Epigenetics and Child Neurodevelopment and ADHD

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DISCLOSURE

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What is neurodevelopmental disorder?

Attention-deficit/hyperactivity disorder (AD/HD)
Learning and language disorders (LD)
Autism spectrum disorder (ASD)

Not shown:
- Epilepsy
- Intellectual disability
- Cerebral Palsy
- Schizophrenia

Behavioral components
- “inattentive-disorganized”
- “Hyperactive-impulsive”

Mechanistic Theories widely varying

Complicating problems (MBD)
- Motor control (developmental coordination dis)
- Language development (learning disorders)
- Aggression and defiance
- Cognitive problems (IQ, Executive function)
- Accidents, injuries, health problems

ADHD HIGHLY HERITABLE: TWIN STUDIES

Heritability (% of variance attributed to genetic factors)
Qualifications: parent vs teacher, ratings vs diagnosis

Low birth weight; maternal nutrition; maternal stress; extreme family conflict
Genotype; gene expression; temperament; social supports
Obesity, poor fitness

Child ADHD; gateway
School failure; underachievement
Un- or under-Employment
Drug use, delinquency
Depression; suicide attempts
Serious accidents; injuries; bad driving
Marital problems, conflicts, divorce

Breast cancer
Asthma
Schizophrenia
ADHD

Heritability (% of variance attributed to genetic factors)
Qualifications: parent vs teacher, ratings vs diagnosis

Hudisak, 2000
Nadder, 1998
Levy, 1997
Sherman, 1997
Silberg, 1996
Gijos, 1990
Thaper, 1995
Schmitz, 1995
Edelbrock, 1993
Gillis, 1992
Goodman, 1989
Wilkens, 1973
BUT AT LEAST 3 REASONS TO RECONSIDER SUSCEPTIBILITY X EXPERIENCE EFFECTS

1) Ongoing identification of environmental risk factors (toxicants, nutrition, stress)
2) G x E effects hidden in twin heritability term And keep appearing (Nigg, Nikolas, Burt, 2010)
3) Epigenetic effects largely unexplored

EXAMPLE OF HERITABILITY OF LIABILITY: TUBERCULOSIS

<table>
<thead>
<tr>
<th>Study</th>
<th>MZ</th>
<th>DZ</th>
<th>Approx a²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kallman &amp; Reiser, 1943</td>
<td>62%</td>
<td>18%</td>
<td>.84</td>
</tr>
<tr>
<td>Harvald &amp; Hauge, 1956</td>
<td>38%</td>
<td>19%</td>
<td>.38</td>
</tr>
<tr>
<td>AVG 5 studies</td>
<td>51%</td>
<td>16%</td>
<td>.68</td>
</tr>
</tbody>
</table>

Source: Fine, 1981

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

WHICH ENVIRONMENTS FOR ADHD?

Sociological Effects
- Collapse of civilization?
- Too much pharma marketing?

Caregiver Problems
- Over-indulgent Parenting?
- Under-trained or inexperienced teachers?

Developmental and Biological Context
- Screen Media; Starting school too young; season of birth.
- Prenatal toxicants, diet, stress
- Postnatal toxicants, diet, early family conflict

SOME STRATEGIES IN HUMAN STUDIES FOR STRENGTHENING CAUSAL INFERENCE (LEWIS ET AL 2013)

*comparisons across settings with differential selection biases
*negative controls and natural experiments (migration studies, sibling comparisons)
*individuals conceived using in vitro fertilization
*Mendelian randomization (e.g., using functional genotype; includes some G x E interactions)

Omega 3 supplementation improves ADHD symptoms.
Hawkey & Nigg, 2014

Lower omega-3 blood level in ADHD than non-ADHD youth (g = 0.423). The size of the square indicates the study weight, and the width of the diamond is the 95% confidence interval (CI). Source: Hawkey & Nigg, 2014
Prenatal DHA supplementation trial, infant look time on habituation task (shorter look time predicts better learning and long term cognitive outcome; i.e., high IQ=faster habituation)(n=50)

Columbo et al 2004, Child Development 75, 1254

Toddler attentional development altered by prenatal DHA supplementation (single object free-play session; increasing look time predicts stronger cognitive development later; i.e., high IQ=growing ability to sustain focus)

Columbo et al 2004, Child Development 75, 1254

**MENDELIAN RANDOMIZATION LOGIC**

Confounders (e.g., parent ADHD, SES, etc.)

Prenatal experience (e.g., stress, diet, toxicant)

Mediator (e.g., GC level, toxicant metabolism)

ADHD

Functional variation in genes affecting response to event, e.g., GC production, toxicant uptake, metabolism of toxicant or of nutrients

Confounders (e.g., parent ADHD, SES, etc.)

**WHAT IS EPGENETICS AND WHY IS IT ASKING ME ALL THESE QUESTIONS?**

Same genome therefore can have different health or disease outcomes depending in part on early experience

Source: Adapted from Lewis et al., 2013, Journal of Child Psychology and Psychiatry, 54, pages 1095-1108, 5 SEP 2013

Source: Adapted from Lewis et al., 2015, Journal of Child Psychology and Psychiatry, 56, pages 1109-1118, 15 SEP 2015
**Identical twins with dramatically different phenotype based on epigenetic change caused by feeding different diet to the mother of these animals (source: R. Jirtle, Duke University; Mol Cell Biology, 23, p. 5293, 2003)**

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

**Epigenetics and GxE**
- Types of epigenetic change
  - Genetic
  - Stochastic
  - Environmental (=gxe)
- Types of GxE
  - Non-functional
  - Functional (Mendelian randomization)(below)
- Potential mechanism is epigenetic

**Types of epigenetic change**
- Genetic
- Stochastic
- Environmental

**Types of GxE**
- Non-functional
- Functional (Mendelian randomization)

**Potential mechanism is epigenetic**

**Bootstrapping logic**
- Animals
  - Experimental design
  - Brain methylation
  - Gene expression
- Humans
  - Simple environmental association studies
  - Mendelian randomization designs
  - Peripheral tissue methylation studies
  - Related to exposure (toxicants, stress, diet)
  - Related to disorder (primarily these are in SZ)

**CORD BLOOD PCB TO CHILD ADHD SX**

**Effect by Exposure Quartile**

- N=567 initial cohort (% loss)
- New Bedford MA (1990's)
- Conners Teacher
- Age 7-11
- Umbilical cord PCB/DDDT
- OR

Source: Sagiv et al (2010), Am J Epidemiology, 171, p. 103

**Cord Blood PCB to Child ADHD SX**

<table>
<thead>
<tr>
<th>Exposure Quartile</th>
<th>OR</th>
<th>Effect</th>
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<tbody>
<tr>
<td>Q1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q2</td>
<td></td>
<td></td>
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<tr>
<td>Q3</td>
<td></td>
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<tr>
<td>Q4</td>
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**Related to exposure (toxicants, stress, diet)**

**Related to disorder (primarily these are in SZ)**

**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
  - 24 month no effect
  - 6-9 yr no interaction
  - Paraoxynase 1 enzyme susceptibility

<table>
<thead>
<tr>
<th>PON1 genotype</th>
<th>Age 12 months Bayley score</th>
</tr>
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<tbody>
<tr>
<td>Slow</td>
<td>Log10 B</td>
</tr>
<tr>
<td>Fast</td>
<td></td>
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</tbody>
</table>

- Maternal metabolism
  - "fast"

Source: Engel et al 2011, Environ Health Perspect. 119, 1182

**Log10 β**

**Mendelian Randomization: PON1 Gene Affects Risk for Cognitive Outcomes**

- Lane network of genes differentially expressed vinclozolin* lineage F3 generation germ cells.

Explanation: F3=Grandpups of mice exposed to vinclozolin* and control mice. Epigenetic (methylation) effects enter the germ line and expressed in 3rd generation, including in male germline.

Red=upregulated, Blue=downregulated

*Fungicide used in agriculture

**Environmentally Induced Transgenerational Epigenetic Reprogramming of Primordial Germ Cells and the Subsequent Germ Line.**


**BLOOD LEAD AND ADHD 2005-2007**

Nigg et al (2008), Bio Psych, 63, 325-330

- F(2,124)=8.57, p<.001
- $\beta=0.84$, [0.38-1.1], p<.001
- $\beta=0.30$, [0.17, 0.43], p<.001

**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

**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 $\beta$-actin in hippocampus


**Human DNA Methylation studies in ADHD**

- Candidate gene studies of ADHD methylation
  - Park et al 2015: SLC6A4 methylation-MRI, n=102
  - Xu et al (2015), 50 ADHD, 50 control, 7 genes
  - *Van Mil et al 2014, GenR n=540, 7 genes, CBCL

- First Methylation-wide study at OHSU
  - Wilmot et al, under review
  - N=92/88 cohort 1, all boys, half treated
  - N=20 cohort 2, all boys, med naive
  - Positive finding in VIPR2 and in nicotinic receptor pathways

VIPR2 probe cg 13444538 methylation values of ADHD and non-ADHD boys in a discovery cohort (n=88, panel A, p=0.03) and a replication cohort (n=20, panel B, p=0.006) showing decreased methylation in ADHD. Source: Wilmot, Fry, Musser, Mill, & Nigg, 2015, under review.
VIPR2

- VIPR2 [7q36.3] encodes VPAC2, a G-protein coupled receptor
- Mediates anti-inflammatory effects
- Expressed in cerebellum
- Previously associated with schizophrenia
- Animal model knock out is hypo-active
  - Suggesting demethylation would lead to hyperactivity

Issues and limitations in this effort to date

- Blood/saliva versus brain
  - But newer studies are promising
- Methylation versus gene expression
  - But methylation is potential GxE discovery
- Methylation vs histone modification
  - Looking under the lamp-post
- Sample sizes
  - Large effects for specific inputs (toxins, stress)
  - Small effects for multifactorial outcomes

Conclusions

- Neurodevelopmental conditions and psychiatric conditions may be epigenetic conditions
- Initial evidence suggests causal influences of environment modified by genotype
- Epigenetic mechanisms only now beginning to be examined in this context

THANK YOU