Early Exposure to Bisphenol A and Lead: Effects on Metabolic Homeostasis and the Epigenome

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Presentation Overview

- Conceptual Framework of UM SPH P20 Center; Intro environmental and nutritional epigenetics

- **Example:** Early bisphenol A (BPA) exposure and metabolic homeostasis

- **Example:** Early lead (Pb) exposure, metabolic homeostasis and neuropathology
Conceptual Framework

Perinatal exposures, epigenetics, child obesity and sexual maturation

Perinatal Exposures
- Lead (animal, human)
- Bisphenol A (animal, human)
- Phthalates (human)

Childhood Exposures
- Lead (human)

Adolescent Exposures
- Bisphenol A (human)
- Phthalates (human)

Epigenetic Regulation
DNA methylation of genes associated with growth

Weight Gain
Weight Status
- Childhood
- Adolescence

Sexual Maturation
- Tanner stage
- Hormonal biomarkers

Adult Chronic Disease
- Obesity
- Type 2 diabetes
- CVD
- Some cancers

Center PI: Karen E. Peterson
Epigenetics in a Genetic Context

DNA (human): 3.2 billion bases (haploid), ~23,000 genes, 2 meters; A typical cell: 10-100 micro meters

Epigenetic marks:
- DNA methylation
- Histone modifications

DNA is “packed.” ...But creates challenges and opportunities for regulation of gene transcription

Environmental epigenetics and the developmental origins of disease

Epigenetic plasticity may allow for pharmacological or nutritional intervention/prevention/treatment approaches
Why We Care: Early Origins of Disease

The Barker Hypothesis (1992)

Poor nutrition during gestation alters the development of an unborn child such that it will be prepared for survival in an environment in which resources are likely to be short, resulting in a **thrifty phenotype**.

However, often an **environmental mismatch** occurs. Those who develop in an affluent environment may be more prone to metabolic disorders, such as obesity and type II diabetes.

**Miracle:** Epigenetic Modifications
Epigenetic Susceptibility

Gametogenesis

Pre-implantation stage of embryogenesis

Fetal and neonatal periods of development

Puberty

Old age

Jirtle and Skinner Nature Reviews Genetics 2007
Mouse to Human Experimental Approach

$A^{vy}$ Model (multiple doses)

Human Clinical Samples

Population-based Cohorts

Perinatal Bisphenol A (BPA) Exposure, Epigenetics, and Metabolic Homeostasis

![BPA molecule structure](image-url)
Viable Yellow Agouti Mouse Model: Epigenetic Biosensor

DNA unmethylated
Histone acetylation
Ectopic expression
Adult onset obesity

DNA methylated
H4K20 methylation
Little to no expression
Lean

Methyl Donors
(Waterland et al. 2003)

Genistein
(Dolinoy et al., Environ Health Perspect 2006)
Maternal Bisphenol A (BPA) Exposure

Bisphenol A
(50 mg BPA/kg diet)

Maternal Nutritional Supplementation

Bisphenol A
(50 mg BPA/kg diet)

Methyl Donors
Genistein

Dolinoy, et al. 2007 PNAS
Goals of Current Research

1) Expand dose-response assessment
2) Move from candidate gene driven to full epigenome technologies
3) Link epigenetically labile loci with biological pathways or phenotypes/health outcomes
4) Move from animal models to human clinical samples to human population approaches
(1) Moving from Single to Multiple Doses

2 Weeks Prior to Mating - 1 of 4 Diets:
1) AIN 93G Control
2) 50 mg BPA/kg Diet
3) 50 ug BPA/kg Diet
4) 50 ng BPA/kg Diet

50% a/a offspring

50% A^vy/a offspring
Environmentally Relevant Levels?
Liver Tissue Levels in ng/g

Work in Progress! Collaboration with K. Kannan, Wadsworth Institute in Albany, NY
(Fetal samples from BDRL at Univ. Washington)

Nahar M., Anderson O. In Preparation
Dose Assessment - Coat Color Shift
Milligram (50 mg/kg diet) Dose Level

(p=0.006);

Mirrors 2007 PNAS findings

Anderson O. et al. In Press
**A<sup>vy</sup> Methylation Analysis:**
Milligram (50 mg/kg diet) Dose Level

<table>
<thead>
<tr>
<th>Exposure</th>
<th>Mean</th>
<th>Mean: <em>PNAS</em> 2007</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg</td>
<td>24.3</td>
<td>27</td>
</tr>
<tr>
<td>control</td>
<td>35.63</td>
<td>39</td>
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</tbody>
</table>

Anderson O. et al. In Press
Dose Assessment - Coat Color Shift
Microgram (50 ug BPA/ kg diet)
Nanogram (50 ng BPA/ kg diet)

Anderson O. et al. In Press
Cabp<sup>AP</sup> Methylation Analysis:
Microgram (50 ug BPA/ kg diet) Dose Level

\begin{tabular}{|c|c|c|c|}
\hline
\textbf{Diet} & \textbf{N} & \textbf{Mean} & \textbf{SD} \\
\hline
ug & 67 & 85.80 & 5.62 \\
control & 82 & 83.12 & 8.23 \\
\hline
\end{tabular}

p=0.02

Anderson O. et al. In Press
Avy locus as an Epigenetic Biomarker

Milligram Exposure

Microgram and Nanogram Exposure

Unmethylated  Methylated  Unmethylated  Methylated

Non-monotonic
(2) Moving from Candidate Gene to Whole Epigenome - Multi-Platform (Multi-Tissue) Approach

**Avy Model - Liver tissue plus blood**

**Human Clinical Samples – Fetal liver, placental tissues, cord blood**

**Perinatal Bisphenol A (BPA) Exposure**

Methylation Deep Sequencing followed by validation with quantitative bisulfite sequencing

(+) Unbiased

(-) Expensive, complex bioinformatics
Differential Promoter Methylation by Dose

Kim J. et al. In Preparation
## Pathway Enrichment Analysis

<table>
<thead>
<tr>
<th>GOID</th>
<th>GO_term - Function</th>
<th>Corrected P-value</th>
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<tbody>
<tr>
<td>GO:0005488</td>
<td>binding</td>
<td>1.4528E-18</td>
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<td>GO:0005515</td>
<td>protein binding</td>
<td>9.2311E-10</td>
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<td>GO:0003824</td>
<td>catalytic activity</td>
<td>1.8317E-08</td>
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<td>GO:0043169</td>
<td>cation binding</td>
<td>6.9366E-05</td>
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<td>GO:0046872</td>
<td>metal ion binding</td>
<td>7.7010E-05</td>
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<td>GO:0043167</td>
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<td>GO:0016787</td>
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<td>GO:0008233</td>
<td>peptidase activity</td>
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<td>GO:0016491</td>
<td>oxidoreductase activity</td>
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<td>GO:0070011</td>
<td>peptidase activity, acting on L-amino acid peptides</td>
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<table>
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<th>GO_term - Process</th>
<th>Corrected P-value</th>
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<tbody>
<tr>
<td>GO:0009987</td>
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<td>GO:0008152</td>
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<td>GO:0065007</td>
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<td>GO:0044238</td>
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<td>GO:0050789</td>
<td>regulation of biological process</td>
<td>1.8678E-09</td>
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<td>GO:0044237</td>
<td>cellular metabolic process</td>
<td>3.9161E-08</td>
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<td>GO:0050794</td>
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<td>GO:0043170</td>
<td>macromolecule metabolic process</td>
<td>2.8598E-06</td>
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<tr>
<td>GO:0019222</td>
<td>regulation of metabolic process</td>
<td>1.3345E-05</td>
</tr>
</tbody>
</table>

Enriched in binding activity

Enriched in metabolic processes

Kim J. et al. In Preparation
(3) Linking Epigenetic Effects to Adverse Phenotype

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2) 50 mg BPA/kg Diet
3) 50 ug BPA/kg Diet
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50% a/a offspring
50% A^vy/a offspring

Life-Course analysis of phenotypes related to obesity/metabolic disorders/cancer
Life-Course Phenotyping (ongoing)

- D22 - Adiponectin and leptin
- D90 - Free fatty acids; oxidative stress markers (NI EHS BPA Supplement Award to V. Padmanabhan)
- 3, 6 and 9 months - Body composition; energy intake/expenditure; spontaneous activity
- 9 mo - Glucose tolerance test
- 10 months - Tissue collection; adiponectin & leptin levels; epigenomics (tiling arrays); tumor burden
Life-Course Activity Patterns Associated with Perinatal BPA Exposure

Results

- No difference in food intake
- Increased oxygen consumption and activity in female offspring
- Exposed females weigh less (with decreased fat mass) than the controls over each visit, but not statistically significant
- Female-specific results mirror Braun et al. findings in human population cohorts

Caveats

- Phytoestrogen free background diet
- Mice were not challenged with high-fat diet

Future Directions

- Candidate gene methylation/Promoter tiling arrays
(4) Moving from Animals to Humans
Clinical and Population Samples

Human Clinical Samples

- Human Clinical Samples
  - PI: Vasantha Padmanabhan
    Project: Maternal and term Cord Blood from UM Hospital

Population-based Cohorts

- Population-based Cohorts
  - PI: Dana Dolinoy
    Project: NIH-funded fetal tissue bank (Univ. of Washington)
  - PI: Karen Peterson
    Mexico City Birth Cohort (NIH/EPA Children’s Env. Health Formative Center P20)
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Bisphenol A (BPA) exposure

- PI: Dana Dolinoy
  Project: NIH-funded fetal tissue bank (Univ. of Washington)

- PI: Vasantha Padmanabhan
  Project: Maternal and term Cord Blood from UM Hospital

- PI: Karen Peterson
  Mexico City Birth Cohort (NIH/EPA Children’s Env. Health Formative Center P20)

- Pilot Project funded by UM NIEHS P30 Core Center
  Collaborators: Amr Soliman, Laura Rozek
  Project: Egyptian Girls
Early Life Exposures in Mexico to Environmental Toxicants (ELEMENT)

- ELEMENT is >15-year birth cohort comprised of mother-child pairs recruited from Mexico City during pregnancy and followed throughout childhood and adolescence.

- Biomarkers of exposure are available at various developmental time points (e.g., urinary BPA/phthalate measures; blood lead levels).

- Growth parameters and sexual maturation (tanner staging/hormones) are monitored overtime in the children.

- Epigenetic analyses is ongoing including methylation analysis of LINE1 repetitive elements and, key growth genes and hormone receptors ($IGF2$, $H19$, $HSD11B2$, $PPARA$, $PPARG$) using DNA from birth and later time points.

- For P20 Target Sample Size = 200; Currently recruited ~100 pre-adolescent/early adolescent offspring.
Leasure et al. report increased BW in 1 year old males following maternal Pb exposures with peak BLL ~10 ug/dL and ~25 ug/dL.

Puzas et al. observe increased adipocyte differentiation in stem cells exposed to Pb.

We expand to humans and lower doses in animal model with sophisticated measurements.

Incorporate blood, fat, and brain concordance of DNA methylation and gene expression (animal model).
Perinatal Lead Exposure

2 Weeks Prior to Mating:
1) Control
2) 3 ppm (~peak BLL 2 ug/dL)
3) 27 ppm (~peak BLL 10 ug/dL)
4) 55 ppm (~peak BLL 25 ug/dL)

50% a/a offspring

50% A^vy/a offspring

Life-Course analysis of phenotypes related to obesity/metabolic disorders

A^vy epigenetic biomarker
Preliminary Results: Lead (Pb) and Coat Color Shifts

N = 6 to 8 litters per group

** Significant coat color shifts toward yellow are observed among offspring from the 27 ppm and 55 ppm Pb groups compared to controls ($\chi^2$ p-value=0.009 and 0.006).
Perinatal Lead Exposure

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Life-Course analysis of phenotypes related to obesity/metabolic disorders

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50% A^vy/a offspring
Conclusion and Future Direction

- Dose and full epigenome studies are crucial to deciphering the role of the environment on the epigenome.

- Identification of epigenetically labile genes in the Mouse and Human (and other model species).

- Link epigenetically labile loci with biological pathways and phenotypes/human health outcomes.

- DNA methylation in concert with other factors such as histone modifications and ncRNAs.
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