Environmental Toxins and Autism Spectrum Disorders (ASD)

Pamela J. Lein, Ph.D.
Department of Molecular Biosciences
Center for Children’s Environmental Health
UC Davis School of Veterinary Medicine

What is the evidence that environmental factors contribute to ASD risk?

What is the evidence that environmental factors contribute to ASD risk? continued

What is the evidence that environmental factors contribute to ASD risk? continued

What is the evidence that environmental factors contribute to ASD risk? continued

However, Hertz-Picciotto and Delwiche (2009) Epidemiology 20: 84-90:

600% increase in cases:
24% due to earlier diagnosis
56% due to inclusion of milder cases
120% due to changes in diagnostic criteria

400% of increased cases cannot be attributed to diagnostic substitution
Environmental risk factors for ASD

• Rubella infection during the first trimester of pregnancy
• In utero exposure to thalidomide or valproic acid
• Paternal age
• Environmental chemicals?

Epidemiological Data Linking Environmental Chemicals to Increased Risk of ASD

• Data recently critically reviewed
• Of 58 articles identified in the peer-reviewed literature published prior to March 1, 2014, 32 met inclusion criteria
  – Individual-level data on autism diagnosis
  – Exposure measures during pregnancy or 1st year of life
  – Valid comparison groups
  – Controls for confounding variables
  – Adequate sample size

Why focus on Environmental Chemicals as Risk Factors for ASD?

• In contrast to genetic risks, which are currently irreversible, environmental factors are modifiable risk factors
  – Therefore, identifying specific environmental factors that increase risk for neurodevelopmental disorders may provide rational approaches for the primary prevention of the symptoms associated with these disorders.

Summary of Kalkbrenner et al., 2014 Review of Published Epidemiological Data

• Environmental chemicals studied in the 32 articles reviewed by Kalkbrenner et al., 2014
  – Tobacco and alcohol
  – Air pollutants, volatile organic compounds and solvents
  – Metals, PCBs, PBDEs
  – Pesticides, BPA and phthalates
• The most strongly and consistently associated with increased ASD risk
  – Traffic-related air pollutants
  – Some metals
  – OP and OC pesticides
• Environmental chemicals NOT associated with increased ASD risk
  – Tobacco and alcohol

Environmental Chemicals Postulated to Confer Risk for ASD

• Legacy chemicals known to interfere with normal neurodevelopment
  ❖ Lead
  ❖ Methyl mercury
  ❖ Polychlorinated biphenyls (PCBs)
• Contemporary contaminants
  ❖ Pesticides
    • Organophosphorus (OP), organochlorine (OC), pyrethroids
  ❖ Flame retardants
    • Polybrominated diphenyl ethers (PBDEs)
  ❖ Plasticizers
    • Phthalates, bisphenol A (BPA)
• Complex environmental mixtures
  ❖ Air pollution

Major Conclusion in Critical Analyses of Epi Data by Kalkbrenner et al., 2014

The relevant publications that are currently available, with the possible exception of studies of tobacco and alcohol, are too limited in scope to either infer causality or to rule out the possibility that these or additional environmental chemicals confer risk for ASD.
The Challenge of Identifying Environmental Risk Factors for ASD

How do environmental chemicals interact with genetic mechanisms to increase ASD risk?

- Heritable deficits in xenobiotic metabolism
  - Decreased ability to detoxify environmental chemicals might effectively increase the neurotoxic potential of an environmental chemical
- Endocrine disruption
  - ASD occurs predominantly in boys and many hormones are required for normal neurodevelopment (sex steroids and thyroid hormones) or have significant effects on neurodevelopment (glucocorticoids)
- Disruption of the gut microbiome
  - Emerging evidence indicates that the gut microbiome regulates host response to pathogenic microbial or xenobiotic exposures, and the gut microbiota in children with autism differs from that of neurotypical children

The Challenge of Identifying Environmental Risk Factors for ASD, continued

A significant challenge, particularly for epidemiological studies:

The complexity of heritable factors contributing to ASD susceptibility creates a range of sensitivities to environmental factors

How do environmental chemicals interact with genetic mechanisms to increase ASD risk?

- Epigenetic
  - Environmental chemicals have been demonstrated to alter DNA methylation, histone acetylation and miRNA expression profiles, and these parameters are altered in at least some children with ASD
- Immune Dysregulation
  - Crosstalk between the nervous and immune systems is essential for normal neurodevelopment, environmental chemicals can alter immune function, and there is significant clinical evidence of immune dysregulation in ASD
- Convergence of environmental and genetic factors on common signaling pathways critical in neurodevelopment
  - Heritable genetic vulnerabilities amplify adverse effects triggered by environmental exposures if genes and environment converge to dysregulate the same signaling system at critical times of neurodevelopment

The Challenge of Identifying Environmental Risk Factors for ASD, continued

ASD Pathology

Autism reflects altered patterns of neuronal connectivity within the developing brain
Genes associated with ASD susceptibility: Neuronal connectivity

<table>
<thead>
<tr>
<th>Gene</th>
<th>Symbol</th>
<th>Type of Protein</th>
<th>Expression</th>
<th>Function</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTP4B</td>
<td>672</td>
<td>Synaptic protein</td>
<td>M</td>
<td>Increased with ASD</td>
<td>PFM, KRG</td>
</tr>
<tr>
<td>FMRP</td>
<td>165</td>
<td>RNA-binding protein</td>
<td>M</td>
<td>Associated with ASD</td>
<td>FMRP1, KRG</td>
</tr>
<tr>
<td>TCF4</td>
<td>TCF4</td>
<td>Transcription factor</td>
<td>A</td>
<td>Associated with ASD</td>
<td>TCF4, KRG</td>
</tr>
<tr>
<td>ZNF469</td>
<td>ZNF469</td>
<td>Zinc-finger protein</td>
<td>A</td>
<td>Associated with ASD</td>
<td>ZNF469, KRG</td>
</tr>
<tr>
<td>NRXN1</td>
<td>NRXN1</td>
<td>Neurexin protein</td>
<td>M</td>
<td>Increased with ASD</td>
<td>NRXN1, KRG</td>
</tr>
<tr>
<td>UNC5C</td>
<td>UNC5C</td>
<td>Neurotrophin receptor</td>
<td>L, A</td>
<td>Increased with ASD</td>
<td>UNC5C, KRG</td>
</tr>
<tr>
<td>CNTNAP2</td>
<td>CNTNAP2</td>
<td>Contactophilin protein</td>
<td>M</td>
<td>Increased with ASD</td>
<td>CNTNAP2, KRG</td>
</tr>
<tr>
<td>COMMD1</td>
<td>COMMD1</td>
<td>Contactophilin protein</td>
<td>M</td>
<td>Increased with ASD</td>
<td>COMMD1, KRG</td>
</tr>
<tr>
<td>CHD8</td>
<td>CHD8</td>
<td>Chromodomain protein</td>
<td>L, A</td>
<td>Increased with ASD</td>
<td>CHD8, KRG</td>
</tr>
<tr>
<td>ERBB2IP</td>
<td>ERBB2IP</td>
<td>ERBB2-interacting protein</td>
<td>L</td>
<td>Increased with ASD</td>
<td>ERBB2IP, KRG</td>
</tr>
<tr>
<td>SYNGR1</td>
<td>SYNGR1</td>
<td>Synaptic protein</td>
<td>M</td>
<td>Increased with ASD</td>
<td>SYNGR1, KRG</td>
</tr>
<tr>
<td>SHANK2</td>
<td>SHANK2</td>
<td>Shank protein</td>
<td>L, A</td>
<td>Increased with ASD</td>
<td>SHANK2, KRG</td>
</tr>
<tr>
<td>RPS6KB1</td>
<td>RPS6KB1</td>
<td>Ribosomal protein</td>
<td>L</td>
<td>Increased with ASD</td>
<td>RPS6KB1, KRG</td>
</tr>
<tr>
<td>LAMA1</td>
<td>LAMA1</td>
<td>Lamin protein</td>
<td>L, A</td>
<td>Increased with ASD</td>
<td>LAMA1, KRG</td>
</tr>
<tr>
<td>ARL3</td>
<td>ARL3</td>
<td>Small GTPase</td>
<td>L</td>
<td>Increased with ASD</td>
<td>ARL3, KRG</td>
</tr>
</tbody>
</table>

Persico and Bourgeron, 2006, TINS

Imaging studies of autistic brains: Neuronal connectivity

Typically developing children

Children with autism

Hyper-connected neurons in autism

Increased connectivity in local circuits of the visual cortex

Keown et al. 2013, Cell Reports 5: 567-72

Neurodevelopmental processes that determine neuronal connectivity

and are thus likely to be altered in ASD:

- Neuronal migration
- Interneuron development
- Neuronal programmed cell death
- Axonal growth and branching
- Dendritic growth and plasticity
- Synaptogenesis and synaptic plasticity

PCB Developmental Neurotoxicity

- Human epidemiological data indicate a negative association between developmental exposure to environmental PCBs and cognitive function in infancy or childhood
  - Decreased IQ, impaired learning and memory, attentional deficits, lowered reading comprehension, psychomotor problems
- Comparable cognitive and behavioral deficits observed in primate and rodent models following developmental PCB exposures
  - Developmental neurotoxic effects of PCBs have been observed at relatively low exposure levels corresponding to between 1 and 10x the background levels observed in humans

PCBs: A current public health concern

- Exposure from legacy sources as well as contemporary unintentional sources of PCBs, most notably commercial paint pigments
- PCB levels in the indoor air of elementary schools in the United States exceed the EPA’s 2009 public health guidelines
- Latest NHANES study confirmed widespread exposure to PCBs among U.S. women of childbearing age
- Levels of NDL PCBs are NOT decreasing rapidly in the environment and human tissues
- PCB153 levels in plasma of at risk MARBLES mothers are 7 to 20-fold higher than those reported in the 2007-2008 NHANES report
Non-dioxin-like vs. dioxin-like polychlorinated biphenyls (PCBs)

<table>
<thead>
<tr>
<th>Arylhydrocarbon Receptor (AhR)</th>
<th>Non-dioxin-like congeners</th>
<th>Dioxin-like congeners</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cancer</td>
<td>Low to no affinity</td>
<td>High affinity</td>
</tr>
<tr>
<td>Developmental Neurotoxicity</td>
<td>+++</td>
<td>+/-</td>
</tr>
</tbody>
</table>

NDL predominate in environmental samples and human tissues

2,2',3,3'-A-pentachlorobiphenyl (PCB 95) 2,3',4,4'-tetrachlorobiphenyl (PCB 66)

Overarching Hypothesis:
Non-dioxin-like PCBs disrupt neuronal connectivity via RyR-mediated mechanisms that modulate Ca^{2+}-dependent signaling pathways linked to activity-dependent dendritic growth and plasticity.

Postulated molecular mechanism(s) of PCB developmental neurotoxicity

- Decreased dopamine content
- Interference with thyroid hormone signaling
- Increased levels of intracellular calcium Ca^{2+}
  - Sensitization of the ryanodine receptor (RyR)

Ca^{2+}-dependent signaling regulates dendritic growth in the developing brain

- Dendritic branching patterns influence the number, types and distribution of synaptic inputs
- Structural plasticity of dendrites is thought to be the cellular substrate of learning and memory
- Perturbations of normal patterns of dendritic growth and plasticity are associated with functional deficits

Golgi analyses of the hippocampus of weanling rats exposed developmentally to PCBs in the maternal diet

PCB 95 alters dendritic growth in primary cultures of hippocampal neurons

Wayman et al. (2012) Environmental Health Perspectives 120:997-1002.
The dendrite-promoting activity of PCBs is RyR-dependent

PCB 95 enhances dendritic growth in cultured hippocampal neurons via RyR-dependent mechanisms

Increased Dendritic Spine Density in ASD

Experimental approaches for investigating Ca\textsuperscript{2+}-dependent signaling pathways in PCB-induced dendritic growth

PCB 95 Triggers Dendritic Spine Formation in Cultured Hippocampal Neurons
PCB-induced spine formation coincides with increased frequency of mini-excitatory postsynaptic currents (mEPSC).


Relevance of these findings to ASD?

- **Animal studies**
  - Perinatal exposure to a mixture of the non-dioxin-like PCB 47 and dioxin-like PCB 77 shown to alter social behaviors in rats
  - PCB 95 found in significantly higher levels in postmortem brains of children with a syndromic form of autism (maternal 15q11-q13 duplication or Dup15q), but not idiopathic autism as compared to neurotypical controls
    [Mitchell et al. (2012) Environmental and Molecular Mutagenesis 58:589-98]

- **Human exposure studies**

A significant proportion of genes linked to ASD risk encode for proteins that regulate Ca\(^{2+}\) signaling.

Ca\(^{2+}\)-dependent signaling is a critical determinant of dendritic growth in the developing brain.

At least some forms of autism result from an imbalance in the ratio of excitatory and inhibitory circuits within the developing brain.

Developmental exposure to PCB 95 in the maternal diet interferes with the topographic organization of the auditory cortex in rats.

Fig. 1. Exposure to PCB95 alters A1 maps. (Upper Left) Tonotopic map from a typical control rat pup. (Upper Right, Lower Left, and Lower Right) Examples of maps from PCB95-exposed rat pups. □ indicates an unresponsive site. Color bar, CF (kilohertz).


PCBs: Environmental Risk Factors for ASD

Environmental exposures X Genetic susceptibility X Timing (non-dioxin-like PCBs) (heritable defects in Ca\(^{2+}\) signaling)

↓

Altered dendritic arborization

↓

Altered Neuronal Connectivity

↓

ASD risk, severity and treatment outcome

Environmental exposures X Genetic susceptibility X Timing (non-dioxin-like PCBs) (heritable defects in Ca\(^{2+}\) signaling)

↓

Altered dendritic arborization

↓

Altered Neuronal Connectivity

↓

ASD risk, severity and treatment outcome
What do these findings mean to parents and clinicians?

• Chemical exposure both pre- and postnatal can influence clinical outcome (types and severity of behaviors, co-morbidities)
• Chemical exposures are more readily controlled than genetic factors to prevent or mitigate the expression of ASD-related traits


What do these findings mean to parents and clinicians?

• Minimizing or preventing exposure to chemical contaminants during pregnancy or early childhood may improve clinical outcome
  – Do not use brilliantly colored paints in the home
  – Work with local agencies to determine levels of PCBs in public buildings
  – Limit dietary consumption of fatty fish, red meats
  – Remove skin, fat from fish and meats

Acknowledgements

Collaborators
Isaac Pessah, UC Davis
Gary Wayman, Washington State University
Adam Lesiak
Lein Laboratory
Christopher Barnhart
Donald Bruun
Hao Chen
Dongren Yang

Funding Sources
NIHES
USEPA
Jane Johnson Foundation