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Epigenomic insights to the interplay between the Environment and Health

13th July 2023

The Exposome in Health & Disease

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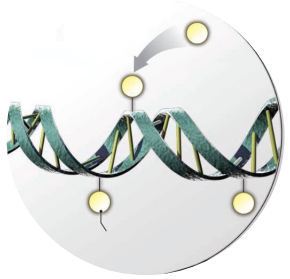
‘Epidemiological Epigenetics’

- How the **Epigenomic** Marker of

DNA methylation

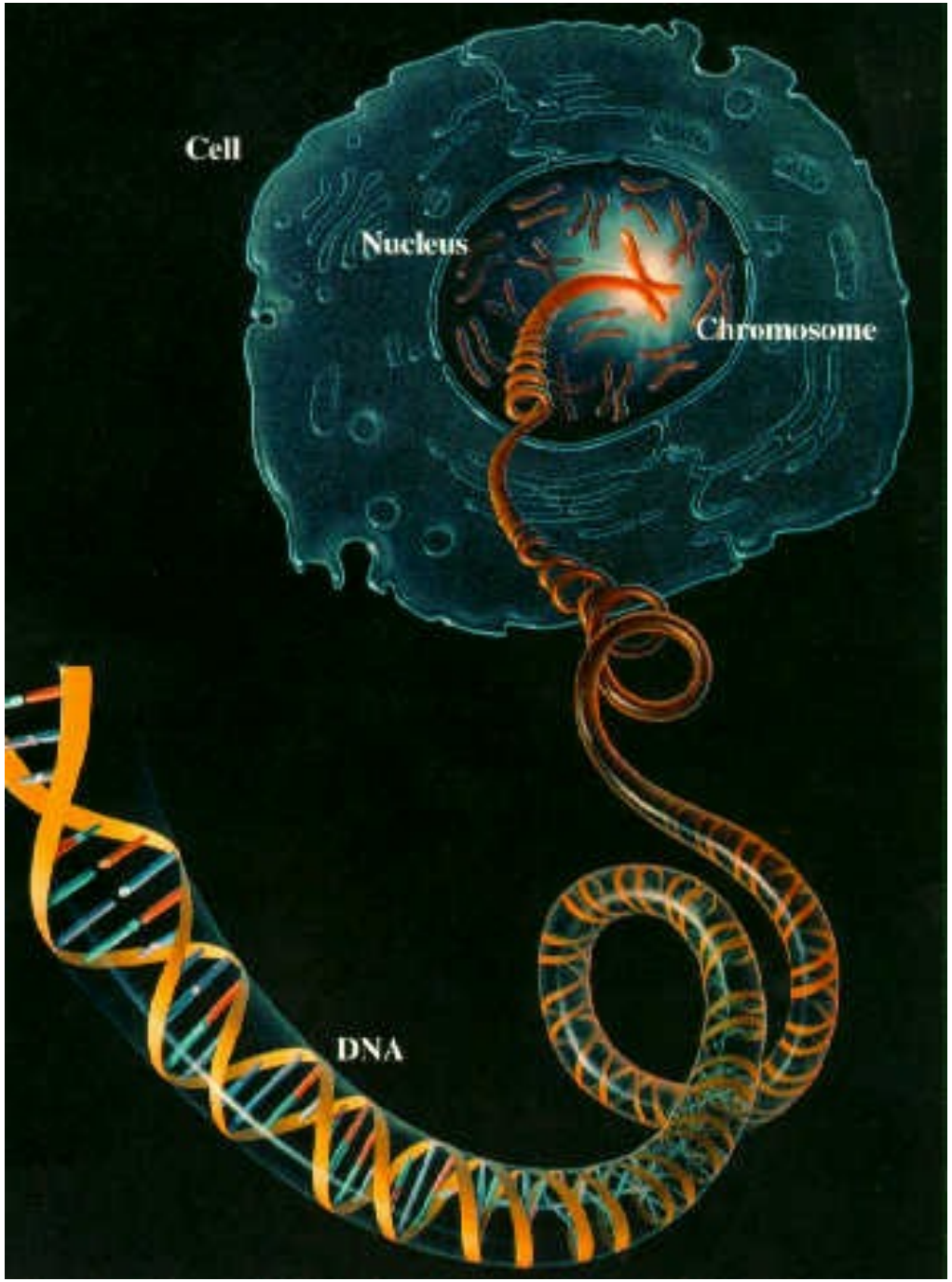


- = A powerful **Biomarker** in Human Epidemiology
- = Potential **Functional** insights to pathophysiology

Informative about Environmental Factors & Health

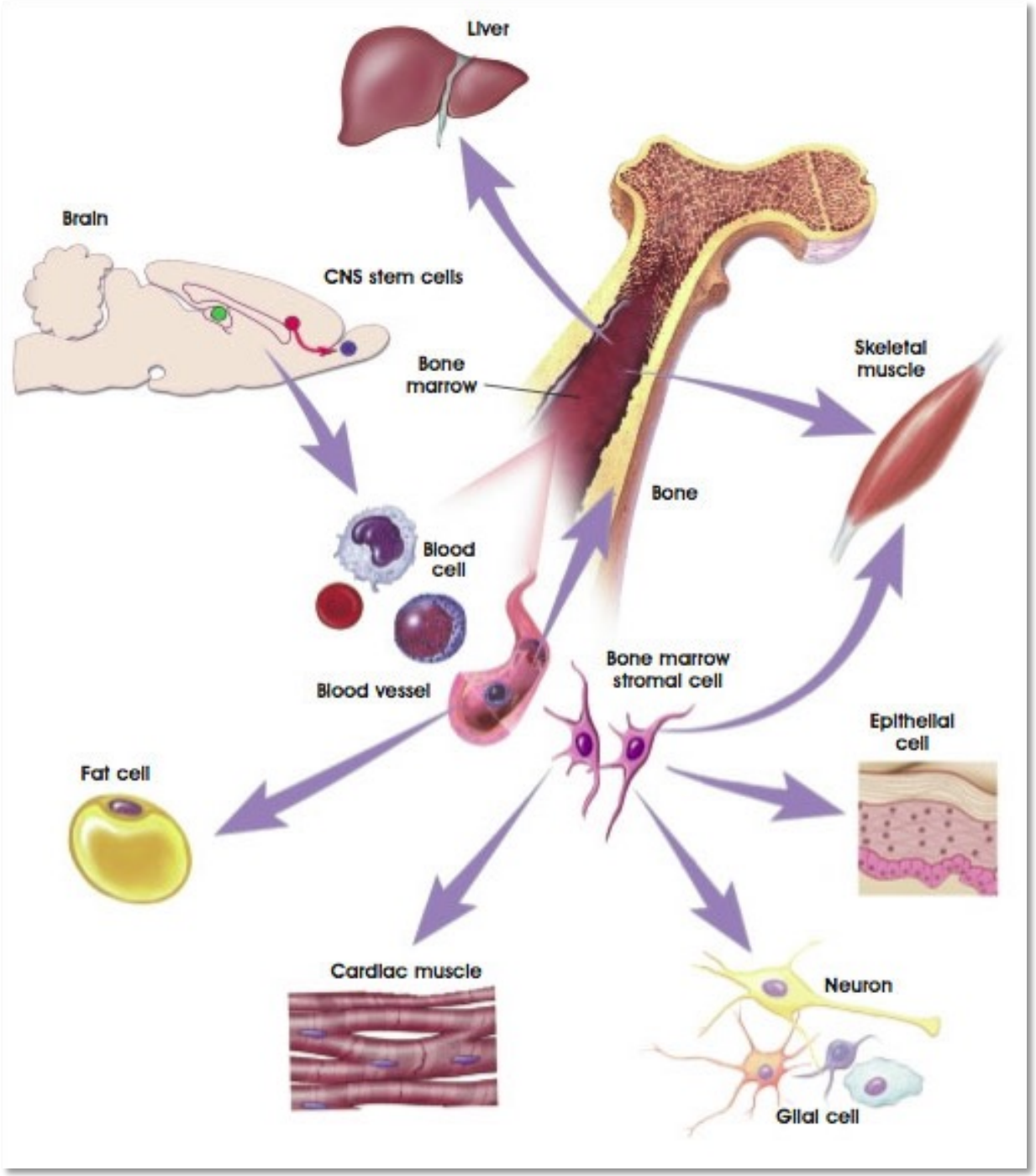
Outline

- What is **Epigenetics**?
- What is the **Epigenome**?
  - DNA Methylation
- DNA methylation Signatures
  - Environmental Exposures
  - Biomarkers of Disease
  - 'Biological' Age



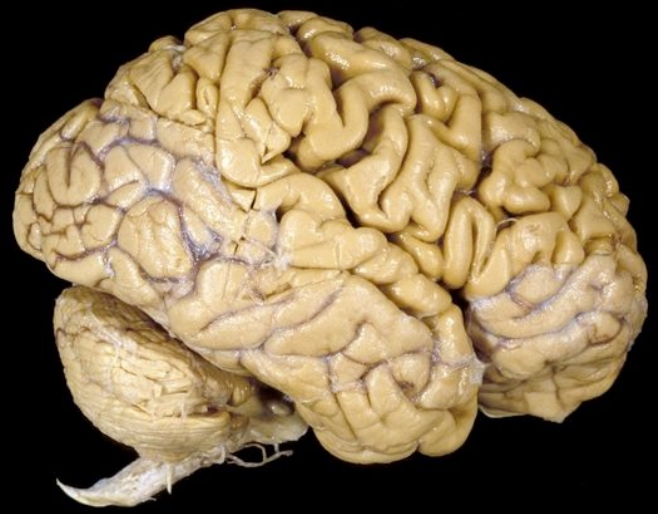
© The National Human Genome Research Institute

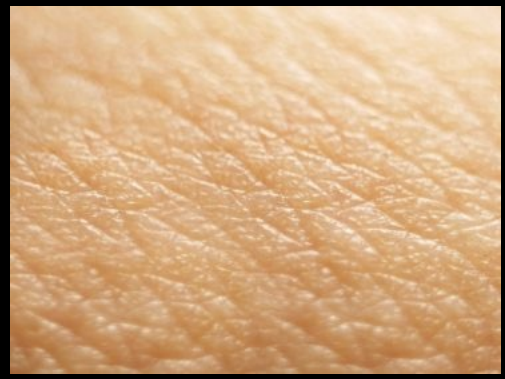
- All cells
  - ~ Same Genome



- Multiple Cell Types

stemcells.nih.gov





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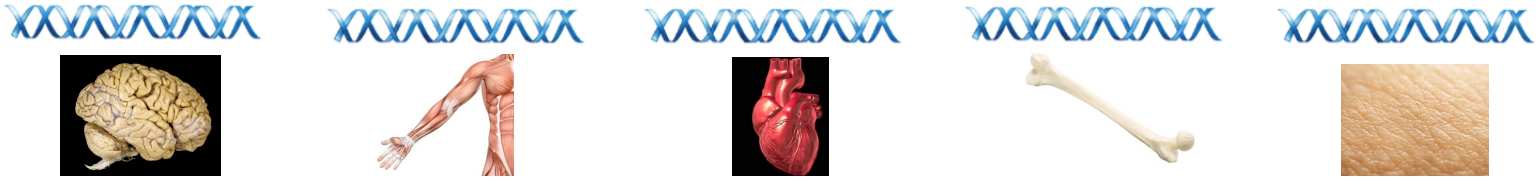
GENOME



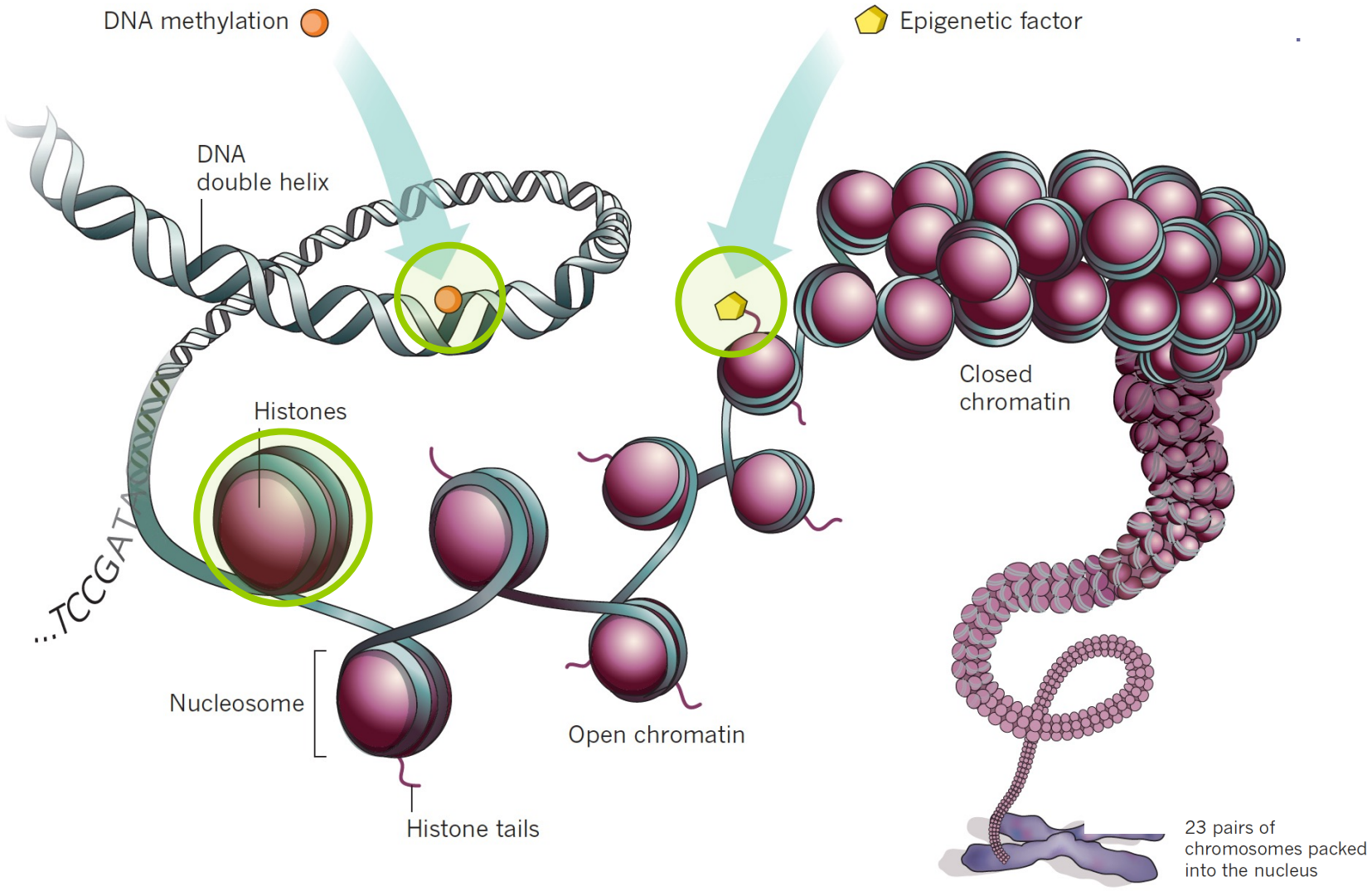
EPIGENOMES

Epigenetic Mechanisms

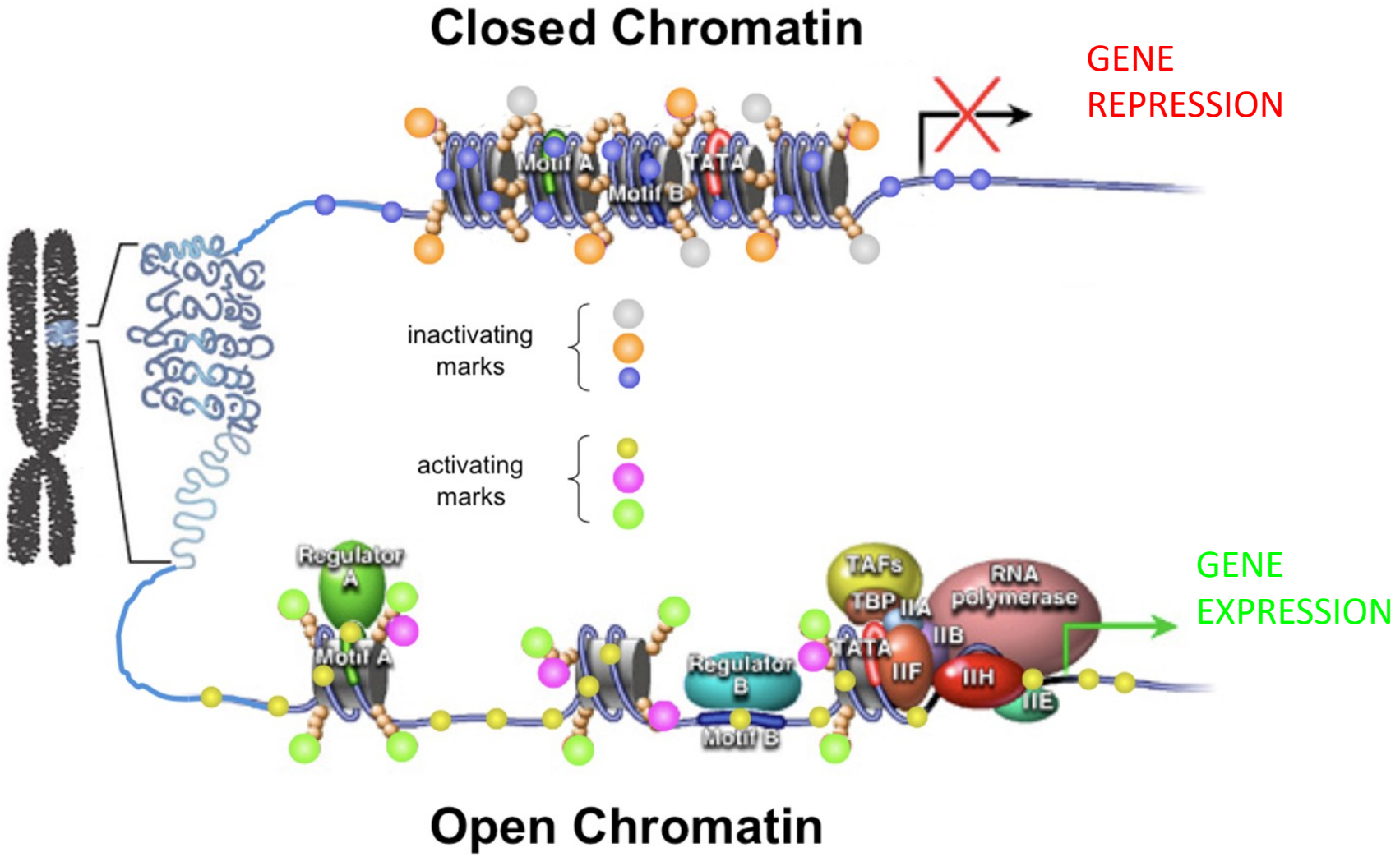
- Packaging
- Chemical Modifications of DNA

⇨ Influences &/or Informs about Gene Expression

⇨ Molecular Insight to Cell & Organ-Specific Activity

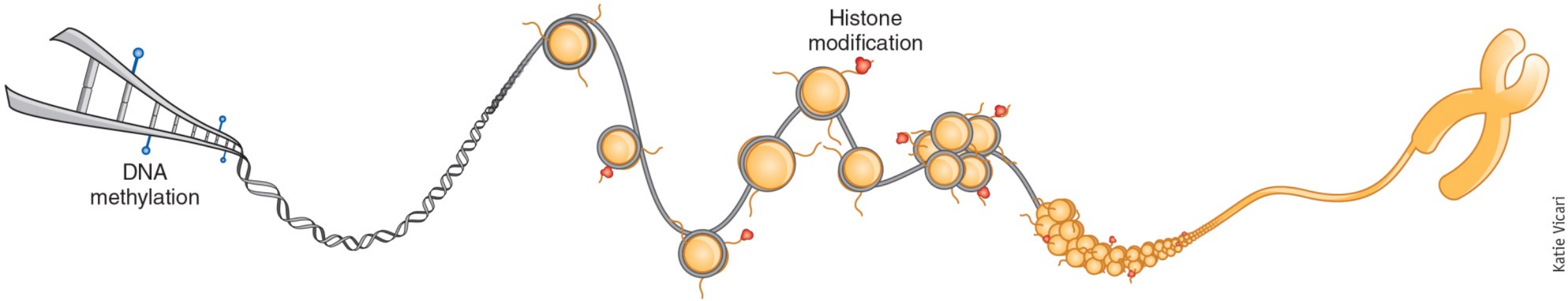


Chromatin State Influences Gene Expression



Epigenetics Definition

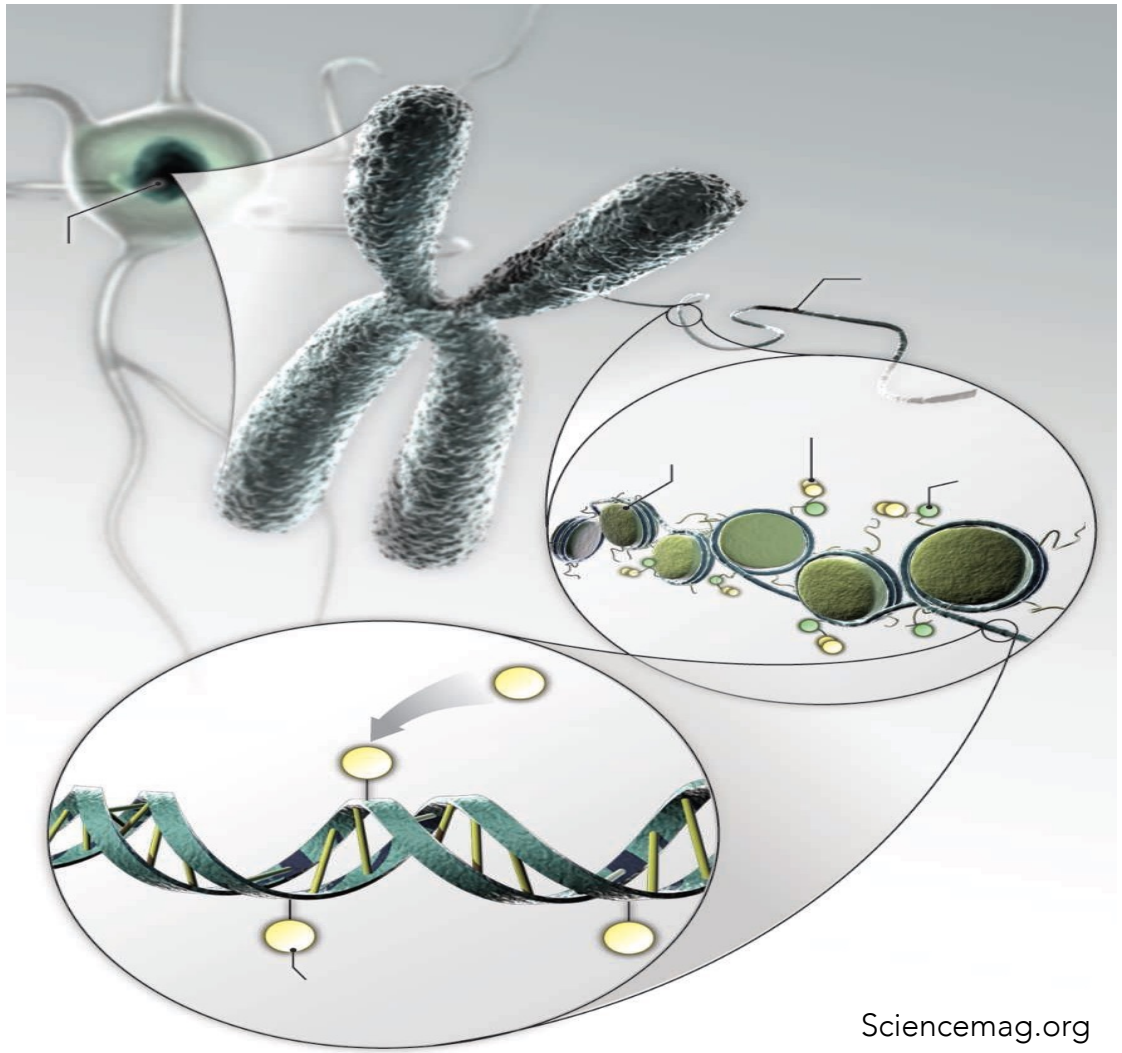
- "Stable heritable information transfer that does NOT require Mutagenic Change of the underlying nucleotide sequence"



Baker (2010) *Nature Methods*

The Epigenome

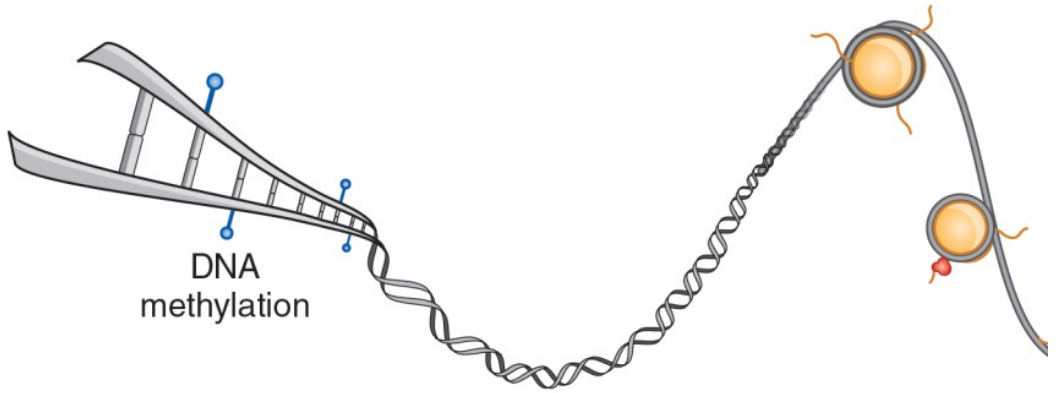
- The ‘Genome-Wide’ Epigenetic State
- All of the Epigenetic Modifications within the Cell’s Genome



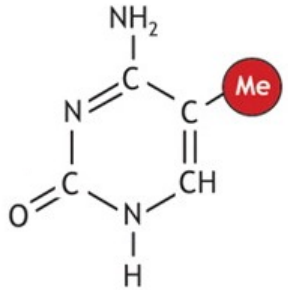
Sciencemag.org

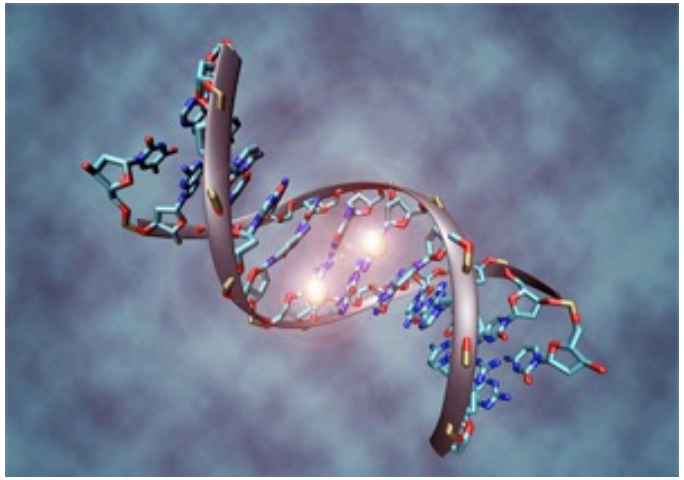
Modifications of DNA

- DNA methylation
  - Methylcytosine (5mC)
    - Addition of Methyl group onto 5 Carbon of Cytosine
      - Highly Stable Mark
      - Most Common
      - Most Studied

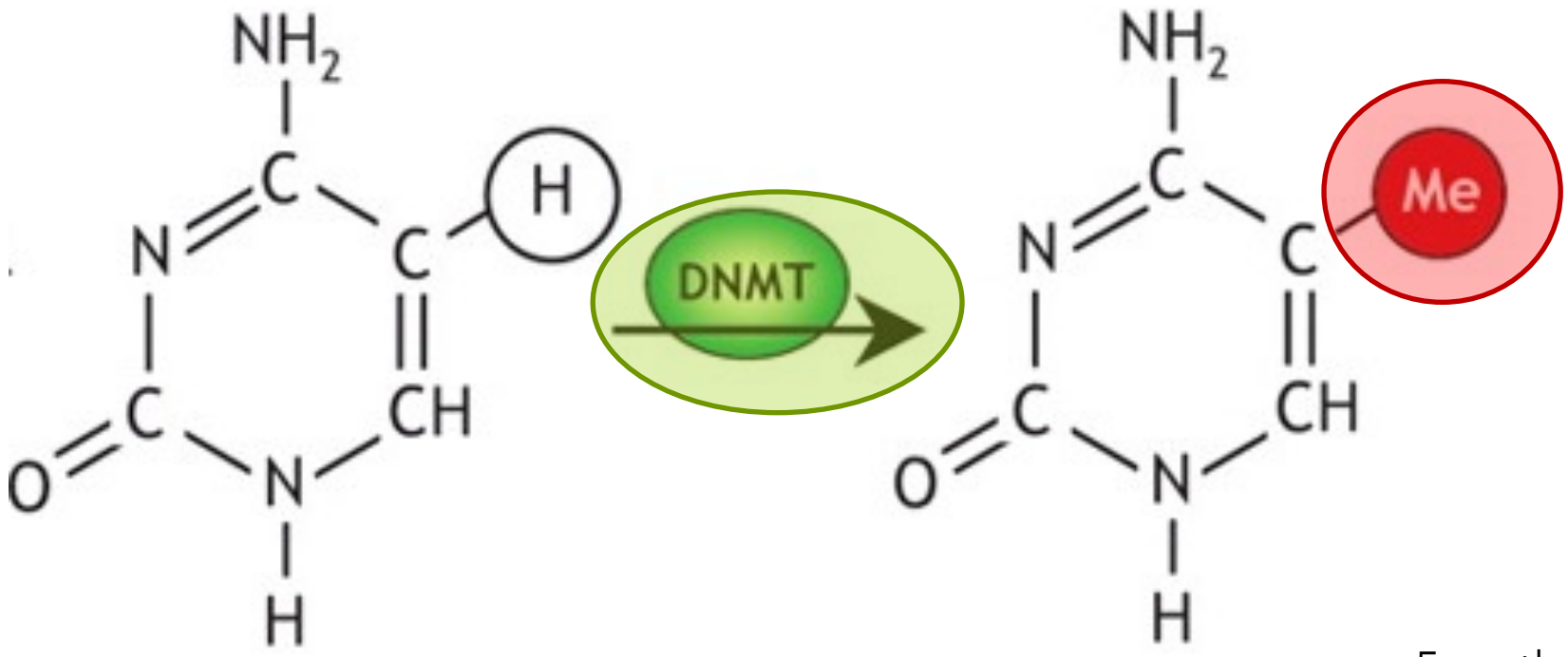


- Additional Rarer DNA Modifications
  - Hydroxymethylcytosine(5hmC)
  - Formylcytosine (5fC)
  - Carboxylcytosine (5cC)





DNA methylation

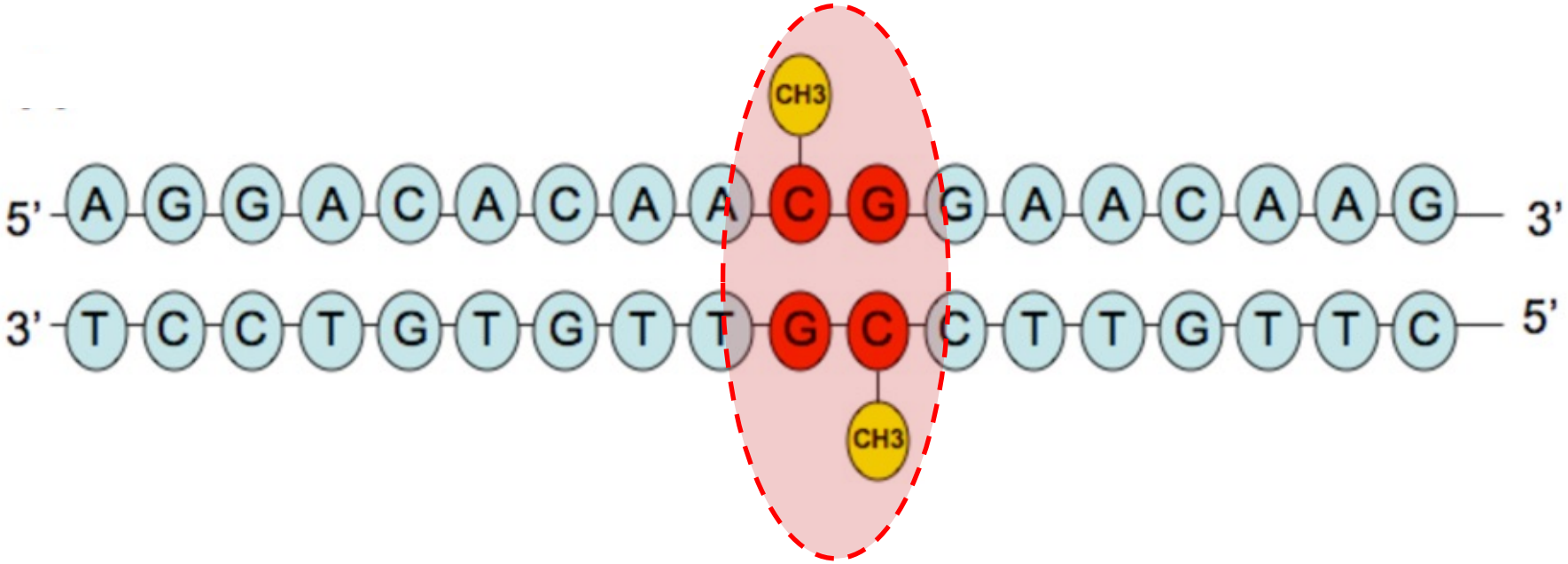


Cytosine

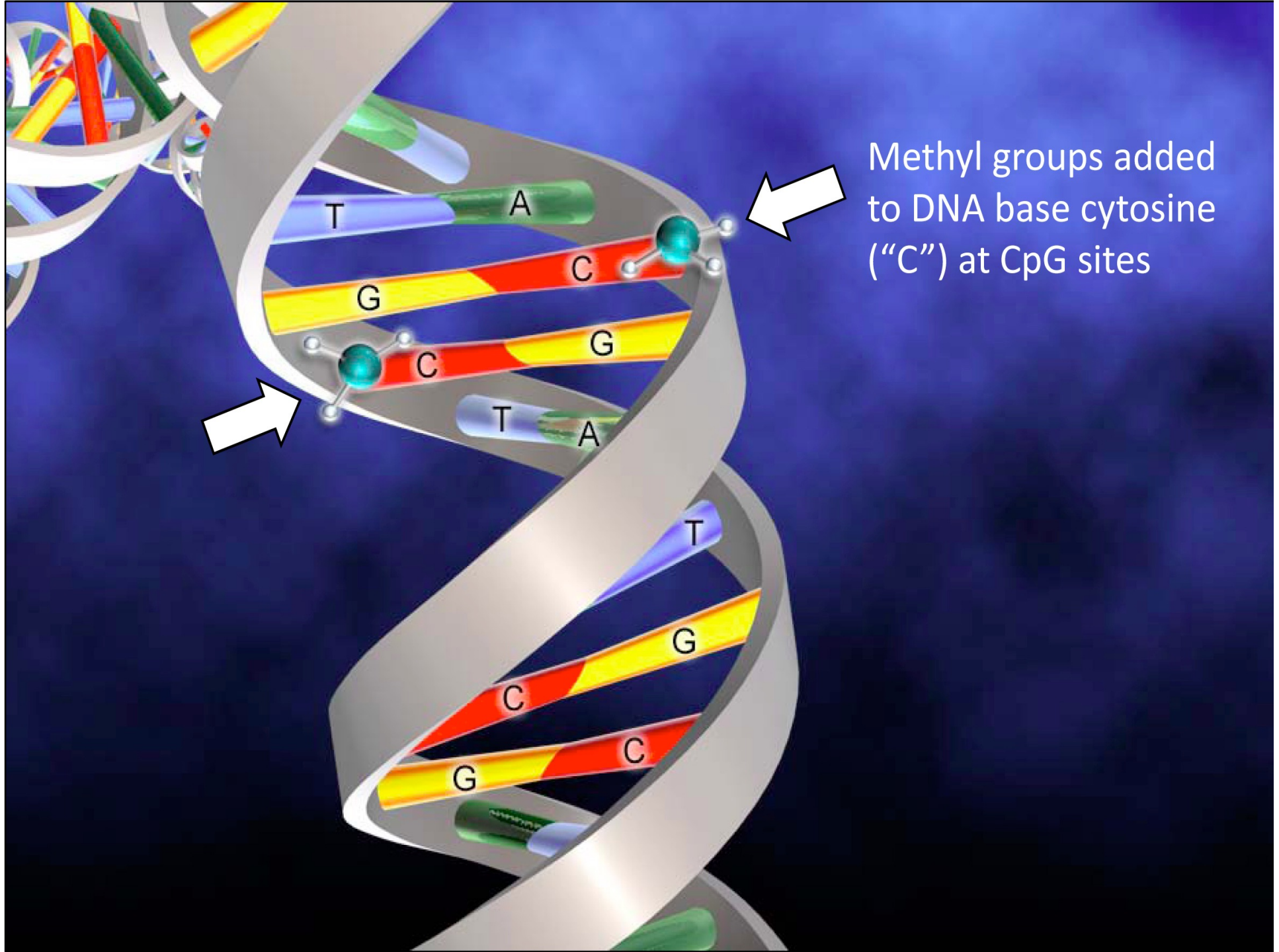
5-methylCytosine

DNA Methyltransferase (DNMT) Enzyme

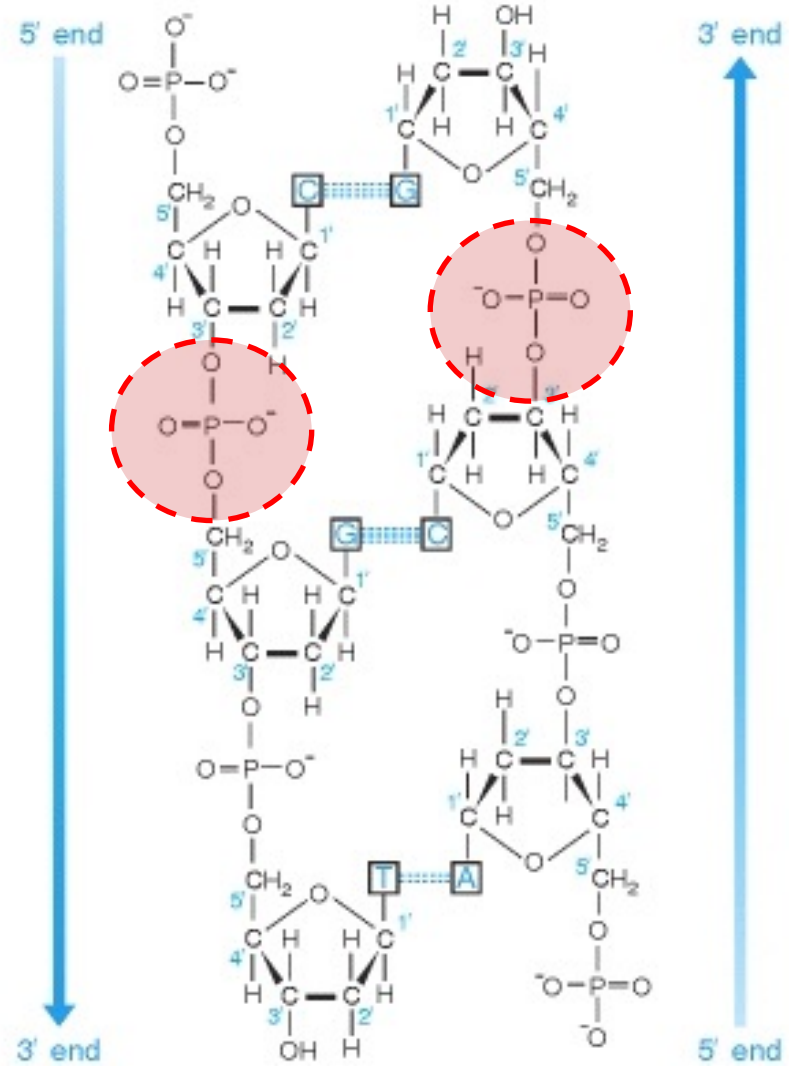
DNA Methylation



- Occurs usually at a Cytosine followed by Guanine base
- Palindromic Motif
  - C then G from 5’ to 3’ on both strands
  - = CpG dinucleotide

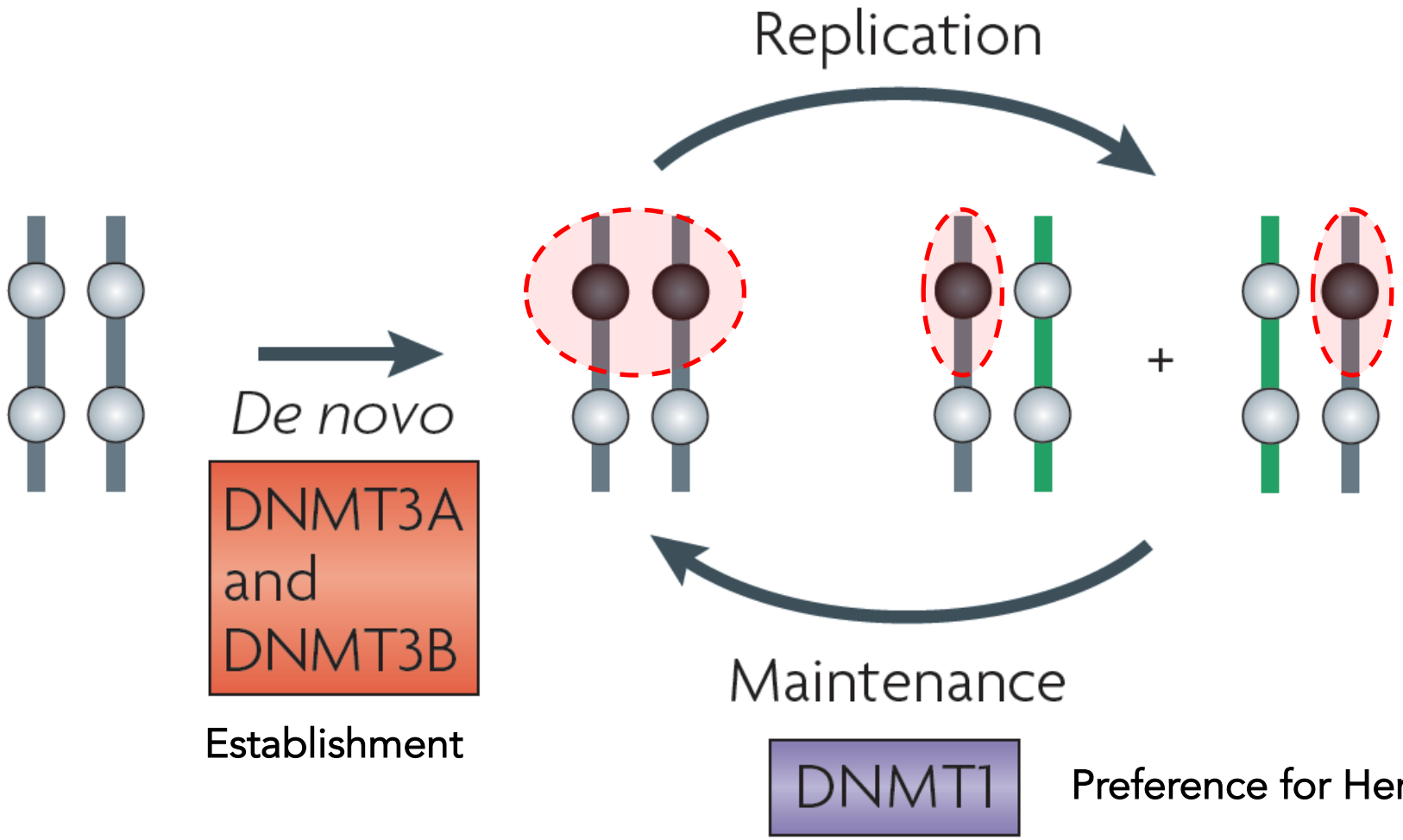

Methyl groups added to DNA base cytosine (“C”) at CpG sites
T
A
C
G
C
G
T
A
T
G
C
C
G

CpG Dinucleotide



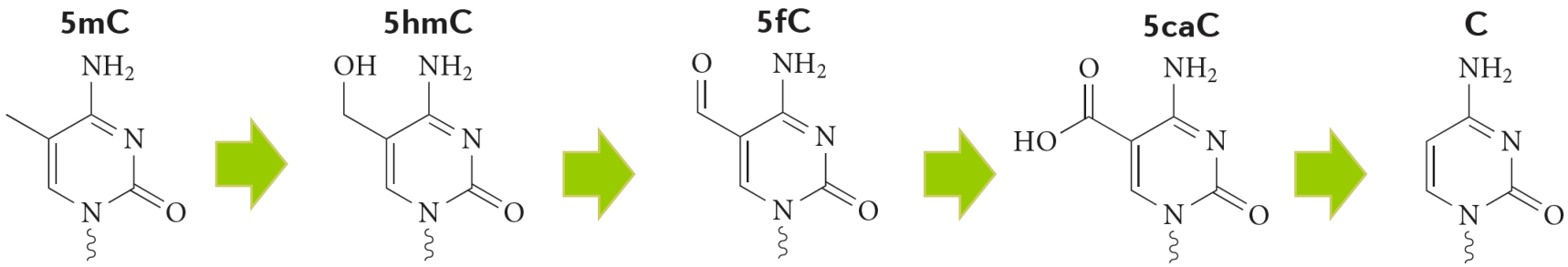
- Cytosine followed by Guanine in 5′→3′ Direction
- Via the Phosphodiester bond
- *i.e.* ‘CpG’

Establishment & Inheritance of DNA methylation



Jones & Liang (2009) *Nature Reviews Genetics*

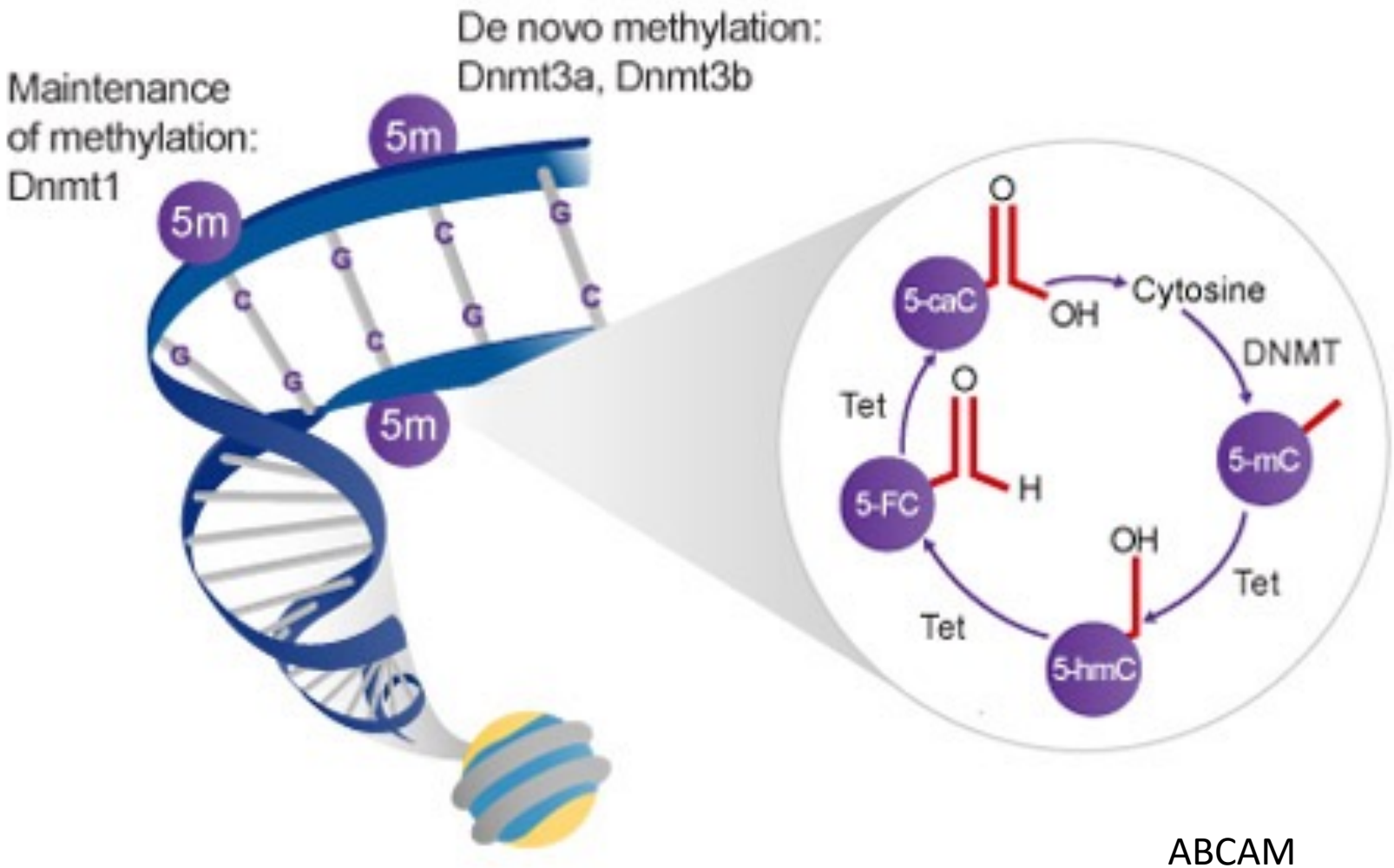
Potential Pathways of DNA demethylation



- Active Removal
  - Oxidised to 5hmC (TET Enzyme)
  - 5hmC further oxidised to 5fC & 5caC
  - Base Excision Repair Machinery → Unmodified Cytosine
    - Deformation Or TDG cleaves
- Passive Removal
  - Hemimethylated DNA is not methylated by DNMT1 during replication

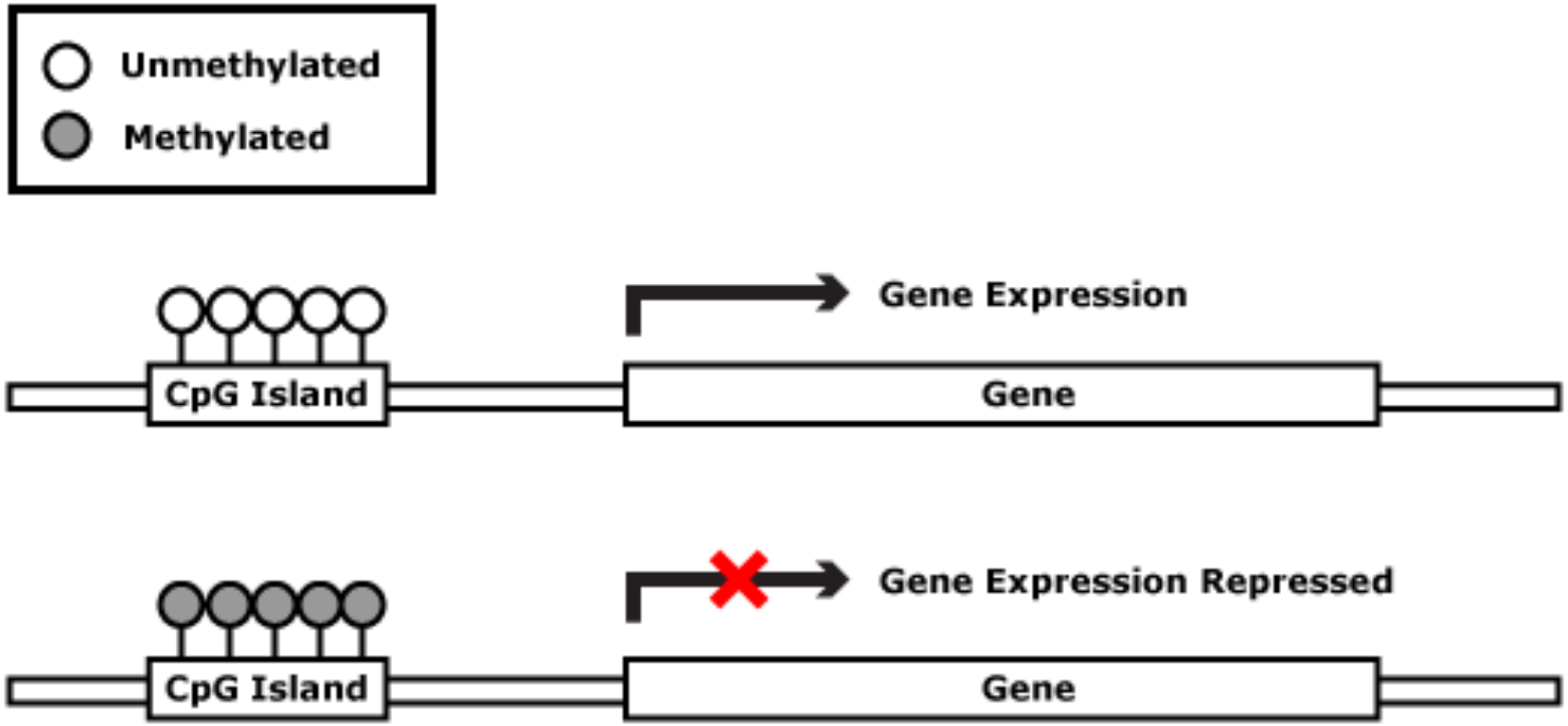
Branco & Reik (2012) *Nature Reviews Genetics*

DNA methylation Cycle



ABCAM

DNA Methylation Repressive in Promoters

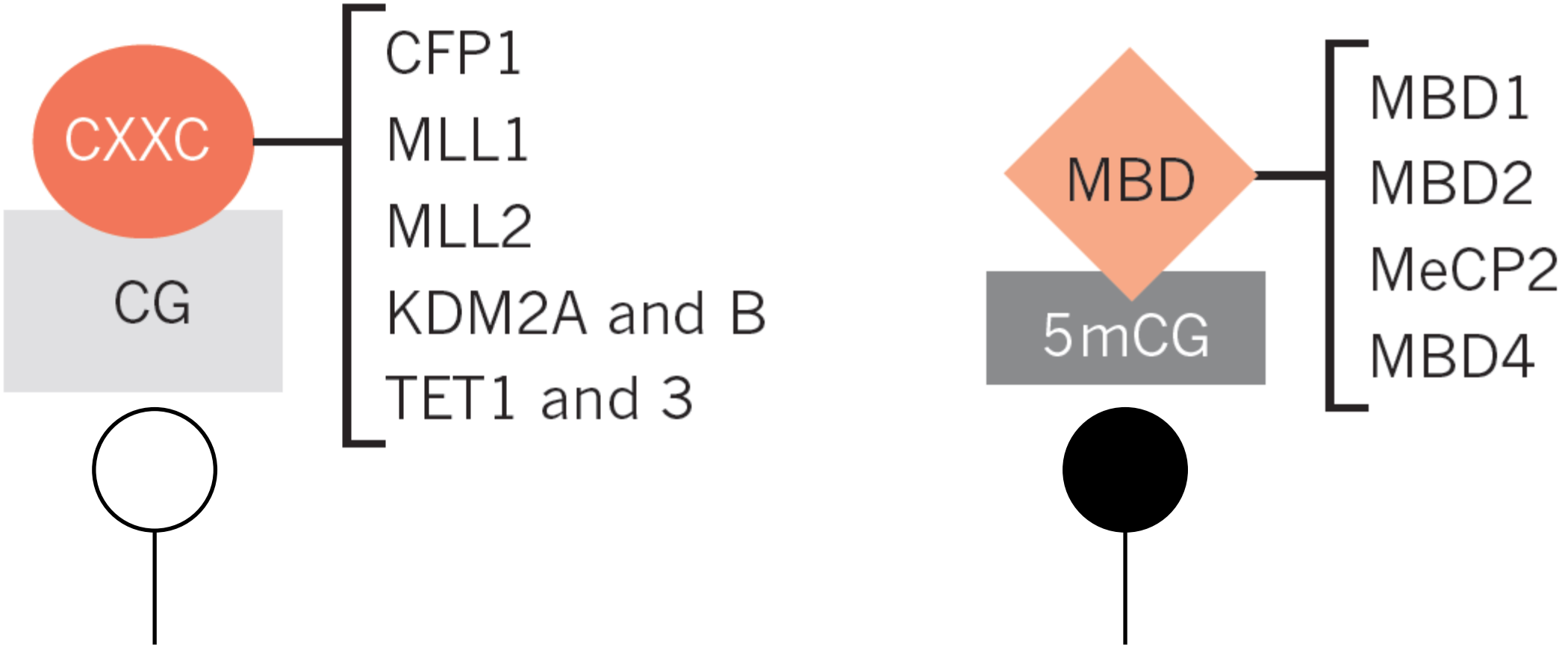


Suzuki & Bird. (2008) DNA methylation landscapes: provocative insights from epigenomics. *Nature Reviews Genetics*

CpG Dinucleotide Signalling Molecule

ACTIVATING

REPRESSIVE

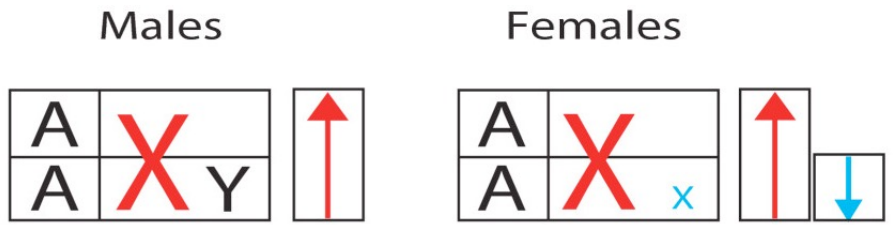


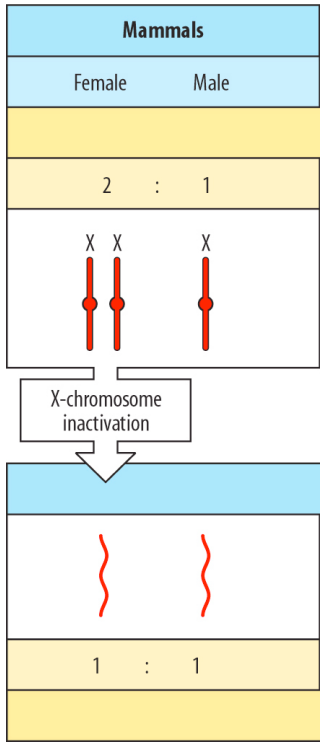
Schubeler (2015) Function and information content of DNA methylation. *Nature*

X Inactivation

- Epigenetic Dosage Compensation Mechanism
  - As Females have 2 X Chromosomes
  - Males only 1
  - Random Switch-Off

Hypermethylation of 1 X chromosome in Female





GENE COPY

EXPRESSION

X Inactivation

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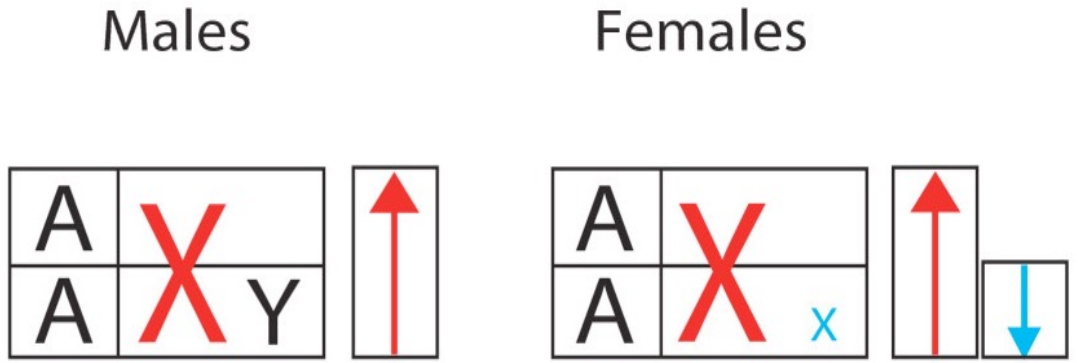

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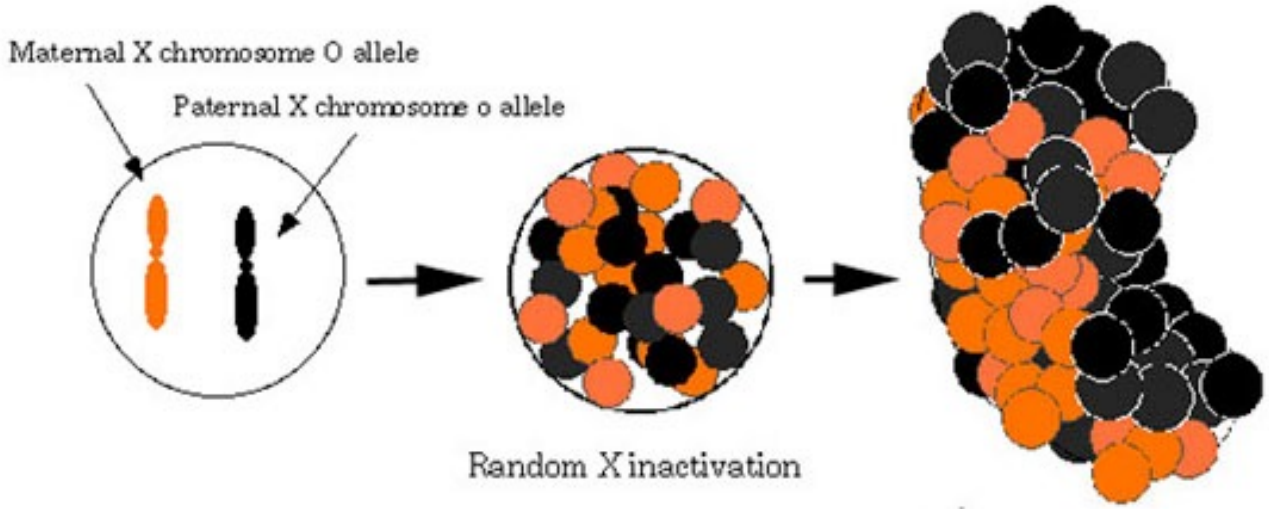
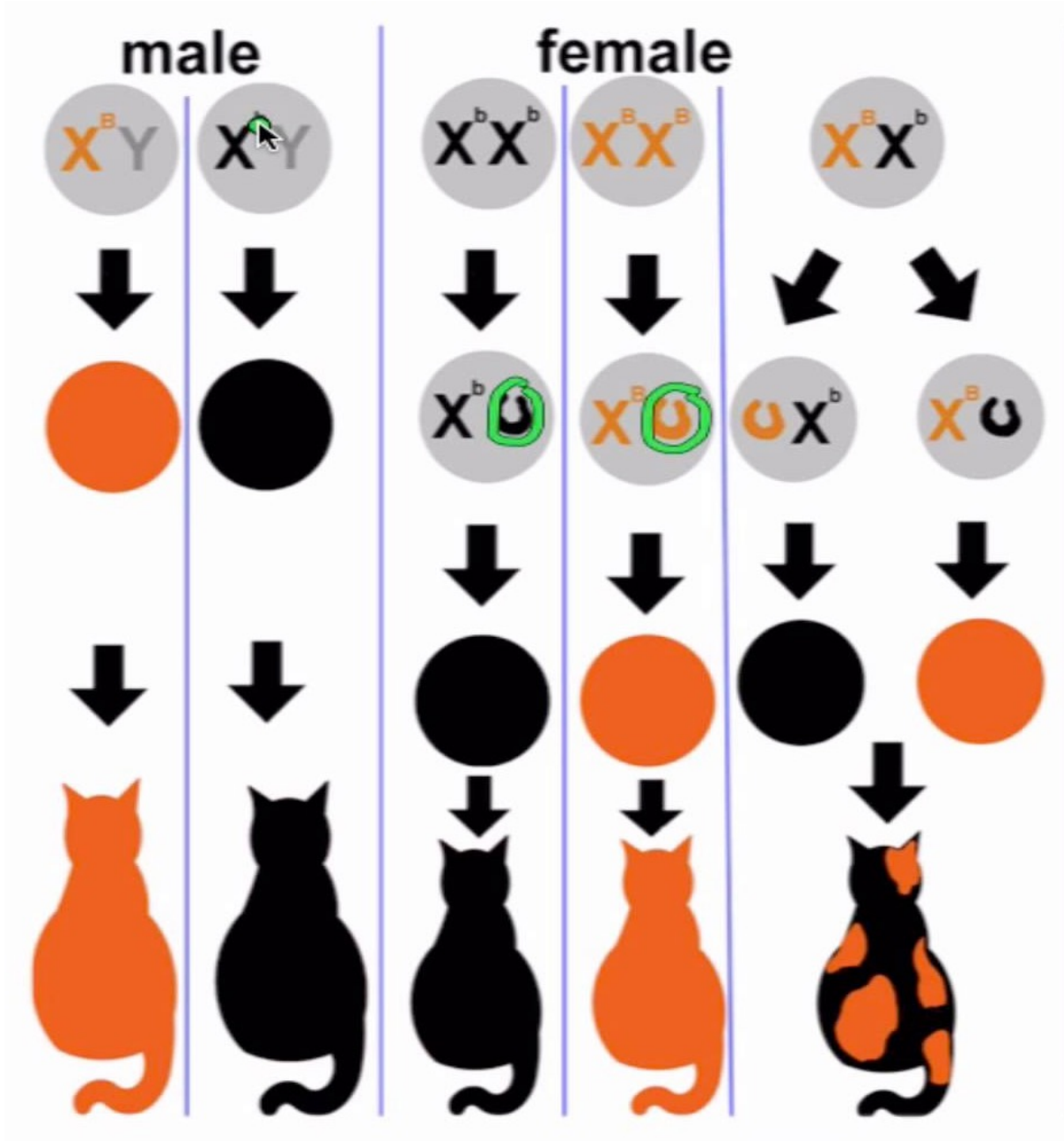
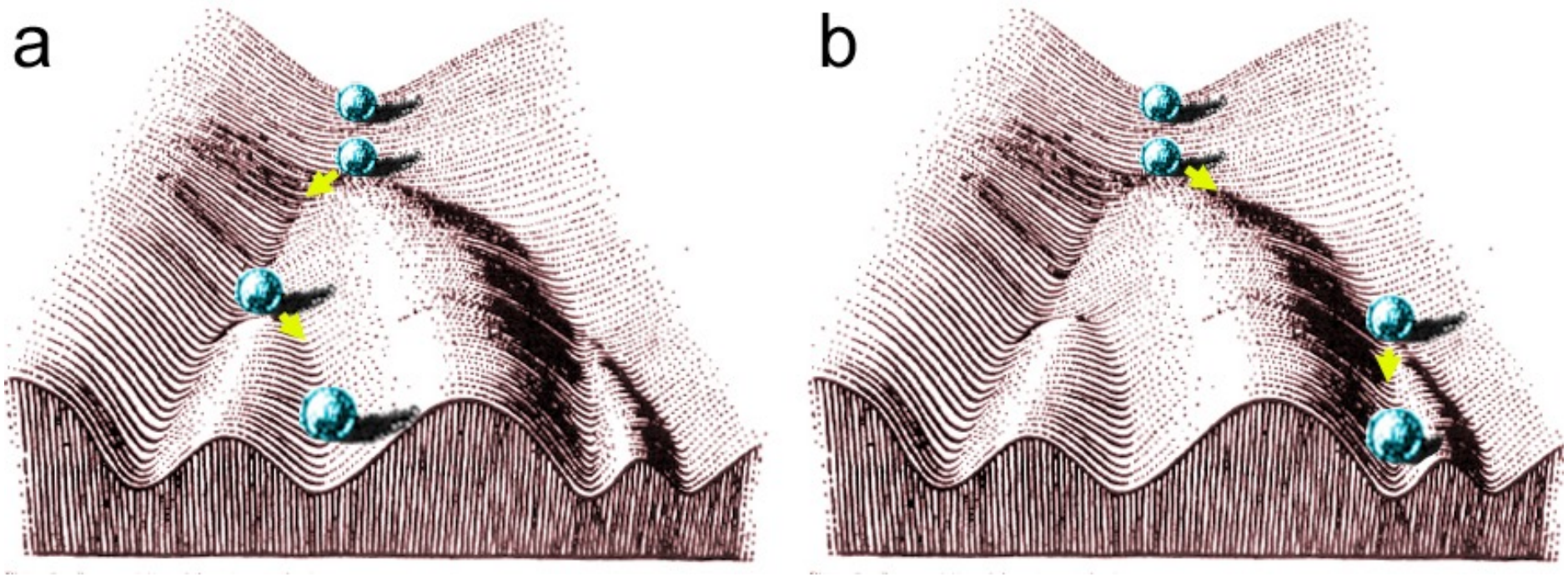




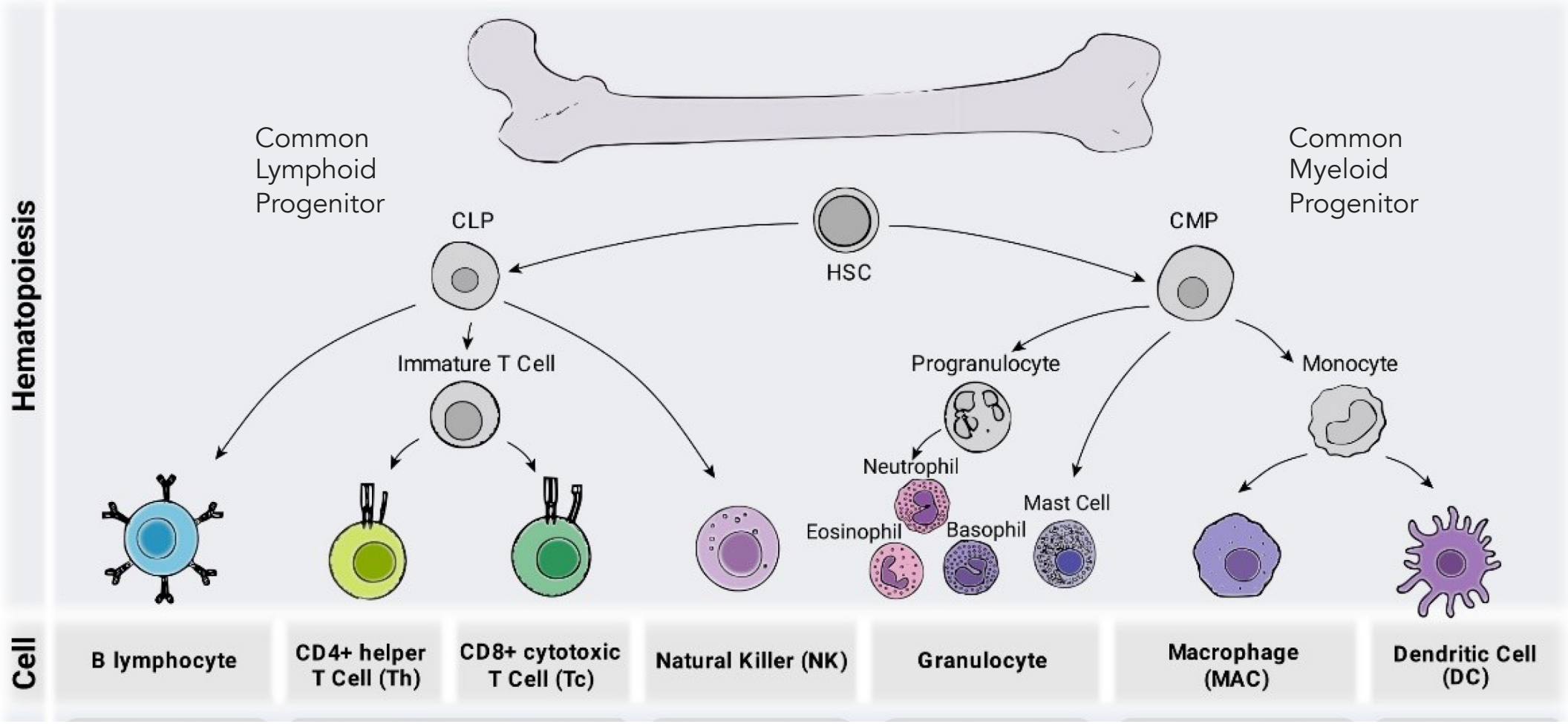
Random X-Inactivation

Waddington's Epigenetic Landscape



In 1957, Conrad Waddington proposed the concept of an Epigenetic Landscape to represent the process of cellular decision-making during Development

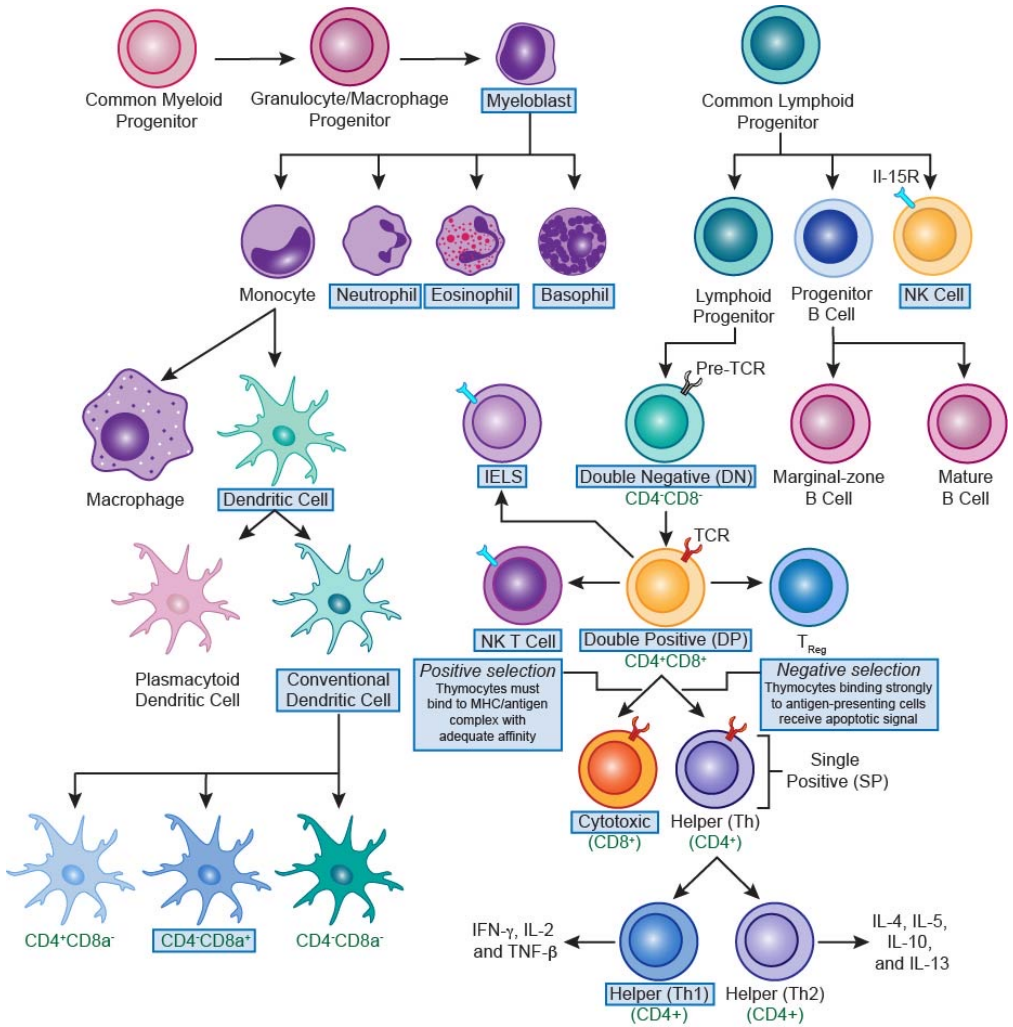
Haematopoiesis: Haematopoietic Stem Cell →



de la Calle-Fabregat *et al.* (2020) Understanding the Relevance of DNA Methylation Changes in Immune Differentiation and Disease. ***Genes***

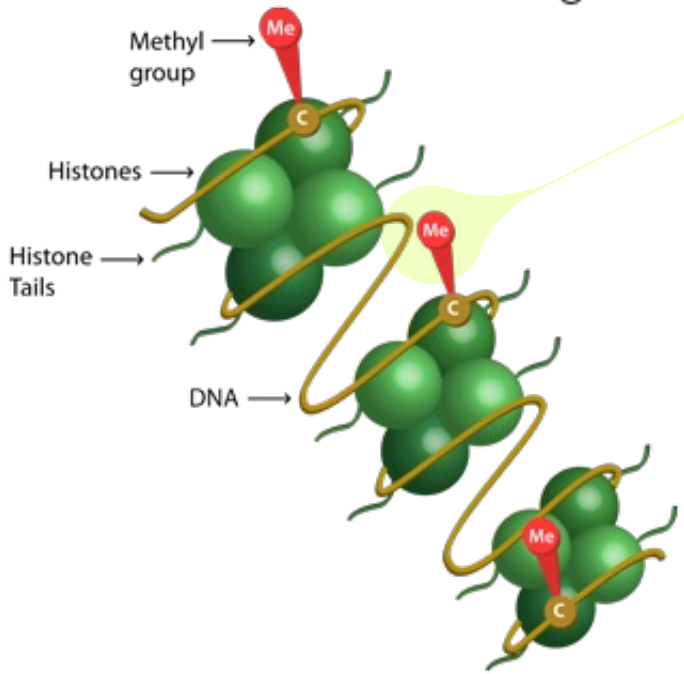
Heterogeneous Blood Tissue = Meta-Epigenome

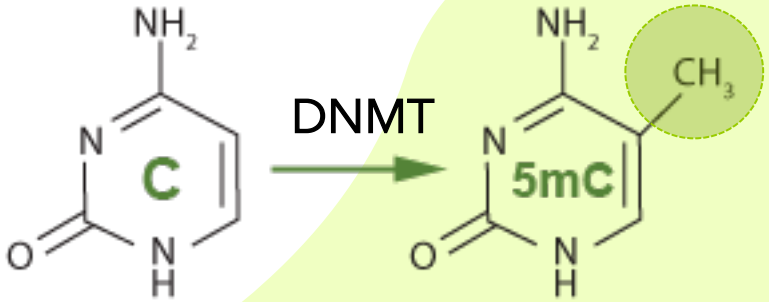
- Meta-Epigenome
  - Mixed Signal
    - from all the cell-types that comprise sample
  - Blood DNA = Leukocytes
    - Mature Red Cells
      - Nucleus expunged
  - Use Epigenomic Information
  - ⇨ Constituent cell type proportions
  - ⇨ Deconvolution Leukocyte Cell Types



Houseman *et al.*, (2012) BMC Bioinformatics;

DNA modifications = Extremely Stable





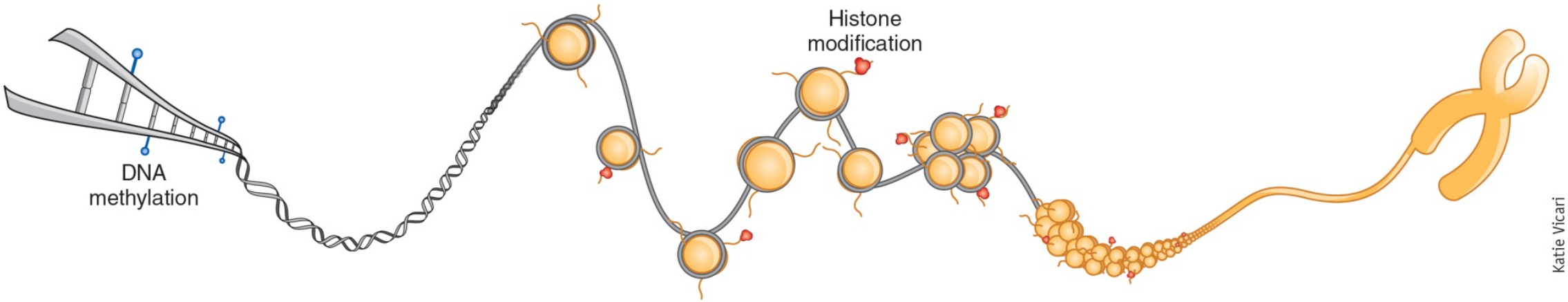
Cell Type Proportions

DNA from Mixed Cells
⇨ DECONVOLUTE

e.g. Blood DNA (Houseman *et al.*)

Houseman *et al.* (2010) DNA methylation arrays as surrogate measures of cell mixture distribution ***BMC Bioinformatics***

Analysing the Epigenome



Assessing the DNA methylome

DNA Methylome

= Total set of DNA methylation modifications in an organism's genome
or in a particular cell

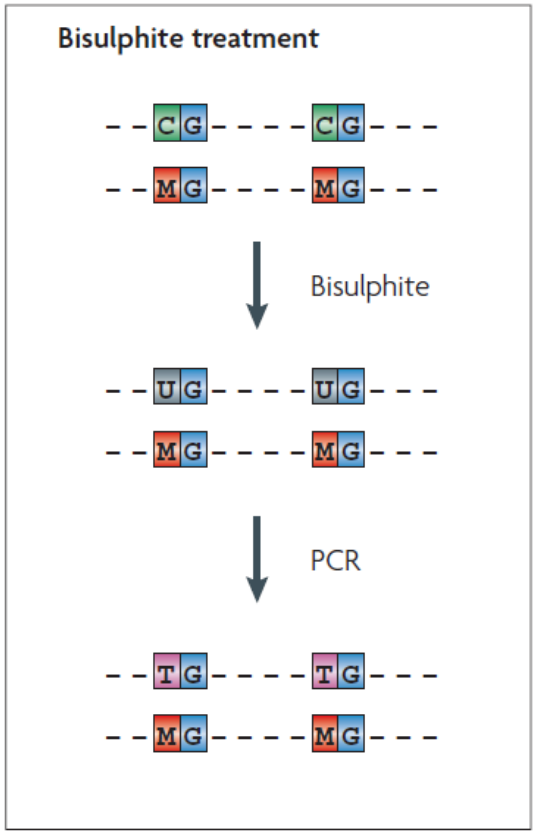
- Predominately at CpG dinucleotides
  - ~32 million CpGs in Human genome sequence (Gershman *et al.*)
- Although low level non-CpG Cytosine methylation occurs
  - Particularly In Developmental and Brain tissue

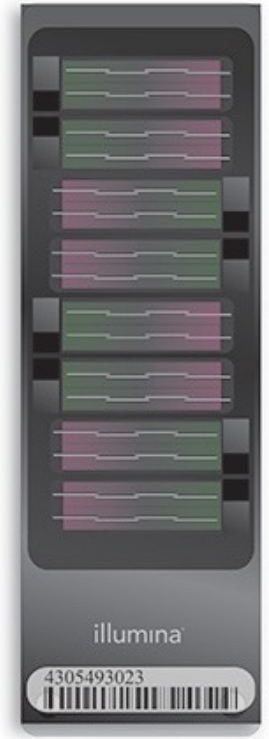
Gershman *et al.* (2022) Epigenetic patterns in a complete human genome. ***Science***

High-Throughput DNA methylation Array Analysis

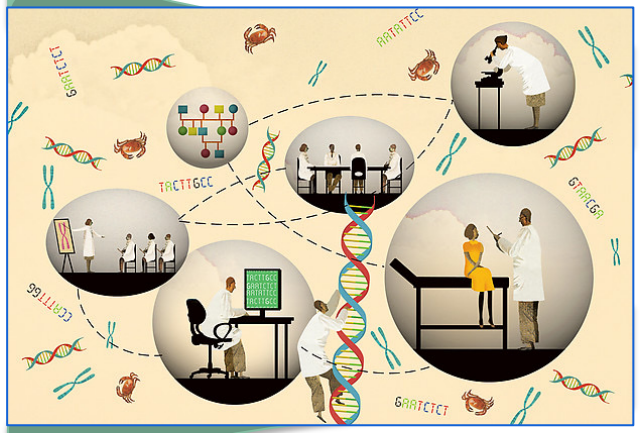
- BiSulphite Reaction
  - Convert Cytosine in CpG
  - → Pseudo-SNP = C/T SNP
  - Reflecting Methylation State
- Illumina Arrays
  - Adapted SNP array technology
    - 27k, then 450k, 850k (EPIC)
    - Now: 900k (EPIC v2)

      ~3% of all CpGs
  - Stringent Quality Control
    - Probe; BiS Conversion; Batch; Normalisation; Cell Type Heterogeneity; Genetic Confounders



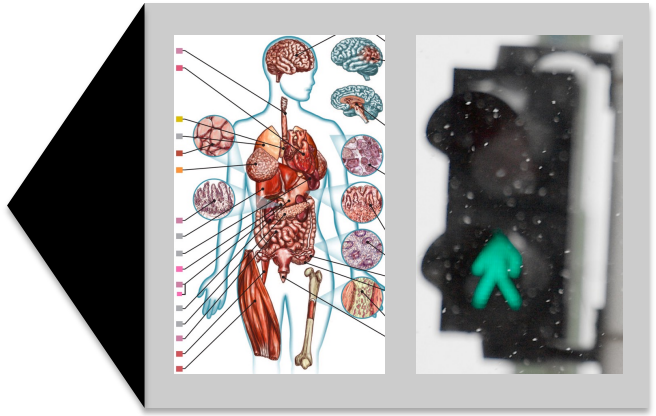


Analysing the Epigenome



FUNCTION

- Gene Activity &/or
- Informative of Function
- Cell-Type Specific



BIOMARKER

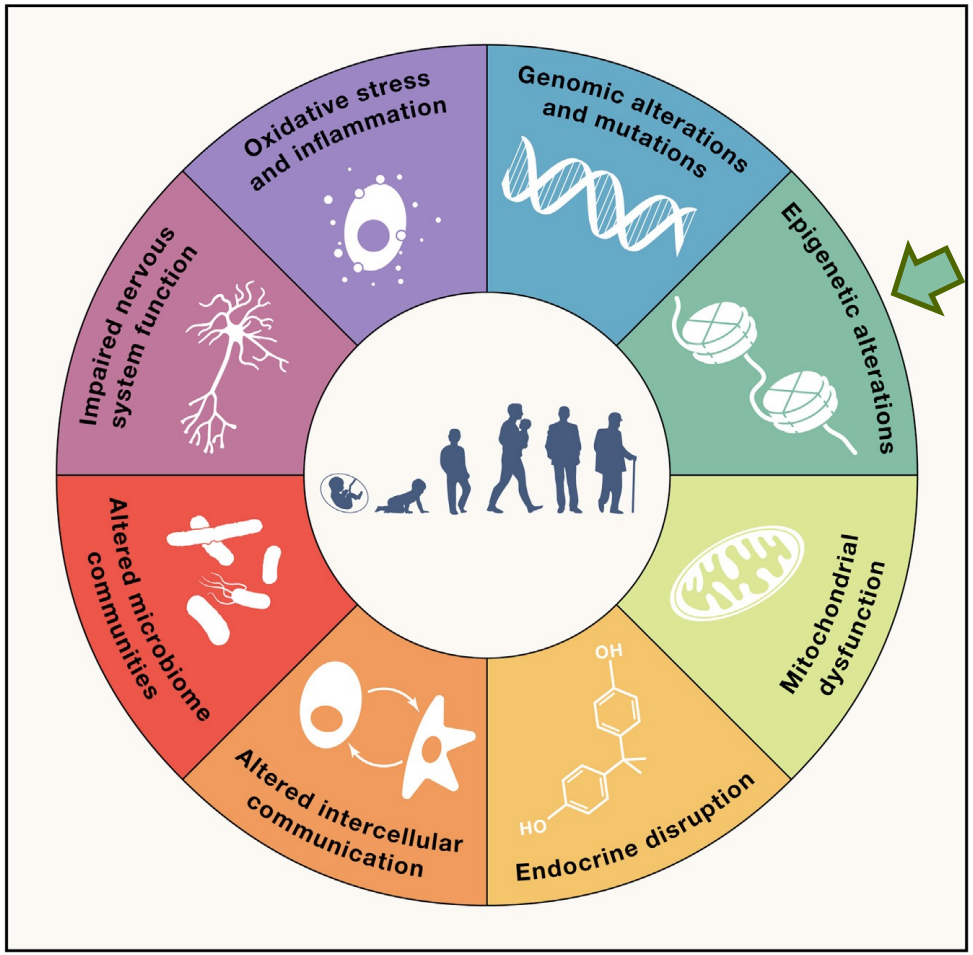
- Passive Marker
- Exposure → Smoking
- Disease Outcome
- Biological Marker

Breitling *et al.* (2011); Horvath (2013)

Hallmarks of Environmental Insults

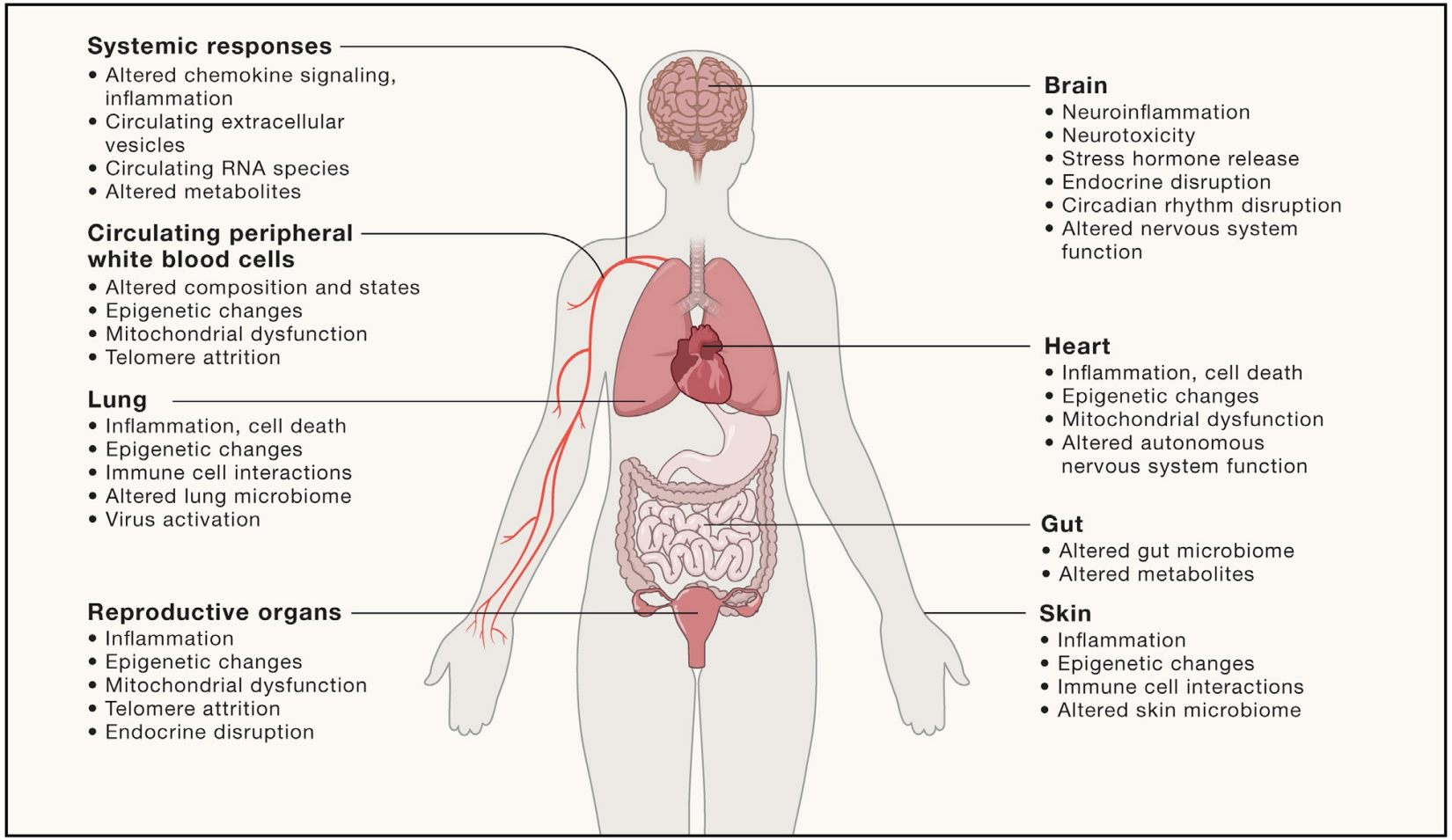
- Hallmarks of environmental insults
  - Cellular & Molecular processes

    involved in:
    - Essential cellular mechanisms & activities
    - Linking environmental exposures to chronic diseases
      - Cancer
      - Respiratory
      - Cardiovascular
      - Metabolic diseases
      - Nervous system



Peters *et al*. (2021) Hallmarks of environmental insults. ***Cell***

Organ-specific impacts based on Hallmarks of Environmental Insults



Peters *et al*. (2021) Hallmarks of environmental insults. ***Cell***

External Exposures

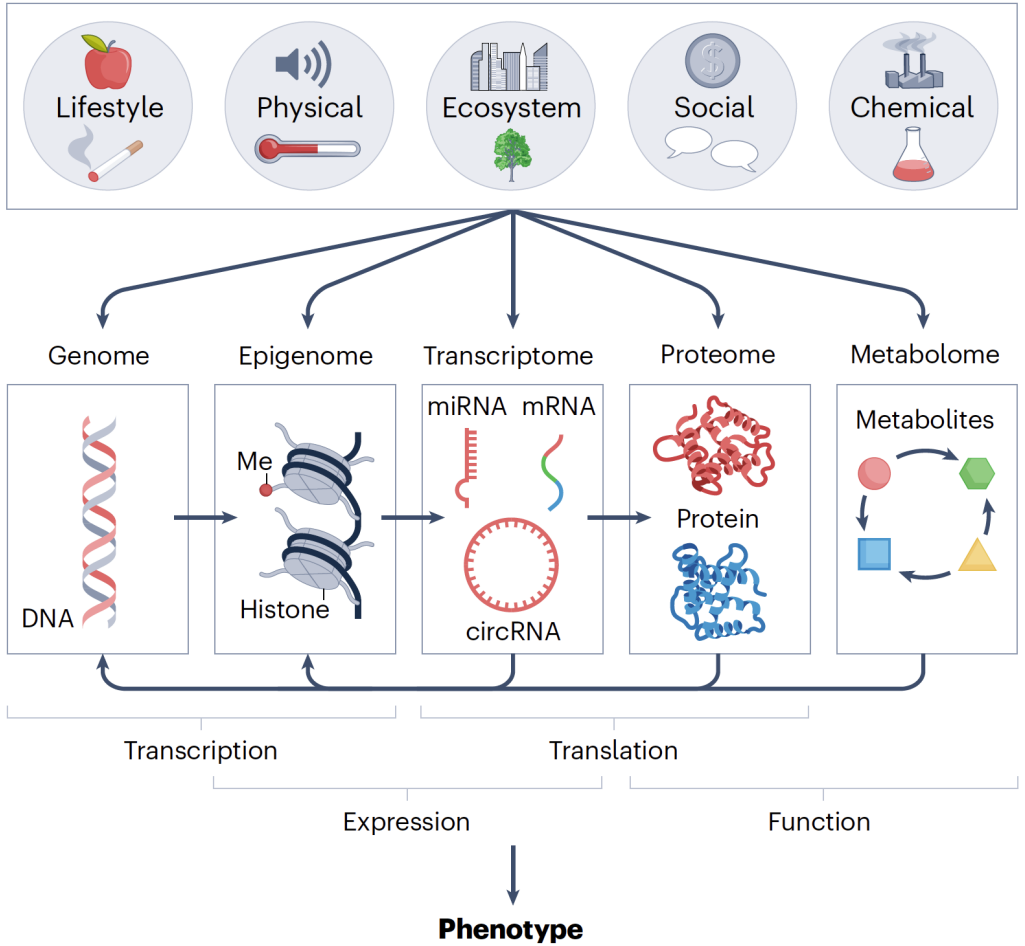
- Any Outside Stimulus
  - That body can Detected
  - Potential to cause Epigenetic Modifications
- Which Exposures?
  - Affect which epigenetic marks?
  - What are the mechanisms and downstream effects?

Table 1 | **Chemicals and pollutants**

Compound	Species
Tobacco smoke	Human
Particulate air pollution	Human, Mouse
Asbestos	Human
Bisphenol A (BPA)	Mouse
Diethylstilbestrol (DES)	Mouse
Metal ions (such as chromium, cadmiun, nickel, arsenic and methylmercury)	Multiple species
Vinclozolin	Mouse, rat
Methoxychlor	Mouse
Silica	Human
Benzene	Human
Di- and trichloroacetic acid, trichloroethylene	Mouse

Feil & Fraga (2012) *Nat Rev Genet*

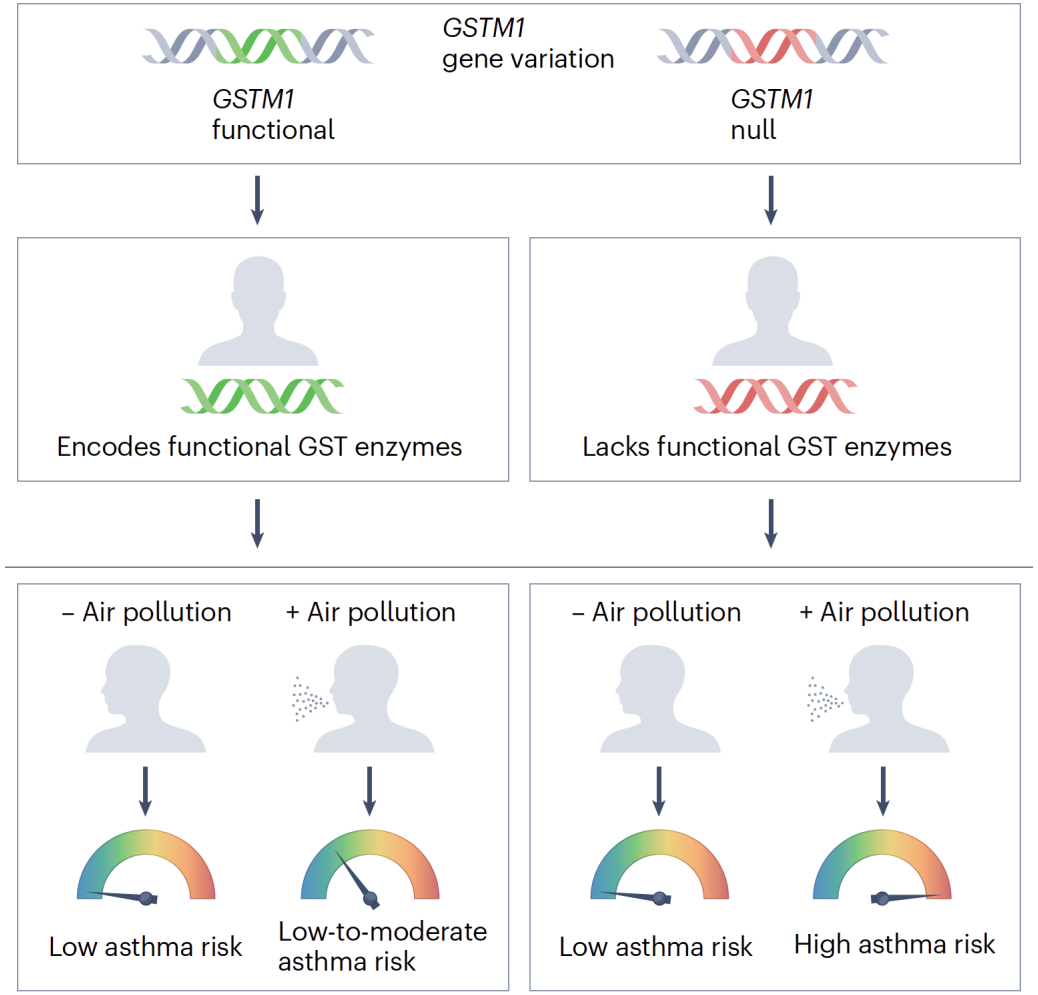
The Exposome



= Cumulative measure of environmental influences over the lifespan

Wu *et al.* (2023) Molecular mechanisms of environmental exposures and human disease. ***Nat Rev Geneti***

Gene-Environment Interaction → Impact on Disease Risk



- *GST* encodes glutathione S-transferase
  - Detoxifying Enzyme
  - Protects against pollution-related oxidative stress
- Carriers *GST* null genotypes
  - > susceptibility indoor air pollution
  - ↑ risk of asthma

Wu *et al.* (2023) Molecular mechanisms of environmental exposures and human disease. ***Nat Rev Genetics***

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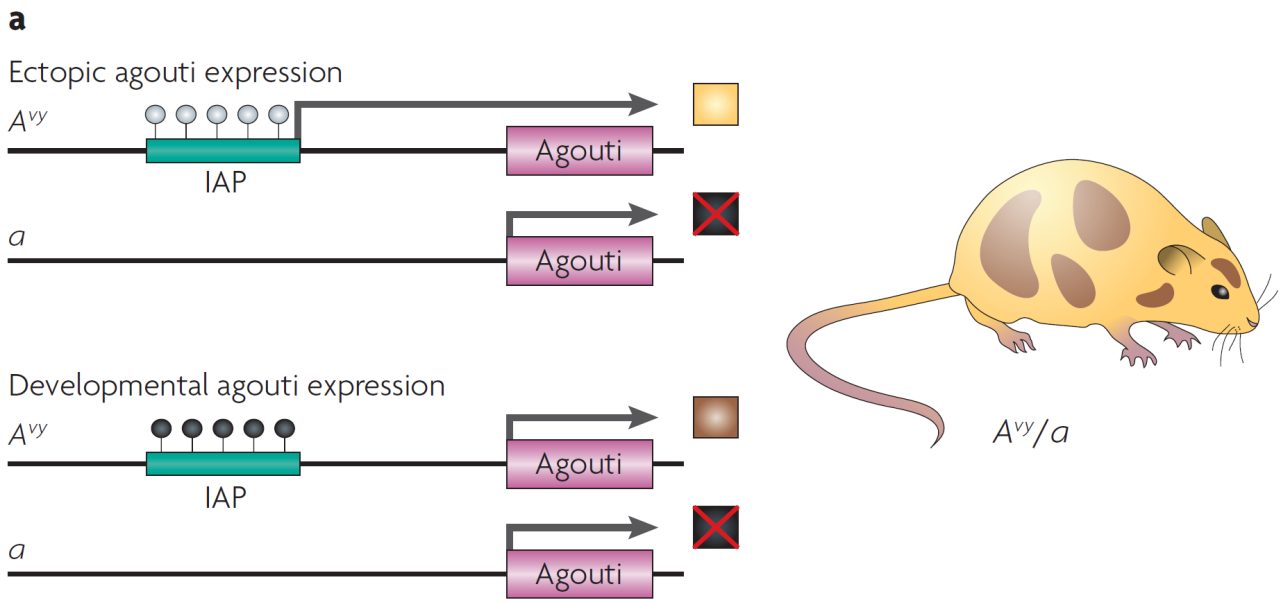
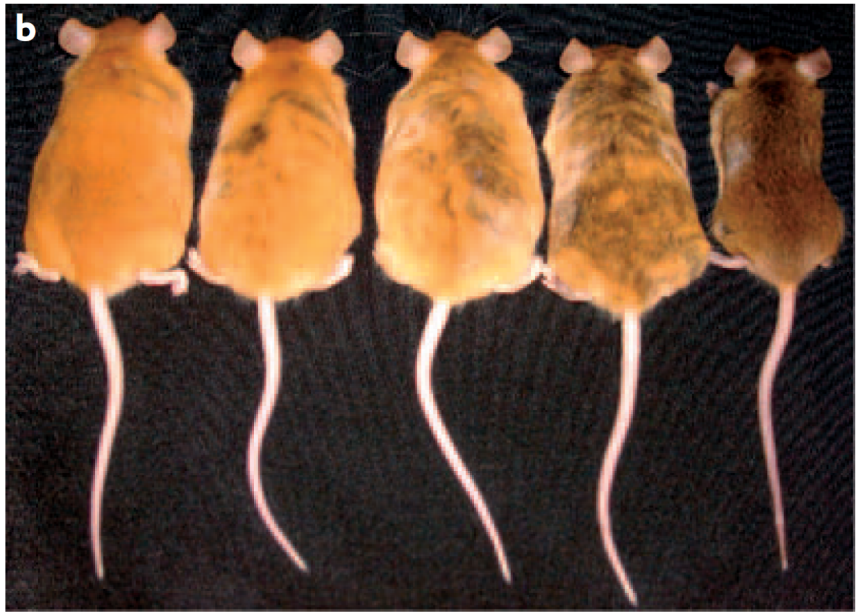
Agouti Mouse

Metastable Epialleles

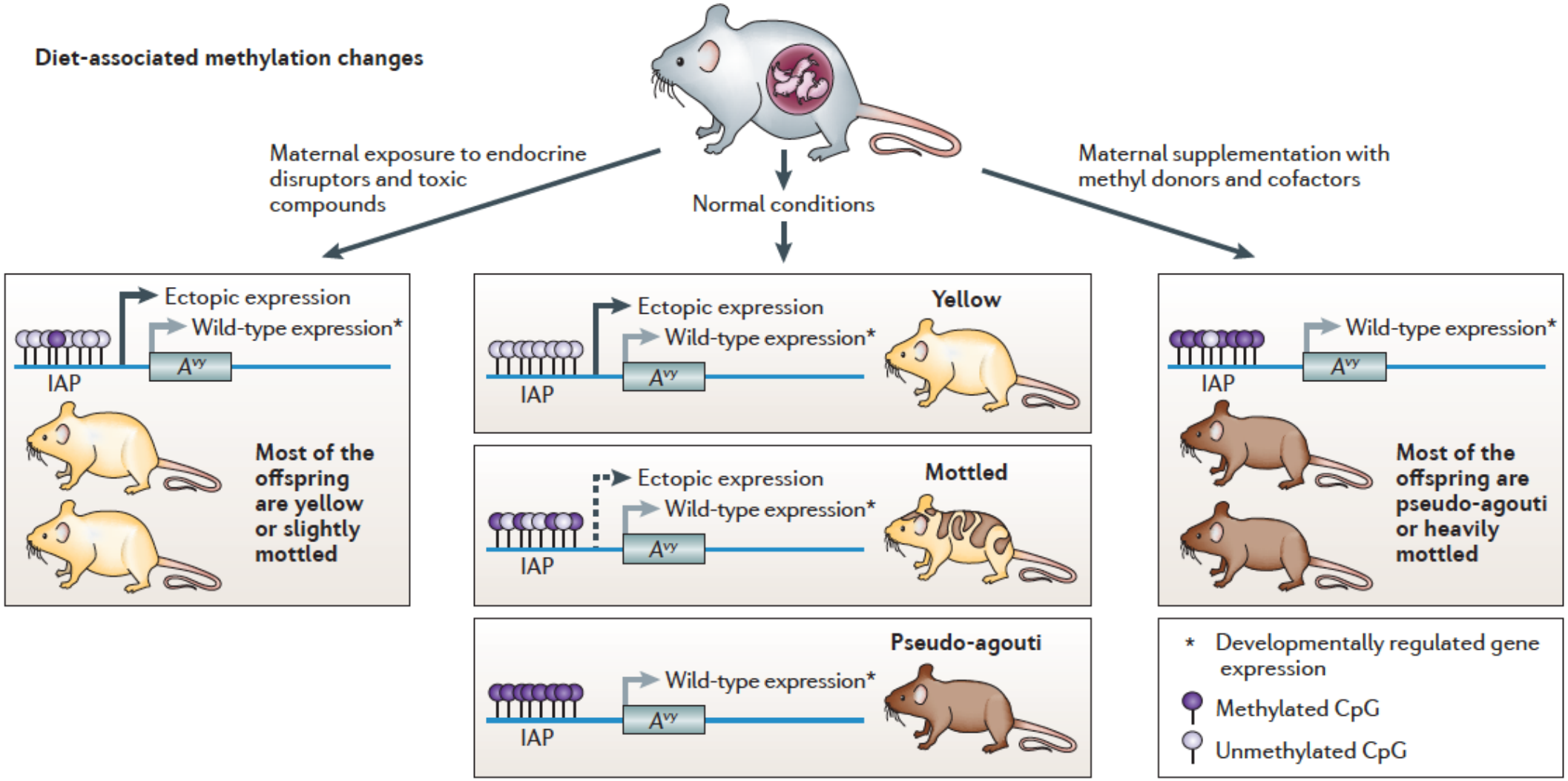




- Dietary Influence on Agouti Locus
  - Intracisternal A particle ~ Variable methylation
    - Influences Agouti Promoter
  - Ectopic Agouti Expression → Yellow Coat
    - Also Obesogenic
  - Controlled expression → Brown Coat
  - Methylation late/partial → Mottled Coat

Jirtle & *Skinner.* (2007) ***Nat Rev Genet***

Maternal Dietary Influence



Feil & Fraga (2012) *Nat Rev Genet*

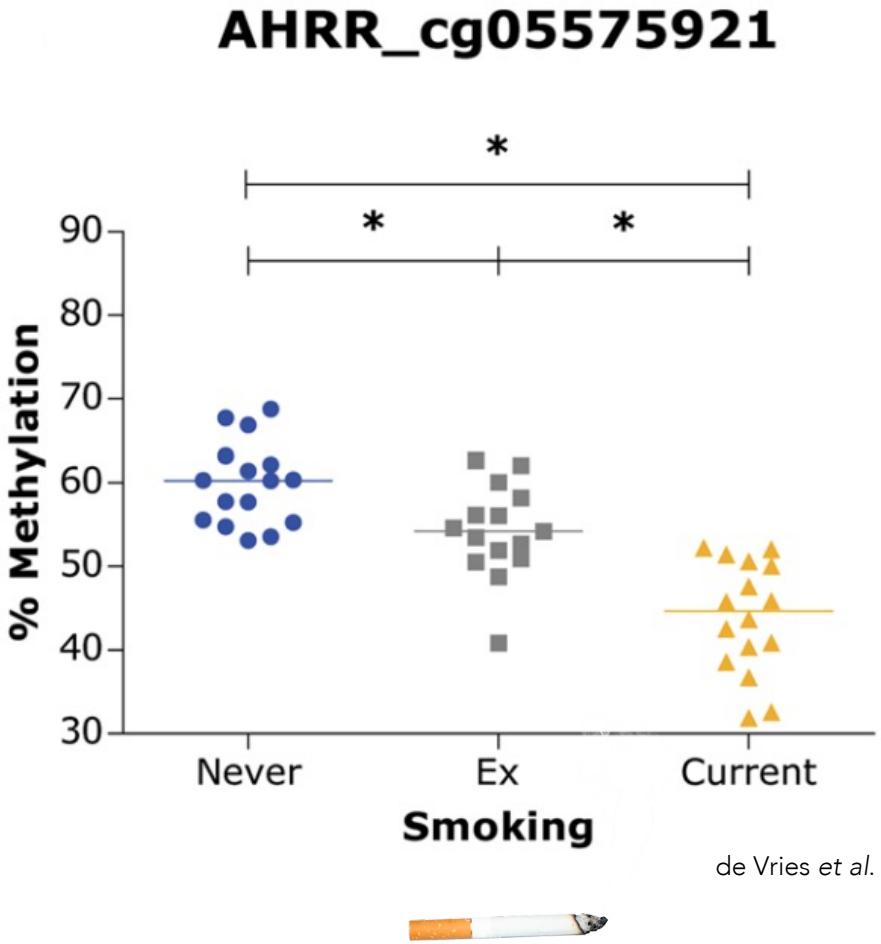
Tobacco Smoking

- → Significant Change
  in the Blood DNA methylome
  - Maternal – *In utero* effects
  - Passive Effects
  - Ex-smoker Effects

Joubert et al. (2012) EHP

Environmental Exposure: Tobacco Smoke

- **DNA methylation** = Quantitative **Biomarker** of Tobacco Exposure
  - Smokers
  - Ex-smokers
  - Passive Smoking
  - Prenatal exposure Infants (Jorbert *et al.*)
- **Strong signal 1 locus**
  - *AHRR*
    - Aryl Hydrocarbon Receptor Repressor
    - Detoxification process of Tobacco productions

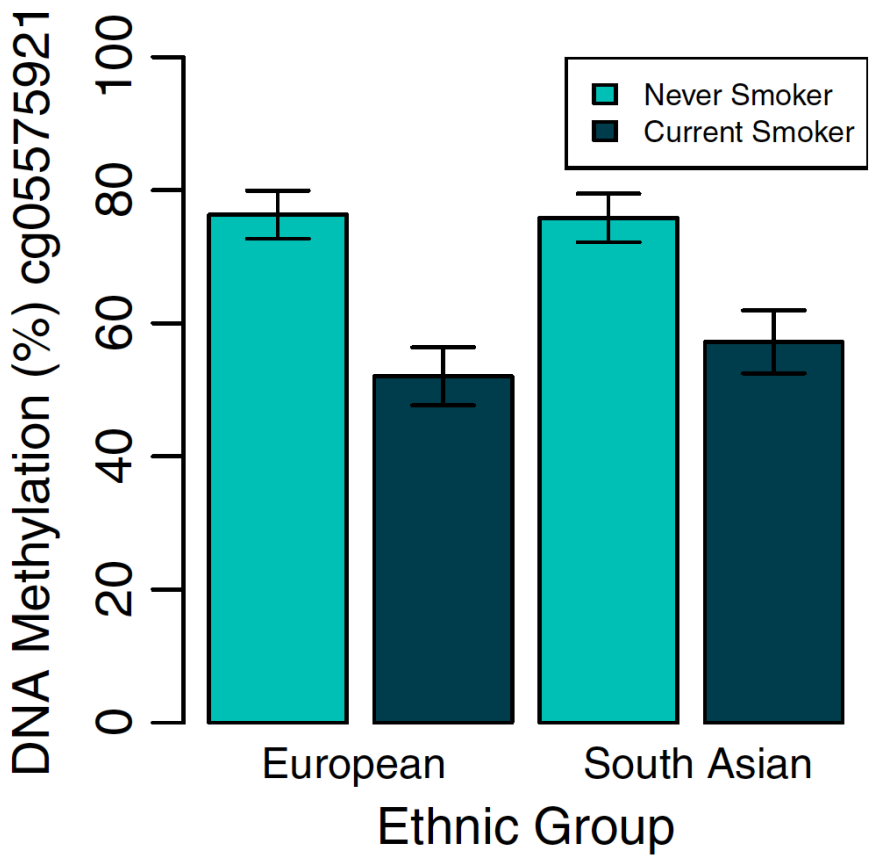


de Vries *et al.* (2018)

Jorbert *et al.* (2016) DNA methylation in Newborns and Maternal Smoking in Pregnancy: Genome-Wide Consortium Meta-analysis. ***AJHG***; de Vries *et al.* (2018) ***Respir Res***

AHRR
cg05575921

Aryl Hydrocarbon Receptor Repressor (*AHRR*) CpG



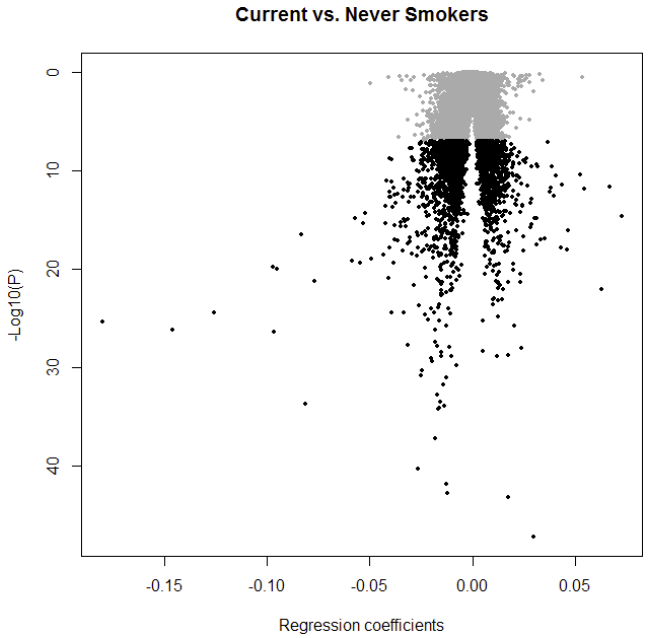
Elliot *et al.* (2014) *Clinical Epigenetics*

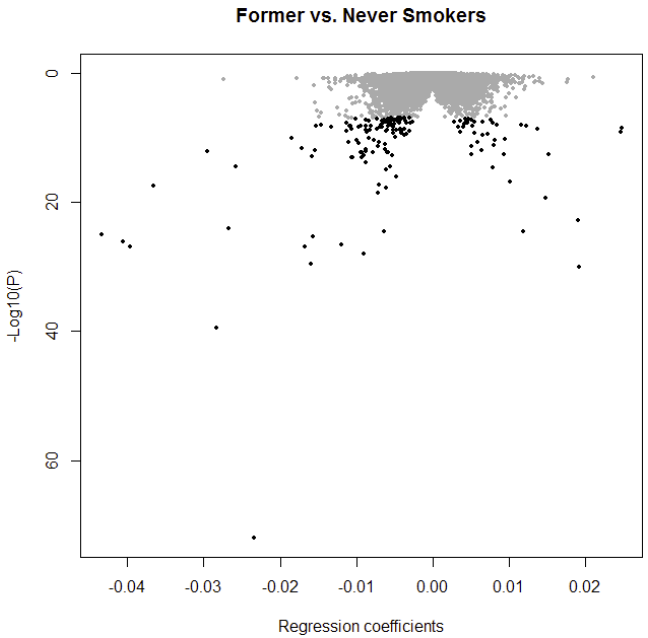
DNA methylome Signatures of Cigarette Smoking

- 15,907 blood DNA samples (16 cohorts)
  - 2,433 current
  - 6,518 former
  - 6,956 never

  } Smokers (Joehanes *et al.*)

  - **Current** versus **Never** smokers
    - **2,623 CpGs** at Bonferroni $p<1x10^{-7}$
      - annotated to 1405 genes
      - 18,760 CpGs at FDR < 0.05
  - **Former** versus **Never** smokers
    - **185** of the current v never CpGs, $p<1x10^{-7}$
      - 2,623 CpGs at FDR <0.05
      - Pattern of persistent altered methylation

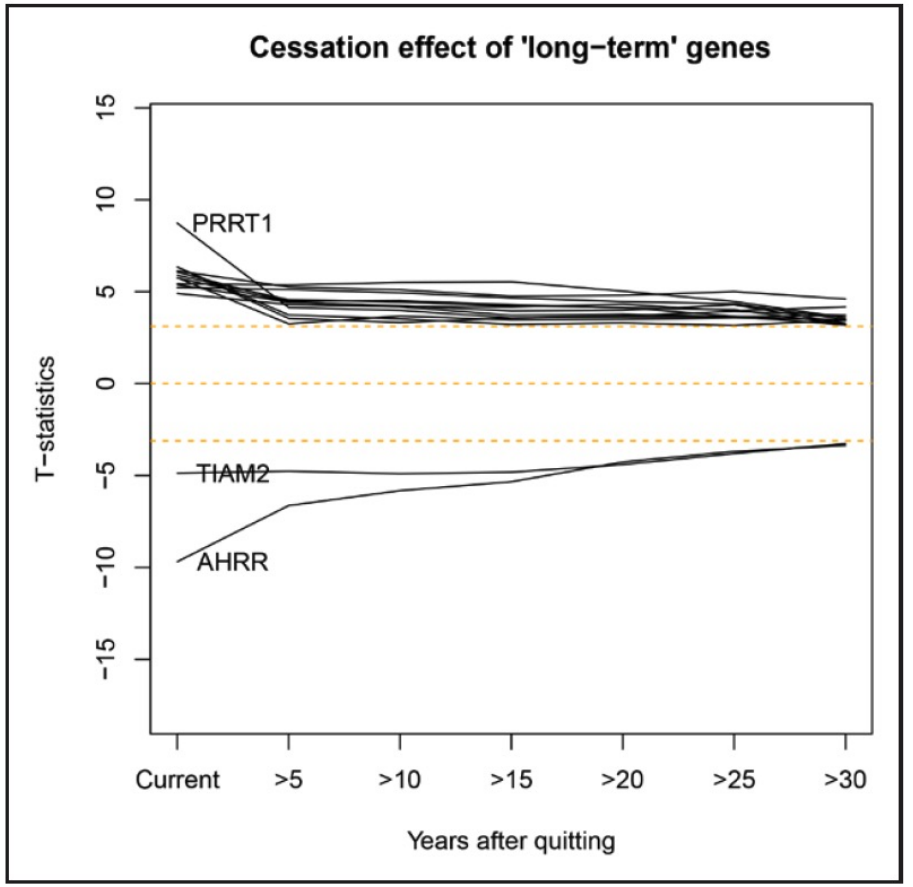




Joehanes *et al.* (2016) Epigenetic Signatures of Cigarette Smoking ***Circ Cardiovasc Genet***

Long term maintenance of Smoking DNAm changes

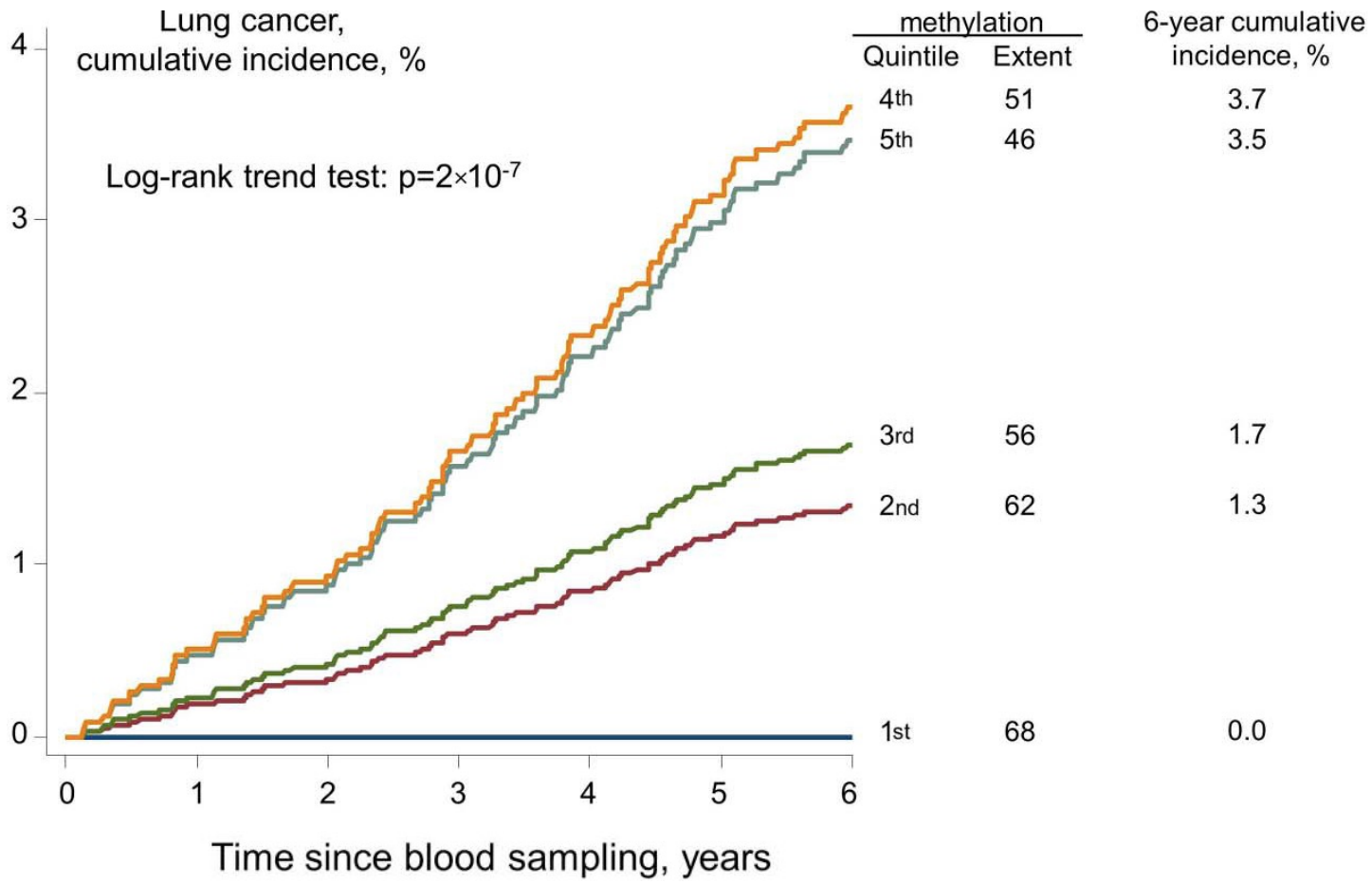
- Majority of differentially methylated CpGs
  - Observed in **Current** versus **Never** smokers
  - Returned to the level of never smokers within 5 years of smoking cessation
- However, Trajectories of 36 CpGs (19 genes)
  - Did Not Return to Never-Smoker Levels
    - 30 years After Smoking Cessation in the Framingham Heart Study (n=2648)
      - *e.g. PRRT1, TIAM2, AHRR*



Joehanes *et al.* (2016) Epigenetic Signatures of Cigarette Smoking ***Circ Cardiovasc Genet***

AHRR DNA Methylation ⟶ Future Lung Cancer Risk

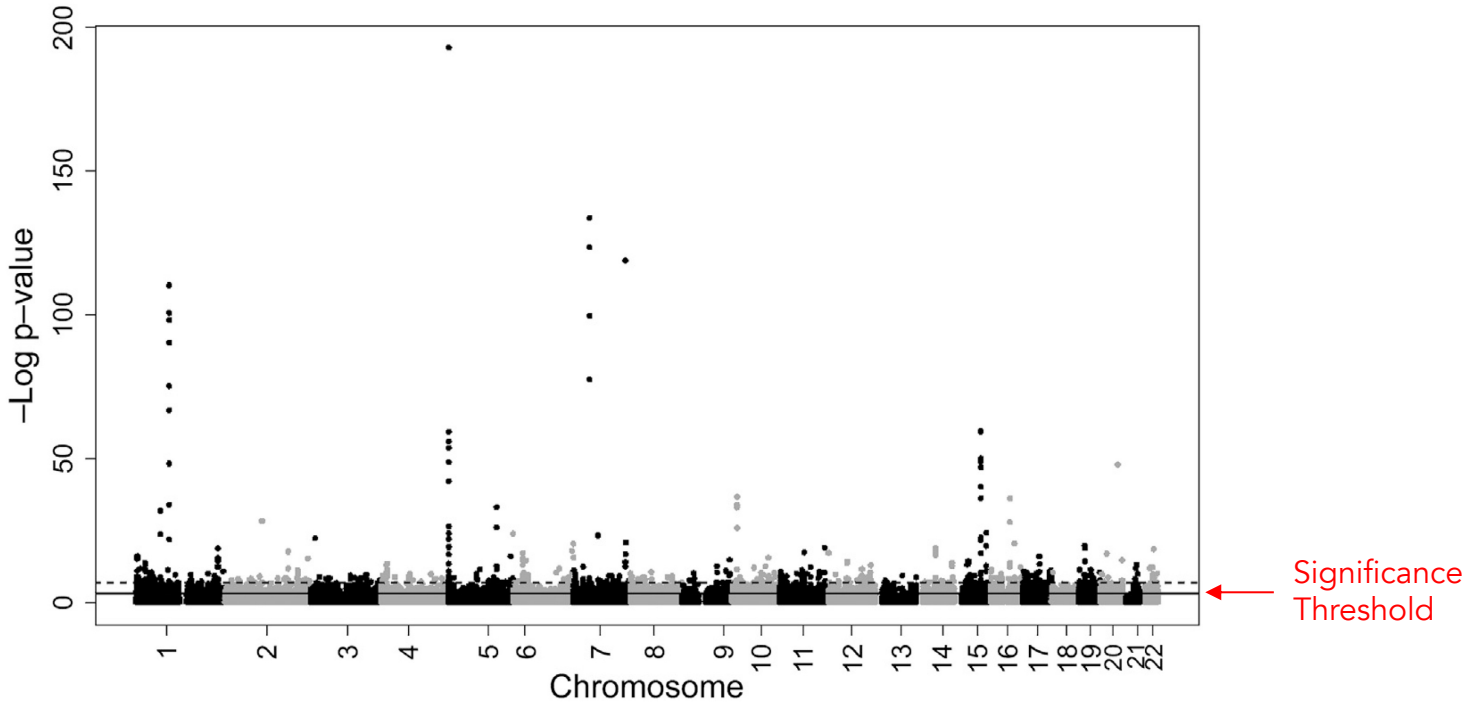
- *AHRR* CpG Biomarker
  - cg05575921
    - = Marker of smoking behaviour
  - Future Lung Cancer Risk
    - ~2k High Risk smokers
    - Adults mean ~60 years
    - Cumulative incidence
      - Lung cancer
      - Predicted 6-year risk by *AHRR* DNA methylation Quintiles



Bojesen *et al.* (2017) *AHRR* (cg05575921) hypomethylation marks smoking behaviour, morbidity and mortality. ***Thorax***

In Utero Smoking Exposure → Newborn DNA methylation

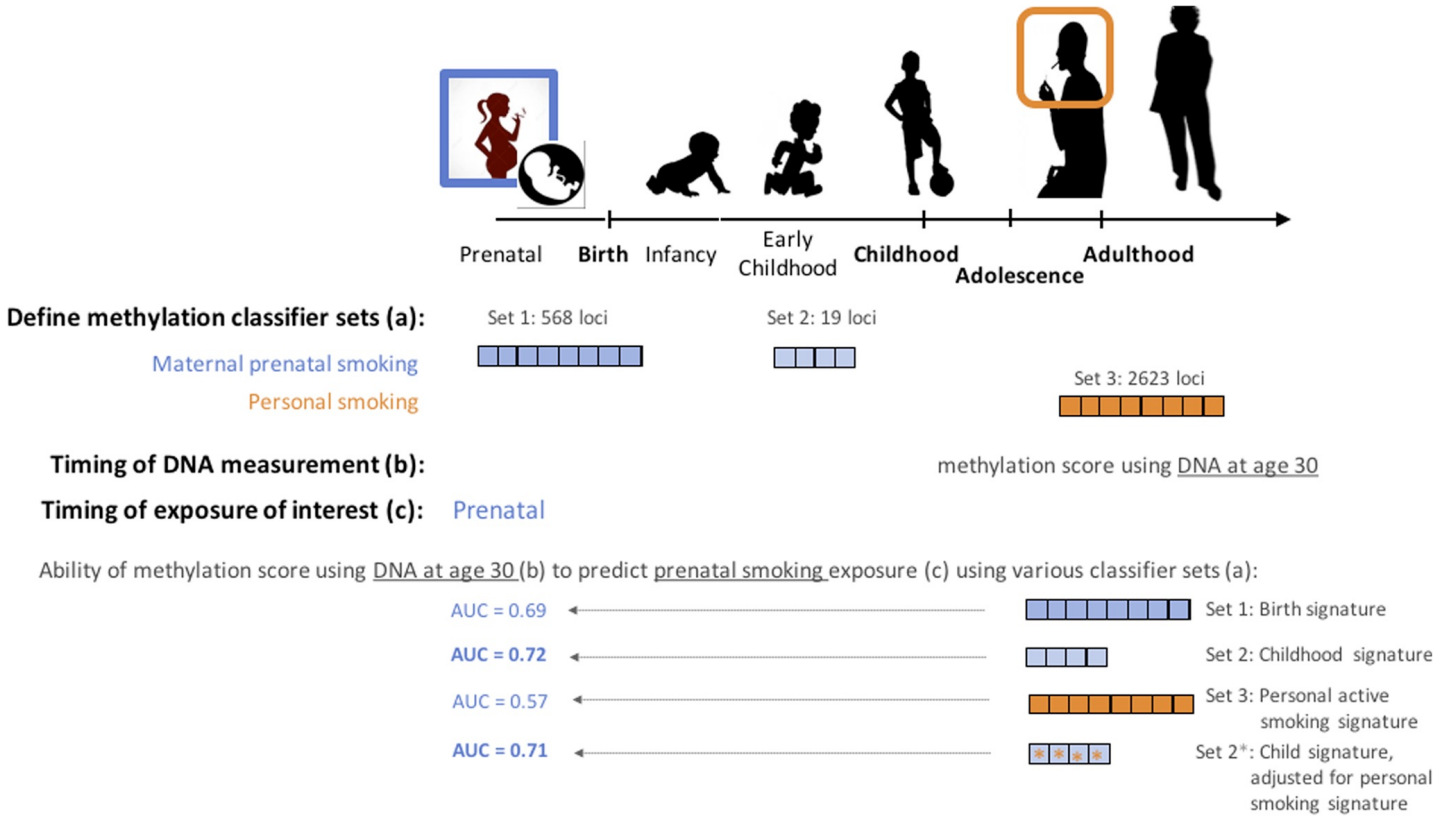
- Meta Epigenome Wide Association Study
  - 13 cohort (~6.5k individuals)
    - Newborn Blood
  - ~6,000 CpG DNAm Δs
    - Methylation Variation Relevant to Diseases influenced by Maternal Smoking
      - *Incl.* Asthma and Orofacial Clefts
    - & Can persist into childhood



Joubert *et al.* (2016) DNA methylation in Newborns and Maternal Smoking in Pregnancy: Genome-Wide Consortium Meta-analysis. ***AJHG***

Able to Distinguish Smoking Exposure Periods

- DNA methylation patterns/ scores
  - Accurate biomarker past exposure
    - ⟶ Environmental & Gene-environment interaction studies in existing banked samples
  - 2 Types of exposures
    - Prenatal & Personal exposure isolated (Richmond *et al.*)
      - DNA collected at age 30
    - Predict Prenatal exposure to smoking with ~72% accuracy
    - Postnatal personal smoking
      - **Not** good predictor of Prenatal smoking exposure (AUC=0.57)
      - Suggesting Methylation patterns differ by exposure window



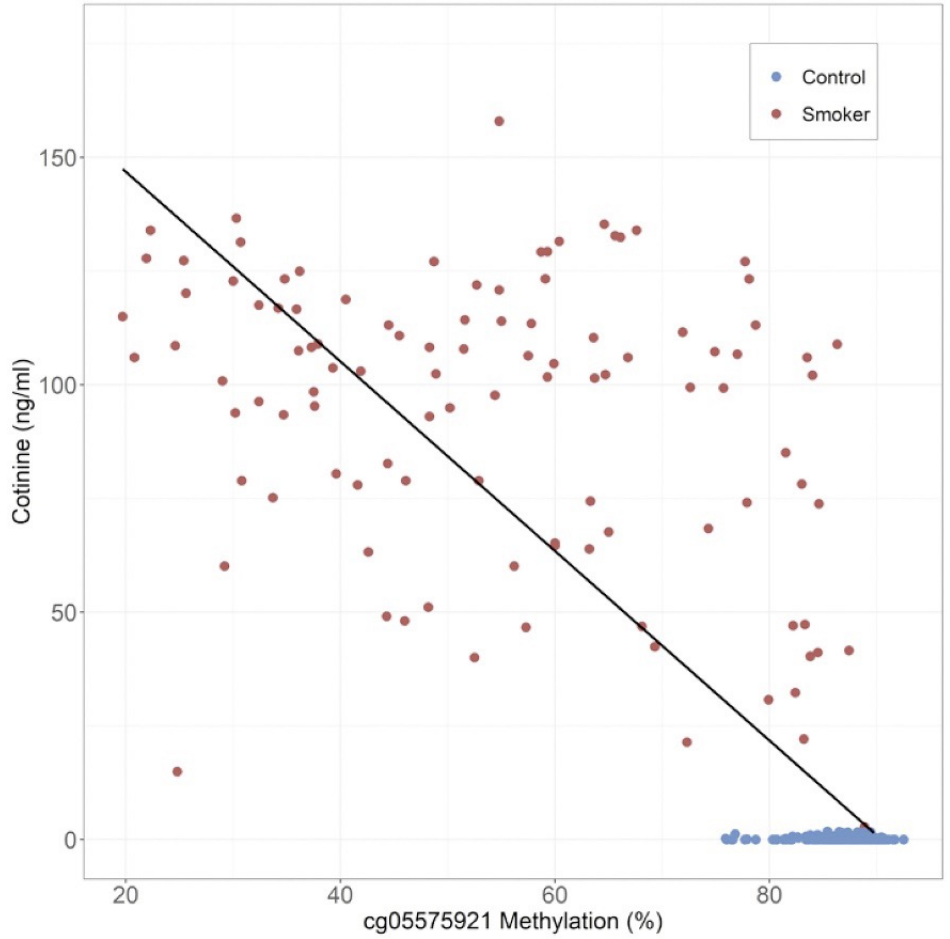
Ladd-Acosta & Fallin (2019) DNA Methylation Signatures as Biomarkers of Prior Environmental Exposures ***Genetic Epidemiology***

Richmond R, *et al*. (2018) DNA methylation as a marker for prenatal smoke exposure in adults. ***Int J Epidemiol***.

AHRR CpG (cg05575921) correlation with Cotinine & CO

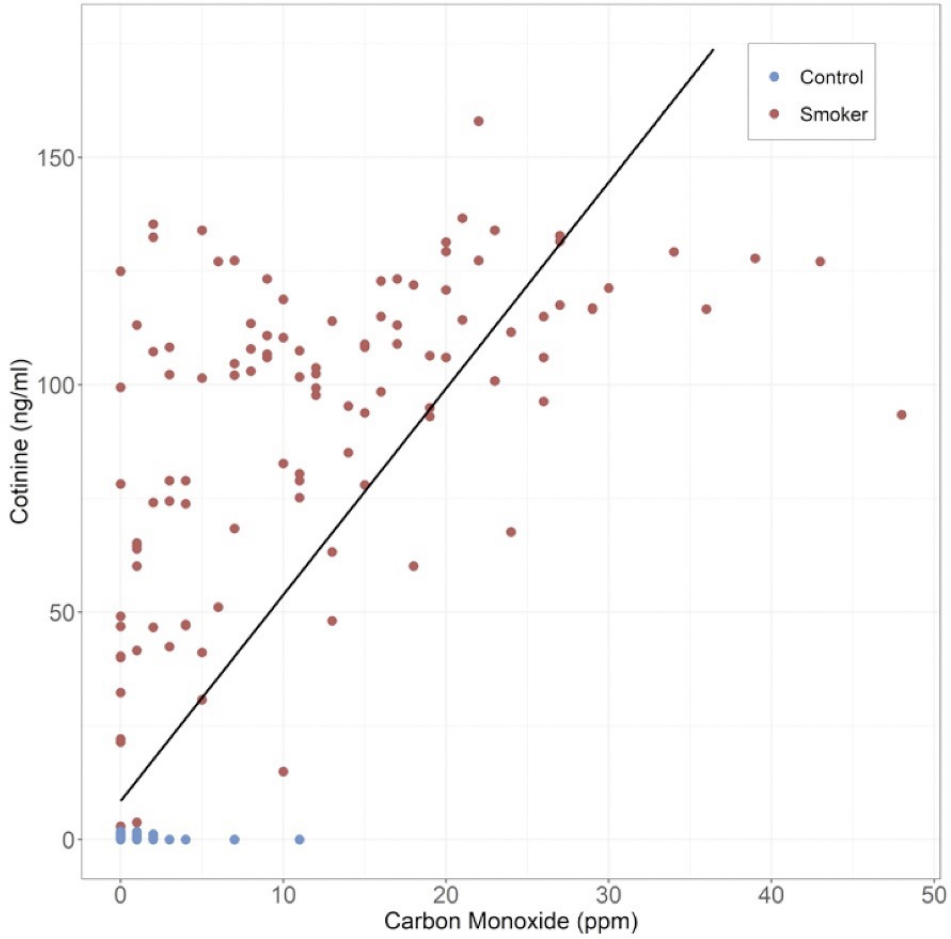
Serum Cotinine

Control & Smoking (n = 366, adj-R2 = 0.68)



Exhaled Carbon Monoxide Levels

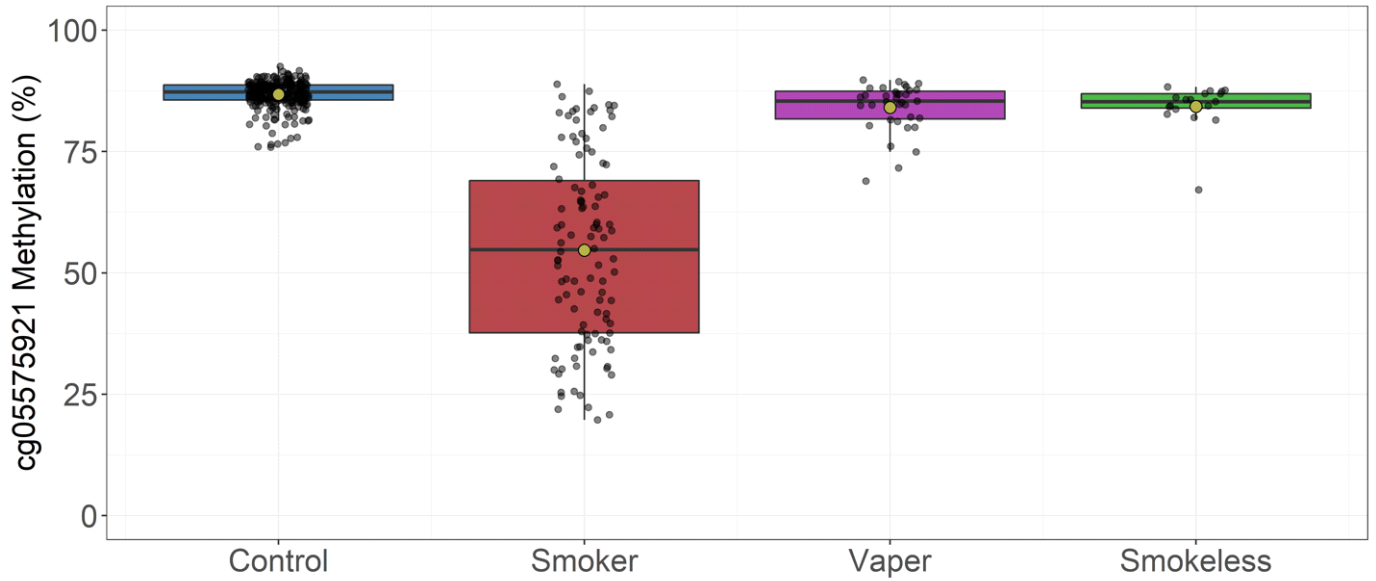
(n = 368, adj-R2 = 0.60)



Andersen *et al*. DNA methylation differentiates smoking from vaping and non-combustible tobacco use ***Epigenetics***

DNA methylation at cg05575921 Specific Biomarker of Combusted Tobacco Smoke Exposure

- DNA methylation at *AHRR* cg05575921
  - Biomarker of Combusted Tobacco → ⇩ DNAm
  - Can differentiates exposure to combusted tobacco smoking
  - From vaping (e-cigarettes) & non-combustible tobacco use

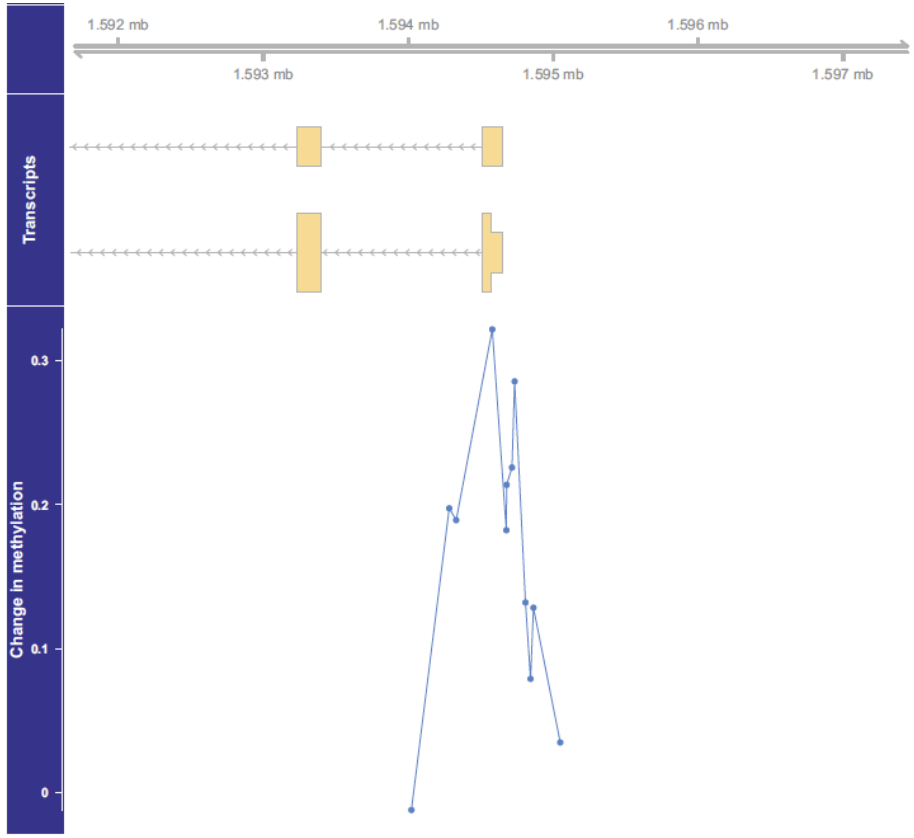


Andersen *et al*. DNA methylation differentiates smoking from vaping and non-combustible tobacco use ***Epigenetics***

Pollution → DNA methylation → Atherosclerosis

- Air pollution effect on atherosclerosis
  - Epigenome-wide association study (EWAS)
    - In CD14+ Monocytes
      - Cell Type critical in atherosclerosis pathology
    - Long-term ambient air pollution exposure
      - Adults: Multi-Ethnic Study of Atherosclerosis (MESA) - n = 1,207
      - 1-year average concentrations outdoor
        - Fine particulate matter (PM2.5)
        - Oxides of nitrogen (NOX)
        - Estimated at participants' homes
    - PM2.5 = 4 differentially methylated regions (DMRs)
      - within/near *SDHAP3*, *ZFP57*, *HOXA5*, & *PRM1*
    - NOX = 2 DMRs
      - at *SDHAP3* & *ZFP57*
    - Some DMRs associated with gene expression
      - *e.g. HOXA5* DMR with *HOXA5*, *HOXA9*, & *HOXA10*
      - Novel insights air pollution → cardiovascular disease

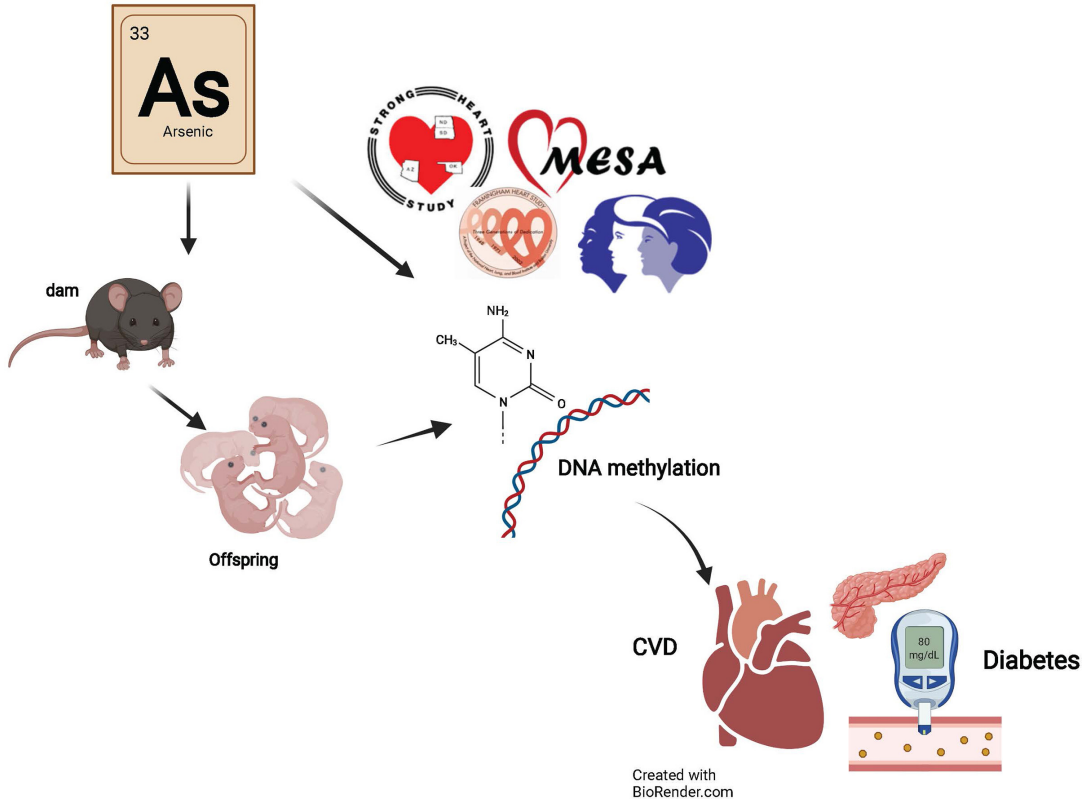
SDHAP3



Chi *et al*. (2021) Epigenome-wide analysis of long-term air pollution exposure and DNA methylation in monocytes: results from the Multi-Ethnic Study of Atherosclerosis. ***Epigenetics***

Arsenic Exposure associated DNA methylation Changes

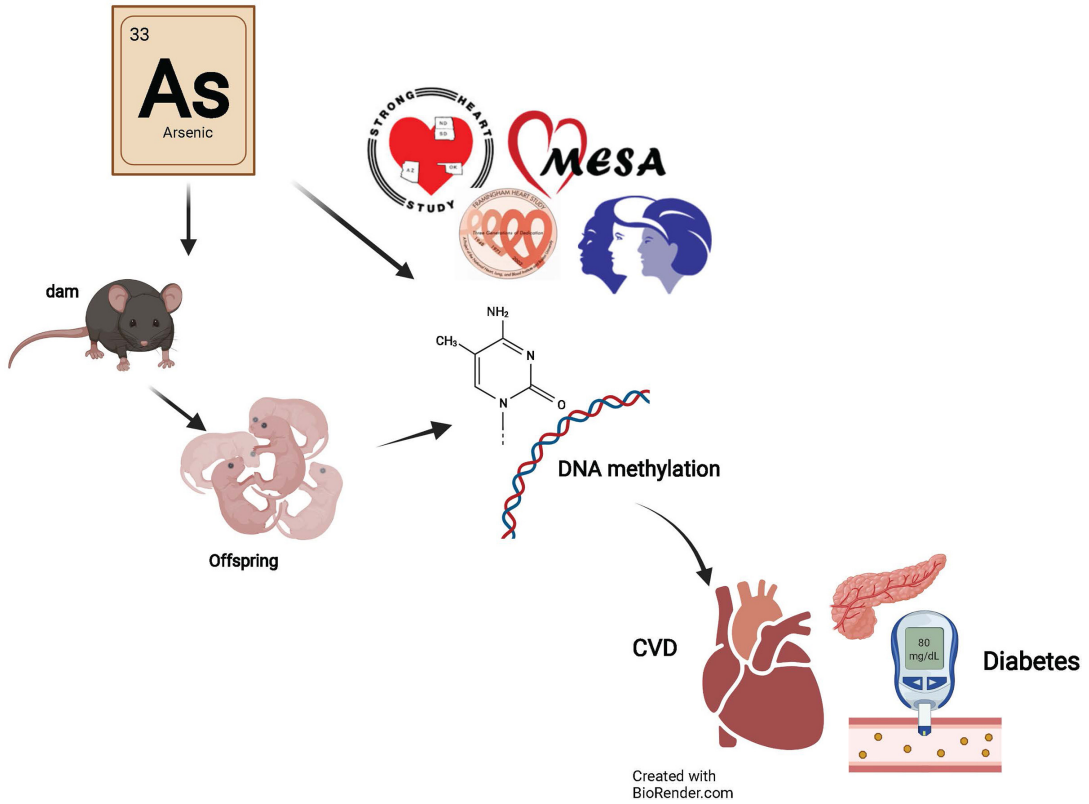
- Arsenic related to multiple health outcomes
  - Even at low exposure levels in water & food
  - Including:
    - Atherosclerotic Cardiovascular Disease (CVD)
    - Coronary Heart Disease
    - Stroke
    - Peripheral Arterial Disease
    - Overall CVD mortality
  - Also prospectively
    - Changes in Blood Pressure
    - Carotid Atherosclerosis



Domingo-Relloso *et al.* (2022) Arsenic Exposure, Blood DNA Methylation, and Cardiovascular Disease. ***Circ Res***

Arsenic Exposure associated DNA methylation Changes

- **Arsenic** induces epigenetic modifications in experimental models
  - DNA methylation
    - Proposed as intermediate mechanism between environmental exposures and disease
- **Blood DNA methylation** analysed
  - 2,321 participants
    - Strong Heart Study:
    - American Indian prospective cohort
    - Mean age 56.2, 58.6% ♀
  - **Urinary arsenic** species were measured
    - Using high-performance liquid chromatography coupled to inductively coupled plasma mass spectrometry



Domingo-Relloso *et al.* (2022) Arsenic Exposure, Blood DNA Methylation, and Cardiovascular Disease. ***Circ Res***

Arsenic Exposure associated DNA methylation Changes

- Arsenic DNA methylation changes
  - 20 & 13 Differentially Methylated Positions (DMPs) were potential mediators for CVD incidence & mortality, respectively,
    - Several in/near genes related to Diabetes
    - 11 of these DMPs associated with incident CVD in 3 diverse prospective cohorts
      - Framingham Heart Study, Women's Health Initiative, & Multi-Ethnic Study of Atherosclerosis
  - Mouse model arsenic-induced atherosclerosis
    - Differential liver DNA methylation following early-life arsenic exposure
    - DMPs in 10 genes overlap
  - Possible biological link b/t arsenic & CVD
    - Gene functions support that diabetes & redox signalling are involved in arsenic-induced CVD

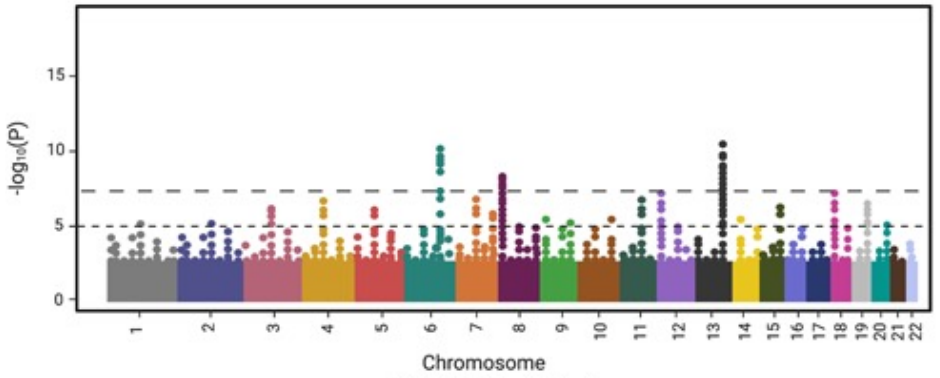
Table 2. HRs (95% CIs) of the Common Differentially Methylated Positions for Cardiovascular Disease Incidence and Mortality Comparing the 90th vs the 10th Percentile of Methylation Obtained From the Cox ISIS-Aenet

CpG	Chr	Gene	Function	Location	CVD incidence HR (95% CI)	CVD mortality HR (95% CI)
cg13251119	1	*EPS8L3*	Unknown function	Body	0.51 (0.29–1.00)	0.18 (0.06–0.63)
cg00841849	2	*ID2*	Cellular growth, senescence, differentiation, apoptosis, angiogenesis, neoplastic transformation	Intergenic	0.57 (0.40–0.84)	0.63 (0.32–1.01)
cg14066163	17	Unknown	…	Intergenic	0.63 (0.39–1.00)	0.67 (0.31–1.17)
cg25371036	11	*AMOTL1*	Endothelial cell migration, capillary formation	TSS1500	0.71 (0.54–0.92)	0.42 (0.27–0.73)
cg03362418	22	*TYMP*	Angiogenesis and endothelial cell growth. Proposed as therapeutic target for CVD	Body	0.73 (0.50–1.02)	0.51 (0.29–0.94)
cg25452273	15	*PPCDC*	Biosynthesis of coenzyme A. Metabolism of water-soluble vitamins	Body	1.25 (0.96–1.81)	1.80 (1.00–3.42)
cg18130370	22	*NCF4*	Arterial remodeling and advanced atherosclerosis	Body	0.79 (0.48–1.12)	0.44 (0.19–0.99)
cg00451635	16	*EMP2*	Blood vessel endothelial cell migration and angiogenesis	TSS1500	1.11 (0.86–1.33)	0.68 (0.46–1.00)
cg06970472	4	*APBB2*	Beta-cell function, insulin secretion impairment in mice	Body	1.22 (0.93–1.61)	0.69 (0.43–1.05)

Domingo-Relloso *et al.* (2022) Arsenic Exposure, Blood DNA Methylation, and Cardiovascular Disease. ***Circ Res***

Integrating Genetic & Epigenetic Risk

Genetic Variants



GWAS Risk Loci

Environmental Risk Factors

DISEASE RISK

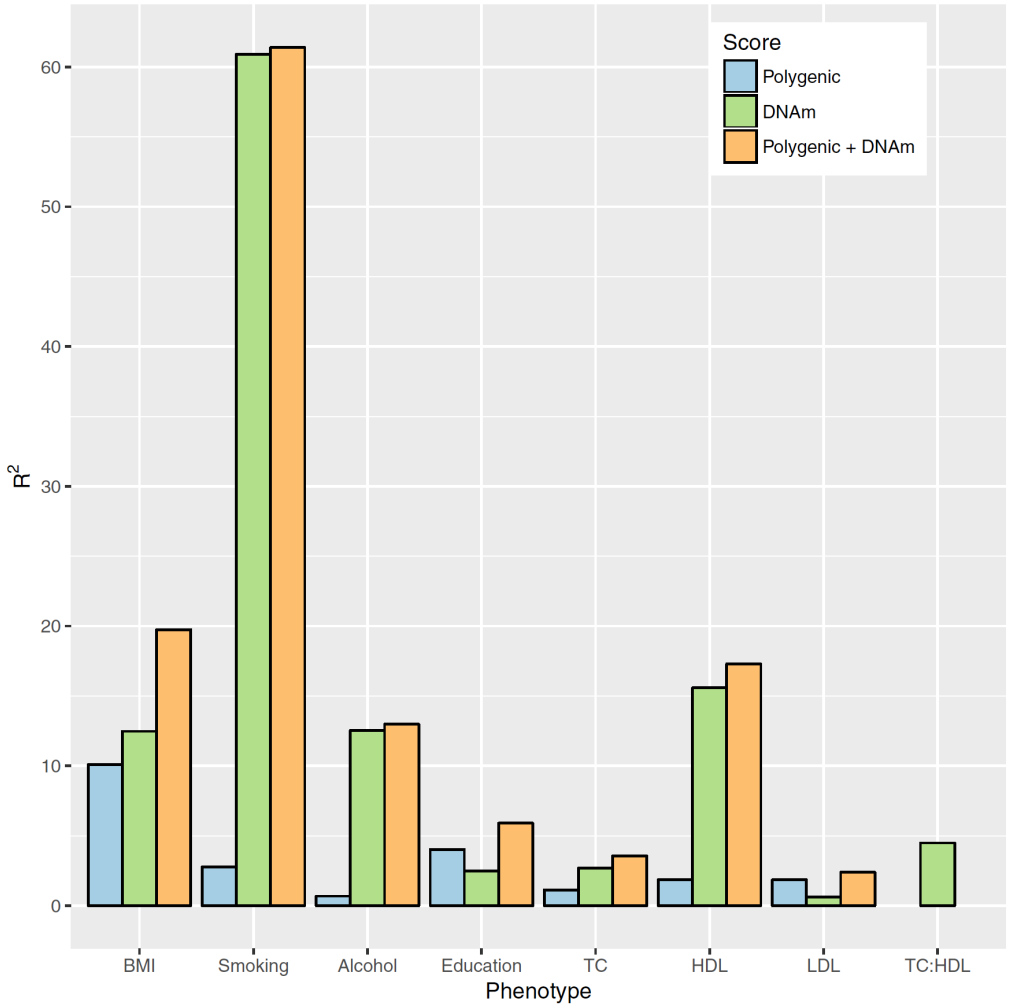
Virolainen *et al*. (2023) ***Genes & Immunity***

DNA methylation + Polygenic Predictors of Trait & Lifestyle

BMI

Combined ~19.7%
Polygenic ~10.1%
DNAm ~12.5%

= Proportion of Phenotype Variance Explained



- Prediction of Complex Traits
  - Combining Genetic Risk (Polygenic Risk Score) + Epigenetic predictors

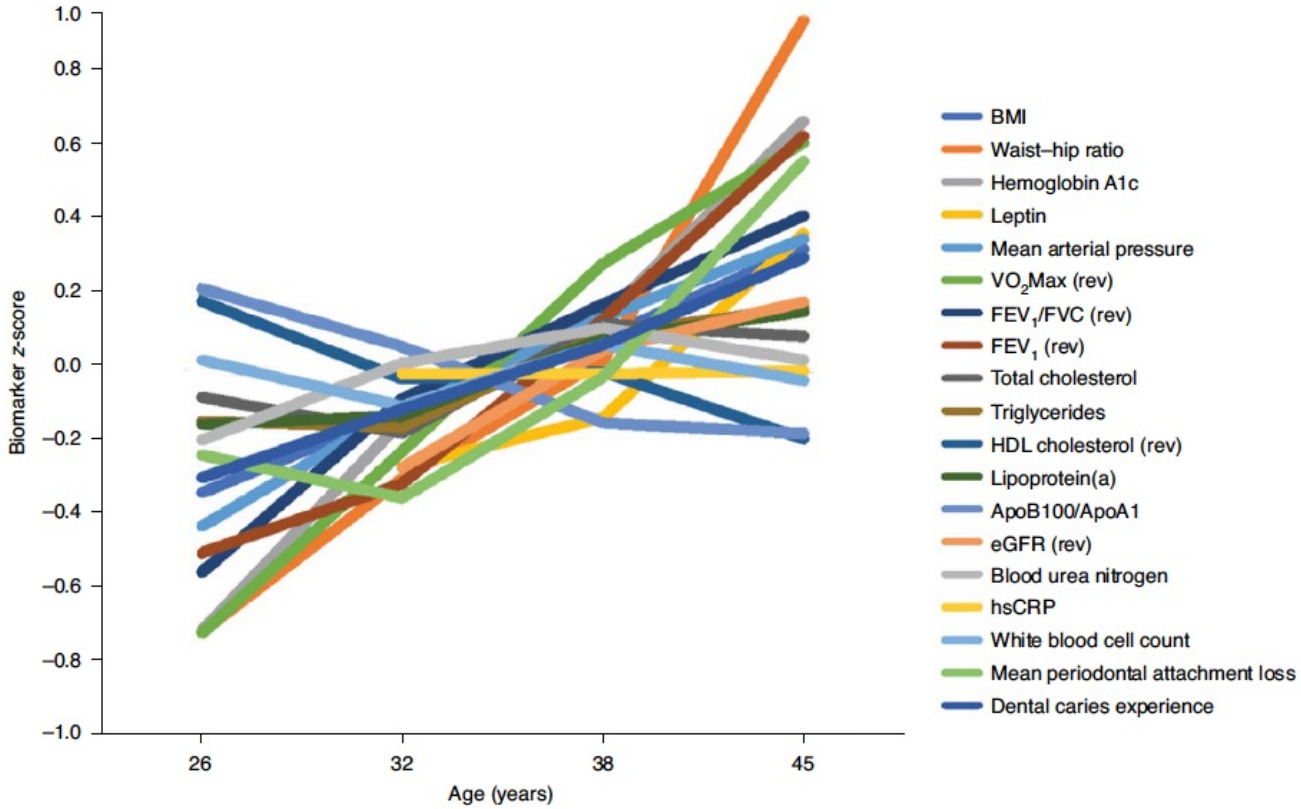
McCartney *et al.* (2018) Epigenetic prediction of complex traits and death. ***Genome Biology***

Queen Mary University of London

Ageing & The Epigenome

Ageing: Multisystemic Changes

- Biological Measures change with Age
  - ↑ $p16^{ink4a}$ tissue levels
  - ↑ Circulating CRP
  - ↑ Creatinine
  - ↑ Fasting Glucose
  - ↓ Telomere Length

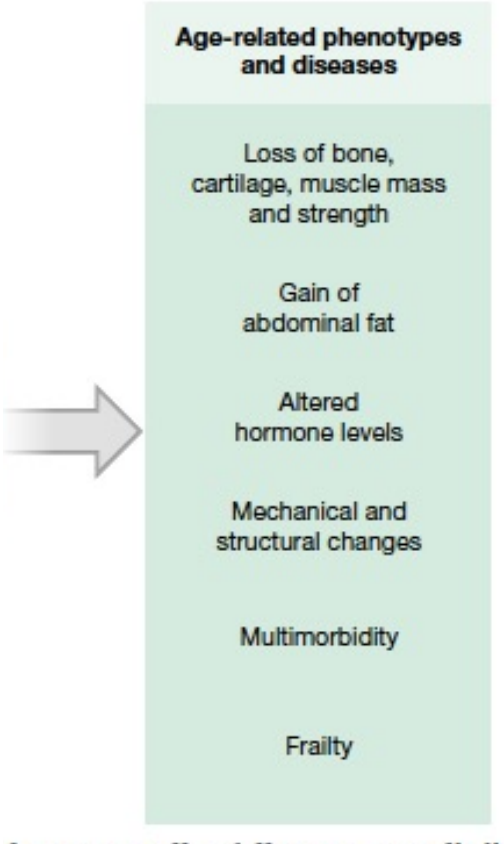


Elliot *et al.* (2021) *Nature Aging*

Burden of Age-related Disease

- Ageing is a major risk factor for
  - Cancer
  - Heart Disease
  - Dementia
  - Type 2 Diabetes *etc.*

- ∴ ↑ Understanding Pathological changes occurring with Ageing
  - ⟶ ↑ 'Healthspan' (Partridge *et al.*)



Partridge *et al.* (2018) Facing up to the Global Challenges of Ageing ***Nature***

Burden of Age-related Disease

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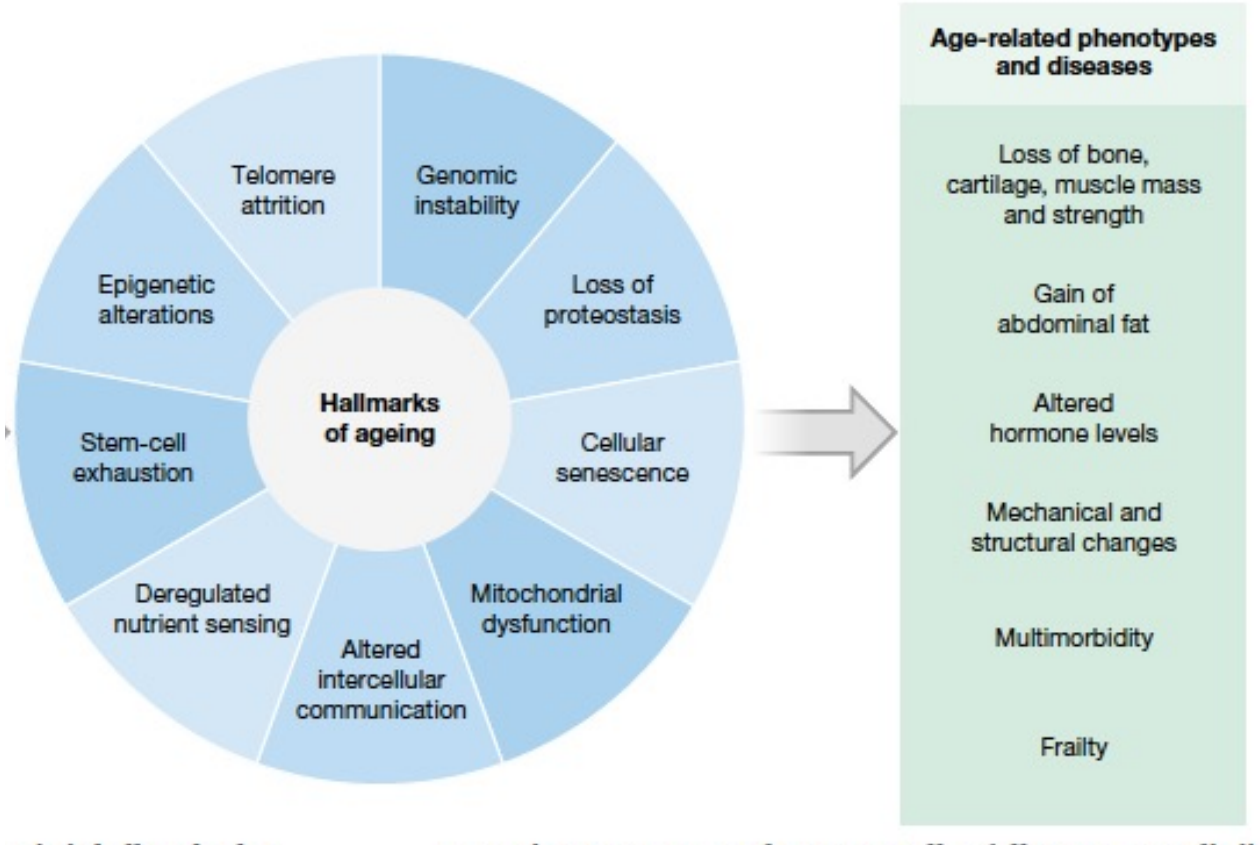
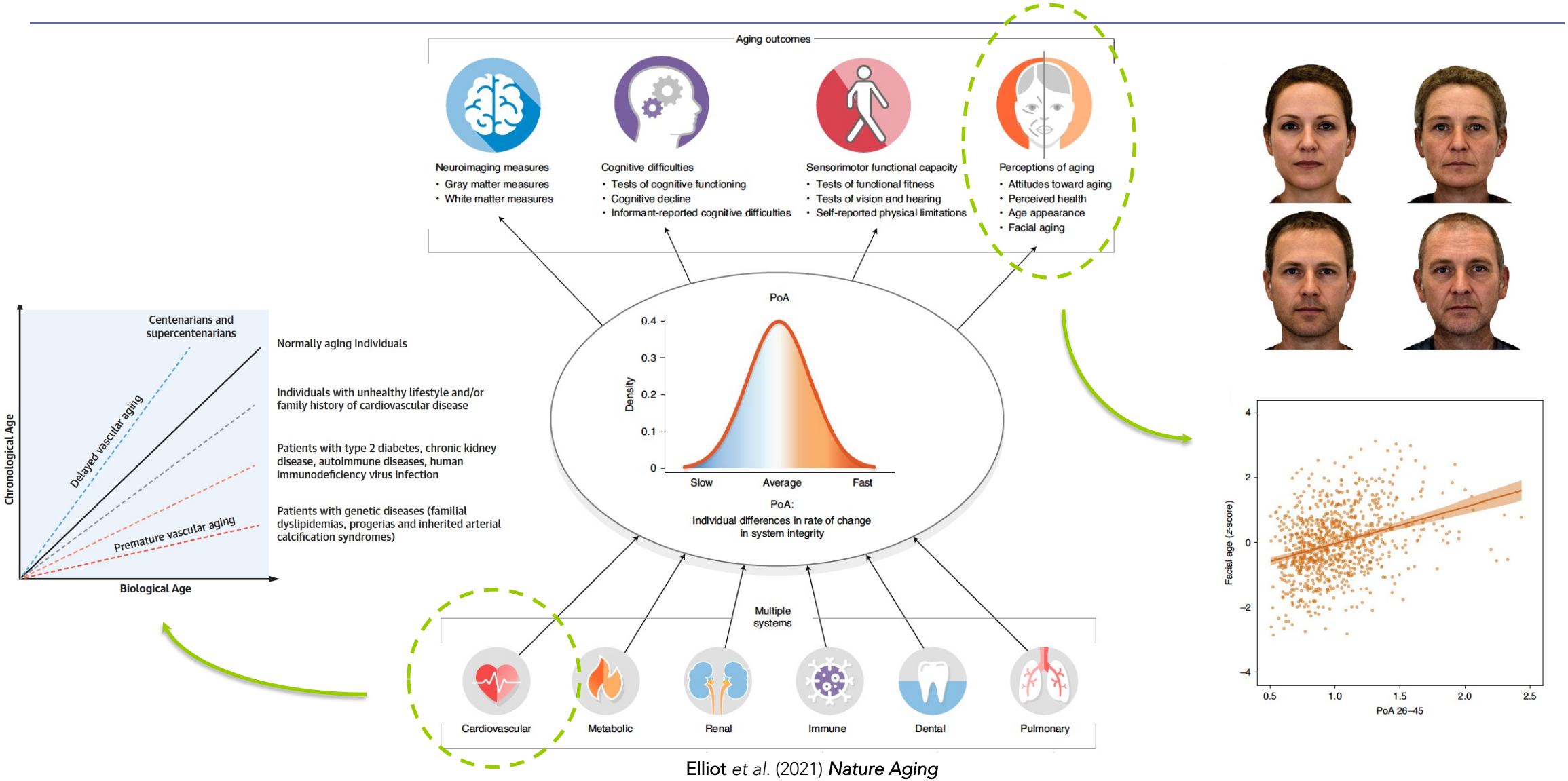
  - ⟶ ↑ 'Healthspan' (Partridge *et al.*)

9 Hallmarks of Ageing



Partridge *et al.* (2018) Facing up to the Global Challenges of Ageing ***Nature***

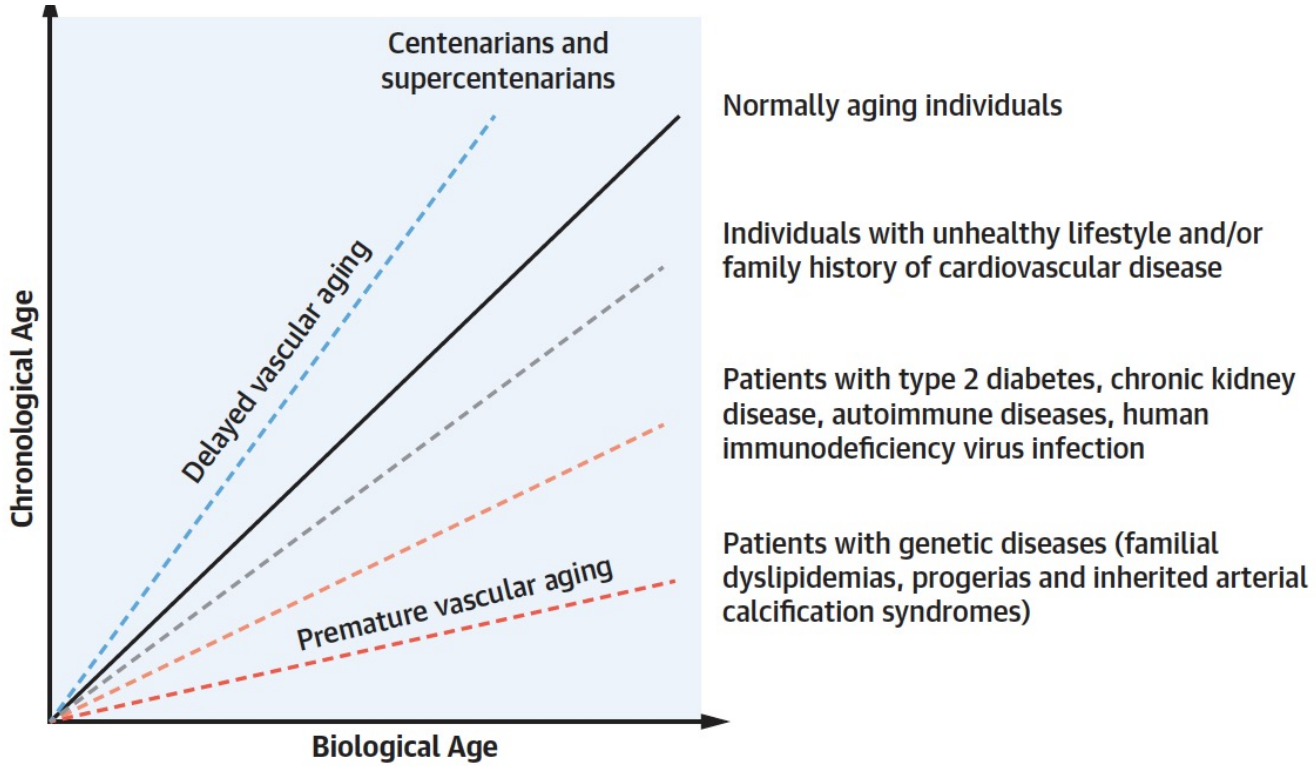
Individual Variation in Rates of 'Biological' Ageing



Elliot *et al.* (2021) ***Nature Aging***

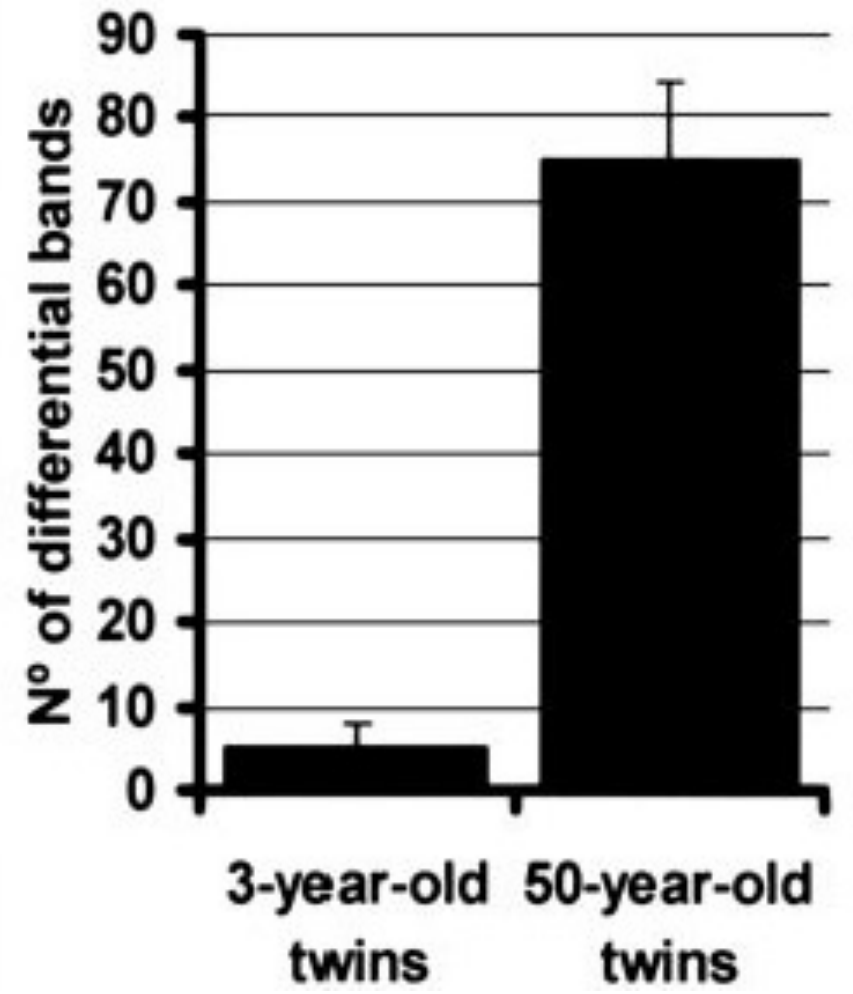
‘Biological’ versus Chronologic Ageing

- Early Life Biomarkers
  - High Risk Vascular Ageing
- Chronological Age
  - Suboptimal for Estimating Vascular Ageing
- ‘Biological’ Ageing
  - Functional/Physiological Ageing
  - Loss of Function



Hamczyk *et al.*(2020) Biological versus Chronological Ageing ***JACC***

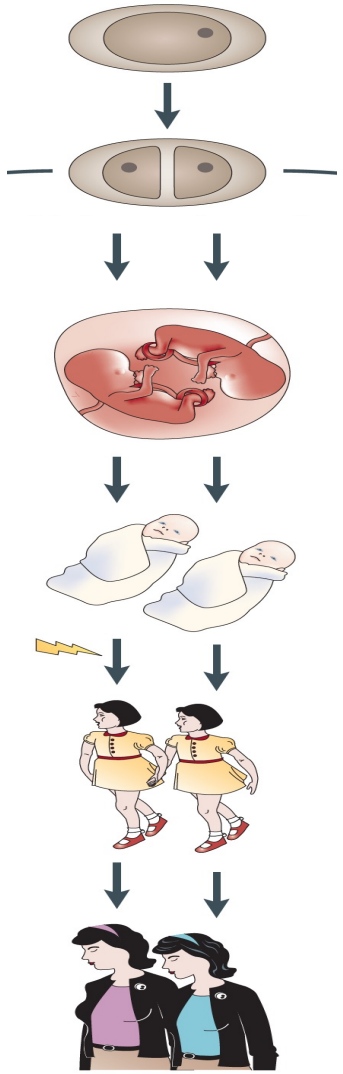
Differences arising during Identical Twins Lifetimes

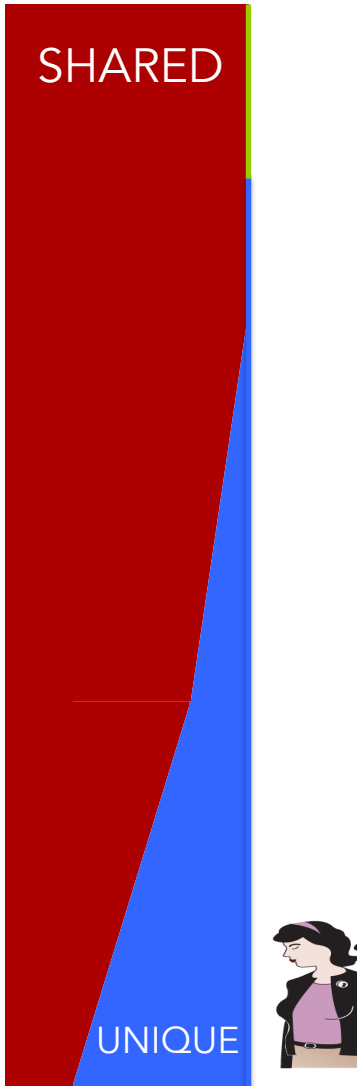


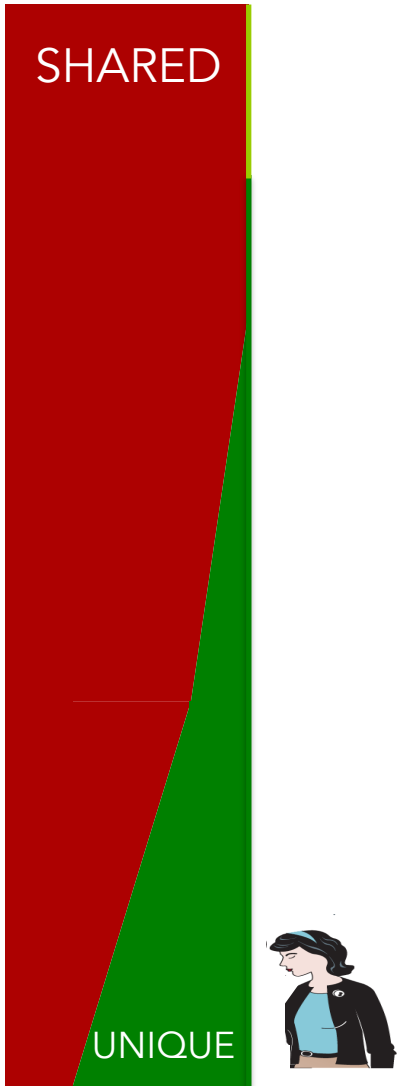
- Landmark Paper
  - Fraga et al. (2005)
- ↑ Variation in DNA methylation levels between
  - Old MZ twins
  - *cf.* Young MZ Twins
    - Amplification of intermethylated sites

Fraga *et al* (2005) Epigenetic differences arise during the lifetime of monozygotic twins ***PNAS***

Monozygotic Twins: Environmental Change with Age







Mill & Heijmans (2013) ***Nat Rev Genet***

Monozygotic versus Dizygotic Twins Disease Concordance

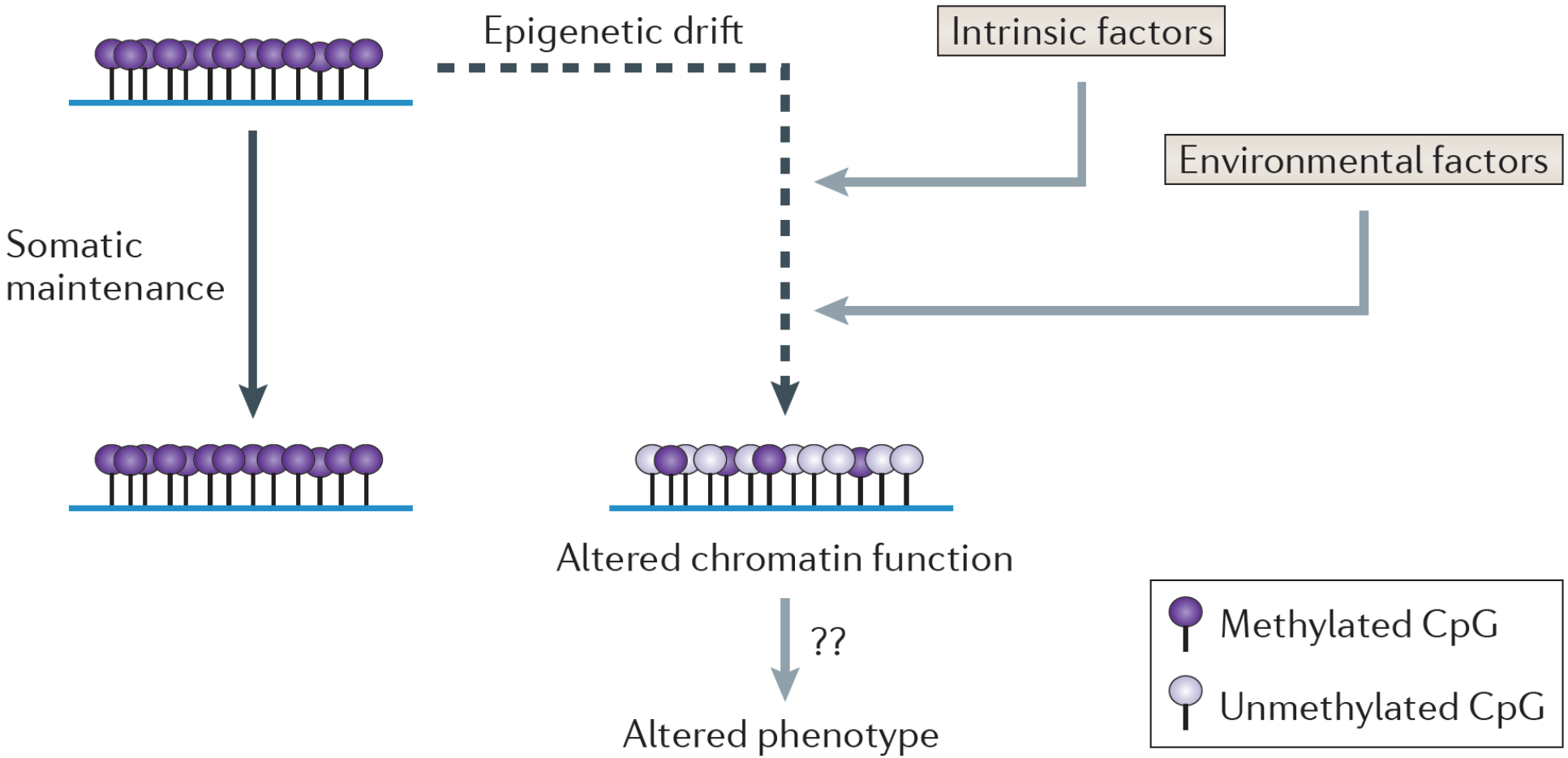
	Probandwise concordance* (%)	
	MZ twins	DZ twins
Type 1 diabetes	42.9	7.4
Type 2 diabetes	34	16
Multiple sclerosis	25.3	5.4
Crohn's disease	38	2
Ulcerative colitis	15	8
Alzheimer's disease	32.2	8.7
Parkinson's disease	15.5	11.1
Schizophrenia	40.8	5.3
Major depression	31.1‡ or 47.6§	25.1‡ or 42.6§
Attention-deficit hyperactivity disorder	82.4	37.9
Autism spectrum disorders	93.7	46.7
Colorectal cancer	11	5
Breast cancer	13§	9§
Prostate cancer	18	3

‡Concordance in male twin pairs. §Concordance in female

↑ MZ concordance
↑ Genetic influence

Van Dongen *et al.* (2012) ***Nat Rev Genet.***

‘Epigenetic Drift’ with Age



Feil & Fraga. (2012) *Nat Rev Genetics*

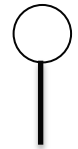
DNA methylome Ageing Changes

Early Life

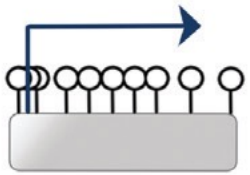
Old Age

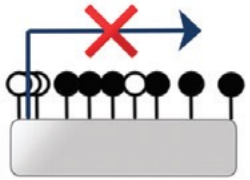
METHYLATED

UNMETHYLATED

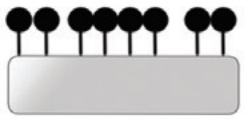


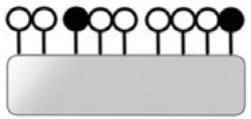
PROMOTERS





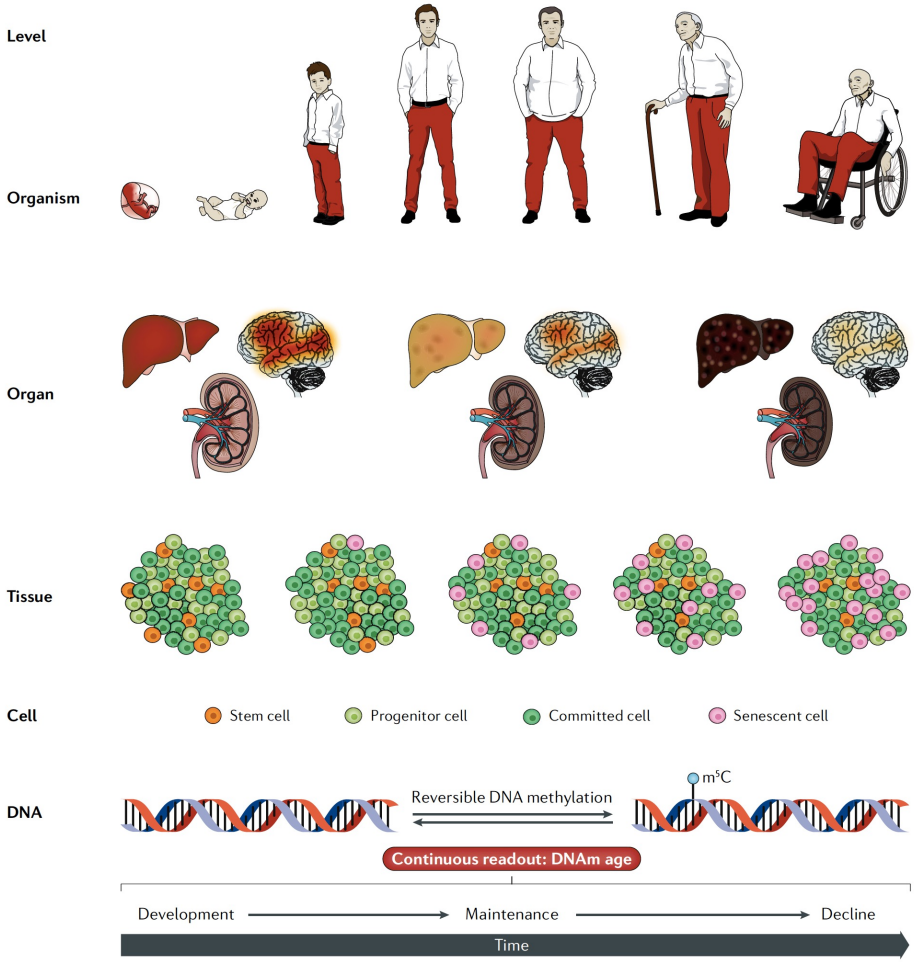
REPETITIVE ELEMENTS





Adapted from Amenyah *et al.* (2020) ***Curr Dev Nut***

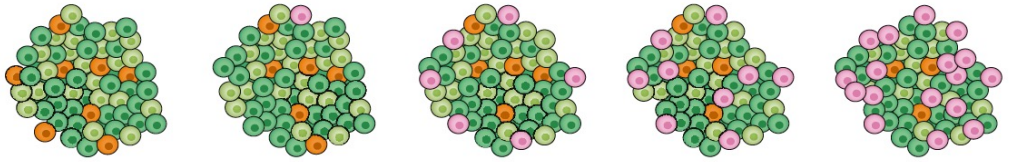
Detect Ageing from Whole Organism to Cell



Horvath & Raj. *et al.* (2018) ***Nat Reviews Genetics***

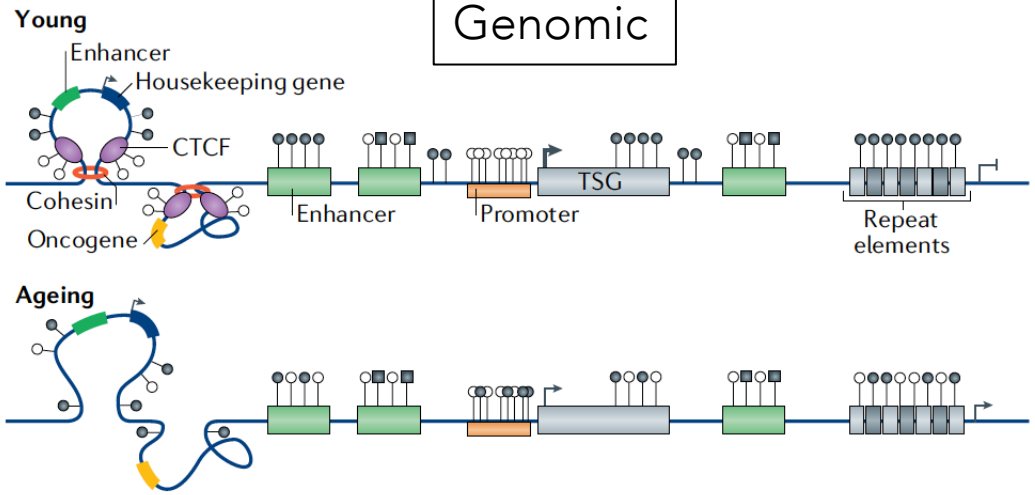
DNA methylation Changes with Age

Cellular Proportion



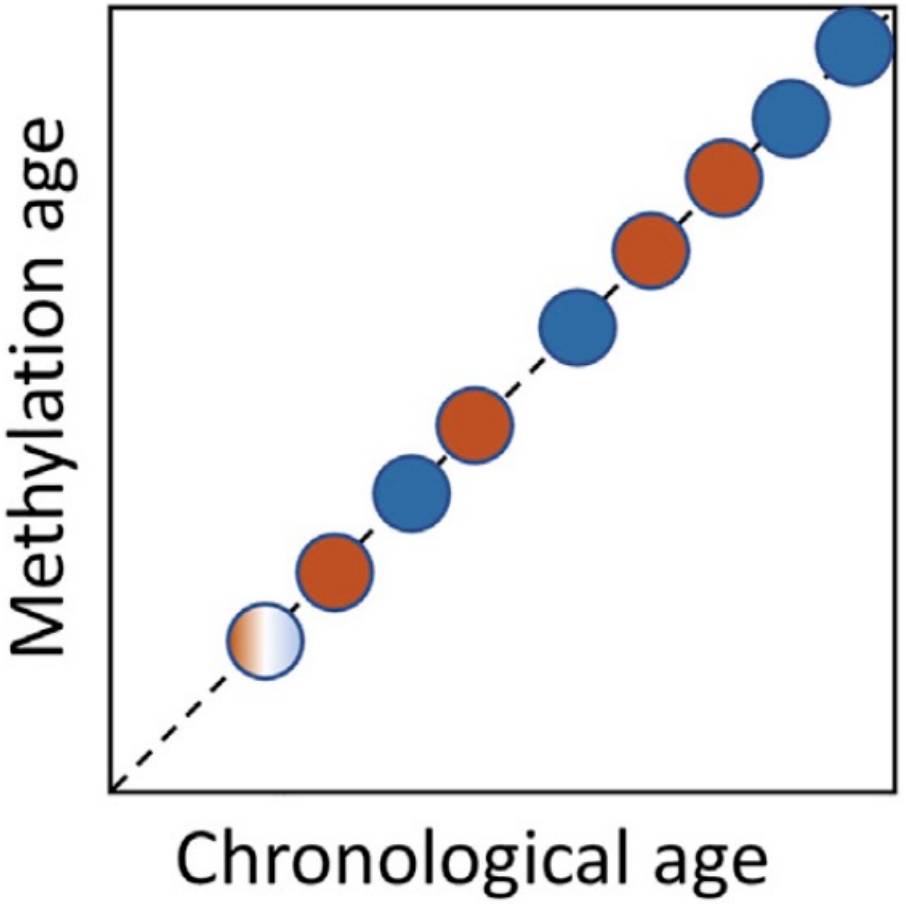
AGEING

Genomic



Horvath & Raj (2018) *Nat Rev Genet*; **Michalak** *et al.* (2019) *Nat Rev Mol Cell Biol* ; Field *et al.* (2018) *Mol Cell*; **Zhang** *et al.* . (2020) *Nat Rev Mol Cell Biol*

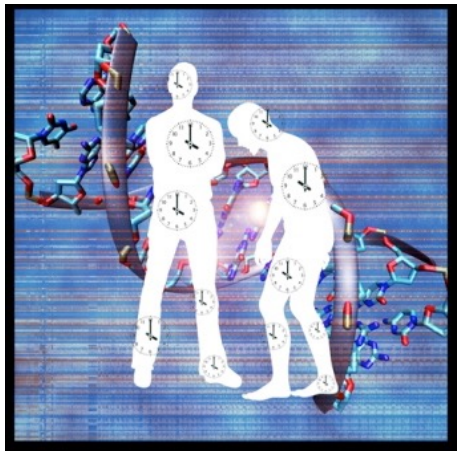
Chronological Clock



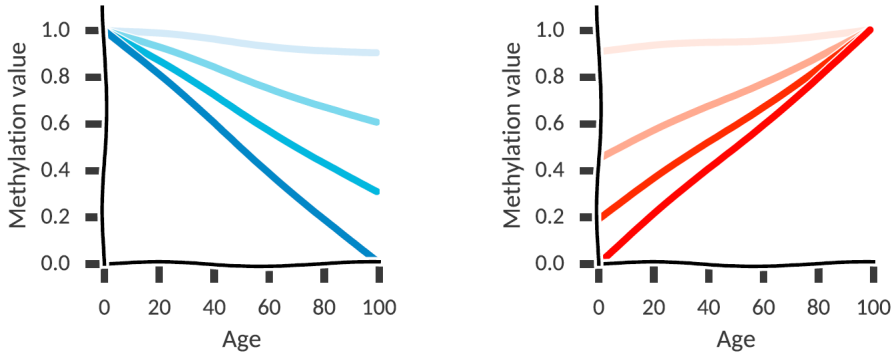
Field et al.. (2018) *Mol Cell*

1st Epigenetic Clocks: The Horvath Clock

- Epigenetic DNA methylation 'Clock'
  - To predict Age across all Tissues
    - with High Accuracy
  - = '**Pan-Tissue**' Predictor
    - Horvath (2013)
- Trained across
  - 51 Healthy Tissue/Cell Types
  - Used Elastic Net Regression
    - Penalized regression model
- Selected **353 CpGs** for this 'clock'
  - Correlation = 0.96; Error 3.6 years



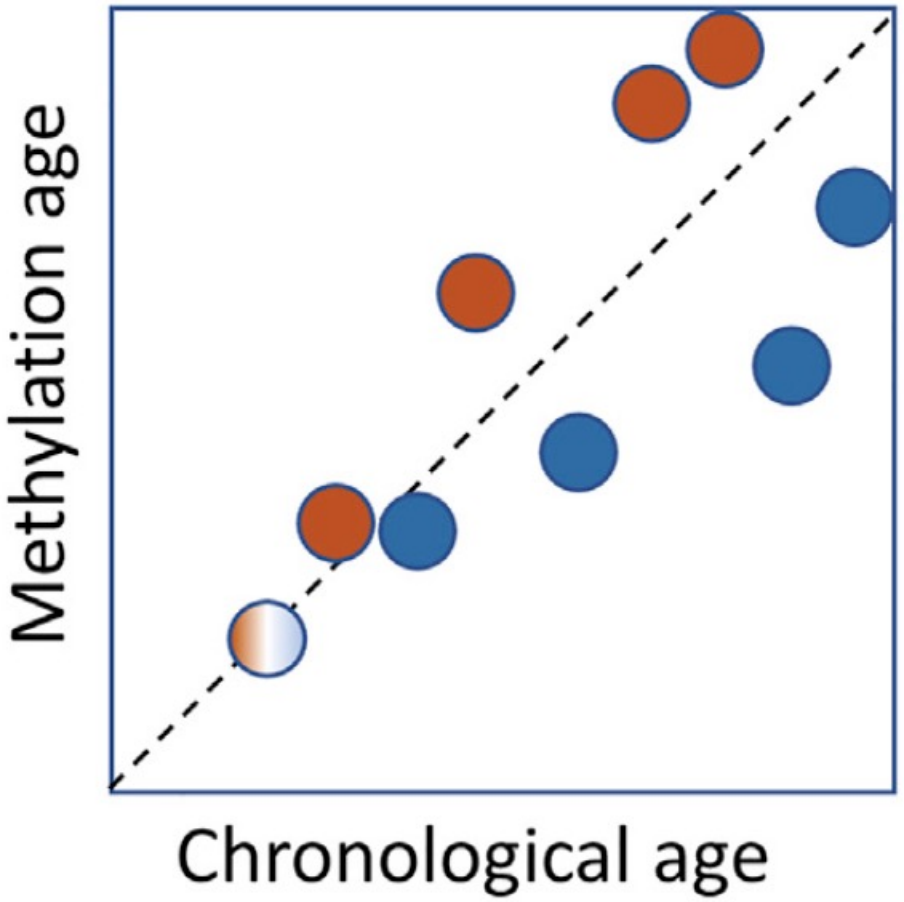
Horvath (2013)



Field *et al.* (2018) ***Mol Cell***

Horvath (2013) DNA methylation age of human tissues and cell types. ***Genome Biology***

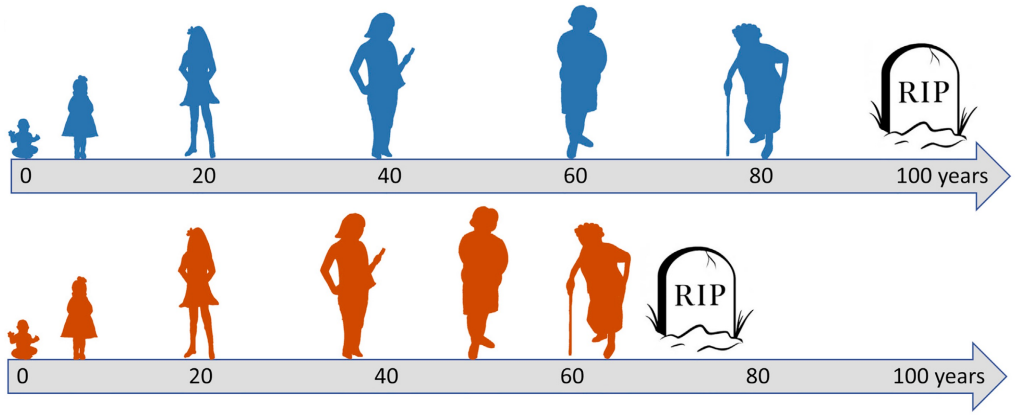
Biological Clock capture 'Biological' Age



Age Prediction

Accelerated Methylation Age

Deaccelerated Methylation Age

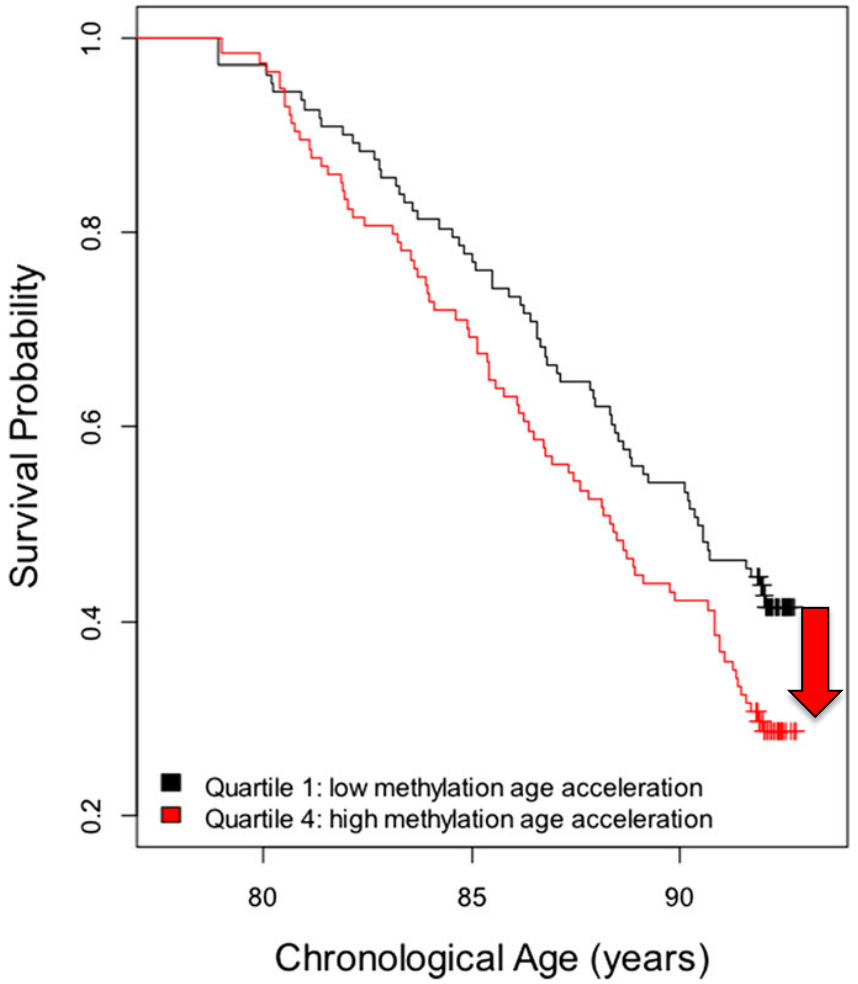


Field et al.. (2018) *Mol Cell*

Predict Mortality Risk

- Accelerated 'Biological' Age
  - DNA methylation Age
    - versus
  - Actual Chronological Age

- = Risk factor
  - All-Cause Mortality
    - in later life
  - Accounting for
    - Known Risk Factors



Marioni *et al*. (2015) DNA methylation age of blood predicts all-cause mortality in later life. ***Genome Biology***

Epigenetic clock is correlated with Physical & Cognitive Fitness in the Lothian Birth Cohort 1936

Table 2. Associations between age acceleration at wave 1 and fitness variables adjusted for age and sex

	Age acceleration		
	Beta[a]	SE	*P*
g_f[b]	−0.07	0.03	0.024
Grip strength (kg)	−0.05	0.02	9.7×10^{-3}
FEV_1 (l)[c]	−0.06	0.02	6.4×10^{-3}
6-m walk time (s)	0.03	0.03	0.45

SE, standard error.

Fluid Type General Intelligence

Forced expiratory volume in 1 second

Marioni *et al.* (2015) The epigenetic clock is correlated with physical and cognitive fitness in the Lothian Birth Cohort 1936. ***Int J Epidemiol.***

Epigenetic Age 'Acceleration'

Conclusions

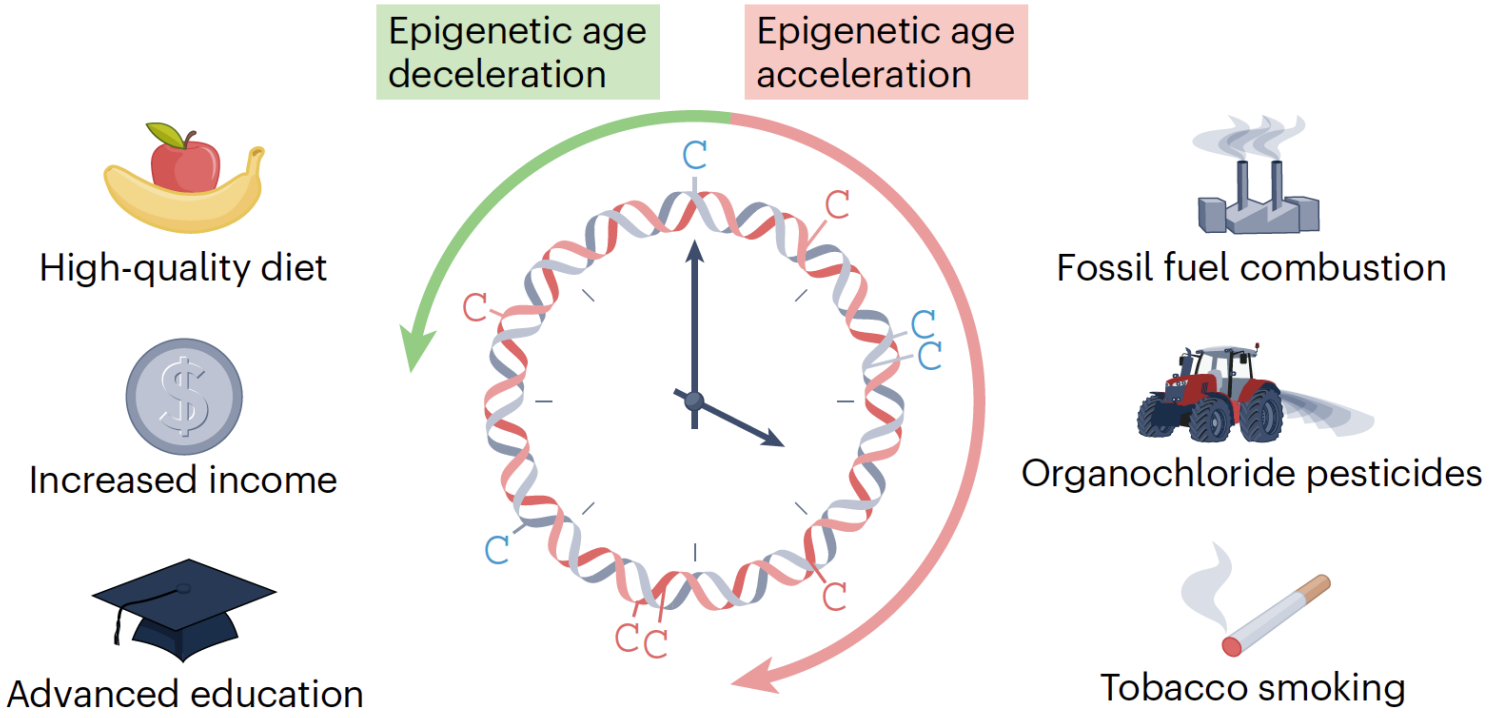
There is continued interest in identifying new risk factors, environmental, genetic, and epigenetic that can improve our ability to predict disease and mortality. Epidemiological studies have identified numerous measures from across the human life-course that are associated with an increased risk of mortality. These include health factors such as cardiovascular disease, diabetes, and hypertension [27], genetic factors such as presence of the *APOE* ε4 allele [29], lifestyle variables such as smoking [30] and education [31], behavioral traits such as cognitive ability [31,32], the personality trait of conscientiousness [33], and candidate biomarkers of age such as telomere length [34,35]. Here, we report on an epigenetic biomarker that is predictive of human mortality, after accounting for known risk factors. We found that two heritable DNA methylation-based measures of the difference between epigenetic age and chronological age are significant predictors of mortality in our meta-analysis of four independent cohorts of older people.

*But Potential Influence of Minor Cell Type Fractions

- *i.e.* senescent T cells ($CD8^+CD28^-$)
- Yang *et al.* (2019) *Genome Med*

Marioni *et al.* (2015) ***Genome Biology***

Epigenetically Predicted 'Biological' Age



Wu *et al.* (2023) Molecular mechanisms of environmental exposures and human disease. ***Nat Rev Genetics***

PhenoAge Clock

- To improve capture of 'Biological Age'
  - Prediction of a Surrogate Measure of "Phenotypic Age"
    - Instead of training on Chronological Age
      - From clinical data from National Health Nutrition Examination Survey (NHANES)
  - PhenoAge strongly *outperforms* 1st Clocks
    - Predictions for Ageing outcomes, including:
      - All-cause Mortality, Cancers, Healthspan, Physical functioning & Alzheimer's disease
      - Blood derived *but* correlates strongly with age in every tissue/cell tested

Variable		Units	Weight
Albumin	Liver	g/L	-0.0336
Creatinine	Kidney	umol/L	0.0095
Glucose, serum	Metabolic	mmol/L	0.1953
C-reactive protein (log)	Inflammation	mg/dL	0.0954
Lymphocyte percent	Immune	%	-0.0120
Mean (red) cell volume	Immune	fL	0.0268
Red cell distribution width	Immune	%	0.3306
Alkaline phosphatase	Liver	U/L	0.0019
White blood cell count	Immune	1000 cells/uL	0.0554
Age		Years	0.0804

PhenoAge Clock

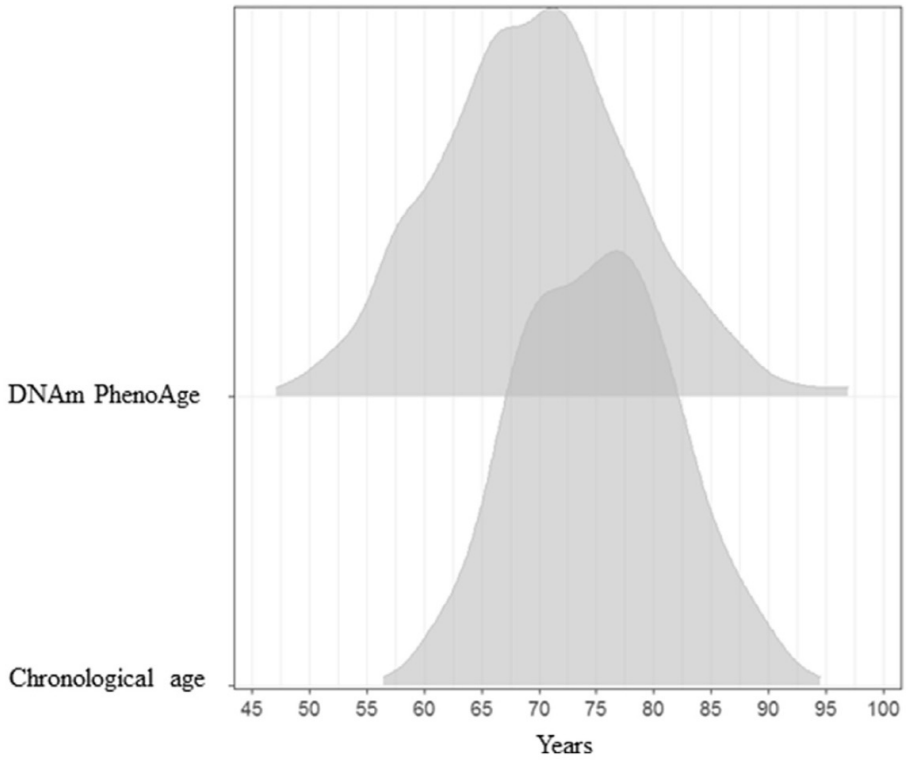
513 CpGs

- + PhenoAge Acceleration associated with:
  - ↑ Activation of Pro-Inflammatory & Interferon Pathways
  - ↓ Activation of transcriptional/translational machinery
  - ↓ DNA damage response & mitochondrial signatures.

Levine *et al.*. (2018) 'An epigenetic biomarker of aging for lifespan and healthspan' ***Aging***

Associations of annual ambient PM2.5 components with DNAm PhenoAge acceleration in elderly men

- 683 elderly men (Normative Aging Study)
- Daily concentrations of PM2.5 species
  - Measured at a fixed air-quality monitoring site
  - 1-year moving averages were computed
- DNA methylation (DNAm) array analysis
  - PhenoAge calculated



Wang et al. (2020) Associations of annual ambient PM2.5 components with DNAm PhenoAge acceleration in elderly men: The Normative Aging Study ***Environmental Pollution***

Associations of annual ambient PM2.5 components with DNAm PhenoAge acceleration in elderly men

- Interquartile Range (IQR) ↑ in $PM_{2.5}$ levels
  - 2.0 mg/m3
  - → ↑ 0.16 years DNAm PhenoAge
- Lead (Pb) component of $PM_{2.5}$
  - ↑ IQR in 1-year 0.0011 mg/m^3
  - → ↑ 1.45-year DNAmPhenoAccel
    - 95% CI: 0.46, 2.46
- Calcium (Ca) component
  - ↑ IQR in 1-year 0.0073 mg/m^3
  - → ↑ 0.62-year DNAmPhenoAccel
    - 95% CI: 0.19, 1.06
- ∴ Annual ambient PM2.5 components
  - → ↑ DNAm PhenoAge acceleration in elderly ♂

Table 2
Summary of one-year moving average of $PM_{2.5}$ mass, and its species from the Normative Aging Study, 1999–2013.

	Min	Mean	Median	Max	IQR	SD
$PM_{2.5}$ (μg/m^3)	6.2	10.1	10.4	12.6	2.0	1.7
BC (μg/m^3)	0.54	0.73	0.73	0.89	0.18	0.10
SO_4^{2-} (μg/m^3)	1.40	2.94	3.06	3.54	0.40	0.53
Na (μg/m^3)	0.0878	0.1941	0.2025	0.2231	0.0128	0.0232
Mg (μg/m^3)	0.0058	0.0511	0.0526	0.0622	0.0051	0.0088
Al (μg/m^3)	0.0174	0.0487	0.0480	0.0672	0.0099	0.0107
Si (μg/m^3)	0.0363	0.0713	0.0653	0.1065	0.0236	0.0185
S (μg/m^3)	0.4680	1.0513	1.1207	1.3019	0.1057	0.2094
Cl (μg/m^3)	0.0038	0.0135	0.0102	0.0405	0.0074	0.0095
K (μg/m^3)	0.0350	0.0404	0.0400	0.0524	0.0029	0.0019
Ca (μg/m^3)	0.0192	0.0303	0.0290	0.0425	0.0073	0.0060
Ti (μg/m^3)	0.0017	0.0034	0.0032	0.0044	0.0009	0.0006
V (μg/m^3)	0.0004	0.0038	0.0038	0.0062	0.0017	0.0015
Fe (μg/m^3)	0.0417	0.0658	0.0628	0.0896	0.0128	0.0125
Ni (μg/m^3)	0.0006	0.0034	0.0035	0.0060	0.0012	0.0015
Zn (μg/m^3)	0.0063	0.0120	0.0120	0.0166	0.0047	0.0028
Sb (μg/m^3)	0.0001	0.0049	0.0051	0.0059	0.0006	0.0009
Pb (μg/m^3)	0.0021	0.0056	0.0057	0.0068	0.0011	0.0010

Wang et al. (2020) Associations of annual ambient PM2.5 components with DNAm PhenoAge acceleration in elderly men: The Normative Aging Study ***Environmental Pollution***

GrimAge Clock

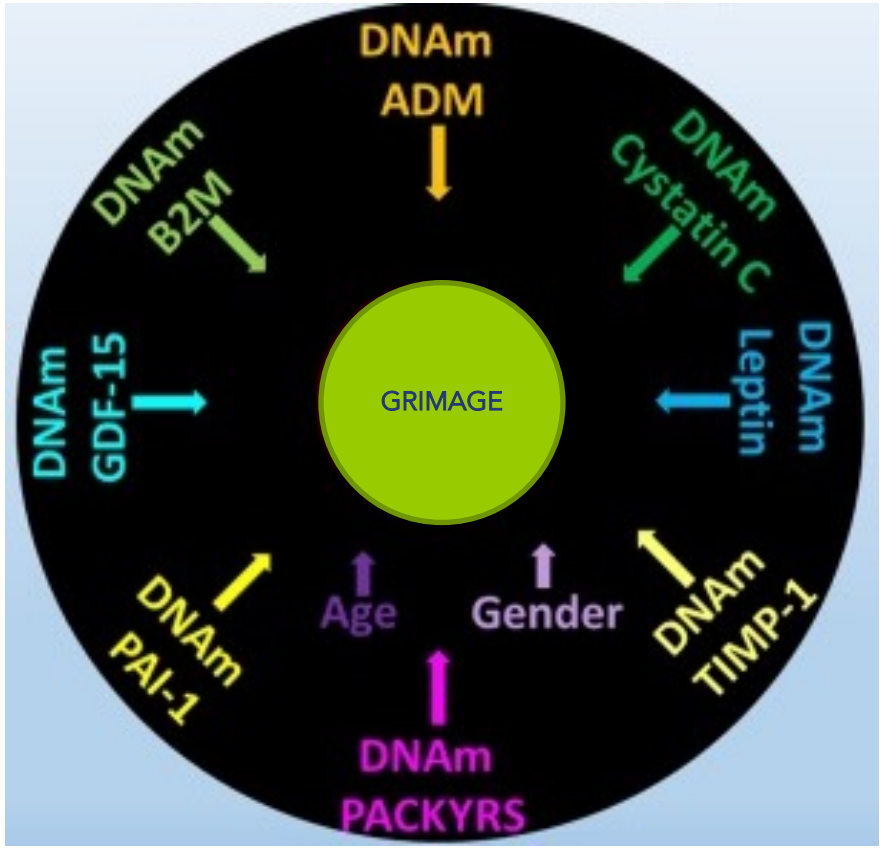
- More Powerful Predictive 'Biological' Clock
  - Strongly predicts Lifespan & Healthspan
  - Includes 7 Plasma protein levels + Smoking (PackYears)
    - **Estimated using DNA methylation levels**
    - Predictor of lifespan: DNAm GrimAge (units of years)
      - Accelerated DNAm GrimAge *cf.* Actual Chronological
      - 1,030 CpGs
  - Predict
    - Time-to-death
      - Even in Never-smokers
    - Time-to-Coronary Heart Disease
    - Time-to-Cancer
  - Outperforms other Clocks (McCory *et al.*)

1. Adrenomedullin Levels
2. Beta-2 microglobulin
3. Cystatin C
4. Growth differentiation factor 15
5. Leptin
6. Tissue inhibitor metalloproteinase 1
7. Plasminogen activation inhibitor 1 (PAI-1)
8. Smoking (PackYears)

Lu *et al.*. (2019) 'DNA methylation GrimAge strongly predicts lifespan and healthspan' ***Aging.***
McCory *et al.* (2020) GrimAge Outperforms Other Epigenetic Clocks in the Prediction of Age-Related Clinical Phenotypes and All-Cause Mortality ***The Journals of Gerontology***
Lu *et al.*. (2022) 'DNA methylation GrimAge version 2' ***Aging***

Type 2 Diabetes → GrimAge Acceleration

- Age-related conditions include
  - Type 2 Diabetes (T2D)
- GrimAge + incident T2D investigated in
  - Coronary Artery Risk Development in Young Adults (CARDIA) study (n=1,057)
    - Stratified: Normal weight, Overweight, & Obese.
  - Each 1-year of GrimAge associated with
    - Higher 10-year (study years 15–25) incidence of T2D
    - OR 1.06 (95% CI 1.01–1.11)
  - Accelerated GrimAge (> Chronological Age)
    - Higher odds of 10-year incidence of T2D
    - In obese = OR 2.57 (95% CI 1.61–4.11)
- ∴ Epigenetic DNA methylation 'clock'
  - = biomarker of T2D development.



Kim *et al.* (2021) DNA Methylation GrimAge and Incident Diabetes: The Coronary Artery Risk Development in Young Adults (CARDIA) Study. ***Diabetes***

Conclusion

- DNA methylation
  - Stable & Robust Tool for Epidemiology
    - Biomarker of Disease
    - Potential Insights to Pathology
  - Environmental Exposures
    - Strong Data to Date with Smoking
      - Including *In Utero* Exposure
    - Other Contaminates
      - Arsenic
      - $PM_{2.5}$ and components
  - Environmental effect on 'Biological' Age
    - Assess with DNA methylation 'Clocks'
      - Capture **Multisystemic** Ageing Effects
      - ↑ Age Related Disease Risk

Questions

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