Environmental Exposures and Epigenetics

Ian Yang

Thoracic Physician
Department of Thoracic Medicine
The Prince Charles Hospital
Brisbane, Australia

Associate Professor
Head, Northside Clinical School
The University of Queensland

Disclosures

Project grants to my institutions:
- National Health and Medical Research Council (NHMRC)
- Australian Research Council (ARC) Discovery Grant
- Cancer Council Queensland
- Queensland Government Health and Medical Research
- The Prince Charles Hospital Foundation
- Asthma Foundation of Queensland/UQ School of Biomedical Sciences Honours Bursary
- Novartis – unrestricted grant to establish a severe asthma service at the hospital (to be paid to The Prince Charles Hospital Foundation)

Fellowships:
- NHMRC Career Development Fellowship

No consultancy fees or honoraria from pharmaceutical companies, or membership of advisory boards

Motivating patient example

Mrs A, 35 y.o.
Asthma since childhood
2 children with asthma
Maximal inhaler therapy

1. Is asthma in my genes? Or was it the environment?
2. Could any exposures during my pregnancy have caused asthma in my children?

Overview

DNA methylation is an epigenetic mechanism that can result in gene silencing

Environmental exposures alter DNA methylation
Examples of epigenetic-environmental interaction:
- Diet
- Allergens
- Cigarette smoke
- Air pollution
- Ageing

This interaction has implications for studies of asthma pathogenesis, and in the future, possibly for the clinic

Pulmonary Perspective

Environmental Epigenetics and Asthma
Current Concepts and Call for Studies
Rachel L. Miller1 and Shuk-mei Ho2

‘There is immense promise that the study of environmental epigenetics will help us understand a theoretically preventable environmental disease’

Miller and Ho. Am J Respir Crit Care Med 2008;177:567–573

Heritability, or responsive to the environment?
Potential susceptibility genes for allergic disease

Ozone challenges

Primary outcome: FEV pre FEV post

250 ppb, 3 hr (n=44)
200 ppb, 4 hr (n=4)
400 ppb, 2 hr (n=3)

Ozone

Filtered air

Screening

Tissue Response


Ozone challenges

200 ppb, 4 hr (n=4)
400 ppb, 2 hr (n=3)

15 min rest, 15 min exercise

Potential susceptibility genes for allergic disease

TNF -308 polymorphism is associated with ozone-induced lung function change

N=51 subjects having ozone exposures

Change in FEV1 (%)

-308 G>A

G/G

G/A or A/A

P=0.024


Gene silencing by DNA methylation

Stable gene silencing can occur through:

- Interference with transcription factor binding
- Recruitment of repressors that specifically bind sites containing methylated CpGs

Neonatal DNA methylation profile in human twins is specified by a complex interplay between intrauterine environmental and genetic factors, subject to tissue-specific influence


Methylation in neonatal twins

**Twin study**
- Cord blood mononuclear cells: 18 MZ, 9 DZ
- Human umbilical vascular endothelial cells: 14 MZ, 10 DZ
- Placenta: 8 MZ, 7 DZ
- Illumina Human Methylation27 BeadChip microarray

**Results**
- Overall, low discordance in methylation in MZ twins
- MZ twins more similar than DZ twins in methylation profile = genetic factors (but small effect)
- Some unrelated individuals had more similarity than some co-twins = non-shared environmental factors
- Tissue-specific variability


In utero supplementation with methyl donors enhances allergic airway disease in mice

- C57BL/6J mice – high methyl diet (HMD) vs low methyl diet (LMD) in utero supplementation with folic acid, vitamin B12, choline, l-methionine, zinc, betaine


Pet keeping and tobacco smoke exposure influence CD14 methylation in childhood

- 157 children – birth cohort, Oslo, Norway
- Age 2 yr and 10 yr; asthma (ever) in 35%
- CD14 methylation in blood DNA vs environmental exposures

- **CD14 methylation:**
  - 2 yr – not associated with exposures
  - 10 yr – some association with exposure to pets and tobacco smoke

- Δ methylation from 2 to 10 yr – smaller increases in children living in homes with pets greater effect of SNPs on sCD14 when low methylation?


Diet

In utero supplementation with methyl donors enhances allergic airway disease in mice

- C57BL/6J mice – high methyl diet (HMD) vs low methyl diet (LMD) in utero supplementation with folic acid, vitamin B12, choline, l-methionine, zinc, betaine


Allergens
DNA methylation levels within the CD14 promoter region are lower in placentas of mothers living on a farm

- Placentas from ALADDIN study
  Assessment of Lifestyle and Allergic Disease During Infancy - Sweden

**CD14 methylation and mRNA expression**

- Farm environment
- CD14 methylation (methylation-specific high resolution melt method) both related to CD14 mRNA expression on fetal side of placenta

**CD14 methylation and living on a farm**

- Lower CD14 methylation (TaqMan method) in placentas from mothers living on a farm (n=28) vs non-farm (n=30)

Epigenetic regulation of CD14 (lower methylation) in response to living on a farm could increase CD14 expression, and provide a protective effect

Slaats et al. Allergy 2012;67:895–903

---

**Cigarette smoke in vitro**

- Cigarette smoke condensate (CSC) exposure to immortalised human bronchial epithelial cells (HBECs)
- Experimental conditions:
  - No CSC in culture
  - No CSC in soft agar clones
  - CSC-derived soft agar clones ± CSC
  - CSC-exposed HBECs

**Results:**

- Short-term culture (5d): no effects
- 9 month culture: stable heritable changes in methylation:
  - 48 genes demethylated
  - 56 genes increased methylated
- Also histone modifications and ‘cancer-associated’ epigenomic modifications

Liu et al. Oncogene 2010;29:5

---

**In utero tobacco exposure epigenetically modifies placental CYPIA1 expression**

- Placentas from smoking and non-smoking mothers
- CYPIA1 expression and methylation
- CYP1A1 – metabolises xenobiotics to reactive intermediates

Hypomethylation:
- 46% in smokers, vs 56% in non-smokers (P=0.027)

Negative correlation between CYPIA1 methylation and mRNA expression

In utero tobacco smoke increases CYPIA1 methylation, and decreases CYPIA1 expression in placentas

Suter et al. Metab Clin Exp 2010;59:1481–90

---

**Cigarette smoke**

---

**Prenatal tobacco smoke exposure affects global and gene-specific DNA methylation**

- Children’s Health Study
- Buccal DNA
- Maternal smoking (49%), ETS (35%)

**Effects of in utero tobacco smoke exposure may occur via DNA methylation**

Breton et al. Am J Respir Crit Care Med 2009;180:462-7

---

**Perinatal nicotine exposure induces asthma in second generation offspring**

- Pregnant Sprague-Dawley rats (F0): nicotine, placebo or nicotine+rosiglitazone (PPARγ agonist) – pregnancy to D21 postnatal

**Nicotine:**

AFFECTED LUNG FUNCTION in F1 and F2 rats:
- airway resistance
- airway hyper-reactivity
- tracheal constriction (males)

**Altered global DNA methylation (and histone H3 acetylation) in F1 rats:**
- T in testes, l in ovaries
- & prevented by rosiglitazone
- no change in lungs

In utero-effects of nicotine can be transmitted to subsequent generations, potentially through epigenetic mechanisms, and can be blocked

Rehan et al. BMC Medicine 2012;10:129
Air pollution

Particulate matter, DNA methylation in nitric oxide synthase, and childhood respiratory disease

Breton et al. Environ Health Perspect 2012;120:1320–1326

• Children’s Health Study – 940 children: Buccal DNA, FeNO, PM$_{2.5}$

PM$_{2.5}$ levels$^1$ and NOS haplotypes$^2$ were associated with NOS methylation (which was associated with wheeze and medication use)


Multiple exposures

Combined inhaled diesel exhaust particles and allergen exposure alter methylation of T helper genes and IgE production

Combined inhaled diesel exhaust particles and allergen exposure alter methylation of T helper genes and IgE production

Inhaled diesel particles + A. fumigatus increased IFN-γ and decreased IL-4 methylation, associated with increased IgE production in mice


Secondhand smoke and ambient air pollution – TCGp methylation and expression of IFN-γ in T effector cells and Foxp3 in T regulatory cells in children

- Children: Fresno (n=62, high air pollution), Stanford (n=40, low air pollution)
- Secondhand smoke exposure (SHS); subgroup verified by urinary cotinine

Children: Fresno (n=62, high air pollution), Stanford (n=40, low air pollution)

Kohli et al. Clinical Epigenetics 2012;4:17

Effect of age on methylation

Distinct DNA methyloes of newborns and centenarians

- Newborn male (‘NB’) - Umbilical cord blood CD4+ T cells
- 103 y.o. Caucasian male (‘Y103’) - Peripheral blood CD4+ T cells
- Whole genome bisulphite sequencing of DNA (13x coverage)

Heyn et al. PNAS 2012;109:10522-27

Methylation at extremes of age

- Hypomethylation with increasing age

Heyn et al. PNAS 2012;109:10522-27
Hypothesis and aims

**Hypothesis:** The human lung exhibits a tissue-specific, age-dependent DNA methylation profile

**Main aim:** To identify an age-related methylation signature in the human lung, using:
- global methylation profiling
- validating high priority candidate genes, using an independent method, EpiTYPER, in:
  - the training set (technical replication), and
  - an independent test set of patients (biological validation)

Methylation microarray profiling in the training set

- 83 lung tissue samples
- Illumina Infinium Methylation27 V1.0
- age analyses: median (below or above), quartiles, tertiles, polarised quartiles and tertiles, continuous age
- prioritisation by Venn approach
- removed genes: FDR>10%, \( P > 0.01 \), not expressed in lung
- ranked on FDR, \( P \)-value, \( \Delta \beta \), correlation coefficient

Top 14 CpG sites (10 genes) selected for technical replication

Study participants

<table>
<thead>
<tr>
<th>Cohort</th>
<th>Training set (Technical replication)</th>
<th>Test set (Biological validation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>83</td>
<td>146</td>
</tr>
<tr>
<td>Sex</td>
<td>n (%): Male 47 (57%) Female 36 (43%)</td>
<td>Male 69 (80%) Female 57 (70%)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>mean ± SD</td>
<td>67 ± 9</td>
</tr>
<tr>
<td>Smoking history</td>
<td>pack years mean (range)</td>
<td>59 (0.25-243)</td>
</tr>
<tr>
<td>Never smokers</td>
<td>n (%): Male 30 (36%) Female 26 (32%)</td>
<td>Male 39 (49%) Female 31 (46%)</td>
</tr>
<tr>
<td>Lung function</td>
<td>FEV&lt;sub&gt;1&lt;/sub&gt; (litres) mean ± SD</td>
<td>2.24 ± 0.72</td>
</tr>
</tbody>
</table>

Methylation microarray profiling

- Bioinformatic analysis
- Candidate gene selection

'Gene A' CpG 20 - replication

Above and below the median age

<table>
<thead>
<tr>
<th>Microarray</th>
<th>EpiTYPER</th>
</tr>
</thead>
<tbody>
<tr>
<td>( P = 0.0000055 )</td>
<td>( P = 0.021 )</td>
</tr>
</tbody>
</table>

Significant hypermethylation with age
Biological validation in an independent cohort with matched lung and blood DNA

**Lung tissue DNA**

**Blood DNA**

11 EpiTYPER fragments (4 genes) in lung tissue, and 10 EpiTYPER fragments (2 genes) in blood DNA, significantly ($P<0.05$) differentially methylated between patients above and below the median age.

**Comments**

- This is one of the first systematic studies of an age-dependent DNA methylation profile in the human lung.
- A robust study design was used, involving microarray profiling, technical replication and biological validation.
- Several candidate genes may represent potential markers for lung ageing.
- A more detailed appreciation of how age shapes the lung methylome may help understand the pathogenesis of chronic lung diseases.

**Implications for the clinic and translational research**

**Models for future studies**

- **Epigenetic regulation**
  - **Karmaus et al.** J Clin Endocrinol Metab 2013;98:475-85
  - **Martino and Prescott. Chest 2011;139:640-7**

**Models for future studies**

- Martino and Prescott. Chest 2011;139:640-7
DNA methylation is responsive to the environment, heritable, and can result in gene silencing.

Examples of epigenetic-environmental interaction include:

- **Diet**
  - methyl diet - \( \uparrow \) Runx3
- **Allergens**
  - pets, farm living - \( \downarrow \) CD14
- **Cigarette smoke**
  - in utero - \( \uparrow \) AXL, PTPRO, CYP
- **Air pollution**
  - PM\(_{2.5}\) DEP+A.fumig - \( \uparrow \) NOS2A
  - AAP+SHS - \( \uparrow \) IFN-\( \gamma \), \( \downarrow \) IL-4

DNA methylation changes with age – requires further confirmation to understand pathogenesis of lung disease.

Future discovery studies in asthma should identify genome – epigenome – environment interaction.

Summary: Environmental epigenetics

**Environmental Exposures and Epigenetics**

Mrs A, 35 y.o.

- Asthma since childhood
- 2 children with asthma

1. **Is asthma in my genes? Or the environment?**
   - Probably both

2. **Could any exposures during my pregnancy have caused asthma in my children?**
   - Not yet proven, but some exposures could have epigenetic effects during pregnancy.

Acknowledgments

**Patients and staff of The Prince Charles Hospital**

UQ Thoracic Research Centre

- Prof Kwun Fong (Director)
- A/Prof Rayleen Bowman
- Postdoctoral fellows: Vardhana Ralhan, Felicia Goh
- Research assistant: Marie Martin, Joanne Robinson, Janet Shae, Kate Chee
- PhD student: Sancy Sivaramanu, Casey Gough, Kaveria String, Morgan Davidson, Jessica Yeo, Eran Qaved, Anneke Dent, Patrick Davisk
- Honours student: Emily Saway, Ellen Shear
- Research nurses: Linda Passmore, Lisa McCul, Luckie Fong, Susan Davidson, Joanne McNamara, Wendy Burnham
- Admin officer: Jessica Tointon, Vickie Parkins, Louise Leahey
- Student casual scientific: Brede Feni, Tasha Doolan, Leon de Coster
- Surgeon: Morgan Windsor, Ashwin Raboo, Kevin Maris
- Pathologists: Ethan Dwyer, Linda Clarke
- Respiratory Investigations Unit

**Funding support to the Centre**

- NHMRC project grants (asthma: 552046, lung cancer: 539695)
- NHMRC Career Development Fellowship
- NHMRC PhD Scholarships
- ARC Discovery Grant
- Cancer Council Queensland
- Cancer Australia
- Dual Diseases Board
- The Cancer Genome Atlas (NCEC)
- The Prince Charles Hospital Foundation
- Queensland Government’s Health and Medical Research
- Asthma Foundation of Queensland/US School of Biomedical Sciences Honours Bursary