The Epigenome and Asthma: Interactions Between Imprinted Genes and Environmental Exposures

Rachel L. Miller M.D., FAAAAI
Associate Professor of Medicine (In Pediatrics) and Environmental Health Sciences
Director, Allergy and Immunology Fellowship
Division Chief, Allergy and Immunology
Columbia University College of Physicians and Surgeons

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Epigenome and asthma—What we know/Don’t know:

Do prenatal environmental exposures influence the phenotype in offspring?
Is there a parent of origin effect?
Can asthma be imprinted?
Is epigenetic regulation the mechanism for imprinting?
Does epigenetic regulation of asthma genes explain the link between prenatal environmental exposures and asthma in children?

Epigenome and asthma—What we know/Don’t know:

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Epigenome and asthma—What we know/Don’t know:

3 examples: Cigarette smoke, air pollution, acetaminophen

Prenatal exposure to environmental tobacco smoke and traffic-associated air pollution have been associated with:
- wheezy illnesses and asthma
- reduced lung function
- respiratory infections in children
- Prenatal intake of acetaminophen has been associated with wheeze at age 5 years

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Parent of origin??

Intrauterine environment matters

+ Stronger associations of maternal (vs paternal) inheritance of asthma phenotype
+ Development of childhood asthma (<5 yrs old)
+ Development of elevated IgE levels in cord blood, 6 months.


Maternal smoking transmitted asthma risk across multiple generations

+ Children’s Health Study: N=338 children with asthma and 570 matched controls
  – Grandmaternal smoking during pregnancy increased the odds of asthma in grandchildren (OR, 1.8; 95% CI, 1.0 to 3.3)
  – Grandmaternal and maternal smoking during pregnancy increased the odds of asthma in grandchildren (OR, 2.6; 95% CI, 1.6 to 4.5)

Li et al. Chest 2005

Epigenome and asthma – What we know/Don’t know:

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Many asthma genes have exhibited evidence of imprinting

+ Transmitted paternally:
  + Stat6 haplotype ATTCAA
+ Transmitted maternally:
  + Association between chromosomal 8p23, 5q13 regions and mite sensitization
  + β-chain of the high-affinity receptor for IgE: mixed results*
  + Association between glutathione-S-transferase P1 and lung function

Godava Biomed Pap Med Fac Univ Palacky Olomouc CZ 2012
Kurz JACI 2000
*Cookson AJRCCM 2000
Carroll PM 2003

Imprinted genes

+ Genes whose expression is determined by the parent that contributed them.
+ Most are repressed; either:
  + the maternal allele is expressed exclusively because the paternal allele is imprinted or
  + vice versa
+ Process begins during gamete formation in developing sperm, egg
+ Aberrant imprinting disturbs development and can cause preclinical and disease syndromes

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**Epigenetic regulation may be mechanism for imprinting**

- DNA Methylation, histone alterations, may "mark" the allele destined to be inactive in the new embryo (gamete)
  - the other is fully expressed
  - father’s or the mother’s
- Classical example in agouti mice.

**Epigenetic inheritance at the agouti (hair color) locus**

- Epigenetic marks not completely erased on passage through the germline in mammals.

**Environmental Epigenetics and Allergic Disease**

- Environmental exposures
  - Allergens
  - Antibiotics
  - Air pollution
  - Diet
  - ETS

**DNA methylation**

Covalent addition of methyl group to $5'$ position of cytosine pyrimidine ring, primarily at CpG sites

- Inhibits gene transcription by:
  - blocking transcription factor binding to recognition sites
  - attracting methyl CpG-binding proteins (MBPs)
- MBPs can interact with chromatin forming proteins modifying the surrounding chromatin
Post-translational modification of histones

- Acetylation, methylation, and phosphorylation
- Key elements in the chromatin packaging of DNA
- Acetylation: DNA around histone core unwinds, activators of transcription obtain access to DNA, and gene expression proceeds.
- Deacetylation: Acetyl group removed and gene expression inhibited
- Extent determined by histone acetyltransferase (HAT) vs histone deacetylases (HDAC) activity.

Methylation patterns

- Gene-specific:
  - promoter, untranslated region (junk DNA), etc.
- Global:
  - overall level of methylation of all CpGs in genome
  - influences genomic instability, loss-of-imprinting, etc.
- Repetitive elements are indicators
  - LINE-1: Long interspersed nuclear element
  - AluYb8: Short interspersed nucleotide element

MicroRNA

- Single-stranded small RNA molecules 21-23 nucleotides in length
- Non-coding
- Important roles:
  - tissue development and differentiation, cellular proliferation, and tissue repair
- Induce degradation of target mRNA
- Implicated in asthma, eosinophilic chronic rhinosinusitis, CF, Influenza virulence, lung cancer, IPF

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Epigenetic regulation and asthma, allergy: Publications over time

Prenatal diet: methyl donors (i.e. folate)

- Animal studies:
  - Increased methylation of Runx3 and suppression of Runx3 mRNA and protein associated with allergic airway disease
- Human studies:
  - Mixed results: Maybe increased risk of asthma, lower respiratory tract infections in children

Listed in PubMed, as of 01/10

Hollingsworth et al. JCI, 2008
Miller JCI, 2008
Whitmore, M et al AAnnal of Epidemiology, 2009
Gestational exposure to dietary methyl donors regulated severity of allergic asthma

Prenatal A. fumigatus associated with changes in CpG methylation at the IL-4 and IFN-γ promoters in grandoffspring

Prenatal polycyclic aromatic hydrocarbon exposure associated with methylated Acyl-CoA synthetase long-chain family member 3 (ACSL3)

Methylation of ACSL3 associated with asthma

Legend:
- ACSL3 5'CGI Methylation Status
- Methylation
- Unmethylated
- All

ACSL3 5'CGI

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>PAH</th>
<th>All</th>
<th>Odds Ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>12 A (N=30)</td>
<td>12 A (N=26)</td>
<td>12 A (N=56)</td>
</tr>
<tr>
<td>Ethnicity (% AA vs Dominican)</td>
<td>15 (50%)</td>
<td>15 (58%)</td>
<td>30 (54%)</td>
</tr>
<tr>
<td>Gender (% Males vs Females)</td>
<td>12 (40%)</td>
<td>12 (40%)</td>
<td>24 (43%)</td>
</tr>
<tr>
<td>ACSL3 5'CGI (% Methylated vs Unmethylated)</td>
<td>7 (23%)</td>
<td>21 (81%)</td>
<td>28 (50%)</td>
</tr>
</tbody>
</table>

PAH cutpoint value 2.41ng/ml obtained from an ROC curve
p<0.001

Hollingsworth et al. JCI, 2008

Saline         62.5 ug/ml
A. fumigatus    125 ug/ml
A. fumigatus    1250 ug/ml

Gestational exposure to A. fumigatus [early (left) and late (right)] regulated IgE levels in grandoffspring

Human studies

Odds ratio 3.9 95% CI=[1.1, 14.3] of asthma given ACSL3 methylated

P<0.001

Perera, Miller et al. PLoSONE 2009

Perera, Miller et al. PLoSONE 2009
Prenatal tobacco smoke exposure not associated with strong clustering of 1,031 Illumina CpG loci

DNA extracted from buccal cells of 58 children participating in the Children's Health Study
No strong clustering of methylation patterns by prenatal smoke exposure

Prenatal tobacco smoke exposure associated with lower methylation for AluYb8 repetitive elements

<table>
<thead>
<tr>
<th>Prenatal tobacco smoke</th>
<th>AluYb8</th>
<th>LINE1</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>ref</td>
<td>ref</td>
</tr>
<tr>
<td>Yes</td>
<td>ref</td>
<td>ref</td>
</tr>
</tbody>
</table>

*Z-score transformed estimate is interpreted as a change in SD units.
All models were adjusted for gender, race, and Hispanic ethnicity

Prenatal tobacco smoke exposure associated with lower methylation in the GSTM1-null children but higher methylation in the GSTM1-present children

<table>
<thead>
<tr>
<th>GSTMs</th>
<th>Prenatal tobacco smoke</th>
<th>AluYb8</th>
<th>LINE1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present</td>
<td>No</td>
<td>ref</td>
<td>ref</td>
</tr>
<tr>
<td>Present</td>
<td>Yes</td>
<td>-0.31</td>
<td>0.44</td>
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<tr>
<td>Null</td>
<td>No</td>
<td>ref</td>
<td>ref</td>
</tr>
<tr>
<td>Null</td>
<td>Yes</td>
<td>-0.39</td>
<td>0.27</td>
</tr>
</tbody>
</table>

Z-score-transformed estimate is interpreted as a change in SD units

Prenatal PAH exposure associated with differences in IFNγ promoter methylation

Smoothed plots relating predicted values of percent methylation of regions 1 (left) and 2 (right) to PAH levels; n = 53 children

Maternal farm exposure modulated neonatal immune mechanisms through DNA methylation of Tregs

Back to imprinting: gene-environment interactions and complex diseases

- Usually others focused on linkage designs with pedigrees or case-parent designs with case-parent trios when parent-of-origin information is available in offspring
- Few data on how environmental exposures modify the effects of imprinted genes.
- Can apply environmental-imprinted gene interactions to cohort research
Imprinting tests using mother (no paternal)-offspring pairs

- Columbia Center for Children's Environmental Health birth cohort
- Tested interactions between imprinted genes and environmental exposures
- Assumed a bi-allelic marker with a high-risk allele (minor allele in cohort) and a low-risk allele
- Mathematically derived probability of father having certain allele
- Derived code to deal with mother-offspring pairs with ambiguous parent-of-offspring information, including omitting those pairs and many simulation studies
- Obtained better power than assuming Mendelian dominant model when the true underlying genetic model is imprinting.

Cord PAH-adduct levels interacted with CYP1A1-78, CYP1B1-05, CYP1B1-06 and IL-13-01

<table>
<thead>
<tr>
<th>Marker</th>
<th>Imprinting Tests</th>
<th>Test assuming Dominant Model</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Paternal Imprinting</td>
<td>Paternal Imprinting</td>
<td></td>
</tr>
<tr>
<td></td>
<td>mPair</td>
<td>pPair</td>
<td></td>
</tr>
<tr>
<td></td>
<td>mPair.sub</td>
<td>pPair.sub</td>
<td></td>
</tr>
<tr>
<td>CYP1A1 109</td>
<td>0.296</td>
<td>0.412</td>
<td>0.153</td>
</tr>
<tr>
<td>CYP1A1 14</td>
<td>0.002</td>
<td>0.035</td>
<td>0.006</td>
</tr>
<tr>
<td>CYP1A1 15</td>
<td>0.063</td>
<td>0.083</td>
<td>0.121</td>
</tr>
<tr>
<td>CYP1A1 78</td>
<td>0.578</td>
<td>0.228</td>
<td>0.111</td>
</tr>
<tr>
<td>CYP1B1 05</td>
<td>0.071</td>
<td>0.071</td>
<td>0.330</td>
</tr>
<tr>
<td>CYP1B1 06</td>
<td>0.132</td>
<td>0.091</td>
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<tr>
<td>IL13 01</td>
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<td>0.926</td>
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<tr>
<td>IL4R 06</td>
<td>0.812</td>
<td>0.625</td>
<td>0.890</td>
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Environmental Epigenetics and Allergic Disease

- Environmental exposures
- Allergens
- Antibiotics
- Air pollution
- Diet
- ETS
- Epigenetic modifications
- DNA methylation
- Covalent modification of cytosine in CpG’s
- Posttranslational modifications of histones
- Baby’s alleles
- Environmental Epigenetics and Allergic Disease

So what?

- Do we all potentially possess a diagnostic epigenome that will mark our risk for clinical allergic disease?
- Or, can we ‘fix’ our DNA before we develop our epigenome with targeted environmental interventions?