The role of epigenetics in the developmental origins of human metabolic disease

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Genotype

Early life environment

Adult life style factors
Eg. Energy rich diet, physical inactivity

Phenotype

Chronic disease susceptibility
(CVD, obesity, type II diabetes, cancer)
The prenatal environment determines future disease risk

Infant mortality rates per 1000 live births during 1901-10

Standardised mortality ratios for coronary heart disease among men aged 65-74 (1968-78)

Barker DJP (1998) Mothers, babies and health in later life
Developmental origins of health and disease

- In 1986 David Barker showed that low birth weight is associated with an increased incidence of CVD and metabolic syndrome in later life.
• Subsequent epidemiological studies have confirmed a link between low birth weight and CVD

• and showed that smaller babies have an increased risk of

  Hypertension
  Raised serum cholesterol
  Type 2 diabetes
  Obesity
  Cancer
Animal Models

To study how the intrauterine environment may influence development and later risk of disease, a number of animal models have been established.

Eg Low protein diet
    global dietary restriction

- high blood pressure
- Dyslipideamia
- Impaired glucose tolerance
- Vascular dysfunction
Early life environment

Nutrition
Hormones
Oxygen

How does this work?
Predictive adaptive responses (PAR)

- During development organisms can respond to an environmental stimulus which allows the organism to shift its developmental path to produce a phenotype which confers a survival/reproductive advantage in postnatal life.

- Environmental Cue: e.g. Poor Nutrition
  - Developmental Plastic Stage: “prenatal environment”
    - Reduce energy demands
    - Increase capacity for fat storage
    - Less investment in muscle mass
  - Response: Alternative Developmental Path

- Future Actual Postnatal Environment: e.g. High Calorie Diet
  - Inc Disease Risk
    - Inappropriate PAR MATCH

- Future Actual Postnatal Environment: e.g. Poor Nutrition
  - Low Disease Risk
    - Appropriate PAR MATCH
A second developmental pathway to obesity?

Over-nutrition in early life can also increase obesity in later life.

- A J/U shaped relationship has been seen between BW and rates of obesity/diabetes.

In animal models:
- High fat feeding during pregnancy
- Over-feeding in early postnatal life

![Graph showing birthweight and type-2 diabetes prevalence.](attachment:image.png)

Type-2 diabetes
1179 Pima Indians aged 20-39 yrs

Obesity
Diabetes
CVD disturbances
Developmental plasticity

Genotype

Fixed – Mutations rate and influence populations over evolutionary time scales

Interaction with the (uterine) environment

Phenotype

Phenotype

Phenotype

Phenotype
Defining epigenetics as processes that induce heritable changes in gene expression potential without a change in gene sequence:

- DNA methylation
- Histone modification
- microRNAs
DNA Methylation

- Cytosine is methylated to 5-methyl cytosine
- 90% of methylated cytosine is found as a dinucleotide CpG
- CpGs are not randomly distributed throughout the genome but are clustered at the 5' ends of genes

Gene activity

Exon1 Exon 2
- 

Exon1 Exon 2
+

Exon1 Exon 2
++

Exon1 Exon 2
++++

= methylation
= no methylation
Methylation patterns are largely establishment during development and early life.

Once established, these methylation patterns are then stably maintained throughout the life of an organism.

1-carbon metabolism, which provides the methyl groups for virtually all methylation reactions, is highly dependent upon dietary methyl donors and cofactors.

(adapted from Santos & Dean)
Are epigenetic processes involved in the developmental origins of chronic diseases?

Protein-restricted rat model

50% reduction in maternal protein intake during pregnancy, normal diet during lactation

Glucocorticoid receptor (GR)
Peroxisome proliferator activated receptorα (PPARα)

Hypertension, dyslipidaemia, insulin resistance, altered kidney structure, increased cancer risk
The PR diet induces altered epigenetic regulation

**PPARα methylation**

DNA methylation compared to control (%)

- Control: 100%
- PR: 70%

**PPARα expression**

mRNA concentration compared to control (%)

- Control: 0%
- PR: 10%

**AOX expression**

mRNA concentration compared to control (%)

- Control: 0%
- PR: 10%

**B oxidation**

β-hydroxybutyrate concentration (μmol/l)

- Control: 0 μmol/l
- PR: 50 μmol/l

**GR methylation**

DNA methylation compared to control (%)

- Control: 100%
- PR: 70%

**GR expression**

mRNA concentration compared to control (%)

- Control: 0%
- PR: 10%

**PEPCK expression**

mRNA concentration compared to control (%)

- Control: 0%
- PR: 10%

**Gluconeogenesis**

Glucose concentration (mmol/l)

- Control: 0 mmol/l
- PR: 5 mmol/l

*Note: Data from Lillycrop et al. 2007*
Maternal diet alters methylation of specific CpGs in the PPARα promoter

Lillycrop et al. 2007
What about other nutritional challenges?

- Global undernutrition (UN) also induces GR hypomethylation
Over feeding in early life also induces altered DNA methylation

Litter size reduced to 3 pups ➔ Rapid fat accumulation ➔ Obesity
CVD disturbances

● Over-feeding leads to the hypermethylation of the POMC promoter

POMC acts to reduce food intake
Normally induced by leptin and insulin

Methylation blocks POMC activation
By hyperleptineamia and hyperinsulineamia

Plagemann A et al. 2009
Epigenetic mark as predictive biomarkers?

Epigenotype

- Protein restriction
  - Global restriction

- Over-nutrition

Altered DNA methylation

Detect changes in methylation

Pregnancy

Lactation

Weaning

Long term changes in gene expression

Altered metabolic capacity

Predict metabolic capacity & future disease risk

Altered susceptibility to obesity
Epigenetic marks as biomarkers?
In humans limited tissue availability?
Available tissues: Umbilical cord
Cord blood
Placenta
buccal cells
Blood

Rat Liver PPAR\(\alpha\)
DNA methylation compared to control (%)

<table>
<thead>
<tr>
<th>Maternal dietary group</th>
<th>Control</th>
<th>PR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>PR</td>
<td>*</td>
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Rat umbilical cord PPAR\(\alpha\)
Promoter methylation relative to Control diet (%)

<table>
<thead>
<tr>
<th>Maternal dietary group</th>
<th>Control</th>
<th>PR</th>
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</thead>
<tbody>
<tr>
<td>Control</td>
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<td></td>
</tr>
<tr>
<td>PR</td>
<td>90</td>
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</table>

P < 0.0001
Strategy for finding epigenetic biomarkers?
Are methylation marks at birth associated with later metabolic capacity?

Extracted DNA from umbilical cords from babies within the normal birth weight range

Genome wide screen of gene promoter methylation using a MeDIP-promoter array

Correlated methylation levels to phenotypic outcomes of the children age 9

Associations between methylation of CpGs and outcome confirmed by bisulfite sequencing (Sequenom)
Stability of methylation patterns?

Methylation in blood samples taken 11-20 years apart

Methylation in recent blood versus recent buccal samples

Heijmans et al., 2010
Can we intervene to prevent or reverse these methylation marks?
Folate supplementation of the protein restricted diet restores normal phenotype.

Control

Protein restricted

Protein restricted + folic acid

Hypertension
Dyslipidemia
Vascular dysfunction
**Increased maternal folic acid intake prevents induction of altered epigenetic regulation**

**PPARα**

- **DNA methylation** compared to control (%)
  - Control: Blue bar, PR: Red bar, PRF: Green bar
  - * indicates significant difference

- **mRNA concentration** compared to control (%)
  - Control: Blue bar, PR: Red bar, PRF: Green bar
  - * indicates significant difference

**β-oxidation**

- **β-hydroxybutyrate concentration** (μmol/l)
  - Control: Blue bar, PR: Red bar, PRF: Green bar

**Glucocorticoid Receptor**

- **DNA methylation** compared to control (%)
  - Control: Blue bar, PR: Red bar, PRF: Green bar
  - * indicates significant difference

- **mRNA concentration** compared to control (%)
  - Control: Blue bar, PR: Red bar, PRF: Green bar
  - * indicates significant difference

**Gluconeogenesis**

- **Glucose concentration** (mmol/l)
  - Control: Blue bar, PR: Red bar, PRF: Green bar

Lillycrop et al., 2005
Increasing maternal folic acid intake induces hypermethylation of specific CpG dinucleotides

Day 34

Methylation (% Relative to Control)

<table>
<thead>
<tr>
<th>CpG</th>
<th>2</th>
<th>3</th>
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<th>5</th>
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<tr>
<td></td>
<td>Sp1</td>
<td>AHR</td>
<td>CREB</td>
<td>SP1</td>
<td>HESF</td>
<td>NRF</td>
<td>PAX9</td>
<td>CREB</td>
<td>WHNF</td>
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Lillycrop et al. 2008
Gene expression changes in PR and PRF offspring

1.3% up-regulated
0.7% down regulated
Can supplementation with folic acid after weaning reverse the changes in phenotypes and in gene methylation induced by the PR diet?
Post weaning folic acid supplementation alters the phenotype and epigenotype

Burdge et al., 2009
**Conclusions**

- Increasing evidence that many chronic diseases originate at least in part in utero.
- Epigenetic mechanisms are likely to play a key role in the developmental origins of disease.

![Diagram showing Environmental Influences from conception to aging with stages of development and environmental factors such as In vitro culture, Maternal behaviour, Folic acid, Maternal nutrition.]  

- Initial studies suggest that these altered epigenetic marks may be used as predictive markers of future metabolic capacity and potential disease risk.
- Animal studies have also shown that it is possible to prevent/reverse these epigenetic marks, offering the opportunity for intervention.
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