Exploring Gene-Environment Interactions in Autism Spectrum Disorder

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University of Michigan
Kelly Bakulski
Today’s Talk

• Epidemiology of autism and related traits

• Main effects of environmental exposures on autism, related traits, and brain development

• Gene-environment interaction

• Challenges and opportunities ahead in population-based research of autism, genes, and the environment
Autism

Impairments in social communication and social interaction

Restricted and repetitive behaviors and interests

- Inclusion of sensory interests and aversions
- Severity levels

Picture from: http://www.cdc.gov/ncbddd/autism/facts.html
US Prevalence Estimates Continue to Increase

Percentage of 8-year-old children identified with ASD by ADDM Network Site

1 in 44
8-year-old children were identified with ASD in 2018 by the ADDM Network

More children are being identified with ASD by 48 months

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ADDM Community Report on Autism, 2021
Genetic Variation in Neurodevelopmental Disorders and Traits

- Heritability moderate to high for many neurodevelopmental outcomes
  - Autism (50-80%)
  - ADHD (77-88%)
  - Cognition (20-70%)
  - IQ (60-80%)

- Genetic variation includes:
  - Functional Polymorphisms
  - Common Variation (GWAS)
  - Genomic Instability (Copy Number)
  - Rare Variation (WES or WGS)
Family History

More likely to have ASD diagnosis if have a sibling with an ASD diagnosis

Original Investigation

The Familial Risk of Autism

Sven Sandin, MSc; Paul Lichtenstein, PhD; Ralf Kuja-Halkola, MSc; Henrik Larsson, PhD; Christina M. Hultman, PhD; Abraham Reichenberg, PhD

Figure 1. Age-Cumulative Probabilities for ASD Diagnosis in Siblings With a Full Sibling With ASD and in Siblings With a Full Sibling Without an ASD Diagnosis

ASD indicates autism spectrum disorder. Shaded areas represent 95% 2-sided point-wise confidence interval bands. The siblings who had a full-sibling with ASD were followed for 76 481 person-years resulting in 634 ASD events. The siblings who had a full sibling without ASD were followed for 35 486 922 person-years resulting in 17 327 ASD events.
Autism is more common in full siblings
- ~7-8 fold increase
- Male sex and number of affected siblings are predictors

Ozonoff et al., 2011
Trait Distributions Are Also Shifted in Siblings

- Mean MSEL scores suggest decreased performance in autism and non-autism siblings
  
  Messinger et al., 2015

- Greater proportion of “delayed” VABS scores in autism and non-autism siblings
  
  Charman et al., 2018
Social Responsiveness Scale (SRS) in Siblings

- SRS Scores Elevated in familial instances
- Distribution of trait shifted across autism and non-autism

Frazier et al., 2015
Genetic Influences on Autism

- Factors responsible for severity may diverge from those responsible for disease occurrence
- Suggests role of the early, non-shared environment

Fig. 1 Distributions of MZ twin-co-twin differences for autistic trait severity in superimposed density plots for the three respective samples in the study

Castelbaum et al., 2020

Fig. 2 Scatter plots of MZ twin-co-twin data: a General population, Social Responsiveness Scale (SRS) scores; b Clinically-ascertained MZ twins, SRS; c Clinically-ascertained MZ twins, Autism Diagnostic Observation Scale (ADOS)
Many Target Outcomes For Study in Autism

<table>
<thead>
<tr>
<th>DEVELOPMENTAL QUANTITATIVE TRAITS</th>
<th>Informs influences on child development, related, but not restricted to ASD</th>
</tr>
</thead>
<tbody>
<tr>
<td>• SRS</td>
<td></td>
</tr>
<tr>
<td>• SCQ</td>
<td></td>
</tr>
<tr>
<td>• CAST</td>
<td></td>
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</table>

<table>
<thead>
<tr>
<th>ASD DIAGNOSIS</th>
<th>Informs ASD etiology</th>
</tr>
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<table>
<thead>
<tr>
<th>ASD SEVERITY</th>
<th>Informs influences on severity for people with ASD</th>
</tr>
</thead>
<tbody>
<tr>
<td>• SRS</td>
<td></td>
</tr>
<tr>
<td>• VABS-2</td>
<td></td>
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</table>

<table>
<thead>
<tr>
<th>ASD CONDITIONS</th>
<th>Informs influences on severity for people with ASD</th>
</tr>
</thead>
<tbody>
<tr>
<td>• GI symptoms/conditions</td>
<td></td>
</tr>
<tr>
<td>• CBCL</td>
<td></td>
</tr>
<tr>
<td>• Mental health</td>
<td></td>
</tr>
<tr>
<td>• Sleep</td>
<td></td>
</tr>
<tr>
<td>• Obesity</td>
<td></td>
</tr>
</tbody>
</table>
Environmental Chemicals and Neurodevelopment

Figure 1. Effect of neurotoxicants during early brain development
Exposures in early life to neurotoxic chemicals can cause a wide range of adverse effects on brain development and maturation that can manifest as functional impairments or disease at any point in the human lifespan, from early infancy to very old age.

Grandjean and Landrigan, 2015
What Can Environmental Epidemiology Tells Us About Neurodevelopment?

• Environment = chemical/physical, social, lifestyle, ecosystems
  • Possible target for intervention

• Population based studies can inform on:
  • Frequency of environmental factor
  • Magnitude of risk in the population

• Environmental studies can inform on:
  • Mechanism and pathways
  • Biomarkers of effect
Environments of Interest in NDDs

Toxicants
- Air Pollution
- Metals
- Pesticides
- Flame Retardants
- Phthalates

Maternal Medical Factors
- Pregnancy Complications
- Mode of Delivery
- Prenatal Vitamins / Folate / Vitamin D / PUFAS
- Infections / Fever
- Pre-pregnancy BMI / Diabetes

Social Factors
- SES
- Discrimination

Demographics
- Maternal Age
- Paternal Age
- Inter-pregnancy Interval

Ecosystems
- Greenspace
- Neighborhood Deprivation
Investigated Modifiable Risk Factors

**Toxicants**
- Air Pollution
- Metals
- Pesticides
- Flame Retardants
- Phthalates

**Maternal Medical Factors**
- Pregnancy Complications
- Mode of Delivery
- Ultrasound
- Prenatal Vitamins / Folate
- Vitamin D
- PUFAs
- SSRIs
- Infections / Fever
- Pre-pregnancy BMI / Diabetes

**Demographics**
- Maternal Age
- Paternal Age
- Inter-pregnancy Interval
How Do You Measure the Environment?

• Questionnaires
  • In the last 12 months have you experienced…? 
  • During your pregnancy did you….? 
  • What was your address at the time of your child’s birth? 
  • Does your child have difficulty falling asleep? 
  • Does your child frequently demonstrate abdominal pain?

• Medical Records
  • Has your child been diagnosed by a doctor with…? 
  • Gestational age: _____ weeks 
  • Birth weight: _____ lbs ___ oz 
  • Maternal major depression diagnosis       yes     no 
    • Date of diagnosis ______ 
    • Medication history ______  ______  ______
How Do You Measure the Environment?

- Measures of Burden / Metabolism
  - Blood
  - Urine
  - Stool
  - Placenta
  - Hair
  - Nails
  - Teeth

- Geographic Linkages
  - US Census, ACS, Air Pollution
Air Pollution Has Broad Effects on Neurodevelopment

Review article

Prenatal air pollution exposure and neurodevelopment: A review and blueprint for a harmonized approach within ECHO

Heather E. Volk a,*, Frederica Perera b, Joseph M. Braun c, Samantha L. Kingsley c, Kimberly Gray d, Jessie Buckley e, Jane E. Clougherty f, Lisa A. Croen g, Brenda Eskenazi h, Megan Herting i, Allan C. Just j, Itai Kloog k, Amy Margolis l, Leslie A. McClure m, Rachel Miller n, Sarah Levine b, Rosalind Wright o, on behalf of program collaborators for Environmental influences on Child Health Outcomes l
A Systematic Review and Meta-Analysis of Multiple Airborne Pollutants and Autism Spectrum Disorder

Juleen Lam¹*, Patrice Sutton², Amy Kalkbrenner³, Gayle Windham⁴, Alycia Halladay⁵,⁶, Erica Koustas⁷, Cindy Lawler⁸, Lisette Davidson⁹, Natalyn Daniels¹⁰, Craig Newschaffer¹¹, Tracey Woodruff¹²

Conclusion

After considering strengths and limitations of the body of research, we concluded that there is "limited evidence of toxicity" for the association between early life exposure to air pollution as a whole and diagnosis of ASD. The strongest evidence was between prenatal exposure to particulate matter and ASD. However, the small number of studies in the meta-analysis and unexplained statistical heterogeneity across the individual study estimates means that the effect could be larger or smaller (including not significant) than these studies estimate. Our research supports the need for health protective public policy to reduce exposures to harmful airborne contaminants among pregnant women and children and suggests opportunities for optimizing future research.

Published: September 21, 2016
Air Pollution and Adaptive Function

Pre- and Postnatal Fine Particulate Matter Exposure and Childhood Cognitive and Adaptive Function

Laura A. McGuinn 1,*, Lisa D. Wiggins 2, Heather E. Volk 3, Qian Di 4, Eric J. Moody 5, Eric Kasten 6, Joel Schwartz 7, Robert O. Wright 1, Laura A. Schieve 2, Gayle C. Windham 8 and Julie L. Daniels 9

Figure 1. Adjusted mean difference (95% CI) in the scores of the Vineland Adaptive Behavior Scales associated with a 1 μg/m³ increase in PM$_{2.5}$, among ASD cases.
Infection and Fever

Boston Birth Cohort
N=1,257; 86 ASD Cases

Figure 1. Forest plot showing adjusted odds ratio (OR) and 95% confidence intervals for the association between prenatal GU infection, flu (overall and trimester-specific), and fever (overall and trimester-specific) and Autism Spectrum Disorder in the Boston Birth Cohort (BBC).

Brucato et al., 2017
Maternal Infection During Pregnancy

Fever appears to be consistent in context

Brucato et al., Autism Research 2017
Maternal Infection During Pregnancy

Prenatal maternal infection and risk for autism in offspring: A meta-analysis

Nina Tiroleco, Anna E. Silberman, Katharine Stratigos, Sharmila Banerjee-Basu, Marisa N. Spann, Agnes H. Whitaker, J. Blake Turner.

Consistency across 36 studies
Folate, Multi-Vitamins, and ASD

Case-cohort in Israel
N=45,300; 572 ASD Cases
Folate and ASD

Are high doses deleterious?

Boston Birth Cohort
N=1,257; 86 ASD Cases

Raghavan et al. 2018
Multivitamin Intake and ASD

Figure. Proportion of Mothers Who Reported Prenatal Vitamin Supplement Intake From 6 Months Before Pregnancy Through the End of Pregnancy

Modifiable factor operating on high-familial risk background!

MARBLES
N=55 ASD Cases; 60 non-TD; 126 TD

ASD indicates autism spectrum disorder; non-TD, nontypical development; and TD, typical development. Vertical bars represent 95% exact CIs (Clopper-Pearson).
Maternal Antidepressant Use During Pregnancy

Use of Selective Serotonin Reuptake Inhibitors during Pregnancy and Risk of Autism

Anders Hviid, Dr. Med. Sci., Mads Melbye, M.D., Dr. Med. Sci., and Björn Pasternak, M.D., Ph.D.

But what about the role of mental health itself?

Table 3. Association between Period of SSRI Use and Autism Spectrum Disorders in Offspring.*

<table>
<thead>
<tr>
<th>Period of Maternal SSRI Use</th>
<th>Offspring with Autism Spectrum Disorder</th>
<th>Follow-up</th>
<th>Rate Ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>no.</td>
<td>no. of person-yr</td>
</tr>
<tr>
<td>No use from 2 yr before pregnancy through delivery</td>
<td>3752</td>
<td>4,948,903</td>
<td>Reference</td>
</tr>
<tr>
<td>Use during pregnancy†</td>
<td>52</td>
<td>42,400</td>
<td>1.62 (1.23–2.13)</td>
</tr>
<tr>
<td>From 2 yr before pregnancy through delivery</td>
<td>29</td>
<td>25,436</td>
<td>1.50 (1.04–2.17)</td>
</tr>
<tr>
<td>Only during pregnancy</td>
<td>23</td>
<td>16,964</td>
<td>1.79 (1.19–2.69)</td>
</tr>
<tr>
<td>During first trimester</td>
<td>40</td>
<td>28,947</td>
<td>1.82 (1.33–2.49)</td>
</tr>
<tr>
<td>Use 2 yr to 6 mo before pregnancy but not during pregnancy</td>
<td>88</td>
<td>65,978</td>
<td>1.76 (1.42–2.27)</td>
</tr>
</tbody>
</table>
Maternal Psychiatric Conditions, Treatment With Selective Serotonin Reuptake Inhibitors, and Neurodevelopmental Disorders

Jennifer L. Ames, Christine Ladd-Acosta, M. Daniele Fallin, Yinge Qian, Laura A. Schieve, Carolyn DiGuiseppi, Li-Ching Lee, Eric P. Kasten, Guoli Zhou, Jennifer Pinto-Martin, Ellen M. Howerton, Christopher L. Eaton, and Lisa A. Croen


<table>
<thead>
<tr>
<th>SSRI Use</th>
<th>ASD</th>
<th>DD</th>
<th>POP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any Psychiatric Condition</td>
<td>128 (25.6)</td>
<td>167 (28.2)</td>
<td>94 (20.7)</td>
</tr>
<tr>
<td>ADHD</td>
<td>10 (19.2)</td>
<td>12 (20.0)</td>
<td>4 (13.3)</td>
</tr>
<tr>
<td>Anxiety Disorder</td>
<td>83 (38.1)</td>
<td>118 (42.9)</td>
<td>46 (27.1)</td>
</tr>
<tr>
<td>Bipolar Disorder</td>
<td>16 (32.7)</td>
<td>26 (40.6)</td>
<td>14 (42.4)</td>
</tr>
<tr>
<td>Depression</td>
<td>118 (31.7)</td>
<td>147 (35.2)</td>
<td>88 (27.4)</td>
</tr>
<tr>
<td>Eating Disorder</td>
<td>11 (31.4)</td>
<td>10 (22.7)</td>
<td>5 (11.1)</td>
</tr>
<tr>
<td>OCD</td>
<td>8 (34.8)</td>
<td>9 (28.1)</td>
<td>4 (28.6)</td>
</tr>
<tr>
<td>Personality Disorder</td>
<td>1 (11.1)</td>
<td>7 (50.0)</td>
<td>1 (16.6)</td>
</tr>
<tr>
<td>Schizophrenia</td>
<td>2 (40.0)</td>
<td>6 (85.7)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Sleep Disorder</td>
<td>25 (22.9)</td>
<td>30 (25.9)</td>
<td>11 (16.2)</td>
</tr>
<tr>
<td>Self-injuring Behavior</td>
<td>9 (42.9)</td>
<td>6 (30.0)</td>
<td>4 (19.0)</td>
</tr>
<tr>
<td>Suicide Attempt</td>
<td>14 (28.6)</td>
<td>20 (33.3)</td>
<td>8 (29.6)</td>
</tr>
</tbody>
</table>

Mothers with a history of anxiety disorder or eating disorder were more likely to have a child with ASD.

Mothers with a history of any psychiatric condition, anxiety, or depression were more likely to have a child with DD.
No significant association for ASD when comparing SSRI use among mothers with a psychiatric disorder
Exposure x Exposure Interaction

Fig. 1  Key pathways that may link environmental exposures, nutrients, and neurodevelopmental outcomes

Bragg et al., 2022
Environment x Social Factors

Distribution of Absolute burden of PM(2.5) emissions from NEI facilities by race/ethnicity & poverty

Air pollution, neighborhood deprivation, and autism spectrum disorder in the Study to Explore Early Development

Laura A. McGuinn\textsuperscript{1,}\textsuperscript{2}, Gayle C. Windham\textsuperscript{3}, Lynne C. Messer\textsuperscript{4}, Qian Di\textsuperscript{5}, Joel Schwartz\textsuperscript{6}, Lisa A. Croen\textsuperscript{7}, Eric J. Moody\textsuperscript{8}, Ana G. Rappold\textsuperscript{9}, David B. Richardson\textsuperscript{10}, Lucas M. Neas\textsuperscript{11}, Marilie D. Gammon\textsuperscript{12}, Laura A. Schieve\textsuperscript{13}, Julie L. Daniels\textsuperscript{13}

\textbf{Results:} Neighborhood deprivation modified ($P_{\text{int}} = 0.08$) the association between PM$_{2.5}$ exposure during the first year of life and ASD, with a stronger association for those living in high (OR = 2.42, 95% CI = 1.20, 4.86) rather than moderate (OR = 1.21, 95% CI = 0.67, 2.17) or low (OR = 1.46, 95% CI = 0.80, 2.65) deprivation neighborhoods. Departure from additivity or multiplicativity was not observed for roadway proximity or exposures during pregnancy.

\textbf{Conclusion:} These results provide suggestive evidence of interaction between neighborhood deprivation and PM$_{2.5}$ exposure during the first year of life in association with ASD.

Visual representation of long-term average PM2.5 exposures for four racial/ethnic groups by urbanicity and region
Moving Toward GxE in ASD

**Genetics**
- Inherited
  - Common SNP
  - Common CNV
  - Rare SNV
  - Rare CNV
- *De novo*
  - Rare SNV
  - Rare CNV

**Environment**
- Parental Characteristics
  - Age
  - Medical Conditions
  - Perinatal/Obstetric
  - Nutrition
- Toxicants
  - Chemical
    - Behavioral
    - Environmental
    - Occupational
    - Pharmaceutical
    - Biological

**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**

Bakulski KM, Singer AB, Fallin MD. 2014. *Frontiers in Autism Research*
Gene-Environment Interaction in ASD

Different Types of Genetic Variation
- Functional Polymorphisms
- Common Variation (GWAS)
- Genomic Instability (Copy Number)
- Rare Variation (WES or WGS)

Gaugler et al., 2014
Limited Work on GxE in ASD: ~12 Published Studies

Genetic Factors Studied:
- Functional Polymorphisms (6 studies)
- Copy Number Variants (3 studies)
- De Novo Mutations (1 study)
- ASD associated Variants (1 study)
- Oxidative Stress Risk Score (1 study)

Environmental Factors Studied:
- Air Pollution (2 studies)
- Folate / Prenatal Vitamins (2 studies)
- 1st Trimester Ultrasound (1 study)
- Infection / Fever During Pregnancy (1 study)
- Phthalates (1 study)
- Familial Psychiatric Disorders (1 study)
- Metals (3 studies)
- Mix of Environmental Chemicals (1 study)
Folic Acid and MTHFR

FIGURE 2. ORs (95% CIs) for associations between mean maternal daily folic acid intake (≥600 μg compared with <600 μg) during the first month of pregnancy and autism spectrum disorder by maternal and child MTHFR genotype. ORs were adjusted for maternal educational level and child’s birth year. Categories of folic acid intake were created on the basis of the recommended intake during pregnancy (600 μg/d). Analyses were based on 272 children with autism spectrum disorder and 275 of their mothers, and 154 children with typical development and 163 of their mothers with MTHFR 677 genotype and folic acid intake data. The frequencies of participants in each category of folic acid intake and MTHFR 677 genotype are presented in Supplemental Table 1 under “Supplemental data” in the online issue.
Air Pollution and MET in ASD

Prenatal Benzo(a)Pyrene Exposure Reduces MET Protein Expression in Mouse Cortex

Sheng et al., 2010

Joint Effect of MET and Air Pollution in CHARGE

<table>
<thead>
<tr>
<th>Near Roadway Air Pollution</th>
<th>MET rs1858830 Genotype</th>
<th>C/C</th>
<th>C/G or G/G</th>
</tr>
</thead>
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<tr>
<td>Exposed</td>
<td>2.9 (1.0-10.4)</td>
<td>1.3 (0.73-2.2)</td>
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<tr>
<td>Unexposed</td>
<td>0.80 (0.47-1.4)</td>
<td>reference</td>
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<table>
<thead>
<tr>
<th>Regional Nitrogen Dioxide</th>
<th>MET rs1858830 Genotype</th>
<th>C/C</th>
<th>C/G or G/G</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exposed</td>
<td>3.6 (1.3-12.7)</td>
<td>1.2 (0.71-2.1)</td>
<td></td>
</tr>
<tr>
<td>Unexposed</td>
<td>0.72 (0.41-1.3)</td>
<td>reference</td>
<td></td>
</tr>
</tbody>
</table>

Volk et al., 2014
Copy Number Burden x Air Pollution in CHARGE

Kim et al., 2017
Maternal Infection X CNV Burden

Figure 1. Autism symptomatology and cognitive and adaptive functioning of children with autism spectrum disorder–associated copy number variants (CNVs) and history of maternal infection or fever during pregnancy (N = 1971). Error bars = 95% confidence interval. As shown in graph C (p = .010), a main effect for presence of infections is demonstrated. As shown in graphs G (p = .019) and H (p = .049), main effects for presence of CNV are demonstrated. As shown in graphs A–E: (A) p = .006; (B) p = .006; (C) p = .017; (D) p = .012; (E) p = .014, significant interactions are observed. No significant interactions are observed in graphs F and H.

Mazina et al., 2015
Antidepressant Use \(\times\) Large Gene Disrupting Mutations

**Fig. 1** Interaction between antidepressant (AD) exposure and LGD mutation on ASD symptoms (ADOS CSS): significant interactive effect: \(F(1, 2542) = 4.882, p = 0.027.\) *Error bars 95% CI*

**Fig. 2** Interaction between antidepressant (AD) exposure and LGD mutation versus ASD severity (ADI-R): significant interactive effect in ADI-R verbal communication domain: \(F(1, 2397) = 4.554, p = 0.033.\) *Error bars 95% CI*

Ackerman et al., 2018 JADD
Gene-Environment Interaction in Neurodevelopmental Disorders

• What’s the goal?
  • Identification of new genes / environments associated with an outcome?
  • Account for inherited risk?
  • Study joint effects of gene and environment?

Sample size needs increase as E and G become more rare!
GWAS Exist For Many Neurodevelopmental Outcomes

Figure 1. Manhattan plot depicting results of genome-wide association study meta-analysis for general cognitive function. Green arrows indicate loci attaining genome-wide significance (red line, $P < 5 \times 10^{-8}$). Gray arrow indicates locus at chromosome 17q21.31 approaching genome-wide significance.

General Cognitive Function
Trampush et al., 2017

ADHD
Demontis et al., 2019

Digit Symbol (Executive Function)
Ibrahim-Verbaas et al., 2016

ASD
Grove et al., 2019
ASD GWAS...Now With Hits!

N=2503 / 7271

N=7387 / 8567
PGC

N=18,381 / 27,969
iPSYCH + PGC
PGC+iPSYCH GWAS findings:
- 18,381 ASD cases
- 27,969 controls
- >9 million SNPs

From GWAS to Polygenic Score (PGS)

From Discovery . . .

From GWAS to Polygenic Score (PGS)

From Discovery . . . To Target Sample

Kelly Benke

ASD-risk SNPs >20k

Compute ASD-PGS in familial samples

p-value threshold & pruning

<table>
<thead>
<tr>
<th>SNP</th>
<th>SNP 1</th>
<th>Weighted ASD-PGS</th>
<th>Weighted Cross-Disorder PGS</th>
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<tbody>
<tr>
<td>0</td>
<td>1.03</td>
<td>5.34</td>
<td>7.34</td>
</tr>
<tr>
<td>1.2</td>
<td>2.06</td>
<td>10.69</td>
<td>25.6</td>
</tr>
<tr>
<td>0</td>
<td>1.03</td>
<td>7.4</td>
<td>2.03</td>
</tr>
<tr>
<td>2.4</td>
<td>2.06</td>
<td>22.94</td>
<td>15.31</td>
</tr>
</tbody>
</table>
PGS x Environment Example: Obstetric Complications and Schizophrenia PRS

- PGS + ELCs helped both be characterized
- Prediction implications are important!
Sidenote: PGS Might Predict Progression Over Time

Chaudhury et al., 2019

PGS for Alzheimer’s Disease
• Controls vs. Late Onset AD
• Non-Converters vs. Late Onset AD
• Higher Deciles Have More Late Onset AD
Genetic Susceptibility for ASD and Related Traits

- Build polygenetic scores (PGS) from genome-wide data in several ASD studies:
  - Longitudinal Sibling Cohort Designs
    - EARLI Autism Risk Longitudinal Investigation (EARLI)
    - Markers of Autism Risk in Babies Learning Early Signs (MARBLES)
    - Infant Brain Imaging Study (IBIS)
    - Baby Sibling Research Consortium (BSRC)
**Current ASD PGS Analysis in Siblings**

**Diagnosed with Autism (CBE/DSMIV/DSM5) at 36 or 24 months**

- **Yes = ASD**
- **No or Missing**

**ADOS Comparison Score (36 months or 24 months) >= 3**

- **Yes = Non-TD**
- **No Concerns or Missing**

**Mullen[Expressive Language, Fine Motor, Receptive Language, Visual Reception]**

(At 36 or 24 months):

- One subset is <30 OR
- Two or more subsets are <35

- **Yes = Non-TD**
- **No Concerns = TD**

---

*IF Participant is missing ADOS and missing all 4 subscales THEN they are excluded. IF they are missing one subscale, they must score > 40 on the other three subscales in order to be included.*

---

<table>
<thead>
<tr>
<th></th>
<th>ASD</th>
<th>NTD</th>
<th>TD</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>IBIS</strong></td>
<td>67</td>
<td>67</td>
<td>202</td>
<td>336</td>
</tr>
<tr>
<td><strong>EARLI</strong></td>
<td>34</td>
<td>55</td>
<td>74</td>
<td>163</td>
</tr>
<tr>
<td><strong>MARBLES</strong></td>
<td>51</td>
<td>45</td>
<td>171</td>
<td>267</td>
</tr>
<tr>
<td><strong>All three</strong></td>
<td>124</td>
<td>195</td>
<td>447</td>
<td>766</td>
</tr>
</tbody>
</table>
Genes and Environment in SPARK

• Data source: Simons Powering Autism Research for Knowledge (SPARK)
  • Database of 50,000 families with genetic and phenotypic data available
  • All participants re-contactable through SPARK Research Match

• Mothers contacted to participate in “Genes and Environment Autism Research Study (GEARS)”
  • Eligibility: children 2-12 years old, genetic data available
Exposure & Outcome Measurement

Exposures

- Custom, 30-item “GEARS Questionnaire”
- Lifetime and pregnancy medical, and lifestyle history
  
  “Before your pregnancy with [child name], did you have any of the following conditions as an adult?” (severe mental illness, diabetes, asthma…)
  
  “During the 3 months before you became pregnant with [child name], were you taking a prenatal vitamin, multi-vitamin, or folic acid supplement?”

Outcomes

- Mother-reported diagnosis of ASD
- Three autism-related quantitative trait measures:
  
  - Social Communication Questionnaire-Lifetime (SCQ)
  - Repetitive Behavior Scale-Revised (RBS-R)
  - Developmental Coordination Disorder Questionnaire (DCDQ)
GEARS in SPARK so far…

Genetic and exposure data for:
• N = 2,290 mother-child pairs with genetic & exposure data
• N = 1,752 of European ancestry

<table>
<thead>
<tr>
<th>Variable</th>
<th>N (%) or Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Child Male Sex</td>
<td>1422 (81.2)</td>
</tr>
<tr>
<td>Child age at registration</td>
<td>5.55 (2.35)</td>
</tr>
<tr>
<td>Child age at ASD diagnosis</td>
<td>3.82 (1.91)</td>
</tr>
<tr>
<td>Mother age at birth (years)</td>
<td>30.01 (5.49)</td>
</tr>
<tr>
<td>Mother reported race</td>
<td></td>
</tr>
<tr>
<td>African American</td>
<td>15 (0.9)</td>
</tr>
<tr>
<td>Asian</td>
<td>6 (0.4)</td>
</tr>
<tr>
<td>Native American</td>
<td>32 (2.0)</td>
</tr>
<tr>
<td>Other</td>
<td>10 (0.6)</td>
</tr>
<tr>
<td>White</td>
<td>1571 (96.1)</td>
</tr>
<tr>
<td>Mother ethnicity (non-Hispanic)</td>
<td>1627 (92.6)</td>
</tr>
<tr>
<td>Mother highest education completed</td>
<td></td>
</tr>
<tr>
<td>H.S. or Less</td>
<td>187 (10.9)</td>
</tr>
<tr>
<td>Some College</td>
<td>262 (15.3)</td>
</tr>
<tr>
<td>Tech./Assoc. Degree</td>
<td>246 (14.4)</td>
</tr>
<tr>
<td>Bachelor’s Degree</td>
<td>567 (33.1)</td>
</tr>
<tr>
<td>Advanced Degree</td>
<td>450 (26.3)</td>
</tr>
</tbody>
</table>
SCQ Scores in SPARK GEARS Sample

SCQ Final Scores for N = 1,685 children

- ASD screen result
  - Negative
  - Positive
# Reported Exposures from SPARK GEARS

<table>
<thead>
<tr>
<th>Exposure</th>
<th>N total</th>
<th>N endorsed</th>
<th>% endorsed</th>
<th>N missing</th>
<th>% missing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamins</td>
<td>1701</td>
<td>1651</td>
<td>97.1</td>
<td>51</td>
<td>3</td>
</tr>
<tr>
<td>Pre-natal vitamins</td>
<td>1668</td>
<td>938</td>
<td>56.2</td>
<td>84</td>
<td>5</td>
</tr>
<tr>
<td>Fever</td>
<td>1429</td>
<td>769</td>
<td>53.8</td>
<td>323</td>
<td>22.6</td>
</tr>
<tr>
<td>Alcohol</td>
<td>1463</td>
<td>644</td>
<td>44</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Depression</td>
<td>1667</td>
<td>663</td>
<td>39.8</td>
<td>85</td>
<td>5.1</td>
</tr>
<tr>
<td>Flu</td>
<td>1519</td>
<td>435</td>
<td>28.6</td>
<td>233</td>
<td>15.3</td>
</tr>
<tr>
<td>Smoke</td>
<td>720</td>
<td>167</td>
<td>23.2</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Pre-term labor</td>
<td>1693</td>
<td>349</td>
<td>20.6</td>
<td>59</td>
<td>3.5</td>
</tr>
<tr>
<td>Asthma</td>
<td>1705</td>
<td>330</td>
<td>19.4</td>
<td>47</td>
<td>2.8</td>
</tr>
<tr>
<td>Pre-eclampsia</td>
<td>1693</td>
<td>307</td>
<td>18.1</td>
<td>59</td>
<td>3.5</td>
</tr>
<tr>
<td>Hyperemesis</td>
<td>1699</td>
<td>255</td>
<td>15</td>
<td>53</td>
<td>3.1</td>
</tr>
<tr>
<td>Gestational diabetes</td>
<td>1694</td>
<td>249</td>
<td>14.7</td>
<td>58</td>
<td>3.4</td>
</tr>
<tr>
<td>Mental illness</td>
<td>1707</td>
<td>164</td>
<td>9.6</td>
<td>45</td>
<td>2.6</td>
</tr>
<tr>
<td>Eclampsia</td>
<td>1697</td>
<td>103</td>
<td>6.1</td>
<td>55</td>
<td>3.2</td>
</tr>
<tr>
<td>Diabetes</td>
<td>1708</td>
<td>60</td>
<td>3.5</td>
<td>44</td>
<td>2.6</td>
</tr>
</tbody>
</table>
### Relationship of ASD PGS and Environment in Children with Autism

<table>
<thead>
<tr>
<th>Exposure</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asthma</td>
<td>1705</td>
<td>19.4</td>
</tr>
<tr>
<td>Lupus</td>
<td>1695</td>
<td>3.9</td>
</tr>
<tr>
<td>Depression</td>
<td>1667</td>
<td>39.8</td>
</tr>
<tr>
<td>Mental illness</td>
<td>1707</td>
<td>9.6</td>
</tr>
<tr>
<td>Pre vitamin</td>
<td>1668</td>
<td>56.2</td>
</tr>
<tr>
<td>Vitamin</td>
<td>1701</td>
<td>97.1</td>
</tr>
<tr>
<td>Flu</td>
<td>1519</td>
<td>28.6</td>
</tr>
<tr>
<td>Fever</td>
<td>1429</td>
<td>53.8</td>
</tr>
<tr>
<td>Diabetes</td>
<td>1708</td>
<td>3.5</td>
</tr>
<tr>
<td>Eclampsia</td>
<td>1697</td>
<td>6.1</td>
</tr>
<tr>
<td>Gest diabetes</td>
<td>1694</td>
<td>14.7</td>
</tr>
<tr>
<td>Hyperemesis</td>
<td>1699</td>
<td>15</td>
</tr>
<tr>
<td>Low BP</td>
<td>1702</td>
<td>4.7</td>
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<tr>
<td>Pre–eclampsia</td>
<td>1693</td>
<td>18.1</td>
</tr>
<tr>
<td>Prerupture</td>
<td>1699</td>
<td>11.6</td>
</tr>
<tr>
<td>Preterm labor</td>
<td>1693</td>
<td>20.6</td>
</tr>
<tr>
<td>Smoking</td>
<td>720</td>
<td>23.2</td>
</tr>
<tr>
<td>Tobacco</td>
<td>134</td>
<td>10.4</td>
</tr>
<tr>
<td>Alcohol</td>
<td>1463</td>
<td>44</td>
</tr>
</tbody>
</table>

#### Odds ratio (95% CI)

- Odds ratio for each exposure indicates the risk associated with the presence of a particular condition.
Interaction of PGS and Exposure

<table>
<thead>
<tr>
<th>Exposure</th>
<th>SCQ score</th>
<th>N</th>
<th>Beta (95% CI)</th>
<th>Unadjusted p-value</th>
<th>FDR-adjusted p-value*</th>
<th>2df LRT Adjusted R2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever</td>
<td>Final</td>
<td>1376</td>
<td>-0.98 (-1.82, -0.05)</td>
<td>0.038</td>
<td>0.627</td>
<td>0.017</td>
</tr>
</tbody>
</table>

* p-values adjusted for 76 total exposure-outcome tests

**Fever**

![Graph showing the interaction of PGS and SCQ score for Fever exposure]

**Mental illness**

![Graph showing the interaction of PGS and SCQ score for Mental illness exposure]
Interaction of PGS and Exposure

<table>
<thead>
<tr>
<th>Exposure</th>
<th>SCQ score</th>
<th>N</th>
<th>Beta (95% CI)</th>
<th>Unadjusted p-value</th>
<th>FDR-adjusted p-value*</th>
<th>2df LRT Adjusted R2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-natal vitamin</td>
<td>Stereotyped</td>
<td>1235</td>
<td>0.24 (-0.01, 0.50)</td>
<td>0.058</td>
<td>0.627</td>
<td>0.028</td>
</tr>
</tbody>
</table>

*p-values adjusted for 76 total exposure-outcome tests
Further Considerations in GxE

Genotyping
- Other Types of Burden Scores
  - Copy number, rare variant, etc.
- Mom or Child Genotype?
- Comparability of discovery and target samples

Environment
- Exposure Assessment Harmonization
- Exposure Misclassification

Analytic Challenges
- Categorial outcomes or continuous traits? Subgroups?
- Outcome harmonization
- Which test to use?
  - Discovery? Joint effects? Prediction?
  - 1, 2, or 3 degree of freedom tests
Infrastructure for GxE:

- 18 Network Sites (plans to add more)
  - ~175,000 individuals
- Translation to Laboratory Models
  - Mini-brain Models
- Community Advisory Board and Outreach

R01 ES034554 (MPI Volk, Ladd-Acosta)
Flexible Structure for Study of GxE in Epidemiologic Data

- Genetics: SNP, CNV, RV, PRS, RVb, CNVb
- Environments: E1, E2, E3, Emix, Esum, Elatent

**Developmental Quantitative Traits**
- SRS
- SCQ
- CAST
  - Informs influences on child development, related, but not restricted to ASD

**ASD Diagnosis**
- Informs ASD etiology

**ASD Severity**
- SRS
- VABS-2
  - Informs influences on severity for people with ASD

**ASD Conditions**
- GI symptoms/conditions
- CBCL
- Mental health
- Sleep
- Obesity
  - Informs influences on severity for people with ASD

GEARs Network
Combining advances in Genomics and Environmental science to accelerate Actionable Research and practice in ASD
Repository for Study of GxE in Laboratory Models
# Planned Analyses

<table>
<thead>
<tr>
<th>ASD outcome domain (measure)</th>
<th>Participants</th>
<th>Environment</th>
<th>Genetic measure</th>
<th>Detectable effect size*</th>
<th>Power increase**</th>
</tr>
</thead>
<tbody>
<tr>
<td>(i) Cognitive ability (MSEL)</td>
<td>All</td>
<td>Air pollutants</td>
<td>16p11.2 deletion</td>
<td>1.13</td>
<td>15x</td>
</tr>
<tr>
<td>(ii) Diagnosis (ASD)</td>
<td>All</td>
<td>Maternal infection</td>
<td>SNP genotypes</td>
<td>1.13</td>
<td>16x</td>
</tr>
<tr>
<td>(iii) Severity (SRS)</td>
<td>ASD cases</td>
<td>Folic acid/vitamins</td>
<td>Rare variant burden</td>
<td>1.14</td>
<td>10x</td>
</tr>
<tr>
<td>(iv) Health conditions (anxiety)</td>
<td>ASD cases</td>
<td>Air pollutants</td>
<td>ASD polygenic burden</td>
<td>1.07</td>
<td>10x</td>
</tr>
</tbody>
</table>

*Using anticipated total number of ~170,000 participants in total and ~85,000 ASD cases (see details in Table 2)

**Increase from largest sample size for published GxE result to date (n=2,514)

ASD= autism spectrum disorder; SRS = social responsiveness scale; MSEL = Mullen Scales of Early Learning;
Develop and implement a pipeline for outreach and dissemination of GxE findings that informs action. Using a multi-pronged communication strategy, we will:

- Develop and transmit material to community partners, stakeholders, clinicians, and educators to translate our findings into actionable public health messaging.
- Actively seek input from stakeholders, including autistic people and their families, to foster collaborative partnership and design effective and valued communication.
Moving Toward Intervention and Prevention

- Disorder severity and trait measures
- Co-occurrence of physical health conditions
- Many types of exposures (together!)
- Diverse populations

With Collaboration We Can Effectively Address Challenges and Improve Children’s Health!
Thank you!

Thank you to our funders: NIH (OD023342, ES032469, HD103538, ES029511, ES030893, ES026961, HD055741, ES025531, ES023780), CDC (DD00129), Autism Speaks (#7785, 8463), Autism Science Foundation