Developmental Plasticity – Sensitive Periods and Risk of Obesity

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Genotype

Chronic disease susceptibility
(CVD, obesity, type II diabetes, cancer)

Environment

THE GLOBAL OBESITY PROBLEM

An obese adult is classified as having a Body Mass Index equal to or greater than 30. SOURCE: World Health Organization, 2006.
Early life environment can alter the epigenome

Gametogenesis → conception → birth → weaning → Growth → maturation → aging

Establishment of imprinting → Erasure of parental methylation → Cell lineage specific methylation

Epigenome

Prenatal → Neonatal → Pubertal

Nutrition/Body composition
Maternal stress/childhood adversity
Endocrine disruptors/pollution
What is the evidence that early life environment can induce epigenetic and phenotypic changes?
Maternal protein restriction induces the hypomethylation of the PPARα Promoter in the liver of the offspring

Maternal protein restriction → Hypertension  
Dyslipidemia  
Insulin resistance

Hepatic PPARα methylation  
PN34

PPARα expression  
PN34

B oxidation  
PN34

Lillycrop et al., 2005, 2007
Maternal protein restriction induces a change in PPAR α methylation in fetal liver

![Graph showing changes in PPAR α methylation](image)

- **Con**: Control
- **PR**: Protein Restriction

% Methylation (mean ±SD)

- E18
- PN34
- PN84

Significance:
- *: p < 0.05
- **: p < 0.01
Maternal high fat feeding alters the methylation of the FADS2 gene

Female liver 22: 6n-3

Female Liver 20:4n-6

Female Hepatic FADS2 expression

CpG -394

\[ r^2 = 0.56, \ P<0.0001 \]
Overfeeding in early postnatal life leads to obesity and the hypermethylation of the POMC promoter.

Rat pups raised in small litters (SL)

**POMC methylation in the hypothalamus (PN21)**

Plagemann et al., 2009
Nutrition in early life can induce long term changes in DNA methylation.
The Dutch Hunger Winter

A period of severe food shortage in the Netherlands in 1944.

Energy intakes dropped from 1800 to between 400 and 800 kcal per day (equivalent 100 - 200g pasta).

Individuals born to women exposed to famine during pregnancy have an increased risk of CVD, T2D and obesity in later life.

Alterations in DNA methylation has been shown in individuals from mothers who were exposed to famine during pregnancy compared to their non exposed siblings.

Tobi et al., 2009
Developmental plasticity – Same genome, different phenotype

**Developmental Plastic Stage “prenatal environment”**
- Reduce energy demands
- Increase capacity for fat storage
- Less investment in muscle mass

**Environmental Cue**
- e.g. Poor Nutrition

**Response**
- Alternative Developmental Path

**Future Actual Postnatal Environment**
- e.g. High Calorie Diet
- **High Disease Risk MISMATCH**

**Future Actual Postnatal Environment**
- e.g. Poor Nutrition
- **Low Disease Risk MATCH**

**Early embryo**
- Normal

**Adapted**
conception  birth  weaning  Growth  maturation  aging

Epigenome

GR, PPAR, PEPCK
P16, p21, HNF4, ATR1

GR, PPARα

FADS2
p16

POMC

FADS2

Protein restriction

Global restriction

High fat

Over feeding

Folic acid

Long term changes in gene expression & metabolism

Detect changes in methylation

Predict metabolic capacity & future disease risk

Altered disease risk
Epigenetic marks as biomarkers?

In humans limited tissue availability?

Available tissues:  Umbilical cord
   Cord blood
   Placenta
   buccal cells
   Blood
Methylation at the retinoid X receptor α (RXRA) promoter at birth vs child’s fat mass

PAH children age 9 yrs

\[ r = 0.32 \quad P = 0.009 \]
\[ n = 64 \]

SWS children age 6 yrs

\[ r = 0.20 \quad P = 0.002 \]
\[ n = 239 \]

Godfrey et al., 2011
Differentially methylated Regions (DMRs – 100 nt) identified (Fishers Exact test) associated with % fat mass age 6 yrs

Methyl DNA capture
Genomic DNA from SWS subjects sonicated and precipitated using His-tagged MBD2b to enrich for methylated sequences

Hybridised to Human Promoter Array
60 mer probes spaced across promoter regions of 17,000 best characterised transcripts covering -8kb to +2kb downstream of TSS

DATA analysis
Methylation levels of 100nt regions were estimated using the Bayesian algorithm BATMAN

Differentially methylated Regions (DMRs – 100 nt) identified (Fishers Exact test) associated with % fat mass age 6 yrs

93 DMRs identified, associated with changes in % fat mass age 6 years
Among the identified DMRs, the top pathway enriched was DNA replication & repair.

This pathway includes a DMR linked with cyclin-dependent kinase inhibitor 2A (CDKN2A).
- Encodes for 2 cell cycle inhibitors $\text{p14}^{\text{ARF}}$ and $\text{P16}^{\text{INK4a}}$, which play roles in cell proliferation, differentiation and senescence
Lower umbilical cord CDKN2A methylation is associated with higher child’s % fat mass (SWS children)

At 4 years

- **CpG1**
  - P=0.003
  - n=208

- **CpG3**
  - P=0.003
  - n=204

- **CpG4**
  - P=0.005
  - n=231

- **CpG6**
  - P=0.015
  - n=230

At 6 years

- **CpG1**
  - P=0.007
  - n=221

- **CpG3**
  - P=0.007
  - n=215

- **CpG4**
  - P=0.007
  - n=247

- **CpG6**
  - P=0.04
  - n=244
Lower CDKN2A methylation at birth associated with greater ponderal index at age 18 months in 161 GUSTO infants, reflecting higher adiposity

\[ P=0.015 \]

Perinatal CDKN2A methylation in an independent cohort from Singapore with adiposity data at age 18 months

Lillicrop & Godfrey et al, unpublished
Temporal stability of DNA methylation

Recent data suggests that DNA methylation can be dynamically regulated
Methylation stability over time

The Early Bird Study

Longitudinal non-intervention study of healthy children between ages 5 to 16.

Annual measurements include physical activity, body composition and insulin sensitivity and annual whole blood samples taken.

**Sirt1/PGC-1α (PPAR gamma co-activator (PGC))-1α**

- Sirt1 (energy sensor)
- HNF4α
- Energy homeostasis
- Glucose metabolism
- Muscle fiber-type switching
- Insulin secretion
- Mitochondrial biogenesis
- Adipogenesis
Methylation not affected by the proportion of neutrophils or lymphocytes (all $P > 0.1$; neutrophils $r = -0.002$ to 0.34, lymphocytes $r=0.001$ to 0.23).
Methylation of CpG loci in PGC-1α was associated with adiposity at 14 years

- Significant association between the methylation of 4 of the 7 CpG’s at 5-7 years and % body fat from 9-14 years (P<0.05).

- For each 10% difference in methylation at 5-7 years, % body fat differed by 6.3% to 12.5% (P<0.03) at age 14.
Does differential methylation of the CpG loci within PGC1a have functional significance?

Methylation increases binding of the pro-adipogenic HOXB9/PBX1 heterodimer.
1. Early life environment is an important determinant of later obesity risk.

2. Early life environment can alter the epigenome and these epigenetic changes induced in early life make a significant contribution to later phenotype and disease susceptibility.

3. Detection of such altered epigenetic marks in early life may allow the identification of individuals at increased risk of metabolic disease in later life.
Pathways to obesity

Undernourished
Dietary restriction
IUGR

Rapid catch up growth

Poor educational attainment
Poor diet
Take less exercise
Obese

Ill-prepared for pregnancy.
Minimal changes in diet and health behaviours

Over nutrition
Maternal high energy diet
Maternal obesity
GDM

Epigenetic signatures?
CDKN2A?

Growth

Receive low energy diet
Obese

Epigenetic signatures?
PGC1a/Sirt1

Maternal high energy diet

Greater fat mass, less lean mass and lower IQ at age 4

Are there modifiable epigenetic marks?

Maternal drivers?

Mis-match pathway?

Minimal changes in diet and health behaviours

Are there modifiable epigenetic marks?
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