Environmental exposure and autism risk: narrowing the knowledge gaps
Acknowledgements

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The EARLI investigators
The SEED investigators
The ASD-ER investigators
The EEARN team

Funding:
R01 ES016443 (NIEHS, NINDS, NICHD, NIMH)
R21 ES025559 (NIEHS)
R01 ES026903 (NIEHS)
UG3 OD023342 (NIH OD)
R01 ES017646 (NIEHS, OD, NICHD)
R01 ES017646 (Fallin)
U20 DD000183 (Fallin)

Thanks to:
Staff at EARLI, SEED, ASD-ER field sites, DCCs, and biorepositories.
All the EARLI, SEED, ASD-ER participating families.

Conflicts:
No other conflicts to declare.
Outline

• Autism spectrum disorders
• Importance of genes and the environment
• Evidence supporting environmental ASD risk factors
• Why aren’t we further?
  • Tradeoffs between retrospective and prospective designs
  • Potential benefits of prospective designs
  • Examples from the EARLI study
  • Thoughts on streamlining prospective designs
• How can we approach GxE?
  • Challenges of GxE
  • Exposomics
  • GWIS
  • Polygenic risk scores
Since 1938, there have come to our attention a number of children whose condition differs so markedly and uniquely from anything reported so far, that each case merits—and, I hope, will eventually receive—a detailed consideration of its fascinating peculiarities.

Kanner, L. Autistic Disturbances of Affective Contact. Nervous Child, (2) 217-250, 1943
Economic costs of ASD in the United States

Annual US costs

- Children: 61 Billions of 2012 Dollars
- Adults: 175 Billions of 2012 Dollars

Lifetime individual costs

- w ID: 2.4 Millions of 2012 Dollars
- w/o ID: 1.4 Millions of 2012 Dollars

Buescher et al. JAMA Pediatr 2014
Sensory dysregulation
Sleep disturbance
Cognitive impairment
Seizures
Minor dysmorphology
GI disturbance
Impairment in social interaction and communication
Restricted & repetitive behavior/interests/activities
Outline

• Autism spectrum disorders
• Importance of genes and the environment
• Evidence supporting environmental ASD risk factors
• Why aren’t we further?
• How can we approach GxE?
<table>
<thead>
<tr>
<th>Study</th>
<th>Country</th>
<th>Concordance</th>
<th>(N pairs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Folstein and Rutter (1977)</td>
<td>UK</td>
<td>36% 0%</td>
<td>(21)</td>
</tr>
<tr>
<td>Ritvo et al (1985)</td>
<td>U.S.</td>
<td>96% 24%</td>
<td>(40)</td>
</tr>
<tr>
<td>Steffenberg (1989)</td>
<td>Nordic (5)</td>
<td>91% 0%</td>
<td>(21)</td>
</tr>
<tr>
<td>Bailey et al (1995)</td>
<td>UK</td>
<td>60% 5%</td>
<td>(44)</td>
</tr>
<tr>
<td>Taniai et al (2008)</td>
<td>Japan</td>
<td>95% 31%</td>
<td>(45)</td>
</tr>
<tr>
<td>Rosenberg et al (2009)</td>
<td>US</td>
<td>88% 31%</td>
<td>(227)</td>
</tr>
<tr>
<td>Lichtenstein et al (2010)</td>
<td>Sweden (male)</td>
<td>47% 14%</td>
<td>(62)</td>
</tr>
<tr>
<td>Hallmayer et al (2011)</td>
<td>US (male)</td>
<td>77% 31%</td>
<td>(90)</td>
</tr>
<tr>
<td></td>
<td>US (female)</td>
<td>50% 36%</td>
<td>(22)</td>
</tr>
<tr>
<td>Frazier et al (2014)</td>
<td>US</td>
<td>69% 35%</td>
<td>(568)</td>
</tr>
<tr>
<td>Sandin et al (2014)</td>
<td>Sweden</td>
<td>54% 25%</td>
<td>(466*)</td>
</tr>
<tr>
<td>Colvert et al (2015)</td>
<td>UK</td>
<td>75% 40%</td>
<td>(203)</td>
</tr>
</tbody>
</table>

* Discordant pairs only – no count of total pairs given

Heritability estimates from recent (yellow) studies tend to be 50%-60%, with some exceptions
GENES versus ENVIRONMENT
3% de novo (N)
4% non-additive (D)
3% rare inherited (A)
49% common inherited (A)
41% unaccounted

Gaugler et al. Nature Gen 2014
AUTISM IN THALIDOMIDE EMBRYOPATHY: A POPULATION STUDY

Stromland et al. Dev Med Child Neurol 1994
Neuroanatomy: Minicolumn disorganization

Brain gene expression: ASD vs non-ASD brains

Brain gene expression: ASD risk gene networks

Amaral et al. *Trends Neurosci* 2008


Willsey et al. *Cell* 2013
Gene x Environment
Statistical and Epidemiologic Interactions
- Exposure modified by genetics
- Genetics modified by exposure
- Genetic and environmental synergism

Biological Interactions and Molecular Targets
- Exposure mediated by genetic alterations
- Gene product contact with exposure
- Epigenetics

Risk of Autism Spectrum Disorders
Outline

• Autism spectrum disorders
• Importance of genes and the environment
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• How can we approach GxE?
The Changing Epidemiology of Autism Spectrum Disorders

Annual Review of Public Health
Vol. 38:81-102 (Volume publication date March 2017)
First published online as a Review in Advance on December 21, 2016
https://doi-org.ezproxy2.library.drexel.edu/10.1146/annurev-publhealth-031816-044318

Kristen Lyall,1 Lisa Croen,2 Julie Daniels,3 M. Daniele Fallin,4,5 Christine Ladd-Acosta,4,6 Brian K. Lee,7,8 Bo Y. Park,4,5 Nathaniel W. Snyder,1 Diana Schendel,9,10,11 Heather Volk,4,5 Gayle C. Windham,12 and Craig Newschaffer2

Lyall et al. Ann Rev Pub Health 2017
Quantitative reviews of specific non-genetic risk factors as late as 2011 were not finding sufficient statistical evidence -though “implicated” at that time were:

- Older parental age
- Preterm/LBW birth
- Prenatal infection
- Maternal medication use
- Pregnancy complications (bleeding, gestational diabetes)
Relative risk of ASD in relation to maternal prenatal infection of any kind


1.13 (1.03, 1.23)
A Systematic Review and Meta-Analysis of Multiple Airborne Pollutants and Autism Spectrum Disorder

Juleen Lam¹ *, Patrice Sutton², Amy Kaikbrenner³, Gayle Windham⁴, Alycia Halladay⁵,⁶, Erica Koustas⁵, Cindy Lawler⁵, Lisette Davidson⁴, Natalyn Daniels⁴,⁷, Craig Newschaffer¹, Tracey Woodruff⁸

Abstract

Background

Exposure to ambient air pollution is widespread and may be detrimental to human brain development and a potential risk factor for Autism Spectrum Disorder (ASD). We conducted a systematic review of the human evidence on the relationship between ASD and exposure to all airborne pollutants, including particulate matter air pollutants and others (e.g. pesticides and metals).

Objective

To answer the question: “Is developmental exposure to air pollution associated with ASD?”

Received: May 31, 2016
Accepted: August 14, 2016
Published: September 21, 2016

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Data Availability Statement: All relevant data are within the paper and its Supporting Information files.
• Applied Navigation Guide systematic review criteria
• 23 included studies
• Only PM$_{10}$ and PM$_{2.5}$ had sufficient studies rated of adequate quality for meta-analysis
• Heterogeneity suggested clustered analysis
• Statistically significant pooled effects
• Overall, still concluded available literature provided only “limited evidence” – due to concern over exposure assessment, residual confounding

PM$_{10}$: OR=1.07 (per 10ug/m$^3$) [1.06, 1.08]

PM$_{2.5}$: OR=2.32 (per 10ug/m$^3$) [2.15, 2.51]
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Case-control study

- Efficient outcome dependent sampling
- Less expensive, less time-consuming
- Difficult to accurately assess prenatal exposures

Pregnancy cohort

- Access to the proper etiologic window for exposure assessment
- Large sample size for rare outcomes
- Challenges to assessing outcome
- Expensive, time-consuming
Examples from the EARLI Study (an enriched ASD risk pregnancy cohort)

Proband
- Medical records
- Behavioral assessments
- Physical examinations
- Biological samples

Dad
- Biological samples
- Self-report

Sibling
- Medical records
- Behavioral assessments
- Physical examinations
- Biological samples

Home
- Environmental samples
- Environmental surveys

Mom
- Biological samples
- Exposure questionnaires

Prenatal PBDE exposure as an ASD risk factor

- Most highly used flame retardant in consumer products
- Migrate to the environment, are persistent and lipophilic
- PBDE body burdens are an order of magnitude higher in US than Europe & Asia
- US exposure levels had doubled every 4-6yrs US penta- and octa-BDEs production phased out in 2004, deca-BDEs in 2009

Figure from: Herbstman and Mall. Curr Envir Hlth Rep 2014

Hites RA. Environ Sci Technol 2004
Results from pilot analysis of biomarkers of PBDE exposure in the CHARGE case-control study

Exposure biomarker measured at age of diagnosis in child blood

Hertz-Picciotto et al Environ Hlth 2011
Preliminary (unpublished) results from analysis of biomarkers of PBDE exposure in the EARLI study

*Exposure biomarker measured in prenatal maternal blood (n=143)*

**Additional analyses pending:**

- Sequential multiple imputation by chained equations (MICE) for <LOD congeners – designed for use with multiple correlated values <LOD

- Unadjusted and covariate-adjusted models of association between continuous ln(SRS total score) and log-transformed continuous lipid-adjusted congener concentrations (using MIANALYZE in R) for full sample and stratified by sex. Mixed effects model to account for non-independent twin pairs
Mechanistic biomarkers: results from analysis of cord blood testosterone levels and 36mos SRS scores (n=137)

Park et al. *Molec Autism* 2017
How to build larger prospective cohorts?

Create new general population cohorts with more streamlined data collection

- Focus on biomarkers at delivery (maternal blood, cord blood, placenta, meconium) and EMR data
- Use two-stage, streamlined case-finding with nested schemes for biomarker based exposure assessment / deep phenotyping

Create larger synthetic cohorts from smaller existing cohorts

- Combine existing cohorts
- Overlay an array of low-collection-burden common measures

Pregnancy cohort

- Access to the proper etiologic window for exposure assessment
- Large sample size for rare outcomes
- Challenges to assessing outcome
- Expensive, time-consuming
Feasibility of two-stage, streamlined ASD case finding

- Given known performance characteristics of M-CHAT-R/F, simulate effect of different Stage II case confirmation test performance characteristics (non-differential with respect to exposure) on relative risk estimation bias
- Conclusion was that if Stage II cutpoints set to keep specificity above 80%, even if sensitivity drops to 50%-60%, RR bias is <10%
- In sample of 386 3-5 year olds recruited from evaluation at eight ASD and NDD clinics, used two-fold cross validation to estimate sensitivity and specificity for three candidate streamlined Stage II case confirmation approaches (ASI, STAT, E-VAS).
- Both STAT and E-VAS found to have a cutpoint that yielded ~80% specificity at ~50% sensitivity

Newschaffer et al. Aut Research 2017
Getting to 50,000 ECHO Children - Data from Milestone Accrual Plans
Outline

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Where are we now with GxE in ASD research?

- <10 published candidate GxE studies published to date
- Example: air pollution exposure and MET genotype in the CHARGE case-control study

Air pollution exposure modeled from residential distance to roads

MET: CC vs CG/GG

Air pollution: top 25%ile exposed vs others (5 pollutant measures)

Statistically significant interaction for one pollutant (NO2) - driven by a protective estimate for the CC unexposed and risky effect for CC exposed in a very small cell (n=4)

Volk et al Epidemiol 2014
Candidate GxE research in psychiatric epidemiology – the first ten years

- Median sample size of all (103) candidate GxE studies reviewed was 345
- Low statistical power at this sample size

- Combining low statistical power with weak hypotheses ("priors" in Bayesian parlance) leads to large expected false discovery rates

Duncan and Keller. *Amer J Psych* 2011
Candidate GxE research in psychiatric epidemiology – the first ten years

Challenges are amplified by publication bias:

• The first report on a GxE hypothesis is more publishable if positive, less publishable if null
  o 96% (45/47) of reviewed first reports were positive

• Replications of positive first report: more likely to be published regardless of whether there is a null finding
  o 27% (10/37) replication studies were positive

• If we assume that initial findings were false, would expect sample size of positive replication studies to be smaller on average than negative replication studies

Duncan and Keller. *Amer J Psych* 2011
Summary of the BIG challenges of GxE research in ASD

• Our “priors” are fairly weak
• Exposures are difficult to measure accurately in the proper etiologic window
• We will likely need larger sample size – especially if we want to approach discovery studies
The promise of exposomics

- Measure the internal chemical environment (potentially with dense arrays of markers) in biosamples
- Better reflect internal dose
- Account for heterogeneous individual metabolism
- Look for persistent signals
  - Those that accurately reflecting *past* exposure during critical etiologic windows

Rappaport and Smith, *Science* 2010
DNAm as a potential exposomics biomarker

- DNAm patterns in **cord blood** has been associated with prenatal maternal smoking

- Is this pattern replicated in blood of children around the age of ASD dx (3-5)?
The tooth exposome

• Temporally assignable record of exposure to xenobiotics in tooth dentine and enamel possible back to the start of the second trimester
• Microspatial sampling
• A range of mass spectroscopy techniques
• Validated for metals
• Validation for POPs underway

Can we do discovery GxE discovery?

• Gene-environment wide interaction studies (GWIS)
• Requires available genomics and environmental exposure data
• Even with more novel statistical methods intended to maximize efficiency (2df test, empirical Bayes approaches) still need large sample sizes and replication sets
• Still vulnerable to the “GWAS problems” – if real GxE effects are small magnitude, they will be hard to find
A proposed ASD GWIS for ECHO

• Assume ~30,000 subjects with genomics and basic exposure data (i.e., Pb level, air pollution via residential address, prenatal smoking)

• Mega-analyses to overcome cohort-specific differences in ancestry

Table 9. Estimated Minimum Detectable Gene-Environment Interaction Effects for GWIS (p<10^{-8}).

<table>
<thead>
<tr>
<th></th>
<th>Lead (2.6% Prevalence)</th>
<th>Air Pollution (1 SD Change)</th>
<th>Prenatal Smoking (8% Prevalence)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Allele Frequency</td>
<td>Allele Frequency</td>
<td>Allele Frequency</td>
</tr>
<tr>
<td>Minimum Detectable</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Odds Ratio NDD Dx</td>
<td>6,898 cases</td>
<td>2.42</td>
<td>1.79</td>
</tr>
<tr>
<td>Minimum Detectable</td>
<td>0.20</td>
<td>2.01</td>
<td>1.57</td>
</tr>
<tr>
<td>Odds Ratio ASD Dx</td>
<td>978 cases</td>
<td>5.50</td>
<td>1.49</td>
</tr>
<tr>
<td>Minimum Detectable</td>
<td>0.30</td>
<td>1.85</td>
<td>1.49</td>
</tr>
<tr>
<td>Change in Beta</td>
<td>3.95</td>
<td>3.40</td>
<td></td>
</tr>
<tr>
<td>SRS</td>
<td>30,000 subjects</td>
<td>5.27</td>
<td></td>
</tr>
</tbody>
</table>

MAF = Minor Allele Frequency; SD = Standard Deviation; NDD Dx = All Neurodevelopmental Delay (including ASD) Diagnosis; SRS = Social Responsiveness Scale Score
Might polygenic risk scores help us find GxE signals?

- Approach is being used increasingly in cancer epidemiology
- Summarize genetic risk into single value(s)
- Could base score on known ASD risk genes (i.e., from Psychiatric Genetics Consortium)
- Can use 2df test (or other approaches) without multiple-testing penalty
- Proposed this in ECHO for ASD-ER cohort of high-risk cohorts (tooth exposome data)
Environmental exposures and ASD: narrowing the knowledge gaps

• Critically important to consider prenatal environmental risk factors in the study of ASD etiology
• Existing evidence base is thin for most environmental exposures
• Work now underway in small enriched risk pregnancy cohorts
• For the future:
  • Append lighter ASD phenotyping case-finding to ongoing pregnancy/birth cohorts
  • Build *efficient* new birth cohorts
  • Create synthetic cohorts from existing studies
  • Explore exposomics – could support both prospective designs and traditional retrospective case-control designs
  • If valid exposure measures can be collected at scale in samples with genetic data collected/available, GWIS becomes feasible
  • Polygenic risk scores can also be used instead of a single candidate ‘G’ to more efficiently reveal exposures acting through GxE