DISCLOSURES

• None.
• No commercial or industry affiliations.
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How we study ADHD in the OHSU ADHD Program

• Archival data
  – Michigan—~700 families—genetic, environmental cognitive, clinical measures
  – National survey data (~90,000 children) (NSCH)
  – Meta-analyses and detailed literature reviews

• OHSU-Oregon cohort of children
  – ~1200 families surveyed
  – ~600 families followed over time
  – Genetic, epigenetic, brain imaging, cognitive, temperament, environmental, and clinical data in unparalleled detail

• OHSU-Oregon cohort of mother-infant pairs
  – 50 pairs in a pilot effort to identify the earliest signs and causes—an effort we hope to grow
The OHSU ADHD Program relies on a dedicated team of staff and volunteers AND OHSU Scientist and Clinicians: Sarah Karalunas, Ph.D., Psychiatry Beth Wilmot, Ph.D., DMICE Damien Fair, Ph.D., BEHN Elinor Sullivan, Ph.D., ONPRC Michael Mooney, Ph.D., DMICE Shannon McWeeney, Ph.D., DMICE Leeza Maron, Ph.D., Psychiatry Bonnie Nagel, Ph.D., Psychiatry Ajit Jetmalani, M.D., Psychiatry Minkyoung Sun, Ph.D., Nursing Nate Dieckman, Ph.D., Nursing Hanna Gustafson, Ph.D., Fellowship Jeanette Johnstone, Ph.D, Fellowship AND Outside collaborators: Jeffrey Measelle, Ph.D., Univ Oregon Steve Faraone, Ph.D., SUNY Ben Neale, Ph.D., MIT James McCracken, M.D., UCLA Molly Nikolas, Ph.D., UNIV IOWA Erica Musser, Ph.D., FLORIDA INT UNIV Michael Willoughby, North Carolina RTI
Accounting for ADHD and for mental and neurodevelopmental conditions

- **Paradigm=exemplar (Aristotle, Kuhn)**
- **First wrong paradigm: metabolic disease**
  - “ADHD is like Huntington’s; find the gene, solve the disease”
- **Second wrong paradigm: Linear causality**
  - “Human illness is like a machine; mass=force x acceleration. Find the causal chain, solve the disease”
OLD VIEW combined those two ideas into a single paradigms
We have 3 big reasons to change the paradigm to one that is dynamic and integrates the environment with progress in genetics

• Complex disease model with persistence of environmental risk findings is more appropriate
  – And necessary to explain persistent environmental correlates

• Heritability of liability: GxE “hidden” in twin data
  – Heritability of major infectious disease (TB, leprosy) ~ .6-.8
  – GxE now established for some mental disorders

• Epigenetic insight—the recognition that GxE not genotype determines phenotype

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Better Model

- M, P diet, (?adiposity)
- M/P toxicant exposure
- Maternal (paternal?) stress/adversity

Inflammation, oxidative stress, corticosteroid

Dynamic, Experience-expectant, multisystem, Brain Development

CHILD EPIGENOME

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Today

• New data on genetic mediation via endophenotypes
• Recent data on environmental effects supported by Mendelian randomization design to evaluate causality
Pathway of genetic effects: 
Endophenotypes

- Working memory***
- Attention: Arousal/vigilance***
- Mental clock—fell short
- Response inhibition—fell short
- Motor speed—fell short
- Negative affect***—exploring here
We study it the following way

• Obtain molecular genetic correlates of ADHD in a sample of 20,000 ADHD cases and 35,000 non-ADHD comparison youth worldwide

• Use those odds ratios to compute molecular genetic “loading” for ADHD in our sample of 656 genetically unrelated children at OHSU

• Examine the effect of this molecular score on our carefully measured cognitive markers in our sample
ADHD Worldwide Consortium Meta-analysis--Forthcoming--Cohort Summary (OHSU will be in the next “release” –here we do replication

<table>
<thead>
<tr>
<th>Cohort</th>
<th>Cases</th>
<th>Controls</th>
<th>Design</th>
<th>PGC Batch</th>
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<tbody>
<tr>
<td>CHOP</td>
<td>262</td>
<td>262</td>
<td>Trios</td>
<td>PGC ADHD1</td>
</tr>
<tr>
<td>IMAGE-I</td>
<td>700</td>
<td>700</td>
<td>Trios</td>
<td>PGC ADHD1</td>
</tr>
<tr>
<td>IMAGE-II</td>
<td>624</td>
<td>1755</td>
<td>Case/control</td>
<td>PGC ADHD1</td>
</tr>
<tr>
<td>PUWMA</td>
<td>635</td>
<td>635</td>
<td>Trios</td>
<td>PGC ADHD1</td>
</tr>
<tr>
<td>Toronto, Canada</td>
<td>109</td>
<td>109</td>
<td>Trios</td>
<td>PGC CDG</td>
</tr>
<tr>
<td>Barcelona, Spain</td>
<td>572</td>
<td>425</td>
<td>Case/control</td>
<td>PGC CDG</td>
</tr>
<tr>
<td>Cardiff, UK</td>
<td>721</td>
<td>5081</td>
<td>Case/control</td>
<td>PGC CDG</td>
</tr>
<tr>
<td>Germany</td>
<td>487</td>
<td>1290</td>
<td>Case/control</td>
<td>PGC CDG</td>
</tr>
<tr>
<td>Beijing, China</td>
<td>1012</td>
<td>925</td>
<td>Case/control</td>
<td>Solo (Yang et al. 2013)</td>
</tr>
<tr>
<td>Bergen, Norway</td>
<td>295</td>
<td>202</td>
<td>Case/control</td>
<td>New (Zayas et al. 2015)</td>
</tr>
<tr>
<td>Yale-Penn</td>
<td>182</td>
<td>1315</td>
<td>Case/control</td>
<td>New</td>
</tr>
<tr>
<td>Denmark iPSYCH</td>
<td>14584</td>
<td>22492</td>
<td>Case/control</td>
<td>New</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>20183</strong></td>
<td><strong>35191</strong></td>
<td>---</td>
<td>---</td>
</tr>
</tbody>
</table>
PGC + iPSYCH Meta-Analysis: 12 novel "genome wide significant" hits
OHSU ADHD Genetic Cohort

- N=656 genetically unrelated children to 3rd degree relative
- Genotyped on Illumina Infinium “Psych-array-24 bead chip
  - Genome-wide coverage
  - Common single-nucleotide polymorphisms (SNP)
  - selected to maximize representation of both entire genome using 603,132 SNPs (265,000 tag SNPs, 245,000 Exome SNPs) and enriched psychiatrically relevant genes (50,000 markers)
  - Imputation covers ~16 million SNPs on the genome
  - Compute polygenic score using all SNPs with p>.50, multiply number of risk alleles (0,1,2) by odds ratio in discovery data set (used 193,692 SNPS in the polygenic score)
ADHD Genetic risk score → Working memory latent variable

.17*** → ADHD latent variable
ADHD Genetic risk score

Working memory latent variable

ADHD latent variable

.21**

.09*

.39**
ADHD Genetic risk score

Working memory latent variable

Polygenic risk: variance explained 44%
Beginning to parse ADHD molecular etiology and cognitive endophenotype effects on etiology—Working memory

Total ADHD variation

- Environmental, other Genetic, and Gene x Environment (epigenetic) effects
- Polygenic risk score effect
- Other mediators
- Working memory effect
Beginning to parse ADHD molecular etiology and cognitive endophenotype effects on etiology—Attentional vigilance or cortical arousal
ADHD Genetic risk score

Negative Affect latent variable

ADHD SWAN Parent total

.25***
Total Indirect effect $B=.063$, $p=.004$
ADHD Genetic risk score ➔ Working memory latent variable ➔ ADHD SWAN Parent total

.25***
Total Indirect effect
B=.067, p<.001

ADHD Genetic risk score

Working memory latent variable

ADHD SWAN Parent total
ADHD Genetic risk score

SWAN Total ADHD Score

Working memory

Neg Affect

Total indirect effect $B = .11$, $p = .000016$

$.14***$

$.22***$

$.23***$

$.45***$

$r = -.16**$

44% of polygenic score variance explained

N=656, Nigg et al, in preparation
ADHD subtypes

Control
N=221
Inattentive type
N=113
Combined Type
N=312

Temperament types

Control
N=221
Mild emotional profile
N=80
Surgent emotional profile
N=197
Irritable Emotional Profile
N=145
Interim comment

• With molecular genetic composite scores we can now begin to map endophenotypes that are part of the mechanistic story of ADHD

• Now how and why do we look at environmental effects?
ENVIRONMENT—HOW DO WE EVALUATE CAUSALITY?
If we accept a susceptibility model of ADHD: Which Environments do we study and how do we do it?

– **Sociological Effects**
  • Collapse of civilization
  • Too much pharma marketing
  • Performance pressures on child, starting school too young

– **Caregiver Problems**
  • Over-indulgent or else hostile/intrusive parenting
  • Under-trained or inexperienced teachers

– **Developmental and Biological Context**
  • Rare events (may be non-shared environments)
    – Perinatal problems, teratogens (alcohol, drugs); micro-ischemias
    – Extreme toxicant exposures, extreme neglect (Romanian orphans)
  • Common but harmful contexts (more likely to be shared env)
    – *Moderate psychosocial stress/distress (esp. prenatal)
    – *Common poor diet
    – *Common Toxicant/pollutant exposures (pre-natal, post-natal)
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Substantial literatures show early exposures are risk factors for ADHD: But are these risk factors part of a causal process?

- Plausibility (can “low amounts” do harm?)
- rGE has to be considered. How?
- Animal experiments help: But
- Unmeasured confounders have to be addressed somehow in human studies
Blood Lead and ADHD 2005-2007

![Bar graph showing blood lead levels in parts per billion for ADHD-C (n=50), ADHD PI (n=47), and Control (n=53).]

F(2,124)=8.57, p<.001

Data from Nigg et al (2008) *Bio Psych, 63, 325-31; © Elsevier Inc., and Society of Biological Psychiatry*
Plausibility: EX: Relative Lead level in human children’s blood (parts per billion or ppb)

1970 average: ~200 ppb

CDC current Action level: 50 ppb

2010 national average: ~9 ppb

Not to scale

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Relative Lead level in human children’s blood (parts per billion or ppb)

- 1970 average: ~200 ppb
- CDC current Action level: 50 ppb
- 2010 national average: ~9 ppb
- Estimated prehistoric average: < 0.2 ppb
Some Strategies in Human Studies for strengthening Causal inference (adapted from Lewis et al 2013)

- Experimental manipulations (possible with, e.g., diet)
- Comparisons over settings w/ differential selection biases
- negative controls and natural experiments
  - migration studies
  - sibling comparisons
  - individuals conceived using in vitro fertilization gestated by surrogate mothers
- Mendelian randomization (requires functional genotype)
Random assignment: Cognitive /attention development responds to maternal supplementation w DHA (Columbo et al 2004)

Distractability index for high and low docahexaenoic-acid supplementation (DHA) infants as duration of look time at distractor showing causal effect of DHA on development of toddler attentional control (Columbo et al, 2004 Child Development 75, 1254 © John Wiley&Sons, Inc.)
MENDELIAN RANDOMIZATION LOGIC

Experience (stress, diet, toxicant) → Biological Mediator (e.g., toxicant metabolism) → Outcome (ADHD)

Unmeasured confounders (parent ADHD, SES, etc.)

Measured covariates

Source: Adapted from Lewis et al., 2013, Journal of Child Psychology and Psychiatry, 54, pages 1095-08; © ACAH, JCPP. Slide © Joel Nigg, Ph.D.
MENDELIAN RANDOMIZATION LOGIC

Experience (stress, diet, toxicant) → Biological Mediator (e.g., toxicant metabolism) → Outcome (ADHD)

Functional variation in genes affecting biological pathway (e.g., toxicant metabolism)

Source: Adapted from Lewis et al., 2013, Journal of Child Psychology and Psychiatry, 54, pages 1095-08; © ACAH, JCPP. Slide © Joel Nigg, Ph.D.
Schematic of hypothesized effects for lead x HFE interplay in ADHD

Source: Nigg et al., 2016, *Psychological Science*; © Association for Psychological Science
Effect of lead on ADHD depends on child genotype: Example of HFE gene

- \( \beta = 0.84, [0.38-1.1], p<0.001 \)
- \( \beta = 0.30, [0.17, 0.43], p<0.001 \)

Slope difference interaction \( p<0.001 \)

Average child blood lead level in US

Source: Nigg, 2016, *Psychological Science*; © Association for Psychological Science

Nigg et al., 2016, *Psychological Science*; © Association for Psychological Sci.
Lead-Related hyperactivity caused by epigenetic change

A: Greater hyperactivity in lead-exposed rats in open field test (home cage and open field shown)

Lead-Related hyperactivity caused by epigenetic change

A: Greater hyperactivity in lead-exposed rats in open field test (home cage and open field shown)

B: Relative expression of histone H3 acetylation to β-actin in hippocampus

Source: Man Luo et al. (2014), Toxicology Letters, Vol 225, 78-85 © Elsevier
Effect of food additives on hyperactivity in 8 yr olds is moderated by histamine degradation gene (HNMT Thr105Ile and HNMT T939C). On the left (Thr105Ile), note that when the T allele is present, the food additive challenge has no effect. When the T allele is absent, the food additives cause more hyperactivity than the placebo. ((H3 receptors in the brain may be the mechanism.)) Source: Stevenson et al., 2010, Am J Psychiatry, 167, 1108-1115, © American Psychiatric Association
Elements of a Synthetic Approach

- Neurodevelopmental conditions testable model
  - Endophenotypes help identify mechanisms
  - G x E framework with specific constituents
- Strategy and questions to evaluate frame
  - Understand susceptibility/plasticity (genetic, other)
  - Link to differentiated phenotypes $\rightarrow$ individuals
  - Link to psychological mechanisms (emotion/cognition)
  - Map brain correlates
  - Genetically informed studies of developmental context

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• THANK YOU TO

• Collaborators, staff, volunteers, and families

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Example of In Vitro Study: Prenatal Smoking and Offspring ADHD: Probably not causal

- Surrogate mother sample

- Results for smoking

Source: Thapar et al., 2009, Bio Psychiatry, 66, 722. Left Fig © Elsevier and Society of Biological Psychiatry; right fig data from Thapar et al., Figure © Joel Nigg, Ph.d.
Example of Sibling Comparison: Smoking and ADHD has minimal causality

- NLSY (1979)
- N=8889 children from the 2002 assessment age 4-10 yrs
- 35% smoked in at least one pregnancy
- Unrelated comparison (between offspring of smokers/nonsmokers)
- Sibling comparisons when smoking differed

Data from: DeOnoforia et al., 2008, *Development & Psychopathology*, 20, 139. © D&P
### HERITABILITY OF LIABILITY: TUBERCULOSIS, LEPROSY

<table>
<thead>
<tr>
<th>Study</th>
<th>Disease</th>
<th>MZ</th>
<th>DZ</th>
<th>~a²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kallman &amp; Reiser, 1943</td>
<td>TB</td>
<td>62%</td>
<td>18%</td>
<td>.84</td>
</tr>
<tr>
<td>Harvald &amp; Hauge, 1956</td>
<td>TB</td>
<td>38%</td>
<td>19%</td>
<td>.38</td>
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<tr>
<td><strong>AVG 5 studies of TB</strong></td>
<td>TB</td>
<td>51%</td>
<td>16%</td>
<td>.68</td>
</tr>
<tr>
<td>Chakravartti &amp; Vogel, 1973</td>
<td>LP</td>
<td>60%</td>
<td>20%</td>
<td>.80</td>
</tr>
<tr>
<td>Ali &amp; Ramanunam, 1966</td>
<td>LP</td>
<td>83%</td>
<td>17%</td>
<td>&gt;.90</td>
</tr>
<tr>
<td><strong>AVG 2 studies of LP</strong></td>
<td>LP</td>
<td>66%</td>
<td>19%</td>
<td>.94</td>
</tr>
</tbody>
</table>

MZ=monozygotic or identical twins concordance, DZ=dizygotic or fraternal twins concordance.

Major genetic contribution to susceptibility now accepted (Hill, AVS, 1998; Ann Rev Immu.)

### CAVEATS

1) MZ and DZ concordances are higher for ASD, ADHD, than for TB
2) For infectious disease increased co-socialization of MZ twins must be considered

Data from Fine PE(1981), *Int J Lepr Other Mycobact Dis*, 49, 437-454; © Joel Nigg, Ph.D.
Genetically identical animals with very different phenotype based on epigenetic change caused by feeding different toxicant-diet combination to the mother (R Jirtle et al, Duke University; *Mol Cell Biology*, 23, p. 5293, 2003; © Elsevier Inc.) MCB)

Most events affect only the person exposed, but germ line (transgenerational, 2nd or 3rd generation) effects are known. (Jablonka & Raz 2009, *Quarterly Review of Biology*, 84:131–76; © University of Chicago Press)

Epigenetic Effects based on experiences can, in principle, be as large as genetic effects, although this is unknown in humans