Placental DNA Methylation Linking Exposures and Newborn Health

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DNA Methylation is highly tissue specific

Represents functional alteration

Placenta

- First complex organ to form
- Regulates intrauterine environment
- Transport
  - Nutrients
  - Water
  - Gas
  - Waste products
- Immuno-endocrine
  - Hormones
  - Growth factors
Role of Placental Epigenome

MATERNAL ENVIRONMENT
• Growth • Drugs
• Nutrition • Stress
• Exposures

PLACENTAL EPIGENOME

PLACENTAL FUNCTION

• Demonstrate placental molecular features integrate environmental signals
• Link variability in molecular features to Infant Outcomes (and beyond!)
Study Population: Rhode Island Child Health Study

- Hospital-based Birth Cohort
  - Moms enrolled following delivery at Women & Infant Hospital
  - WIH sees 75% of deliveries in RI
  - 2009-2014
  - Total Enrollment n=899

- Mothers
  - 18-40 years old
  - No history of psychological disorders
  - In good physical health

- Infants
  - Viable
  - No known genetic disorders
  - No life threatening illness
  - Term (≥ 37 weeks)

- Oversampled for SGA (small) and LGA (large) infants. Matched to AGA (appropriate)

- Gestation time, infant sex, maternal age (±3 yrs)

Mom
- Medical Chart Review
- Questionnaire
- Maternal blood
- Toenail Samples

Infant
- Clinical Characteristics
- NNNS Assessment
- Placenta
- Cord Blood
- Toenails

Linking Molecular Character with Exposures and Outcomes
Mercury exposures in utero

Sources of exposure
- Dietary (fish, seafood) i.e. methylmercury (MeHg)
  - Biomethylated, biomagnified
- Dental amalgams
- Industrial
  - Minimata disease (Japan) (Harada et al. 1968)
  - Iraqi fungicide contamination incident (Bakir et al. 1973)
- Air Pollution

  - Interferes with placental function (Boadi et al. 1992)

Neurobehavioral effects associated with prenatal and childhood exposure
- Infants: Cerebellum size, CNS damage, poor psychomotor development (Cace et al. 2011; Choi 1989; Llop et al. 2012)
- Children: Memory, attention, language, visual-spatial perception (Faeroe Islands study) (Grandjean et al. 1997)
Preliminary - Discovery Study

Placental DNA Methylation
Illumina HumanMethylation450 array
(192 placental samples, 450,000+ CpG loci)

QA/QC, Normalization, Batch Adjustment (COMBAT),
Limit to Autosomal, non-SNP associated loci only
(192 placental samples, 384,474 CpG loci)

N = 41 infants placental methylation AND Hg data from infant toenail clippings

Identify Loci with Variable Methylation Related to Hg Exposure
Methylation Impacting Neuro-related Genes

- 339 CpG loci associated with infant toenail Hg
- Within genes or gene promoters associated with neurologic outcomes
  - **Neurobehavior** *(CPLX1, LMX1B, ADD2)* (Drew et al. 2007; Glynn et al. 2007; Barreto-Valer et al. 2013; Porro et al. 2010)
  - **Schizophrenia** *(DIXDC1, ARVCF, MAGI2, ZIC2)* (Bradshaw and Porteous 2012; Sim et al. 2012; Mas et al. 2010; Mas et al. 2009; Chen et al. 2005)
  - **ADHD** *(TCERG1L)* (Neale et al. 2010; Karlsson et al. 2012; Hatayama et al. 2011)
  - **Movement disorders** *(NOL3, TP53INP2)* (Russell et al. 2012; Bennetts et al. 2007)
  - **Autism** *(PLXNA4, WNT2)* (Suda et al. 2011; Lin et al. 2012; Kalkman 2012)

Looking at Neurobehavioral Effect

NICU Network Neurobehavioral Scales (NNNS)

- Developed by Lester and Tronick (2004)
  - Built on backbone of Neonatal Behavioral Assessment Scales (NBAS) developed by Brazelton (1973)
- Developed for use in at-risk infant
  - Specifically substance exposed – used in the Maternal Lifestyle Study
  - Designed for broad applicability
    - Generalizable
    - Reproducible
    - Sensitive to variety of infant risk factors
    - Infants from 30 weeks gestation to ~2 months post-partum
- Approx. 30 minute exam performed after 24 hours of life but prior to discharge
- Examine three major area of neurodevelopment
  - Neurological
  - Behavioral
  - Stress/Abstinence
- Summarized into 13 Summary Scales reflecting various aspects of neurodevelopment
  - Can examine individually
  - Used latent profiling strategy to create profiles of neurobehavior (Liu et al Pediatrics 2010)
Focused on “At-risk” profile

Mean-centered score

-1.50
-1.00
-0.50
0.00
0.50
1.00
1.50

Excitability
Arousal
Stress
Handling
Hypertonicity
Hypotonicity
Lethargy
Nonoptimal Reflexes
Habituation
Asymmetric reflexes
Attention
Movement Quality

Not Profile 7
Profile 7
Discovery Scheme

Illumina HumanMethylation450 placental tissue array
(192 placental samples, 450,000+ CpG loci)

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Subset 1:
N = 41 infants placental methylation AND Hg data from
infant toenail clippings

339 Loci Related to Hg Exposure

Subset 2:
N = 151 infants with placental methylation AND infant
Neurobehavioral Assessments

Examine Relationship of Hg-Associated Loci with
NNNS Profiles
6 CpG Hg-associated loci are associated with NNNS High Excitability Profile

<table>
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<tr>
<th>Illumina CpG Designation</th>
<th>Gene Symbol</th>
<th>P Value</th>
<th>UCSC CpG Island Designation</th>
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Placental hypomethylation of EMID2 associated with Risk Behavioral Profile

Non-Risk Profile (n=135)  Risk Profile (n=16)
Methylation Correlated with Expression

EMID2 Expression vs. EMID2 Mean Methylation

EMID2

- Collagen protein, unknown placental function
- Variant associated with asthma/airway hyperresponsiveness in nasal passages (Pasaje et al. 2011; Pasaje et al. 2012)
- A SNP within EMID2 mediates side effects on vision and hearing in response to an antidepressant (Adkins et al. 2012)
- More work needed to understand the functional role of this gene in placenta
DNA Methylation as Toxicant Mechanism

- Alterations to genes or pathways can have long-term consequences on development

- DNA methylation is susceptible to environmental signals
  - Toxicant Exposures
  - Maternal Factors/Lifestyle
  - Stress, Psychosocial adversity

- Can then link altered DNA methylation to critical outcomes

- Ongoing work
  - Better defining what environment can do
  - Consequences of these alterations – Various Health Outcomes
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