

Epigenetic variation and complex disease risk

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Missing heritability



The case of the missing heritability

Brendan Maher. Nature 2008; 456: 18-21

“Even when dozens of genes have been linked to a trait, both the individual and cumulative effects are disappointingly small and nowhere near enough to explain earlier estimates of heritability”

Francis Collins

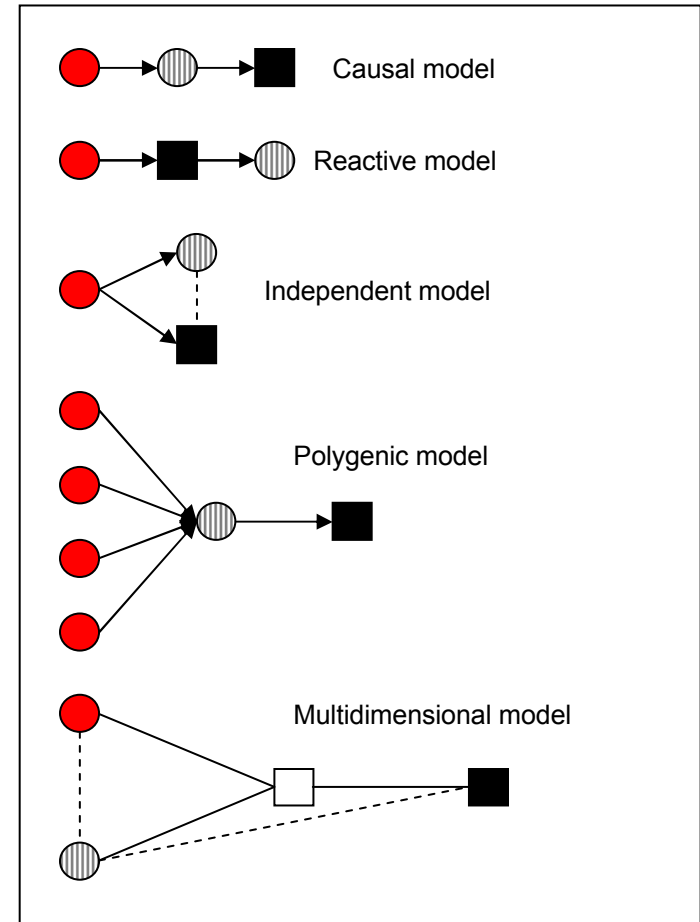
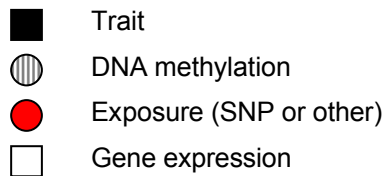
1. Rare variants
2. Copy number variants
3. Epistasis
4. Epigenetics
5. Something else

Epigenetics and complex disease

- Epigenotype is be influenced by environmental factors and stochastic events
- Epigenotype is inherited through the germ-line
- Epigenotype can influence phenotype
 - Central & Eastern European Lung Cancer Case Control Study
 - North Cumbria Community Genetics Project
 - Newcastle Thousand Families Study
 - ALSPAC

Causality

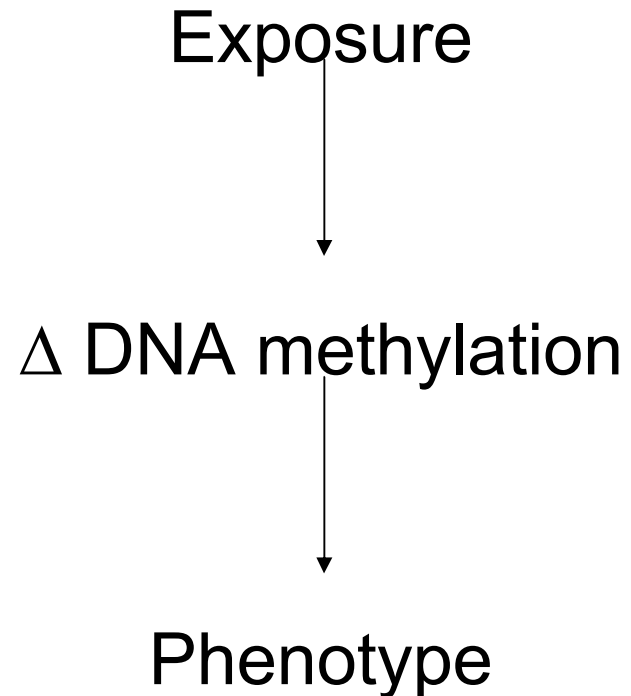
- Epigenotype can be considered an intermediate phenotype
- Is epigenotype on the causal pathway to disease?



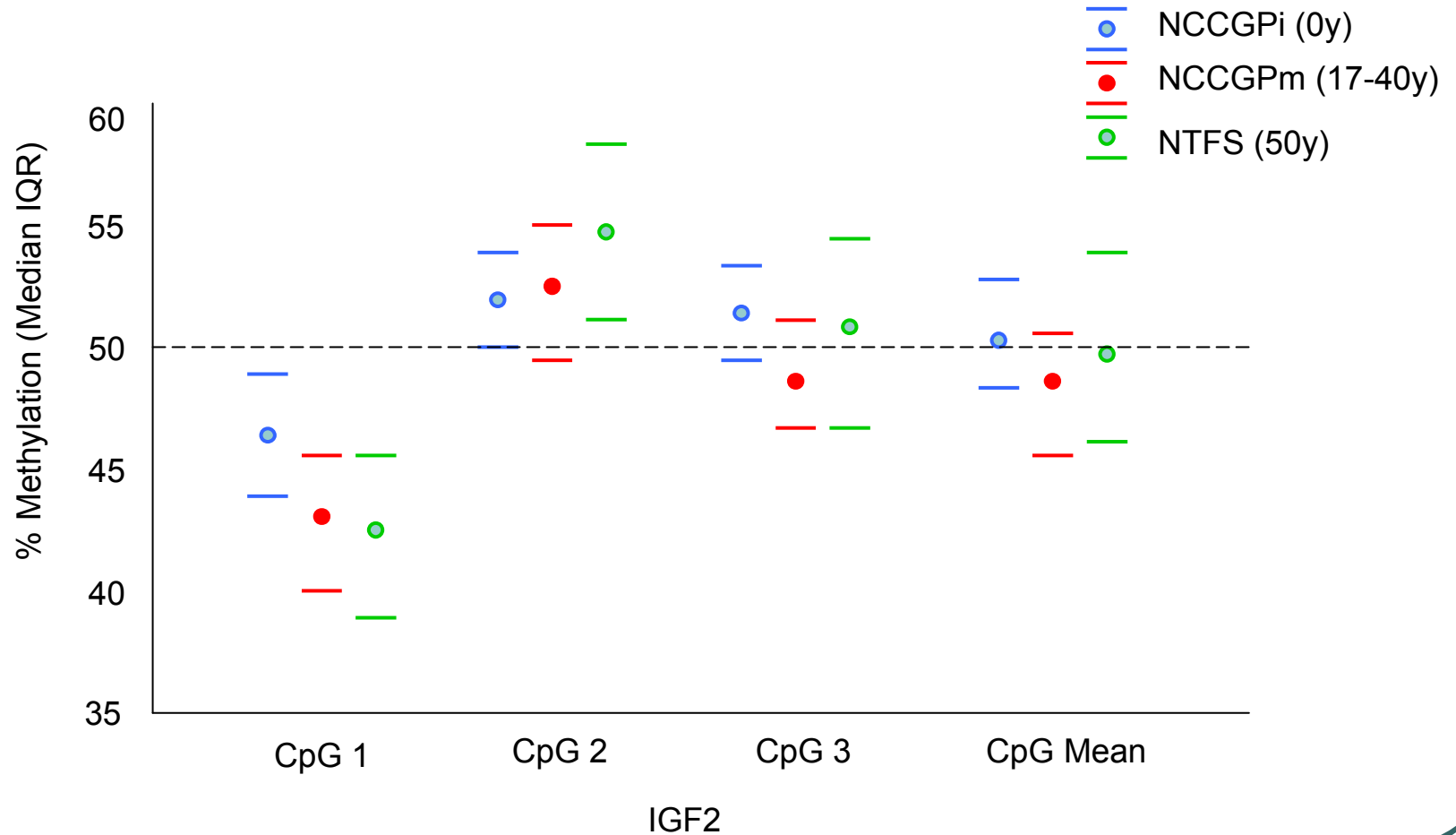
**Epigenotype is influenced by
environmental factors and
stochastic events**

Determinants of DNA methylation

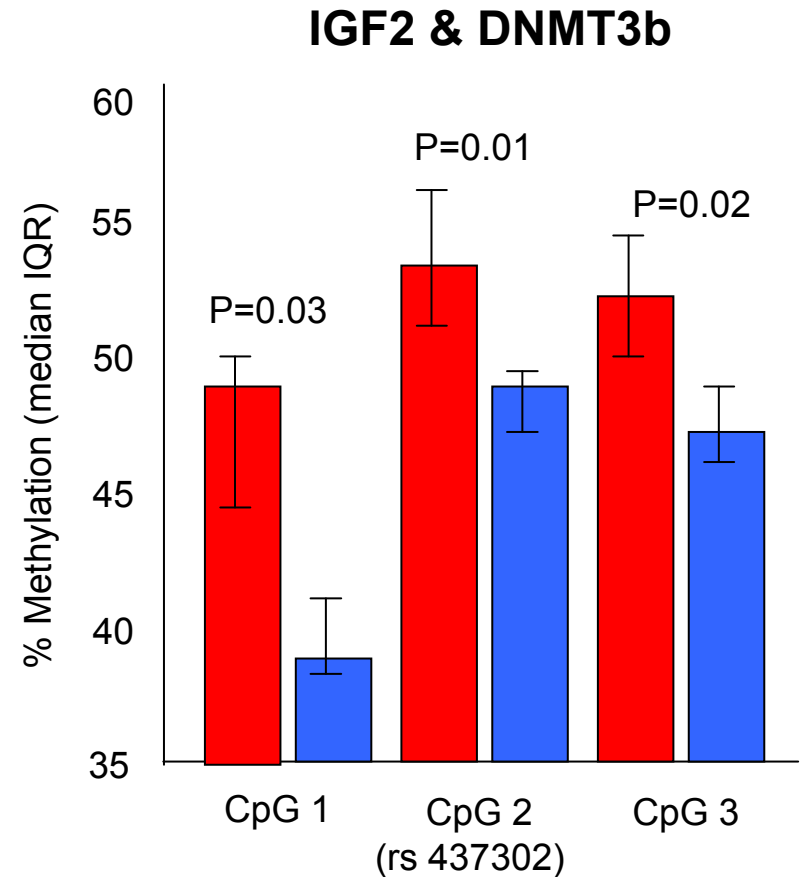
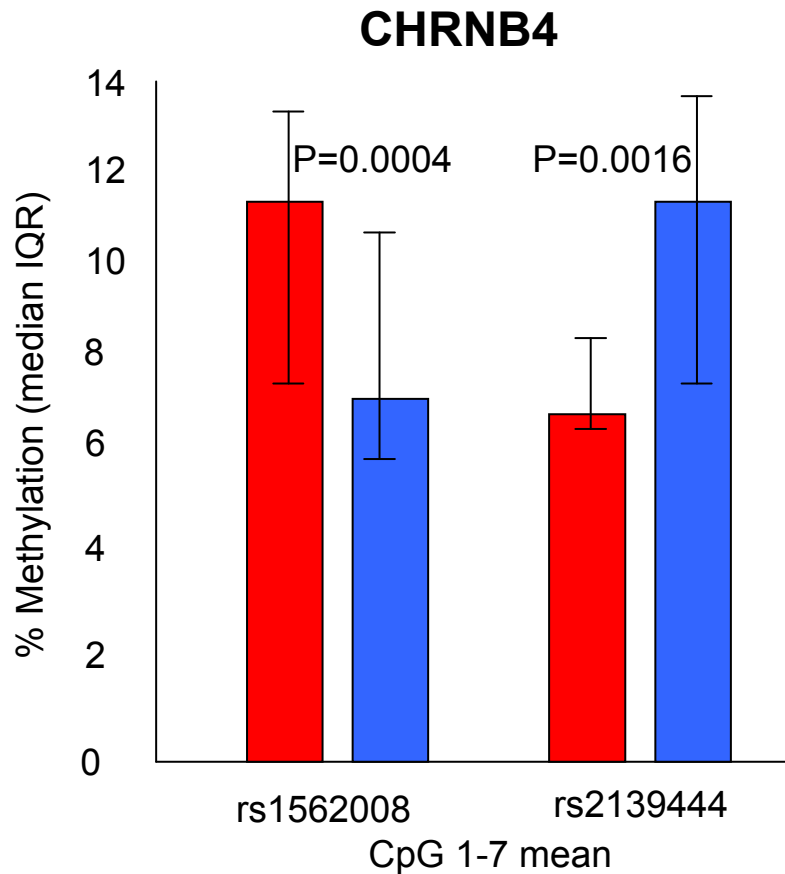
- Nutrition
- Age
- Genetic factors
- Stress
- Smoking
- Infection
- Disease status



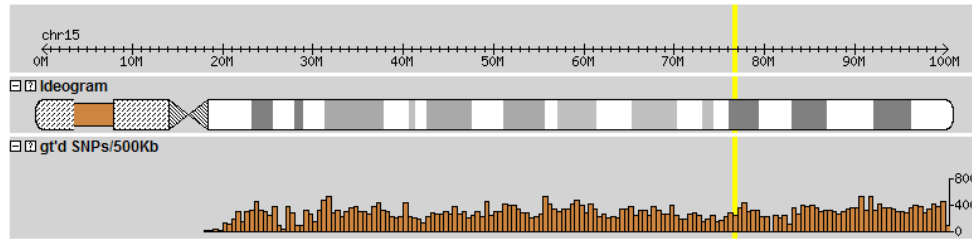
Age-related change in methylation



Genetic influences

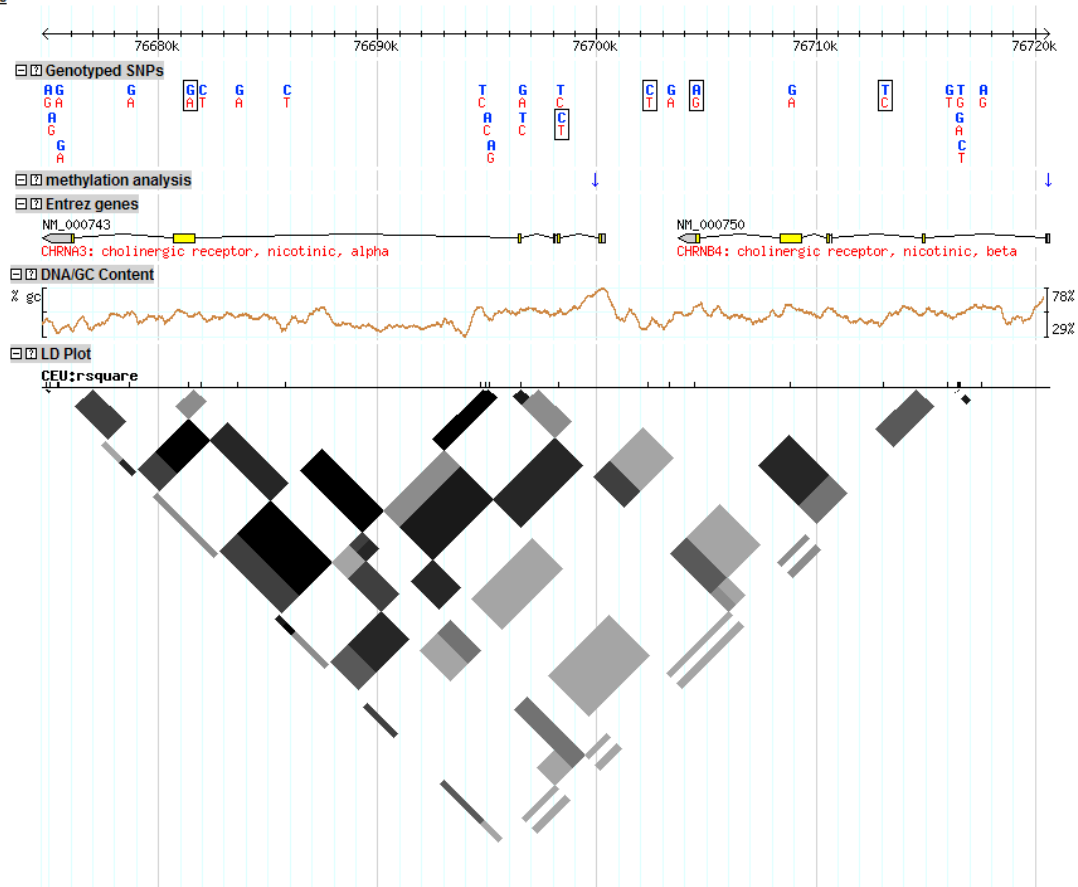


Overview



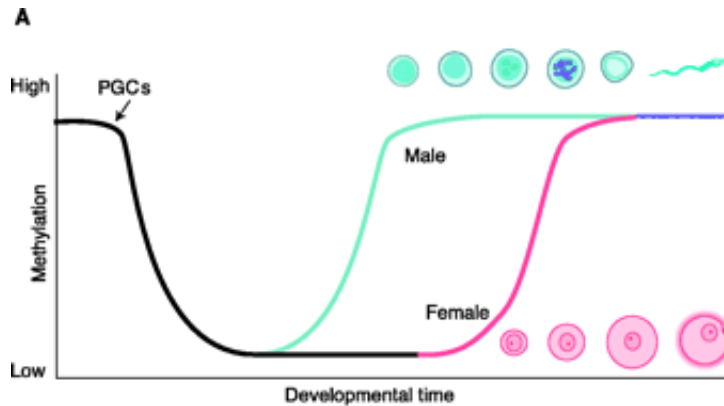
Region

Details

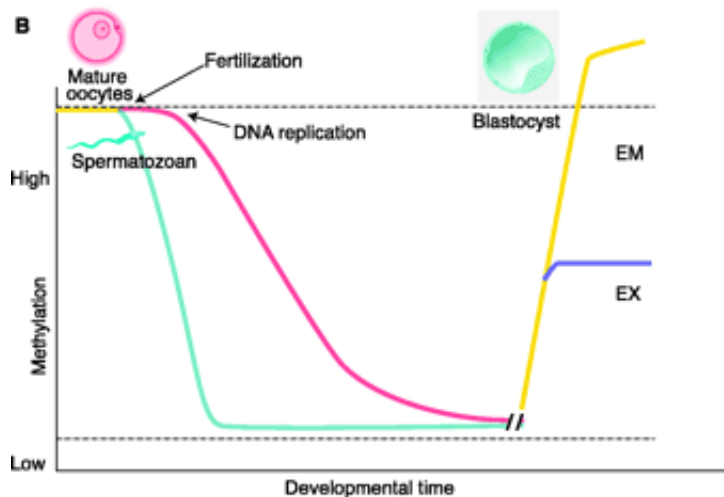


**Epigenotype can be inherited
through the germ-line**

Early epigenetic marking of the genome



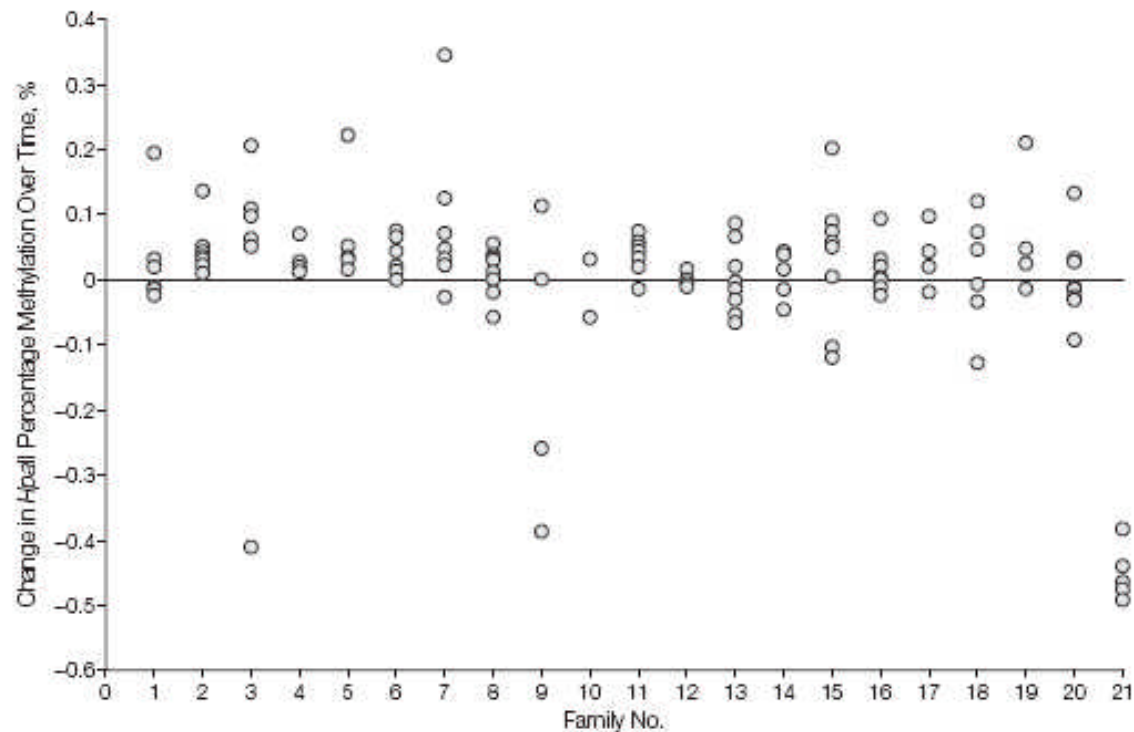
Methylation reprogramming in the germ line



Methylation reprogramming in pre-implantation embryos

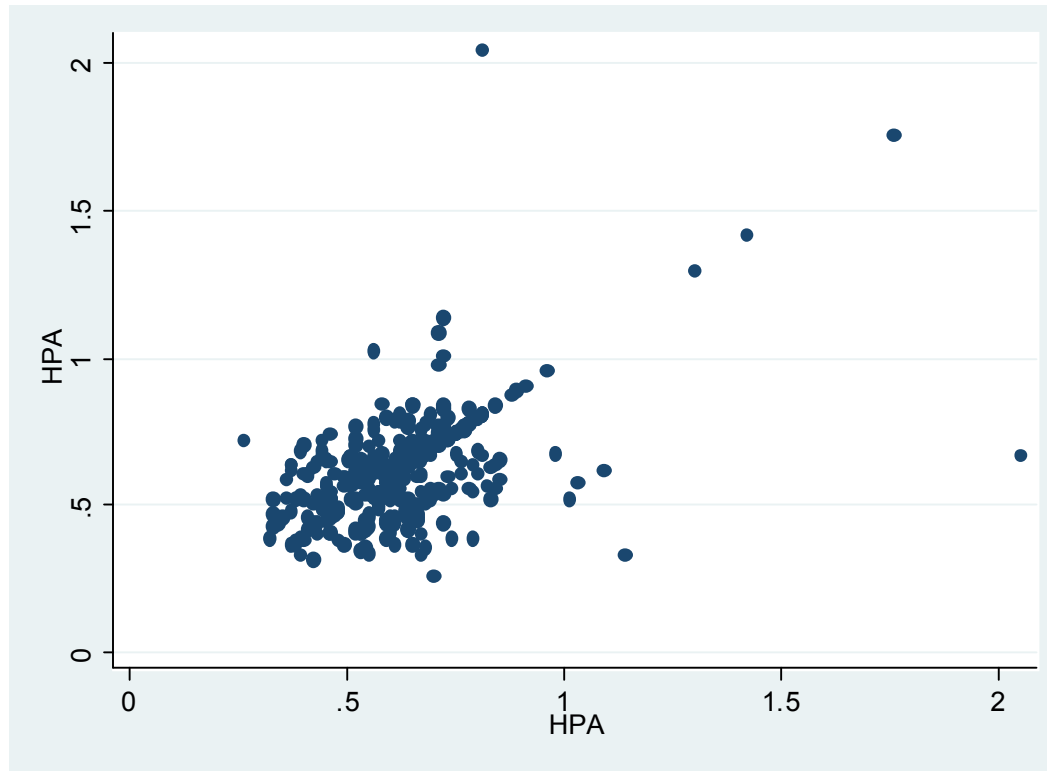
Familial Clustering

Figure 2. Familiarity of Global DNA Methylation Change in the Utah Cohort



Change in *HpaII* methylation as measured by luminometric methylation assay between the 2 time points (average 16-year interval) for the Utah cohort, sorted by family. Each circle represents an individual.

Maternal-child correlation



0.489
 $p < 0.0001$

Epigenotype can influence phenotype

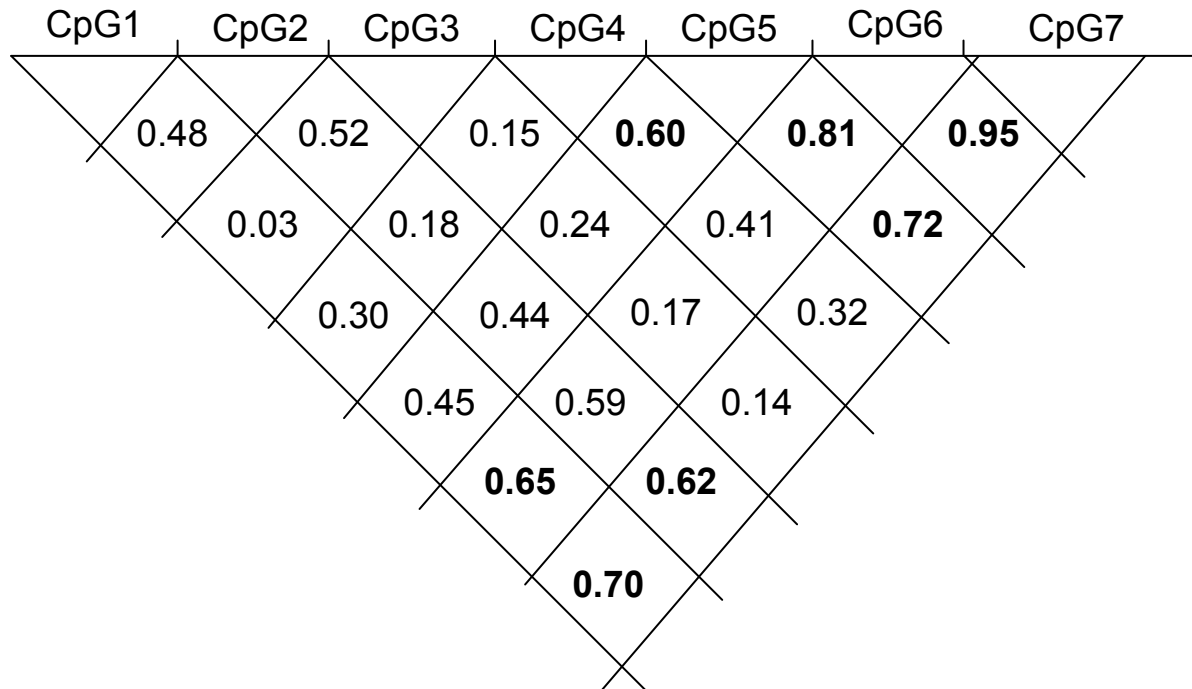
CHRNA/B & lung cancer

	CpG1	CpG2	CpG3	CpG4	CpG5	CpG6	CpG7	CpGmean
CHRNA#3								
Case	6.95	7.00	7.10	4.80	5.80	5.85	2.50	5.86
Control	6.50	7.40	6.80	9.85	5.30	7.80	2.60	6.77
P-value	0.198	0.317	0.594	<0.0001	0.110	0.0001	0.463	0.001
CHRNA#4								
Case	6.05	8.65	3.45	11.40	3.65	6.75	6.30	6.60
Control	9.40	12.60	3.90	11.60	4.60	13.30	21.00	11.20
P-value	<0.0001	<0.0001	0.366	0.9992	0.626	0.0006	0.0001	0.0001

Two-sample Wilcoxon rank sum (Mann-Whitney) test for significant differences in CHRN qCpG in PBL DNA from lung cancer cases and controls

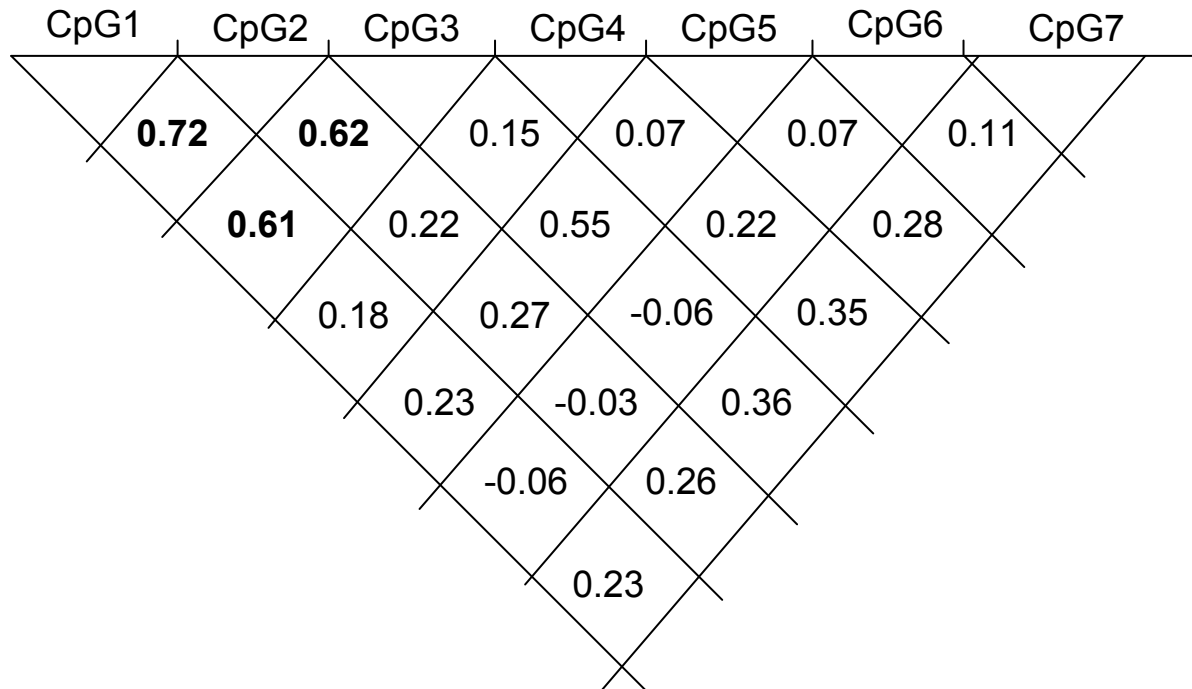
Is methylation of CpG sites correlated?

CHRN4



Is methylation of CpG sites correlated?

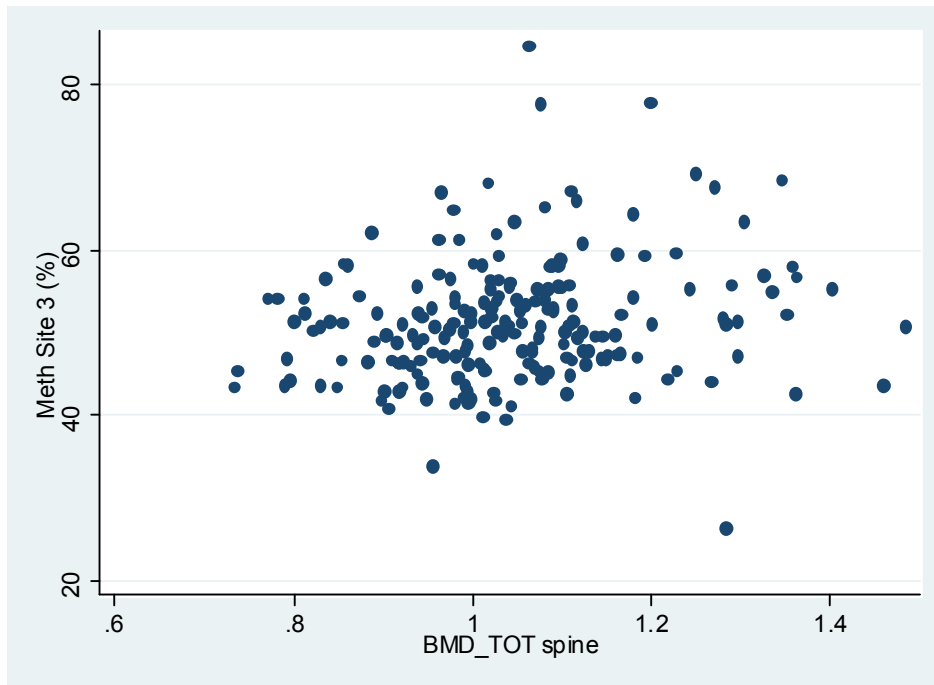
CHRN3A



Cross-sectional study of DNA methylation and phenotype at age 50

- Bone health
- Respiratory health
- Cardiovascular health
- Metabolic health
- Body composition

Newcastle Thousand Families Study



Bone mineral density
Cross-sectional study, age 50y

Regression co-efficient
0.84 (0.76, 0.93) $p=0.001$

Epigenetics and developmental programming

Early life programming mediated by DNA methylation

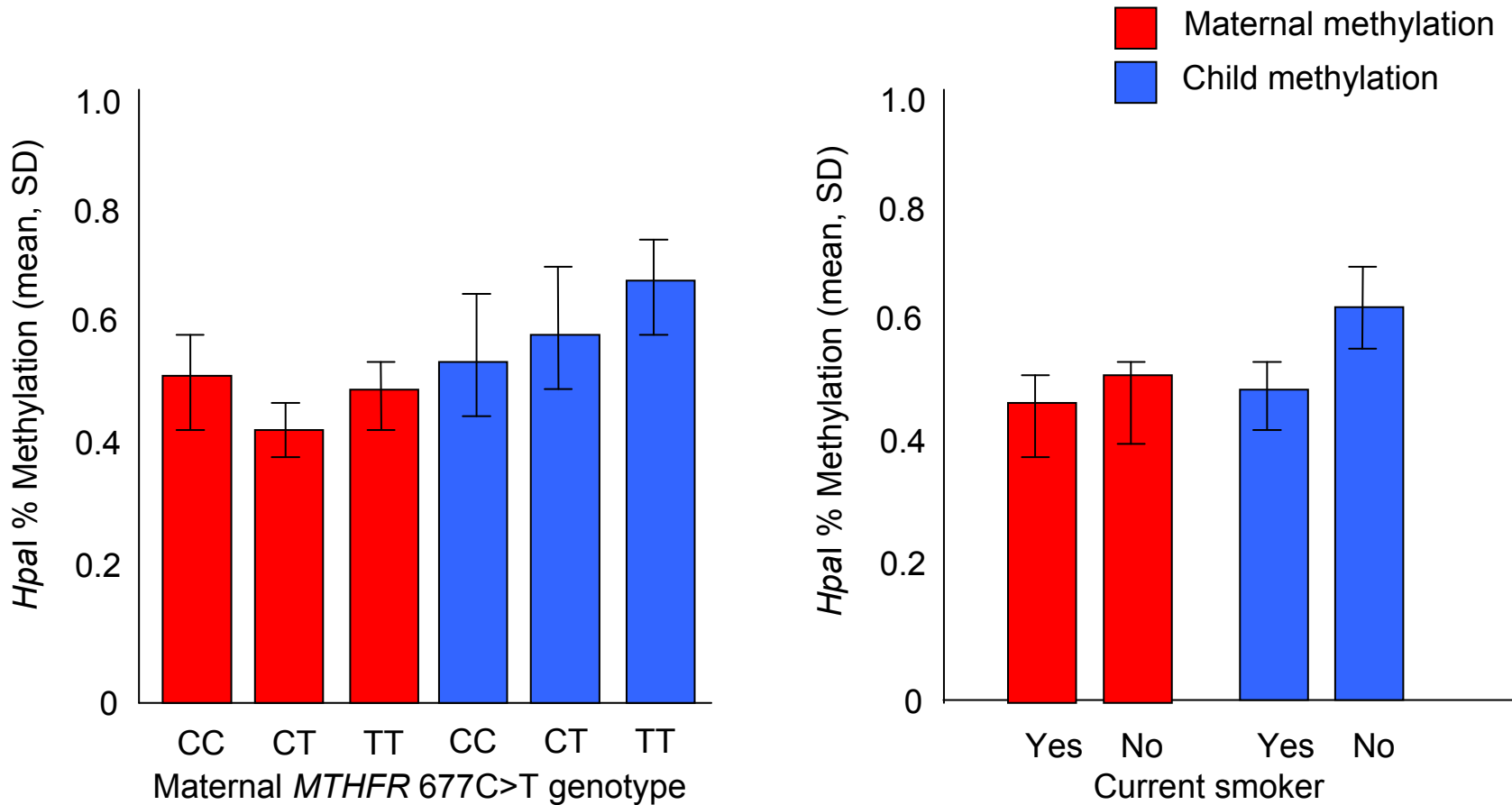
Persistent epigenetic differences associated with prenatal exposure to famine in humans

Bastiaan T. Heijmans^{a,1,2}, Elmar W. Tobin^{a,2}, Aryeh D. Stein^b, Hein Putter^c, Gerard J. Blauw^d, Ezra S. Susser^{e,f}, P. Eline Slagboom^a, and L. H. Lumey^{e,1}

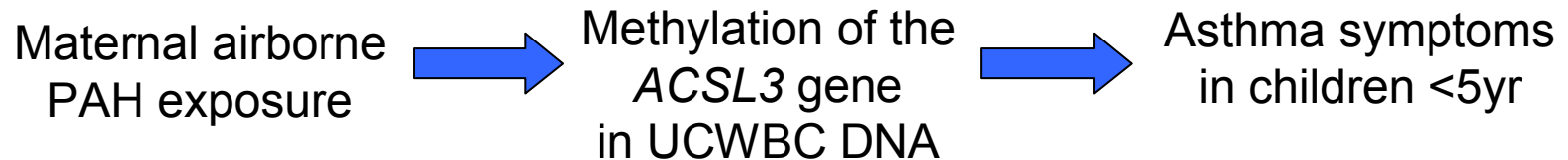
- 5 CpG sites in the *IGF2* gene measured in exposed (n=60) and their same sex siblings (n=60)
- Prenatal early gestation exposure to famine is associated with changes in methylation 6 decades later; ↓5.2% in exposed vs unexposed
- No association observed with those exposed in late gestation
- 122 additional controls analysed to assess influence of age; ↓3.6% with each 10 year increase in age

Proc Natl Acad Sci 2008; 105(44): 17046-49

In utero influences



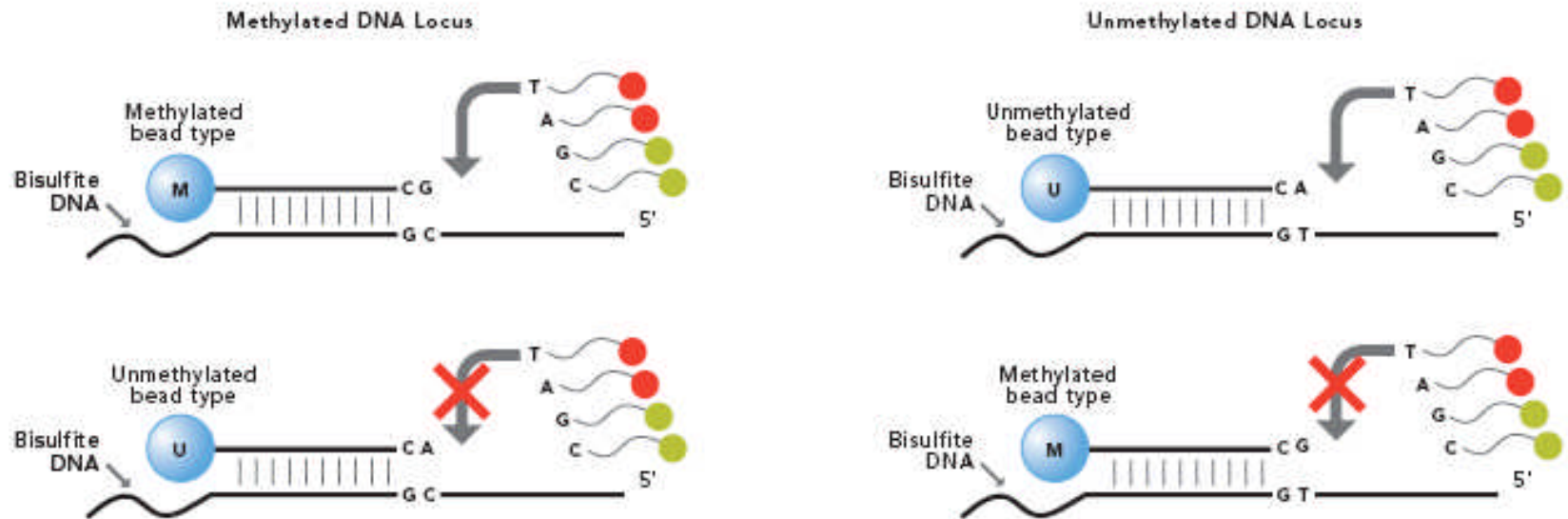
DNA methylation in the *ACSL3* gene, PAH exposure and childhood asthma



- Methylation profiling n=10 exposed & n=10 unexposed
- 31 candidate genes demonstrated differential methylation
- 6/31 genes had CGI in promoter region
- Validated association in 6 promoters in n=20
- Established association between *ACSL3* CGI promoter methylation, PAH exposure and childhood asthma in n=56

Illumina GoldenGate

FIGURE 1: THE INFINIUM ASSAY FOR METHYLATION

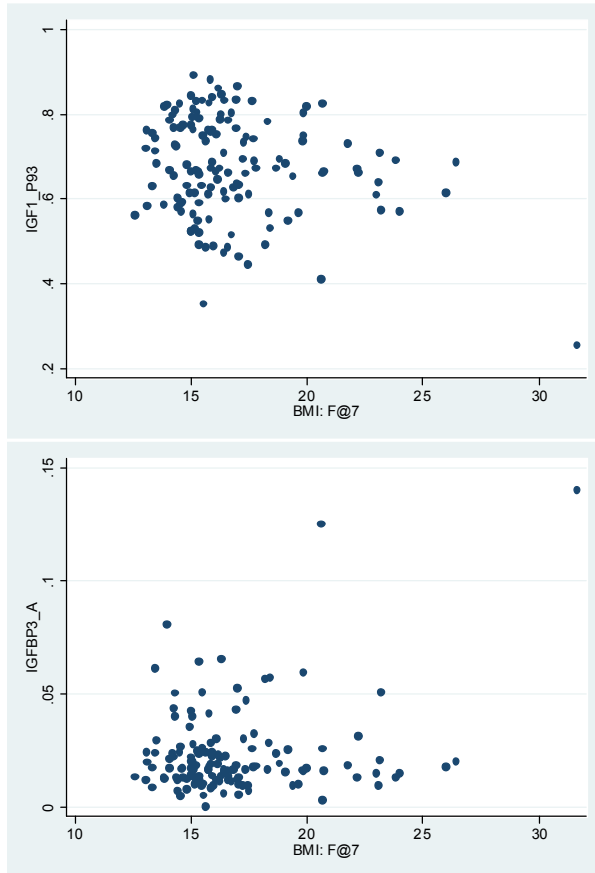


The Infinium Assay for Methylation detects methylation status at individual CpG loci by typing bisulfite-converted DNA. Methylation protects C from conversion (left), whereas unmethylated C is converted to T (right). A pair of bead-bound probes is used to detect the presence of T or C by hybridization followed by single-base extension with a labeled nucleotide.

Relationship between DNA methylation and body composition

- 1,505 CpG sites in 807 genes
- 234 CpG sites in 100 genes associated with body composition [height, BMI] based on Medline evidence and GWAS data
- Informative n=?
- Is methylation status in cord blood DNA associated with;
 - BMI at age 7w, 40w, 1.5y, 3.5y, 7y, 9y, 10y, 11y
 - Height at age 7w, 40w, 1.5y, 3.5y, 7y, 9y, 10y, 11y

DNA methylation in cord blood DNA predicts BMI at age 7y



BMI at age 7y, IGF1 methylation in cord blood DNA

Regression co-efficient
-5.47 (-9.96, -1.53) $p=0.008$
-0.66 (-1.19, -0.18)

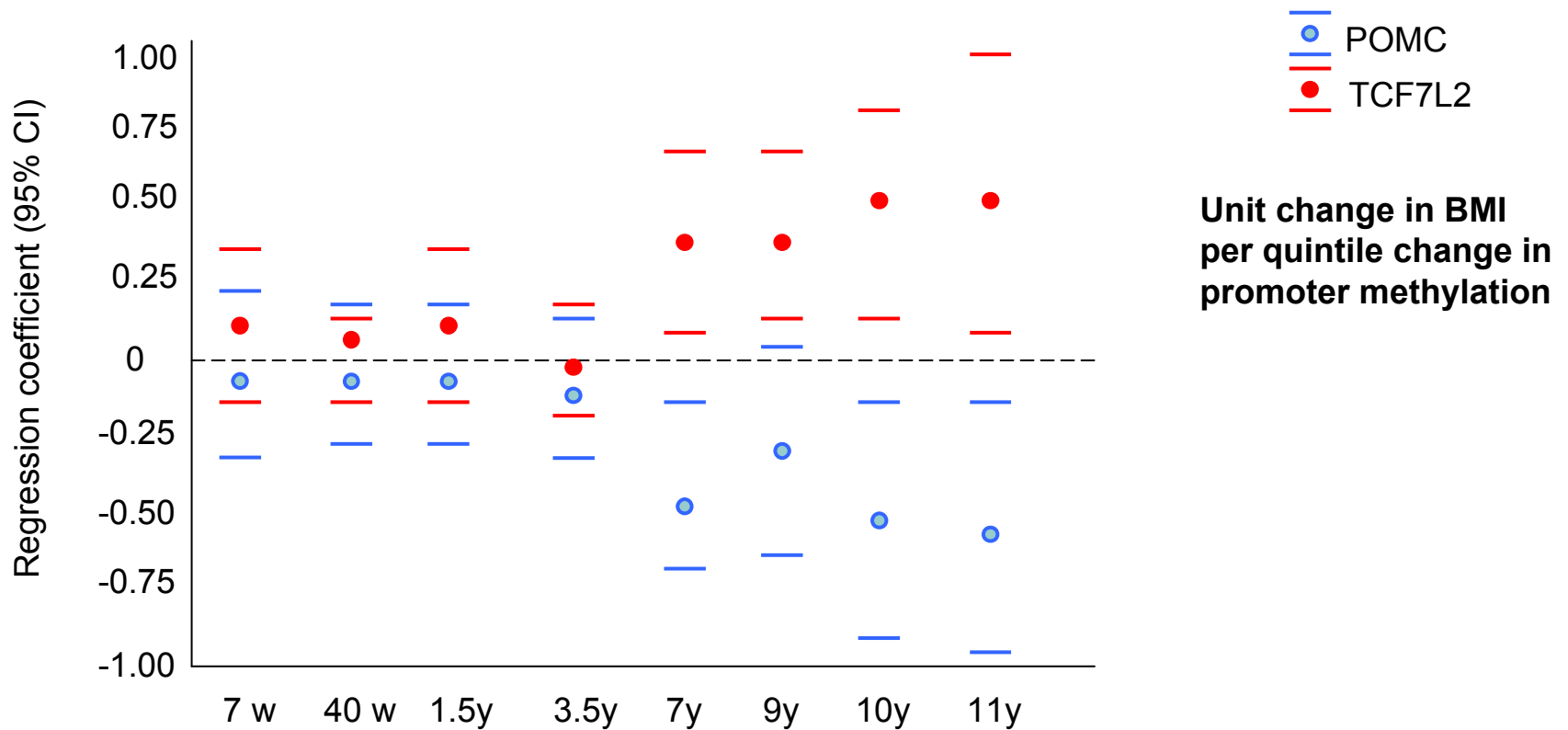
Mean (SD) = 0.676 (0.12)

BMI at age 7y, IGFBP3 methylation in cord blood DNA

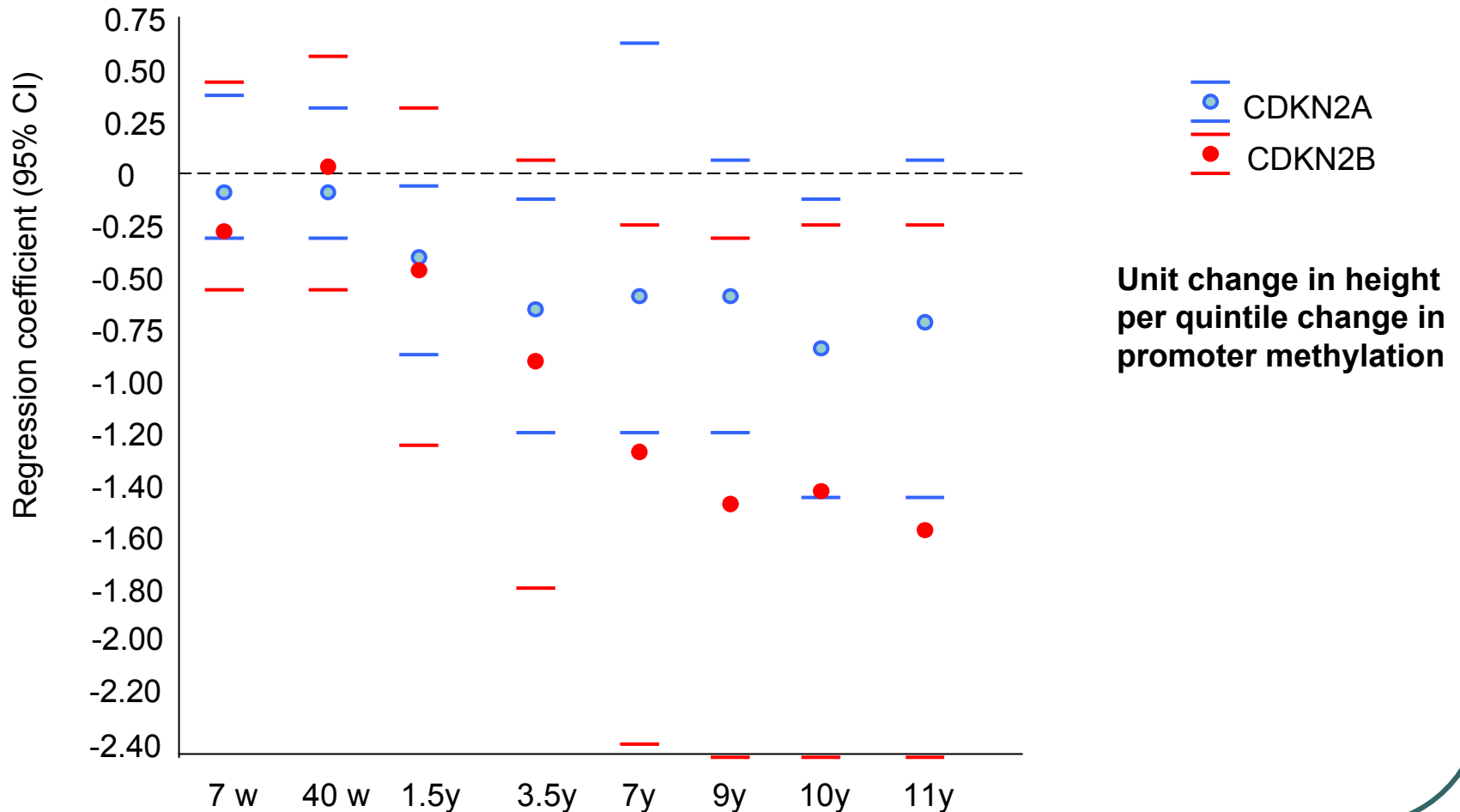
Regression co-efficient
37.97 (12.69, 63.25) $p=0.004$
0.68 (0.23, 1.14)

Mean (SD) = 0.022 (0.018)

Tracking of DNA methylation effects through childhood



Tracking of DNA methylation effects through childhood



Challenges

- Which tissue?
- Which loci?
- Which methods?
- How many CpG sites?
- Are CpG sites correlated (epitypes)?
- How do genetic and epigenetic variation interact (hepitypes)?
- CGIs and CGI-shores?
- Skewed or bimodal distribution
- Subtle exposures – subtle effects
- Establishing causality

Epigenetic biomarker discovery

- **Illumina GoldenGate/Infinium arrays**
 - Cancer Panel (1,505 CpG sites in 807 genes)
 - 27K array (all annotated promoters)
 - 50K array
- **MeDIP-chip**
 - Requires 20-30 cases/controls
- **MeDIP-seq**
 - 12 samples pooled using unique molecular tags. Full methylome sequenced. 1.2Gb sequence = 1 Solexa 1G run

CpG coverage

CDKN2A 21984138 - 21984490

CGCTCAGGGAAGGCGGGTGCGCGCCTGCGGGGCGGAGATGGGCA
GGGGGCGGTGCG**TGGGTCCAGTCTGCAGTTAAGGG**GGCAGGAG
TGGCGCTGCT**CACCTCTGGTGCCAAAGGGCGGCGCAGCGGCTGCC**
GAGCTCGGCCCTGGAGGCGGCGAGAACATGGTGCGCAGGTTCTTG
GTGACCCT**CCGGATTCCGGCGCGCGTGCGGGCCCG**CCGCGAGTGAG
GGTTTTCGTGGTTCACATCCCGCGGCTCACGGGGGAGTGGGCAGC
GCCAGGGGCGCCCGCCCGCTGTGGCCCTCGTGCTGATGCTACTGAG
GAGCCAGCGTCTAGGGCAGCAGCCCG**CTTCCTAGAAGACCAGgtagg**
aaaggccct

TGGT Sequenom (29)

£12 / sample

TGGT Illumina GoldenGate (1)

£0.10 / sample

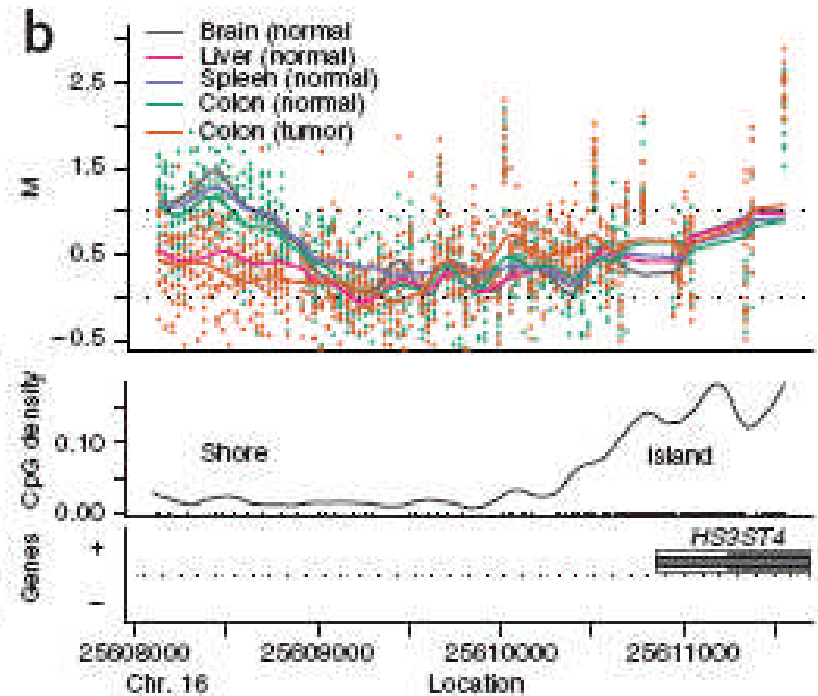
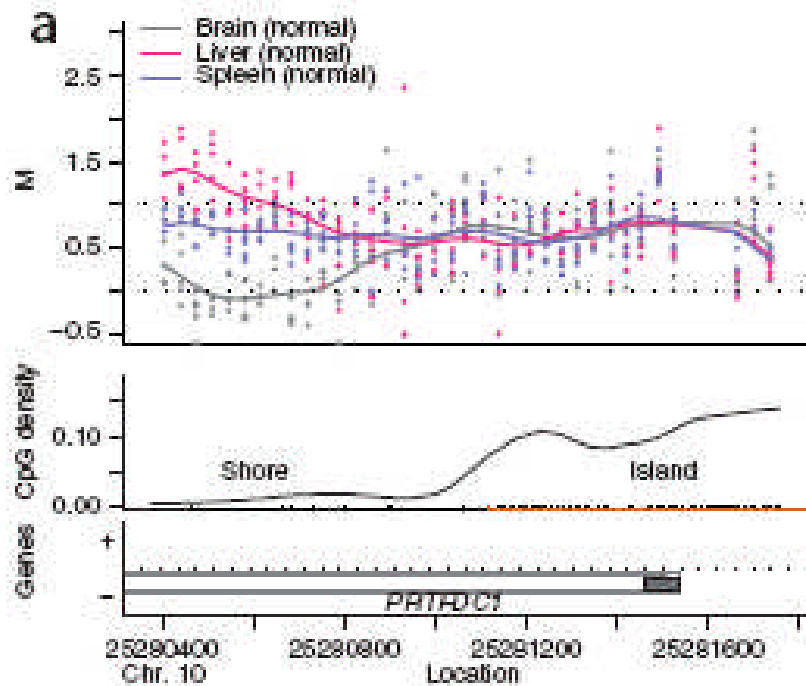
TGGT Pyrosequencer (7)

£3.50 / sample

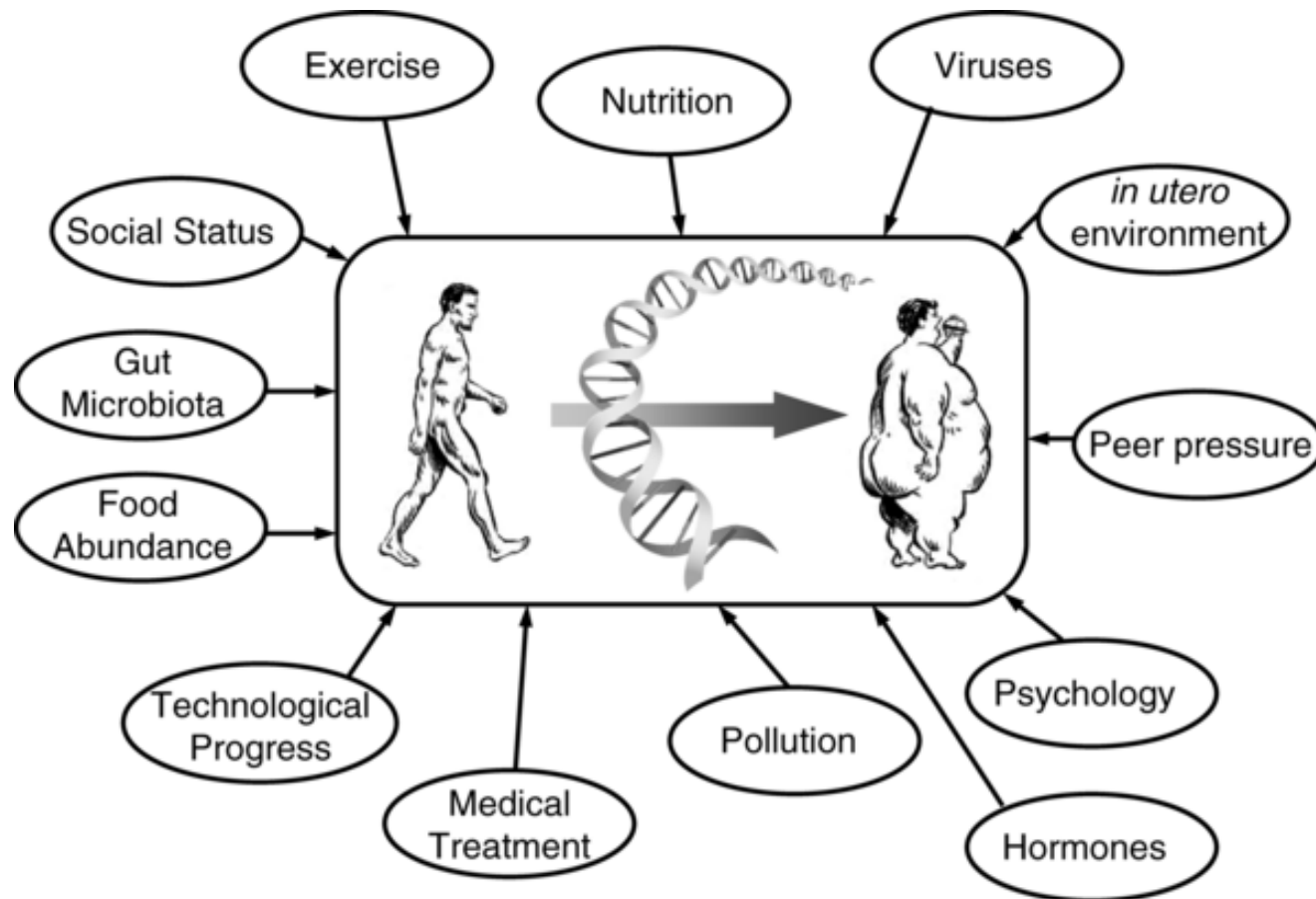
Quantitative DNA methylation analysis in epidemiological studies

Standard Deviation	Independent groups		Paired groups	
	$\alpha = 0.05$	$\alpha = 0.001$	$\alpha = 0.05$	$\alpha = 0.001$
0.10	1238	3095	787	1713
0.25	199	498	128	279
0.50	64	140	34	74
1.00	17	37	10	23

CGIs and CGI shores



Epigenetic mechanisms transduce stimuli to influence body composition



Acknowledgements

- Newcastle University
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