Health Effects Associated with Prenatal Exposure to PAHs: understanding the mechanisms

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1. PAH exposure and how we measure it in the CCCEH
2. Health effects associated with prenatal PAH exposure
3. Understanding possible mechanisms: DNA methylation
4. Ongoing work to study PAH-related methylation
Polycyclic Aromatic Hydrocarbons (PAHs)
carcinogenic combustion-related urban air pollutants generated by multiple sources
Benzo[a]pyrene

CYP1A1
Reductase

Glutathione (GSTs)

BP-7,8-epoxide

EH

BP-7,8-dihydrodiol

CYP3A4

Sulfate esters

BP-7,8-diol 9,10-epoxide

Guanine

BPDE-DNA Adduct

- Chromosomal aberrations
- Mutation (e.g., P53)
- Gene inactivation

Repair

cancer

[adapted from Drs. Rundle and Perera]
• In animals, some PAHs are transplacental carcinogens [Soyka et al. 1980; Vesselinovitch et al. 1975]

• Animal studies estimate that the dose to the fetus is generally an order of magnitude lower than the dose to paired maternal tissues [Srivastava et al. 1986; Withey et al. 1993]

• The amount of DNA damage per unit dose of PAH may be on the order of 10-fold higher in the fetus relative to the mother [Perera et al. 2005]
Why is a fetus or child more susceptible than adults?

- Differential exposure
- Greater absorption and retention of toxics
- Decreased efficiency in detoxification/repair
- Higher rate of cell proliferation
- Time for related adverse effects to develop over the lifecourse
Columbia Children’s Center for Environmental Health: Parallel Studies of in Utero Exposures and Childhood Disease

New York City, USA (1998-present)
N. Manhattan/S. Bronx Cohort
725 mothers & newborns and 60+ siblings
World Trade Center Cohort
329 mothers & newborns

Krakow, Poland (2000-present)
550 mothers & newborns

Chongqing, China (2001-present)
450 mothers & newborns

Asthma
Growth & Development
Cancer Risk

Adult Diseases
CCCEH: Longitudinal Study Design

Prenatal  Birth  Age 1  Age 12
How do we measure prenatal PAHs in our cohorts?

Area Exposure

- Area monitor (and GIS)

Personal Exposure

- Backpack PAH

Internal Dose

- Urinary metabolite (e.g., 1-OH pyrene)

Biologically effective dose

- B(a)P adducts

It is not always possible to collect all measures:

Example 1:
in the WTC study, not possible to recruit before 9/11 to measure when exposure was highest

Example 2:
it is not always feasible to set up an air monitor (e.g., China)
Factors that affect PAH exposure

- Temporal: across years and within years (seasonal)
- Geographical (i.e. residential proximity to pollutant sources)
- Personal/behavioral (i.e. time spent outdoors, use of incense)

[Narvaez et al. 2008; Tonne et al. 2004; Jung et al. submitted]
Associations between prenatal PAH and adverse health outcomes

Prenatal PAH has been associated with:

- Adverse fetal growth
- Adverse neurodevelopment
- Childhood asthma
- Markers of cancer risk (chromosomal aberrations)
- Obesity (preliminary)
- (others?)

[Perera at al., Miller et al., Tang et al., Orjuela et al.]
In NYC, prenatal PAH-DNA adducts and ETS were associated with reduced birth weight (-233 g, \( p=0.04 \)) and head circumference (\( p=0.01 \)).

[Perera et al., 2005]

In the WTC study, PAH–DNA adducts, in conjunction with ETS exposure, were significantly associated with reduced birth weight (-276 g, 8%) and head circumference (-1.03 cm, 3%) (both \( p < 0.05 \)).

[Perera et al., 2005]
Prenatal PAH and adverse neurodevelopment

In NYC, significant inverse association between high PAH exposure and full-scale and verbal IQ
[Perera et al. 2009]

In China, among those exposed prenatally to coal-burning emissions, PAH-DNA adducts were significantly associated adverse effects on motor developmental quotient.

In a second cohort who were not exposed to emissions, no significant effects of PAH-DNA adducts were observed.
[Perera et al. 2008]
In NYC, by 24 months, probable asthma was reported more frequently among children exposed to prenatal PAH and ETS postnatally. [Miller et al. 2004]

In Poland, the frequency of wheeze during the first 2 yrs was positively associated with detectable prenatal PAH-DNA adducts. (IRR = 1.69, 95%CI = 1.52–1.88). [Jedrychowski et al. 2010]
Mechanisms underlying observed associations between prenatal PAH and adverse health effects

- Induction of apoptosis after DNA damage from PAHs?
- Antiestrogenic effects of PAHs?
- Binding to the human aryl hydrocarbon receptor to induce P450 enzymes?
- Binding to receptors for placental growth factors, resulting in decreased exchange of oxygen and nutrients?

- Altered expression of specific genes
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▶ Altered expression of specific genes
Toxicological evidence that PAH alters methylation:

• Decrease global DNA methylation in vitro (Wilson and Jones, 1983)

• Inhibit DNA methyltransferase activity (Wilson and Jones, 1984)

• Interfere with recruitment of methylation machinery (Weisenberger and Romano 1999; Zhang et al. 2005)

• Global hypermethylation of sperm in mice exposed to PAH (Yauk et al. 2008)
How to measure CpG Methylation

CpGs are under-represented across the genome and are not randomly distributed
- clusters in the promoter region of genes ("CpG islands")
- majority found throughout repetitive sequences

Many ways to quantify methylation, 2 categories include:
- global methylation
- gene-specific methylation
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- global methylation
- gene-specific methylation
Prenatal PAH exposure could alter methylation as methylation marks are being established
CCCEH research objectives: understanding the exposure pathway

1. Evaluate whether prenatal airborne PAH exposure is associated with genomic DNA methylation in cord blood:
   - global methylation
   - gene-specific methylation

2. Evaluate whether methylation levels persist in blood collected from the same children at age 3.
CCCEH: Prenatal PAH and global DNA methylation

**Study Population:** CCCEH Mothers and Newborns Cohort
- 725 mother/newborn pairs
- Race/Ethnicity: African American and Dominican
- Residence: Northern Manhattan and South Bronx
- Exclusion: Smokers, Illicit Drug, HIV, Hypertension, Diabetes

n=164 subjects from the CCCEH cohort in NYC with stored cord blood DNA:
- 82 with prenatal PAH exposure above the population median
- 82 with prenatal PAH exposure below the population median

**Methylation assay:**
MethyImprove Methylated DNA Capture Kit
methylated fraction of DNA is quantified through an ELISA-like reaction
1. Methylation and Prenatal PAH (CCCEH)

[unpublished work removed]
• Experimental data indicates that B(a)P DNA adducts preferentially form at guanines 3’ to methylated cytosines [Denissenko et al. 1997]
2. Global methylation level persists from birth to age 3

Study Population:
n= 159 subjects from the CCCEH cohort with stored cord blood DNA and available 3 year blood
Prenatal PAH exposure is inversely associated with global methylation.

Cord B(a)P adducts are positively associated with global methylation.

Methylation levels appear to persist between cord and 3 year blood.

Next question: Is prenatal PAH exposure associated with methylation of specific genes?

How do you know which genes to look at?
Subject selection:
Identify subjects with the most different PAH levels

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Low PAH n=12</th>
<th>High PAH n=12</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Med (min, max)</td>
<td>Med (min, max)</td>
</tr>
<tr>
<td>all</td>
<td>24</td>
<td>2.15 (0.55, 2.69)</td>
<td>17.23 (12.58, 154.04)</td>
</tr>
<tr>
<td>girls</td>
<td>12</td>
<td>2.18 (1.76, 2.69)</td>
<td>14.54 (12.58, 51.09)</td>
</tr>
<tr>
<td>boys</td>
<td>12</td>
<td>2.00 (0.55, 2.53)</td>
<td>23.38 (16.89, 154.04)</td>
</tr>
</tbody>
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• Match on sex
• Only Dominicans
• No ETS exposure

Assay: Illumina’s Infinium Human Methylation27 Bead Chip (>27,000 CpG assays per sample, 12 samples per chip)
Prenatal PAH and gene-specific methylation

[unpublished work removed]
Additional plans to study PAH-related methylation: Identify genes related to PAH and...

**Asthma** (collaboration with S-M Ho)

- Nimblegen promotor array and Affymetrix expression array in n=32 cord blood samples
- Test association between PAH-related differentially methylated genes and asthma
  - Design: case-control study
  - n=40 asthmatics and n=80 controls

**Neurodevelopment**

- Illumina 40K Infinium methylation array followed by RT-PCR n=40 cord blood samples
- Test association between PAH-related differentially methylated genes and neurodevelopment
  - Design: cohort study
  - n=350 children
Potential pitfalls and plans to address them

• Based on our preliminary work, we know we are looking to detect fairly small changes in methylation

• Many factors influence methylation (e.g., maternal diet, genetics, social factors)

How can we better isolate the effects of prenatal exposure (to PAHs) on the epigenome?

• Alter biological matrix
• Statistics to reduce “nuisance” variation
• Alter the study design
Methylation is tissue specific; Blood is a mixture of cells

White blood cells:
85% granulocytes (half-life: hrs to days)
15% mononuclear cells; most are lymphocytes (half-life: ~3 years)

Different cell types likely have different methylation patterns
What if exposures (i.e. PAH) differentially influence methylation?
Composition of the cells for DNA could introduce lots of noise!
Controlling variation by altering the biological matrix: using PBMCs instead of total WBC

Need to plan for this during the study design phase (prior to blood collection)
Controlling variation by design: The Sibling-Hermanos Study

Goal: to recruit newborn siblings of children currently enrolled in the CCCEH Mothers and Newborns Study (NYC)

Use a matched design to better control for shared genes and environment to better isolate the influence of differential prenatal exposures
Summary

- Prenatal exposure is ubiquitous
- Prenatal exposure to PAHs is associated with many adverse health outcomes
- Prenatal PAH appears to impact the epigenome
- Global methylation measured at birth is similar to 3 year methylation levels
- Ongoing work to identify genes that are differentially methylation in relation to prenatal PAH
- How can we better isolate the effects of prenatal exposure (to PAHs) on the epigenome?
  - Alter biological matrix
  - Statistics to reduce “nuisance” variation
  - Alter the study design
Future Plans and Overall Objective

- Explore associations between prenatal PAH and gene-specific methylation levels
  - Use peripheral blood mononuclear cell (PBMC) DNA
  - paired (sibling) and unpaired (overall cohort) approaches in concert

By identifying genes whose methylation is affected by prenatal PAH, we will be able to understand the mechanisms by which prenatal PAH influences adverse health outcomes.

disease prevention
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Study Participants