New directions integrating genetic, environment, and possible epigenetic effects to understand causes of ADHD

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DISCLOSURES

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  - Getting Ahead of ADHD (2017)
  - What Causes ADHD (2007)
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OUTLINE

- Plausibility of an epigenetic model or paradigm for ADHD
  - Lessons from complex disease studies
- What exactly is epigenetic change?
- Are environmental correlates of ADHD causal?
  - Using genetically informed studies as one approach to find out
- Preliminary human DNA methylation findings in ADHD
- Conclusions

PLAUSIBILITY OF AN EPIGENETIC PARADIGM FOR ADHD

What is the paradigm?

- Paradigm=exemplar (Aristotle, Kuhn)
- Wrong paradigm 1: metabolic disease
  - “find the gene, solve the disease”
- Wrong paradigm 2: Linear causality
  - “like a machine; mass=force x acceleration. Find the causal chain, solve the disease”

OLD VIEW combined those two ideas into a single paradigms(1980s-2000’s)
3 reasons to reconsider the environment and integrate it with our progress in genetics

- Complex disease model more appropriate
- GxE (heritability of liability) hidden in heritability
- Epigenetic insight—GxE determines phenotype biologically (if not always statistically)

Better Model

Simple versus complex disease: Is it “genetic”? What does that mean?

- Single gene disorder
- Complex disease

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- Deterministic
- Rare (< 1/10,000)
- Large risk increase in relatives
- PKU, Huntington’s

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Heritability” of ADHD is about 70%, suggesting that ~% of variation in the trait is accounted for by genetic variation. MZ twins more likely to share ADHD than DZ twins
BUT: ADHD does not behave like a single gene metabolic disease

<table>
<thead>
<tr>
<th>Feature</th>
<th>Single Gene Disorder (PKU, Huntington’s)</th>
<th>ADHD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Role of genes</td>
<td>Mostly deterministic</td>
<td>Probabilistic</td>
</tr>
<tr>
<td>Disease incidence</td>
<td>Rare (&lt; 1/10,000)</td>
<td>Common (&gt;1/100)</td>
</tr>
<tr>
<td>Increased sibling risk</td>
<td>Large (~1000x)</td>
<td>Small (~3-10x)</td>
</tr>
</tbody>
</table>

Proposed view of ADHD as epigenetic

- Does NOT mean “all environmental” and not “2 types”
- ADHD heterogeneous
  - but many routes are potentially GxE or epigenetic.
  - Likely very little “all or none”
- Thus: susceptibility (substantially genetic) + experience (epigenetically mediated effects) = complex syndrome
- With
  - varying manifestations
  - temporal variations,
  - multiple routes to emergence and recovery

SHORT DETOUR: WHAT IS EPIGENETICS ANYWAY?

What is it? Epigenetic markings change gene activity, respond to gene and environmental inputs

Epigenetic Effects based on experiences can, in principle, be as large as genetic effects, although this is unknown in humans
Overview of the best-documented examples of epigenetic deregulation in neurodevelopmental and neurodegenerative disorders.

**Johannes Gräff et al.** *Physiol Rev* 2011;91:603-649

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**ARE ENVIRONMENTAL CORRELATES OF ADHD CAUSAL?**

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 children, starting school too young

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

– Developmental and Biological Context
  - Rare events
    - Perinatal problems, teratogens (alcohol, drugs); micro-ischemias
    - Extreme toxicant exposures, extreme neglect (Romanian orphans)
  - **Common but harmful contexts**
    - Modern screen Media
    - Moderate psychosocial stress/distress (esp. prenatal)
    - Poor diet
    - Low grade Toxicant/pollutant exposures (pre-natal, post-natal)

***

Substantial literature links ADHD to environmental risk factors in development

- **Toxicants**
  - Lead; PCB’s; BPA; Pesticides

- **Dietary insults**
  - Western high-fat diet during gestation
  - Western diet (additives) in development

- **Gestational and perinatal risks**
  - Parental stress, BMI, smoking, other exposures
  - Infant distress, birthweight, delivery complications

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But are these causal?

- Plausibility (can “low amounts” do harm?)
- rGE and unexamined genetic effects
- Unmeasured confounders

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Toddler attention altered by prenatal DHA supplementation (single object free-play session; increasing look time predicts stronger cognitive development later; i.e., high IQ-growth ability to sustain focus) from Columbo et al 2004. *Child Development* 75, 1254 © John Wiley&Sons, Inc.

Data from Nigg et al (2008) *Bio Psych*, 63, 325-331; © Elsevier Inc. and Society of Biological Psychiatry

### How can we evaluate causality of environmental influences on ADHD in humans when experimental tests are not possible?

- Surrogate pregnancy—(e.g., smoking, Thapar et al)
- Sibling, twin, adoption designs
- Natural stratifications (e.g., Dutch famine; or one city stops vaccinations)
- Mendelian randomization** (focus today)

### Mendelian Randomization Logic

**Unmeasured confounders (parent ADHD, SES, etc.)**

**Measured covariates**

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

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

### 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

### Lead

- Normal lead effect on iron oxidation
- Relatively small effect on ADHD symptoms

- Accelerated lead effect on iron oxidation
- Relatively large effect on ADHD symptoms

**Mendelian randomization logic**

**Exposure**

- Genetic variation

**Putative biological change**

- Outcome

**Source:** Nigg et al., 2016, *Psychological Science*; © Association for Psychological Science
How we proceeded on lead+ADHD

• Replicated the ADHD-low-lead correlation\(\text{(n=213)}\)
• Then combined both samples, (Total N=363; ADHD+control)
• Mendelian randomization design
  • HFE gene (6p22.2)
    – iron uptake in gut, lead x iron interplay
  • Weakness: Lacked an independent replication
  • Strengths of our study
    – ADHD very well characterized
    – Genotype frequencies matched the regional population
    – Control group blood lead levels matched the population
    – No high blood levels (max=3ug/dL)
    – rGE controlled
    – Race/ethnicity, SES controlled

Effect of lead on ADHD depends on child genotype: Example of HFE gene

\[
\beta = 0.84, [0.38 - 1.1], p < .001
\]

\[
\beta = 0.30, [0.17, 0.43], p < .001
\]

Slope difference interaction \(p < .001\)

Average child blood lead level in US

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

Evidence of causality

Potential to identify responders genetically

Effect of food additives on hyperactivity in 8 yr olds is moderated by histamine degradation gene (\(\text{HNMT}^{\text{Thr105Ile}}\) and \(\text{HNMT}^{\text{T939C}}\)). 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. (\(\text{H3 receptors in the brain may be the mechanism}\)). Source: Stevenson et al., 2010, Am J Psychiatry, 167, 1108-1115, © American Psychiatric Association

Mendelian Randomization: G x E liability effects on organic pollutants and cognitive outcome

• PON1 gene \(7q21.3\) effect (slow vs fast metabolizing)

*NYC Mt Sinai cohort

*1998 – maternal urinary organophosphate metabolism

DAP metabolite

*12 month G x E

Paraoxynase 1 enzyme

Age 12 months Bayley score

Log\(B_0\), B

“fast”

“slow”

PON1 genotype

Published NIH/ES data in public domain; Image © Nigg
Examples Linking findings in ND: causally informative designs

- Lead $\rightarrow$ ADHD (Nigg et al, 2016)
- Lead $\rightarrow$ epigenetic change $\rightarrow$ RNA brain $\rightarrow$ hyperactivity (Luo et al, 2014)
- Prenatal chemical toxicant $\rightarrow$ ADHD, IQ, autism (e.g., Engle et al 2011)
- Prenatal omega-3 intake $\rightarrow$ infant IQ (e.g., Columbo et al 2004)
- Food additives $\rightarrow$ ADHD (Stevenson et al 2010)
- Epigenetic mediation (e.g. Skinner et al 2014)
- We should not be uncritical but should consider these linkages carefully

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Epigenetic studies in ADHD in humans

- Strategies
  - Population based vs enriched case-control
  - Candidate gene/probe vs MWAS
- Cautions
  - Tissue specificity and inaccessibility
    - Biomarker vs causal pathway
  - Dynamic
    - Multi-causal (genetic, experience, random)
    - But may help find mechanism of gene action

Key findings in DNA methylation in ADHD in children

- Van Mil et al J Psych Res 2014: 7 candidate genes examined in cord blood $\rightarrow$ ADHD sx at age 6 (CBCL); DRD4, 5HTT.
- Walton et al (2016), altered DNA methylation at birth associated with future ADHD trajectory (AVON study, n=872)
- Barker et al 2017 (Child Dev)(n=671, ALSPAC), cord blood MWAS related to ODD and overlap with ADHD symptoms
- Wilmot et al 2016 (JCPP), n=92 boys case control MWAS, VIPR2 (later confirmed for boys) and MYT1L (confirmed in mixed male-female sample later)

Conclusions

- ADHD hypothesized as at least in part an epigenetic response to widespread low grade insults in genetically susceptible children
- At least some exposure effects appear to have causal link to ADHD
- Genetically informed studies of environment can clarify causal effects
- Initial epigenetic effects in ADHD show promise, although many cautions are in order

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