Strategies for Detecting Later Life Effects Following Early Life Stressors in Humans

National Academies Workshop on Use of In Utero and Post-Natal Indicators to Predict Health Outcomes Later in Life

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Developmental Origins of Health Laboratories

The University of Utah
Division of Neonatology

ISIS INTEGRATIVE
SCIENTIFIC
INVESTIGATIVE
SERVICES
Disclosure

- Dr. Lane and his colleagues have documented that they have nothing to disclose
WHAT IS THE RANGE OF ADULT DISEASES STATES THAT HAVE DEVELOPMENTAL ORIGINS?
Quiz: True or False – are which diseases are potentially later life effects of early life stressors?

- Heart Disease
- Cancer
- Chronic Lower Respiratory Diseases
- Accidents
- Alzheimer's
- Diabetes
- Influenza
- Renal Disease
Range of Exposures

- Environmental toxins exposure
- Maternal exposures
  - Tobacco smoke exposure *vs.* nicotine
  - Medications
- Nutrition
  - Uteroplacental insufficiency
  - Maternal diet
Dutch Famine of 1944–1945

- The famine lasted 5 months
  - Calories dropped from 1800/day to 600/day
  - After liberation, calories increased > 2000 calories/day
- Dutch Famine Birth Cohort Study
Dutch Famine – An Example of the Fidelity of the Biology

- First Trimester
  - CV Disease
  - Hypertension
  - Dyslipidemia
  - Obesity

- Second Trimester
  - Pulmonary Disease
  - Renal Disease

- Third Trimester
  - Diabetes
  - Depression
  - Schizophrenia
  - Anti-Social Personality Disorder
Current Issues: Prematurity

- In the US, > 1 in every 8 babies are premature
  - √ Prematurity has increased by 36% since the early 80s
  - √ Over the last 20 years, more and more of these infants are surviving
Prematurity and Associated Later Life Effects

- Neurodevelopmental Delay
- ADHD
- SIDS
- Chronic Lung Disease
- Cardiac Disease
- Obesity (↑Visceral Adiposity)
- Renal Disease
- Hypertension
Adjusted Risk Among Young Swedish Men (n = 329,477)

Adjusted OR

2

1

0

24 – 28
29 – 32
33 – 36

SBP > 140 mm Hg
DBP > 90 mm Hg

Adjusted for:
- Current BMI
- Maternal Age
- Parity
- Parental Education
- Parental Occupation

Johansson et al., Circulation - 2005
WHAT ARE THE POSSIBLE MECHANISMS FOR LATER LIFE EFFECTS OF EARLY LIFE STRESSORS?
Multiple Mechanisms are Involved

- **Cell Number**
  - √ Differentiation
  - √ Proliferation
  - √ Apoptosis

- **Epigenetics**
  - Neural Stem Cells
  - Lung Mesenchyme
  - Nephrons
  - The rest of the story...
Covalent Modifications to Chromatin Alter Gene Expression

Heterochromatin = gene silencing
Euchromatin = gene activation

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WHAT EARLY LIFE EPIGENETIC BIOMARKERS HAVE BEEN USED TO PREDICT LATER LIFE DISEASE?
DNA Methylation as a Biomarker

- DNA methylation studies lend themselves toward high throughput techniques
  - Mass spectrometry
  - Restriction enzyme analysis
  - Bisulfite modification
  - CpG Microarrays
- DNA methylation studies require little sample
Prenatal Maternal Nutrition

- Individuals exposed to Dutch Famine 6 decades latter....

![Diagram showing the expression levels of various genes (IL10, LEP, ABCA1, GNASAS, MEG, INSIG) in early and late stages.]

Heijmans et al., PNAS 2008
Tobi et al., Human Molecular Genetics 2009
Periconceptual Nutrition – part deux

- Countries worldwide recommend peri-conceptual maternal folic acid intake for 400 μg/day

- Focused upon the DMR of IGF2 in offspring from 86 supplemented mothers vs. 37 unsupplemented mothers

Steegers-Theunissen et al., PLoS ONE 2009

IGF2 DMR DNA Methylation
FAS 49.5%
UNSUP 47.4%

p = 0.014

IGF2 DNA Methylation independently correlated with birth weight (p = 0.034)
Conception: *In Vitro* or *In Vivo*

- Pregnancies conceived *in vitro* have a greater relative risk of LBW and rare disorders (controversial)

- DNA methylation measured at 1356 CpG sites in 700 genes

Changes in DNA methylation associated changes in mRNA levels

The range of inter-individual variation in expression of the two groups overlapped substantially

Katari *et al*., Human Molecular Genetics 2009
Gomes *et al*., Molecular Human Reproduction 2009
Does being wombmates help us anticipate an association between early life stressors and latter life effects?

- MZ twins exhibit less phenotype differences than DZ twins.
- DNA methylation analysis performed on 114 MZ twins and 80 DZ twins using CpG microarrays.
- Less epigenetic differences in buccal cells of MZ co-twins versus DZ co-twins.

Kaiminsky et al., Nature Genetics 2008
Baranzini et al., Nature 2009
Transplacental Exposure: Asthma

- Polycyclic aromatic hydrocarbons (PAH) exposure hypothesized to increase the risk of asthma
- Methylation sensitive restriction footprinting analyzes DNA sequences with differential DNA methylation dependent upon PAH exposure
- In a cohort of 56 children, methylation of the 5' CpG Island ACSL3 was significantly associated with...
- Maternal report of PAH exposure
- Asthma symptoms < 5 years of age

Perea et al., PLoS One 2009
Intrauterine Growth Restriction (IUGR)

- IUGR predisposes toward multiple adult diseases
  - No global differences
- Used CD34+ cells \( (n = 10) \) from cord blood
  - No differences in 4 ‘imprinted regions’ – including IGF2
  - Identified specific loci /gene – many of which were intergenic
  - Network analysis consistently pointed towards HNF4α

Einstein et al., PLoS ONE 2010
Model of Deliver: Caesarean Section

- C-section increases the risk for allergy, diabetes, and leukemia
- DNA methylation measured in WBC in cord blood and peripheral blood

\[ \downarrow \text{DNA methylation in cord blood} \ (p < 0.001) \]
Postnatal Effects on DNA Methylation

- Maternal care influences glucocorticoid receptor expression (GR) expression and epigenetic characteristics.

<table>
<thead>
<tr>
<th></th>
<th>Abuse</th>
<th>Without Abuse</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>GR mRNA</td>
<td>↓</td>
<td>+/−</td>
<td>+/−</td>
</tr>
<tr>
<td>NR3C1 DNA</td>
<td>↑</td>
<td>+/−</td>
<td>+/−</td>
</tr>
<tr>
<td>Methylation</td>
<td></td>
<td>+/−</td>
<td>+/−</td>
</tr>
<tr>
<td>(limited # of sites)</td>
<td></td>
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</tbody>
</table>

Suicide victims with childhood abuse

Suicide victims without childhood abuse

Controls

McGowan et al., Nature Neurosci 2009
What would you expect the DNA methylation profile from whole blood to be in a child ...

- whose mother was on a low calorie – high protein diet
- whose mother was taking folic acid supplementation
- who was conceived via *in vitro* techniques
- who was delivered by c-section
- who was born IUGR
- who was born prematurely
- who lived initially in LA, but then moved to NYC
- who was abused (sadly)
- whose teen age persona was a blend of Goth and “EMO”
## Ponderal Index and Adult Insulin Resistance

Uppsala men at age 60 years: What is the best predictor of insulin resistance?

<table>
<thead>
<tr>
<th>Independent Variable</th>
<th>Relative Odds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ponderal Index at Birth (lowest 5th)</td>
<td>5.0 (2.4–10.5)</td>
</tr>
<tr>
<td>Birth Weight (&lt; 3250 grams)</td>
<td>1.9 (1.0–3.8)</td>
</tr>
<tr>
<td>BMI at age 50 (1 SD increase)</td>
<td>2.9 (2.1–4.0)</td>
</tr>
<tr>
<td>60 minute Acute Insulin Response at 50 y</td>
<td>3.5 (1.8–6.6)</td>
</tr>
</tbody>
</table>

Lithell HO et al, BMJ–1996
Epigenetics as a Mechanism

- Maternal – Paternal imprinting
- Developmentally regulated expression
- Cancer biology
- Adaptation to the environment

Fidelity
Early model for gene expression

TF = transcription factor
RNAP = RNA polymerase

DNA
DNA embedded in chromatin (a set of nucleosomes)
National Academies Workshop on Use of In Utero and Post–Natal Indicators to Predict Health Outcomes Later in Life

OUR SCIENTIFIC UNDERSTANDING: LIMITATIONS AND CHALLENGES
The Whole Gene is Used: IGF–1

- IGF–1 is relevant
  - Affected by early life events
    - *e.g.* IUGR
  - Plays a role in postnatal processes affected by early life events
    - *e.g.* insulin resistance, obesity, neurodevelopment, chronic lung disease
IGF-1 Gene (conserved)

Exon 1  Exon 2  Exon 3  Exon 4  Exon 5  Exon 6

Transcript from P1

Transcript from P2

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H3 Acetylation and Methylation along Control Female Hepatic Rat IGF–1 Gene

% of P1

P1  P2  Exon5  3’UTR proximal  3’UTR distal

AcK14  Me^2K4  Me^3K4  Me^3K9  Me^3K36

* p<0.05  ** p<0.01  *** p<0.001

Fu et al, FASEB – 2009
UPI IUGR Affects the IGF–1 Histone 3 Code Along the Length of the Gene

<table>
<thead>
<tr>
<th></th>
<th>P1</th>
<th>P2</th>
<th>Exon 5</th>
<th>3’UTR proximal</th>
<th>3’ UTR Distal</th>
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<tr>
<td>UTR</td>
<td>♂</td>
<td>♂</td>
<td>♂</td>
<td>♂</td>
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</tr>
<tr>
<td>P1</td>
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<td>♂</td>
<td>♂</td>
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</tr>
<tr>
<td>P2</td>
<td>♂</td>
<td>♂</td>
<td>♂</td>
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</tr>
<tr>
<td>Exon 5</td>
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<tr>
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</tr>
</tbody>
</table>

Fu et al, FASEB – 2009
The Whole Gene Really Matters: Glucocorticoid Receptor (GR)

- Human and rat studies suggest that IUGR reprograms the HPA axis
- A regulator of the HPA axis is the hippocampal glucocorticoid receptor (GR)
- Is GR only a “promoter”?
  - Mammals use isoforms for diversity
  - Human and rat GR has multiple isoforms
Human GR gene

Rat GR gene

Exon 1 Variants
- Exon 1.5 and 1.7 are present in hippocampus, but not liver or lung
- Exon 1.11 is present in hippocampus, as well as other tissues
- GRα, GRβ, GRA, and GRP are associated with glucocorticoid resistance
Ke et al., Physiologic Genomics 2010
Our Challenge is in Understanding how Fidelity Occurs

- We know fidelity exists through the wide range of responses that can be generated …
  - How do we integrate early life stressors?
  - How does a gene generate a continuum of responses?
  - How does gender influence generate these varied responses?
  - How does tissue specificity generate these varied responses?
WHERE ARE WE NOW AND WHERE DO WE GO?
Present

- Epigenetic characteristics (e.g. DNA methylation) can potentially be used to predict latter life effects following early life stressors...
  - Thoughtfully and carefully planned
  - Rigorously assessed using large populations
  - Limitations need to be acknowledged
Future

● Depends on our understanding ...

✓ How does epigenetics generate fidelity?
  ▪ DNA methylation
  ▪ Histone code
  ▪ Nucleosome positioning
  ▪ miRNA
Next set of human studies

- The next set of human studies need to tie specific epigenetic findings with tissue phenotype / function.....
  - So that we can directly measure the impact of the epigenetic findings upon expression and phenotypes
  - So that we can focus interventions to be as specific as possible
  - So that we can directly assess the consequences of interventions
Cautionary Note

● Non-specific treatments must be considered with a great deal of caution

● If you turn a good gene “up”, you may do the same thing with a bad gene

   and you may not know about it for years

● The future is taking advantage of the fidelity intrinsic to environmental epigenetics to predict and intervene between early life stressors and latter life effects
Thank You

Developmental Origins of Health Laboratories

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