Emerging Knowledge about Environmental Exposures & Risk for Autism

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What Doesn’t Cause Autism?

Neurobiologic basis: aberrant brain development
What Causes Autism?

- Genes
  - Syndromes
  - Single genes
  - Copy number variants

- Environmental factors
‘Root’ Causes

• Distinguish from factors that may exacerbate symptoms
• Occur prior to diagnosis

• Public health: ways to intervene
  Goal is prevention
Genetics & Environment

- Heritability estimates 35%-60%

Two largest studies of twins
Estimate the contribution of environment: 38%-57%

Hallmayer et al, Arch Gen Psychiatry 2010
Rosenberg et al, Arch Ped Adolesc Med 2009

Environment is modifiable
Multifactorial causation

-across the population and within an individual

Sufficient Causes Model
Genes & Environment

Multifactorial causation

Gene 1

Gene 2

A
B
C
D

Pesticide

Metabolic condition

Maternal
Nutrition

Household
chemical

Medical
procedures

Air
pollution

Maternal
metabolic
condition

Gene 2
Timing matters!!

Developmental Biology Perspective:

Conception

Birth

Diagnosis
Time Trends in Autism

Comparing 1990 births to 2001 births in California,

...autism incidence, by 5 years of age:

rose 7-fold (=600%)
How much of the 600% increase could have resulted from:

- Change in DSM criteria: 120%
- Trend towards younger age at diagnosis: 24%
- Broadening to include milder cases: 56%
- Older ages of mothers: 4%

Total (from these 4): 204%

“...the possibility of a true increase in incidence deserves serious consideration.”

Hertz-Picciotto & Delwiche, Epidemiol 2009; Shelton et al, Autism Res 2010
Time Trends of Other Conditions

- asthma, obesity, diabetes, ADHD, and mental health disorders (OCD, bipolar disorder)

Is there a common set of environmental exposures that provides a unifying explanation for increased incidences of these conditions in recent decades?
*CHildhood Autism Risks from Genetics and the Environment
Environmental Exposures & Ways to Assess

1. Pesticides
2. Metals
3. Organic pollutants (PCBs, PBDEs, etc.)
4. Viruses, bacteria & other infections
5. Medical procedures & pharmaceuticals
6. Nutritional factors

Biospecimens:
- Blood
- Baby lock (first year of life)
- Mother’s hair
- Urine
- Newborn blood spot (prenatal)
- Teeth

Interviews:
- Diet
- Residential information
- Lifestyle
- Consumer products
- Medical history
- Linkage to exposure databases
- Air, water, pesticides, haz waste
- Medical records
To identify causes and contributing factors for autism:

- Environmental exposures
- Genetic susceptibility
- Interactions of the two
Case-control - three 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
Case-control sampling design
*Population-based recruitment
*Standardized clinical confirmation of dx
*Linkage to state-of-the-art laboratories
All 3 groups:

- 24-60 months
- Born in California
- Live with biologic parent
- English / Español
- Reside in study catchment area
Clinic visit:

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

Medical examination and history

Broad assessment of behavior, development, comorbidities
- Interview: lifestyle, demographics, medical conditions, repro hx, residential hx, etc.

- Specimens collected from family members: blood urine hair

- Permission to obtain medical charts: 
  - prenatal
  - labor and delivery
  - pediatrician
  - fertility clinics
Be in **CHARGE**!

http://beincharge.ucdavis.edu/
State of the Science on Autism Etiology: 2013

- Genes?
- Environmental pollutants? (air pollution)
- Household chemicals (pesticides, phthalates)
- Vitamins, nutritional deficiencies?
- Maternal reproductive characteristics (IPI)?
- Obstetric, perinatal conditions (chronic & acute) ?
- Infections, inflammation, immune regulation?
- Fertility treatments & other medical interventions?
- Medications?
- Modern technology?
1. Maternal Metabolic Conditions
Goal:
To determine differences between mothers of children with typical development and those of children with autism or with developmental delay, in relation to:

* pre-pregnancy obesity,
* Type 2 or gestational diabetes, or
* underlying hypertension
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
Maternal Conditions and Child’s Dx: ASD, Other Developmental Delay (DD)

<table>
<thead>
<tr>
<th>Conditions in index pregnancy:</th>
<th>ASD vs. TD</th>
<th>DD vs. TD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR† 95% CI</td>
<td>OR† 95% CI</td>
</tr>
<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, 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, Pediatrics 2012*
Among ASD and among non-ASD, separately, maternal diabetes also associated with poorer:

- Receptive language
- Expressive language

Among non-ASD maternal diabetes also associated with reduced:

- Socialization

*Krakowiak et al, Pediatrics 2012*
Discussion

• Prior studies of diabetes show associations with intellectual disability

What influences metabolic conditions??

• Cross-sectional studies show associations of several endocrine disruptors with diabetes/obesity (bisphenol A, anti-bacterials)

• High fructose corn syrup (Stanhope et al 2012)
1. Maternal Metabolic Conditions
2. Maternal Nutrition
Goals:
To determine whether mothers of children with typical development differed from those of children with autism or with developmental delay
* intake of prenatal vitamins
* timing of intake
* combination of genes and prenatal vitamin intake
Folic Acid

• Required for DNA: synthesis, repair, and methylation
• Supplementation prevents 50-70% of neural tube defects (NTDs)
• Prenatal supplements recommended before and during early pregnancy
• Folic acid fortification mandated for cereal grains by 1998
• Several studies have since shown declining prevalence of NTDs in US
Prenatal Vitamin Supplements

OR for autism in child
= 0.6 (95% CI: 0.4 to 0.9)

Schmidt et al, Epidemiology 2011; Amer J Clin Nutrition 2012
Average Daily Folic Acid Intake

<table>
<thead>
<tr>
<th>Folic Acid P1</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Reference</td>
</tr>
<tr>
<td>&lt; 500</td>
<td>0.73 (0.31, 1.69)</td>
</tr>
<tr>
<td>500 – 800</td>
<td>0.64 (0.24, 1.71)</td>
</tr>
<tr>
<td>800 – 1000</td>
<td>0.57 (0.24, 1.35)</td>
</tr>
<tr>
<td>1000+</td>
<td>0.41 (0.17, 0.96)</td>
</tr>
</tbody>
</table>

$P_{\text{trend}} = 0.001$
Maternal Nutrition

• Estimated intake from diet and supplements → similar results

• Inter-pregnancy interval
  - <12 months: 3.7-fold
  - 12-24 months: 2.1
  - 24-36 months: 1.4

• Replication of prenatal supplement result in Norway

*Schmidt et al, Epidemiology 2011; Amer J Clin Nutrition 2012
Cheslack-Postava Pediatrics 2011
Suren et al JAMA 2013*
Confirmation in a prospective study

- Norwegian mother-baby cohort: MoBa Study
- 85,000 children followed to mean age of 6.4 years (Range= 3.3 to 10.2 years)
- Recruited during pregnancy, children born 2002-2008
- Asked about dietary supplements at 18 weeks gestation for the period 4 weeks before LMP to 8 weeks post LMP
- Also found a 40% reduction in risk for ASD for folic acid consumers
- Rate for autism was substantially lower than in the U.S., and prenatal supplements were 400 mcg folic acid.

Suren et al JAMA 2013
Folate, Methionine, and Transmethylation Pathways

Maternal and Child Gene Variants Associated with Increased Autism Risk in Combination with No Intake of Prenatal Vitamins
Gene x Nutrition Interaction

<table>
<thead>
<tr>
<th>ODDS RATIOS</th>
<th>Periconceptional supplementation:</th>
</tr>
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<tbody>
<tr>
<td>COMT genotype</td>
<td>Yes</td>
</tr>
<tr>
<td>GG+GA</td>
<td>Referent</td>
</tr>
<tr>
<td></td>
<td>No</td>
</tr>
<tr>
<td>AA</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>7.2</td>
</tr>
</tbody>
</table>

Child’s COMT gene exerts a **synergistic** effect in combination with prenatal vitamin supplements taken around the time of conception

*Schmidt et al, Epidemiology 2011, Amer J Clin Nutrition 2012*
Impact of Folic Acid Intake according to genotype for MTHFR 677

MTHFR 677 gene regulates folate metabolism

All participants
- Maternal: CC
  - OR: 0.46 (0.25-0.85)
  - OR with CT/TT: 0.62 (0.42-0.92)
- Child: CC
  - OR: 0.48 (0.27-0.88)
  - OR with CT/TT: 1.15 (0.55-2.38)
- Both are CC: 1.20 (0.61-2.34)
- One is CT/TT: 0.49 (0.16-1.50)
- Both are CT/TT: 1.29 (0.54-3.10)
1. Maternal Metabolic Conditions
2. Maternal Nutrition
3. Medications
1. Maternal Metabolic Conditions
2. Maternal Nutrition
3. Medications
4. Air Pollution
Goal

• To determine whether mothers of cases were more likely during pregnancy to have greater exposures to ambient air pollution.

• Distance to freeway

• Estimated ambient concentrations of pollutants in air
## Residential Proximity to Freeways

<table>
<thead>
<tr>
<th>Distance to freeway</th>
<th>Adjusted Odds Ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;2/10 mile</td>
<td>closest 10 %</td>
<td>1.86</td>
</tr>
<tr>
<td>2/10 – 4/10</td>
<td>10&lt;sup&gt;th&lt;/sup&gt; to 25&lt;sup&gt;th&lt;/sup&gt; %</td>
<td>0.96</td>
</tr>
<tr>
<td>4/10 – 9/10</td>
<td>25&lt;sup&gt;th&lt;/sup&gt; to 50&lt;sup&gt;th&lt;/sup&gt; %</td>
<td>1.11</td>
</tr>
<tr>
<td>&gt;9/10 mile</td>
<td>farthest 50%</td>
<td>reference</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*
• Particle concentrations in ambient air high near freeways, and drop to background at about 300m

• Objective exposure measure for prenatal period

• Proximity to traffic ~ asthma

• Components of traffic-related AP (benzo(a)pyrene, diesel, ozone) induce neurodevelopmental deficits in rodents

• PAHs → oxidative stress, inflammation, endocrine disruption
Early Life Air Pollution

• Windham et al 2006, San Francisco
• Kalkbrenner et al 2010, North Carolina & West Virginia
• Roberts et al 2013, Nurses Health Study II
• Volk et al 2011, 2012, northern & southern Calif selected areas
• Becerra et al 2013, Los Angeles County
• Jung et al 2013, Taiwan
Discussion

- PAHs (polycyclic aromatic hydrocarbons), chlorinated semi-volatile compounds, fine and coarse particles, ozone, metals, nitric oxide, nitrogen dioxide,
- Most components of cigarette smoke and ambient air pollution are in both
cigarette smoking literature
- How to explain discrepancy?
1. Maternal Metabolic Conditions
2. Maternal Nutrition
3. Medications
4. Air Pollution
5. Infection/Inflammation/Immune Responses
### Adjusted OR (95% CI) associated with Fever During Pregnancy stratified by use of anti-fever or anti-inflammatory medication

<table>
<thead>
<tr>
<th></th>
<th>Autism/ASD</th>
<th>Developmental Delay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Had fever, took anti-fever medication</td>
<td>1.30 (0.59 – 2.84)</td>
<td>2.05 (0.78 – 5.36)</td>
</tr>
<tr>
<td>Had fever, did not take anti-fever medication</td>
<td>2.55 (1.30 – 4.99)</td>
<td>2.73 (1.19 – 6.28)</td>
</tr>
<tr>
<td>Did not have fever, took no anti-fever med.</td>
<td>Referent group</td>
<td>Referent group</td>
</tr>
</tbody>
</table>

1 Adjusted for maternal report of flu, private vs. public health insurance for delivery, race/ethnicity, and the matching variables (child age, sex, and maternal place of residence at child birth)

Zerbo et al 2011
Self reports – recall bias?

Results are consistent with a role for an acute inflammatory reaction

Influenza rodent model

Is autism a neuroinflammatory condition?

Also consistent with seasonality data
Infection, Fever, Inflammation

- Atladottir et al 2010a, 2010b: Maternal hospitalizations for infection, Denmark
- Zerbo et al 2012, Fever in pregnancy
- Vargas et al, 2005: Neuroinflammation and autism
- Patterson et al 2011, Huang et al
Up to 23% of mothers of children with autism may produce specific antibodies to fetal brain tissue.

• Direction of connection to autism unclear:
  – immune aberrations could be downstream or upstream of neuropathology
  …or neither

• Possibility that environmental chemicals may influence neurodevelopment indirectly, through immune dysregulation
Take-home messages from the CHARGE & other studies

Take prenatal vitamin supplements – before pregnancy (best: 3 months prior)!

Space pregnancies (best: 36 months or more from conception to next conception)

Consult with your physician regarding risks & benefits from SSRI use

Limit exposure to air pollutants from traffic, cigarettes, and other sources

Reduce weight, control blood glucose, exercise moderately to reduce blood pressure

Avoid flu/prolonged fever, take anti-fever meds
The CHARGE Study

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
A study of pregnant moms, who already have a child with autism, following their pregnancies and new child
Prospective Studies of Younger Siblings Starting in Pregnancy

- Complement case-control studies
- Eliminate problem of differential reporting
- Permit examination of temporally relevant exposures

Prospective Studies of Large Populations
NCS, Danish NCS, MoBa
Results from MARBLES

- Trophoblastic inclusions
Results from MARBLES

- Trophoblastic inclusions

Walker et al, Molecular Psychiatr 2013
Postnatal influences?

Early childhood exposures

• Air pollution?
• Nutrition?
• Breastfeeding?
Post-diagnosis influences?

- Behavioral
- Nutritional
- Environmental
  - Address core symptoms
  - Address co-morbidities
- ReCHARGE Study
Investigators & Collaborators

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End