Epigenetics and cardio-metabolic disease

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When exposed to a ‘westernised diet and lifestyle’ some cope better than others?

Not just mutations!!

Effect sizes of known T2D susceptibility loci

The rest must be ‘Environmental Factors’
Early life environment and future disease risk

Mortality from coronary heart disease before 65 years in 15,726 men and women in Hertfordshire

0 50 100
Men Women

Birth weight (lbs)

Environmental factors

Environment

Hypertension & endothelial dysfunction
Obesity
Dyslipidaemia
Increased thrombogenesis
Impaired glucose tolerance and type 2 diabetes
Cancer (breast, leukaemia, hepatocarcinoma)
Osteoporosis

Barker DJP (1998) Mothers, babies and health in later life

Standardised mortality ratio
Environment meets genome

Environment
Epigenetics
Genome
Phenotype
Disease risk

Life course
Epigenetics

Group of processes acting over different time scales that regulate transcription

DNA methylation
Histone modifications
Non-coding RNA

Gluckman et al. NEJM 2009
DNA methylation

DNA methyltransferase

Transcription active

Transcription suppressed
DNA Methylation patterns are induced during development

Cell type-specific genes – Differentially methylated

House-keeping genes (e.g. DNA repair genes) - Unmethylated

Parentally imprinted genes – Methylated according to parental origin

DNA methylation – Not just a switch

Transcription fully active

Transcription suppressed
Bee (*Apis mellifera*) phenotype and epigenotype is controlled by early life nutrition.

Genome

- Royal jelly
- + Royal jelly

Worker

Queen

Lava head dynactin p62 methylation

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<th></th>
<th>Worker</th>
<th>Queen</th>
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<td>(%)</td>
<td>60</td>
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*Kucharski Science 2008*
Analytical techniques
DNA methylation

Bisulphite sequencing

- CpG – specific
- Short sequences (< 500bp)
- Some degradation of DNA (small samples difficult)

Array

- Large coverage (but not methylome-wide)
- Cancer bias
- Semi-quantitative
- Challenging bioinformatics

Samples

Human

- Highly dependent on proxy tissues (blood, buccal cells)
- Leukocyte populations may vary due to infection
- DNA methylation patterns are cell type-specific
- State-of-the-art array technology

Mixed cell populations

- Is apparent variation in DNA methylation actually reflecting variation in the relative sizes of different cell populations?
1. Evidence that early life environment (nutrition) modifies risk of cardio-metabolic disease through altered epigenetic processes (DNA methylation)

2. The effect of a public health intervention on the epigenome

3. Epigenetic marks as biomarkers of disease risk
Early life nutrition and epigenetic outcomes
The Dutch Hunger winter

Autumn 1944 Nazi authorities imposed rationing on the Dutch population as punishment for the resistance movement.

Energy intakes dropped from 1800 to between 400 and 800 kcal per day.

This lasted for 7 months until Holland was liberated.

Despite the famine, recording of detail birth records was maintained.

Pictures: University of Leiden ‘Traces of Dutch 'Hunger Winter' in genetic material’
Compared to those born before, individuals in utero during the famine:

Cardiovascular disease
Impaired glucose tolerance and insulin secretion
Increased incidence of breast cancer
Increased response to psychological stress
Reduced carotid intima media thickness

Roseboom 2006
DNA methylation differences after exposure to prenatal famine are common and timing- and sex-specific

Methylation of peroxisomal proliferator-activated receptor gamma co-activator-1 (PGC-1α) is associated with type 2 diabetes mellitus

Human pancreatic islets from 48 non-diabetic and 12 type 2 diabetic multi-organ donors

The effect of public health policy on the epigenome
Epigenetics

Serine → Homocysteine → THF → CH₃ → Methionine

Source: Gluckman et al. NEJM 2008
Maternal methyl donor / folic acid intake can alter the phenotype of the offspring: The Agouti viable yellow mouse

Supplementation of maternal diet with dietary methyl donors (folic acid, B12, choline and betaine) shifted the coat colour of the offspring from yellow to brown

Maternal folic acid supplementation and health-related outcomes in offspring


Metabolic outcomes

Yajnik Diabetologica (2008) - FA supplementation from 18wks leading to high folate status & low B12 status increases insulin resistance in 6 yr olds

Lewis Br J Nutr (2009) – No association between maternal FA and child’s body composition

Stewart J Nutr (2012) - No association between maternal FA and child’s body composition in Nepal

Lillycrop J Nutr 2005 - Maternal FA supplementation in rats induced altered epigenetic regulation of the PPARa promoter and fatty acid beta-oxidation

Hoile Br J Nutr (2011) - Maternal FA supplementation in rats induced raised fasting blood glucose via altered epigenetic regulation of phosphoenolpyruvate carboxykinase promoter

Liu Mol Biol Repro (2011) - Maternal FA supplementation in rats induced altered epigenetic regulation of hepatic PPAR alpha + gamma, and glucocorticoid receptor in pigs
Allergic outcomes

Matsui & Matsui J Allergy (2009) - ~ 40% decrease in risk of raised IgE, atopy, wheezing (not asthma) in 2yr olds 5th vs 1st quintile maternal serum folate

Bekkers Eur Resp J (2012) – No association between maternal FA supplementation and respiratory outcomes at 8 yrs (transient 20% increase wheeze at 1 yr)

Martinussen AJOG (2012) No association between maternal FA supplementation and respiratory outcomes at 6 years

Whithrow Am J Epidemiol (2009) Increased risk of asthma at 3.5 yrs in children of women who took FA in late pregnancy

Dunstan Allergy (2012) Maternal FA supplementation increased risk of eczema at 1 yr by 85% where mothers took 500μg vs 200μg FA prenatally.

Hollingsworth JCI (2008) Maternal FA supplementation induced increased allergic sensitisation via an epigentic mechanism in mice.
Folic acid supplementation and the occurrence of congenital heart defects, orofacial clefts, multiple births, and miscarriage


Meta-analysis of the effect of multivitamin or folic acid on congenital heart defects

“Insufficient data on which to base firm conclusions”
Increased IGF2 associated with prenatal under nutrition and increased cardiovascular disease risk (Wang 2011)
Epigenetic imprinting at the IGF2/ H19 locus

Maternal

Non-coding RNA

Enricher

Imprinting control region

CTCF

H19

IGF2

Ins

Paternal

Non-coding RNA

CTCF

H19

IGF2

Ins

Enricher

Imprinting control region
Methylation variation at IGF2 / H19 DMR and maternal folic acid use before and during pregnancy

Hoyo et al. (Epigenetics 2011)

Grouped by folic acid intake:
- None (n= 326) vs 200μg (n=335)
  DNA isolated from cord blood leukocytes

2 – 4 % decrease in H19 methylation – altered regulation of IGF2 production at the higher intake compared to no folic acid use

Steegers-Theunissen et al. (Epigenetics 2009)

DNA isolated from blood from children aged 17 months.
  86 mothers took 400μg folic acid periconceptionally
  34 mother did not use folic acid.
  Measured methylation of IGF2 / H19 DMR.

Overall 2% increase in promoter methylation with methylation of specific CpG loci increased by up to 4%
Epigenetic marks as biomarkers of disease risk

(Using the epigenome for public health)
Epigenetic biomarkers:

- Stable over time
- Relevant to health outcome

Ideally:
- Regulate gene function
- Measurements in proxy tissue validated in target tissue

Predict risk

Alter CpG to change disease risk
Study design

Princess Anne Study (PAH)
- Women > 16 years
- 1st Pregnancy
- Children 9 years (n = 78)
- Dual energy x-ray absorptiometry
- Methylation specific chromatin precipitation methylation array (n=15)

Southampton women’s Survey (SWS)
- Women 20 - 34 years
- 1st Pregnancy
- Umbilical cord Stored -80°C
- Children 6 years (n = 239)
- Dual energy x-ray absorptiometry
- CpG methylation by Sequenom MassARRAY

Selection of promoter regions
Epigenetic “marks” in perinatal tissue predicts variance in fat mass in childhood

PAH study children aged 9 years (n=78)
Retinol X receptor methylation and fat mass
$r^2=0.44, P=0.001$

SWS children aged 6 years (n=239)
Retinol X receptor methylation and fat mass
$r^2=0.25, P=0.014$

Godfrey et al. Diabetes Published online 2011
Is risk of childhood obesity associated with variation in DNA methylation?

40 Subjects (20 boys, 20 girls) stratified according to insulin resistance at 14 years.

Isolated DNA from whole blood (5-7 yrs, 8,9 yrs, 10……14 yrs)

Methylation of individual CpGs by pyrosequencing.

Fat mass assessed by skin fold thickness
The relationship between methylation of CpGs in PGC-1α at 5-7 years and adiposity at 14 years in girls

- **CpG 4**: $r^2 = 0.30, P = 0.02$
  
  - Log Sum of skin folds
  - Methylation (%)
  - 60 - 62 63 - 64 65 - 68 69 - 69

- **CpG 5**: $r^2 = 0.41, P = 0.002$
  
  - Log Sum of skin folds
  - Methylation (%)
  - 46 - 53 53 - 57 58 - 63 64 - 70

- **CpG 6**: $r^2 = 0.64, P = 0.001$
  
  - Log Sum of skin folds
  - Methylation (%)
  - 40 - 43 44 - 48 49 - 53 54 - 57

- **CpG 7**: $r^2 = 0.41, P = 0.002$
  
  - Log Sum of skin folds
  - Methylation (%)
  - 25 - 30 31 - 38 39 - 46 47 - 58

Clarke-Harris - Unpublished
More challenges !!!!

What type of epigenetic mark?
- DNA methylation
- Histones
- MicroRNA

When and how to intervene?

Targeting interventions?
- Right mark, right tissue

Proxy tissues?

Epigenetic & / or genetic

Technology & Bioinformatics

Cost !!!!!

Sex differences
Summary

1. Variation in epigenetic may potentially account for a substantial proportion of the variation between individuals in risk of non-communicable diseases.

2. Identification of epigenetic biomarkers may have utility in predicting future disease risk and as therapeutic targets.

3. But there’s a long way to go!!!!

   There are considerable technical, biological and financial challenges to translating knowledge about epigenetics into public health applications.
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But if we can manipulate epigenetic variation ..........