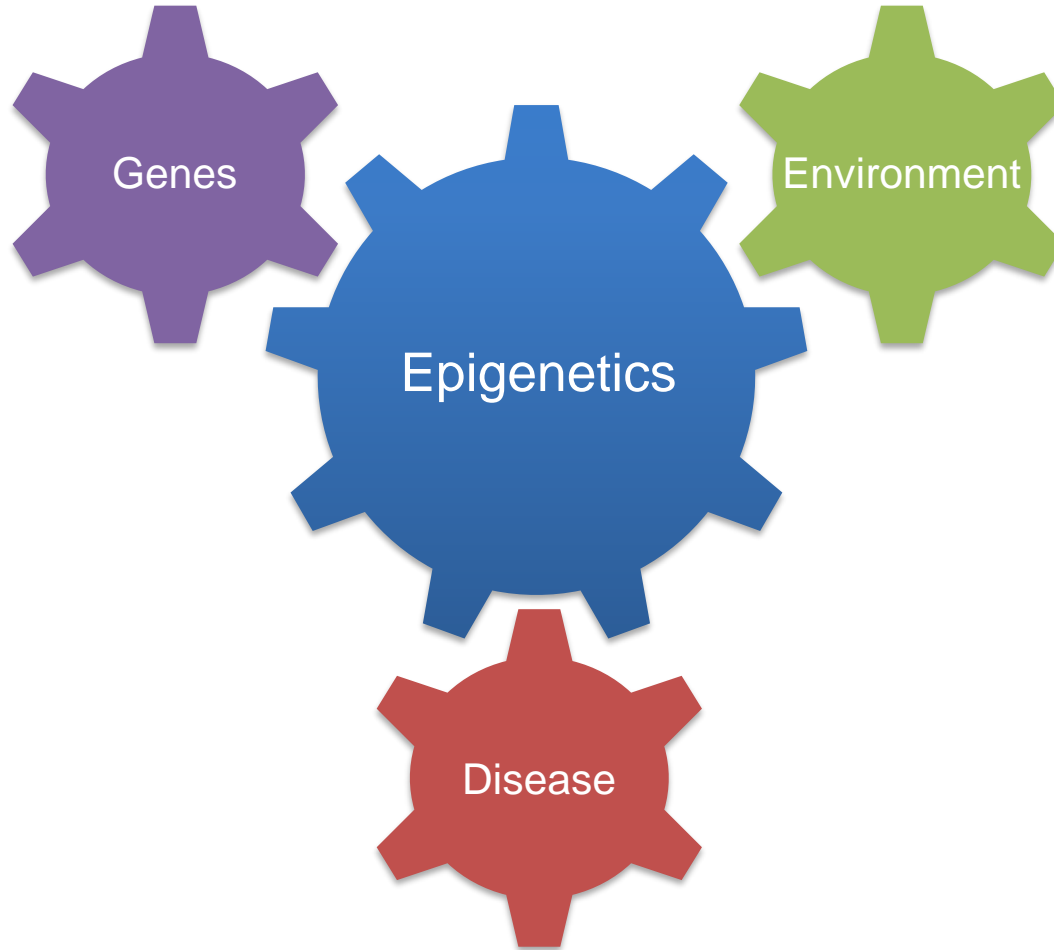


Epigenetics and the Lifecourse

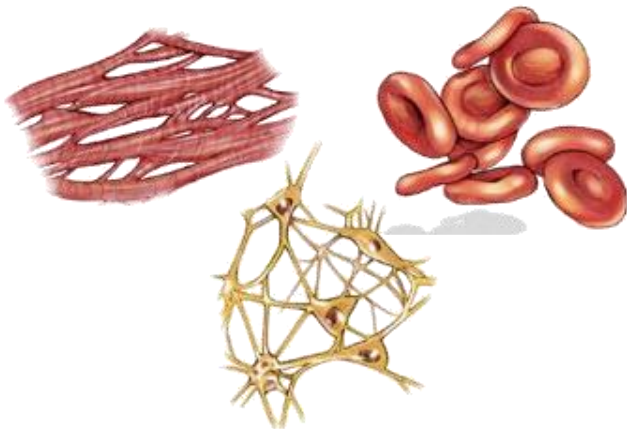
Bas Heijmans
Molecular Epidemiology
Leiden University Medical Center
The Netherlands
bas.heijmans@lumc.nl

FOS MDS – 30 November 2021



Roles epigenetics: variation and memory

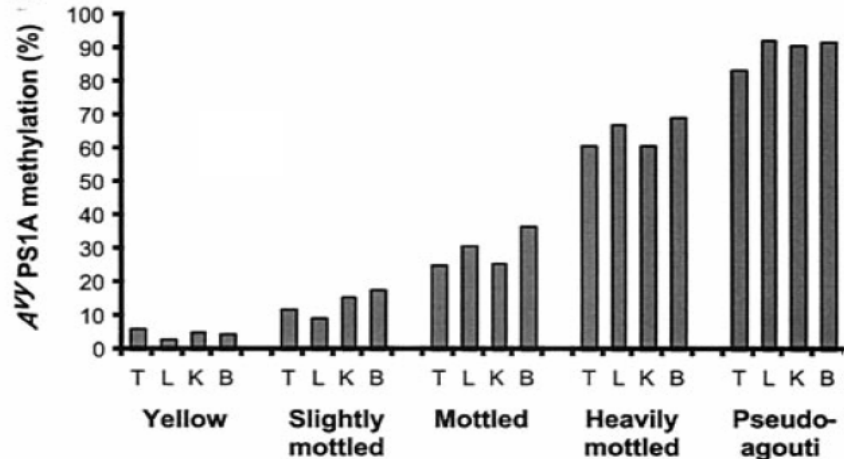
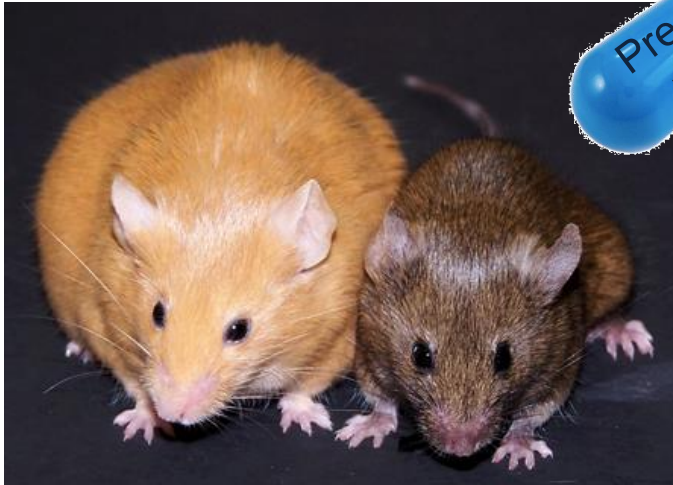
1. Development and cell differentiation
→ 1 DNA molecule, many cell types within an individual.
2. Interface DNA and environment
→ 1 DNA molecule, multiple possible phenotypes.



Epigenetics of coat color

Inbred agouti mice: same DNA sequence

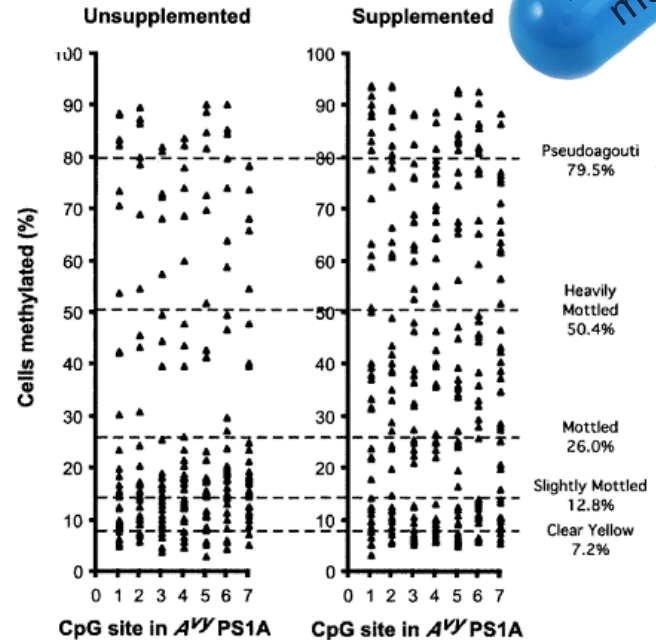
- Methyl supplementation diet pregnant females
- Recorded as higher methylation of *agouti* gene
- Expressed as no synthesis of yellow colour
- Propagated across tissues
- Retained into adulthood



Waterland and Jirtle. *Mol Cell Biol* 2003

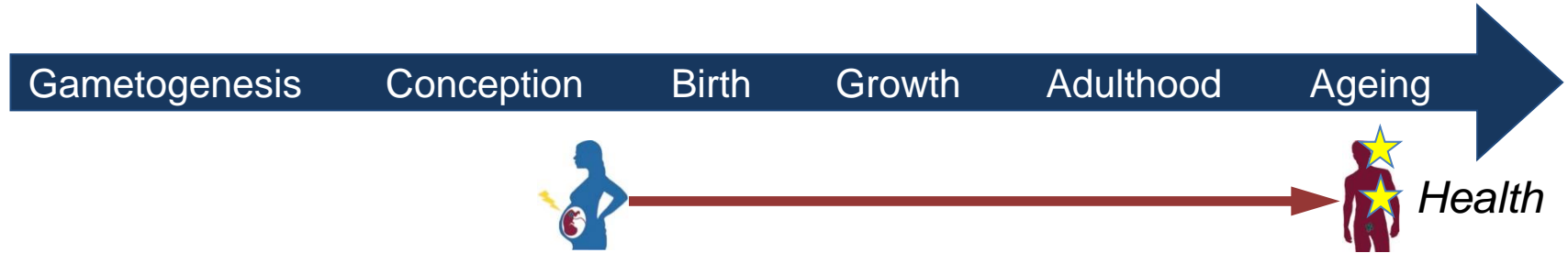
Epigenetics: the memory of the DNA

messy





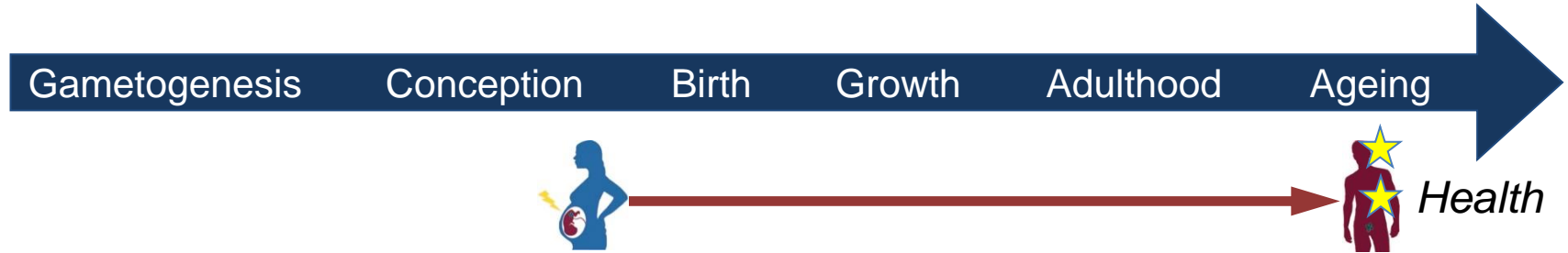
Dutch Hunger Winter



- 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.



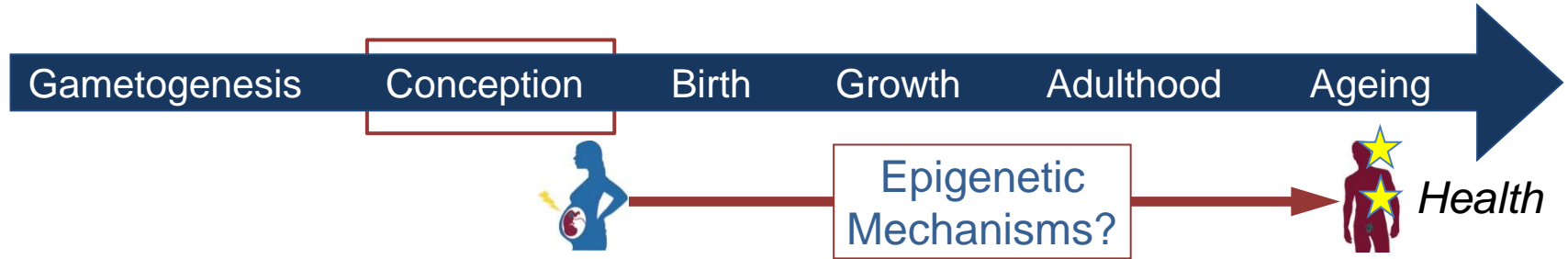
Study design



- **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.

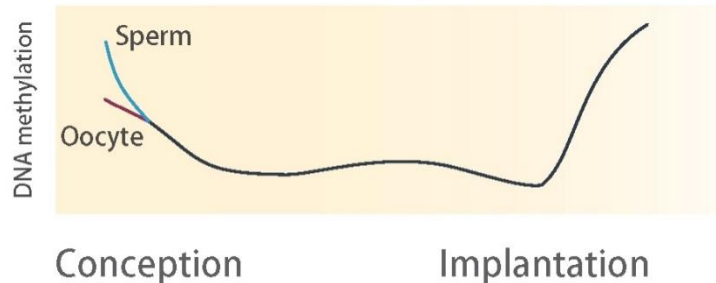


Study design



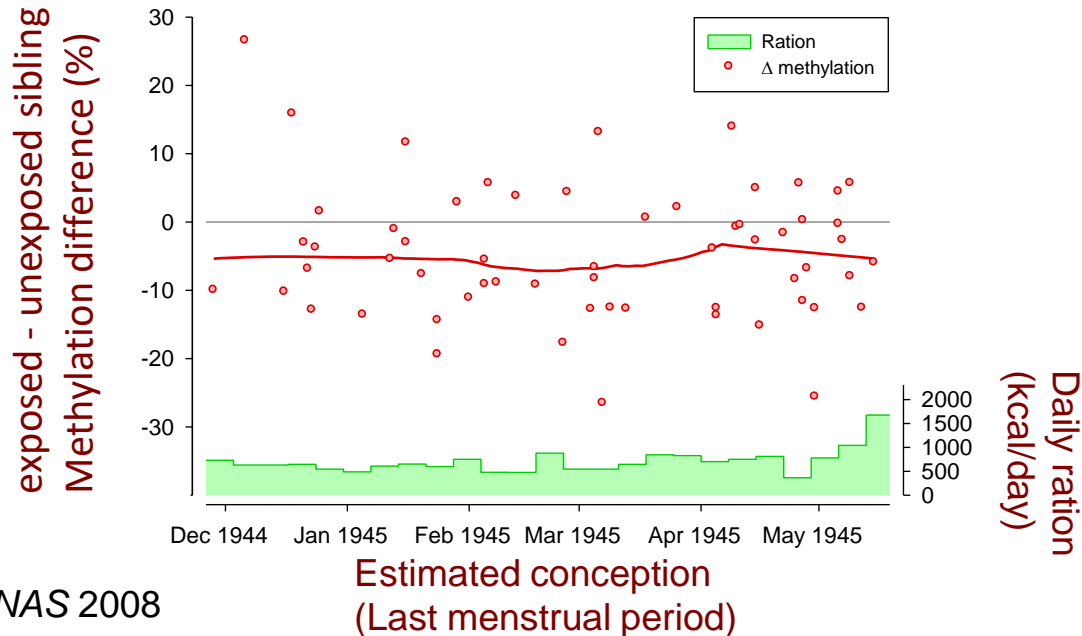
Focus on early gestation (ie. conception during Famine)

- **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

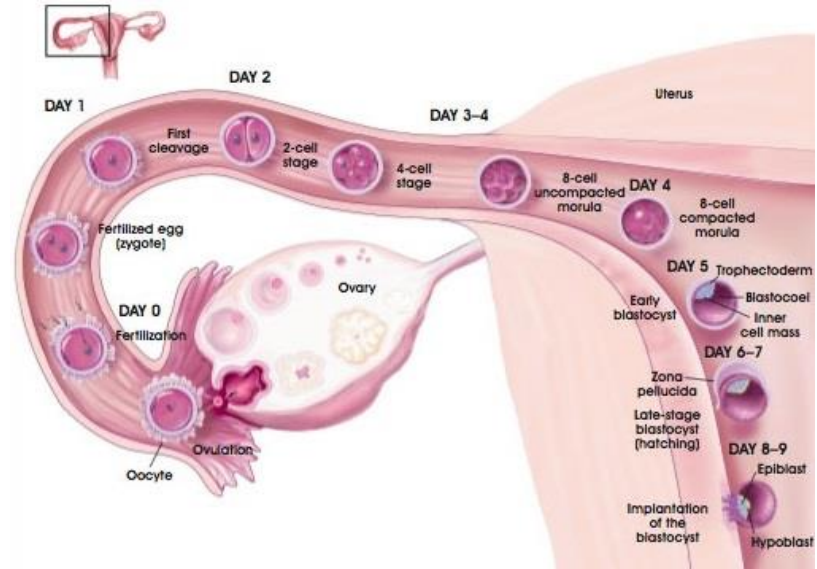
- DNA from blood of 60 individuals who were periconceptionally exposed to the Famine 6 decades ago.
- *IGF2*: Prenatal growth factor, also implicated in metabolic regulation and memory; epigenetically controlled.



Looking across 16 genes

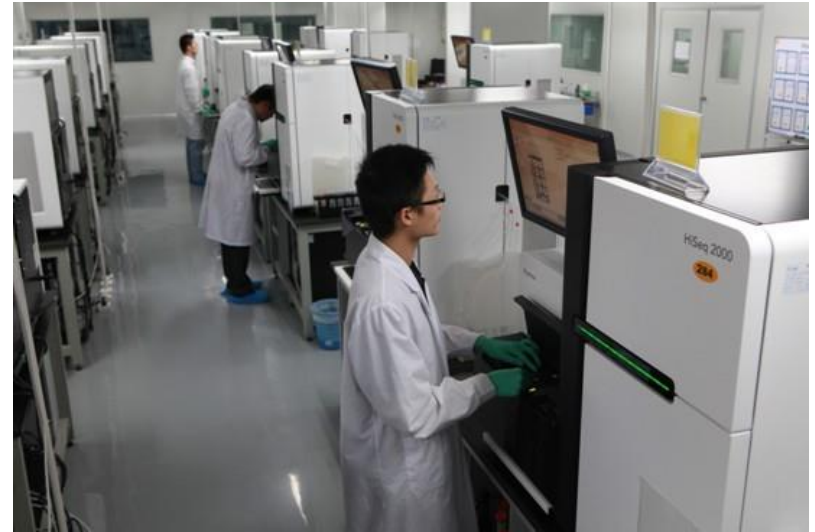
Gene	Early	Late
<i>IGF2</i>	↓	
<i>GNAS</i>	↑	↓
<i>INSIGF</i>	↓	
<i>IL10</i>	↑	
<i>LEP</i>	↑	↑
<i>ABCA1</i>	↑	

Tobi et al. *Hum Mol Genet* 2009



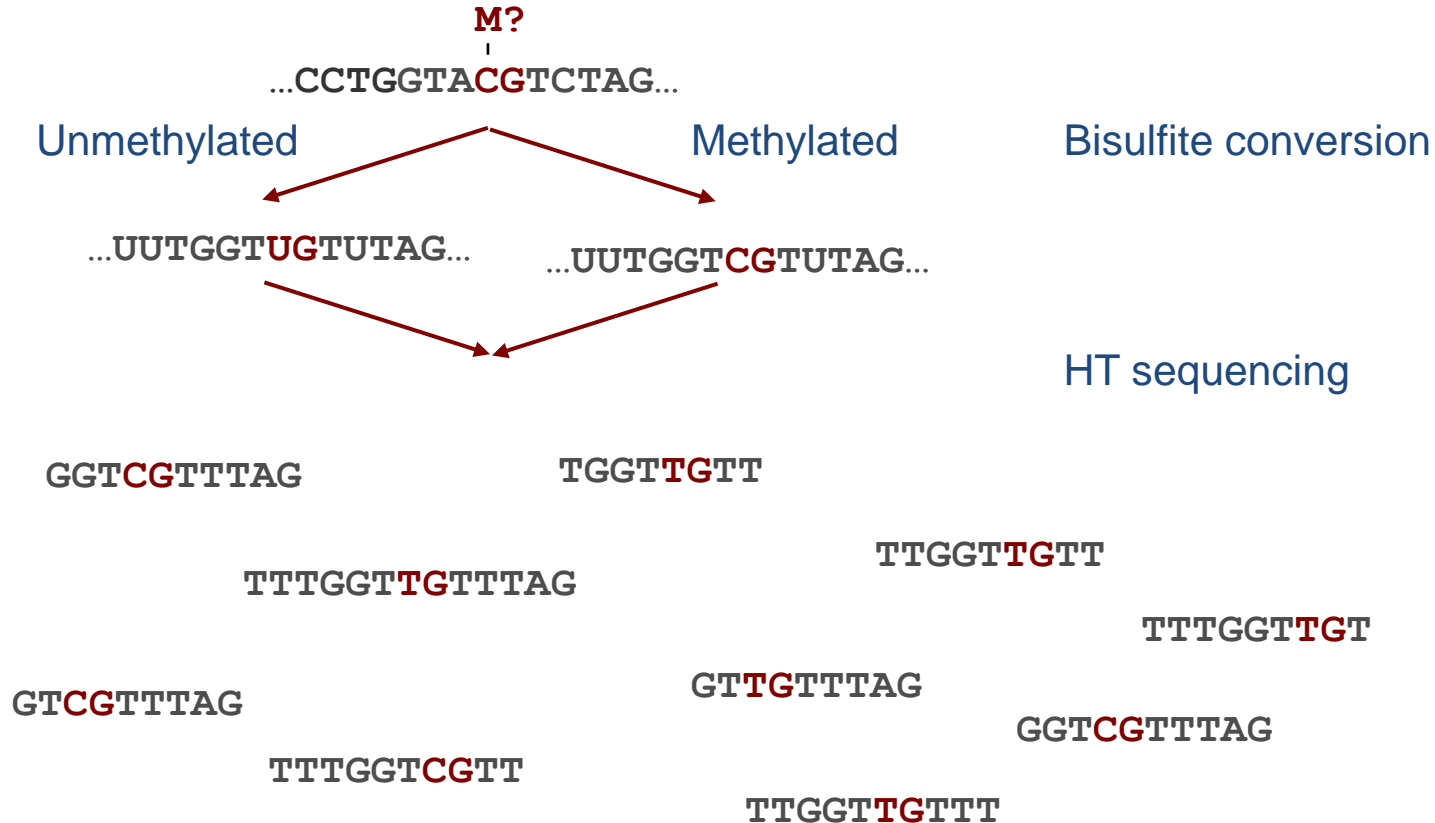
Picture: Terese Winslow, 2001

Genome-scale studies

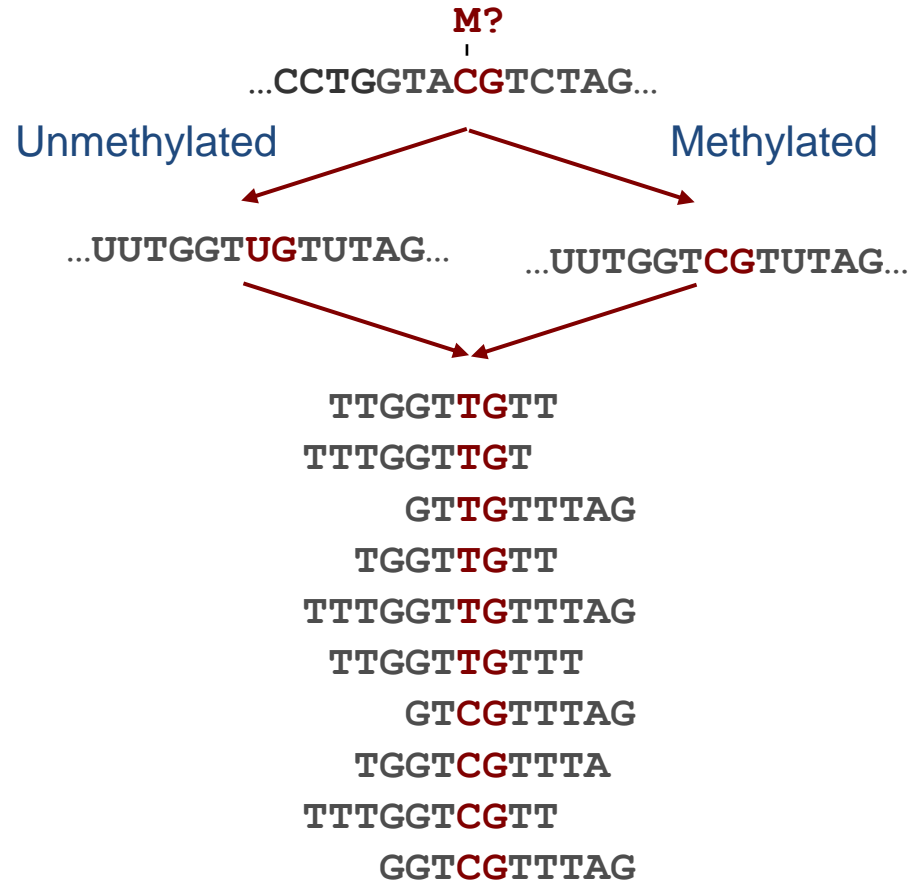


Genes  Genome

Bisulfite sequencing



Bisulfite sequencing



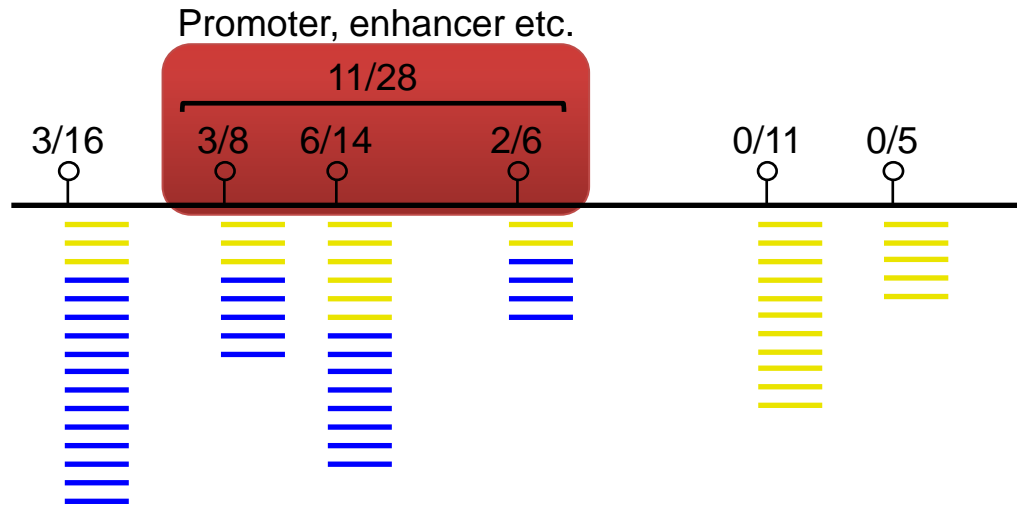
Bisulfite conversion

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**.

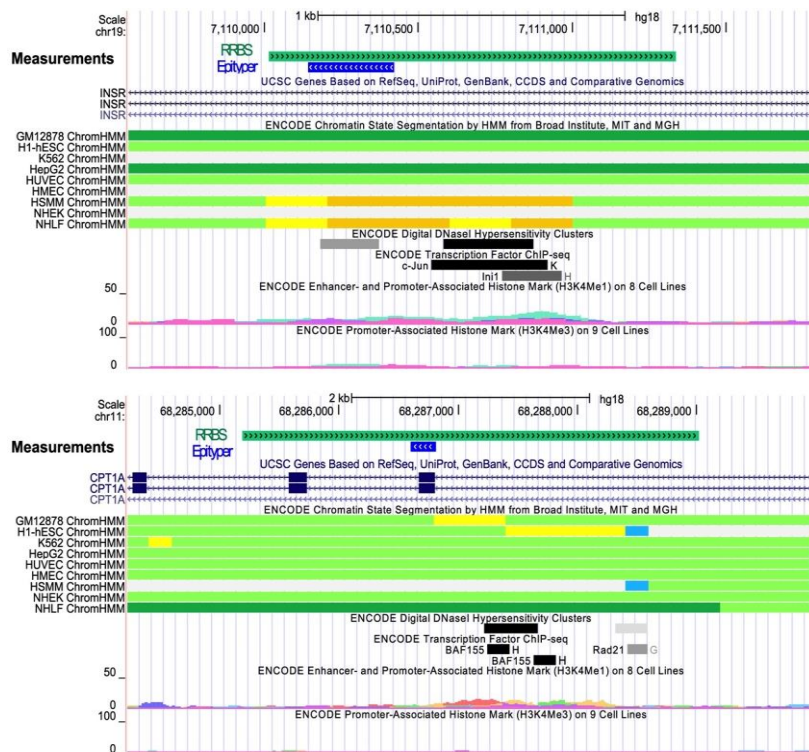


Validated top-hits

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

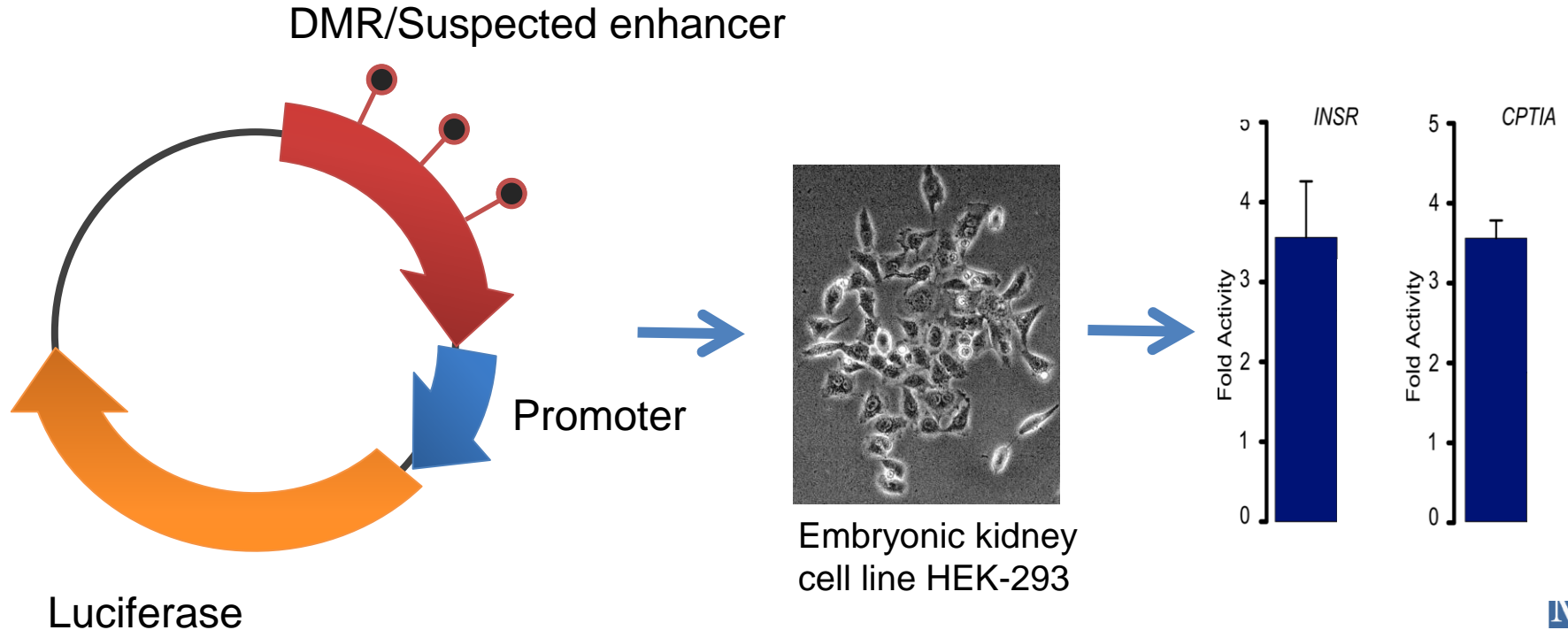
Towards causality

- In silico* annotation-based predictions of DMR functionality

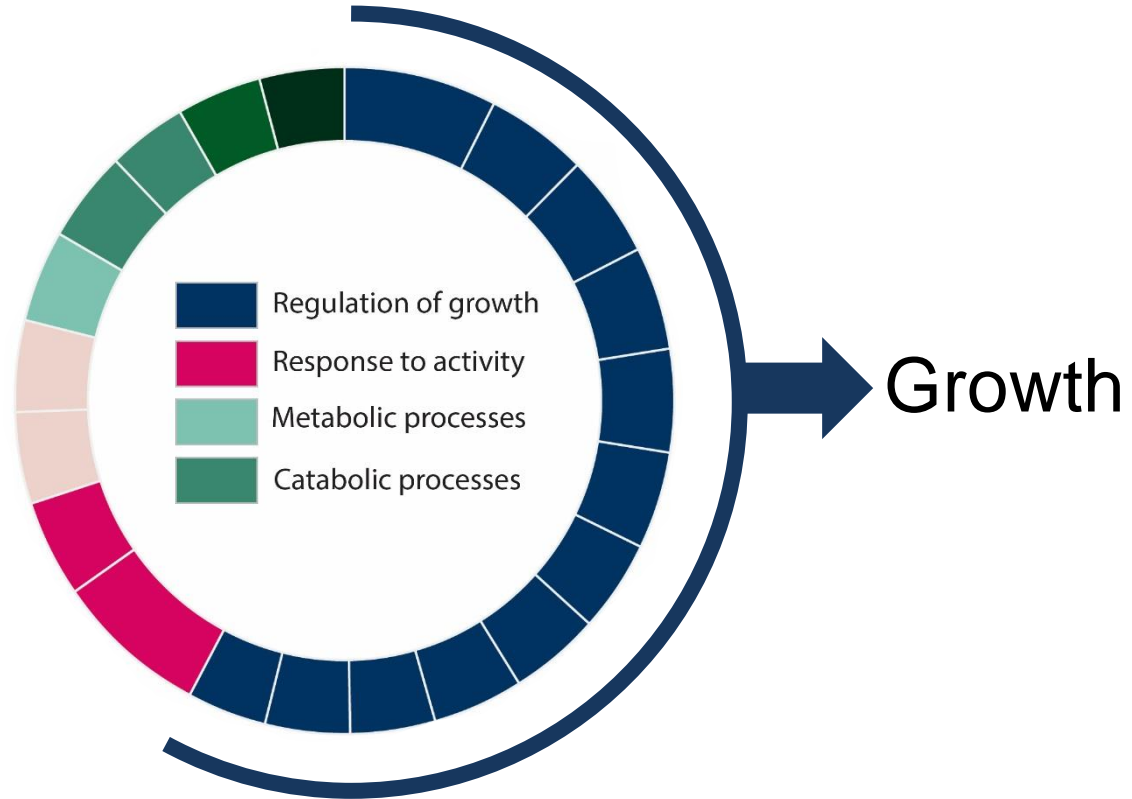


Towards causality

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



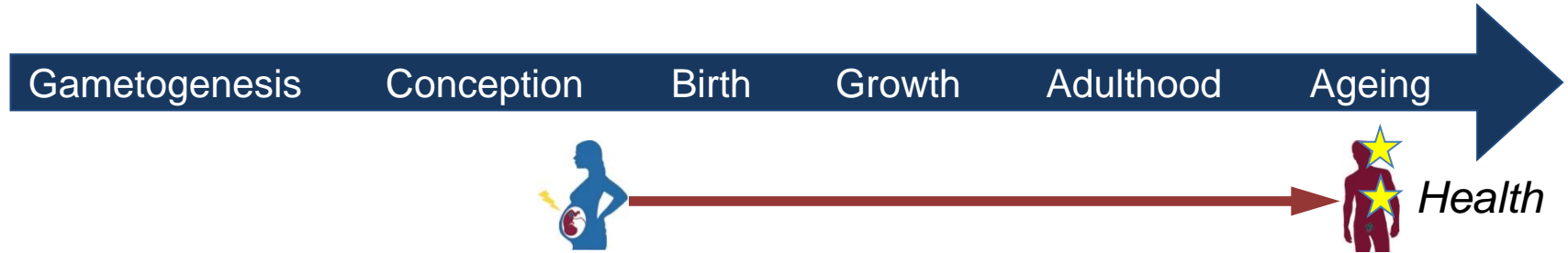
Genome-scale view



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

Epigenetic changes after prenatal famine



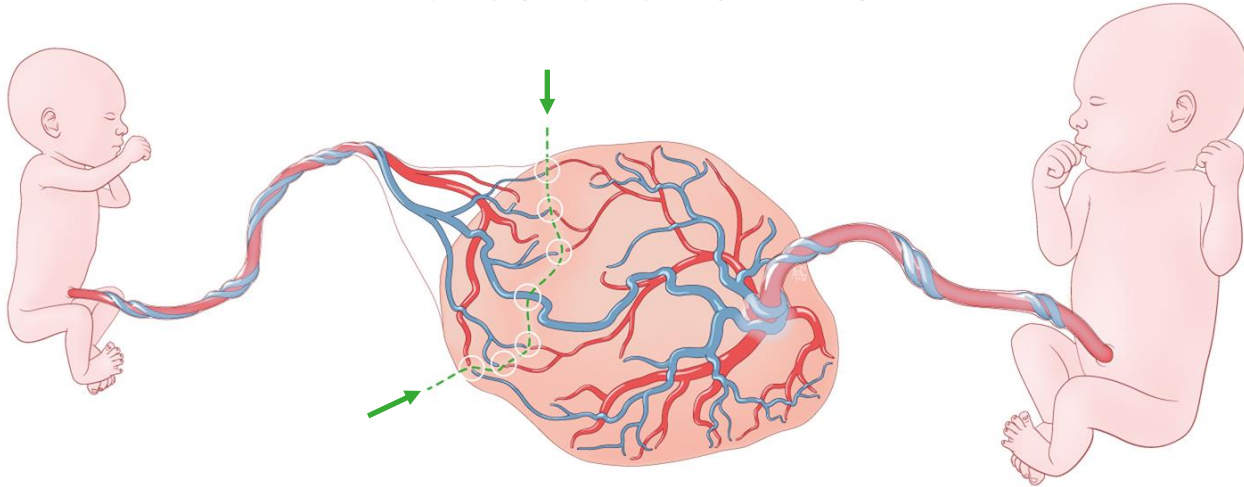
- Exposure to famine during early development is associated with persistent epigenetic differences in humans.
- DNA methylation differences are modest but extend into biological pathways.
- DNA methylation signatures identified link prenatal famine exposure to growth and metabolism.

First 1000 days

Governmental program *Kansrijke start*

A natural experiment

Monochorionic twins



Identical twin, discordant intra-uterine environment

The twin study of the LUMC



National referral center for complicated twin pregnancies (~50 deliveries per year)

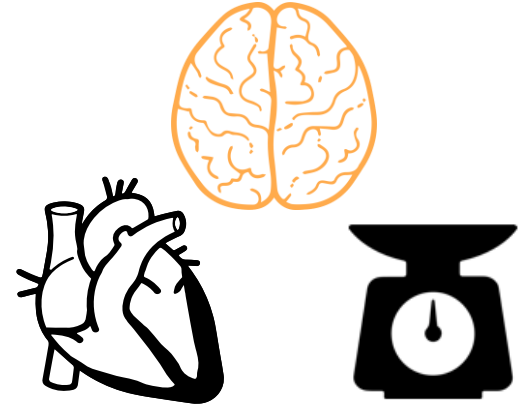




1. Intrauterine
Adversity



2. Epigenetic
Disturbances



3. Childhood health

Mesenchymal stromal cells



2. Epigenetic Disturbances

An advanced proxy for epigenetic dysregulation

- Single cell-type: cellular heterogeneity
- Alive and many: wide range of (epi)genomics and functional assays
- Multipotent: can be differentiated into relevant cell types

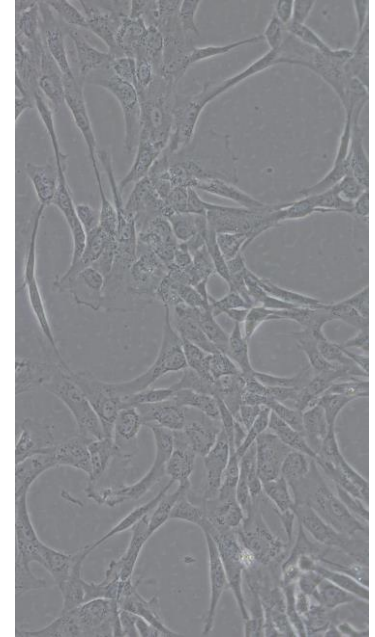
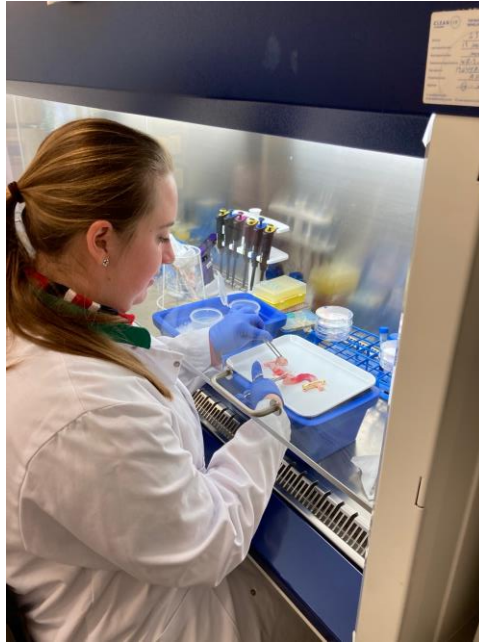
MSCs differences reported for singleton births dependent prenatal environment in regenerative medicine field



Mesenchymal stromal cells



2. Epigenetic Disturbances





1. Intrauterine
Adversity



2. Epigenetic
Disturbances



3. Childhood health

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

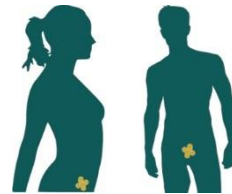
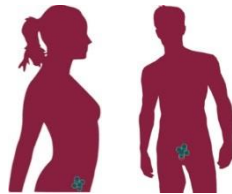
Inability to sustain
normal epigenetic
remodeling

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

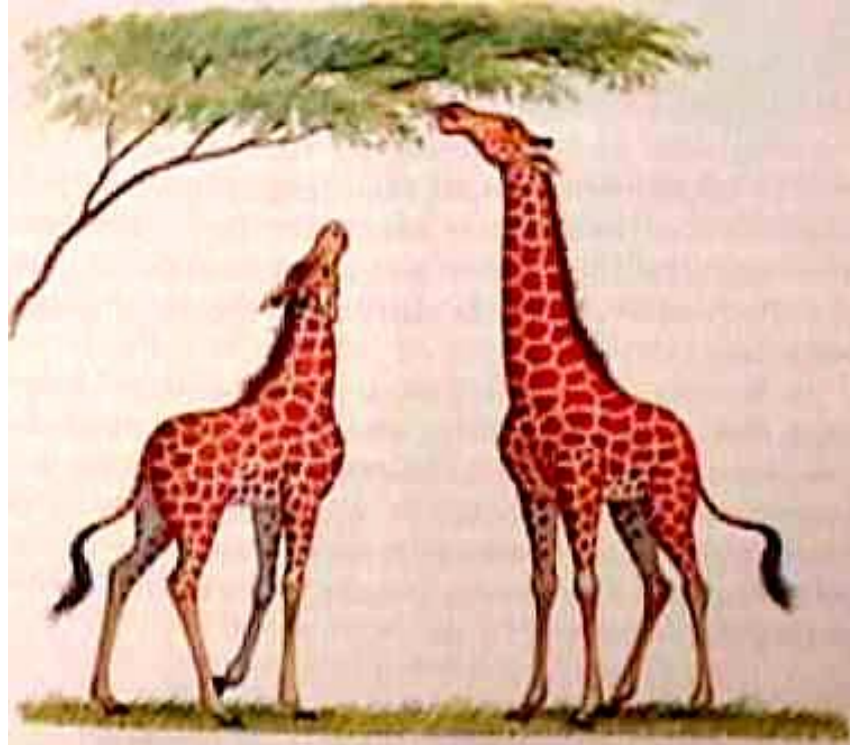


Transgenerational epigenetics?

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')

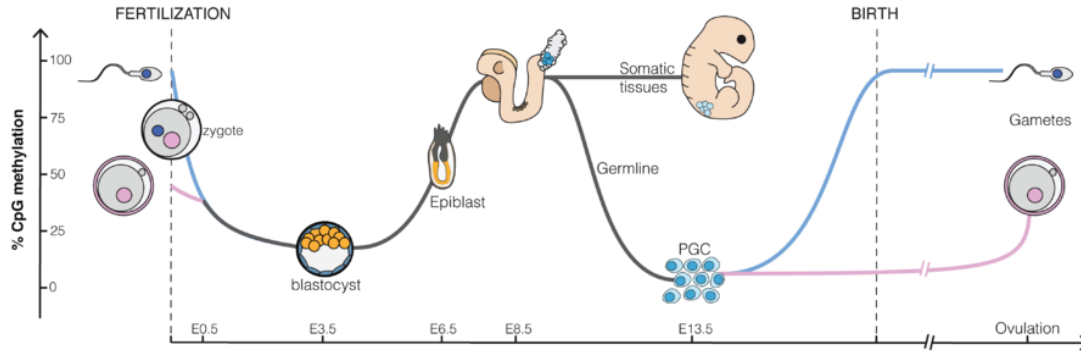
Unlikely, certainly in humans

Evolutionary arguments

- No evidence for advantage

Mechanistic arguments

- Extensive reprogramming





THE
LIFEAGE
OF
WOMAN

STAGES OF WOMAN'S LIFE FROM THE CRADLE TO THE GRAVE

A smiling infant
first she plays
in rambles of
her future days

Her girlish pastimes
sit at her show
The center which woman
life must know

Her ripened beauty
all confess
And wonder
at her loveliness

A husband's arms
in home and pride
Exalt her flow
a lovely bride

A mother's anxious
love and care
With faithful heart
its home to share

Now to the poor
her hand she gives
The blessing of
her love

Abandoned in household
duties now
The weight of toil
contracts her brow

She now resigns
all earthly care
And lifts her soul
in prayer to God

At eighty years
her still abundant
In peaceful blessings
of righteous men

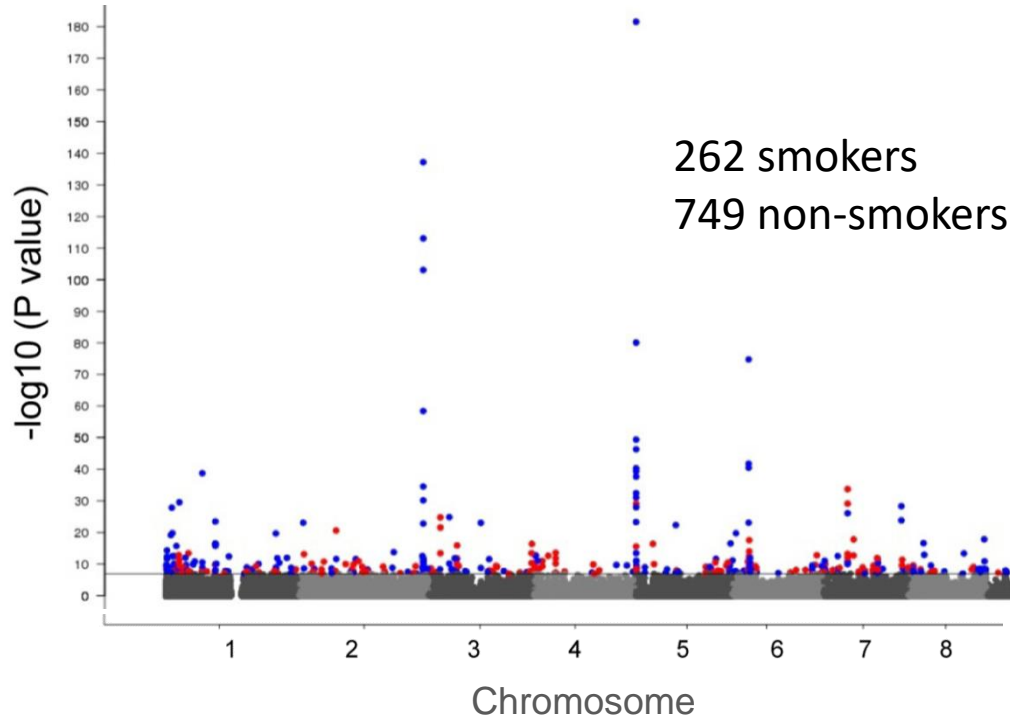
The hoary head
we all should bless
When coming in
of righteous men

The body sinks
and wastes away
The spirit's calm
And serene

Smoking is very unhealthy...



... but a gift to science

[illegible]

Principle methylation array

x 485,000 (out of 28M)

...CCTGGTAC^{M?}GTCTAGC^{M?}CGTAATTAGCT^{M?}CGATTCC^{M?}GGT...

Bisulfite conversion

unmethylated

methylated

...UUTGGT^UGTUTAG...

...UUTGGT^{CG}TUTAG...



unmeth signal

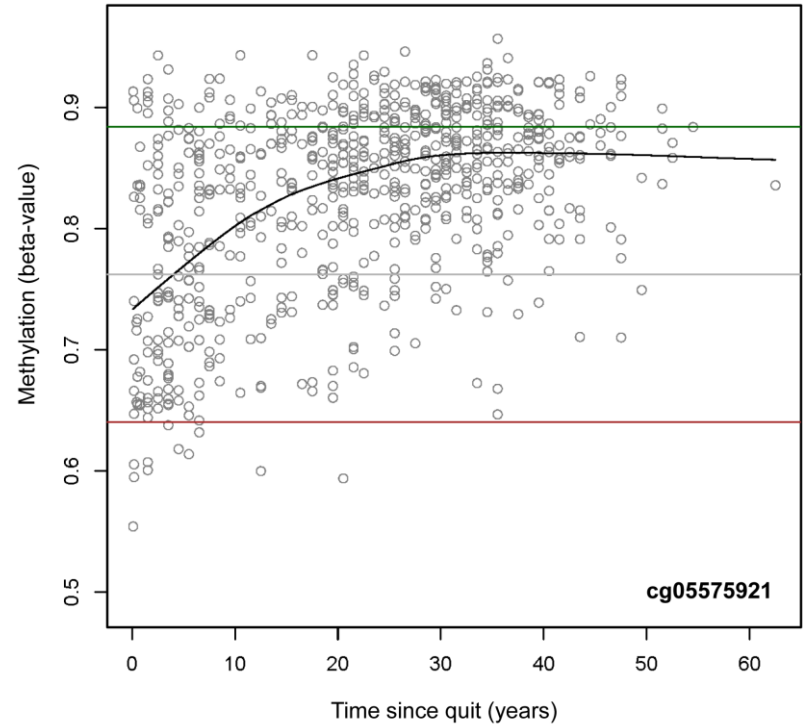
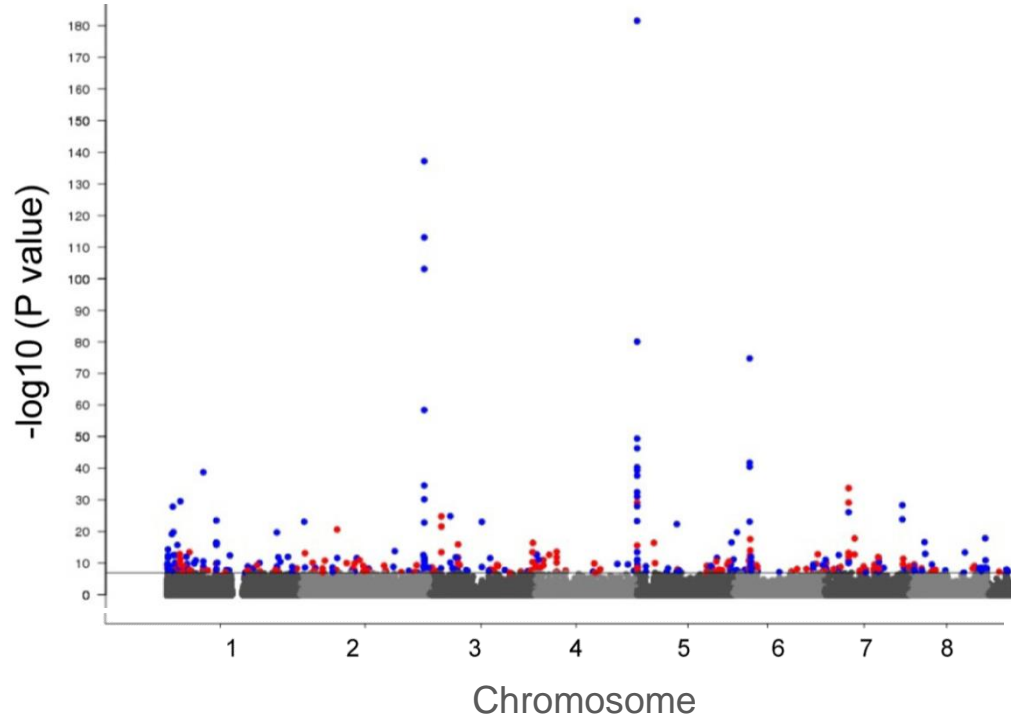
meth signal

$$\beta\text{-value} = \text{meth} / (\text{meth} + \text{unmeth})$$

To think through

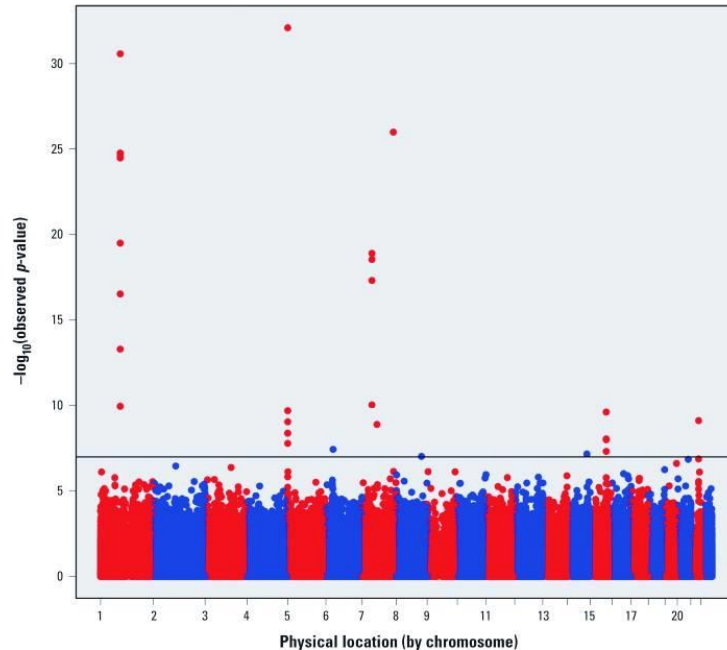
- In the study of smoking-induced DNA methylation changes in blood, counts of the various white cells occurring in blood were measured for every individual and included as confounder in the statistical model. Why?

Smoking sticks epigenetically

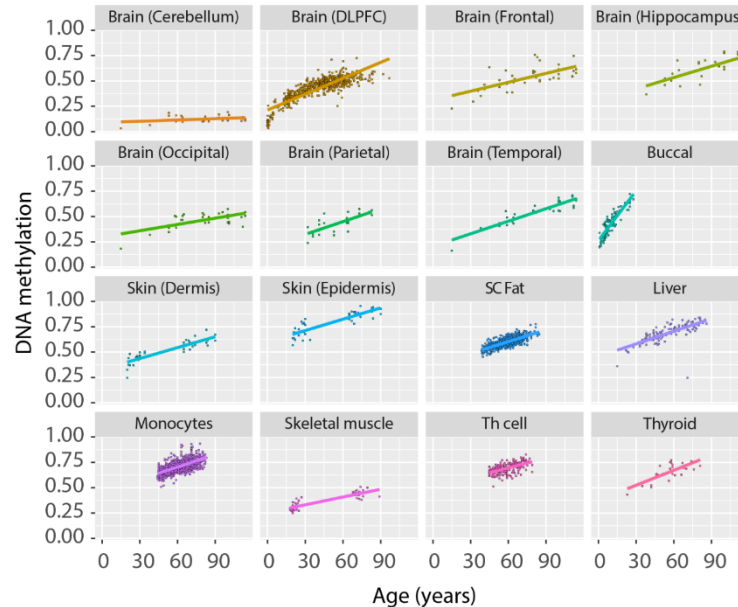


Maternal smoking affects fetus

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



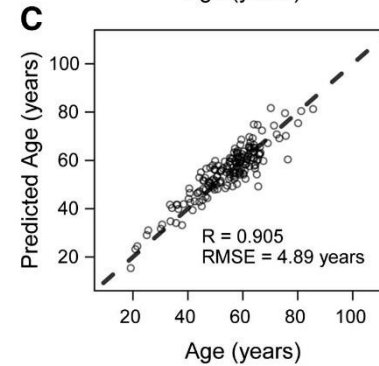
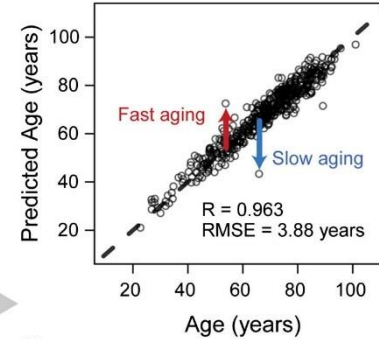
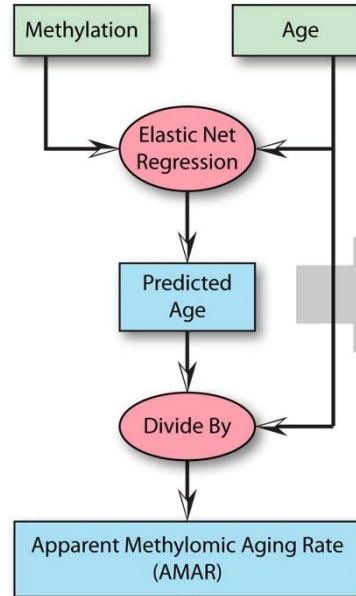
DNA methylation and age



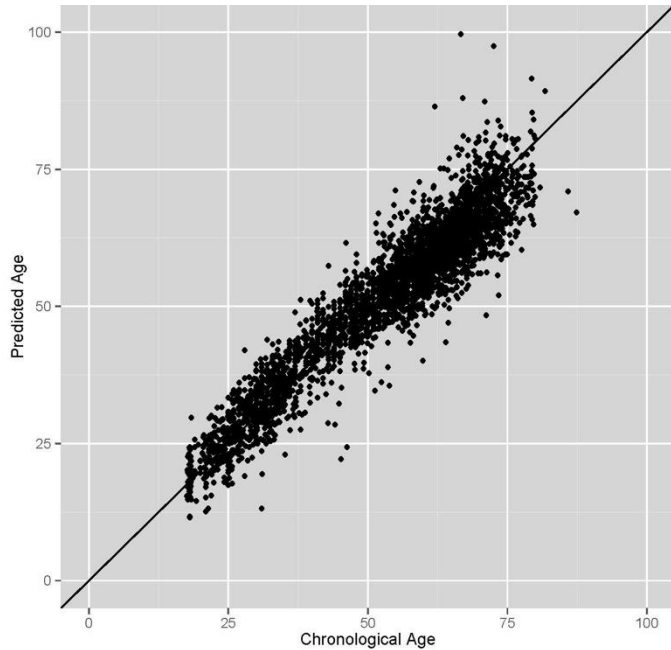
An exceptional case: methylation at CpGs near *ELOVL2* change with age in any tissue.

DNA methylation and age

DNAm at 450 thousand CpGs →



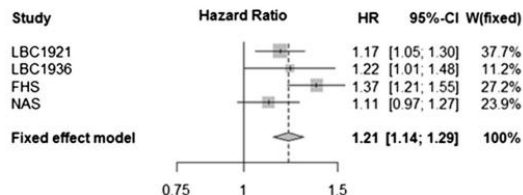
Our age is in our DNA methylation



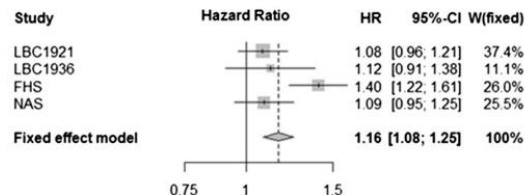
Epigenetic clock by Horvath of 353 CpGs (*Genome Biol* 2013) applied to own data (N>3000).

DNAm changes and mortality

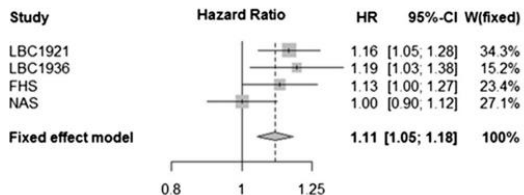
Basic Adjusted Hannum Δ_{age} and Mortality



Fully Adjusted Hannum Δ_{age} and Mortality



Basic Adjusted Horvath Δ_{age} and Mortality



Fully Adjusted Horvath Δ_{age} and Mortality

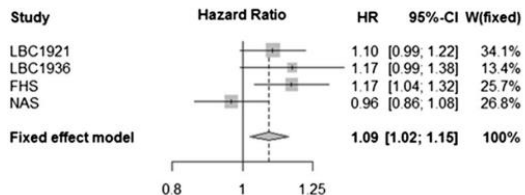
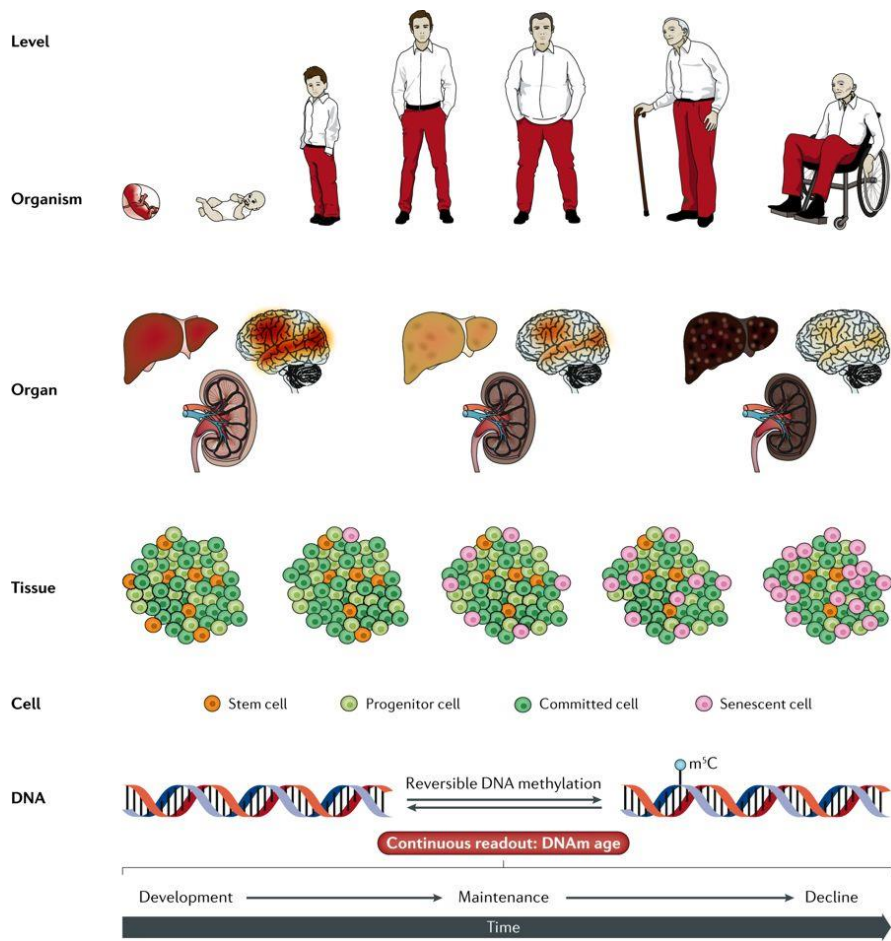


Figure 2 Meta-analysis results of Δ_{age} versus mortality. The basic adjusted models controlled for chronological age, sex (NAS had only male participants), and laboratory batch (FHS only). The fully adjusted models controlled for chronological age, sex, smoking, education, childhood IQ (LBC1921 and 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 Δ_{age} can be attributed to genetic factors.

Biological implications?



Conclusions

- Across the whole lifecourse, from intrauterine life to adulthood, the environment and stochastic effects continuously influence the epigenome.
- DNA methylation changes precisely track chronological age and may also mark biological age.

