trimester
- **Prenatal Stressors**
  - Weeks 25-28

Days = Fetal days after conception. For exposures with more than one study, dark blue indicates overlapping period and light blue indicates timing suggested by one but not all studies.

Reducing Risk for ASD

- Avoid exposures associated with increased risk for ASD:
  - Traffic-related air pollution (don’t live within 100 m of a major freeway)
  - Pesticides (use alternative methods to kill pests, do not have your home sprayed/fogged regularly, do not live next to agricultural fields)
- Mechanisms unknown but could involve direct neurotoxic effects, inflammation, oxidative stress, epigenetic effects
Protective Factors for ASD

- Maternal prenatal vitamins near conception (Schmidt et al 2011)
- Maternal Iron during pregnancy & BF (Schmidt et al 2014)
- Maternal Folic Acid near conception (Schmidt et al 2012, Suren et al 2013)

- Might counter the effects of risk factors
Findings from the

CHARGE CASE-CONTROL STUDY

CHildhood Autism Risks from Genetics And Environment

PI: Irva Hertz-Picciotto
3 Diagnostic Groups

1. Autism
2. Developmental Delay
3. General Population Frequency matched to projected distributions in cases of: age, gender & geography

California DDS
- ASD
- DD

California Birth files
- TD
## Maternal Iron

<table>
<thead>
<tr>
<th>Total Iron Intake (mg)</th>
<th>Maternal Age &lt;35 Years</th>
<th>Maternal Age 35+ Years</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adjusted OR&lt;sup&gt;a&lt;/sup&gt; (95% CI)</td>
<td>Adjusted OR&lt;sup&gt;a&lt;/sup&gt; (95% CI)</td>
</tr>
<tr>
<td>Highest Quintile (86+)</td>
<td>Reference</td>
<td>0.6 (0.2 – 1.7)</td>
</tr>
<tr>
<td>Quintiles 2-4 (30 - &lt;86)</td>
<td>1.5 (0.9 - 2.4)</td>
<td>1.9 (0.98 – 3.5)</td>
</tr>
<tr>
<td>Lowest Quintile (&lt; 30)</td>
<td>1.4 (0.8 - 2.5)</td>
<td>5.0 (2.0 – 12.7)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Total Iron Intake (mg)</th>
<th>No Metabolic Condition During Pregnancy&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Metabolic Condition During Pregnancy&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adjusted OR&lt;sup&gt;a,c&lt;/sup&gt; (95% CI)</td>
<td>Adjusted OR&lt;sup&gt;a,c&lt;/sup&gt; (95% CI)</td>
</tr>
<tr>
<td>Highest Quintile (86+)</td>
<td>Reference</td>
<td>1.5 (0.4 – 5.8)</td>
</tr>
<tr>
<td>Quintiles 2-4 (30 - &lt;86)</td>
<td>1.6 (0.98 - 2.6)</td>
<td>2.2 (1.0 - 4.3)</td>
</tr>
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<td>Lowest Quintile (&lt; 30)</td>
<td>1.6 (0.9 - 2.6)</td>
<td>4.7 (1.7 – 13.2)</td>
</tr>
</tbody>
</table>

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<sup>a</sup> Adjusted for maternal folic acid intake, child birth year, home ownership

<sup>b</sup> Metabolic conditions include obesity (prepregnancy BMI≥30), hypertension, diabetes

<sup>c</sup> Adjusted for maternal race/ethnicity & education; child sex, type of health insurance, & regional center catchment area

**Schmidt et al 2014**
Maternal Folic Acid

- Higher folic acid linked to ~40% lower ASD risk
- Near conception
- Dose-response
- Replicated
- Especially for individuals with inefficient 1-C metabolism

- Mechanisms

Potential Folate Mechanisms

DNA Methylation
↓ folate leads to ↓ intracellular SAM & ↓ DNA methylation

DNA Synthesis & Repair
Folate deficiency leads to imbalance in DNA precursors, uracil misincorporation into DNA, and chromosome damage

DNA Synthesis
Cellular Proliferation
Pyrimidines

Folate Cycle
B6
5,10-CH₂-THF
5-CH₂-THF

Folic Acid
THF
DHF

Methionine Cycle
Methionine
B12
Choline
Betaine
SAH
SAM
Methyl Acceptor

Methylation Epigenetic Alterations
Methylated Product
(DNA, RNA, Protein, Neurotransmitter, Histone)

Transsulfuration Antioxidant Reactions
B6
Homocysteine
Cysteine
Glutathione

Davis & Uthus 2004; Duthie 1999
DNA Methylation

DNA gene sequence:
TAAGTCTACGTAATTAG

CG sites can be methylated

Cell Cytoplasm

UC DAvis
UNIVERSITY OF CALIFORNIA
The DNA Methylation Life Cycle


Folate and Methylation

Maternal dietary methyl donors affect offspring’s:

– DNA methylation
– Gene expression
– Health outcomes

Tobi et al 2009 Hum Molec Genet 18(21):4046–4053
Methyl Pool

Dietary Inputs

Folate
Vitamin B12

One-carbon Cycle

SAM

DNA Methylation

Oxidative Stress
Glutathione Reactions

Genomic Instability

CNV Duplication
DNA Repair

Cellular Proliferation
DNA Synthesis

Environmental Toxicants
SAM Inhibitors (Hcy)

Growth
Inflammation
Immune Reactions
**Animal Evidence: Agouti Mice**

**Bisphenol A (BPA)**
- Industrial chemical used to make certain resins and plastics often used in containers that store food and beverages
- Maternal exposure induces DNA hypomethylation in offspring

Maternal high-methyl (FA-supplemented) diet counteracts BPA-induced DNA hypomethylation in early development *(Dolinoy et al, 2007)*
Model To Investigate: CHARGE

- B Vitamins
- Folate

Nutrient Status

Environmental Exposures

Air Pollution
Pesticides

DNA Methylation

ASD Risk
### ASD ORs for Environment & FA Combinations

#### Indoor Pesticides
- **Sprays or Foggers**
  - None
  - Any

- **Pet Flea & Tick Products**
  - None
  - 1-5 m
  - 6+ m

- **Any Indoor Pesticides**
  - None
  - 1-5 m
  - 6+ m

#### Agricultural Pesticides
- **Chlorpyrifos**
  - None
  - Any

- **Organophosphates**
  - None
  - Any

- **Pyrethroids**
  - None
  - Any

- **Carbamates**
  - None
  - Any

#### Traffic-Related Air Pollution
- **TRP**
  - Low
  - High

- **NO2**
  - Low
  - High

- **PM10**
  - Low
  - High

- **PM2.5**
  - Low
  - High

- **O3**
  - Low
  - High

**aOR**: 0.4, 1.0, 2.7, 7.3, 19.7

- **High FA**
- **Low FA**

*Higher Risk*
Comments

• Nutrient x environmental exposure combined effects in expected directions
• Congruent with animal models, but more complex
• Could work for other contaminants
• Epigenetic mechanisms, or others
DNA Synthesis & Repair
Folate deficiency leads to imbalance in DNA precursors, uracil misincorporation into DNA, and chromosome damage.
Acknowledgements

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Kelly Bakulski (JHU)

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Allen Foundation 2008.604, Autism Speaks 9038, MIND Institute Pilot, IDDRC Pilot
Folic Acid

• Synthetic folate: essential vitamin B9

• Critical for development of the brain and nervous system

• Decreases risk for NTDs by 50-70%
  MRC 1991, Czeizel and Dudas 1992

• Also associated with decreased risk for behavioral, social, and peer problems and language delays & improved attention, verbal, verbal-executive function, and social competence
Prenatal Vitamin Use by Perinatal Month

Autism

TD

N=288

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

Schmidt et al, 2011 *Epidemiology*
Mean Folic Acid Intake Associated with Reduced ASD

Mean FA of mothers of children with ASD was 124 µg/d Less

Schmidt et al, 2012 Am J Clin Nutr
Increased Folic Acid, Decreased ASD Risk

$P$ for trend = 0.001

Adjusted for: maternal education level and child’s birth year (similar trend, reduced ORs when adjusted for other nutrients)

Schmidt et al, 2012 *Am J Clin Nutr*
Folic Acid Stratified by MTHFR 677

Schmidt et al, 2012 *Am J Clin Nutr*
Folate, Methionine, and Transmethylaition Pathways

Findings Replicated

• Norwegian Mother & Child Cohort (MoBa) Study (85,176 children, 114 Autistic Disorder)

• Ohio HOME Study (n=209)
  – Regular prenatal vitamin use & lower odds of clinically elevated SRS scores (autistic traits) OR=0.3 (0.1-0.9)
  – 2nd Trimester whole blood folate not associated

• Netherlands Generation R Study (n=3893)
  – FA supplement & lower SRS autistic traits
  – No significant association for maternal plasma folate concentrations at ~13 weeks gestation

• Denmark Study (n=35,059, 198 Autistic Disorder)

% Reporting Taking Iron Supplements

Typical Development

Autism Spectrum Disorder

Months Before (B3-B1) or During Pregnancy (P1-P9), or During Breastfeeding (BF)
### Mean Iron Intake from All Supplements

<table>
<thead>
<tr>
<th>Mean Iron (mg/day)</th>
<th>OR$^1$ (95% CI)</th>
<th>P</th>
<th>OR$^{1,2}$ (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 30</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 - &lt; 36</td>
<td>0.92 (0.54 – 1.57)</td>
<td>0.76</td>
<td>0.91 (0.56 – 1.47)</td>
<td>0.70</td>
</tr>
<tr>
<td>36 - &lt; 52</td>
<td>0.87 (0.51 – 1.47)</td>
<td>0.60</td>
<td>0.86 (0.53 – 1.39)</td>
<td>0.53</td>
</tr>
<tr>
<td>52 - &lt; 86</td>
<td>0.78 (0.45 – 1.33)</td>
<td>0.35</td>
<td>0.80 (0.50 – 1.26)</td>
<td>0.33</td>
</tr>
<tr>
<td>86+</td>
<td>0.55 (0.31 – 0.96)</td>
<td><strong>0.04</strong></td>
<td>0.55 (0.34 – 0.89)</td>
<td><strong>0.02</strong></td>
</tr>
</tbody>
</table>

$^1$ Adjusted for supplemental periconceptional folic acid intake, child’s year of birth, and home ownership

$^2$ Missing values imputed
CHARGE

Nutrient Status

Environmental Exposures

DNA Methylation

Child Neurodevelopment

Autism, ASD Developmental Delay

Newborn Blood Spots

Air Pollution Pesticides

B Vitamins
Folate

UC Davis
University of California
Air Pollution

- Self-reported residential history

- CALINE4 line-source air quality dispersion model used to obtain model-based estimates of exposure to traffic-related air pollution
<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 Pollution (Total)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; Median</td>
<td>Reference</td>
<td>1.0 (0.6-1.5)</td>
</tr>
<tr>
<td>≥ Median</td>
<td>1.0 (0.6-1.5)</td>
<td><strong>1.6 (1.3-3.3)</strong></td>
</tr>
<tr>
<td>NO₂ (Total)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; Median</td>
<td>Reference</td>
<td>1.1 (0.7, 1.6)</td>
</tr>
<tr>
<td>≥ Median</td>
<td>1.1 (0.7, 1.8)</td>
<td><strong>1.7 (1.1, 2.7)</strong></td>
</tr>
<tr>
<td>PM₁₀ (Total)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; Median</td>
<td>Reference</td>
<td>1.1 (0.7, 1.8)</td>
</tr>
<tr>
<td>≥ Median</td>
<td>1.2 (0.8, 1.9)</td>
<td><strong>1.7 (1.1, 2.6)</strong></td>
</tr>
<tr>
<td>PM₂.₅ (Total)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; Median</td>
<td>Reference</td>
<td>1.2 (0.8, 1.8)</td>
</tr>
<tr>
<td>≥ Median</td>
<td>1.3 (0.8, 2.1)</td>
<td><strong>1.7 (1.1, 2.7)</strong></td>
</tr>
<tr>
<td>Ozone (Total)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; Median</td>
<td>Reference</td>
<td>1.4 (0.9, 2.2)</td>
</tr>
<tr>
<td>≥ Median</td>
<td>1.4 (0.9, 2.1)</td>
<td>1.5 (0.9, 2.3)</td>
</tr>
</tbody>
</table>
Pesticide Use

- Self-reported use of products to control ants, flies, cockroaches or pet pests such as fleas and ticks

- Asked product type (spray, bait, etc.), brand name, whether application was indoors, outdoors, or on pet, and about use of professional pest control services
**FA x Indoor Pesticides**

<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>Sprays or Foggers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>Reference</td>
<td>1.4 (0.96 – 2.1)</td>
</tr>
<tr>
<td>Any</td>
<td>1.7 (1.1 – 2.6)</td>
<td>2.7 (1.6 – 4.4)</td>
</tr>
<tr>
<td>Pet Flea and Tick Products</td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>Reference</td>
<td>1.3 (0.9 – 1.9)</td>
</tr>
<tr>
<td>Some (1-5 Months)</td>
<td>0.6 (0.3 – 1.5)</td>
<td>1.5 (0.6 – 3.8)</td>
</tr>
<tr>
<td>Regular (6+ Months)</td>
<td>1.7 (0.9 – 3.1)</td>
<td>4.3 (1.6 – 11.5)</td>
</tr>
<tr>
<td>Any Indoor Pesticides</td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>Reference</td>
<td>1.4 (0.96 - 2.1)</td>
</tr>
<tr>
<td>Some (1-5 Months)</td>
<td>1.2 (0.7 – 2.1)</td>
<td>1.8 (0.97 - 3.2)</td>
</tr>
<tr>
<td>Regular (6+ Months)</td>
<td>2.3 (1.3 - 4.1)</td>
<td>5.1 (2.3 - 11.4)</td>
</tr>
</tbody>
</table>

Adjusted for home ownership
Agricultural Pesticides: Pesticide Use Report (PUR)

Geocode CHARGE addresses by month (-6 months to birth) (ArcGIS)

Download PUR data (1998-2008)

Map CHARGE addresses to PUR square mile grid (ArcGIS)

Sum pesticides applied to grid within buffer by day (SAS)

Model in linear and logistic regression (SAS)

J. Shelton et al, 2014
### FA x Agricultural Pesticides

<table>
<thead>
<tr>
<th>Environmental Exposure (3 Months Before Pregnancy until Pregnancy Month 3)</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><strong>Chlorpyrifos</strong></td>
<td>None</td>
<td>Reference</td>
</tr>
<tr>
<td></td>
<td>Any</td>
<td>0.7 (0.3 – 1.7)</td>
</tr>
<tr>
<td><strong>Organophosphates</strong></td>
<td>None</td>
<td>Reference</td>
</tr>
<tr>
<td></td>
<td>Any</td>
<td>0.8 (0.5 – 1.5)</td>
</tr>
<tr>
<td><strong>Pyrethroids</strong></td>
<td>None</td>
<td>Reference</td>
</tr>
<tr>
<td></td>
<td>Any</td>
<td>1.0 (0.5 – 2.0)</td>
</tr>
<tr>
<td><strong>Carbamates</strong></td>
<td>None</td>
<td>Reference</td>
</tr>
<tr>
<td></td>
<td>Any</td>
<td>2.0 (0.7 – 5.8)</td>
</tr>
</tbody>
</table>

Adjusted for home ownership