Environmental Epigenetics

Results and Opportunities for Children’s Environmental Health and Disease Prevention Research

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Epigenetics

- Programming of gene expression that:
  - does not depend on the DNA code
  - (relatively) stable, i.e., replicated through:
    - cell mitosis
    - meiosis, i.e. transgenerational (limited evidence in humans)

Characteristics of Epigenetic Programming

- Modifiable (can be reprogrammed)
- Active or poised to be activated:
  - Potentially associated with current health states or predict future events

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A Symphonic Example

DNA

Phenotype

Epigenetics
A Symphonic Example

Marks in ink (permanent)

Pencil marks (can be erased)
Epigenetic Marks

**DNA methylation**
Methyl marks added to certain DNA bases repress gene transcription

**Histone modifications**
A combination of different molecules can attach to the ‘tails’ of proteins called histones. These alter the activity of the DNA wrapped around them

**microRNAs**
Small non-coding RNAs that block translation of messenger RNAs into proteins
Environment, Genetics, Epigenetics

**ENVIRONMENTAL EXPOSURES**

- **GENETICS**
  - **E → G**
    - DNA DAMAGE/MUTATIONS
      - HIGHLY PERSISTENT
      - TRANSMITTED TRANSGENERATIONALLY (if in germline)
  - **G × E**
    - GENETIC MAKE UP
      - HIGH/LOW SUSCEPTIBILITY
      - TRANSMITTED TRANSGENERATIONALLY

- **EPIGENETICS**
  - **E → Epi**
    - EPIGENETIC CHANGES
      - REVERSIBLE
      - TRANSMITTED TRANSGENERATIONALLY (limited human evidence)
  - **Epi × E**
    - EPIGENETIC MAKE UP
      - HIGH/LOW SUSCEPTIBILITY
      - TRANSMITTED TRANSGENERATIONALLY (on selected genes)

**HEALTH EFFECTS**

Adapted from Bollati & Baccarelli, Heredity 2010
Environmental Effects on blood DNA methylation – Results from our lab (1)

- Hypomethylation of non-coding DNA (50% of genome):
  - **Air Pollution**
    - Baccarelli et al., AJRCCM 2009 (elderly in Eastern Massachusetts)
    - Tarantini et al., Environ Health Perspectiv 2009 (PM in steel factory)
    - Controlled human exposure study (Bellavia et al., in preparation)
  - **Bone Lead** (elderly in Eastern Mass, Wright et al., EHP 2010)
  - **Benzene**
    - Bollati et al., Cancer Res 2007 (traffic officers, gas station attendants)
    - Seow et al., under review (petrochemical workers, Bulgaria study)
  - **Pesticides and POPs** (Inuit Greenlanders, Rusiecki et al, EHP 2008)
  - **Psychosocial Stress** (PTSD, Rusiecki et al. Epigenomics 2012)
Environmental Effects on blood DNA methylation – Results from our lab (2)

- Altered methylation of candidate genes
  - Air pollution (Particulate Matter)
    - iNOS methylation (Tarantini et al. EHP 2009)
    - Inflammatory genes in blood
      - Ongoing work in elderly in Eastern Mass (Madrigano et al., others);
      - Steel workers study (Tarantini et al in preparation,).
      - Normative Aging Study (Bind et al IJE 2012)
  - Tumor suppressor genes (Hou L, Part Fiber Toxicology 2011)
  - Arsenic and p16 hypermethylation (Kile et al, under review)
  - PAHs & tumor suppressor genes
    - Polish PAH study (Pavanello et al. IJC 2010 & Carcinogenesis 2011)
    - Ma-Ta-Puth, Thailand (Peluso M et al. under review)
Why Environmental Epigenetics in Children’s Research?

- The epigenome is erased and re-established in-utero
  - Animal models (see Dana Dolinoy’s work) indicate exquisite susceptibility to in-utero exposures
- The epigenome could be used to track environmental effect on tissue and system plasticity during childhood
  - Need for prospective studies
- Fetal/early life experiences may determine lifelong trajectories of health risk
  - Epigenomics provides molecular substrate for biological memory of gene programming

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‘Each living organism has two histories that determine its biology: an evolutionary history whose duration is in the hundreds of thousands of years, and a developmental history that starts at the time of its conception.’

Ze'ev Hochberg, 2011
Developmental history of the epigenome

Before conception
- Preconceptional exposures

Fetal life
- In-utero exposures

Childhood and adult life
- Early life exposures

Lifecourse Environmental Epigenetics

Opportunities for longitudinal studies at each lifestage

Genome (parental) → Genome (offspring)

GxE interactions

Programming of disease risks

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Children Studies and Environmental Epigenetics

- Environmental Epigenetics in PubMed
  - 1245 papers as of March 4th 2012
  - Queried for:
    - (epigenetics OR DNA methylation OR epigenomics OR histone modification OR histone methylation OR histone acetylation OR miRNA OR microRNA) AND (chemicals OR toxicants OR environmental health OR environmental exposure)

- Children and Environmental Epigenetics in PubMed
  - 183 papers as of March 4th 2012
    - 80 were reviews
    - Many not actually related to environmental health
  - Sub-queried for:
    - child OR children OR pediatric OR pediatrics OR "in utero" OR prenatal OR perinatal

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Ongoing Research & Future Trends in Environmental Epigenetics

1. Exposures can modify DNA methylation in easily obtainable tissues, such as blood (and others)
   - Do epigenetic modifications in blood predict the risk of future disease?

2. Can we move beyond surrogate tissues?
   1. Opportunities in perinatal and child research

3. Other epigenetic mechanisms are less studied
   - Histone modifications
   - MiRNAs
LINE-1 Methylation and Incidence of non-fatal IHD or Stroke

Cumulative incidence of ischemic heart disease or stroke (%)

- Low (68.1-77.4 %5mC)
- High (77.5-86.2 %5mC)

Adj. Hazard Ratio
3.6 (95%CI 1.8-7.0)

p<0.001

HR=2.8 (95%CI 1.3-5.9), p=0.009 for IHD
HR=4.3 (95%CI 0.7-25.8), p=0.11 for stroke  Baccarelli et al., Epidemiology 2010

Andrea Baccarelli, Harvard School of Public Health
Blood LINE-1 hypomethylation: When is that happening?

A. Baccarelli, Harvard School of Public Health

Kile ML et al, PlosOne 2010
Ongoing Research & Future Trends in Environmental Epigenetics

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Environmental causes of fetal growth
Ongoing Research in the ELEMENT/PROGRESS study

- Umbilical vessels and placenta are critical to maternal-fetal nutrient transfer
- Ongoing case-control study nested in the ELEMENT/PROGRESS Mexico Cohort
- Fetal growth and potential environmental determinants

Figure 3: Umbilical artery and vein dissection

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Top 20 methylation sites in blood, artery, vein associated with birth weight

<table>
<thead>
<tr>
<th>Rank</th>
<th>Blood SYMBOL</th>
<th>Blood p.value</th>
<th>Artery SYMBOL</th>
<th>Artery p.value</th>
<th>Vein SYMBOL</th>
<th>Vein p.value</th>
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<tr>
<td>1</td>
<td>KLF15</td>
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<td>SFT2D1</td>
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<td>LR8</td>
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<td>SLC22A6</td>
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</tr>
</tbody>
</table>

Pilot Study
20 children with small birth weight vs. 20 children with normal birth weight
Ongoing Research & Future Trends in Environmental Epigenetics

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   - MiRNAs
Metal-rich air particles exposure of steel workers

- Foundry work has been associated with cardiovascular disease and lung cancer
- Chemical exposures are low in modern foundry facilities
- Particulate Matter (PM) Rich in metals 10+ fold higher than ambient levels
Years of Employment and Blood H3 activating modifications

H3K4 Dimethylation

H3K9 Acetylation

p-trend=0.04

p-trend=0.006

-Associations were not due to age effects
-Associations with Nickel and Arsenic contained in particles

Cantone et al., EHP 2011

A. Baccarelli, Harvard School of Public Health
MicroRNA expression & Metal-rich PM (Motta et al., in preparation)

Discovery: profiling of 847 miRNAs on 10 pre-post exposure blood sample pairs

Validation: Real time PCR of 12 miRNAs on 62 blood sample pairs

Figure 1: Volcano-plot representing differential miRNA expression in blood leukocyte RNA of foundry workers pre-and post-exposure.

Table 2: MicroRNA expression measured by RT-PCR.
Environment, Genetics, Epigenetics

**ENVIRONMENTAL EXPOSURES**

- GENETICS
  - E→G: DNA DAMAGE/ MUTATIONS
    - Highly Persistent
    - Transmitted Transgenerationally (if in germline)
  - G×E: Genetic Make Up
    - High/Low Susceptibility
    - Transmitted Transgenerationally

- EPIGENETICS
  - E→Epi: Epigenetic Changes
    - Reversible
    - Transmitted Transgenerationally (limited human evidence)
  - Epi×E: Epigenetic Make Up
    - High/Low Susceptibility
    - Transmitted Transgenerationally (on selected genes)

**HEALTH EFFECTS**

Adapted from Bollati & Baccarelli, Heredity 2010
IGF2 Imprinted Region

IGF2, Insulin-like growth factor 2; ICR, imprinting control region, H19, a non-coding RNA thought to inhibit IGF2 expression, KCN, KCNQ1OT1, an upstream regulator of IGF2 expression.

CpG sites analyzed 1 2 3 4

Sequencing Primer SNP, RS10732516

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Epigene X gene interactions and birthweight

Effect of ICR1 methylation on birth weight stratified by ICR1 SNP rs10732516

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Birthweight difference (β) in grams (95% CI)</th>
<th>Interaction P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minor T allele (ht or hm)</td>
<td>77</td>
<td>-187 (-389, 15)</td>
<td></td>
</tr>
<tr>
<td>Major C allele</td>
<td>136</td>
<td>65 (-61, 190)</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Change in birth weight per an SD increase in DNA methylation
Challenges & Opportunities

- How many epigenomes?
  - Tissue specificity
    - Most studies in humans are on blood DNA
    - Need to investigate tissues relevant for the exposure-disease of interest (challenging in epidemiology)
  - The epigenome changes over time
    - Reverse causation is always a potential issue
    - Need for longitudinal studies

- Current focus on DNA methylation
  - Opportunities for investigating other mechanisms

- Can we keep our epigenome healthy?
  - Opportunities for research leading to primordial, primary, secondary interventions
Thanks to:

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  - Bob Wright
  - Joel Schwartz
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  - Laura Cantone
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  - Lifang Hou

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A. Baccarelli, Harvard School of Public Health