Epigenetic studies of a newborn twins cohort - insights into early development"

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Summary

- Introduction: epigenetics, developmental origins, twins
- Results
  - Part 1. How can twins differ epigenetically at birth and how does this relate to environment?
  - Part 2. How different are twins throughout the rest of the genome?
  - Part 3. How DNA methylation changes between birth and 18 months: Epigenetic dynamics & drift

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Epigenetics and the symphony of life

Genes = instruments
Musicians = epigenetics

www.classicsonline.com

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From chromosomes to genes

1. DNA

2. DNA with histone proteins (yellow)

**3. Two metres of DNA per cell packaged with more proteins into chromosomes**
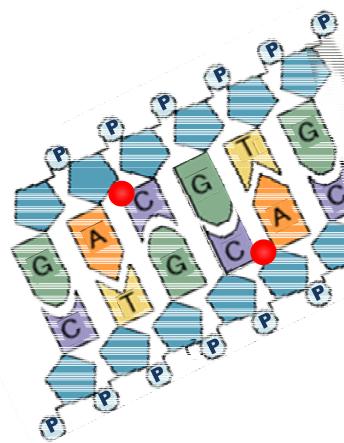
Key to epigenetic marks
- 🔴 Methylation of CpG in DNA
- 🔵 Active marks on histones
- 🟠 Inactive marks on histones

Open and active ⟷ **Closed and inactive**

Development

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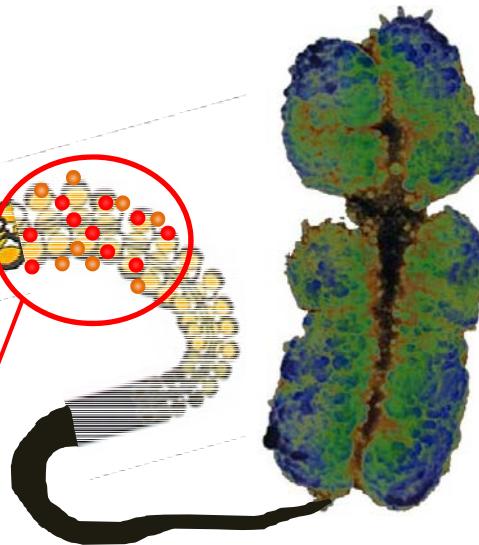
Epigenetic marks can:

- Control gene activity
- Direct development of an organism
- Be heritable though cell division
- Be a way for the environment to influence gene activity & disease
- "the interactions of genes with their environment, which bring the phenotype into being" – Conrad Waddington, 1942

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The role of epigenetics in the Developmental Origins of Health and Disease

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Epigenetics and the Developmental origins of Health and Disease
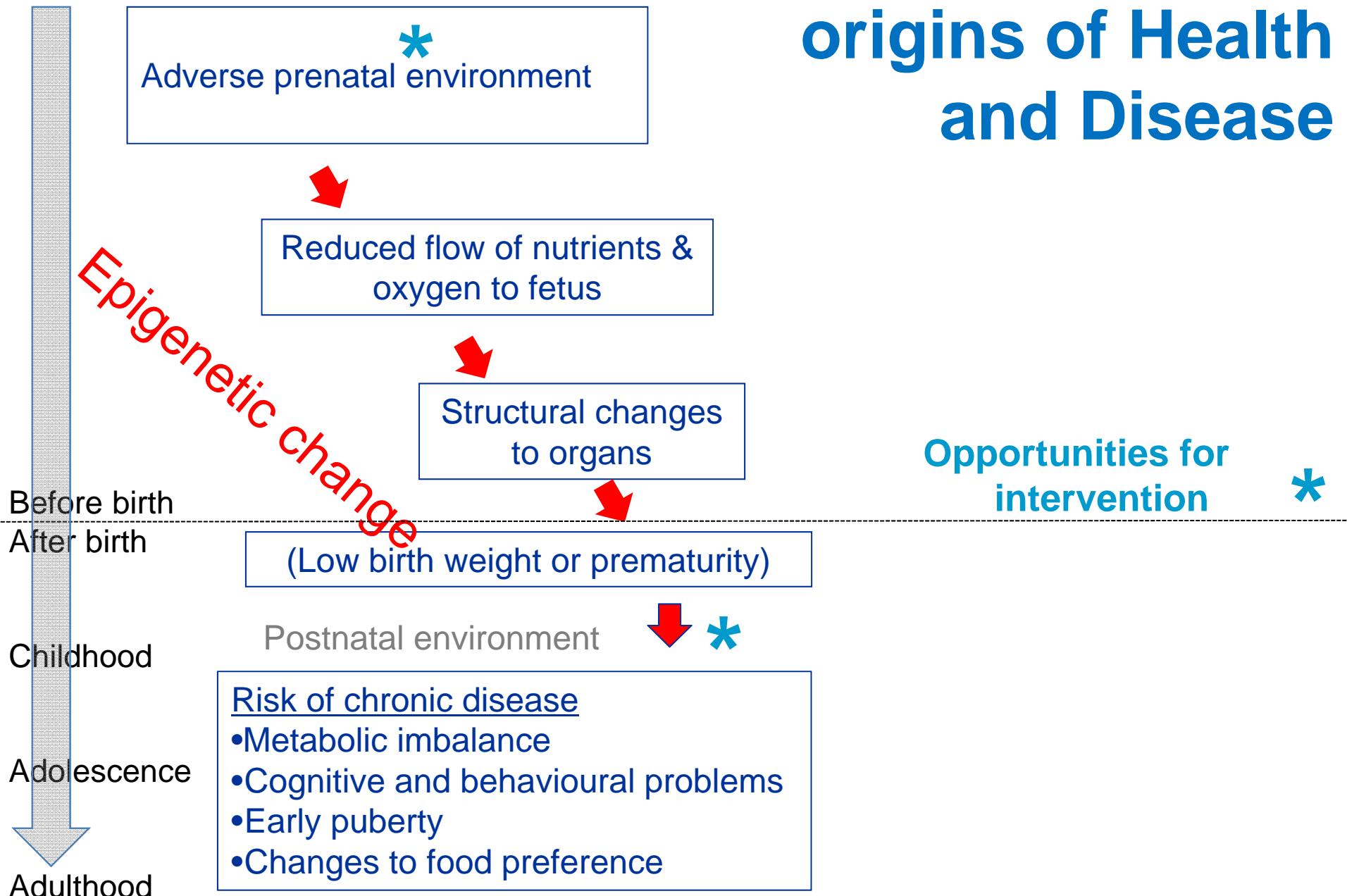
Con-ception

Before birth

After birth

Childhood

Adolescence

Adulthood

Epigenetic change

*Adverse prenatal environment

Reduced flow of nutrients & oxygen to fetus

Structural changes to organs

(Low birth weight or prematurity)

Postnatal environment *

Opportunities for intervention *

Risk of chronic disease
- Metabolic imbalance
- Cognitive and behavioural problems
- Early puberty
- Changes to food preference

Exposure-related epigenetic biomarkers

- Maternal carbohydrate intake (Godfrey et al, 2011)

- Childhood adversity (Kang et al 2013)

- Many more e.g. maternal smoking, alcohol, folate, gestational diabetes

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Outcome-related epigenetic biomarkers

- Cancer
  - a growing number of predictive biomarkers have been validated across many studies and are in clinical trials
- Metabolic and cardiovascular disease:
  - Obesity (Godfrey et al, 2011)
  - Type 1 diabetes (Rakyan et al 2011)
  - Type 2 diabetes (Toperoff et al 2012)
  - cardiovascular disease (Kim et al, 2010)
  - Response to weight loss programs (Cordero et al, 2011, Milagro et al 2011)

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Epigenetics and the symphony of life
Twins: can the same instruments be played differently?

The Peri/Postnatal Epigenetic Twins Study (PETS)

COHORT PROFILE

Cohort Profile: The Peri/post-natal Epigenetic Twins Study

Richard Saffery,[1,4] Ruth Morley,[2,4] John B Carlin,[3,4] Ji-Hoon Eric Joo,[2,4] Miina Ollikainen,[2,8] Boris Novakovic,[1,4] Roberta Andronikos,[2,4] Xin Li,[2] Yuk Jing Loke,[2] Nicole Carson,[2] Euan M Wallace,[5] Mark P Umstad,[6] Michael Permezel,[7] John C Galati[3,4] and Jeffrey M Craig[2,4]*

The Children's
Excellence in clinical care, research and education

The Royal Children's Hospital Melbourne

Murdoch Childrens Research Institute

THE UNIVERSITY OF MELBOURNE

Neonatal epigenetics: main study questions

1. What is the level of epigenetic variation during prenatal development?

2. To what extent is neonatal epigenotype influenced by:

   - DNA sequence?

   - Shared (maternal) factors?

   - Nonshared (supply line) factors?

3. Are different tissues affected the same way by the same environment?

PETS timeline: from Preconception to birth

Murdoch Childrens Research Institute
Healthier Kids. Healthier Future.

conception →

1st trim.

Recruitment & interview 1 (18-20wks)

2nd trim.

Interview 2 (24wks) →

Maternal blood for serum & plasma (28wks) →

3rd trim.

Interview 3 (36wks)

Birth (27-40 wks)
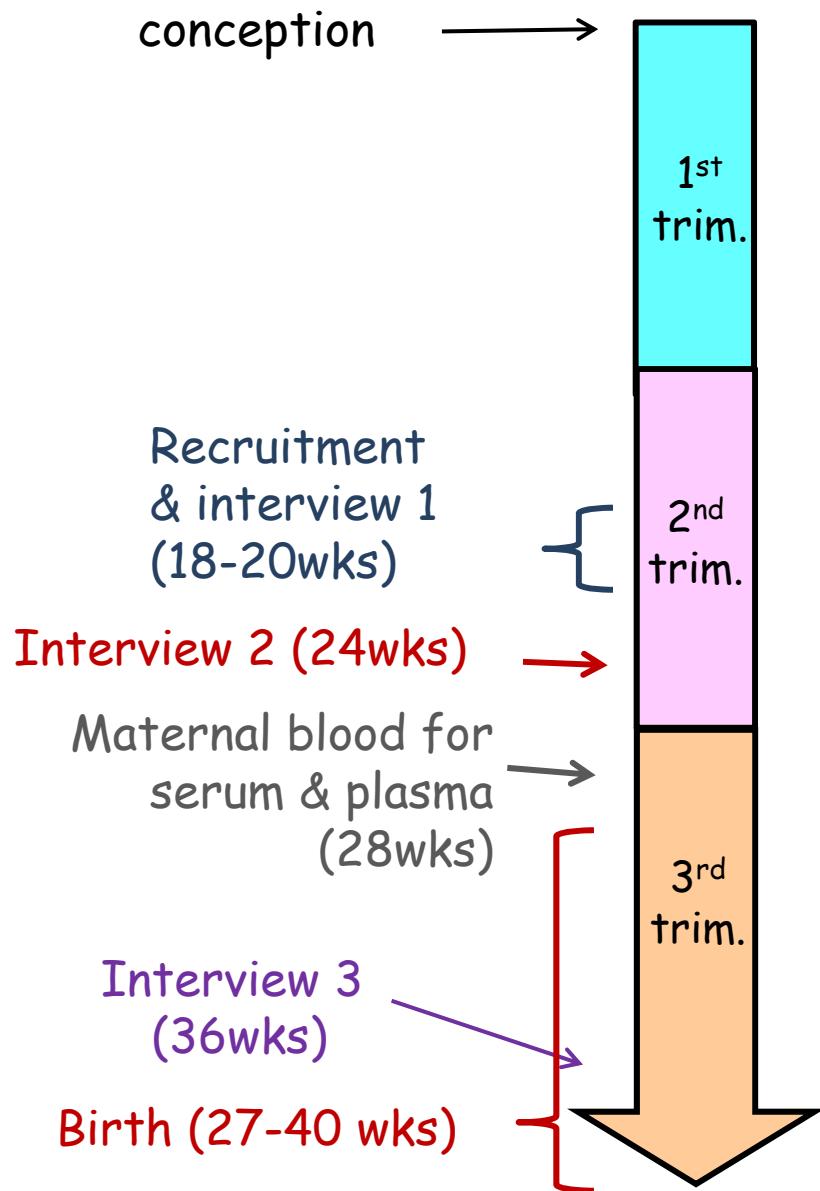
"Environmental" data collected
- Shared (maternal)
  - Diet & supplements
  - Stress
  - Smoking/alcohol
  - ART
  - Illnesses

- Non-shared (supply line)
  - Placental weight
  - Cord insertion

Dichorionic (MZ or DZ)   Monochorionic (MZ)

Separate   Fused
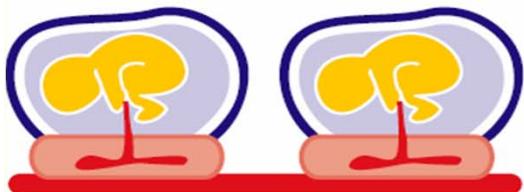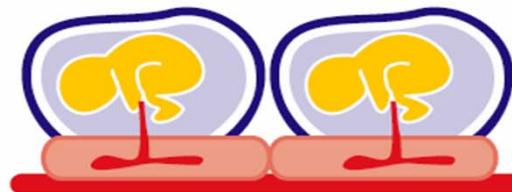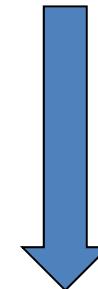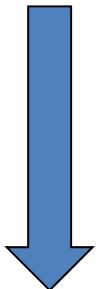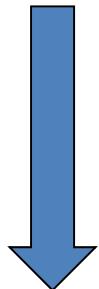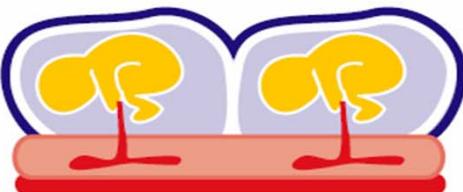
Placental weight

Position of cord insertion

"Good"

"Bad"

Central

Normal central disc insertion

Cord

Placenta

Weerakkody ©

Peripheral

Eccentric cord insertion

Cord

Placenta

Weerakkody ©
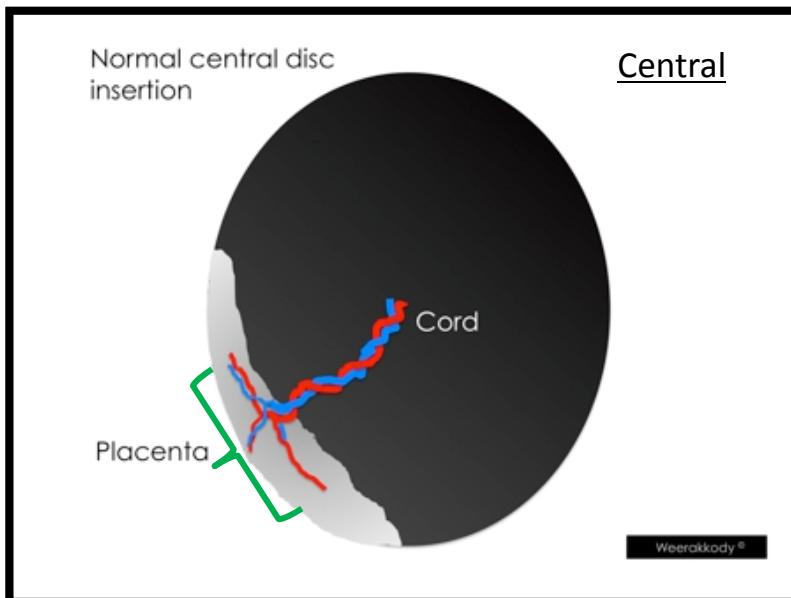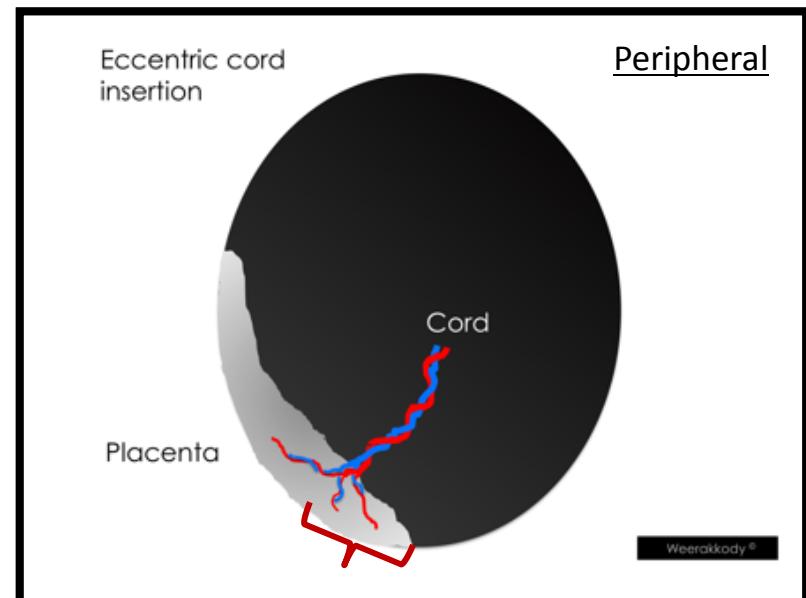
Velamentous

~ 9% in twins
~ 1% in singletons

Velamentous insertion of the cord

Cord

Placenta

Weerakkody ©

http://radiopaedia.org/articles/variation-in-cord-insertion

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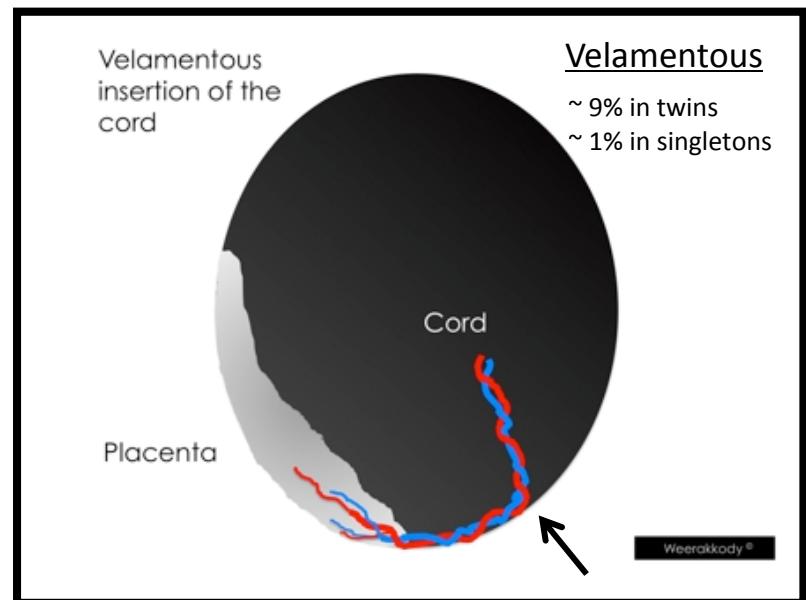
Multiple cell types collected from 251 deliveries

Mesoderm

Extra-embryonic
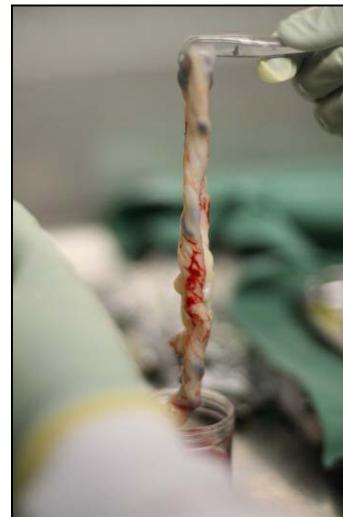
Ectoderm

Cord blood
↓
Mononuclear cells
(CBMCs)

Granulocytes:

Whole blood

Cords
↓
Human
Umbilical vein
Endothelial cells
(HUVECs)
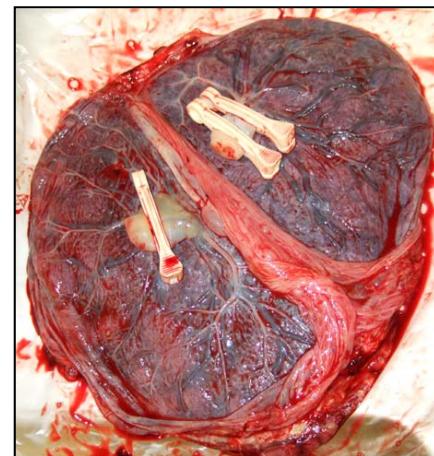
Placenta
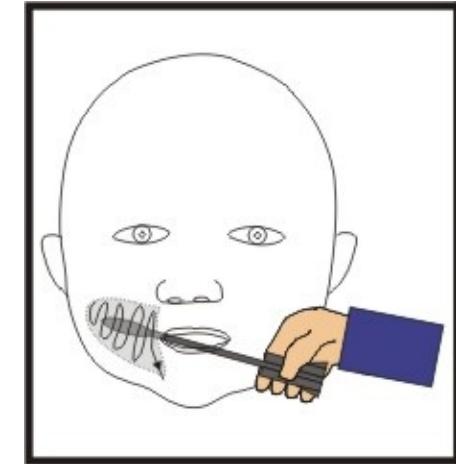
Cheek swabs
(buccal epithelial
cells)

Reading DNA methylation as a sequence

- Sodium bisulphite converts only unmethylated C to T

- Sequenom MassArray EpiTyper measures clusters of CpG units using mass spec.

CG   ^{me}**CG**

Bisulfite Conversion
& PCR

TG   **CG**

Base-change detection
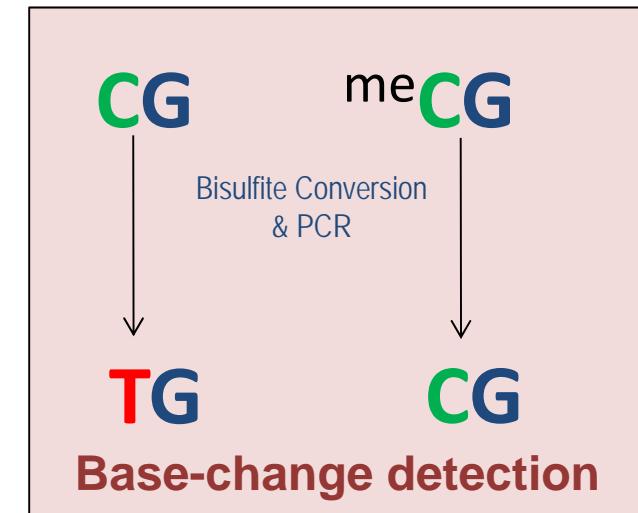
How can twins differ epigenetically at birth and how does this relate to environment?

>100 pairs
5 tissues
1 locus
4 sub-regions

DNA methylation analysis of multiple tissues from newborn twins reveals both genetic and intrauterine components to variation in the human neonatal epigenome

Miina Ollikainen[1,3], Katherine R Smith[2], Eric Ji-Hoon Joo[1,3], Hong Kiat Ng[1,3], Roberta Andronikos[1,3], Boris Novakovic[1,3], Nur Khairunnisa Abdul Aziz[1,3], John B Carlin[2,3], Ruth Morley[1,3], Richard Saffery[1,3,*,†] and Jeffrey M Craig[1,3,†]

Summary of PETS phenotypic data
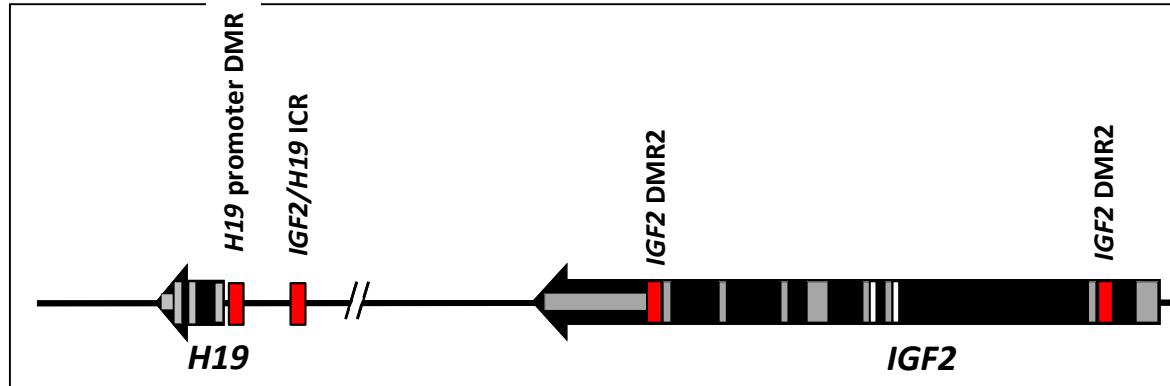
- *IGF2)/H19* locus crucial for prenatal growth but environmentally labile.

- Previous studies focused on 1-2 DMRs & 1 tissue

- Sequenom MassArray EpiTyper methylation analysis:
  - 67 MZ and 49 DZ twin pairs
  - 5 tissues
  - 4 DMRs

Range of Within-pair Methylation Differences at IGF2/H19
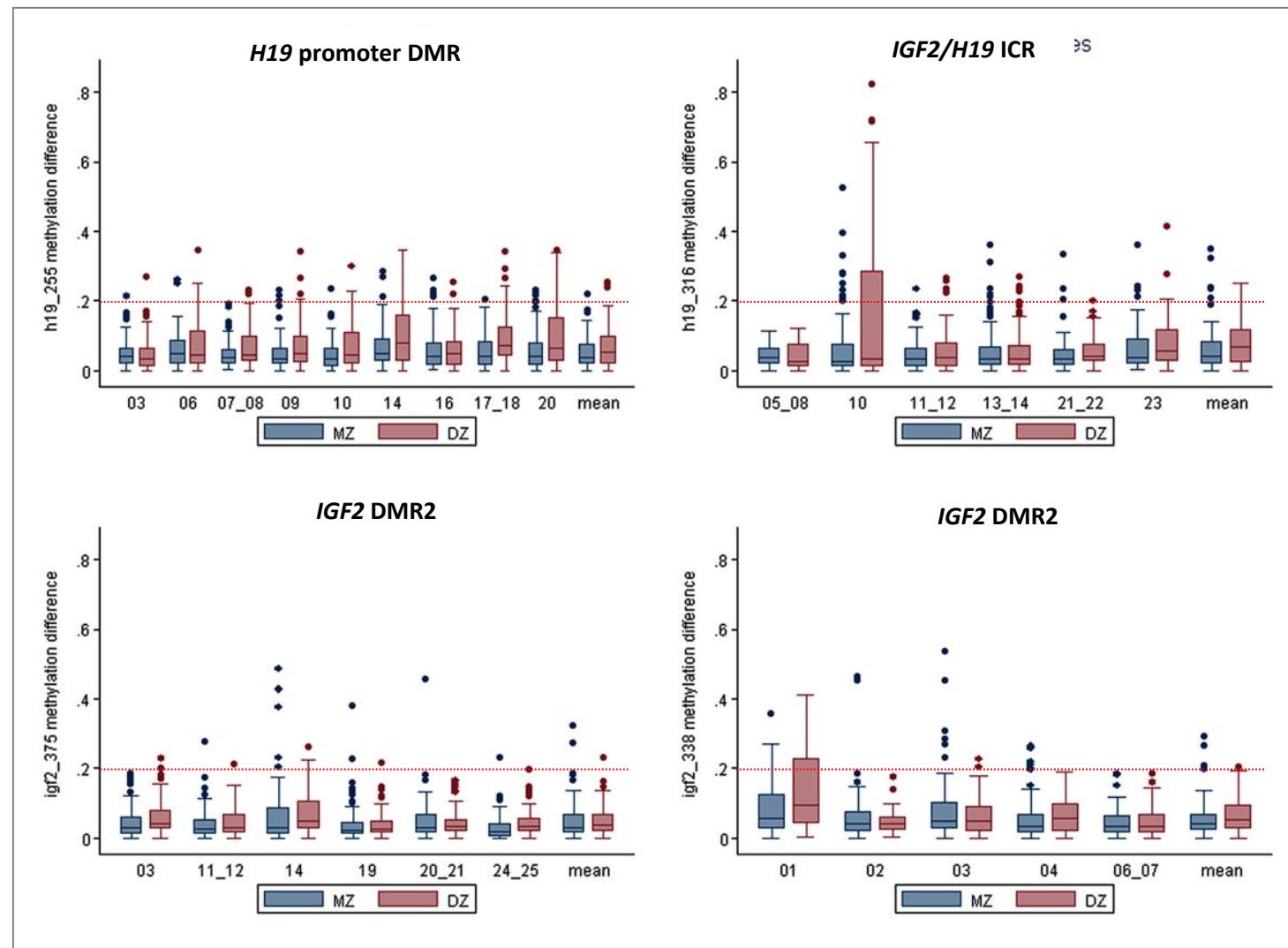
# Methylation similarity within-pair: effects of zygosity
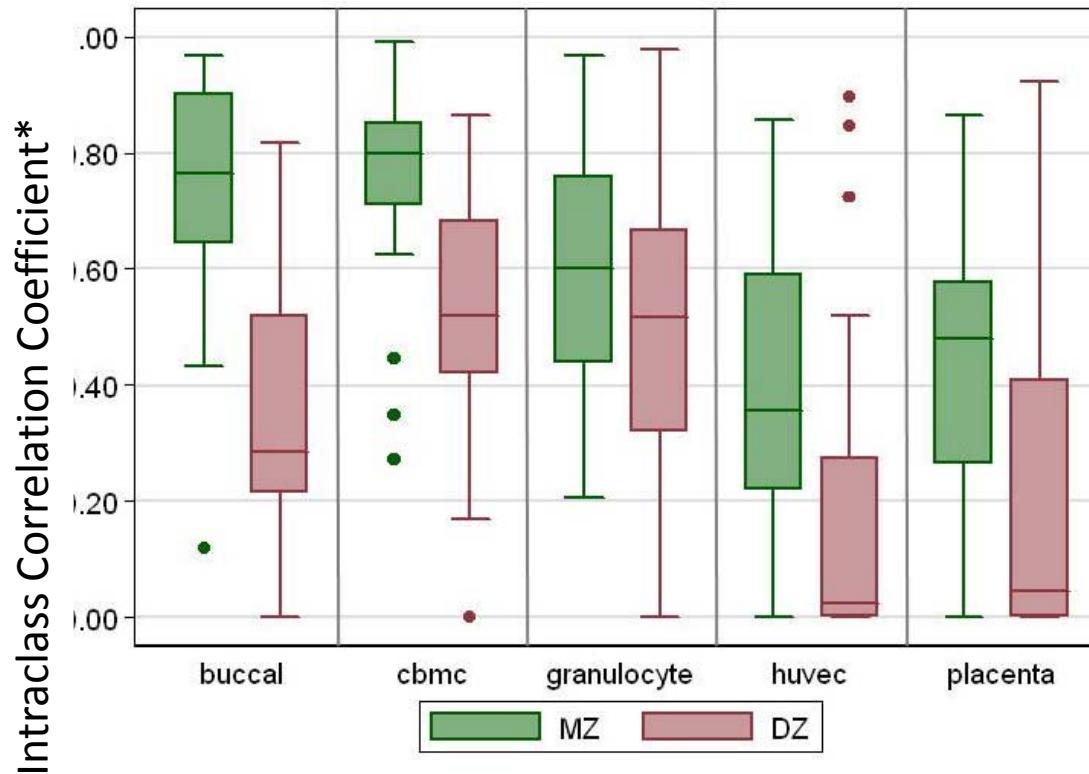
*Intraclass Correlation Coefficient (ICC):
Measures proportion of total variance attributable to within-pair variation within a subset of twins

Correlation with prenatal environment

- Multiple linear regression

- Normalisation to pool all data

- Regression coefficients converted to percentage change in mean methylation

IGF2/H19, 5 tissues, 4 DMRs, 67 MZ 49 DZ, 8 shared, 2 nonshared environments
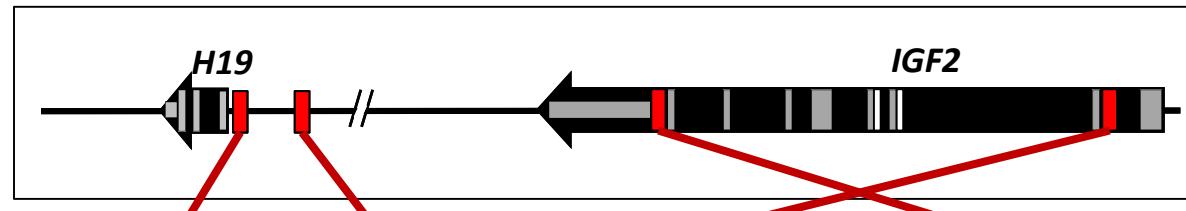
Factor
Had folate
Vitamin B12 (z-score)
Homocysteine (z-score)
Macronutrients (z-score)
Had alcohol
Smoked
Stress (z-score)
Gestational diabetes
Central cord insertion (MC)
Central cord insertion (DC)
Placenta Weight

Associations can be gene- and tissue-specific
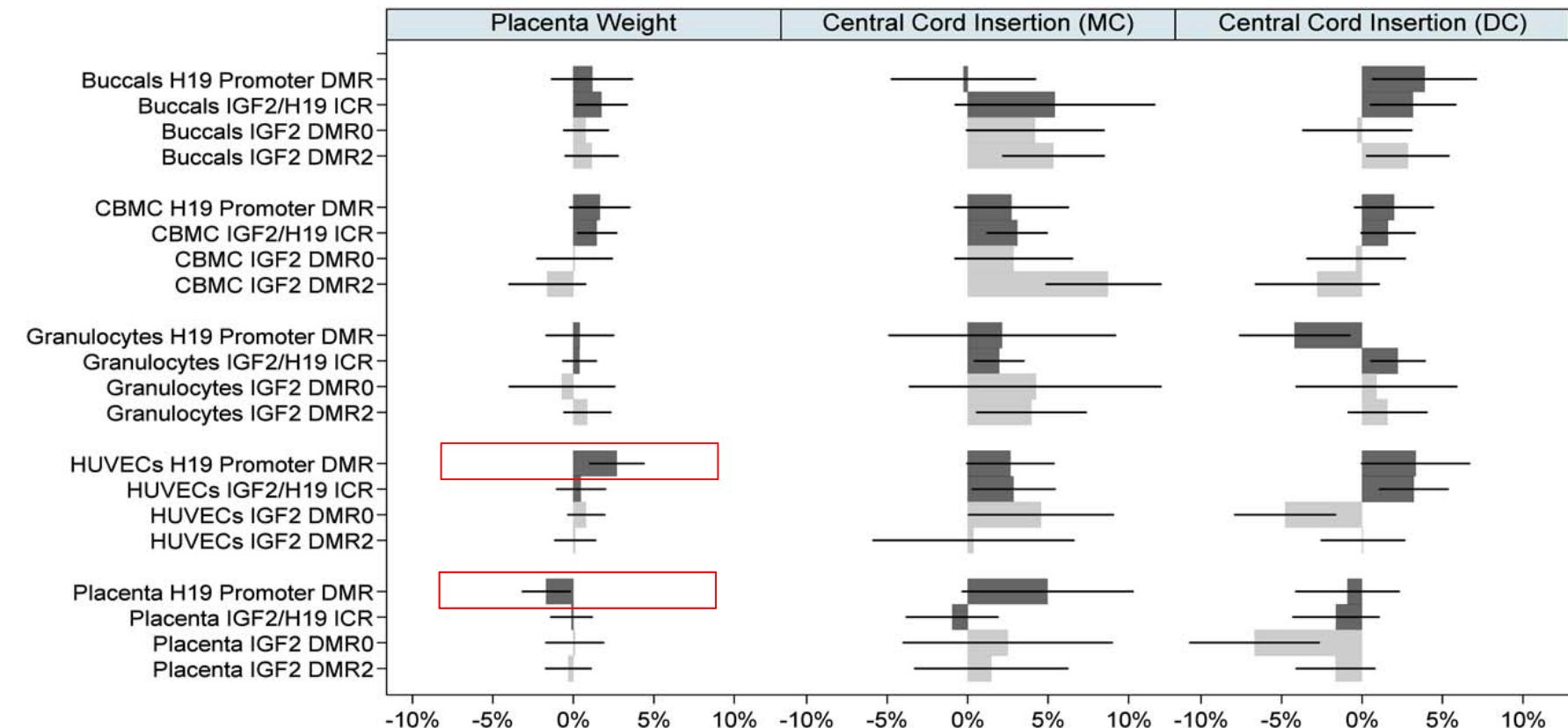
"Supply line factors" influencing nonshared environment

- Placenta (structure and function)

- Umbilical cord

"Supply line factors" influencing nonshared environment
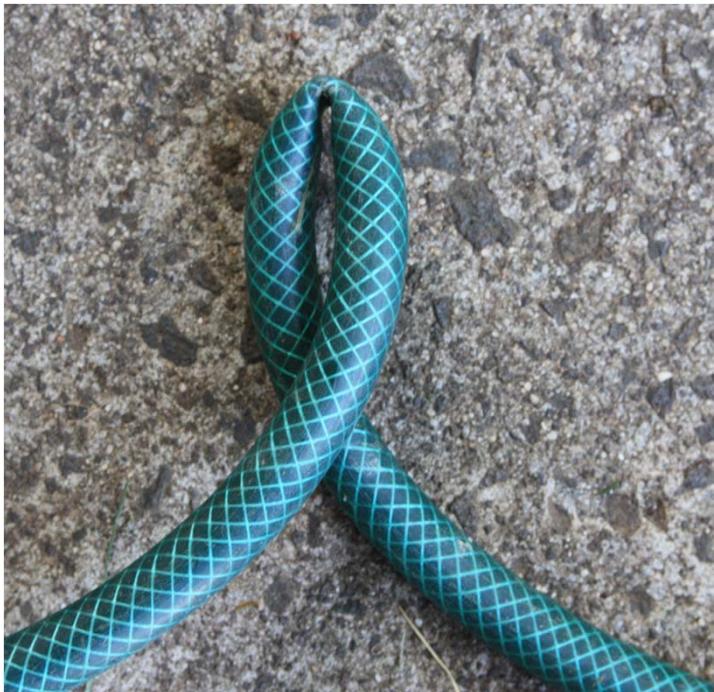
Thin cord

Fat cord

How different are twins throughout the rest of the genome?

Epigenetics 6:5, 1-14; May, 2011; © 2011 Landes Bioscience

Expression discordance of monozygotic twins at birth

Effect of intrauterine environment and a possible mechanism for fetal programming

Lavinia Gordon,[1] Ji-Hoon E. Joo,[2] Roberta Andronikos,[2] Miina Ollikainen,[2,3] Euan M. Wallace,[4] Mark P. Umstad,[5] Michael Permezel,[6] Alicia Oshlack,[7] Ruth Morley,[2] John B. Carlin,[8] Richard Saffery,[2] Gordon K. Smyth[7] and Jeffrey M. Craig[2,*]

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

Lavinia Gordon,[1] Jihoon E. Joo,[2,3] Joseph E. Powell,[4,5] Miina Ollikainen,[6] Boris Novakovic,[2,3] Xin Li,[7] Roberta Andronikos,[3,7] Mark N. Cruickshank,[7] Karen N. Conneely,[8] Alicia K. Smith,[9] Reid S. Alisch,[10] Ruth Morley,[7] Peter M. Visscher,[4,5,11] Jeffrey M. Craig,[3,7,12,13] and Richard Saffery[2,3,12]

CSH PRESS — GENOME RESEARCH

Samples & technology

- CBMCs, HUVECs & placenta from ~24 twin pairs

- Illumina expression arrays

- Illumina Infinium Beadchip arrays

  - Interrogate 27,578 CpG sites

  - Based on bisulphite conversion and genotyping.

 - Probes specific for methylated & unmethylated CpG
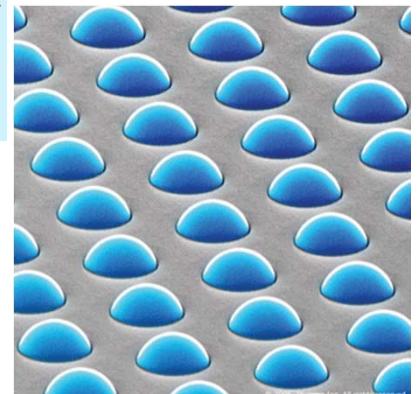
Relationship between within-pair methylation **discordance** and zygosity
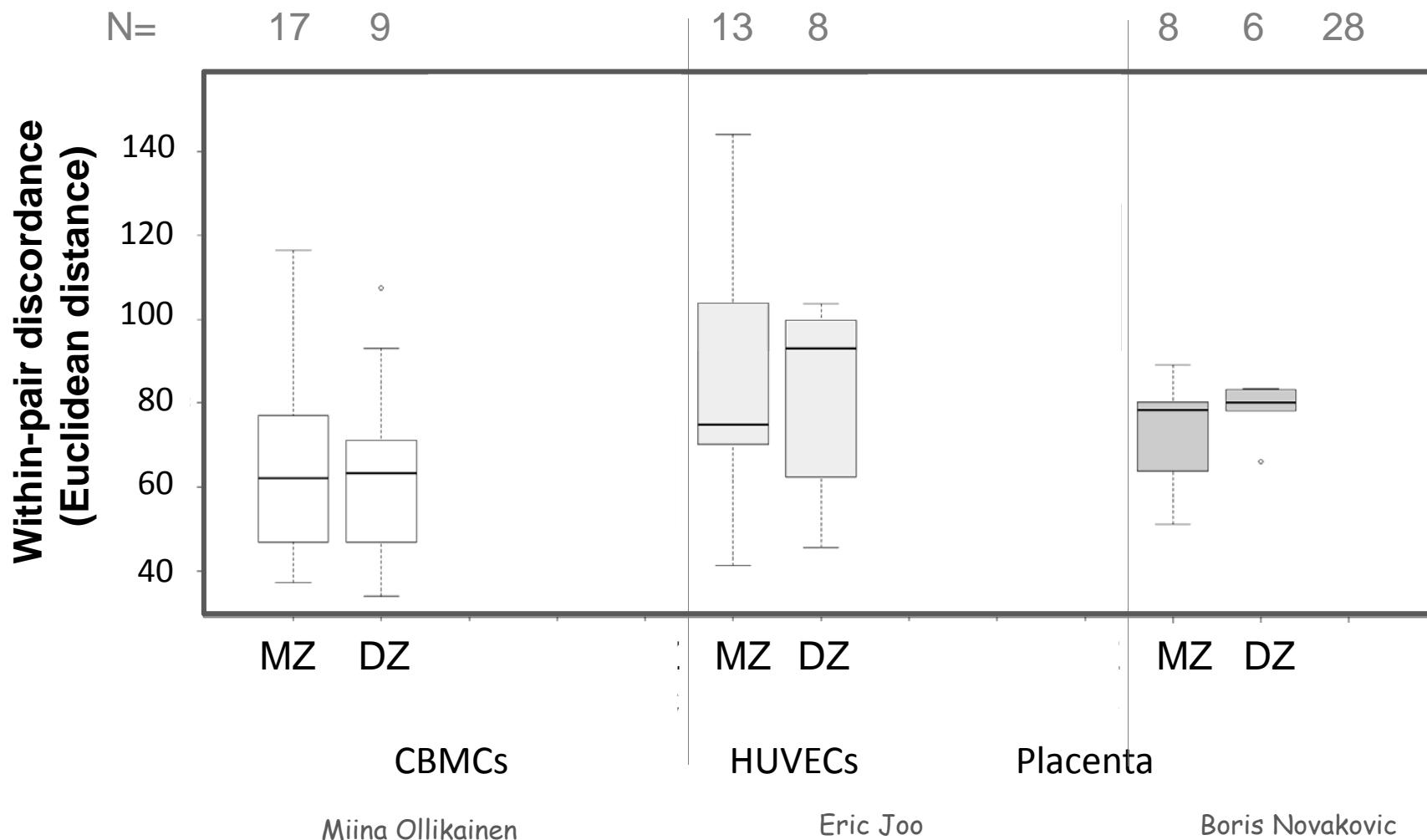
Relationship between within-pair methylation **discordance** and zygosity
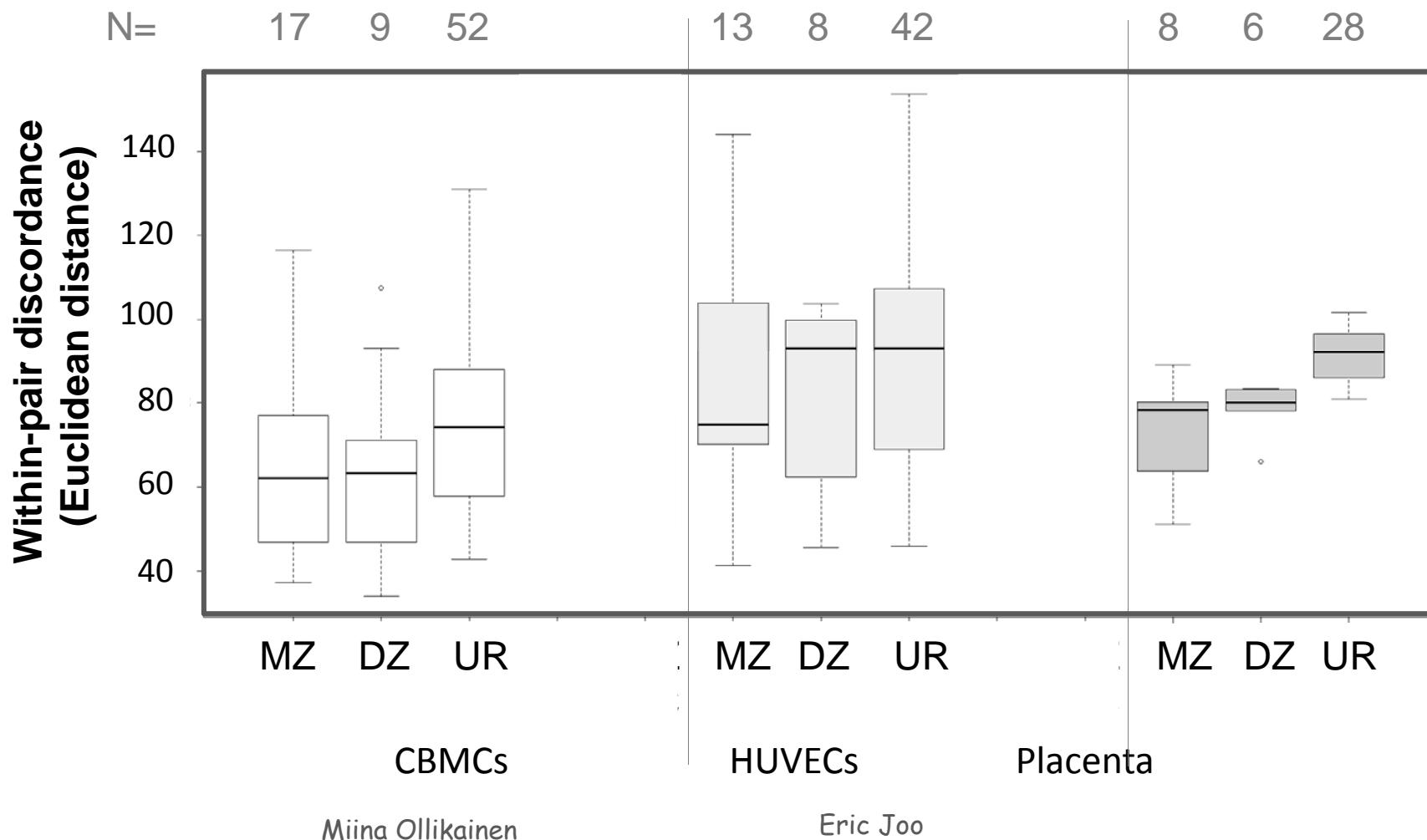
Summary of gene-specific data from expression & methylation arrays

	Expression	Methylation
Arrays	Illumina Expression BeadChip WG-6 CBMCs: 12 MZ pairs HUVECs: 10 MZ pairs	Illumina Infinium HM27 CBMCs: 18 MZ, 8 DZ pairs HUVECs: 12 MZ, 8 DZ pairs Placenta: 8 MZ, 8 DZ pairs
Most discordant within pairs	"response to environment"	"development & morphogenesis" & "response to environment"
Regression for birth weight	Metabolism, biosynthesis, growth, cardiovascular disease/function	Metabolism, biosynthesis, cardiovascular disease/function

Variance components of DNA methylation on a genome-scale

Joseph Powell & Peter Visscher, University of Queensland
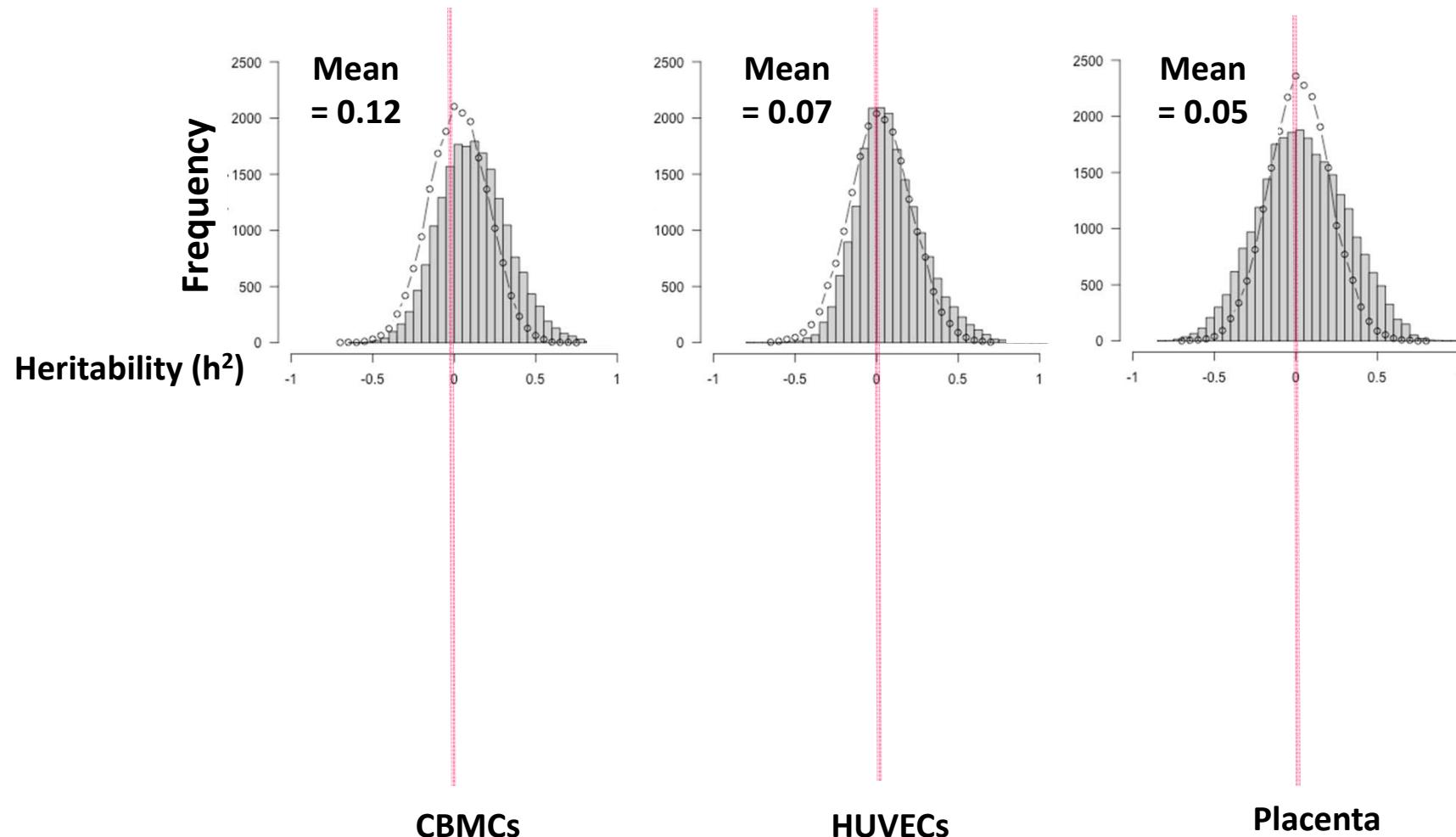
Joseph Powell & Peter Visscher, University of Queensland
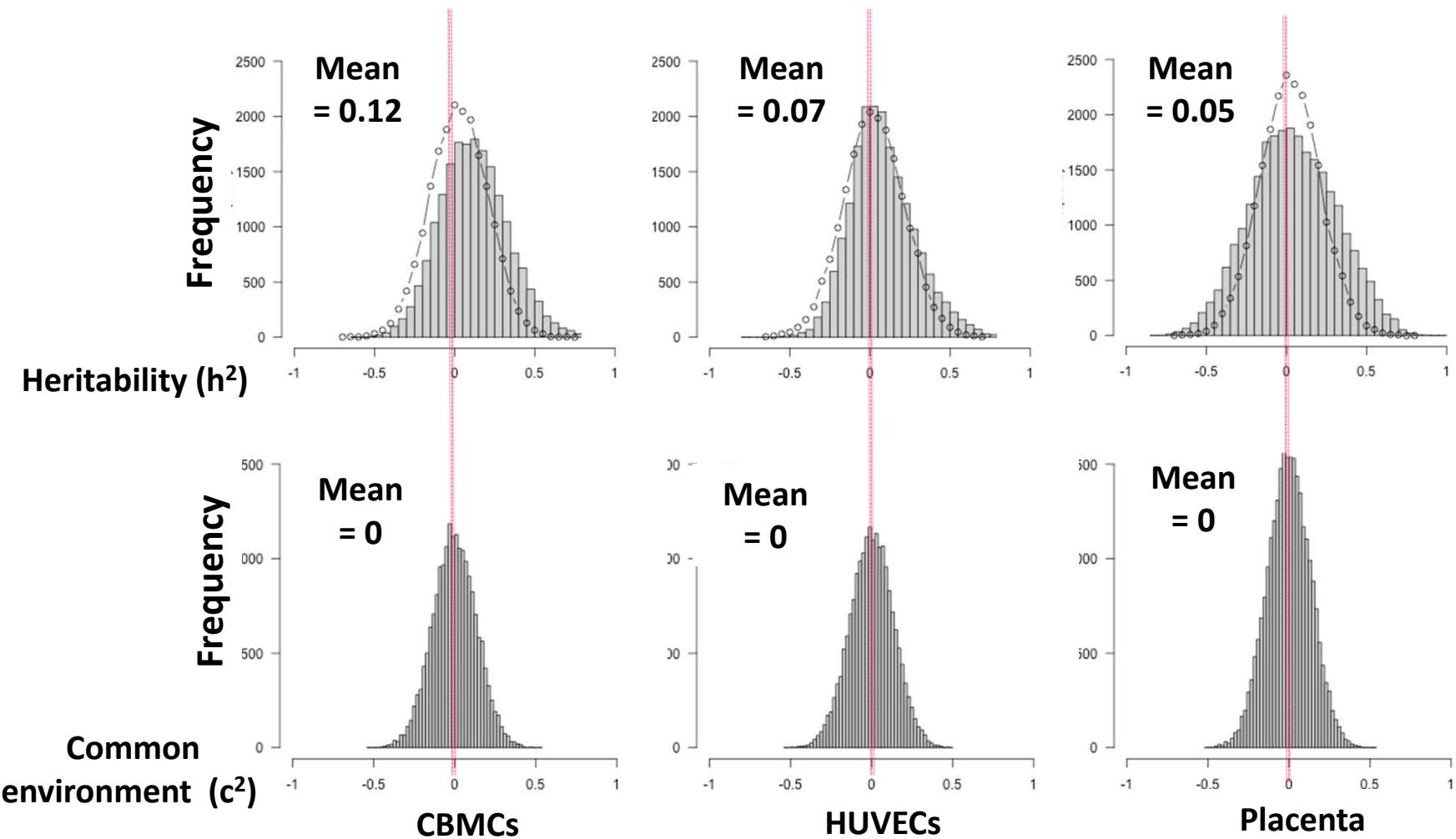
Neonatal variance component conclusions: on average, across the genome:

- Genetic effect present but small and tissue-dependent

- Effect of common environment negligible

- Residual variance is large

    - Nonshared environment

    - Stochastic factors

    - Measurement error

How DNA methylation changes between birth and 18 months: Epigenetic dynamics & drift

Genome Biology

This Provisional PDF corresponds to the article as it appeared upon acceptance. Copyedited and fully formatted PDF and full text (HTML) versions will be made available soon.

Longitudinal, genome-scale analysis of DNA methylation in twins from birth to 18 months of age reveals rapid epigenetic change in early life and pair-specific effects of discordance

Genome Biology 2013, **14**:R42   doi:10.1186/gb-2013-14-5-r42

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Samples and technology

- Buccals from 10 MZ pairs & 5 DZ pairs

- Birth and 18 months

- Infinium HM450 arrays: 485,000 CpGs, genome-wide, regions of functional significance

  - Promoters
  - Enhancers
  - Cancer-associated
  - ES cell-associated
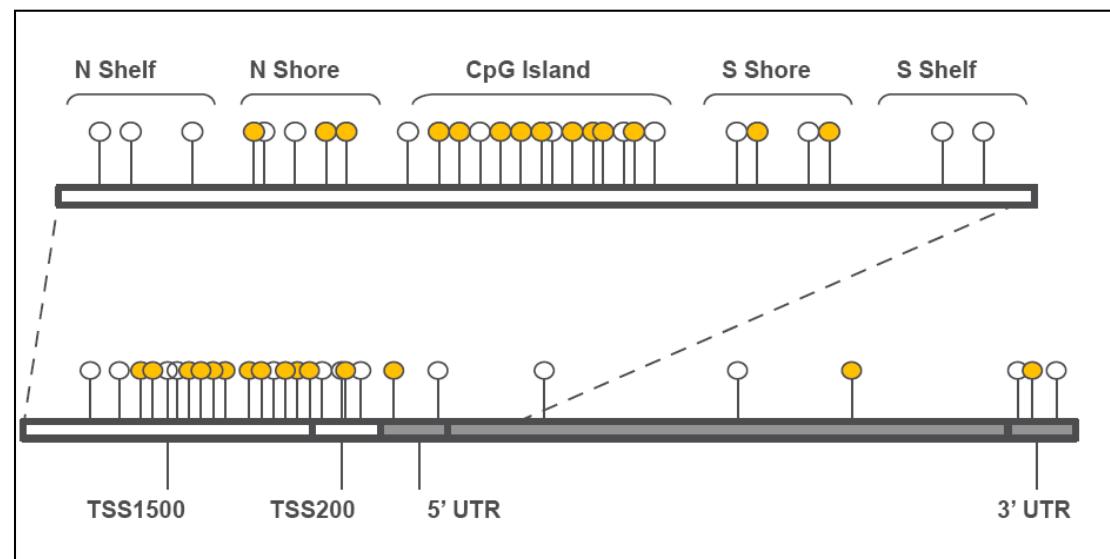
Results

- 1/3 CpGs changed significantly between birth & 18m

- 'age-associated CpGs' enriched in:

  - Genes associated with development & morphogenesis

  - Intergenic regions inc. enhancers

  - Low CpG density promoters

  - Regions surrounding CpG islands

  - Regions associated with stem cell reprogramming

Examination of twin-pair discordance with age
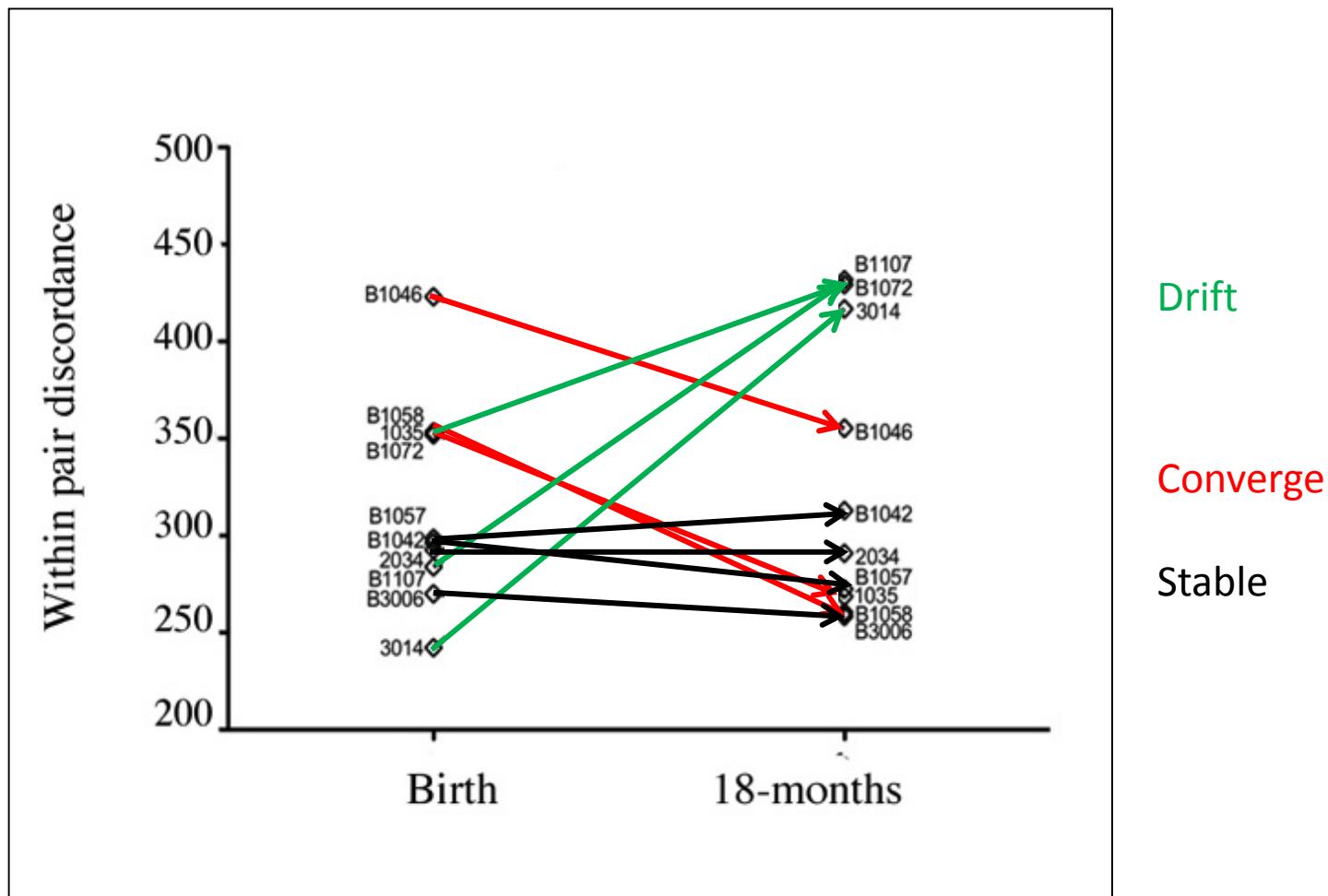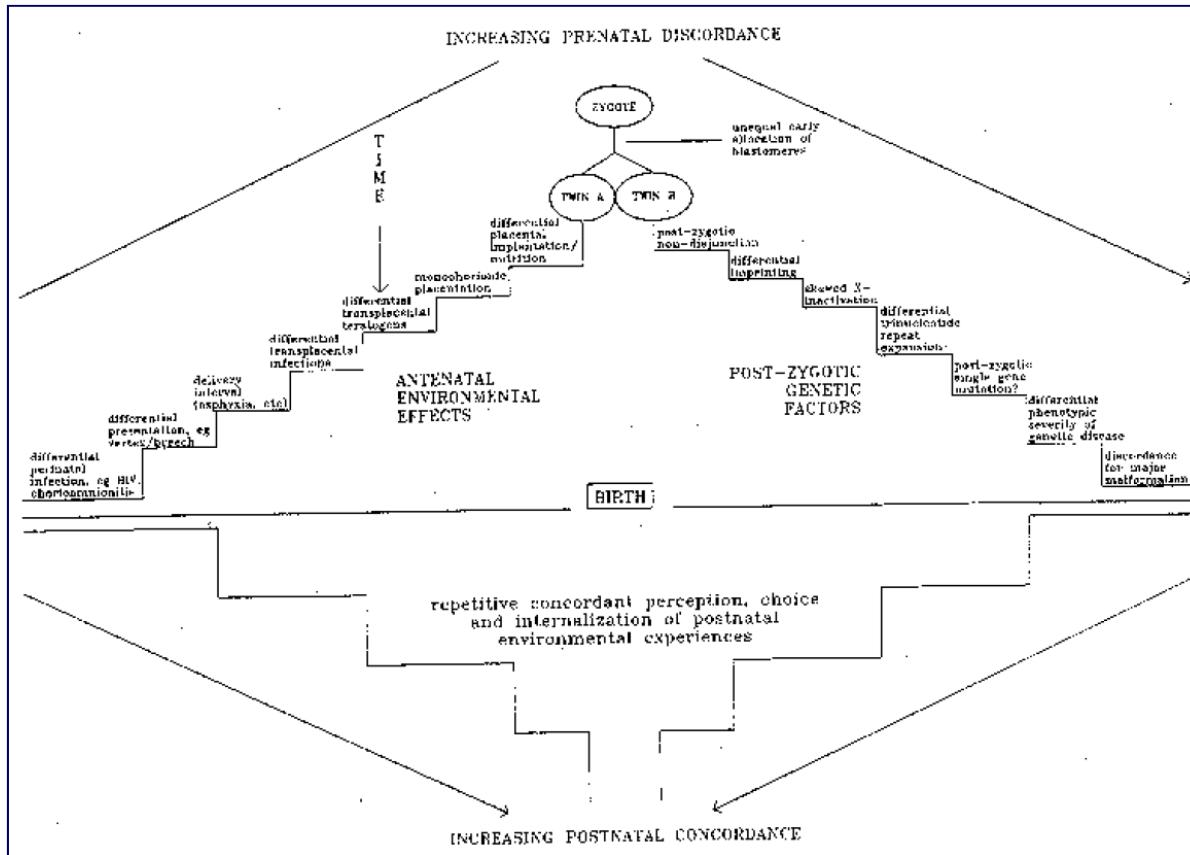
Examination of twin-pair discordance with age

Zygosity Testing
### Current Status and Evolving Issues

Louis Keith, M.D., and Geoffrey Machin, M.D., Ph.D.

Prenatal discordance and postnatal concordance
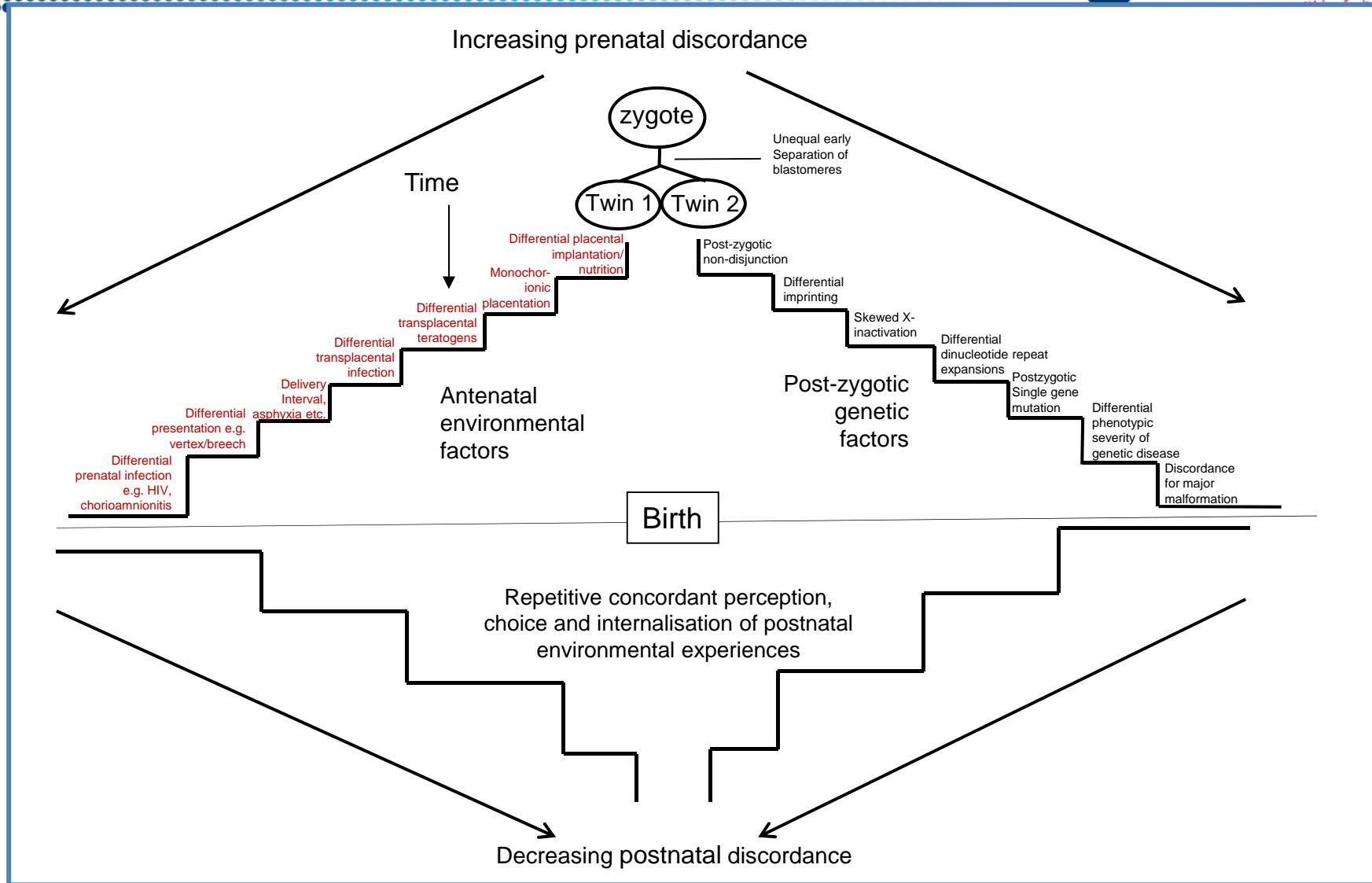
Take-home messages

- Twins are great to tease apart the role of epigenetics in development and health
- Epigenetics can help explain why identical twins (and the rest of us) are different (not forgetting genetics)
- Intrauterine environment can be shared or non-shared
- Environmental affect can differ between genes & tissues
- The first 18 months of postnatal life is extremely epigenetically dynamic
- Prenatal and postnatal environments can differ

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Heritability
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Epigenetic drift
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Kristina Gervin &
Jennifer Harris, Oslo
Jordana Bell & Pei-Chien Tsai, UK, London
Jonathan Mill & Chloe Wong, London
Reid Alisch, Atlanta