Epigenetics: The Interface Between Environment and the Genome

Shuk-mei Ho, Ph.D

Shuk-mei.ho@uc.edu
Chair, Department of Environmental Health
Director, Center for Environmental Genetics
Co-investigator, Breast Cancer and Environment Research Center
Co-Leader, Genetic Susceptibility, Cancer Center
University of Cincinnati College of Medicine, Ohio
Outline:

- Epigenetics and Epigomics: Definitions
- What are the epigenetic mechanisms involved in the development of human disease?
- Study Platforms and Methods
- Examples of epigenetic studies in laboratory animals: Emphasis on early life exposure on adult disease risk
  - i) Estrogen/Bisphenol A and prostate cancer
- Epigenetic epidemiology
  - i) PAHs and childhood asthma
- How can epigenetic data be incorporated into risk assessments?
Question:

Epigenetic changes → Understand the diseases* development?

* Cancer such as lung, prostate, breast, colon cancer, etc.
* Diseases such as Prader-Willi syndrome, Angelman’s syndrome and Beckwith-Wiedemann syndrome

Journals describing epigenetics and disease development* (PubMed)
Epigenetics refers to features such as chromatin and DNA modifications that are stable over rounds of cell proliferation but do not involve changes in the primary DNA sequence of the cell/organism.

Epigenetics keys in on mechanisms that regulate how and when certain genes are turned on and turned off, while epigenomics refers to analysis of epigenetic changes across many genes in a cell or entire organism.

The National Institutes of Health will invest more than $190 million over the next five years to accelerate research in epigenomics.
Relationship between genetics and epigenetics

Epigenetics are markings etched in the "margins" of one's genetic make-up.

Genome: static
Epigenome: dynamic; subjected to changes including those brought on by environmental factors.
Epigenetic mechanisms involved in disease development

Three distinct and interrelated mechanisms:
Non-coding RNAs, DNA methylation, and Histone modification

- These processes affect transcript stability, DNA folding, nucleosome positioning, chromatin compaction, and ultimately nuclear organization.

- Singularly or conjointly, they determine whether and when a gene or a set of genes is silenced or activated, resulting in alterations in cell/organ functions.

Dysregulation of these processes by environmental factors are the bases of disease development.
Epigenetic mechanisms of gene regulation

I. Histone Modification

• N-terminal tails of histones, positioned peripheral to the nucleosome core, are subject to various covalent modifications: acetylation, methylation, phosphorylation and ubiquitination.

• Enzymes including histone deacetylase (HDAC), histone acetyltransferase (HAT), histone methyltransferase (HMTase) are involved.

Daryl C. Drummond et al, 2004
HDAC, HAT, HMTase

• **HDAC**: catalyze [histone deacetylation](#) leading to formation of [repressed](#) chromatin.

• **HAT**: catalyze [histone acetylation](#) at lysine residues at H3 and H4 leading to formation of [open chromatin structure for transcription](#).

• **HMTase**: Methylate histone. Methylation at [lysine 4 (K4) of H3](#) is associated with promoter of active genes, whereas methylation at [lysine 9 (K9) of H3](#) is associated with promoter of inactive genes. Methylation at [arginine (R8H3)](#) correlates with transcriptional activation of a variety of genes.

Aberrant histone modification → affect [chromatin structure and remodeling](#).
Epigenetic mechanisms of gene regulation

II. DNA methylation

- Addition of a methyl group derived from \textit{S-adenosyl-L-methionine} to the \textbf{fifth carbon of the cytosine ring} to form the \textbf{fifth base 5-methyl cytosine} and catalyzed by DNA methyltransferases and accessory proteins.

- It occurs predominantly in cytosines located 5’ of guanines, known as \textbf{CpG dinucleotides (CpGs)}. CpGs are \textit{found as clusters known as CpG islands (CGIs)} in 1-2% of the genome.

- About 70% of CGIs are associated with 1-2 kb long DNA sequences located in the \textit{promoter, the first and second exons, and the first intron regions} of all genes (5’CGIs).
Enzymes & proteins involved in CpG methylation or demethylation

• **DNMT1**: prefers **hemi-methylated DNA substances** and is responsible for maintenance of methylation, which must occur during DNA replication.

• **DNMT3A and 3B**: are responsible for **de novo methylation** and observed in aberrant methylation of tumour suppressor genes in cancer.

• **MeCP1**: requires **densely methylated DNA fragments** (>11 CG) for binding and represses transcription through binding, remodeling and deacetylation of methylated nucleosomes.

• **MeCP2**: binds to **single methylated CG dinucleotides** and represses transcription by binding to methylated DNA and recruiting Sin3 through a transcriptional repression domain.

• **MBDs**: MBD1, 2 and 3 are responsible for gene silencing, whereas MBD4 is responsible for DNA repair by removing deaminated 5-methyl-cytosine bases from DNA.

Example: MBD2 is the methyl binding component of the **MeCP1 complex**.

Stirzaker et al., 2004 Cancer Res
Proposed mechanisms of transcriptional repression mediated by DNA methylation

1. Direct interference with transcription activator factor binding

- Active transcription
- Repression by inhibition of TF binding

2. Specific transcriptional repressors ie. MeCP1 or MeCP2-HDAC complex

- Active transcription
- Repression by MeCP1
- Repression by MeCP2

3. Inactive chromatin structure formation

Methylation sensitive TF: AP-2, E2F, NFκB
Methylation insensitive TF: Sp1

Modified from Singal et al., 1999
Interaction between DNA methylation and histone modification

The underlying mechanisms how they interact are not fully understood!!

TR- trans-acting repressors
TAF- trans-acting activating factors
HDAC- Histone deacetylases
HMTase- Histone methyl-transferase
HAT- Histone acetyltransferase
DNMT- DNA methyltransferase
MeCP- Methylated DNA binding protein

Modified from Szyf M al, 2005, Biochemistry (Mosc) 70(5):533-49
miRNA mediated gene silencing

- **microRNAs (miRNA)** are single-stranded RNAs of about 21-23 nts in length. They are encoded by genes that are transcribed from DNA but not translated into proteins (hence they are referred to as non-coding RNA).

- Mature miRNA molecules are partially complementary to one or more mRNAs (>200), and their primary function is to suppress gene expression.
The epigenome serves as an **interface** between the **environment** and the inherited **genome**.
Study Platforms and Methods: DNA methylation

Bisulfite Sequencing-target gene approach

Unmethylated DNA

gaa gcg gac cgcc

Sodium bisulfite modification

gaa gug gau ugu

Methylated DNA

gaa g\textsuperscript{CM}g gac g\textsuperscript{CM}gc

PCR / Sequencing

gaa gtg gat tgt

Sequencing Data analysis

- Methylation-specific PCR (MSPCR)
- Combined bisulfite restriction analysis (COBRA)
- Methylation-specific single-strand conformation polymorphism (MSSSCP)
- MethyLight
Workflow for DNA methylation profiling - Discovery platform

Methylation profiling tools

Genomic DNA extracted from tissues/ cell lines

Methylation/ Promoter Microarray

Sequencing / BLAST / BLAT search

Promoter / CpG island search

Data confirmation

- Real-time PCR
- Western blot analysis

Known target genes

-Bisulfite sequencing (BS)
-Methylation-specific PCR (MSPCR)
-Combined bisulfite restriction Analysis (COBRA)
- MSSSCP
- MethyLight
Tools for DNA methylation profiling

- Methylation Sensitive Restriction Fingerprinting (MSRF)
- Restriction Landmark Genomic Scanning (RLGS)
- Methylation Sensitive (MS) / Methylation CpG islands Amplification (MCA)
  - Representational Difference Analysis
- Differential Methylation Hybridization (DMH) / Methylation-specific oligonucleotide (MSO) microarray
- Genome-wide methylation microarray / ChIP-chip promoter array
- Luminometric methylation assay (LUMA) using pyrosequencing
- MALDI-TOF - Mass spectrometry / HPLC

Ho and Tang, Repr Tox 2006
## Advantages and limitations of different approaches to methylation profiling

<table>
<thead>
<tr>
<th>Methods</th>
<th>Global / Gene Specific</th>
<th>Sample amount</th>
<th>Advantages</th>
<th>Limitations</th>
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<td>Simple set-up</td>
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<td>Mostly on specific target gene</td>
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</table>

*Ho and Tang 2007*
MSRF (Methylation Sensitive Restriction Fingerprinting)

**A**
- **MseI digest**
  - TTAA
  - TTAA
  - TTAA
  - TTAA
  - TTAA

- **BstUI digest**
  - TTAA
  - TTAA
  - TTAA

- **PCR product**
  - TTAA
  - TTAA

**B**
- **Sample A**
  - MseI: +
  - BstUI: -

- **Sample B**
  - MseI: +
  - BstUI: +

- **PCR product**
  - TTAA
  - TTAA

**Results**
- **Sample A**
  - No difference among both samples
  - Hypermethylation in Sample A
  - Hypomethylation in Sample A

- **Sample B**
  - No difference among both samples
RLGS (Restriction Landmark Genomic Scanning)

Allele 1
NotI
NotI

Allele 2
NotI
mNotI

Cleavage at NotI and Labeling

mNotI
mNotI

EcoRV digestion (1st D)
HinfI digestion (2nd D)

2D gel electrophoresis

1D
2D

1D
2D

1D
2D

Unmethylated
Semi-methylated
Methylated
The software is capable of calculating fragment lengths for the first and second dimensions, visualizing the spot on the two-D pattern, searching sequence data of the spot through database.

I. Crosslink by formaldehyde
II. Isolate genomic DNA
III. Immunoprecipitate with specific Ab ie. Methylcytosine
IV. Reverse crosslinks
V. PCR
VI. Label with Cy5/3
VII. Apply labeled samples to CGI array or promoter array
VIII. Data Analysis
Pyrosequencing

A. Restriction cleavage

EcoRI

AATT

EcoRI + HpaII

or

EcoRI + MspI

HpaII (methylation sensitive) or MspI (methylation insensitive)

C. Pyrosequencing

Step 1

AATT

GC

Step 2

AATT

AA

GC

Step 3

AATT

TTAA

GC

Step 4

AATT

TTAA

GC

+ dATPαS

+ dCTP + dGTP

+ dTTP

+ dCTP + dGTP

Sulfurylase

ATP

Luciferase

Light

HpaII

MspI

A C+G T C+G

Step 1 2 3 4
Tools for database analysis

Sequence analysis
- BLAST search (www.ncbi.nih.gov/BLAST)
- EST homology (www.ncbi.nih.gov/BLAST)
- BLAT search from UCSC Genome Bioinformatics (www.genome.ucsc.edu)

Promoter/ CpG island/Bisulfite genomic sequencing analysis
- MetPrimer (http://www.urogene.org/methprimer/index.html)
- Promoter Inspector (http://www.genomatix.de)
- Data Base of Transcription Start Site (http://dbtss.hgc.jp.com)
- BiQAnalyzer (http://biq-analyzer.bioinf.mpi-sb.mpg.de/)
Tang WY, Ho SM.

Epigenetic reprogramming and imprinting in origins of disease.

Rev Endocr Metab Disord. 2007 8:173-82
Environmental Agents Producing Mother to Fetus Epigenetic Effects

- **Synthetic estrogens**
  - Diethylstilbestrol (DES)
  - DES-daughters/sons
  - Newbold et al., 2001

- **Phytoestrogens**
  - Genistein, Coumestrol, Quercetin
  - Day et al., 2002; Lyn-Cook et al., 1995

- **Endocrine disruptors**
  - Dioxin-Incineration
  - Wu et al., 2004
  - Pesticides
  - Bisphenol A-Polycarbonate plastics, dental sealants
  - Ho, Tang et al., 2006
Critical period for estrogenization effect on rodent reproductive tracts is between days 1-5 of life. It corresponds to the *in utero* 2nd and 3rd trimester development of human counterparts.

Perinatal or neonatal exposure of rats and mice to estrogens leads to "imprinting" of reproductive tracts associated with increased proliferation, inflammation and dysplastic epithelial changes later in life.

*G. Prins et al., 1995, 1997; Newbold et al., 1990, 1997*

**Developmental exposure to estrogens** can be shown to associate with permanent defects in reproductive system and can be transmit to second generation. This reprogramming event includes epigenetic imprinting through DNA methylation.

*McLachan et al., 2001*
Neonatal exposure to estrogen/bisphenol A and prostate cancer

Neonatal estrogen/bisphenol A reprogramming increases later life PCa risk

**No neonatal estrogen treatment**

- Low PCa incidence

**Neonatal estrogen treatment**

- E/BPA Day 1,3,5

- Adult hormonal treatment *T + E Day 90-200

- Sprague Dawley rat

- High PCa incidence

≈ Noble rat
Histological Studies on adult prostate gland

Upper panel: Day200  
Lower panel: Day200w/T+E

High-dose or low-dose EB predispose to PIN with aging while BPA increases the susceptibility of prostate gland to PIN following additional adult hormone exposure.

Ho, Tang & Prins; Can Res 2006
Identification of candidate genes susceptible to E-induced alterations in DNA methylation status

Genomic DNA extracted from tissues/ cell lines

Methylation profiling tools (MSRF)

Sequencing / BLAST / BLAT search

Promoter / CpG island search

Data confirmation

Methylation/ Promoter Microarray

Knowledge mining; selection of candidate genes

-Functional Studies e.g. in vitro and in vivo tumorigenecity; -Western blotting cell growth, movement & migration

-Real-time PCR

-Bisulfite sequencing (BS)

-Methylation-specific PCR (MSPCR)

-Combined bisulfite restriction analysis (COBRA)

-MSSSCP

-MethyLight
Differentially methylated candidates discovered with MSRF

<table>
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<tr>
<th>Clone Name</th>
<th>Primer 1</th>
<th>Primer 2</th>
<th>Hypermethylation</th>
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Graphical View of the Knowledge Mining Results
Intracellular cAMP signaling

PDE4D

HPCAL1

cAMP

Ras

C-raf

PI3K

p38

PKA

B-raf

p90RSK

Gene transcription

Cell cycle progression

Apoptosis

Nucleus

cAMP signaling

MAPK

AKT

ERK

c-myc

Elk-2

Ets

C-fos

CREB

Intracellular cAMP signaling

Gene transcription

Cell cycle progression

Apoptosis

Nucleus
Phosphodiesterase type IV variant 4 (PDE4D4)

- Function of cAMP degradation
- Maintain cAMP in narrow range of concentrations that is critical for growth and differentiation
- Involve in tumor growth suppression by inhibiting its activity in glioma, osteosarcoma and lymphocytic leukemia cells (Chen et al., 2002; Narita et al., 2003; Lerner et al., 2000)
- Recently, concept of phosphodiesterase (PDE) inhibition has gained tremendous interest in field of urology with the use of sildenafil and zaprinast on BPH treatment or erectile dysfunction. (Adolfsson et al., 2000)
Bisulfite Treatment (MSPCR or Bisulfite sequencing)

Unmethylated DNA

\[ \text{gaa g g gae cgc} \]

Sodium bisulfite modification

Methylated DNA

\[ \text{gaa gCmg gae Cmgc} \]

PCR / Sequencing

\[ \text{gaa g g gat tg} \]

Sequencing Data analysis

Methylation-specific PCR

Control

\[ \text{Met} \]

UnMet

M-methylation specific primers set

U- unmethylation specific primers set
PDE4D4 silenced with aging while over-expressed in neonatal E2/BPA treated groups

Gene expression studies

Day 10

Day 90

Day 200

Day 200 w/T+E2

Relative Expression Ratio

MSPCR

Ho, Tang and Prins, Cancer Res 2006
Summary: Identification of PDE4D4 as a target for reprogramming by neonatal E/BPA exposures in rats

PDE4D4 turns off with aging

No neonatal treatment

Low PCa incidence

Neonatal E/BPA treatment

Adult hormonal treatment

High PCa incidence

PDE4D4 fails to shut off with aging

Ho, Tang and Prins, Cancer Res 2006
An E-epigenome?

- *HPCAL-1* has the opposite methylation and expression pattern as *PDE4D4*
- Other genes in the pathway are currently under investigation
- Neonatal DES-exposure and mouse uterine cancer identified additional genes
- Building a repertoire of estrogen/estrogen mimic-affected genes

**Question:** Can these biomarkers be identified in human?
Higher PCa in African-Americans as compared with Caucasians

Hypothesis: *In utero* estrogen exposure may determine PCa risk *Henderson and Ross 1988*

- **Before birth**
  - Caucasians: Lower E
  - African-Americans: Higher E

- **Young**
  - Lower PCa incidence

- **Old**
  - Higher PCa incidence
Clinical studies: PDE4D4 methylation status of LCM samples

Prelesion & cancer specimens → Immunohistochemistry and grading → Laser Capture Microdissection

Individual focus with normal and PCa tumor → Bisulfite Modification

PCRPCR → MSPCR

35 cases of PCa
Race
Gleason Score
Age

Association with methylation status of PDE4D4
PDE4D4 methylation status in Normal and Prostate Cancer cell lines

5’ promoter region of hPDE4D4

Tang, Revelo and Levin (unpublished data)
Methylation status of PDE4D4 5’regulatory region of LCM human prostate samples (analyzed by MSPCR)

Caucasians

African-Americans

Tang, Revelo, Levin & Ho (unpublished data)
Frequencies of methylation status of PDE4D4 by race in normal and tumor tissues for thirty-five prostate cancer patients

*P-Values testing the equality of percents of methylated PDE4D4 between Caucasians and African Americans for each tissue type. †Odd ratios measure the associations between methylation status of PDE4D4 (M, U) and race in normal tissues.

<table>
<thead>
<tr>
<th>Race</th>
<th>Normal</th>
<th>Tumor</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>% N</td>
</tr>
<tr>
<td></td>
<td>Methylated</td>
<td></td>
</tr>
<tr>
<td>Caucasians</td>
<td>14</td>
<td>63.6</td>
</tr>
<tr>
<td>(N=22)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>African Americans</td>
<td>2</td>
<td>15.4</td>
</tr>
<tr>
<td>(N=13)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>p-value*</td>
<td>&lt; 0.01</td>
<td></td>
</tr>
<tr>
<td>(Fisher’s exact test)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Odd ratio†</td>
<td>9.6 [1.7, 54.8]</td>
<td></td>
</tr>
<tr>
<td>(Caucasians: African Americans)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
During pregnancy, maternal conditions such as nutritional deficits, infection, hypertension, diabetes, or hypoxia expose the fetus to hormonal and metabolic cues that induce “fetal programming.” It alters the courses of cellular and organ differentiation in utero and permanently affects the functional capacity of adult organs in later stages of life. (Gluckman PD et al., 2004; Santos F et al., 2004)
Epigenetic Epidemiology

-the study of the relations between epigenetic variation and disease

-epigenetic variation has many sources: genetic and epigenetic inheritance, developmental stochasticity, environmental influences (during development and throughout life), and aging.

-the starting point in epigenetic epidemiology is evidence that inter-individual epigenetic variation affects disease risk.

Waterland and Michels, 2007

Example (unpublished data)
i) PAHs and Childhood Asthma
Minority communities of **New York City** (children residing in urban low-income) experience some of the **highest childhood asthma rates in the U.S.**

*In utero* exposure to the common traffic-related air pollutants, **polycyclic aromatic hydrocarbons (PAHs)**, is a risk factor for development of childhood asthma.

• Cohort study in Krakow, Poland has found a significant relationship between prenatal PAH exposure and respiratory symptoms.

• CCCEH has developed a **comprehensive database on PAH exposure**, allowing us to examine the contribution of PAH-related epigenetic changes to the pathogenesis of childhood asthma.
Unbiased methylation profiling by MSRF (n=10 low PAH; n=10 high PAH)

Identification of 32 candidates showing differential methylation patterns between high and low PAH groups

In silico analysis of candidates:
Identification of 6 candidates genes with 1 or more CGI(s) in the 5' promoter flanking region

Validation of an association between CGI methylation status and PAH exposure level by bisulfite sequencing in n=20 UCWBC DNA samples – association was confirmed for all 6 candidate genes

Evaluation of concordance between degree of CGI methylation in UCWBC and level of transcript expression in n=20 matched FPT samples – the highest concordance gene was ACSL3

Establishment of an association between ACSL3 CGI methylation status (assayed by MS-PCR) and childhood asthma in 60 participants (30 high PAH and 30 low PAH exposure); p<0.05

¹High PAH (>2.3 ng/m³ PAHs), Low PAH (<2.3 ng/m³ PAHs)
Using MSRF, we have analyzed cord white blood cells including T cells (an important source of cytokines and other asthma mediators) from an initial sample of 40 CCCEH cohort children.

<table>
<thead>
<tr>
<th>Clone</th>
<th>Pr1</th>
<th>Pr2</th>
<th>Aberrant Methylation</th>
<th>Chr Band</th>
<th>Gene homology</th>
<th>Location</th>
<th>5' CpG island</th>
<th>Asthma-related pathway</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>A11-1</td>
<td>20</td>
<td>21</td>
<td>highPAH</td>
<td>8q24.3</td>
<td>NIK and IKKbeta-binding protein</td>
<td>IN15</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A12-1</td>
<td>20</td>
<td>21</td>
<td>highPAH</td>
<td>6p25.1</td>
<td>clone IMAGE:4825327</td>
<td>5' end</td>
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<tr>
<td>A12-2</td>
<td>20</td>
<td>21</td>
<td>highPAH</td>
<td>1q41</td>
<td>N/A</td>
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<td>A12-3</td>
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<td>21</td>
<td>highPAH</td>
<td>19p13.3</td>
<td>FLJ4311 fis, clone PLACE3000304</td>
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<tr>
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<td>21</td>
<td>highPAH</td>
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<tr>
<td>A13-3</td>
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<td>21</td>
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<td>8q24.11</td>
<td>protein involved in DNA double-strand break repair, Rad21</td>
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<td>√</td>
<td>oxidative stress</td>
<td>Fortoul et al., 2003</td>
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<td>A21-1</td>
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<td>21</td>
<td>highPAH</td>
<td>6p25.3</td>
<td>LMW-DSP2, dual specificity phosphatase</td>
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<td>√</td>
<td>stress kinase</td>
<td>Zhou et al., 2002</td>
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<tr>
<td>A22-1</td>
<td>22</td>
<td>23</td>
<td>highPAH</td>
<td>8p23.1</td>
<td>beta-defensin 107 (DEFB107)</td>
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<td>B23-1</td>
<td>22</td>
<td>23</td>
<td>highPAH</td>
<td>1q32.2</td>
<td>KIAA0463 protein, partial cds</td>
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<td>B23-2</td>
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<td>23</td>
<td>highPAH</td>
<td>2q24.2</td>
<td>N/A</td>
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<td>D11-1</td>
<td>22</td>
<td>23</td>
<td>low PAH</td>
<td>2p24.2</td>
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<tr>
<td>D11-2</td>
<td>22</td>
<td>23</td>
<td>low PAH</td>
<td>1q32.1</td>
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<tr>
<td>D11-3</td>
<td>22</td>
<td>23</td>
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<td>3q24</td>
<td>WAF1-4002-STS Human THudson EST</td>
<td>IN1</td>
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<tr>
<td>D12-1</td>
<td>22</td>
<td>23</td>
<td>low PAH</td>
<td>15q22.2</td>
<td>VPS13C protein, transcript variant 1A (VPS13C gene)</td>
<td>EX7</td>
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<tr>
<td>D21-1</td>
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<td>23</td>
<td>low PAH</td>
<td>1q41</td>
<td>N/A</td>
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<tr>
<td>D21-2</td>
<td>22</td>
<td>23</td>
<td>low PAH</td>
<td>19p13.2</td>
<td>N/A</td>
<td></td>
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</tbody>
</table>

Using MSRF, we have analyzed cord white blood cells including T cells (an important source of cytokines and other asthma mediators) from an initial sample of 40 CCCEH cohort children.
Network 1
A putative PAH epigenome

**A** ACSL3
- CGI (1058bp)

**B** DSP22
- CGI (927bp)

**C** RAD21
- CGI 1 (704bp)  CGI 2 (272bp)

**D** SCD5
- CGI (1108bp)

**E** SFMBT2
- CGI 1 (935bp)  CGI 2 (667bp)  CGI 3 (309bp)

**F** WWOX
- CGI (955bp)
**5' PROMOTER STRUCTURE OF ACSL3**

### Effects of asthma and race on the difference between mean values of PAH, by methylation status of ACSL3

<table>
<thead>
<tr>
<th>Race</th>
<th>Asthma</th>
<th>Geometric Mean PAH [95% CI]</th>
<th>Methylation Status: M</th>
<th>Methylation Status: U</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dominicans (N=19)</td>
<td>No (N=19)</td>
<td>3.9\textsuperscript{a}</td>
<td></td>
<td>1.4\textsuperscript{a}</td>
</tr>
<tr>
<td>African-Americans (N=27)</td>
<td>No (N=21)</td>
<td>3.6\textsuperscript{b}</td>
<td></td>
<td>1.8\textsuperscript{b}</td>
</tr>
<tr>
<td></td>
<td>Yes (N=6)</td>
<td>3.3\textsuperscript{c}</td>
<td></td>
<td>1.6\textsuperscript{c}</td>
</tr>
</tbody>
</table>

\textsuperscript{a}p<0.001; \textsuperscript{b}p<0.01; \textsuperscript{c}p=0.14. Results obtained from paused comparisons of means obtained from multiple linear regression analysis in which PAH was the dependent variable. Independent variables were methylation status, race, and asthma modeled using indicator variable coding.
## Characteristics of Subjects by Methylation Status

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>M (n=30)</th>
<th>U (n=30)</th>
<th>n=60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asthma (%Yes)*</td>
<td>11 (37%)</td>
<td>4 (13%)</td>
<td>15 (25%)</td>
</tr>
<tr>
<td>Median PAH exposure</td>
<td>3.4</td>
<td>1.7</td>
<td>2.3</td>
</tr>
<tr>
<td>(min, max)</td>
<td>1.1, 34.5</td>
<td>0.5, 3.7</td>
<td>0.5, 34.5</td>
</tr>
</tbody>
</table>

* *p* < 0.05
Summary

- Identified the first epigenetic biomarker associated with transplacental PAH exposure
- Established an association of this biomarker with childhood asthma
Future Prospects

- What are the mechanisms governing selective epigenetic modifications of susceptible genes?
- Can disease-specific epigenetic fingerprints be identified (e.g. cancer, obesity, asthma)?
- Can specific epigenetic “biomarkers” be identified and validated to be associated with an environmental exposure in a qualitative as well as a quantitative manner (a BPA-epigenome, a PAH-epigenome)?
- Could epidemiology facilitate the identification of epigenetic biomarkers to be used for risk assessment in population studies?
- How can epigenetic data be incorporated into risk assessments used by environmental regulatory agencies?
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