Epigenetics and the Life Course

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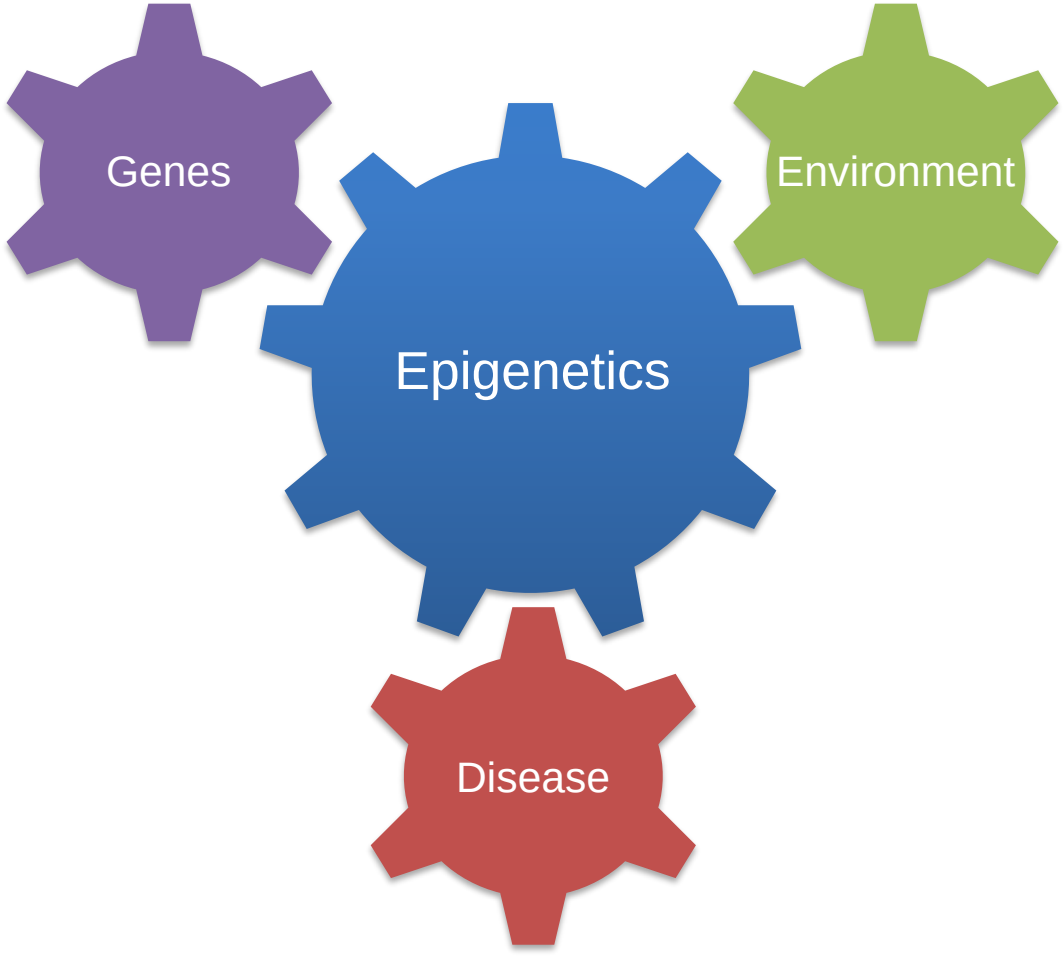
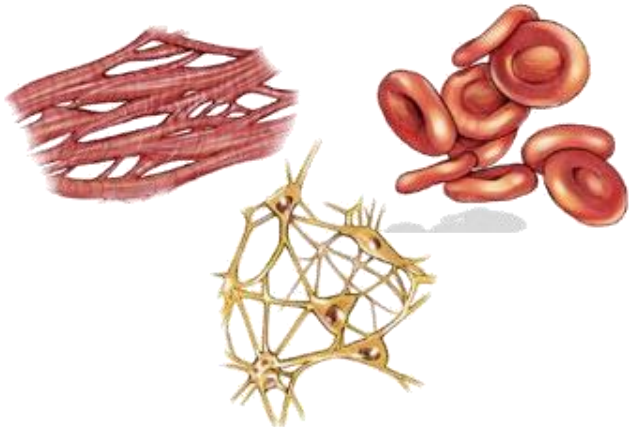

Genes
Environment
Epigenetics
Disease

Roles epigenetics: variation and memory

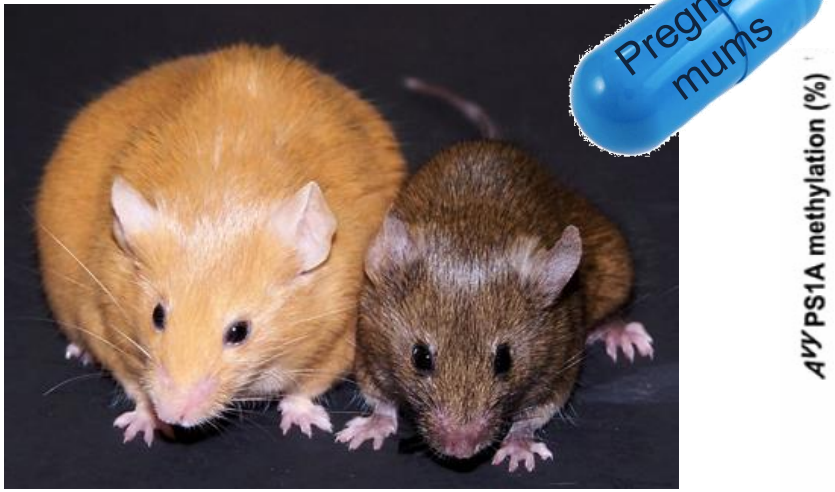
1. Development and cell differentiation
   → 1 DNA molecule, many cell types within an individual.
2. Interface DNA and environment
   → 1 DNA molecule, multiple possible phenotypes.



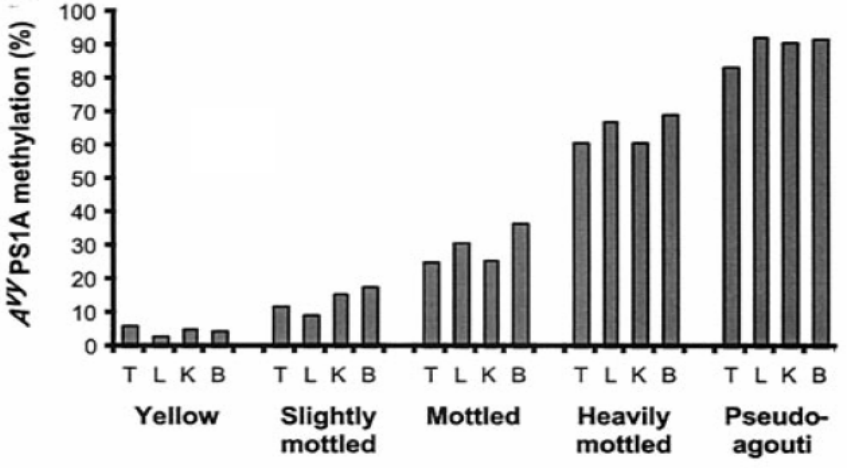
Epigenetics of coat color

Inbred agouti mice: same DNA sequence

- Methyl supplementation diet pregnant females
- Recorded as higher methylation of *agouti* gene
- Expressed as no synthesis of yellow colour
- Propagated across tissues
- Retained into adulthood



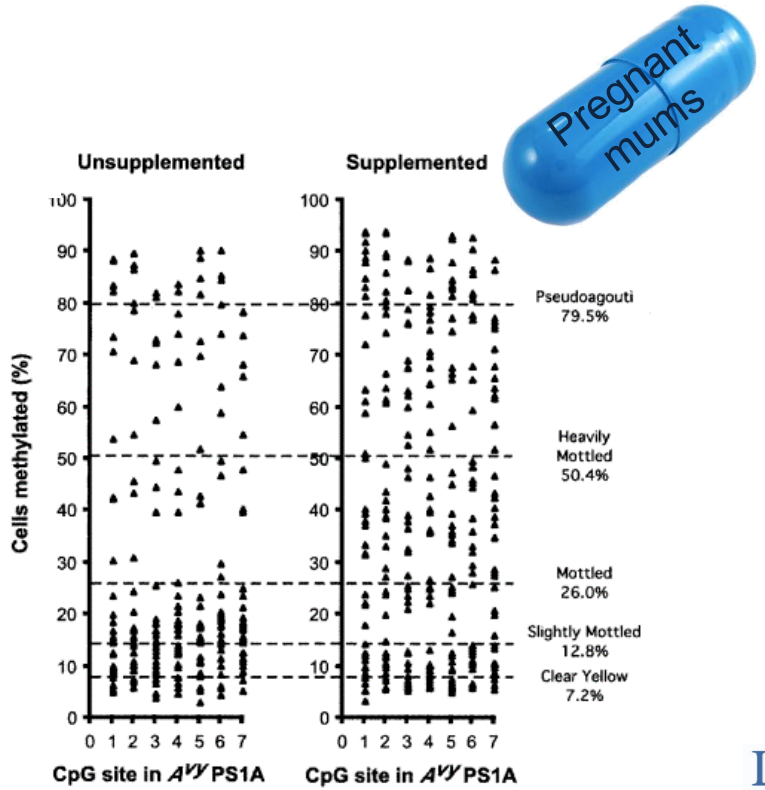
Waterland and Jirtle. *Mol Cell Biol* 2003



Epigenetics: the memory of the DNA

messy

Waterland and Jirtle. *Mol Cell Biol* 2003



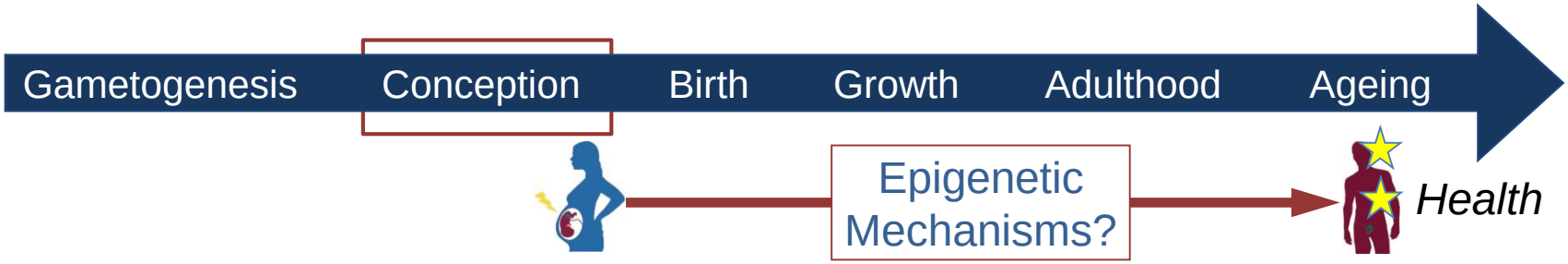
Dutch Hunger Winter

- Severe famine during the winter of 1944-45 in WW2.
- Exposure during intra-uterine life associated with cardiometabolic health (overweight, diabetes, unfavourable lipid levels) and schizophrenia.

Study design

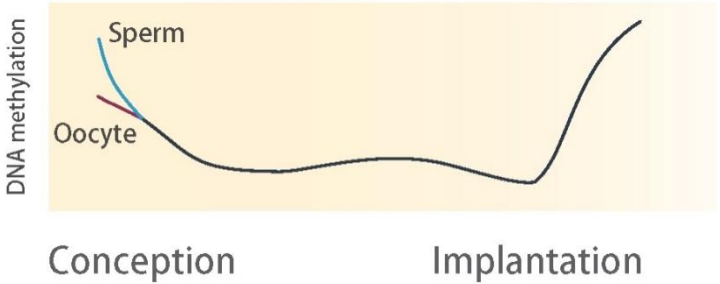
- Quasi-experimental: daily rations <700 kcal/day set for whole population.
- Prospective: traced back exposed individuals at age 60y from records at institutions in affected cities; timing known.
- Best possible controls: unexposed, same-sex siblings.

Study design



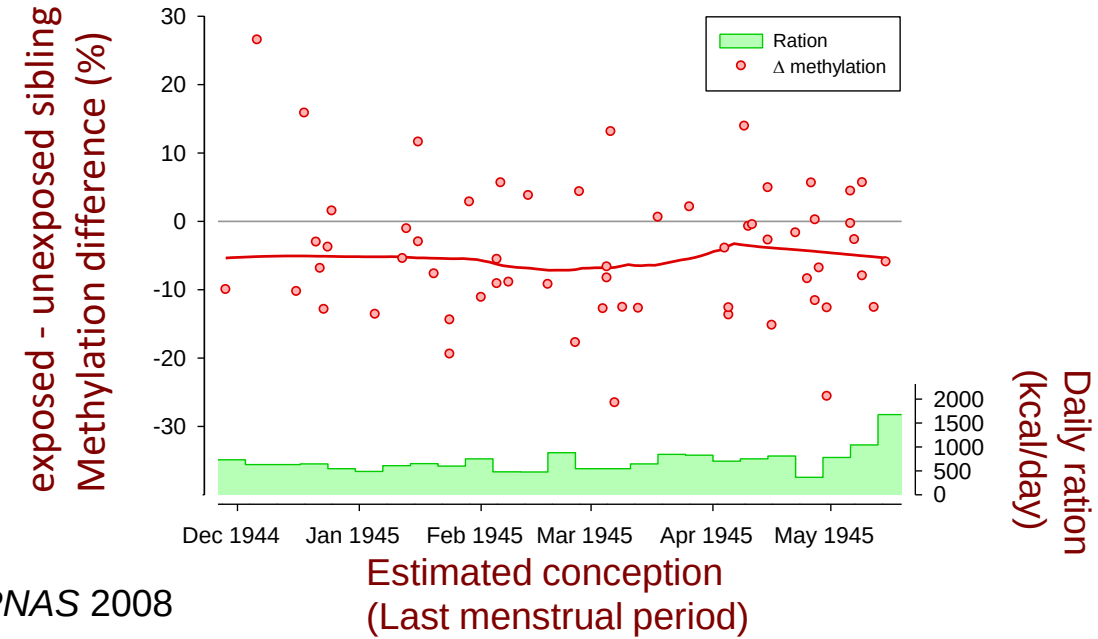
Focus on early gestation (ie. conception during Famine)

- Sensitive window? Critical stage in establishing and maintaining epigenetic marks.
- Soma-wide occurrence? Mitotic inheritance resulting in cross-tissue epigenetic differences (incl. peripheral tissues).



Methylation of a growth gene

- DNA from blood of 60 individuals who were periconceptionally exposed to the Famine 6 decades ago.
- *IGF2*: Prenatal growth factor, also implicated in metabolic regulation and memory; epigenetically controlled.

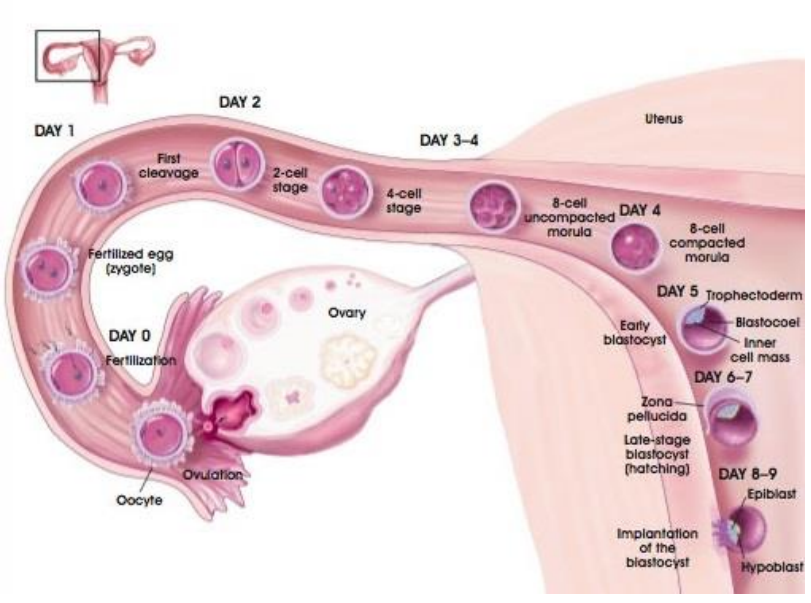


Heijmans et al. *PNAS* 2008

Looking across 16 genes

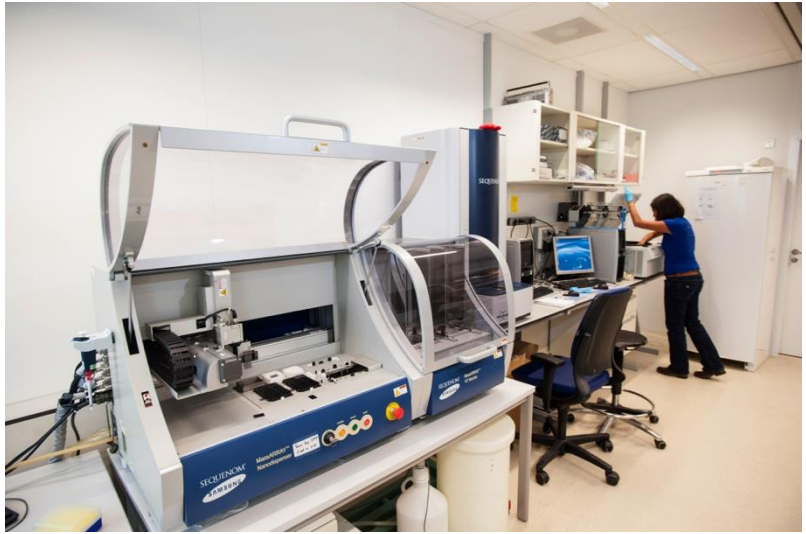
Gene	*Early*	*Late*
IGF2	↓	
GNAS	↑	↓
INSIGF	↓	
IL10	↑	
LEP	↑	↑
ABCA1	↑	

Tobi et al. *Hum Mol Genet* 2009



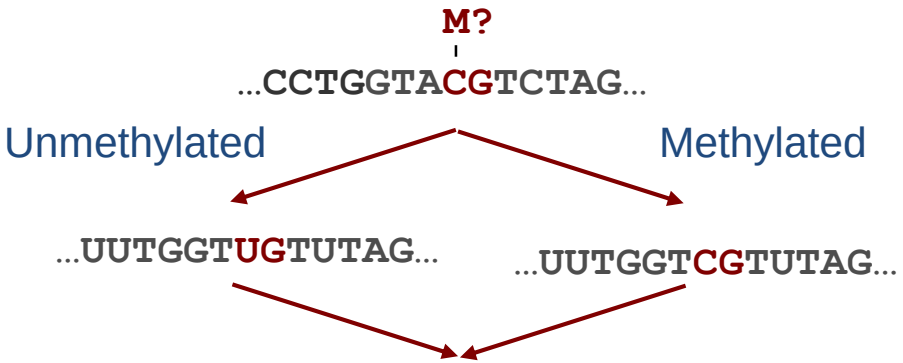
Picture: Terese Winslow, 2001

Genome-scale studies



Genes → Genome

Bisulfite sequencing



Bisulfite conversion

HT sequencing

GGTCGTTTAG

TGGTTGTT

TTGGTTGTT

TTTGGTTGTTTAG

TTTGGTTGT

GTTGTTTAG

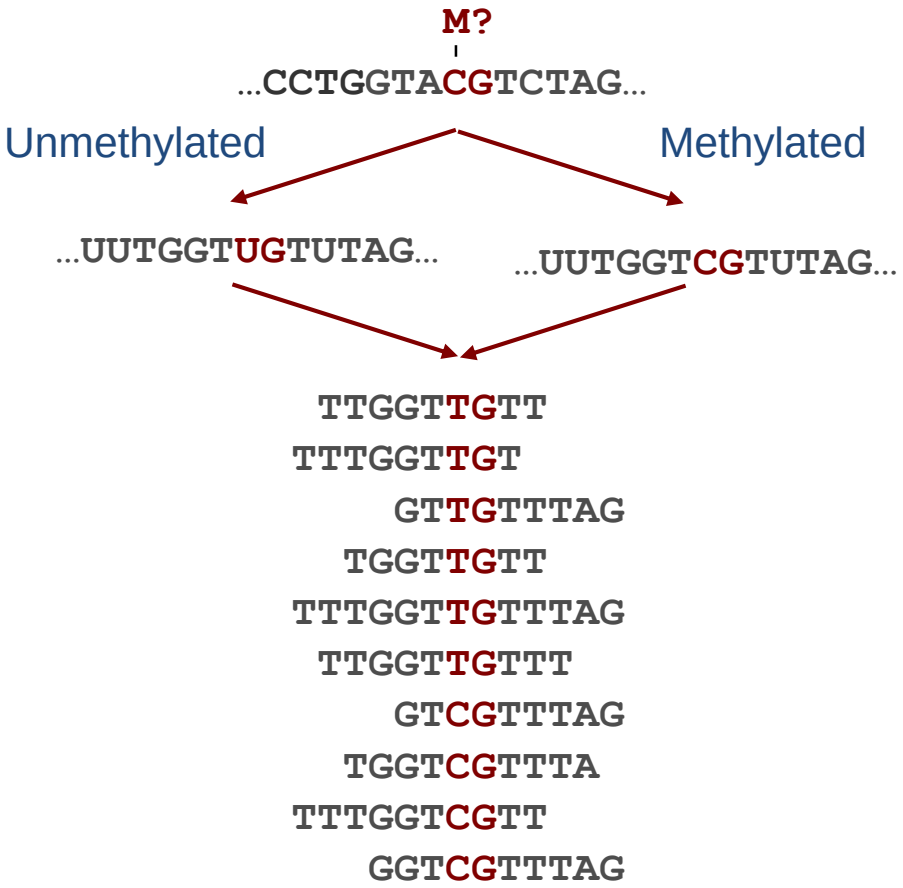
GTCGTTTAG

GGTCGTTTAG

TTTGGTCGTT

TTGGTTGTTT

Bisulfite sequencing



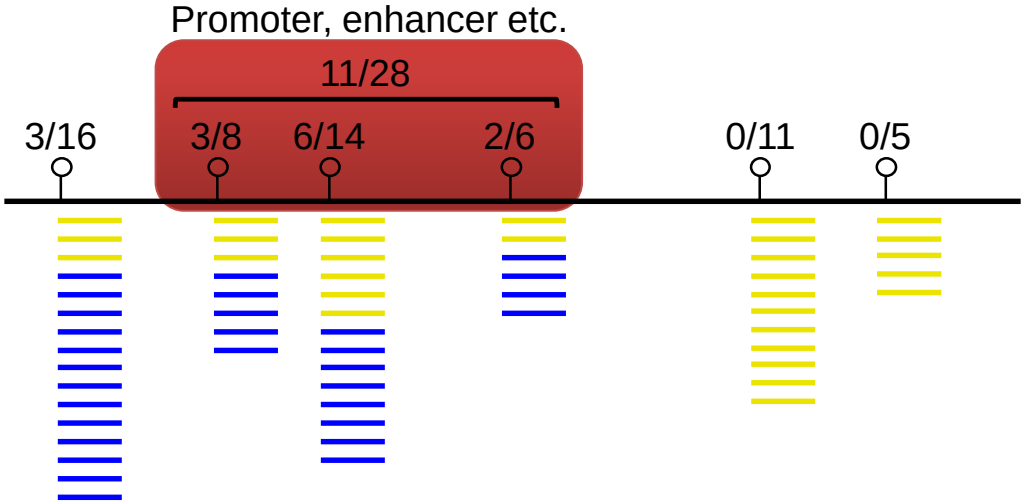
Bisulfite conversion

HT sequencing & Alignment

This fake example
10x coverage, 4/10=40%
(95% CI, 17-70%) methylated

Genome-scale study of prenatal famine

- Focus on periconceptional exposure: 24 exposed + 24 sibling controls
- Reduced-Representation Bisulfite Sequencing (RRBS)
- Methylation of 1.2M CpG sites after QC and exclusion uninformative sites (mean coverage 28x; call rate 0.998)
- Mapping to genomic features to decrease multiple testing, accumulate evidence over adjacent CpGs and increase interpretability.



Tobi et al. *Nat Commun* 2014

Validated top-hits

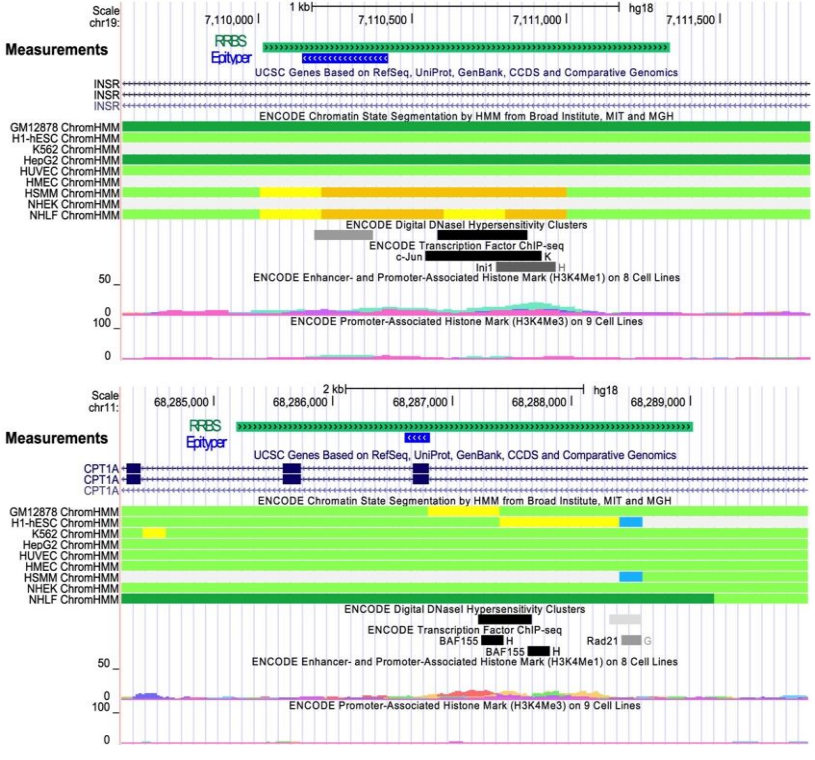
Gene	*Location*	*Function*
SMAD7 SMAD family member 7	25kb downstream	TGFβ signaling, colorectal cancer, β-cell function & development
CDH23 cadherin-related 23	Intragenic	Inner ear development, hearing loss
INSR insulin receptor	Intragenic	Insulin signaling, growth, height
CPT1A carnitine palmitoyltransferase-1	Intragenic	Fatty acid β-oxidation, fatty acid-induced IR and inflammation in adipocytes
KLF13 Krüppel-like factor 13	Intragenic	LDLR regulation, schizophrenia
RFTN1 raftlin	Intragenic	Eye development, obesity

Tobi et al. *Nat Commun* 2014

LUMC

Towards causality

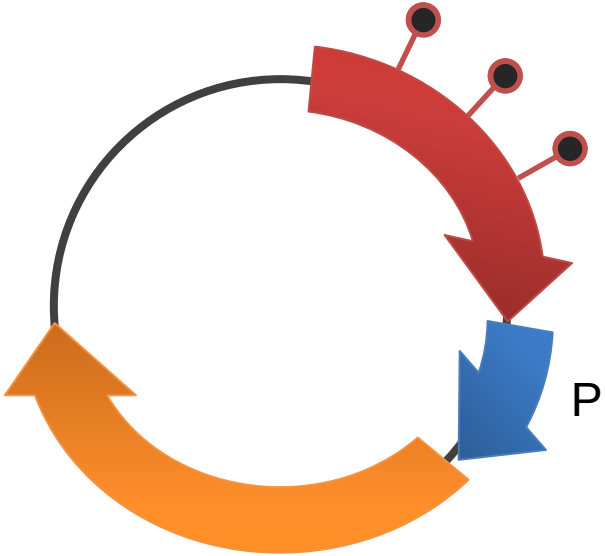
- *In silico* annotation-based predictions of DMR functionality



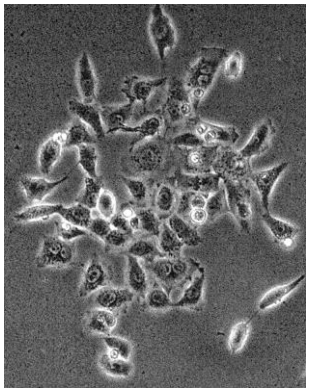
Towards causality

- *In silico* annotation-based predictions of DMR functionality
- *In vitro* testing DMR functionality

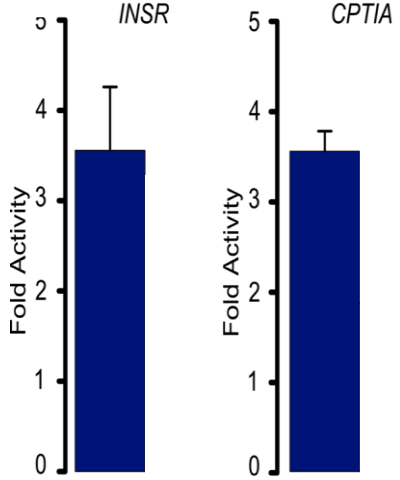
DMR/Suspected enhancer



Promoter



Embryonic kidney cell line HEK-293



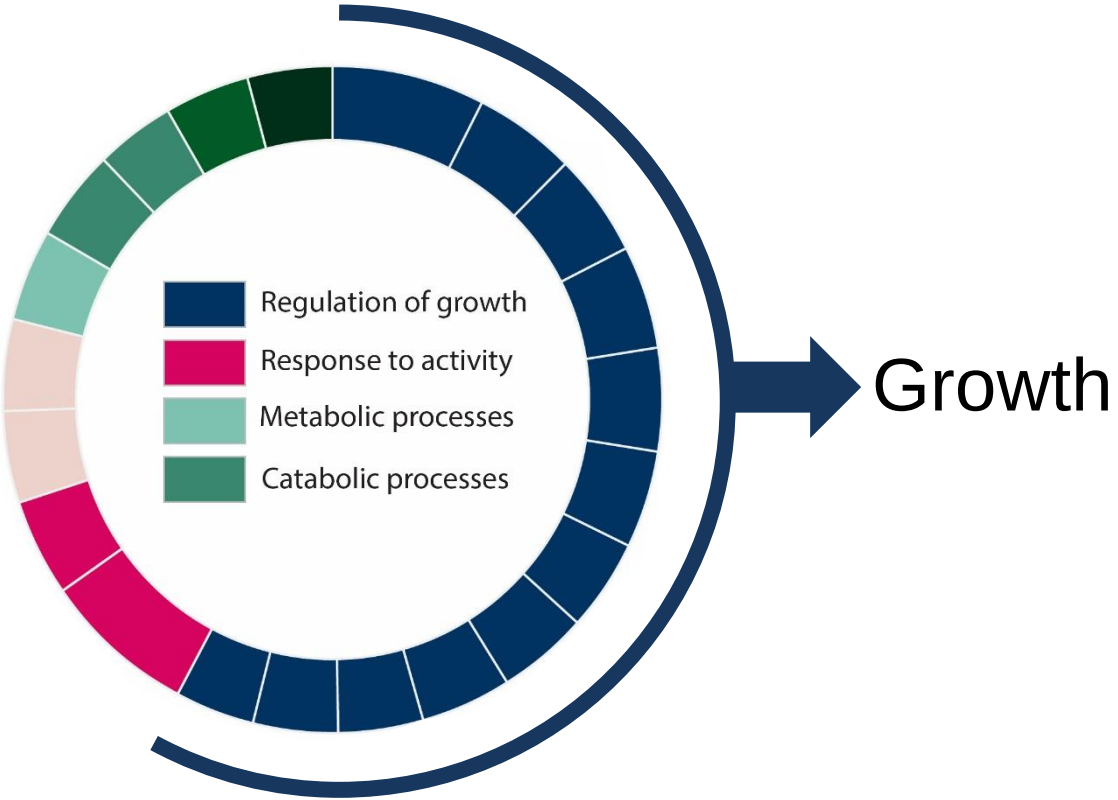
Luciferase

Towards causality

- *In silico* annotation-based predictions of DMR functionality
- *In vitro* testing DMR functionality
- *In vivo* experiments in animals (moving from principles to testing specific human outcomes), short-term interventions in humans, human cells.
- Integrative genomics from genome and epigenome to transcriptome and further
- Causal inference testing statistical approach to evaluate whether DNA methylation mediates associations between prenatal adversity and later-life outcomes

Tobi et al. *Science Adv* 2018

Genome-scale view



Tobi et al. *Nat Commun* 2014

Epigenetic changes after prenatal famine

- Exposure to famine during early development is associated with persistent epigenetic differences in humans.
- DNA methylation differences are modest but extend into biological pathways.
- DNA methylation signatures identified link prenatal famine exposure to growth and metabolism.

The nature epigenetic signatures

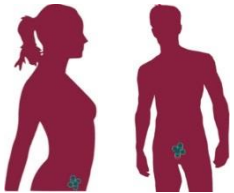
DNA methylation signature of an adverse prenatal environment

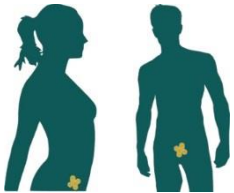
The nature epigenetic signatures

Plasticity

Damage or constraint	Immediate adaptive developmental plasticity	Predictive adaptive developmental plasticity	Epigenetic selection
Inability to sustain normal epigenetic remodeling	Activation of adaptive pathways promoting survival in utero	Activation of adaptive pathways promoting fitness in adulthood	Random variation and selection of fit embryo

DNA methylation signature of an adverse prenatal environment



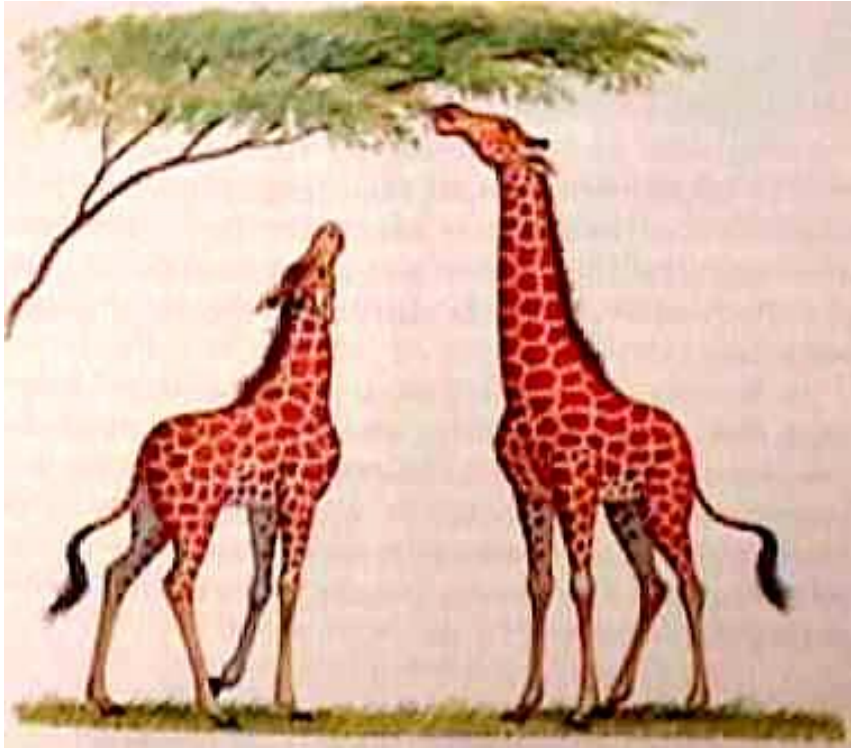


LUMC

Transgenerational epigenetics?

Lamarckism revisited – the inheritance of acquired traits

Jean-Baptiste Lamarck
(1744-1829)



Transgenerational epigenetics?

Intra-generational (or inter-; ‘effects’)

Inter-generational (or multi-; ‘transmission’)

Trans-generational (‘inheritance’)

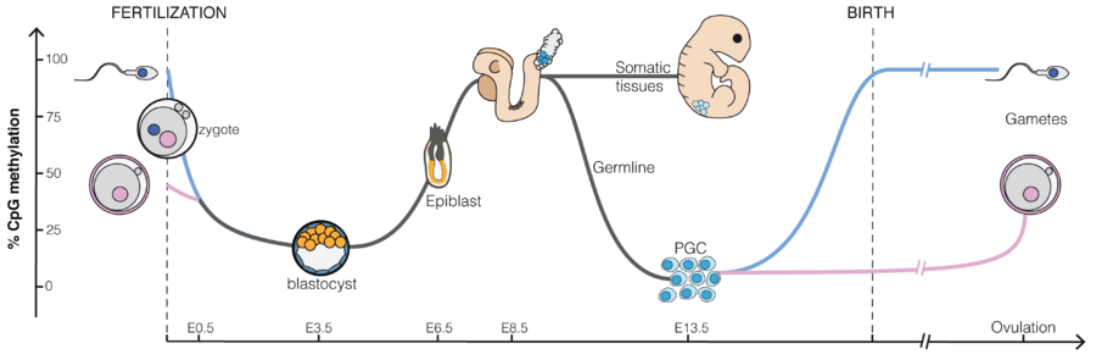
Unlikely, certainly in humans

Evolutionary arguments

- No evidence for advantage

Mechanistic arguments

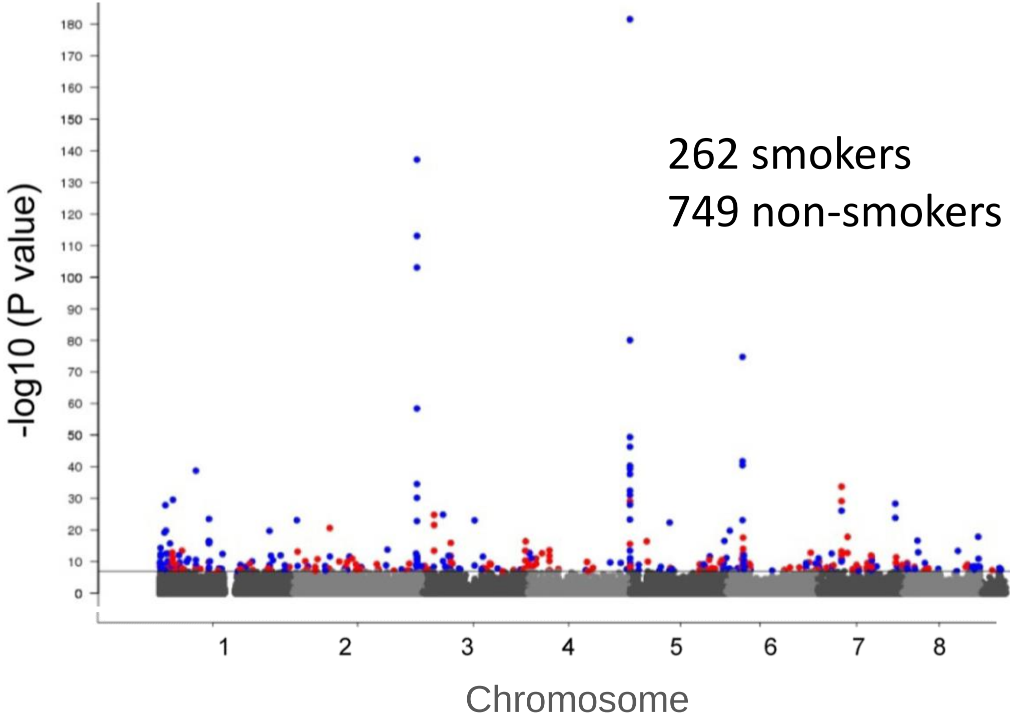
- Extensive reprogramming




THE LIFE & AGE OF WOMAN
STAGES OF WOMAN'S LIFE FROM THE CRADLE TO THE GRAVE
A smiling infant first she plays
Unconscious of her future days
Her girlish gestures next her shows
The cares which woman life must know
Her ripened beauty all confess
And wonder at her loveliness
A husbands arms in hope and pride
Enclose her now a lovely bride
A mothers anxious love and care
With faithful heart its hers to share
Now to the poor her hands she spews
The blessings of benevolence
Absorbed in household duties now
The weight of toil contents her brow
She now resigns all earthly cares
And lifts her soul in fervent prayer
At eighty years her children stand
Imparted blessings to her bind
The hoary head we all should bless
Who found in ways of righteousness
The body sinks and wastes away
The spirit can not know decay.

Smoking

AHRR gene: *P* not 0.05 but 0.000000000000000000000000000000000000000000
0000000000000000000000000000000000000000000000000000000000000000000
0000000000000000000000000000000000000000000000000000000000000000001



Zeilinger et al. *PLoS ONE* 2013

Principle methylation array

M? M? M? M? x 485,000 (out of 28M)

...CCTGGTACGTCTAGCCGTAATTAGCTCGATTCCGGT...

Bisulfite conversion

unmethylated

methylated

...UUTGGTUGTUTAG...

...UUTGGTCGTUTAG...

unmeth signal

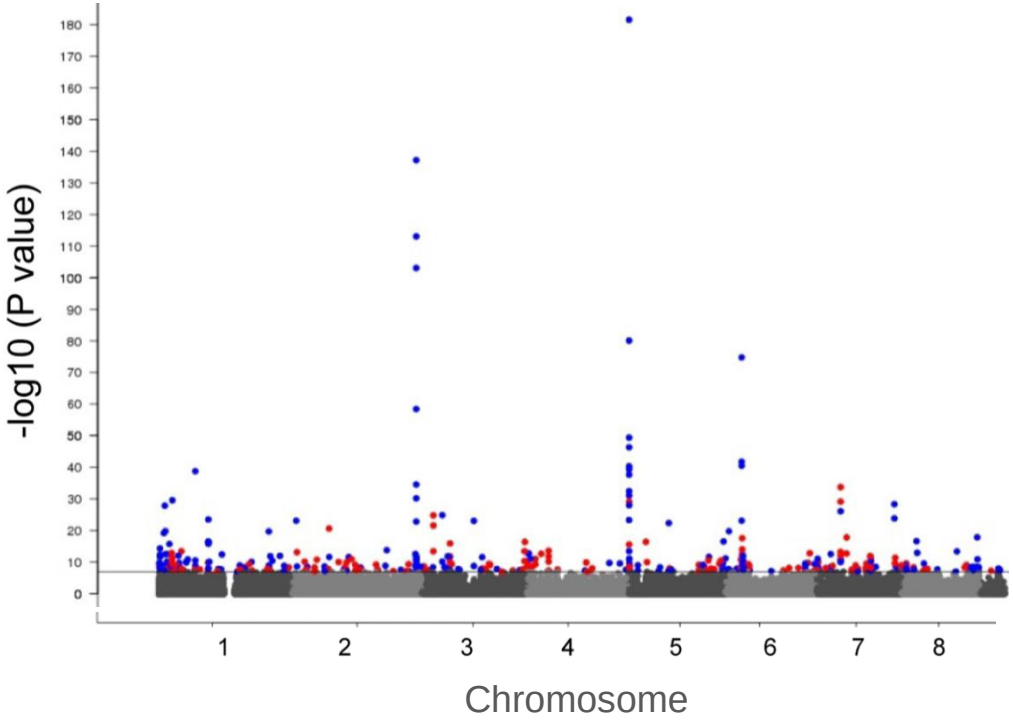
meth signal

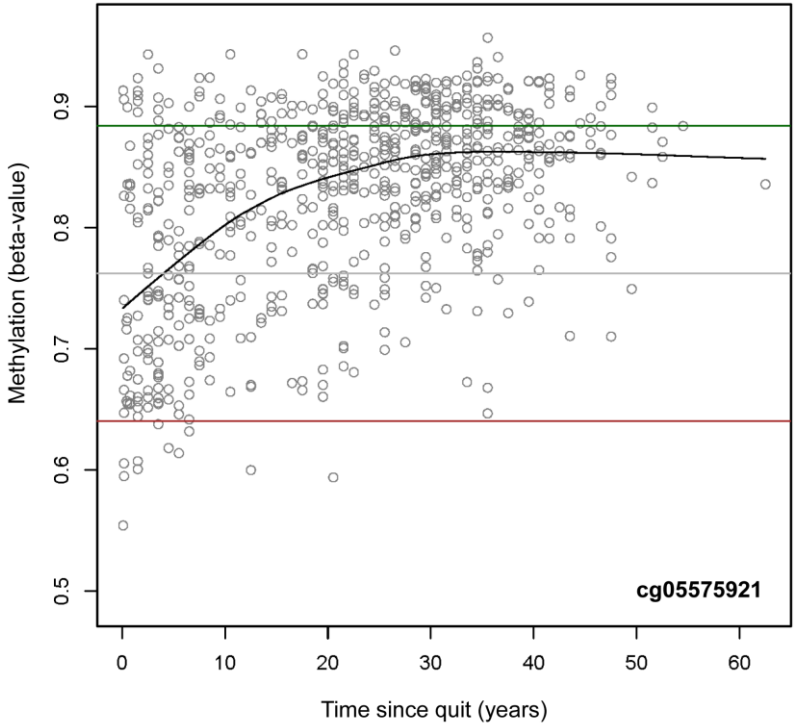
β-value = meth / (meth + unmeth)

To think through

- In the study of smoking-induced DNA methylation changes in blood, counts of the various white cells occurring in blood were measured for every individual and included as confounder in the statistical model. Why?

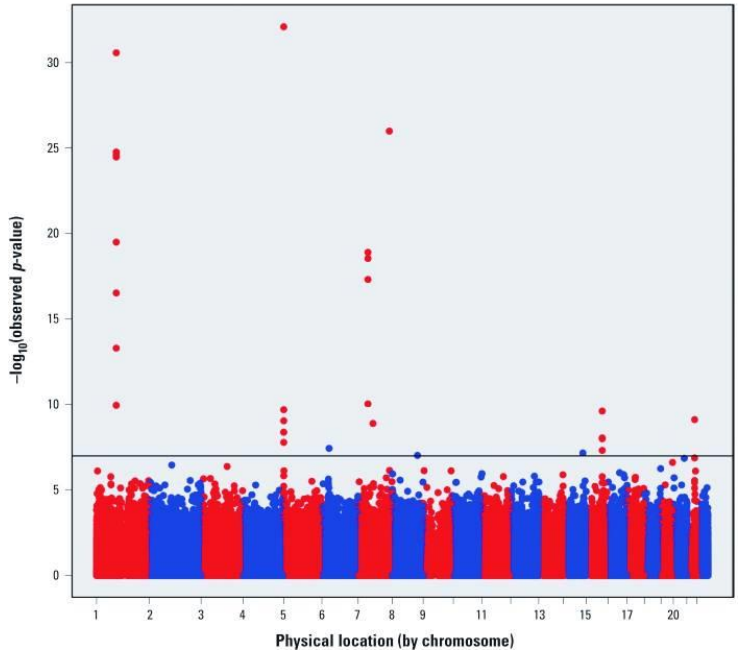
Smoking sticks epigenetically





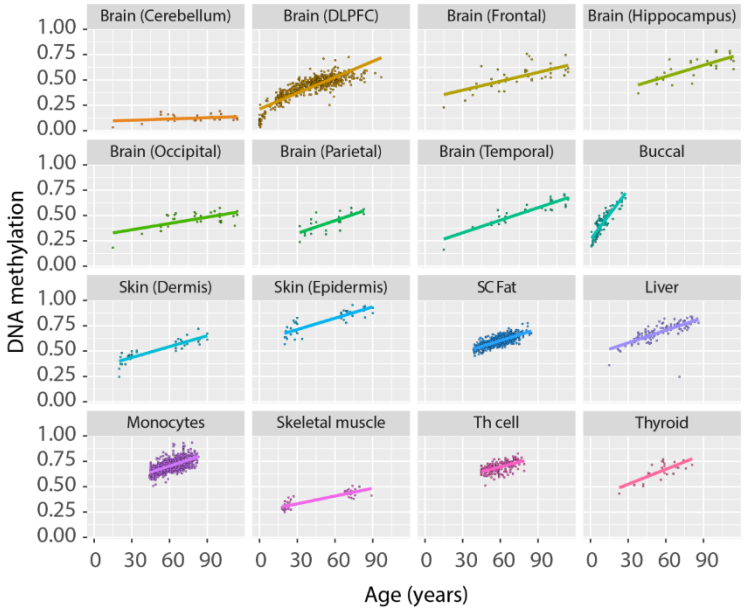
Maternal smoking affects fetus

Methylation (450k array) in 1062 newborns vs. maternal plasma cotinine, a biomarker of smoking.



Joubert et al. *Environ Health Perspect* 2012

DNA methylation and age

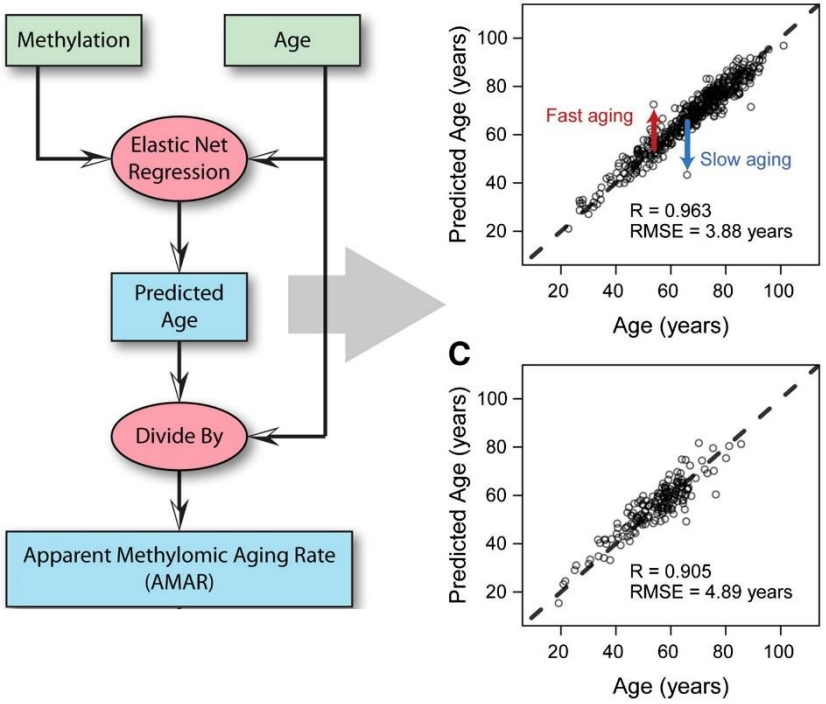


An exceptional case: methylation at CpGs near *ELOVL2* change with age in any tissue.

Slieker et al. *Epigenetics Chromatin* 2018

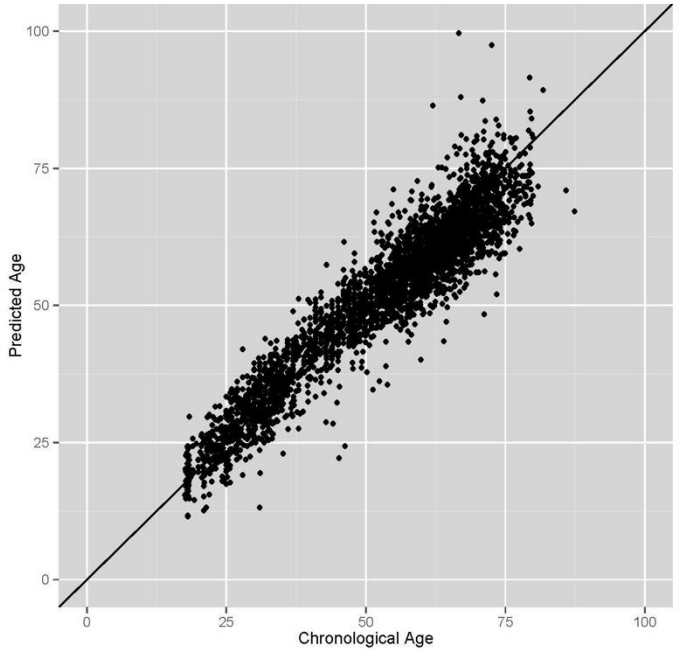
DNA methylation and age

DNAm at 450 thousand CpGs →



Hannum et al. *Mol Cell* 2013

Our age is in our DNA methylation



Epigenetic clock by Horvath of 353 CpGs (*Genome Biol* 2013) applied to own data (N>3000).

DNAm changes and mortality

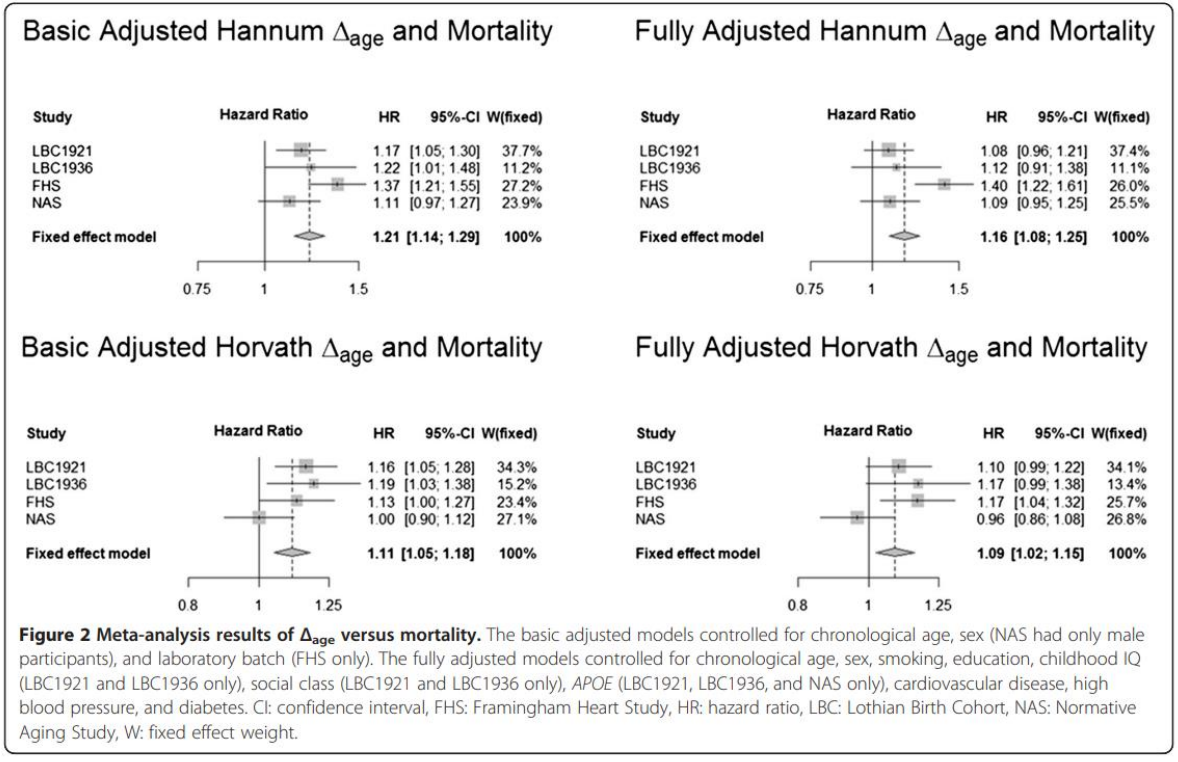
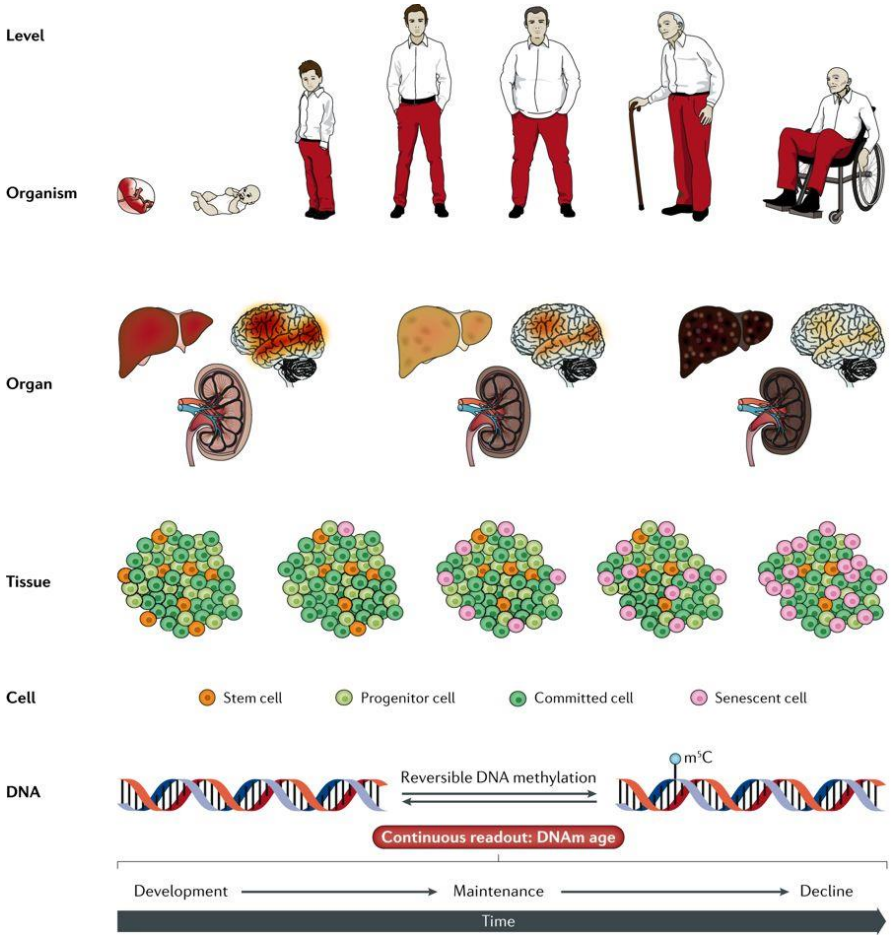


Figure 2 Meta-analysis results of Δ_{age} versus mortality. The basic adjusted models controlled for chronological age, sex (NAS had only male participants), and laboratory batch (FHS only). The fully adjusted models controlled for chronological age, sex, smoking, education, childhood IQ (LBC1921 and LBC1936 only), social class (LBC1921 and LBC1936 only), *APOE* (LBC1921, LBC1936, and NAS only), cardiovascular disease, high blood pressure, and diabetes. CI: confidence interval, FHS: Framingham Heart Study, HR: hazard ratio, LBC: Lothian Birth Cohort, NAS: Normative Aging Study, W: fixed effect weight.

…. 40% of inter-individual differences in Δage can be attributed to genetic factors.

Marioni et al. *Genome Biol* 2015

Biological implications?



Horvath and Raj.
Nat Rev Genet 2018

Conclusions

- Across the whole life course, from intrauterine life to adulthood, the environment continuously influences the epigenome.

- DNA methylation changes precisely track chronological age and may also mark biological age.