Environmental exposure and autism risk:
narrowing the knowledge gaps

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Acknowledgements

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The SEED investigators

The ASD-ER investigators

The EEARN team

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U20 DD000183 (Fallin)

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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

The NERVOUS CHILD

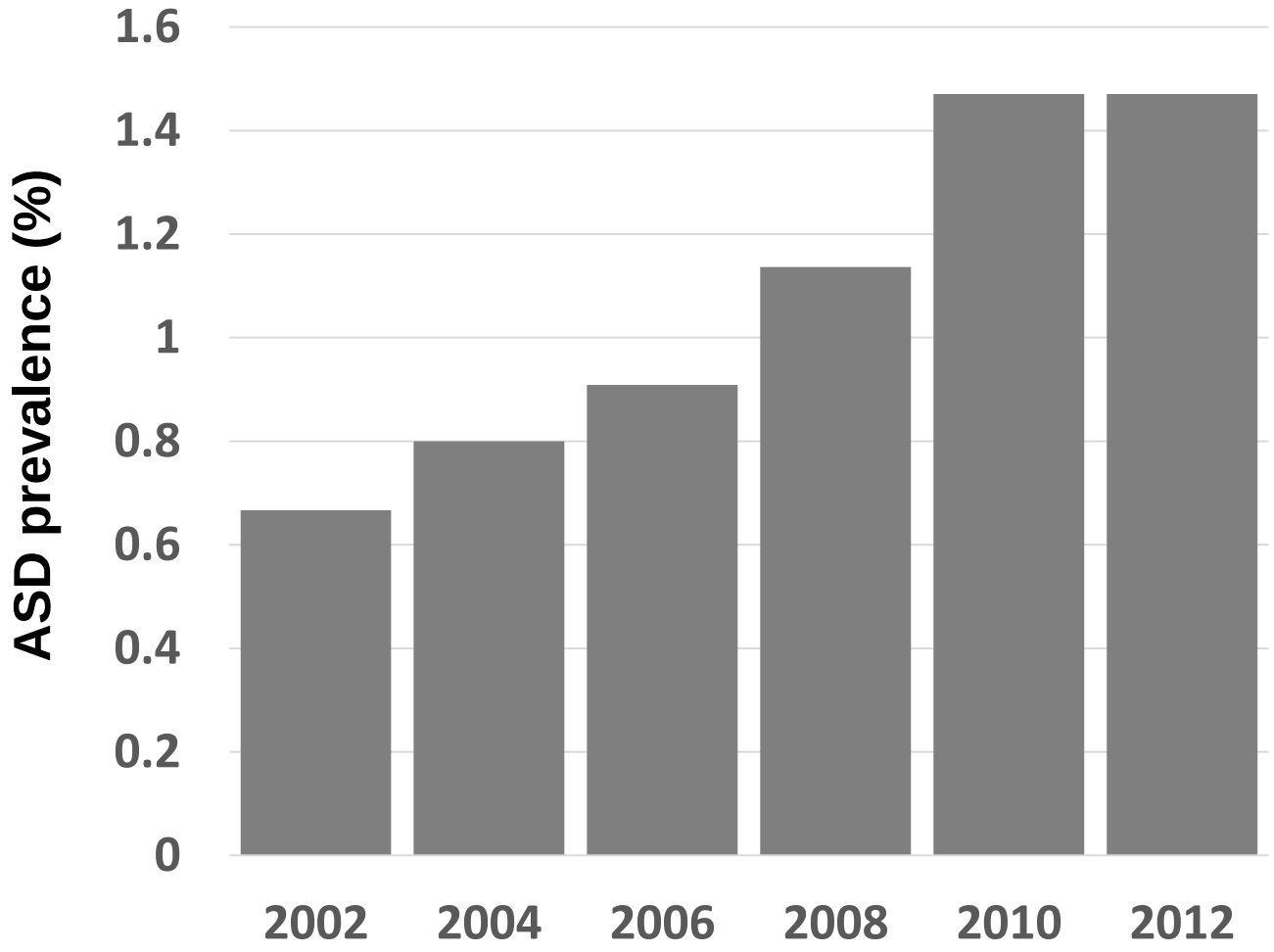
Quarterly Journal of Psychopathology, Psychotherapy,
Mental Hygiene, and Guidance of the Child

AUTISTIC DISTURBANCES OF AFFECTIVE CONTACT

By LEO KANNER

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.

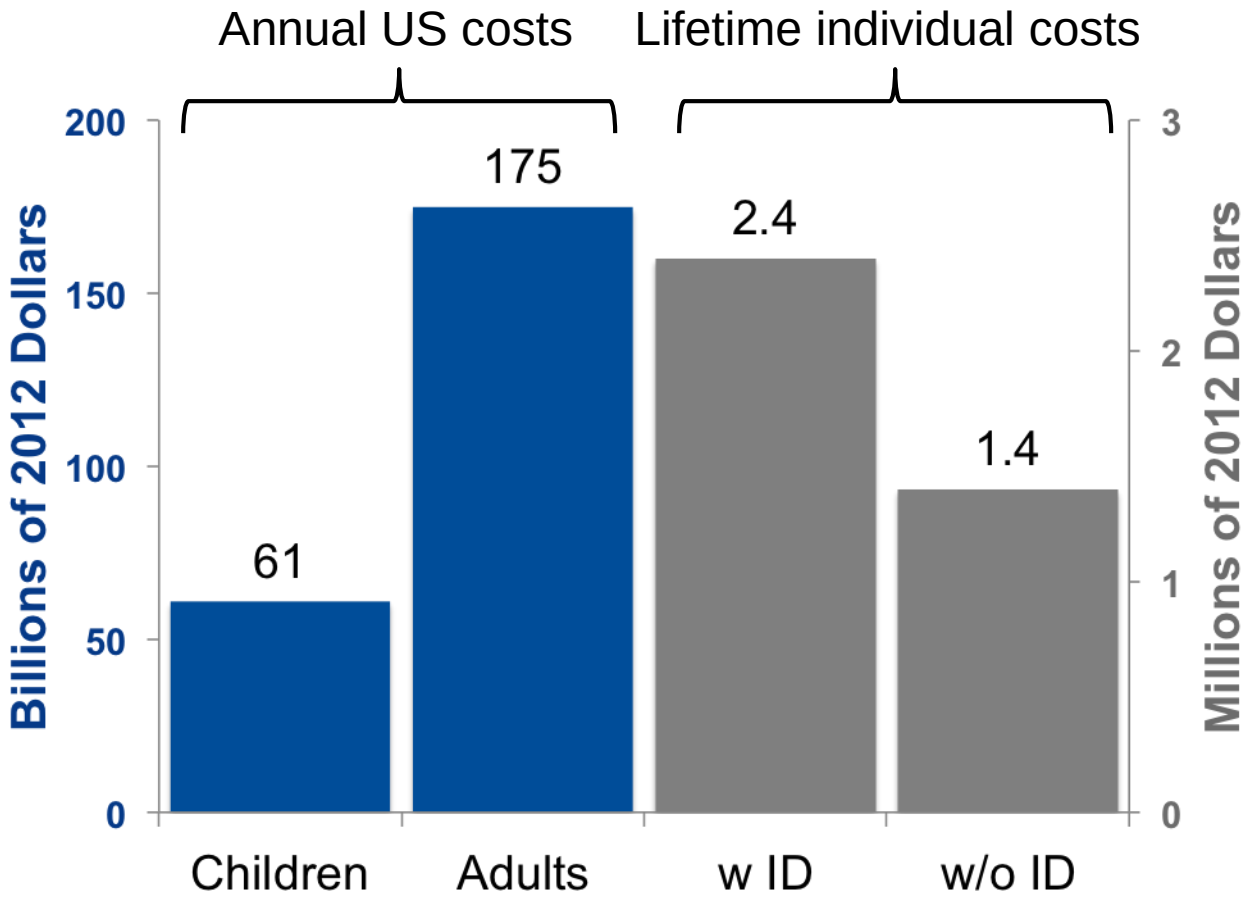
Kanner, L. Autistic Disturbances of Affective Contact. Nervous Child, (2) 217-250, 1943



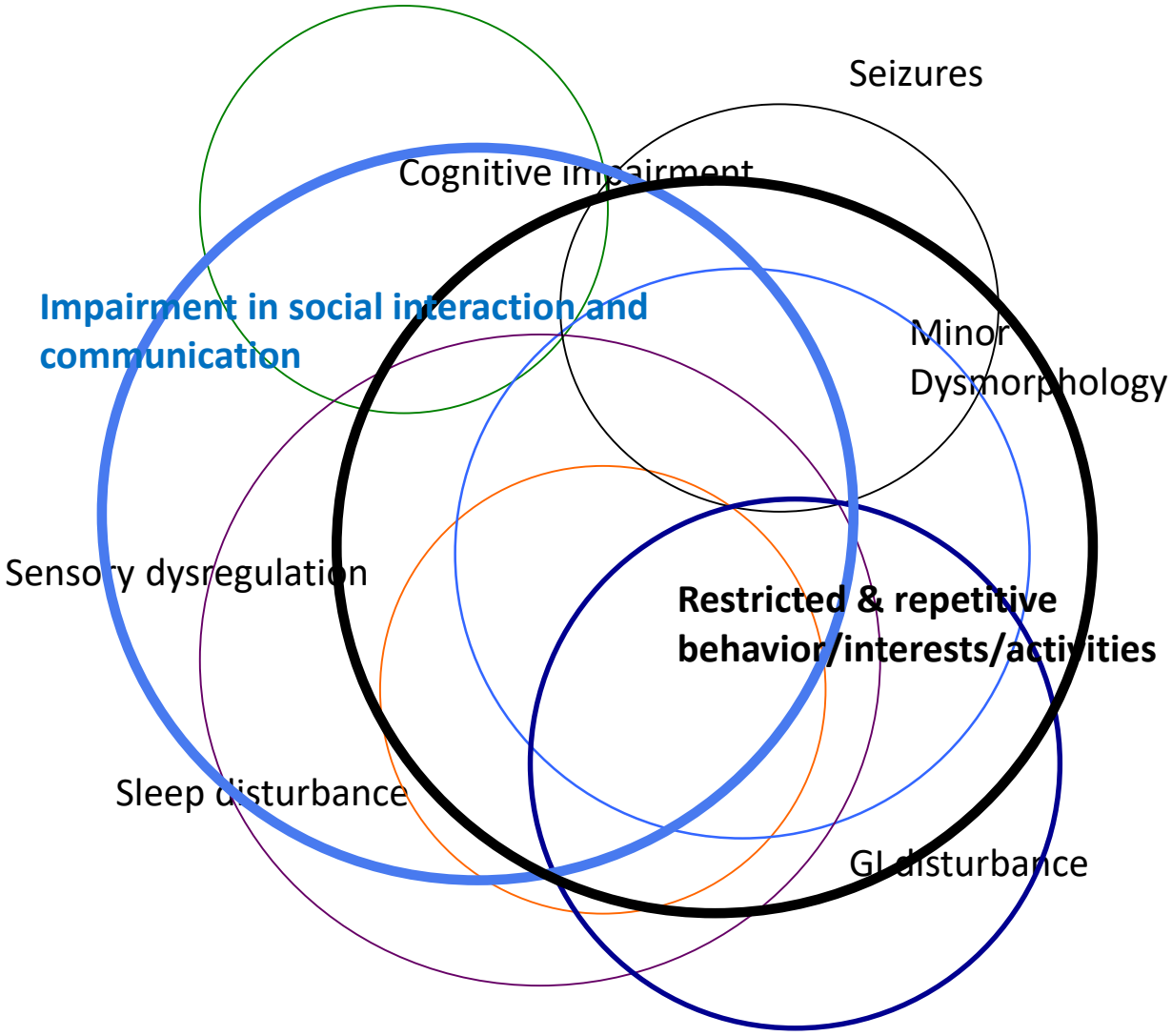
CDC™

ADDM NET
Autism and Developmental Disabilities Monitoring Network

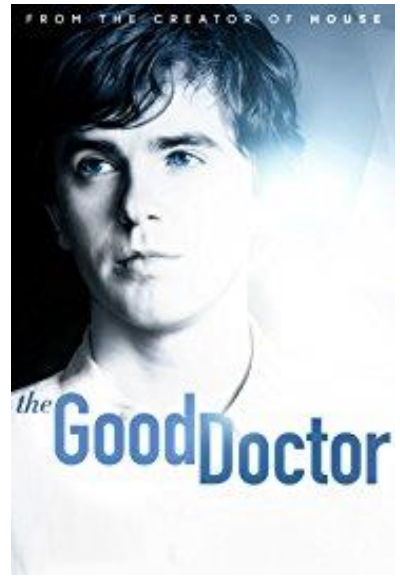
Economic costs of ASD in the United States

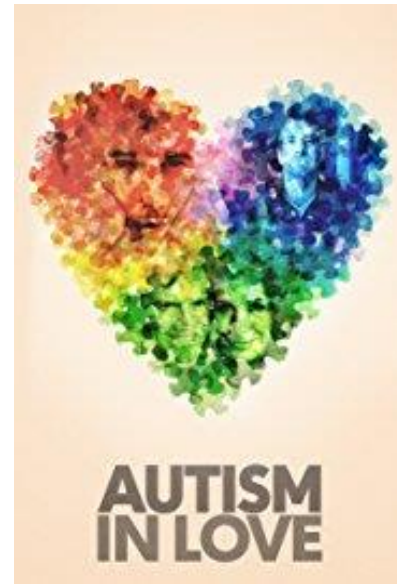


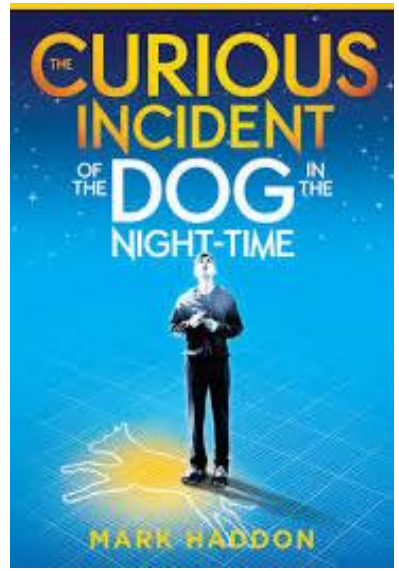
Buescher et al. *JAMA Pediatr* 2014

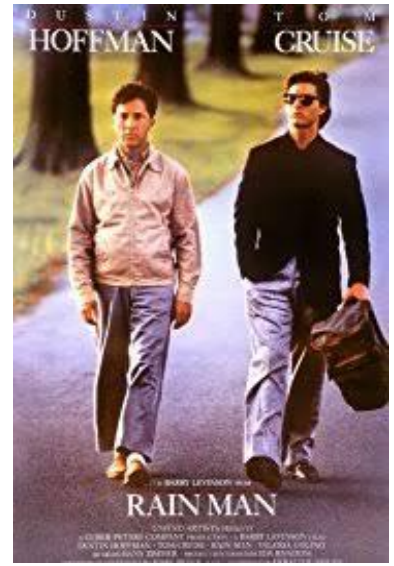

Seizures
Cognitive impairment
Impairment in social interaction and communication
Minor Dysmorphology
Sensory dysregulation
Restricted & repetitive behavior/interests/activities
Sleep disturbance
GI disturbance

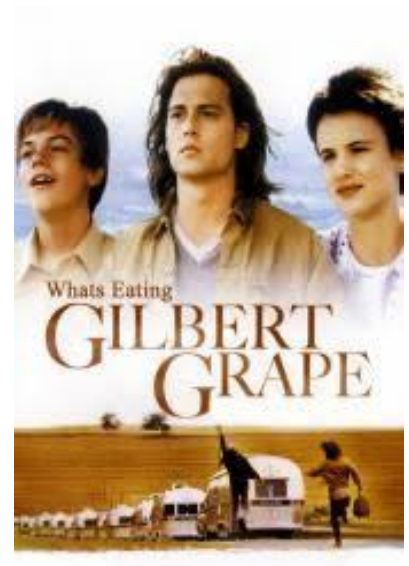

CLAIRE DANES
AUTISM GAVE HER A VISION.
SHE GAVE IT A VOICE.
Temple Grandin
WHAT MADE HER DIFFERENT MADE HER EXCEPTIONAL
SATURDAY, FEBRUARY 6 AT 8 PM HBO


FROM THE CREATOR OF HOUSE
the Good Doctor


AUTISM IN LOVE


THE CURIOUS INCIDENT OF THE DOG IN THE NIGHT-TIME
MARK HADDON


DUSTIN HOFFMAN
TOM CRUISE
RAIN MAN


Whats Eating
GILBERT GRAPE

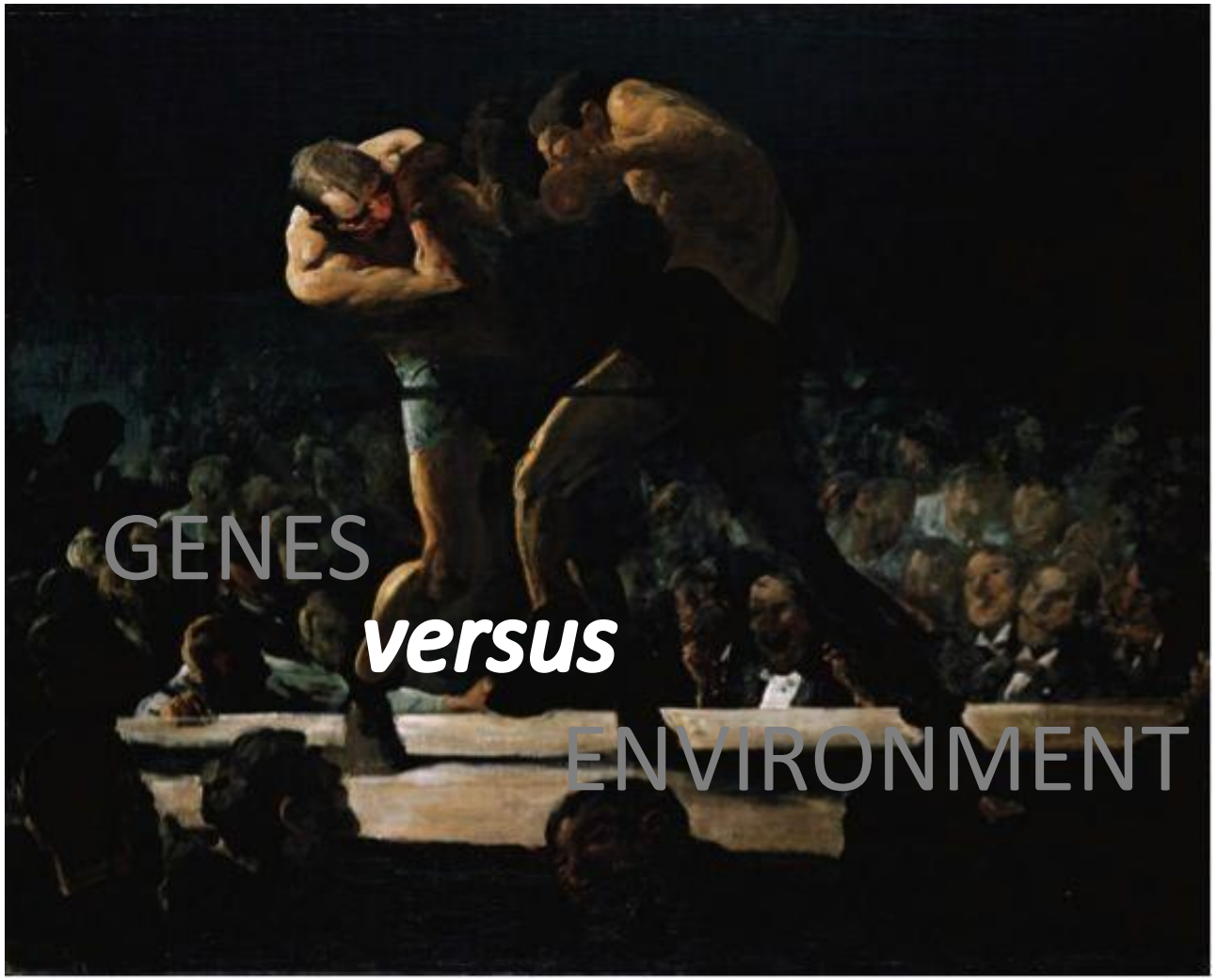
Outline

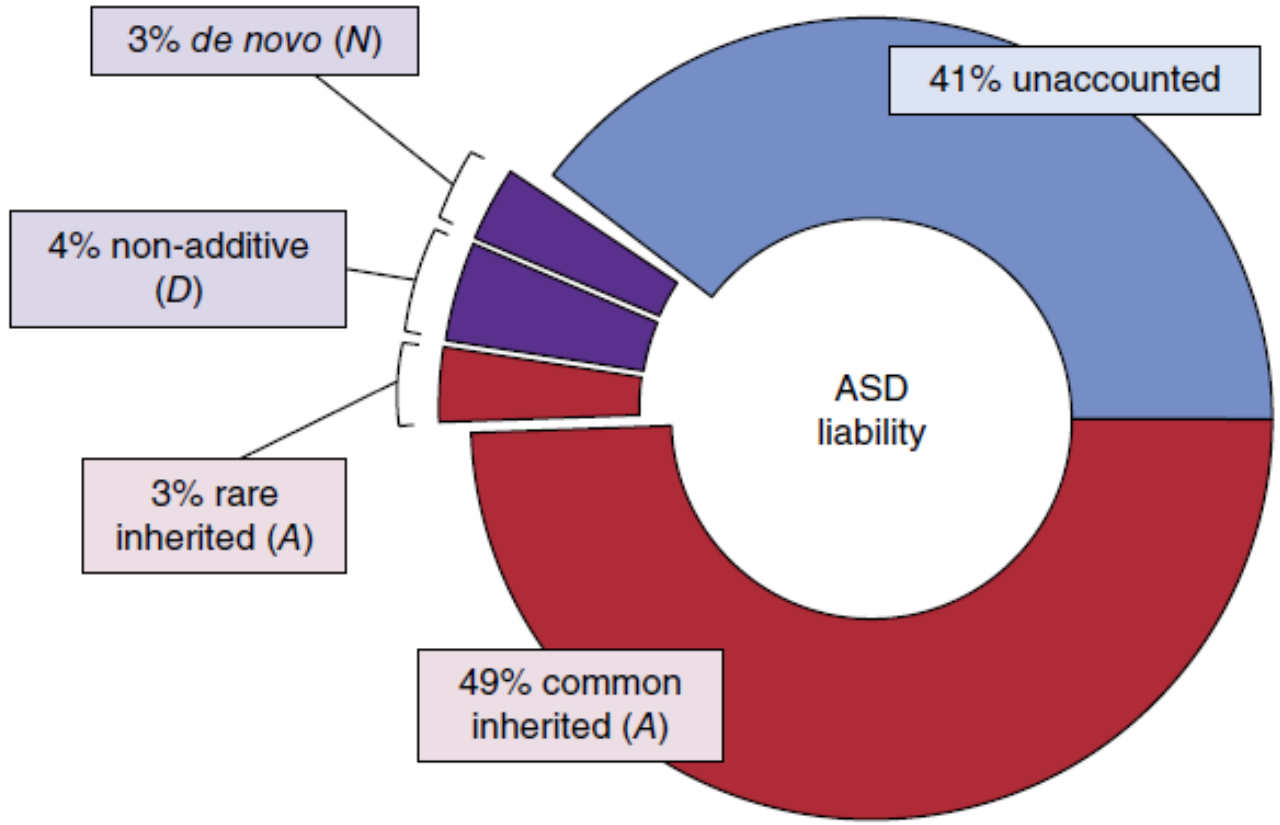
- Autism spectrum disorders
- Importance of genes and the environment
- Evidence supporting environmental ASD risk factors
- Why aren’t we further?
- How can we approach GxE?

Study	Country	Concordance		(N pairs)
		MZ	DZ	
Folstein and Rutter (1977)	UK	36%	0%	(21)
Ritvo et al (1985)	U.S.	96%	24%	(40)
Steffenberg (1989)	Nordic (5)	91%	0%	(21)
Bailey et al (1995)	UK	60%	5%	(44)
Taniai et al (2008)	Japan	95%	31%	(45)
Rosenberg et al (2009)	US	88%	31%	(227)
Lichtenstein et al (2010)	Sweden (male)	47%	14%	(62)
Hallmayer et al (2011)	US (male)	77%	31%	(90)
	US (female)	50%	36%	(22)
Frazier et al (2014)	US	69%	35%	(568)
Sandin et al (2014)	Sweden	54%	25%	(466*)
Colvert et al (2015)	UK	75%	40%	(203)

* 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

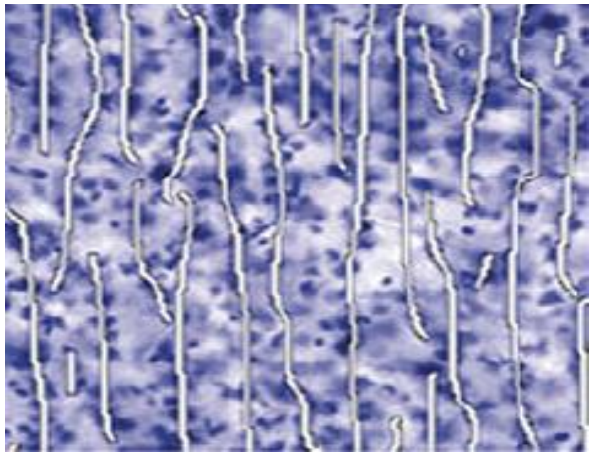


Gaugler et al. *Nature Gen* 2014

AUTISM IN THALIDOMIDE EMBRYOPATHY: A POPULATION STUDY

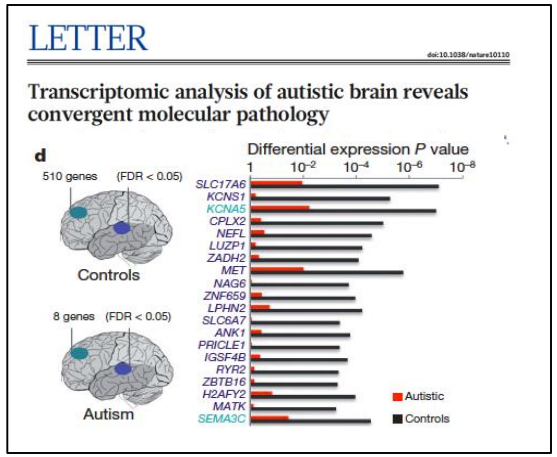
Stromland et al. *Dev Med Child Neurol* 1994

Neuroanatomy:
Minicolumn disorganization



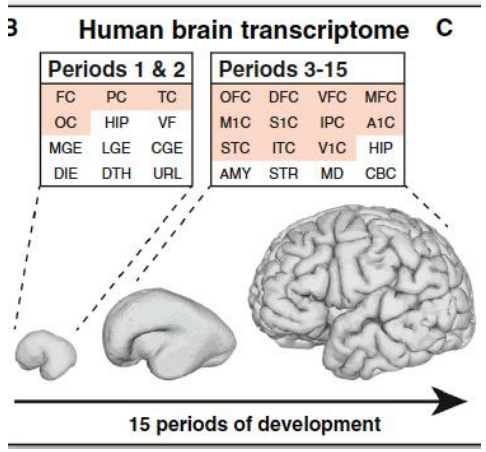
Amaral et al. *Trends Neurosci* 2008

Brain gene expression:
ASD vs non-ASD brains

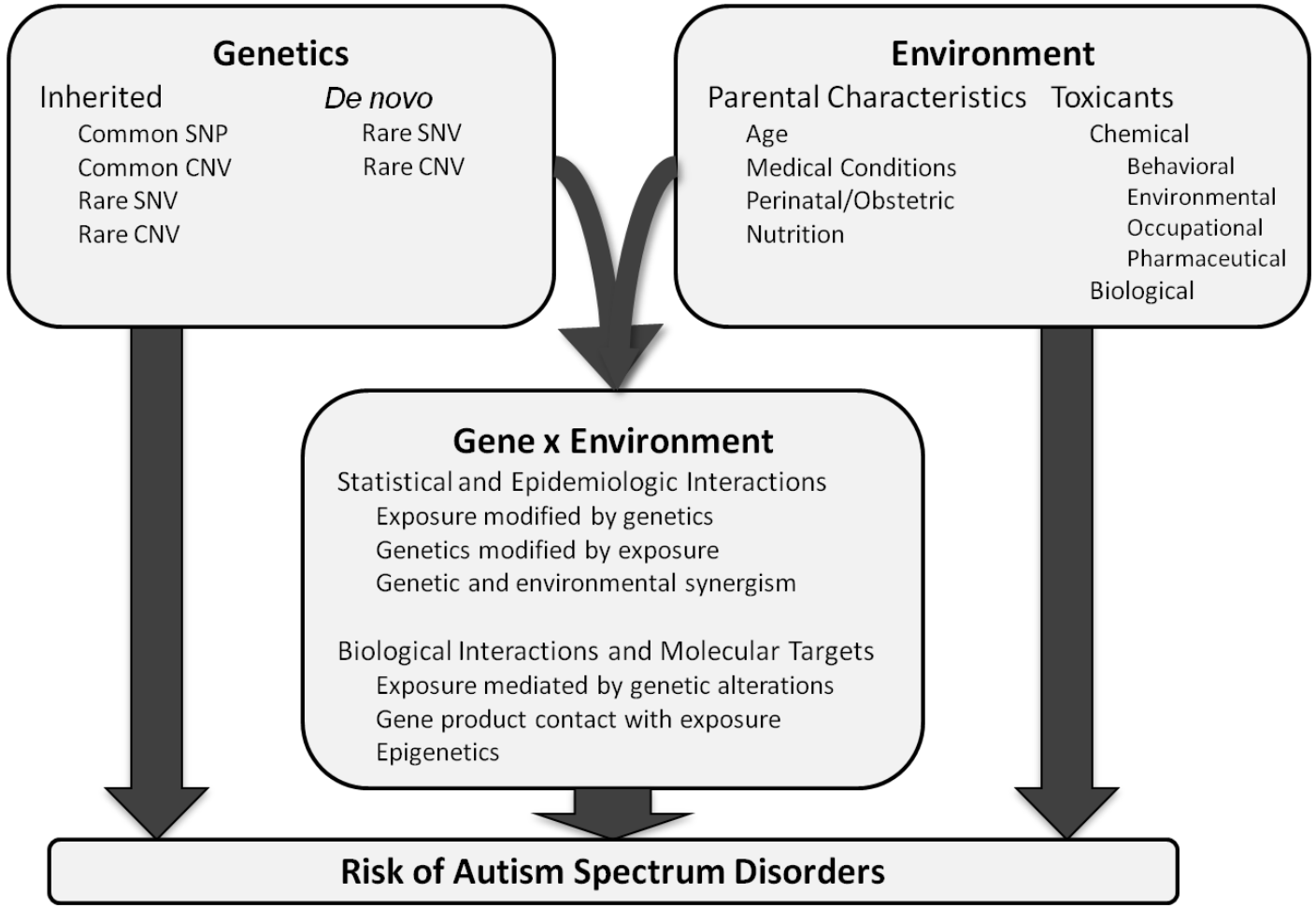


Voineagu et al. *Nature* 2011

Brain gene expression:
ASD risk gene networks



Willsey et al. *Cell* 2013


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

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?

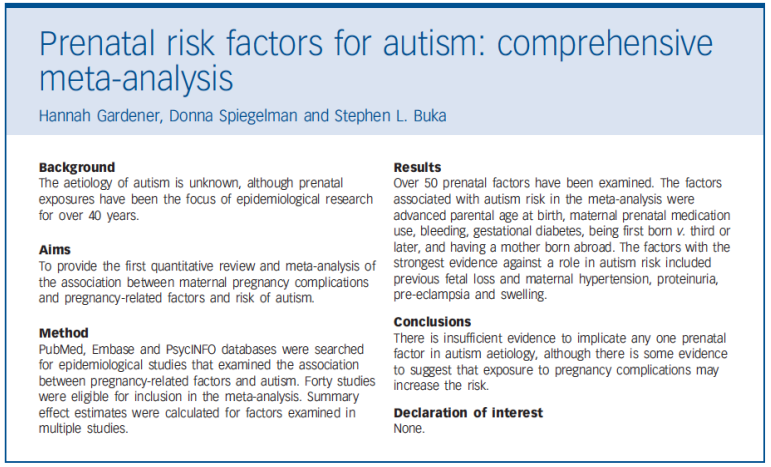
The Changing Epidemiology of Autism Spectrum Disorders

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 Newschaffer[1]

Lyall et al. *Ann Rev Pub Health* 2017



Prenatal risk factors for autism: comprehensive meta-analysis

Hannah Gardener, Donna Spiegelman and Stephen L. Buka

Background
The aetiology of autism is unknown, although prenatal exposures have been the focus of epidemiological research for over 40 years.

Aims
To provide the first quantitative review and meta-analysis of the association between maternal pregnancy complications and pregnancy-related factors and risk of autism.

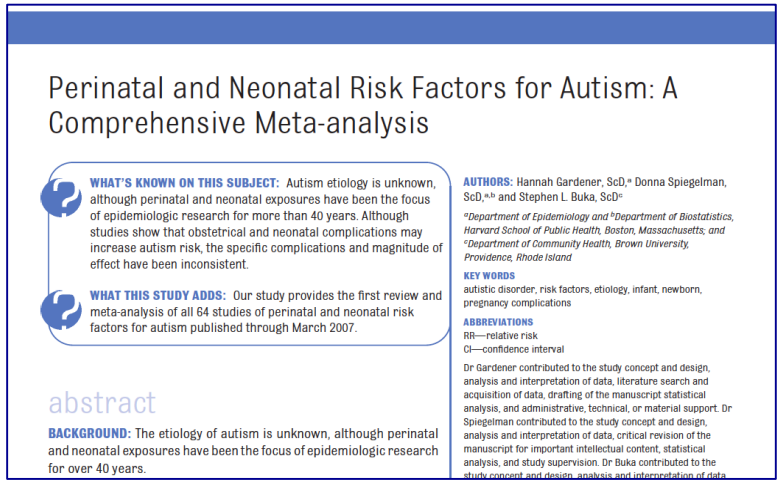
Method
PubMed, Embase and PsycINFO databases were searched for epidemiological studies that examined the association between pregnancy-related factors and autism. Forty studies were eligible for inclusion in the meta-analysis. Summary effect estimates were calculated for factors examined in multiple studies.

Results
Over 50 prenatal factors have been examined. The factors associated with autism risk in the meta-analysis were advanced parental age at birth, maternal prenatal medication use, bleeding, gestational diabetes, being first born *v.* third or later, and having a mother born abroad. The factors with the strongest evidence against a role in autism risk included previous fetal loss and maternal hypertension, proteinuria, pre-eclampsia and swelling.

Conclusions
There is insufficient evidence to implicate any one prenatal factor in autism aetiology, although there is some evidence to suggest that exposure to pregnancy complications may increase the risk.

Declaration of interest
None.

Gardener et al. *Br J Psych* 2009



Perinatal and Neonatal Risk Factors for Autism: A Comprehensive Meta-analysis

WHAT'S KNOWN ON THIS SUBJECT: Autism etiology is unknown, although perinatal and neonatal exposures have been the focus of epidemiologic research for more than 40 years. Although studies show that obstetrical and neonatal complications may increase autism risk, the specific complications and magnitude of effect have been inconsistent.

WHAT THIS STUDY ADDS: Our study provides the first review and meta-analysis of all 64 studies of perinatal and neonatal risk factors for autism published through March 2007.

abstract

BACKGROUND: The etiology of autism is unknown, although perinatal and neonatal exposures have been the focus of epidemiologic research for over 40 years.

AUTHORS: Hannah Gardener, ScD,[a] Donna Spiegelman, ScD,[a,b] and Stephen L. Buka, ScD[c]

[a]Department of Epidemiology and [b]Department of Biostatistics, Harvard School of Public Health, Boston, Massachusetts; and [c]Department of Community Health, Brown University, Providence, Rhode Island

KEY WORDS
autistic disorder, risk factors, etiology, infant, newborn, pregnancy complications

ABBREVIATIONS
RR—relative risk
CI—confidence interval

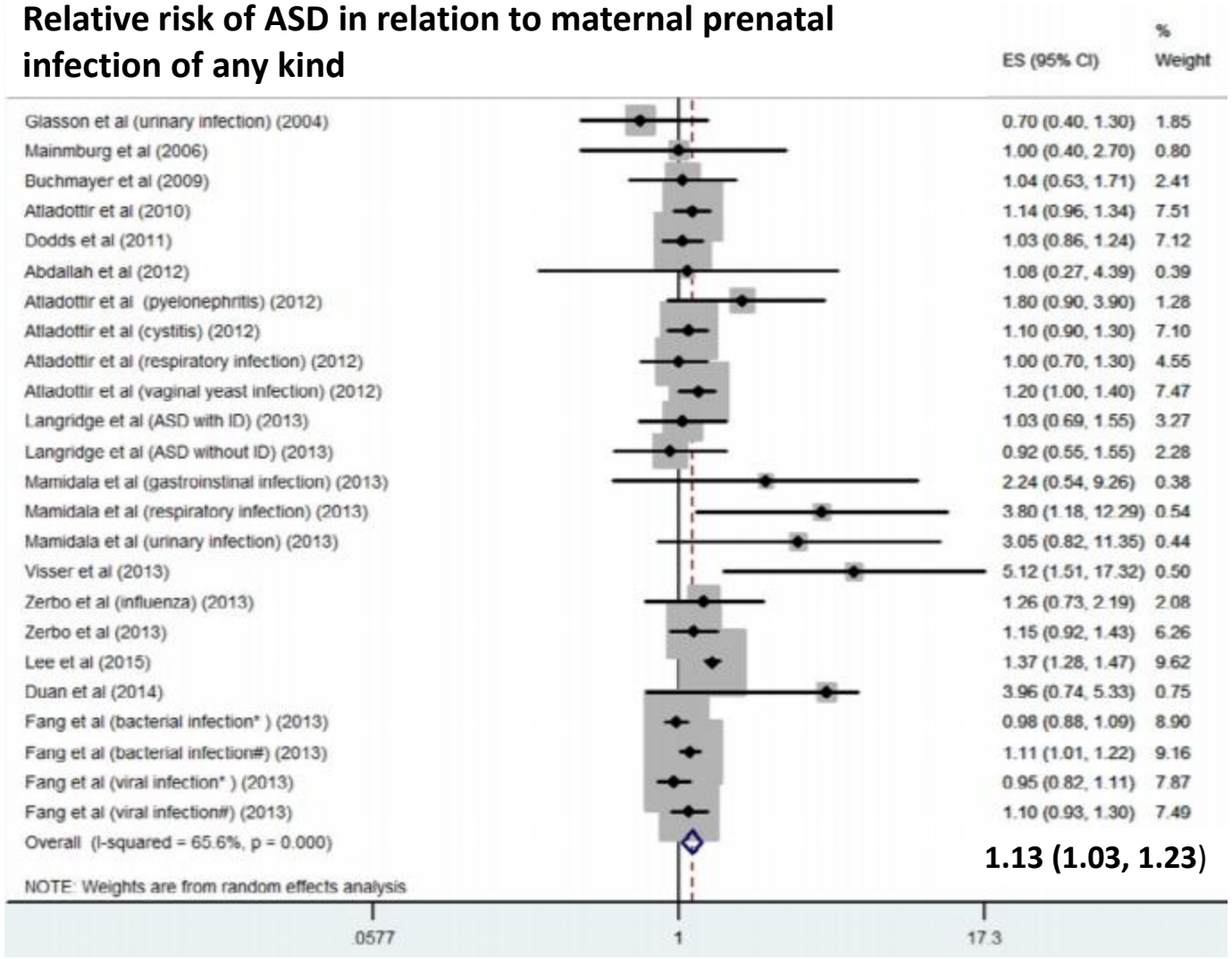
Dr Gardener contributed to the study concept and design, analysis and interpretation of data, literature search and acquisition of data, drafting of the manuscript statistical analysis, and administrative, technical, or material support. Dr Spiegelman contributed to the study concept and design, analysis and interpretation of data, critical revision of the manuscript for important intellectual content, statistical analysis, and study supervision. Dr Buka contributed to the

Gardener et al. *Pediatrics* 2011

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



Jiang et al. *Brain Behav Immun* 2016

PLOS ONE

RESEARCH ARTICLE

A Systematic Review and Meta-Analysis of Multiple Airborne Pollutants and Autism Spectrum Disorder

Juleen Lam[1]*, Patrice Sutton[2], Amy Kalkbrenner[3], Gayle Windham[4], Alycia Halladay[5,6], Erica Koustas[7], Cindy Lawler[8], Lisette Davidson[9], Natalyn Daniels[10], Craig Newschaffer[11], Tracey Woodruff[12]

Received: May 31, 2016

Accepted: August 14, 2016

Published: September 21, 2016

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

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).

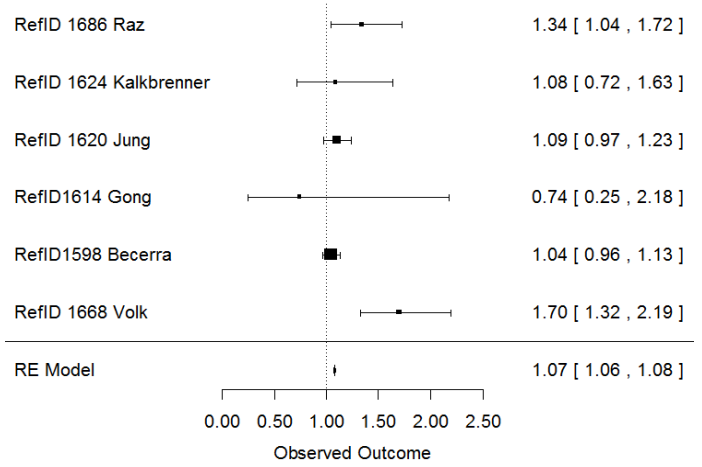
Objective

To answer the question: "is developmental exposure to air pollution associated with ASD?"

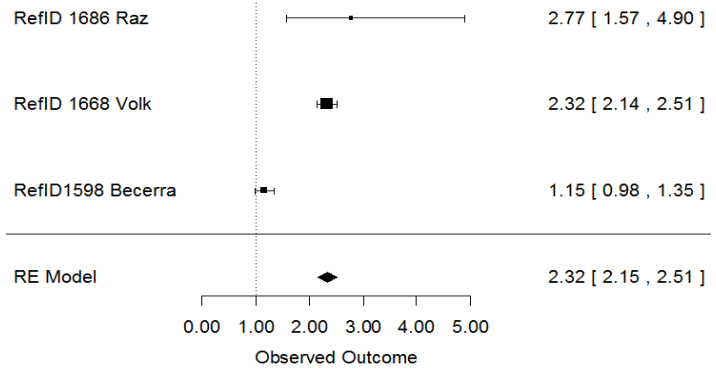
Lam et al. *PLOS One* 2016

- 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]



Lam et al. *PLOS One* 2016

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?

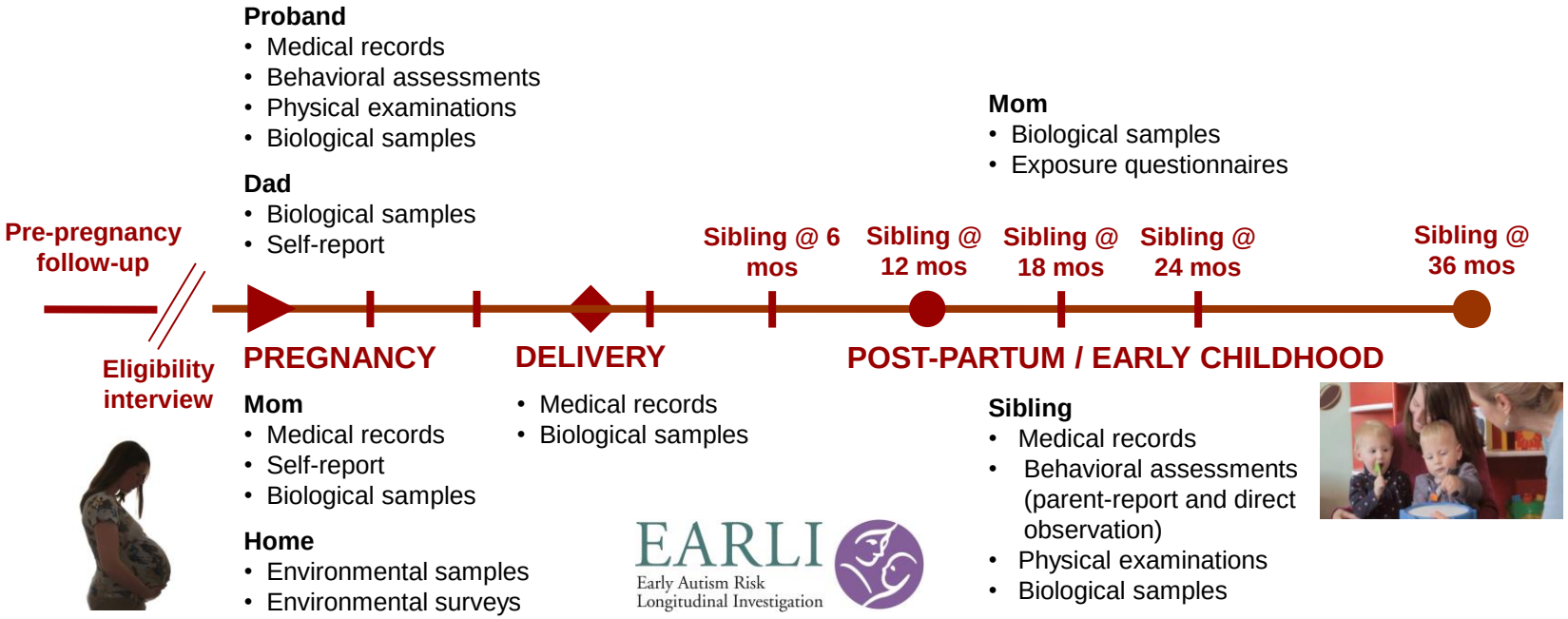
Case-control study

- 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)



Newschaffer et al. *J Neurodev Dis (*2013)

Prenatal PBDE exposure as an ASD risk factor

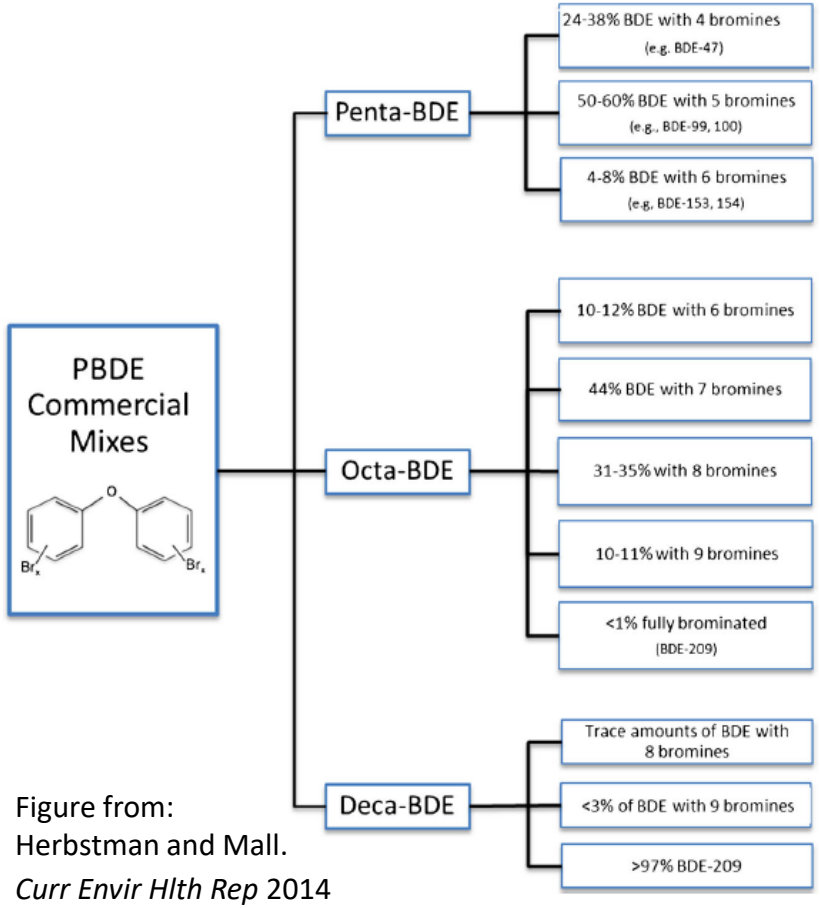


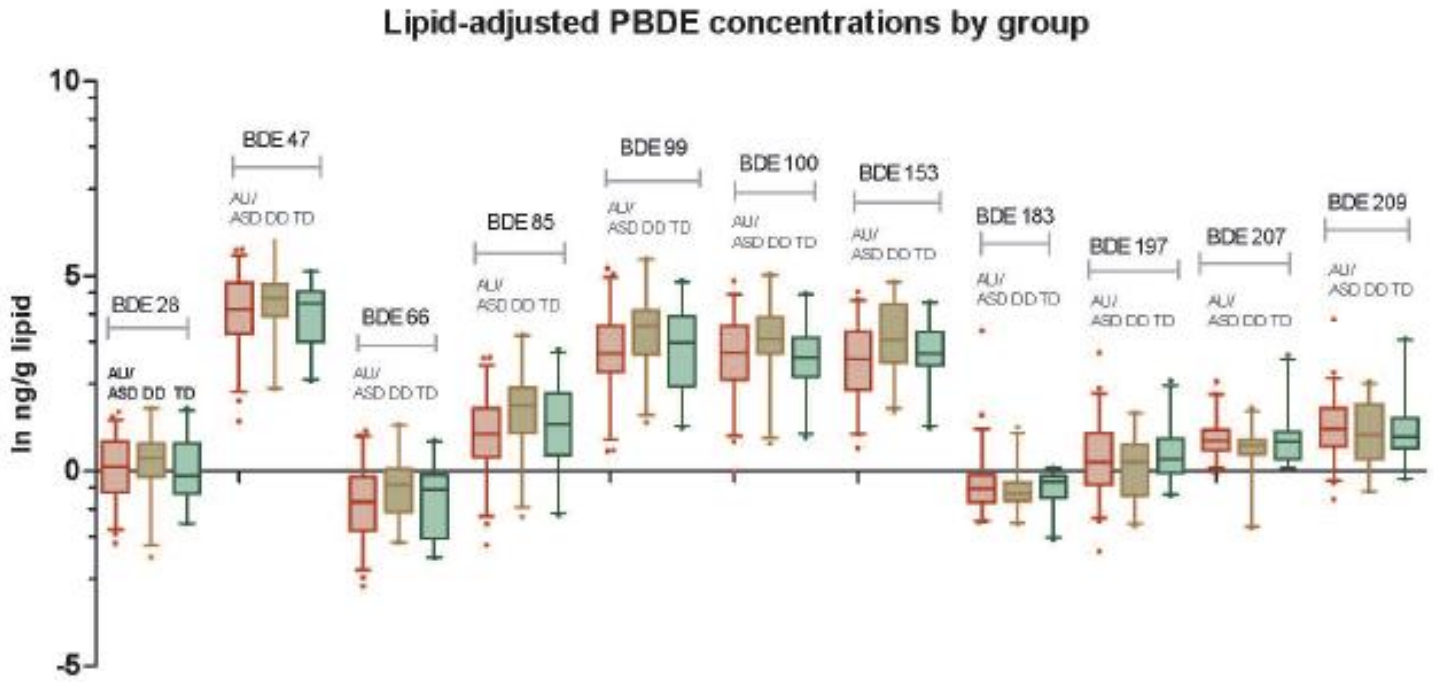
Figure from:
Herbstman and Mall.
Curr Envir Hlth Rep 2014

- 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

Hites RA. *Environ Sci Technol* 2004

Results from pilot analysis of biomarkers of PBDE exposure in the CHARGE case-control study

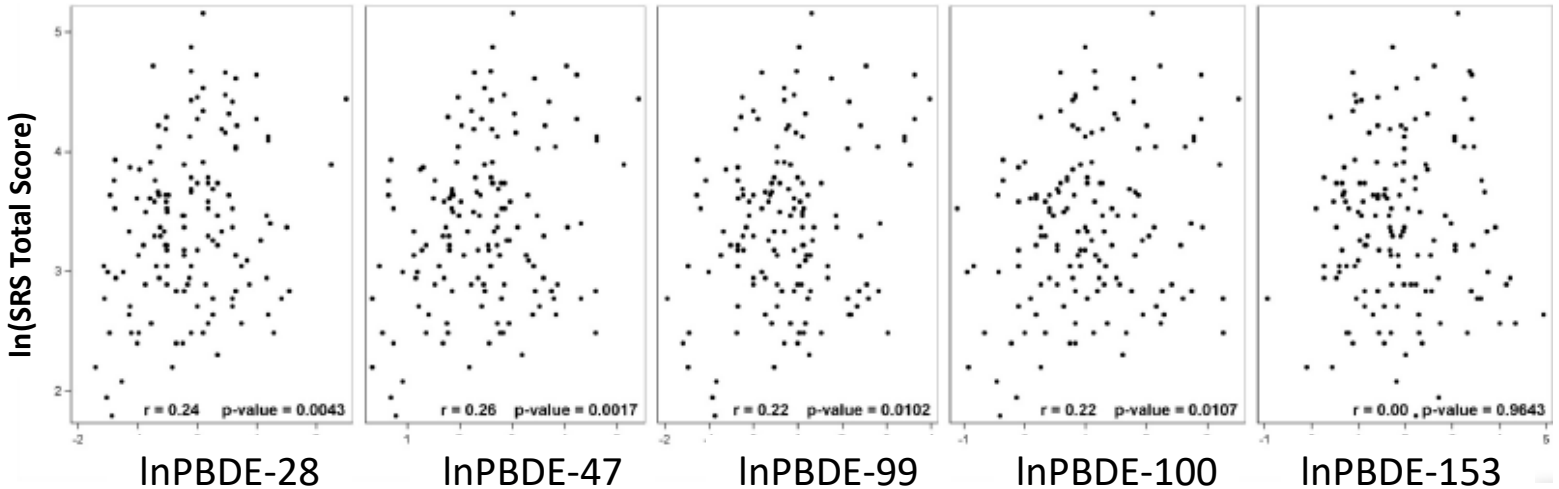
Exposure biomarker measured at age of diagnosis in child blood



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)

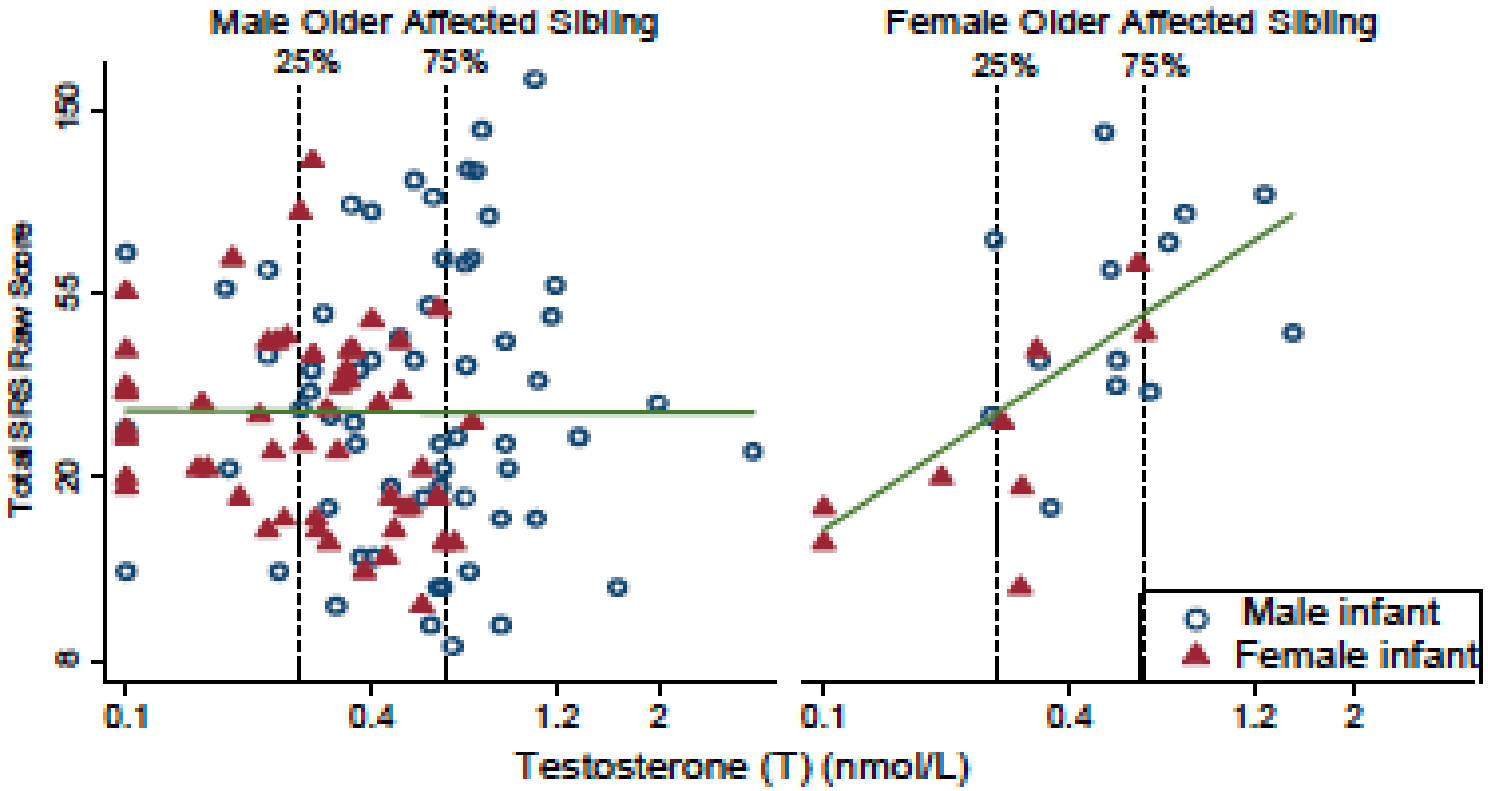


lnPBDE-28	lnPBDE-47	lnPBDE-99	lnPBDE-100	lnPBDE-153
r=0.24 (p<0.01)	r=0.26 (p<0.01)	r=0.22 (p=0.01)	r=0.22 (p=0.01)	r=-0.00 (p=0.96)

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

Pregnancy cohort

- Access to the proper etiologic window for exposure assessment
- Large sample size for rare outcomes
- Challenges to assessing outcome
- Expensive, time-consuming

How to build larger prospective cohorts?

<u>Create new general population cohorts with more streamlined data collection</u>

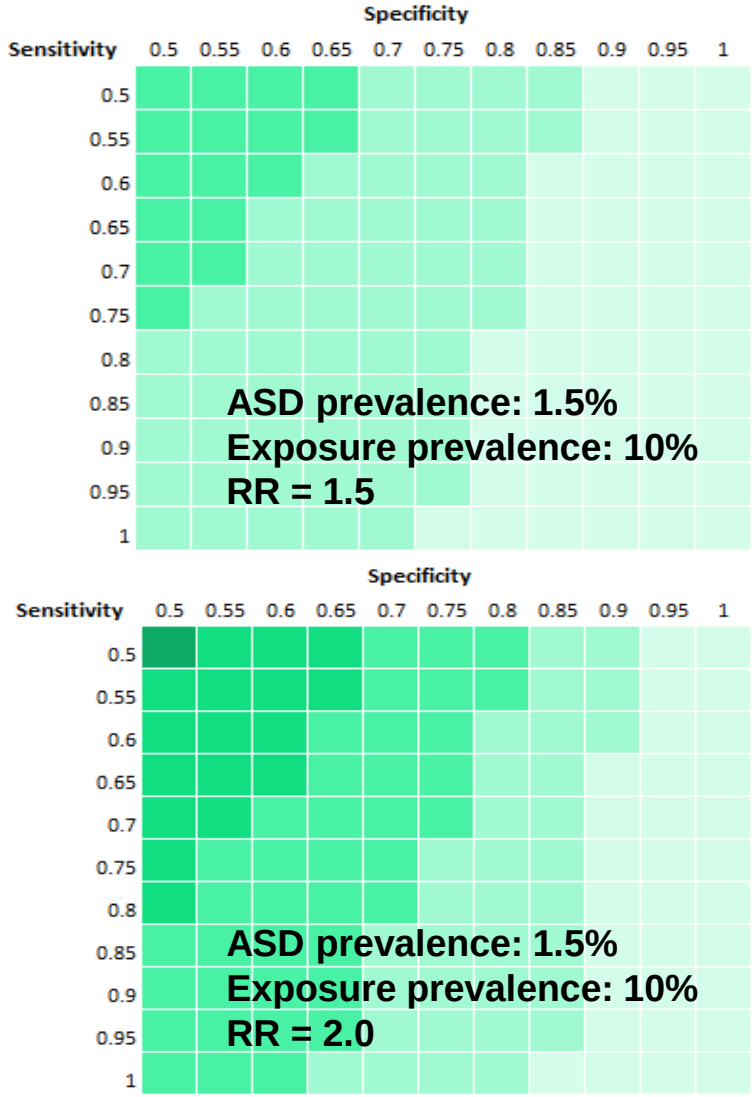
- Focus on biomarkers at delivery (maternal blood, cord blood, placenta, meconium) and EMR data
- Use two-stage, streamlined case-finding with nested schemes for biomarker based exposure assessment / deep phenotyping

<u>Create larger synthetic cohorts from smaller existing cohorts</u>

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

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



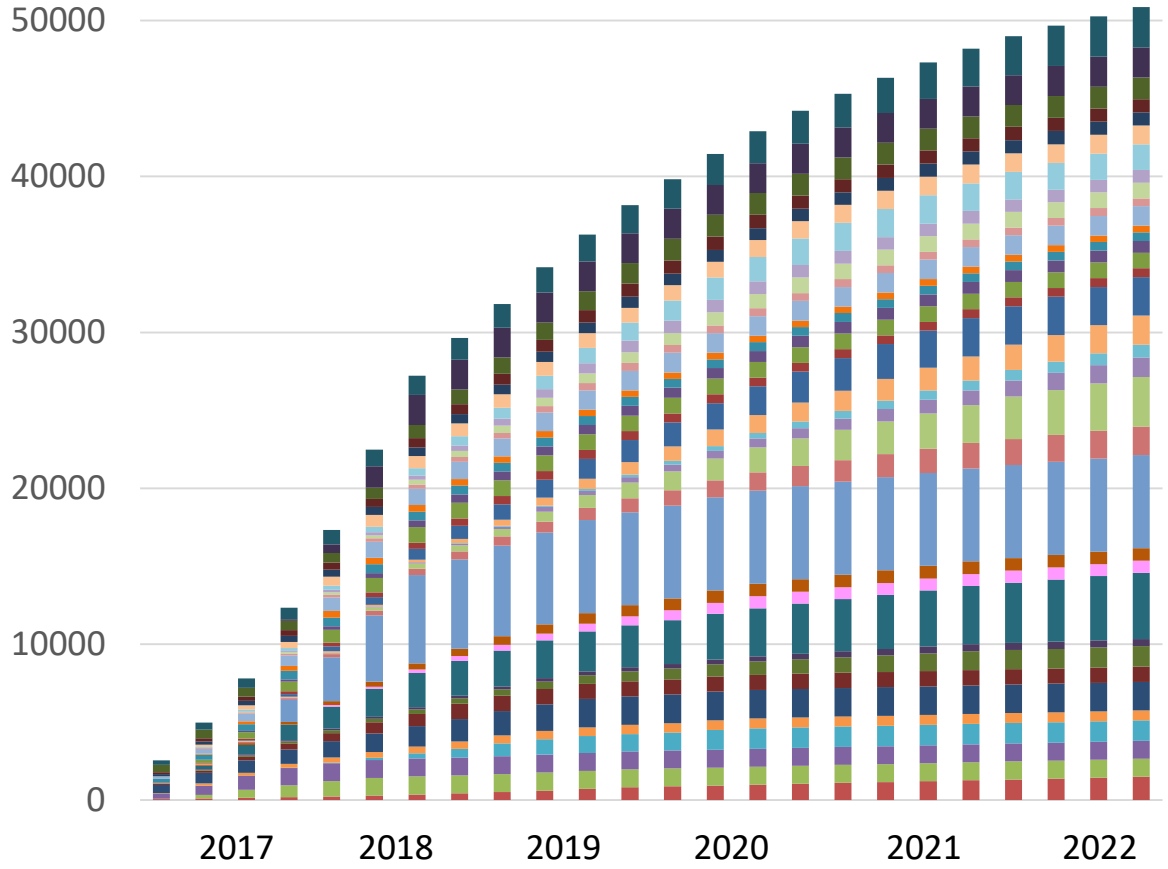
Newschaffer et al. *Aut Research* 2017

ECHO

Environmental influences on Child Health Outcomes

A program supported by the NIH

Getting to 50,000 ECHO Children - Data from Milestone Accrual Plans



Outline

- Autism spectrum disorders
- Genes and environment
- Evidence supporting environmental ASD risk factors
- Why aren't we further?
- How can we approach GxE?

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

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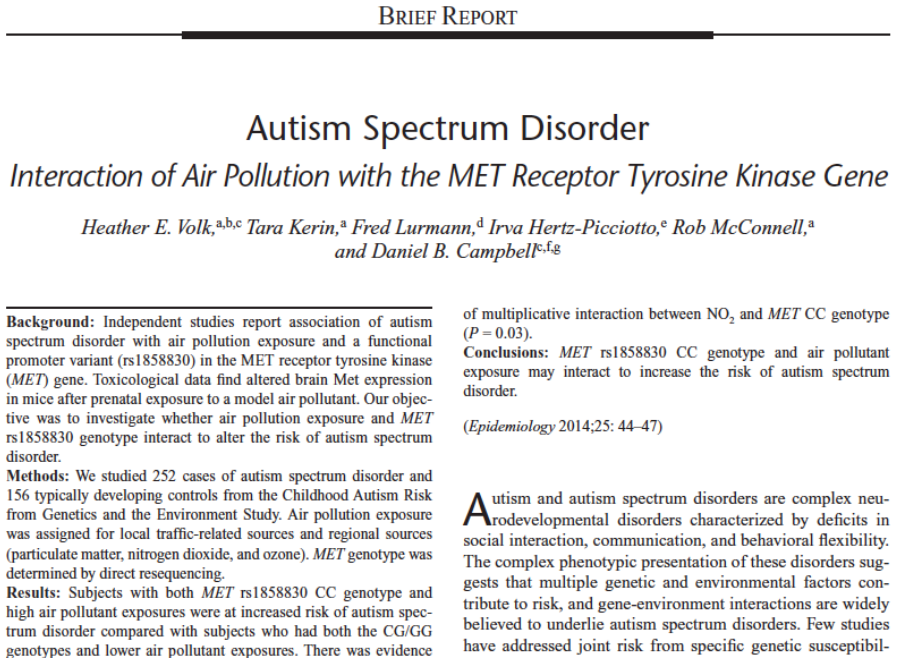


BRIEF REPORT

Autism Spectrum Disorder

Interaction of Air Pollution with the MET Receptor Tyrosine Kinase Gene

Heather E. Volk,[a,b,c] *Tara Kerin,*[a] *Fred Lurmann,*[d] *Irva Hertz-Picciotto,*[e] *Rob McConnell,*[a] *and Daniel B. Campbell*[e,f,g]

Background: Independent studies report association of autism spectrum disorder with air pollution exposure and a functional promoter variant (rs1858830) in the MET receptor tyrosine kinase (*MET*) gene. Toxicological data find altered brain Met expression in mice after prenatal exposure to a model air pollutant. Our objective was to investigate whether air pollution exposure and *MET* rs1858830 genotype interact to alter the risk of autism spectrum disorder.

Methods: We studied 252 cases of autism spectrum disorder and 156 typically developing controls from the Childhood Autism Risk from Genetics and the Environment Study. Air pollution exposure was assigned for local traffic-related sources and regional sources (particulate matter, nitrogen dioxide, and ozone). *MET* genotype was determined by direct resequencing.

Results: Subjects with both *MET* rs1858830 CC genotype and high air pollutant exposures were at increased risk of autism spectrum disorder compared with subjects who had both the CG/GG genotypes and lower air pollutant exposures. There was evidence of multiplicative interaction between NO_2 and *MET* CC genotype ($P = 0.03$).

Conclusions: *MET* rs1858830 CC genotype and air pollutant exposure may interact to increase the risk of autism spectrum disorder.

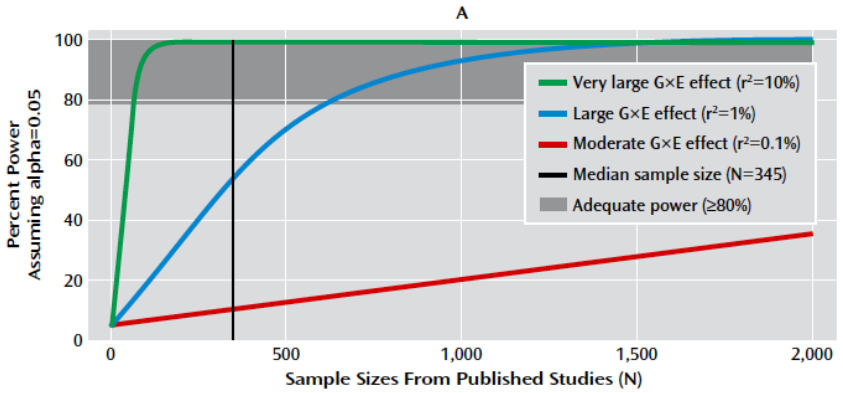
(*Epidemiology* 2014;25: 44–47)

Autism and autism spectrum disorders are complex neurodevelopmental disorders characterized by deficits in social interaction, communication, and behavioral flexibility. The complex phenotypic presentation of these disorders suggests that multiple genetic and environmental factors contribute to risk, and gene-environment interactions are widely believed to underlie autism spectrum disorders. Few studies have addressed joint risk from specific genetic susceptibil-

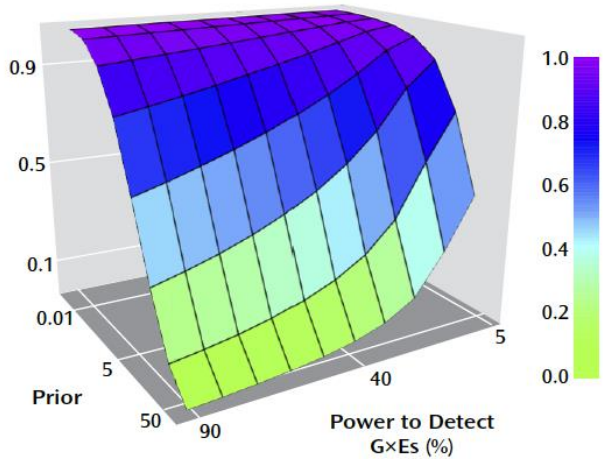
- Air pollution exposure modeled from residential distance to roads
- MET: CC vs CG/GG
- Air pollution: top 25%ile exposed vs others (5 pollutant measures)
- Statistically significant interaction for one pollutant (NO2) - driven by a *protective* estimate for the CC unexposed and risky effect for CC 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



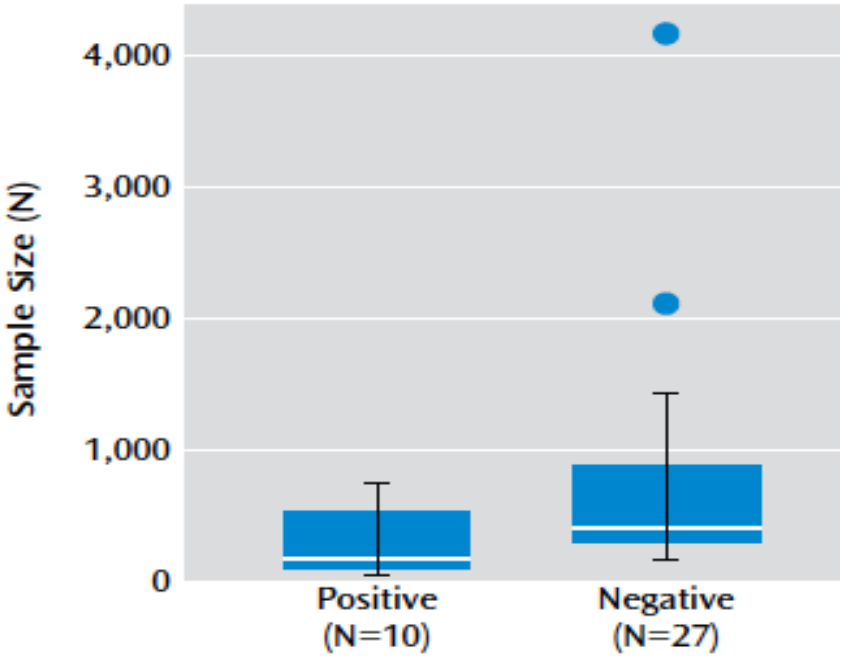
- Combining low statistical power with weak hypotheses ("priors" in Bayesian parlance) leads to large expected false discovery rates

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
    - 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
    - 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



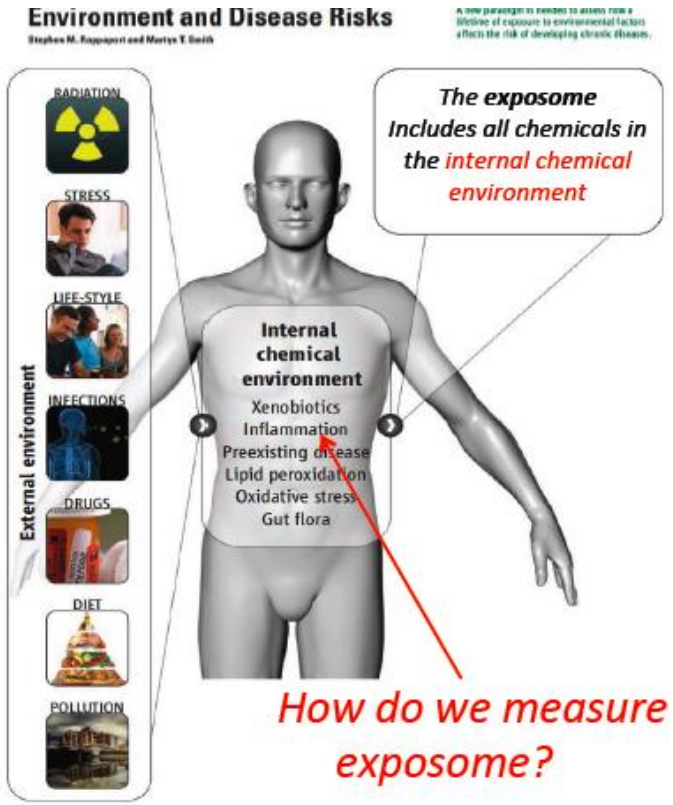
Duncan and Keller. *Amer J Psych* 2011

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

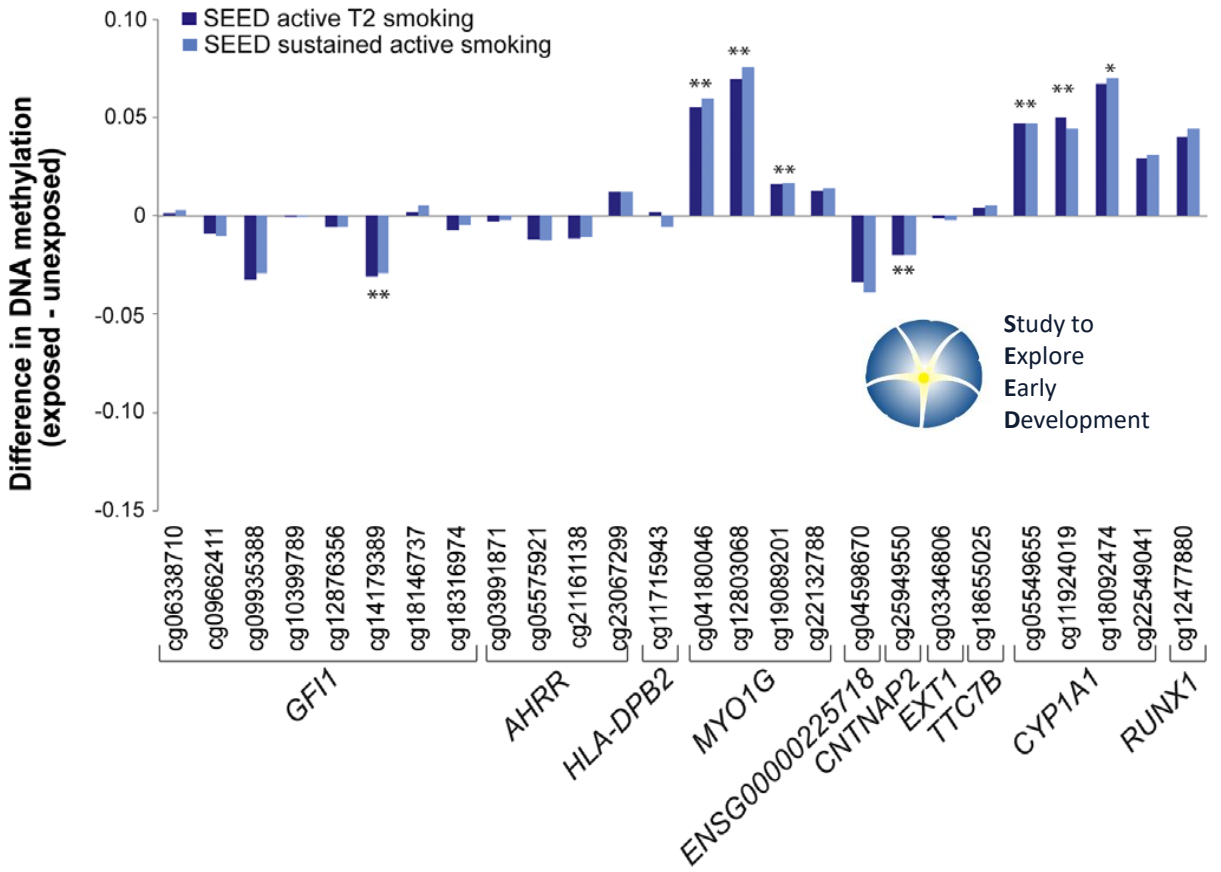
- Measure the internal chemical environment (potentially with dense arrays of markers) in biosamples
- Better reflect internal dose
- Account for heterogeneous individual metabolism
- Look for persistent signals
  - Those that accurately reflecting *past* exposure during critical etiologic windows



Rappaport and Smith, *Science* 2010

DNAm as a potential exposomics biomarker

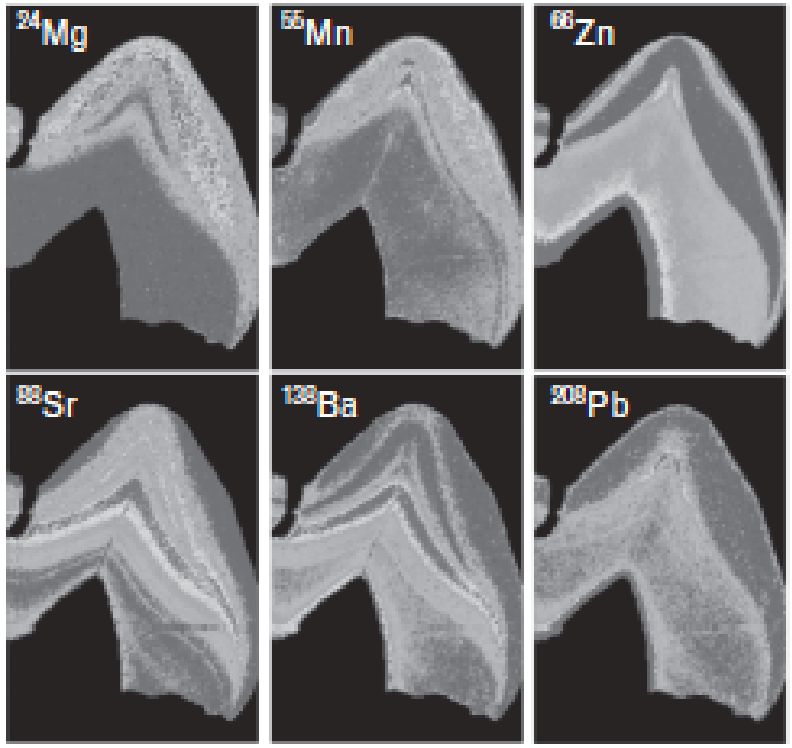
- DNAm patterns in ***cord blood*** has been associated with prenatal maternal smoking
- Is this pattern replicated in blood of children around the age of ASD dx (3-5)?



Joubert et al. *Cancer Epidemiol Biomark Prev.* 2012
Ladd-Acosta et al. *Environ Res*, 2016

The tooth exposome

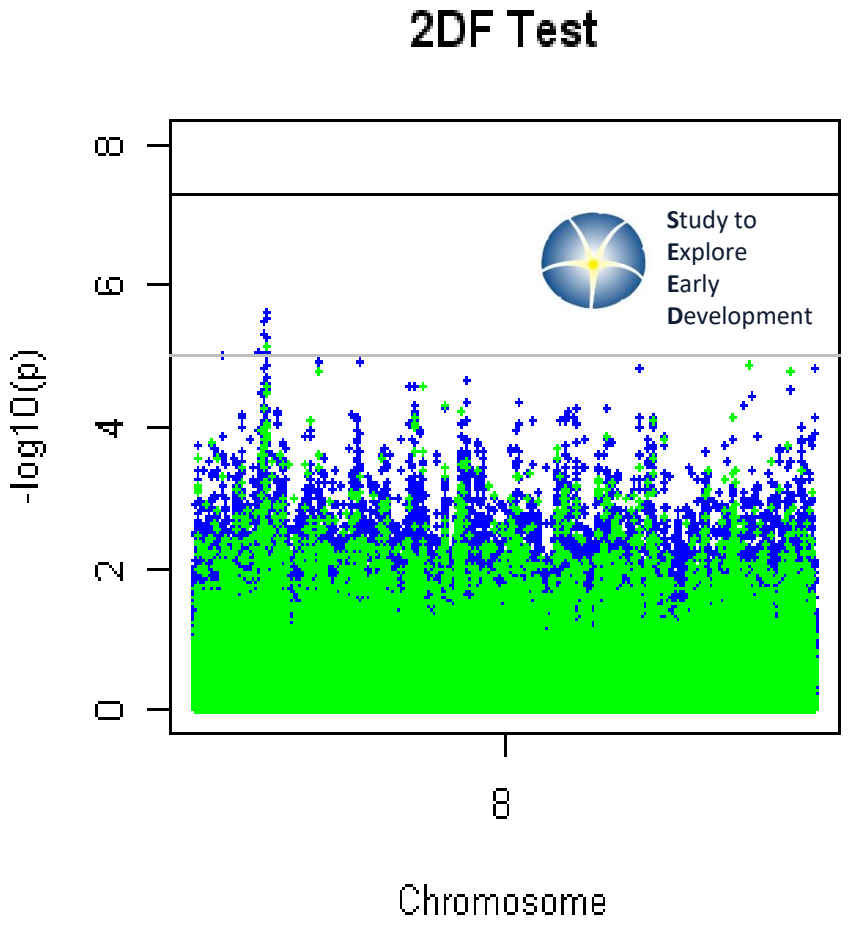
- Temporally assignable record of exposure to xenobiotics in tooth dentine and enamel possible back to the start of the second trimester
- Microspatial sampling
- A range of mass spectroscopy techniques
- Validated for metals
- Validation for POPs underway



Andra et al. *Curr Op Ped*, 2016

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 ($p<10^{-8}$).

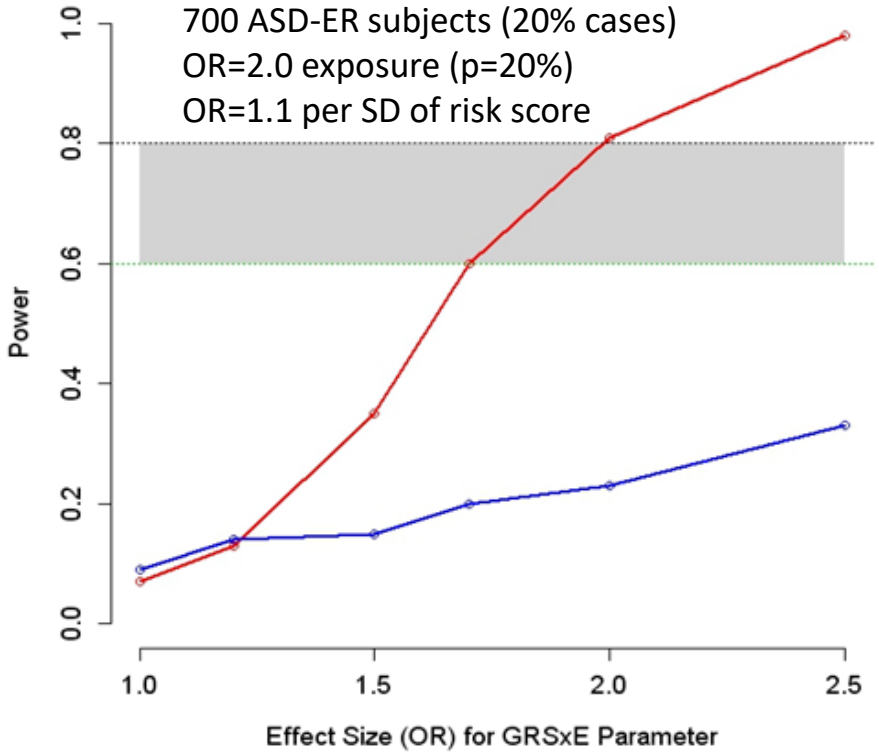
		Lead (2.6% Prevalence) Allele Frequency			Air Pollution (1 SD Change) Allele Frequency			Prenatal Smoking (8% Prevalence) Allele Frequency		
		0.10	0.20	0.30	0.10	0.20	0.30	0.10	0.20	0.30
Minimum Detectable Odds Ratio										
NDD Dx	6,898 cases	2.42	2.01	1.85	1.21	1.15	1.13	1.79	1.57	1.49
Minimum Detectable Odds Ratio										
ASD Dx	978 cases	5.50	3.95	3.40	1.60	1.44	1.38	3.25	2.55	2.30
Minimum Detectable Change in Beta										
SRS	30,000 subjects	5.27	3.95	3.46	0.84	0.63	0.55	3.09	2.31	2.02

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)

Figure 6. Power for detecting environmental effects when there is gene-environment interaction (using a GRS and a 2DF test)



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

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