Epigenetics in asthma

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Asthma is a Heterogeneous Disease

- can be atopic or non-atopic, and demonstrates various subclinical phenotypes

- can provides challenges to treatment because phenotyping asthma is neither easy nor readily available.

Until better matching of phenotypes to driver pathways or molecules is achieved, phenotype specific treatment using expensive biological drugs will not be cost effective.
Asthma has been shown to have a heritable component in large twin studies and by using polygenic heritability estimates.

To date much of the research into the heritability of asthma has focused on the changes to the DNA sequence.

Despite the success of genome-wide association studies (GWASs) in identifying loci associated with asthma, a substantial proportion of the causality remains unexplained.
Asthma Epigenetics comes of Age

Research is becoming more focused on heritable characteristics that are not due to altered DNA, but termed epigenetic modifications, and the sum of these modifications termed the epigenome.

Recent advances in genomic technologies have placed us in a position to initiate large-scale epigenome-wide association studies (EWASs). Such studies present novel opportunities but also create new challenges that are not encountered in GWASs.

Epigenomics (2015) 7(6), 1017–1032
What is Epigenetics?

- The epigenome is a set of heritable modifications and tags that affect the genome without changing the intrinsic DNA sequence.

- These epigenetic processes include modifications to DNA-binding histones, applying methylation marks to cysteine in DNA and noncoding RNAs such as miRNA.
Effect of epigenetic marks (DNA methylation, histone modifications, and miRNAs) on gene expression

Yang IV et al., JACI 2012 review
A Role for Epigenetics in Asthma

- Asthma is heritable.
- Asthma is an immune-mediated disease characterized mainly by skewing toward a Th2 phenotype.
- Asthma is affected by in utero exposures.
- Asthma is influenced by the general environment.
A Role for Epigenetics in Asthma

- Asthma is heritable.
- Asthma is an immune-mediated disease characterized mainly by skewing toward a Th2 phenotype.
- Asthma is affected by in utero exposures.
- Asthma is influenced by the general environment.
Adaptive immune response in asthma
### Mechanism of epigenetic regulation

<table>
<thead>
<tr>
<th>Mechanism of epigenetic regulation</th>
<th>Relevance to asthma</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL4</td>
<td>Demethylation of an intronic sequence that binds GATA-3</td>
</tr>
<tr>
<td></td>
<td>Increase in H3-K9 acetylation and H3-K4 trimethylation</td>
</tr>
<tr>
<td></td>
<td>Extensive demethylation of the 59 flanking region of the IL4 promoter</td>
</tr>
<tr>
<td></td>
<td>Methylation of the 39 end of the IL4 locus</td>
</tr>
<tr>
<td>IFNG</td>
<td>Methylation of an activator protein 1–binding site in the proximal</td>
</tr>
<tr>
<td></td>
<td>promoter resulting in reduced CREB and ATF2/c-Jun binding to this</td>
</tr>
<tr>
<td>IL13, IL5</td>
<td>Increased histone acetylation</td>
</tr>
<tr>
<td>FOXP3+</td>
<td>Class II HDAC inhibitors</td>
</tr>
</tbody>
</table>

**Relevance to asthma**

- Increases IL-4 secretion in Th lymphocytes
- Increases lineage commitment of precursor Th cells to Th2 cells
- Sustains high levels of IL-4 secretion from Th2 cells
- Promotes differentiation of precursor Th cells into Th1 cells
- Associated with the loss of gene expression and the establishment of a Th2 polarization phenotype
- Increases TH2-associated cytokine expression
- Increases Foxp3+ expression and enhances the suppressive function of Foxp3+ Treg cells on Th2 response

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Shuk-Mei Ho, J Allergy Clin Immunol 2010;126:453-65
Are epigenetic alterations in circulating PBMCs associated with allergic asthma?  
Yang et al. JACI 2015

DNA methylation patterns and gene expression in 6-12 years old inner-city children with persistent atopic asthma versus healthy control subjects were compared by using DNA and RNA from PBMCs.

Eightyone differentially methylated regions were identified. Several immune genes were hypomethylated in asthma, including IL13, RUNX3, and TIGIT. Among asthmatic patients, 11 differentially methylated regions were associated with higher serum IgE concentrations, and 16 were associated with percent predicted FEV₁.
Methylation marks involved in T-cell maturation (RUNX3), Th2 immunity (IL4), and oxidative stress (catalase) were validated in an independent asthmatic cohort of children living in the inner city.

These results suggest that epigenetic changes might play a role in establishing the immune phenotype associated with asthma.

Methylation marks involved in T-cell maturation (RUNX3), Th2 immunity (IL4), and oxidative stress (catalase) were validated in an independent asthmatic cohort of children living in the inner city.
Methylomic markers of persistent childhood asthma: a longitudinal study of asthma-discordant monozygotic twins

This study first examined genome-wide patterns of DNA methylation in buccal cell samples collected from 37 MZ twin pairs discordant for asthma at age 10. Their analysis was next stratified by assessing DNA methylation differences in a sub-group of MZ twin pairs who remained persistently discordant for asthma 8 years later, at age 18.

HLX has been identified as an important regulator of Th1 differentiation and a suppressor of Th2 commitment.

A number of probes annotated to the HLX gene were significantly differentially methylated in persistent-asthma twins compared to their unaffected co-twin.
An Epigenome-Wide Association Study of total serum immunoglobulin E concentration
Liang et al. Nature 2015 520:670-4

This study surveyed associations between serum IgE concentrations and DNA methylation in 95 nuclear pedigrees from individuals in their twenties.

Replicated associations between IgE and low methylation were found at 36 loci.

<table>
<thead>
<tr>
<th>CLC, PRG2, PRG3</th>
<th>Eosinophil products</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPCAT2, TMEM86B</td>
<td>Phospholipid inflammatory mediators</td>
</tr>
<tr>
<td>GATA1, ZNF22, RB1,</td>
<td>Specific transcription factors</td>
</tr>
<tr>
<td>IL5RA, IL-4,</td>
<td>Cytokine signaling</td>
</tr>
<tr>
<td>L2HGDH, SLC23A33</td>
<td>Mitochondrial proteins.</td>
</tr>
<tr>
<td>SERPINC1, TFF1, SLC17A4, COL15A, CEL, SPINK4</td>
<td>others</td>
</tr>
</tbody>
</table>
An Epigenome-Wide Association Study of total serum immunoglobulin E concentration

Liang et al. Nature 2015 520:670-4

This EWAS study has discovered reproducible CpG island associations accounting for a variation in the total serum IgE.

The lowest levels of methylation in the subjects with asthma and high IgE was observed and that methylation in asthmatics with low IgE was intermediate to controls.
A Role for Epigenetics in Asthma

- Asthma is heritable.
- Asthma is an immune-mediated disease characterized mainly by skewing toward a Th2 phenotype.
- Asthma is affected by in utero exposures.
- Asthma is influenced by the general environment.
Environmental factors induce immune cell regulation of allergic airway responses
<table>
<thead>
<tr>
<th>Environmental factors</th>
<th>Epigenetic effects</th>
</tr>
</thead>
</table>
| Tobacco smoke                          | • Suppresses HDAC2 expression and overall HDAC activity in macrophages.  
• Induces MAOB promoter hypomethylation in PBMCs  
• Induces hypermethylation of the promoter of p16; CYP1A1, RASSF1A, and FHIT in lung cancer cells |
| Maternal tobacco smoke                 | • Induces global DNA hypomethylation (AluYb8 but not LINE1) and AXL and PTPRO promoter hypermethylation in children                                                                                             |
| Benzo(a)pyrene (BaP)                   | • Induces hypomethylation of a number of genomic repeats and sequence-specific hypomethylation and hypermethylation changes in breast cancer cells  
• Induces H3K9 acetylation at the genome level  
• Decreases global DNA methylation                                                          |
| Maternal polycyclic aromatic hydrocarbons (PAH) exposure | • Increased maternal exposure associated with increased hypermethylation of the ACSL3 promoter in umbilical cord blood DNA of offspring                                                                           |
| Oxidants                               | • Posttranslationally modifies the HDACs and creates HAT/HDAC stoichiometry imbalance                                                                                                                             |
| LPS                                    | • Might be an miRNA-146a target  
• Drives TLR signaling through Akt1-regulated expression of let-7e and miR-155                                                                                                                            |
Increased NF-kB activity and oxidative stress in cigarette smoke lead to the activation of NF-kB, which in turn reduces Glucocorticoid (GR) activity. Changes in HDAC and HAT activity in asthma and COPD are summarized in the table:

<table>
<thead>
<tr>
<th>Condition</th>
<th>HDAC activity</th>
<th>HDAC2</th>
<th>HAT activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asthma</td>
<td>↓</td>
<td>↓</td>
<td>↑</td>
</tr>
<tr>
<td>smoking</td>
<td>↓</td>
<td>↓</td>
<td>-</td>
</tr>
<tr>
<td>COPD</td>
<td>↓</td>
<td>↓</td>
<td>-</td>
</tr>
</tbody>
</table>

Adcock IM et al, Respir Review 2006;7:21
### Environmental factors known to lead to epigenetic changes that influence the asthma phenotype

<table>
<thead>
<tr>
<th>Environmental factors</th>
<th>Epigenetic effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inhaled DEPs</td>
<td>• Induce hypermethylation at specific CpGs of the IFNG promoter and hypomethylation at the IL4 promoter in splenic CD4+ cells</td>
</tr>
<tr>
<td>PM-10</td>
<td>• Increases HAT activity and acetylated histone 4; remolds the IL8 promoter; action mediated through the induction of oxidative stress</td>
</tr>
<tr>
<td>Exposure of elderly to ambient black carbon but not PM2.5 for 4 to 7 d</td>
<td>• Induces hypomethylation of LINE1</td>
</tr>
<tr>
<td>Maternal diet rich in methyl donors</td>
<td>• Favors lymphocyte maturation into a TH2 phenotype</td>
</tr>
<tr>
<td>Methyl donors and coenzymes</td>
<td>• Affects DNMT activities and prevents aberrant global hypomethylation of the genome</td>
</tr>
<tr>
<td>Maternal folic acid supplementation</td>
<td>• Increases the risk of wheeze and lower respiratory tract infections in progeny up to 18 mo of age</td>
</tr>
<tr>
<td>Dust mite antigens</td>
<td>• Induce expression of miRNA-126 and activates TLR4</td>
</tr>
</tbody>
</table>
Prenatal maternal diet affects asthma risk in offspring


**Methyl-rich diet:**
folate, vitamin B$_{12}$, choline, and methionine

Dietary factors can modify the heritable risk of allergic airway disease through epigenetic mechanisms during a vulnerable period of fetal development.
Are phthalate related asthma mediated through alterations in DNA methylation?

Wang IL. et al., Clinical Epigenetics (2015) 7:27

In Taiwan, manufacturers replaced expensive natural emulsifiers with di-ethyl-hexyl phthalate (DEHP) in numerous food and drinks for several decades. Epidemiological studies suggest an association between exposure to DEHP and increased prevalence of asthma.

Urine 5OH-MEHP levels were quantified as an indicator of DEHP exposure.

- Differential methylation in three genes (androgen receptor, TNFα and IL-4) was identified through screening.
- Functional validation revealed that TNFα methylation was inversely correlated with the levels of TNFα protein.
- A lower methylation at CpG island of TNFα was associated with children with asthma (OR = 2.15, 95% CI = 1.01 to 4.62).
We hypothesized that reduced secretion of IL-12 from dendritic cells contributes to the development of allergy.
Subjects and Methods

- **Allergy group**  n=16 : Adult volunteers who have **allergic symptoms** and are **positive** in specific radioallergo-sorbent test (RAST)

- **Control group**  n=10 : Adult volunteers who have **no allergic symptoms** and are **negative** in specific RAST

- Separation of CD14 positive cells using auto-MACS systems from PBMCs

- DNA methylation analysis: Bisulfite-Sequencing analysis

- IL12A gene expression analysis: Quatitative RT-PCR using mRNA from monocytes stimulated with INF-γ and LPS.
DNA methylation ratio in IL12A promoter region in allergy and control group

Length = 242bp
Number of CpG = 26
Number of nonCpG = 48

Methylation ratio %

168\textsuperscript{th} CpG site
188\textsuperscript{th} nonCpG Cytocine

Cytocine position
Methylation ratio at CpG168 between two groups

![Graph showing methylation ratio at CpG168 between controls and allergy patients. The graph indicates a significant difference with a P-value of 0.047.]

- Controls
- Allergy

$P = 0.047$
IL12A gene expression is higher in high IgE group

P=0.004

Hypomethylation of IL12A promoter region may be involved in developing allergic diseases. IL12A gene expression might be regulated by DNA methylation of the promoter region.
DNA methylation changes in 82 genes in naïve T helper cells in accordance with the pathophysiological state in minimal-change nephrotic syndrome (MCNS)

- Minimal change nephrotic syndrome has been reported to involve immunological disturbances.
- The peripheral blood was collected by patients with MCNS both in relapse and following remission phase and healthy controls (n = 5)
- We analyzed genome-wide DNA methylation using the microarray-based integrated analysis of methylation.

We identified 82 co-occurring genes in naïve helper T cells, showing changes in methylation according to disease activity.

Kobayashi Y, et al: submission
The level of DNA methylation was altered according to disease activity.
Two networks diagrams generated by ingenuity pathway analysis with the 82 genes

These suggest that the DNA methylation changes in naïve Th cells are not only concomitant with disease activity, but also functionally relevant.

The intensity of gene (node) color in the networks indicates the degree of hyper-methylation (red) or hypo-methylation (gray).
## Potential asthma therapies targeting epigenetics

Brook PO et al., Epigenomics (2015) 7(6)

<table>
<thead>
<tr>
<th>Epigenetic mark</th>
<th>target</th>
<th>Tool compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histone acetylation</td>
<td>Pan histone deacetylase inhibitors</td>
<td>Trichostatin A, Vorinostat</td>
</tr>
<tr>
<td>Histone methylation</td>
<td>SETD7 methyltransferase</td>
<td>PFI-2, UNC0642, GSK-J1 + GSK-J4</td>
</tr>
<tr>
<td>-H3K4</td>
<td>H3K9 methyltransferase</td>
<td></td>
</tr>
<tr>
<td>-H3K9</td>
<td>H3K27 demethylase</td>
<td></td>
</tr>
<tr>
<td>-H3K27</td>
<td>H3K27 methyltransferase</td>
<td></td>
</tr>
<tr>
<td>DNA methylation</td>
<td>DNMT complexes</td>
<td>DNMT inhibitors</td>
</tr>
<tr>
<td>miRNAs</td>
<td>miR-34 mimic</td>
<td>MRX34, Mir-antagamir</td>
</tr>
<tr>
<td></td>
<td>miR</td>
<td></td>
</tr>
</tbody>
</table>
Trichostatin A suppressed airway hyper-responsiveness, Th2 cytokine production and eosinophilia

Choi et al., Clin Exp Allergy 2005; 35:89–96
Trichostatin A suppressed airway inflammation in mouse asthma model.
DNA methylation of Th1/Th2 cytokine genes affects sensitization and progress of experimental asthma

Brand S et al., J Allergy Clin Immunol 2012;129:1602-10

OVA sensitization/challenge led to an increase in DNA methylation within the IFNG promoter of the CD4+ T-cell subset, which correlated with decreased IFN-γ cytokine expression. Treatment with a DNA methyltransferase inhibitor, 5Aza, suppressed eosinophil number in BALF and inflammation and mucus producing goblet cells in the airways.

This suggests that epigenetic regulation in CD4+T cells contributes to the development of experimental asthma and can be targeted pharmacologically.
Effect of chemically modified IL-13 short interfering RNA on development of an asthmatic phenotype in mice

OVA i.p.
Day 0 and 14

1% OVA neb
Day 28-30

Assay Day 32

60μg IL-13 siRNA
24hr and 3 hr prior to 1st challenge
3hr prior to 2nd and 3rd challenge

% change from baseline

Methacholine (mg/ml)

Control siRNA
IL-13 siRNA
naive

Control siRNA
IL-13 siRNA

Lively J Allergy Clin Immunol 2008;121:88-94
SUMMARY

- Asthma is a heritable heterogeneous disease of the airways.
- Epigenetics may account for the heritability and disease activities of immune diseases, such as asthma and nephrotic syndrome through histone modifications, DNA methylation or interfering RNA.
- Modifying acetylation and methylation of histones and DNA methylation can change expression of inflammatory genes.
- Further work is required before the drugs progress into human clinical trials.

Thank you for your attention!!