Epigenetics as a mediator of environmental influences on disease risk

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- Interrogating epigenetic – outcome associations
- Putting the pathway together
Proposing a causal pathway

Environment → Epigenome → Transcriptome → Proteome → Metabolome → Phenotype

DNA → RNA → Protein → Metabolites → Whole body physiology
Exposure, outcome and mediation

Association between risk factor and epigenetic modification

Epigenetic processes as mediators of risk factor – disease association

Association between epigenetic modification and disease risk

Diet

Heart disease
Epigenetic patterns as environmental sensors

Evidence from *in vitro* or *in vivo* studies:
- Arsenic
- Nickel
- Cadmium
- Chromium
- Aluminium
- Mercury
- Lead
- Pesticides
- Air pollution
- Benzene
- Bisphenol A
- Dioxin
- RDX
- DES
- Chemicals in drinking water

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<th>Exposure</th>
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Epigenetic processes in disease pathogenesis

- Is epigenetic variation associated with disease?
- Cancer
- Imprinting disorders
- Few robust examples in common complex disease
- Generally, associations are just postulated without an evidence base
  – ‘missing heritability…. it must be epigenetic’
Epigenome-wide association studies (EWAS)

N = 180 cases vs 187 controls

N = 48 samples from discordant twin pairs

Maternal Genome-Wide DNA Methylation Patterns and Congenital Heart Defects

N = 15 discordant twin pairs

Identification of Type 1 Diabetes–Associated DNA Methylation Variable Positions That Precede Disease Diagnosis

N = 180 cases vs 187 controls

N = 48 samples from discordant twin pairs
Epigenetic mechanisms as a potential mediator of environmental influences on disease
Confounding and reverse causation

- Epigenetic Inheritance Systems
- Stochastic Events
- Germ-line Genetic Variation
- Environment
- Epigenome
- Intermediate Phenotypes / Biomarkers
- Disease

Relton & Davey Smith *Int J Epidemiol* 2012
Exposure, outcome and mediation

Smoking → [Genetic mechanism] → Heart disease
Epigenetic mediation of smoking and cardiovascular disease

- Identified through EWAS of smoking on DNA methylation
- Smoking associated with ↓ methylation of the coagulation factor II receptor-like 3 gene (F2RL3)
- ↑ expression of the protease-activated receptor-4 (PAR4)
- Induces platelet activation (aggregation)
- Plausible mechanism of smoking induced CVD

↑ smoking ↓ risk CVD

N=177 discovery
N=316 replication

Smoking, *F2RL3* methylation and prognosis in stable coronary heart disease

- **KAROLA study (n = 1206)**
  - Recruited following occurrence of acute cardiovascular event, DNA collected at baseline
  - 3, 4.5, 6 and 8 year follow up
  - 49 non-fatal myocardial infarctions; 41 non-fatal stroke; 64 cardiovascular deaths; 50 other deaths

**Cox’s regression model of *F2RL3* (CpG_4) methylation and prognosis in stable coronary heart disease**

<table>
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<th>Methylation level</th>
<th>Secondary CVD event</th>
<th>CVD mortality</th>
<th>Non-CVD mortality</th>
<th>All-cause mortality</th>
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<td>&gt;0.74 (Q4)</td>
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<td>≤0.74 (Q3)</td>
<td>0.92 (0.57-1.50)</td>
<td>1.06 (0.46-2.46)</td>
<td>1.55 (0.59-4.12)</td>
<td>1.26 (0.67-2.37)</td>
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<td>≤0.67 (Q2)</td>
<td>1.14 (0.71-1.84)</td>
<td>1.89 (0.85-4.16)</td>
<td>2.33 (0.88-6.19)</td>
<td><strong>2.07 (1.12-3.83)</strong></td>
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<td>≤0.54 (Q1)</td>
<td>1.40 (0.83-2.36)</td>
<td><strong>3.49 (1.51-8.04)</strong></td>
<td><strong>5.36 (1.93-14.8)</strong></td>
<td><strong>4.19 (2.20-8.00)</strong></td>
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</table>

Breitling LP et al. *Eur Heart J* Apr 2012
Differential methylation associated with smoking behaviour

- International Genetics COPD Network
  - Former and current smokers (n=1085)
  - Replication cohort of early onset COPD cases (n=369)

- Cross-sectional analysis considering time since quitting

- 15 CpG sites associated with current smoking
  - 9 CpG sites with lower methylation
  - 6 CpG sites with higher methylation

- 2 CpG sites associated with cumulative smoking
  - F2RL3 replicated as a smoking sensitive methylation locus
  - GPR15 identified as a novel smoking sensitive locus

Differential methylation associated with smoking behaviour

- How dynamic are methylation changes in response to smoking?

Gene functions of smoking-sensitive loci

**F2RL3** Platelet activation, cell signalling

**GPR15** Shares homology with angiotensin AT1 and AT2 receptors

**LRRN3** Neurodevelopment

**LIM2** Lens development and cataract formation

**MYLK** Endothelial and epithelial barrier function, asthma

**ADHFE1** Alcohol metabolism

**CNTNAP2** Neurodevelopment

**SLAMF1** Bi-directional T-cell to B-cell stimulation

**APBA2*** Neuronal adaptor protein that stabilises amyloid precursor protein

**CEBPE** Transcription factor

**TIPARP** Adaptive response to chemical exposure?

**TM4SF19** Function unknown; putative bipolar disorder susceptibility region

**ARHGAP25** GTPase activator, signal transduction

**FASLG** Cell signalling, apoptosis

* 2 CpG sites associated

Functional impact of methylation variable loci on gene expression using publicly available data

“To examine the possible impact of differential methylation at loci reported in this manuscript, we conducted exploratory analyses using publicly available data sets to assess for changes in gene expression by smoking status in blood, histologically normal lung tissue and bronchial epithelium.”

- Tissue specificity
- Power
- Increased methylation not always clearly associated with decreased expression – CpG context dependent

Extending the smoking-methylation story

↑ smoking
↓ methylation $F2RL3$
↑ expression PAR4
↑ platelet aggregation
↑ risk CVD

Limitations of existing work
• Retrospective study
• Reporting bias
• No biochemical data (cotinine) to validate smoking report
• No link to phenotype (platelet aggregation or heart disease)
• Illumina 27K array
A study of risk and recovery: Using epidemiology to understand the biology

- Validate exposure measurement
- Analyse intermediate phenotypes
  - British Women’s Heart and Health Study (n = 4,286)
  - Caerphilly Prospective Study (n = 2,959)
- Assess relationship between methylation and CVD risk before the coronary event has taken place
  - Copenhagen General Population Study, n = 77,000 including 1,804 with a CVD event
- Assess causality using a Mendelian randomization approach
- Recall studies to assess response to smoking and recovery
- Assess genome-wide effects of smoking using the Illumina 450K array
Epigenetic studies in ALSPAC

In progress

Alcohol in pregnancy and childhood outcomes – Luisa Zuccolo (University of Bristol)

Conduct problem trajectories – Ted Barker (Birkbeck College London)

Eating disorders – Karen Mitchell (Boston University)

Air pollution and lung function – Paolo Vineis and Paul Elliott (Imperial College London)

Childhood eczema – Lavinia Paternoster (University of Bristol)

Women’s health over the menopausal transition – Debbie Lawlor (University of Bristol)

Childhood body composition – Caroline Relton (Newcastle University)

Maternal weight gain in pregnancy – Eva Morales (CREAL, Barcelona / Newcastle University) & Abi Fraser (University of Bristol)

Planned

Rheumatoid arthritis – Jon Tobias (University of Bristol)

Lung function in children born preterm – John Henderson (University of Bristol)

Endocrine disruptors – Sue Ring & Jean Golding (University of Bristol)

Smoking in pregnancy and childhood outcomes – Jane Ng (Newcastle University)

Autism spectrum disorders – Beate St Pourcain, Jean Golding (University of Bristol) & Ezra Susser (Columbia University)

Pregnancy induced hypertension – Rebecca Richmond (University of Bristol)

Cardiovascular disease – Nic Timpson (University of Bristol)

Breast cancer – Mona Jeffereys (University of Bristol)
Exposure, outcome and mediation

Air pollution → Lung function
Air pollution and lung function

Children in top 5% of air pollution exposure are at increased risk of asthma or wheezing at age 7 (OR 1.44 [95% CI 1.03, 2.02] p=0.031)

Does air pollution exert its influence on lung function through altering the epigenome?

178 CpG sites associated with air pollution

158 CpG sites associated with lung function at age 7

325 CpG sites associated with lung function at age 15

DNA methylation
Exposure, outcome and mediation

In utero exposures

Adiposity
Cord blood methylation and childhood body size

**Low**
- BMI = 16
- FFM = 9.5 kg
- Weight = 35 kg

**High**
- BMI = 26
- FFM = 25 kg
- Weight = 60 kg

- 24 genes differentially methylated
- 24 genes differentially expressed

- PTBGS
  - Birth
  - ALSPAC
- 9/24 genes differentially methylated
- 11-13y
- Gene expression analysis
- Clinical assessment
- Early adolescence

DNA Methylation Patterns in Cord Blood DNA and Body Size in Childhood

Caroline L. Relton¹, Alexandra Groom¹, Beate St. Pourcain², Adrian E. Sayers³, Daniel C. Swan⁴, Nicholas D. Embleton⁵, Mark S. Pearce⁶, Susan M. Ring⁷, Kate Northstone⁵, Jon H. Tobias³, Joseph Trakalo⁸, Andy R. Ness⁹, Seif O. Shaheen¹⁰, George Davey Smith²

Maternal weight gain in pregnancy and childhood adiposity

• Weight gain in pregnancy is increasing
  – Percentage of women gaining >40lb during pregnancy in the US (CDC 2008)

• Greater weight gain in pregnancy is associated with
  – Complications of pregnancy
  – Adverse health outcomes in children
  – Greater adiposity in ALSPAC children at age 9 (Fraser et al. 2010)

• Does greater maternal weight gain mediate its effects through altering fetal DNA methylation patterns?

DNA methylation analysed in cord blood
1505 CpG sites in 807 genes

• Pre-pregnancy weight
  No association with infant methylation

• 0-18 weeks weight gain
  5 genes ↑ >5% methylation (p<0.01)

• 19-28 weeks weight gain
  1 gene ↑ >5% methylation (p<0.01)

• 29 weeks-term weight gain
  No association with infant methylation

MMP7, NFKB1, ABCC2, KCNK4, TRPM5

Vascular disease
PTB, Preeclampsia

Neurodevelopment
PTB

Insulin sensitivity
Epigenetics – One of many possible mechanisms

Obesity /overweight in pregnancy and childhood adiposity

MECHANISMS*

Overnutrition: dysglycaemia +/- aberrant lipid supply

Predictive adaptive phenotype response

Maternal

Epigenetic modification

↑ risk of still birth / miscarriage

↑ risk of congenital anomalies

↑ risk of LGA, greater fatness at birth, shoulder dystocia, difficult labour

↑ risk of later offspring overweight and obesity

Cycling of obesity

OR confounding by:
- Shared lifestyle
- Shared genes

A modifiable causal risk factor [E] for disease [Y] exerts its causal effect (at least in part) via the effect of E on X (the mediator) and through the causal effect of X on Y. U1 and U2 represent all confounders for the association of E with Y and X with Y, respectively. U1 and U2 can include different characteristics. In simple multivariable analyses to test this hypothesis it is tempting to adjust the association of E with Y for U1 and declare that this is the total causal effect of E on Y and then to adjust further for X; any resulting attenuation of the U1 adjusted association of E with Y following further adjustment for X is considered to represent the amount of the causal effect of E on Y that is mediated by X. However, by conditioning on X, a pathway between U2 and E is produced and hence this association (E with Y) is now confounded by U2. Furthermore, measurement error in X will bias the assessment of its mediation. Thus, both U1 and U2 require separate consideration and this can be achieved in the two step epigenetic Mendelian randomization framework.

Relton & Davey Smith *Int J Epidemiol* 2012
References


