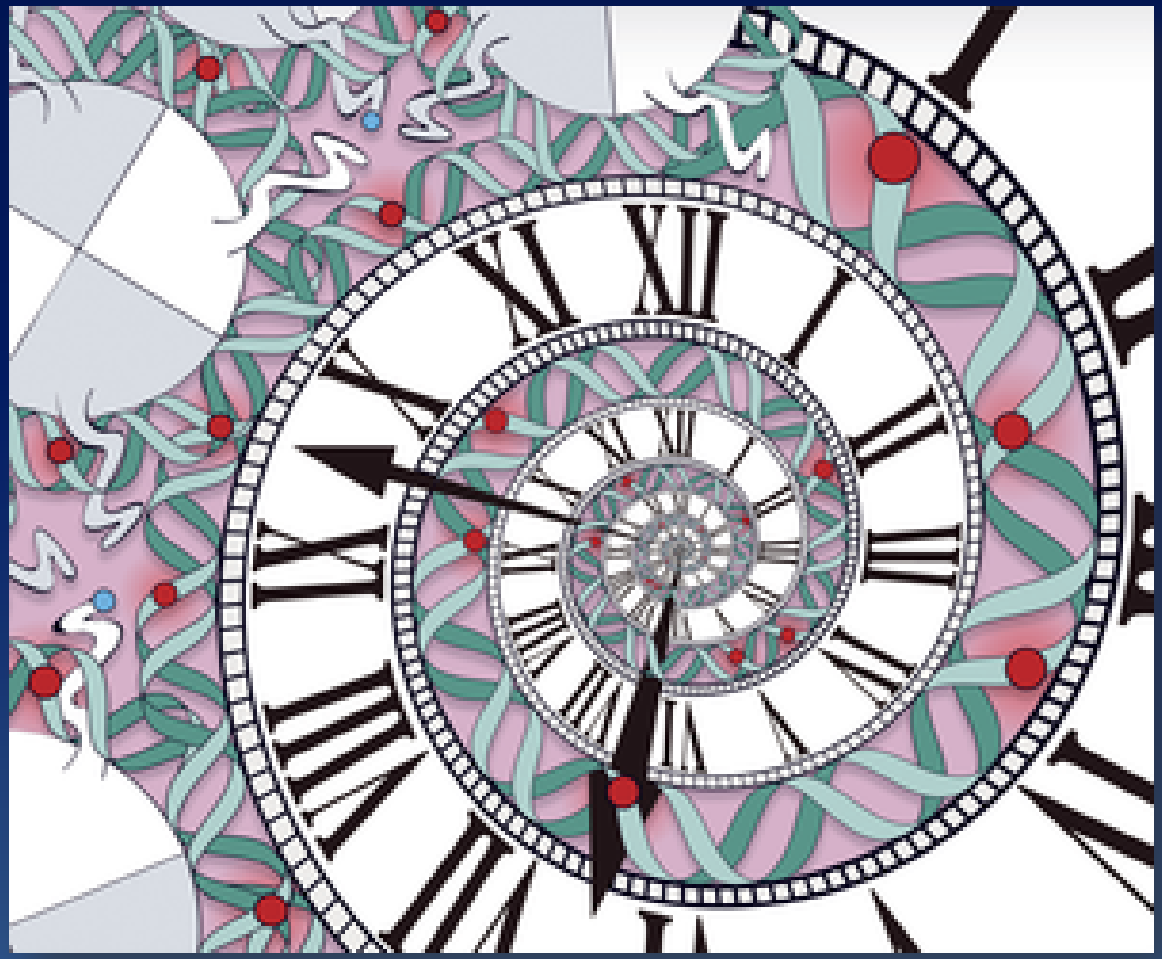
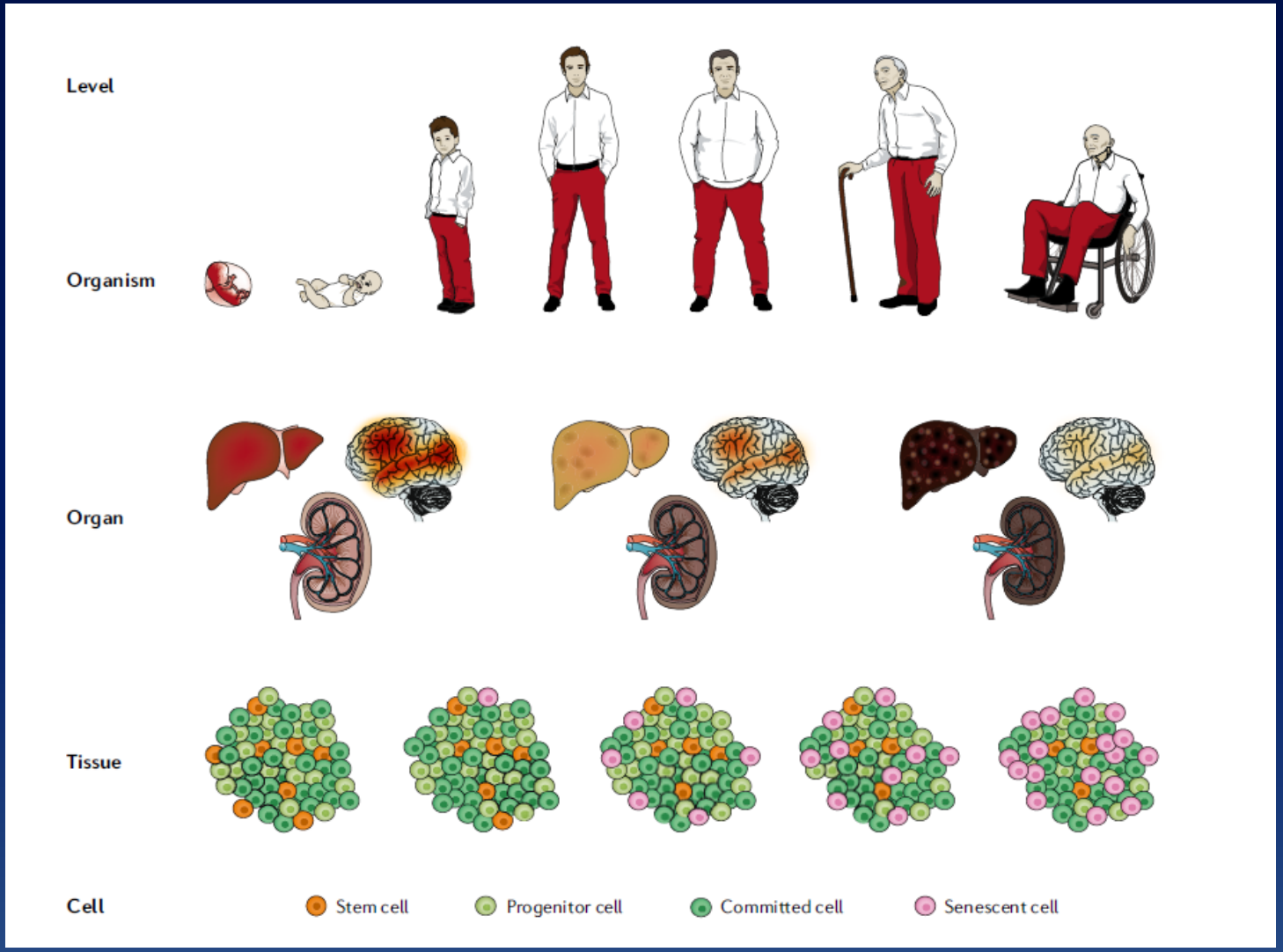
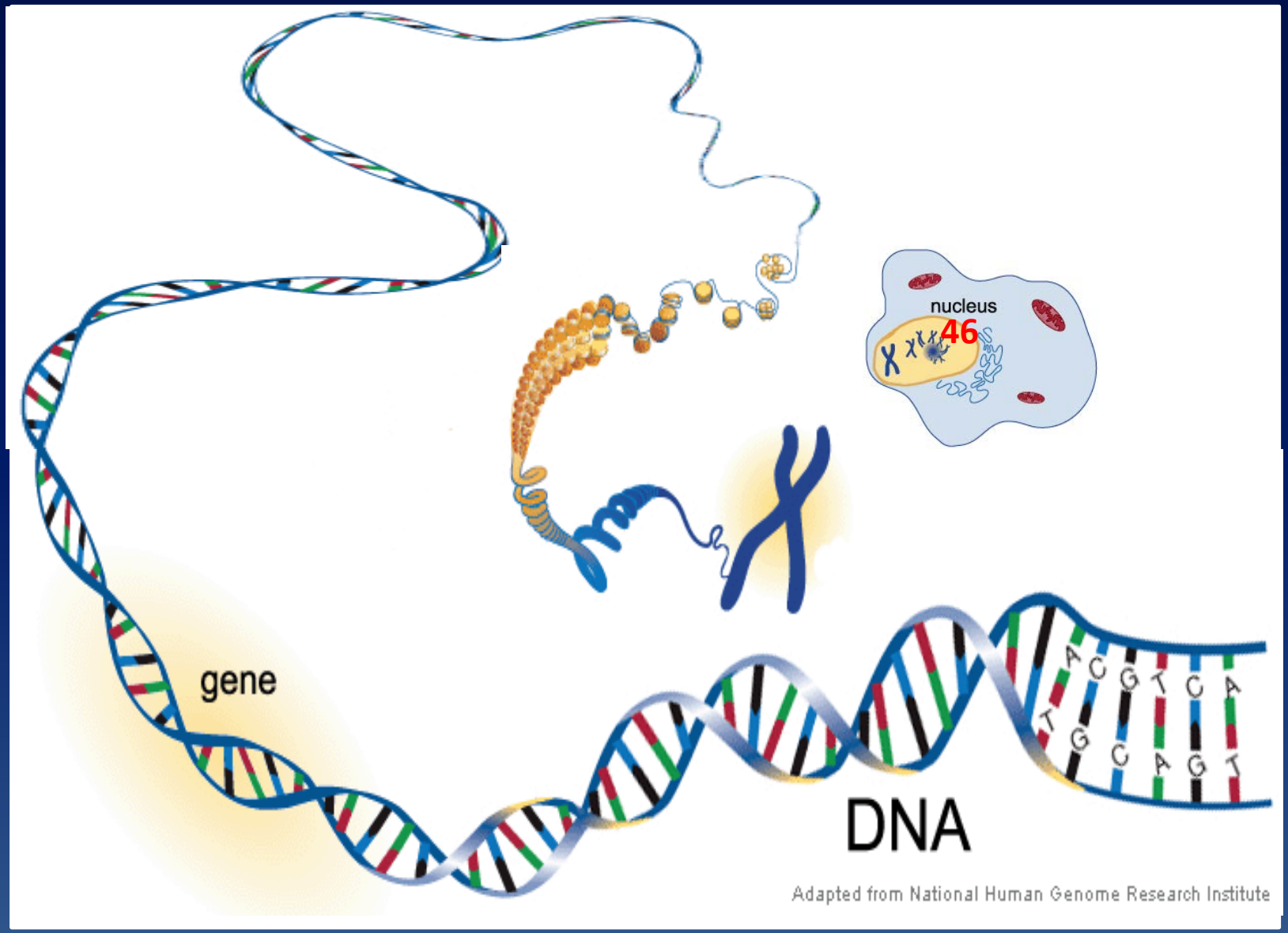
Age another way:
Unexpected discoveries from “Big data”



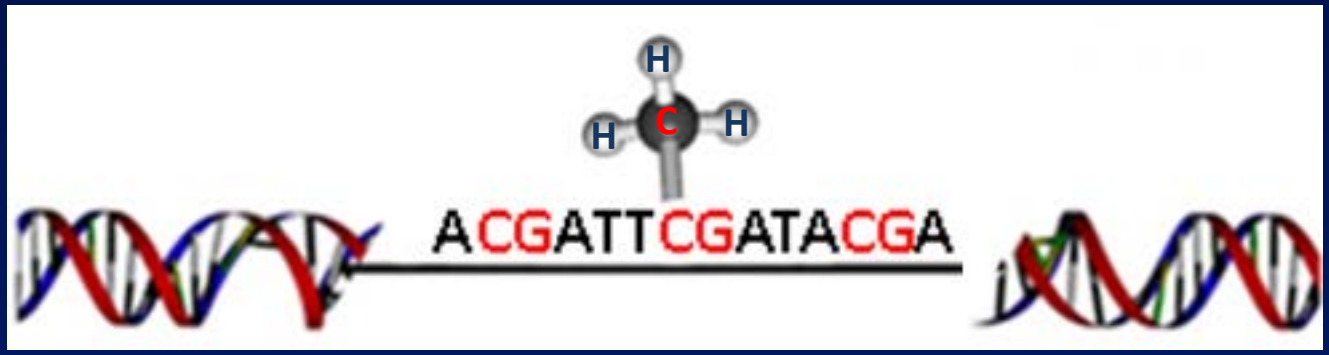
Human Ageing



Sub-Cellular source of human ageing ? DNA

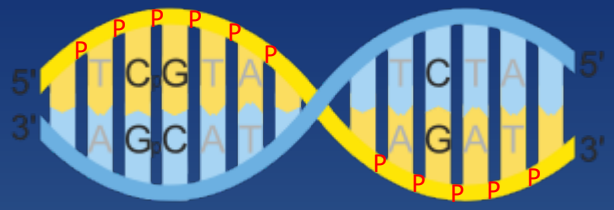

nucleus
46
gene
DNA
Adapted from National Human Genome Research Institute

Methylation of Cytosines at CpG sites



Epigenetic modification

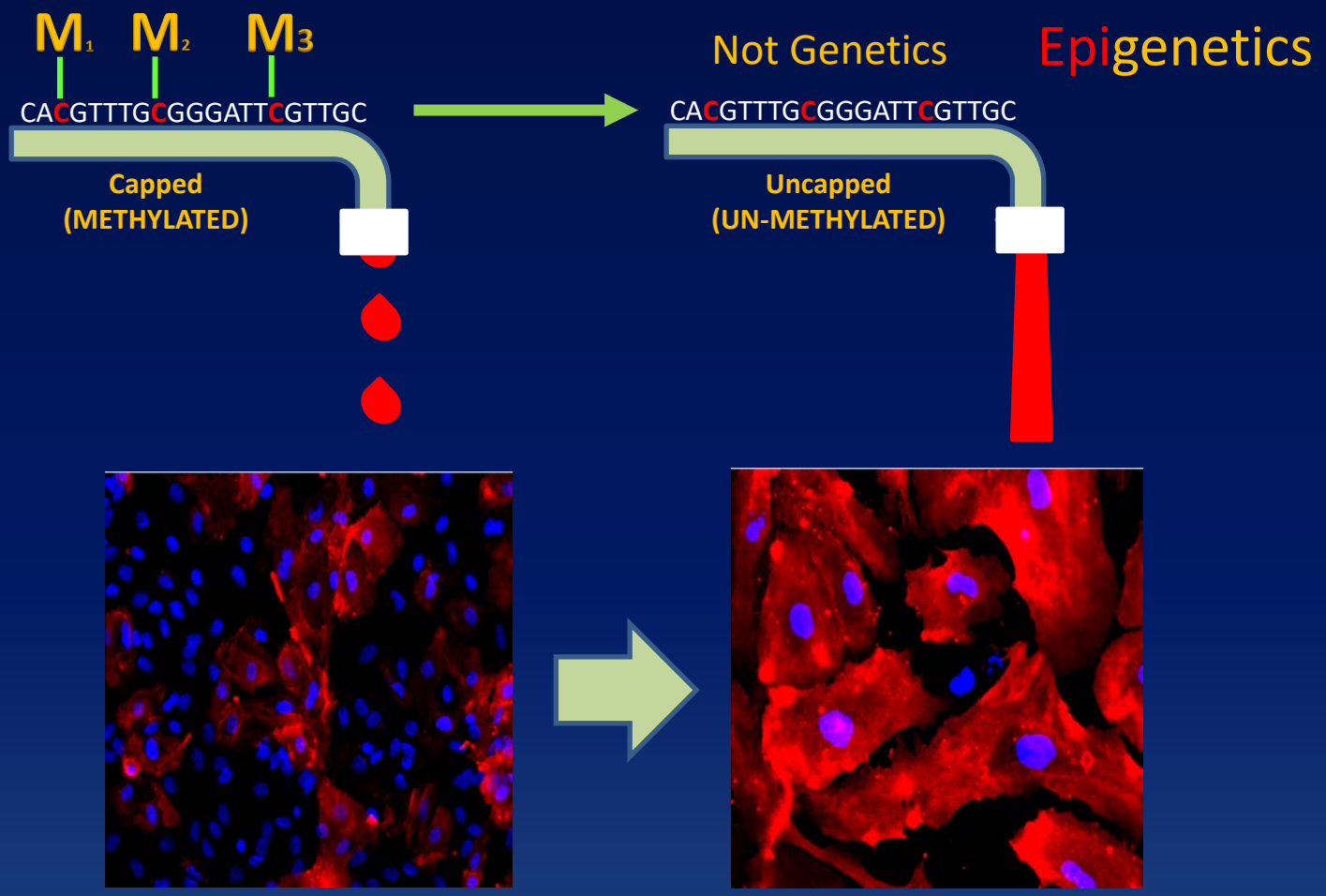
Terminology



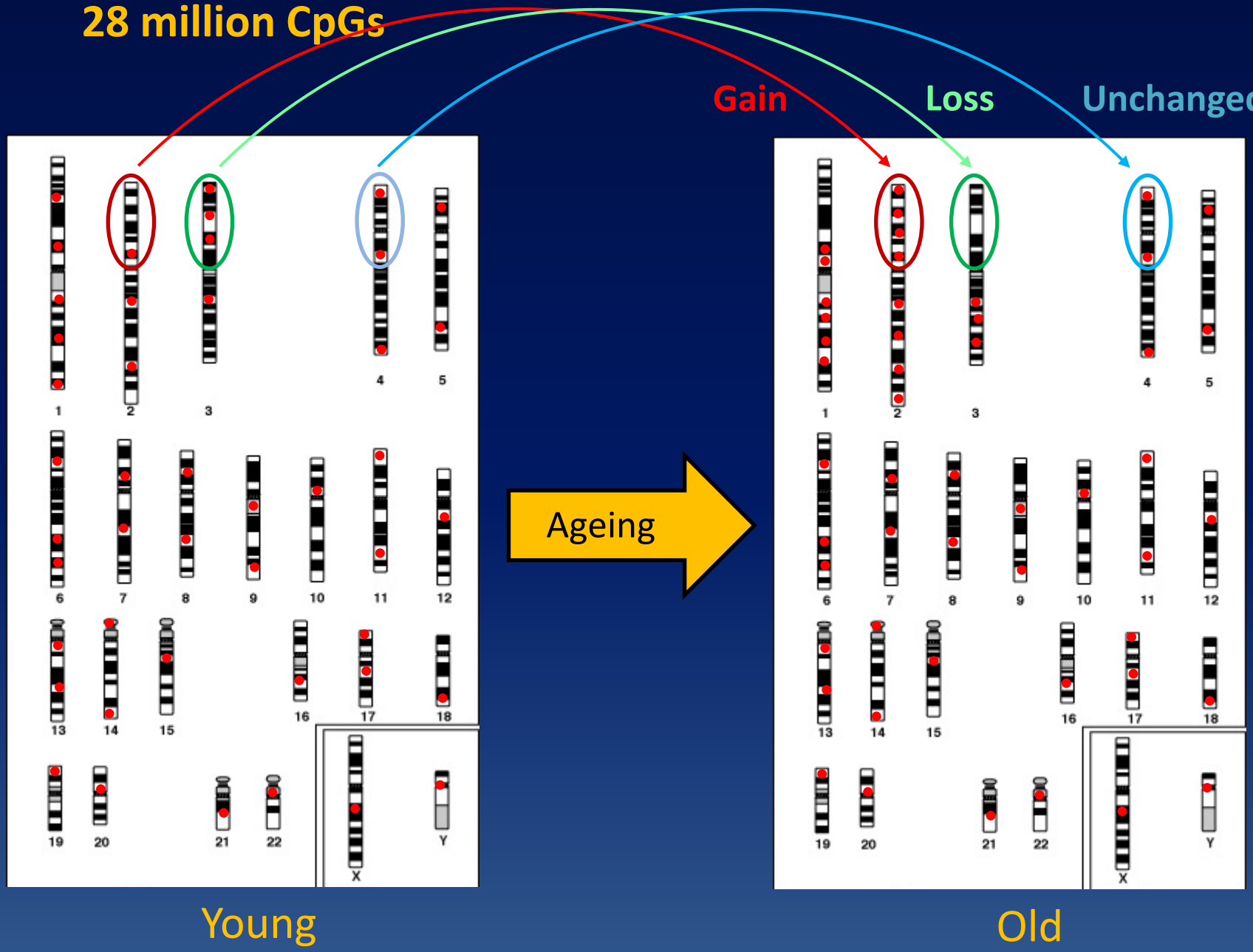
Methylated

Un-methylated

CpG (28 million)



Lowe and Raj *Ageing Cell* 2014 Oct;13(5):900-10.


28 million CpGs
Gain
Loss
Unchanged
Ageing
Young
Old

What is the meaning of age-related DNA methylation changes ?

The challenge of investigating
age-related DNA methylation changes

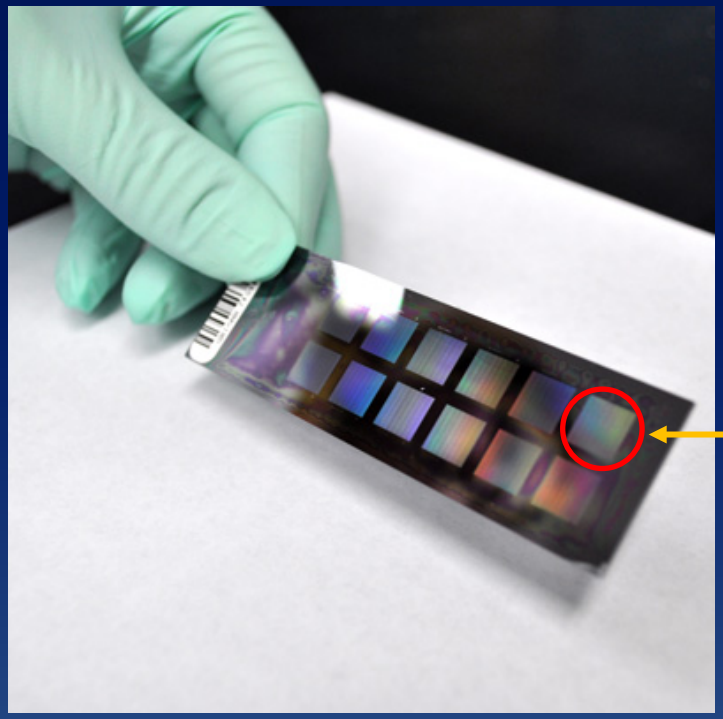
28 million CpGs

Technology to detect methylation changes at specific CpGs

Intelligence to analyse

Technology to detect methylation state of specific CpGs

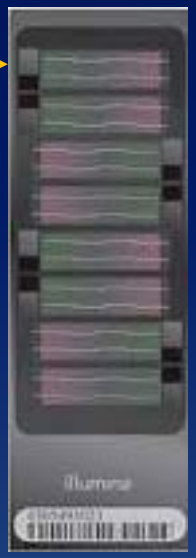
Illumina 450 Array



450,000 CpGs

Illumina 850 Array (EPIC Array)

850,000 CpGs



“Expected Results” from Illumina analysis

Probe ID	Young A	Young B	Young C	Old A	Old B	Old C	
cg00050873	M	M	M	U	U	U	0
cg00212031	U	U	U	U	U	U	0
cg00213748	U	U	U	U	U	U	0
cg00214611	M	M	M	M	M	M	1
cg00455876	M	M	M	M	M	M	1
cg01707559	M	M	M	M	M	M	1
cg02004872	U	U	U	U	U	U	0
cg02011394	U	U	U	U	U	U	0
cg02050847	M	M	M	U	U	U	0
cg02233190	M	M	M	U	U	U	0
cg02494853	U	U	U	U	U	U	0
cg02839557	U	U	U	M	M	M	1
cg02842889	U	U	U	M	M	M	1
cg03052502	U	U	U	U	U	U	0
cg03155755	U	U	U	U	U	U	0
cg03244189	M	M	M	M	M	M	1
cg03443143	U	U	U	U	U	U	0
cg03683899	U	U	U	U	U	U	0
cg03695421	M	M	M	U	U	U	0
cg03706273	M	M	M	U	U	U	0
cg03750315	U	U	U	U	U	U	0
cg03767353	U	U	U	U	U	U	0
cg04016144	M	M	M	M	M	M	1
cg04023335	M	M	M	M	M	M	1

“Actual Results” from Illumina analysis

Probe ID	Young A	Young B	Young C	Old A	Old B	Old C
cg00050873	0.603154	0.581422	0.585725	0.598401	0.612638	0.649252
cg00212031	0.076422	0.067191	0.059793	0.075504	0.070526	0.056024
cg00213748	0.163650	0.157745	0.133008	0.316854	0.121328	0.157084
cg00214611	0.068422	0.072694	0.050413	0.167737	0.077459	0.098686
cg00455876	0.538194	0.569420	0.535995	0.600286	0.576547	0.596032
cg01707559	0.076774	0.095835	0.107720	0.106874	0.108809	0.086575
cg02004872	0.023958	0.043122	0.036053	0.027583	0.037564	0.033150
cg02011394	0.882460	0.877247	0.874077	0.937412	0.917828	0.911204
cg02050847	0.951274	0.938329	0.945382	0.958521	0.963579	0.966982
cg02233190	0.041588	0.035390	0.075974	0.047472	0.048298	0.043000
cg02494853	0.031609	0.028452	0.031908	0.022825	0.027432	0.033366
cg02839557	0.088715	0.070388	0.077480	0.126461	0.081820	0.087572
cg02842889	0.039832	0.043161	0.034498	0.047991	0.054090	0.043330
cg03052502	0.964893	0.966523	0.962055	0.983924	0.976014	0.967884
cg03155755	0.822025	0.814186	0.811199	0.881048	0.826349	0.830599
cg03244189	0.050635	0.084187	0.056970	0.096892	0.082597	0.080470
cg03443143	0.685784	0.678918	0.683631	0.699350	0.692234	0.726183
cg03683899	0.044403	0.035652	0.034824	0.050561	0.045551	0.034855
cg03695421	0.181565	0.216064	0.165855	0.215089	0.174338	0.208848
cg03706273	0.040879	0.037703	0.063889	0.074869	0.047367	0.042911
cg03750315	0.063555	0.069353	0.038697	0.089839	0.075824	0.066402
cg03767353	0.948230	0.952896	0.940580	0.944986	0.942743	0.945600
cg04016144	0.891209	0.884583	0.884982	0.923190	0.919293	0.913679
cg04023335	0.125148	0.119924	0.116954	0.141144	0.143948	0.116678

Intelligence to analyse

(1) Large numbers: 27,000 or 450,000 or 850,000

(2) Non-binary values: 0 ⟷ 1

(3) Very small difference: ~3.2%

Naturally impossible

Artificially possible

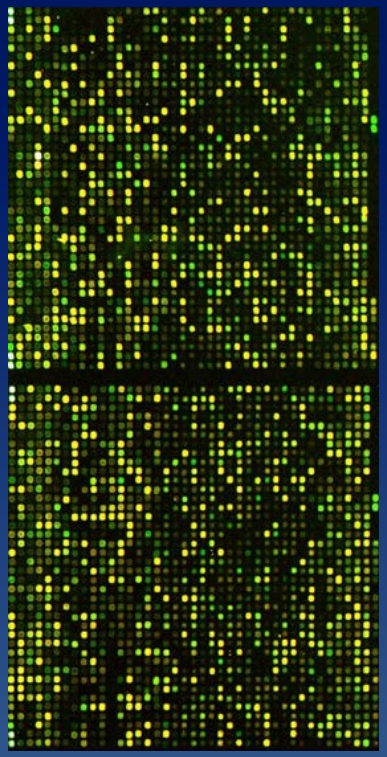
Steve Horvath (UCLA)

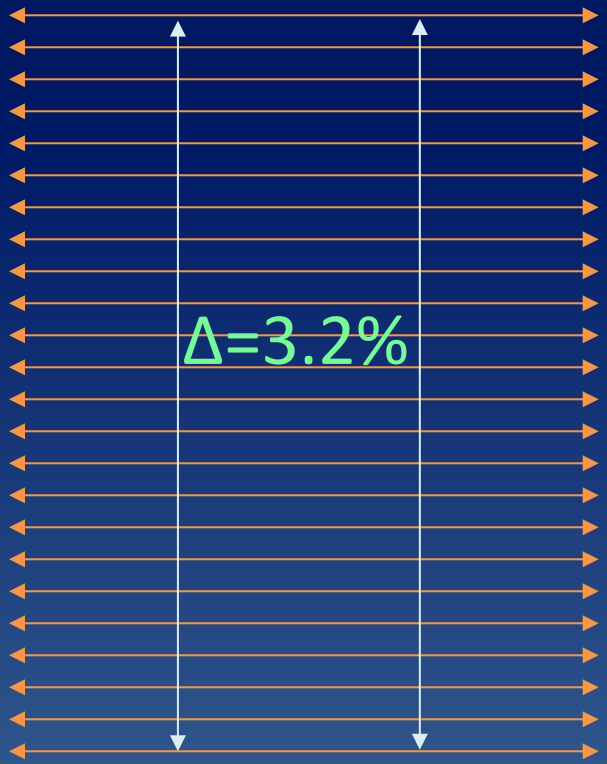
Nature 8th April 2014

Chronological age regressed on CpGs
Using penalised regression model
(Elastic net regression model)

450,000 CpG per human

8,000 X
(82 publicly-available data sets)
(Healthy subjects)





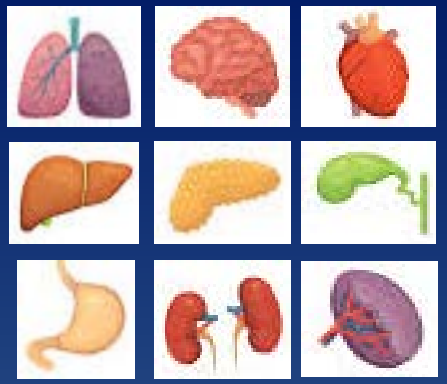
Ages

100

♂ ♀

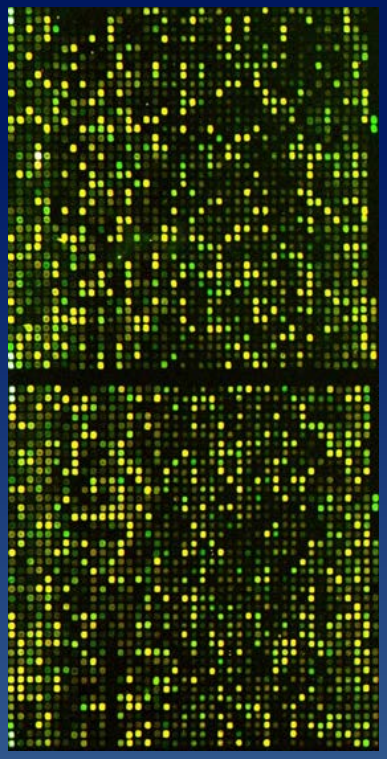
0

Multiple Organs & Tissues (51)



450,000 CpG per human

8,000 X



Age-related CpG: Thousands

Age-predictive CpG: 353

193 CpGs

160 CpGs

$$\mathcal{E}pigenetic\ \mathcal{A}ge = -15.7(\mathcal{C}_p\mathcal{G}_1) + 3.4(\mathcal{C}_p\mathcal{G}_2) + 12.8(\mathcal{C}_p\mathcal{G}_3) - 14.4(\mathcal{C}_p\mathcal{G}_4) \ldots\ldots$$

Epigenetic clock/Horvath clock

Multi-tissue Age Predictor

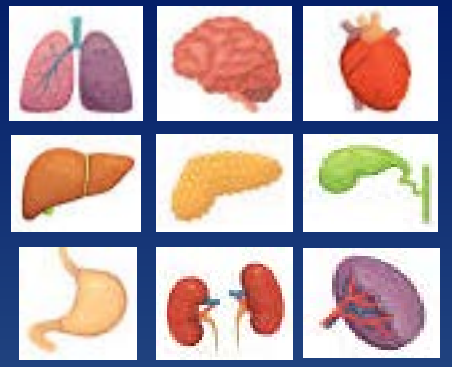
Ages

100

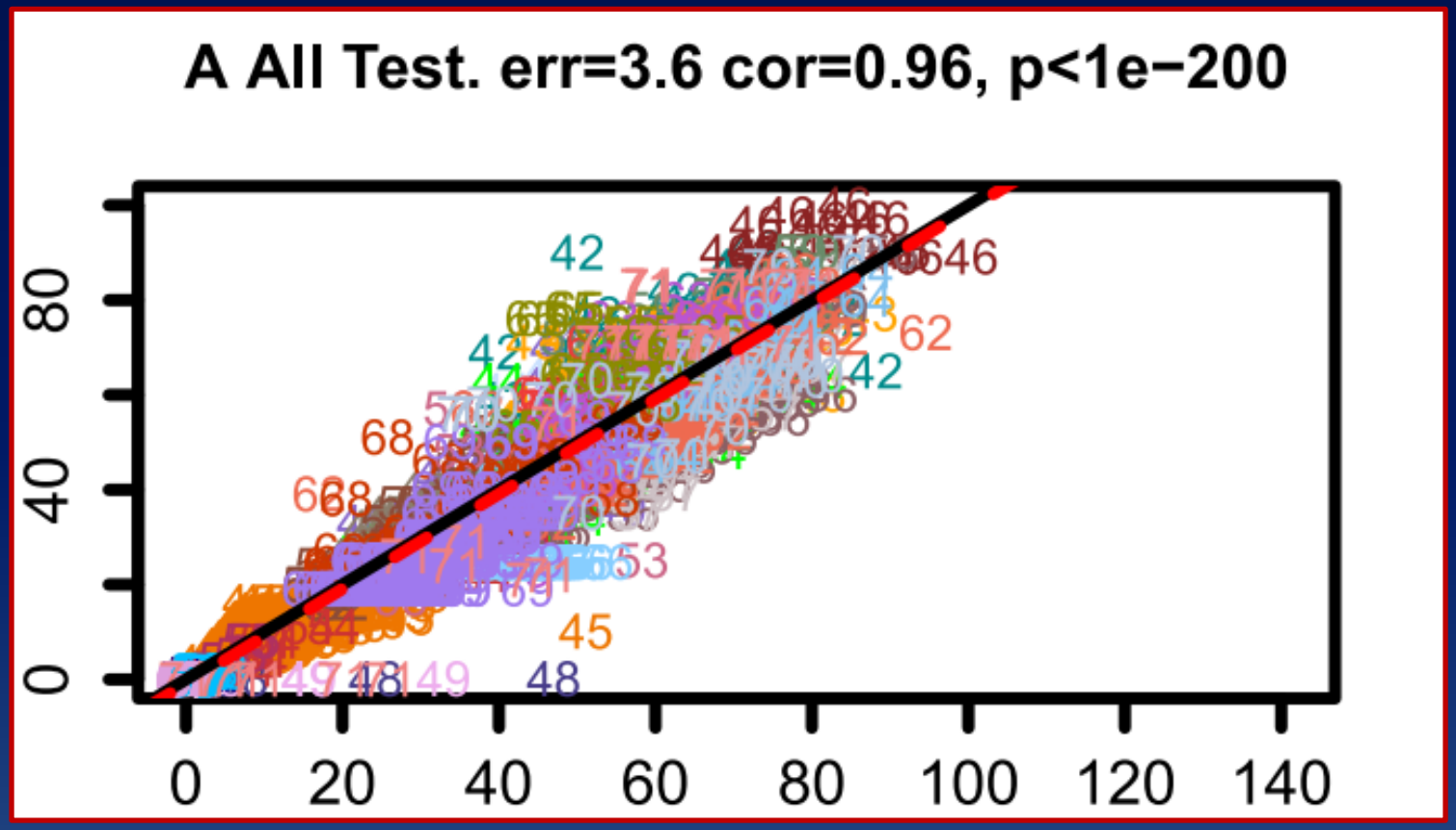
♂ ♀

0

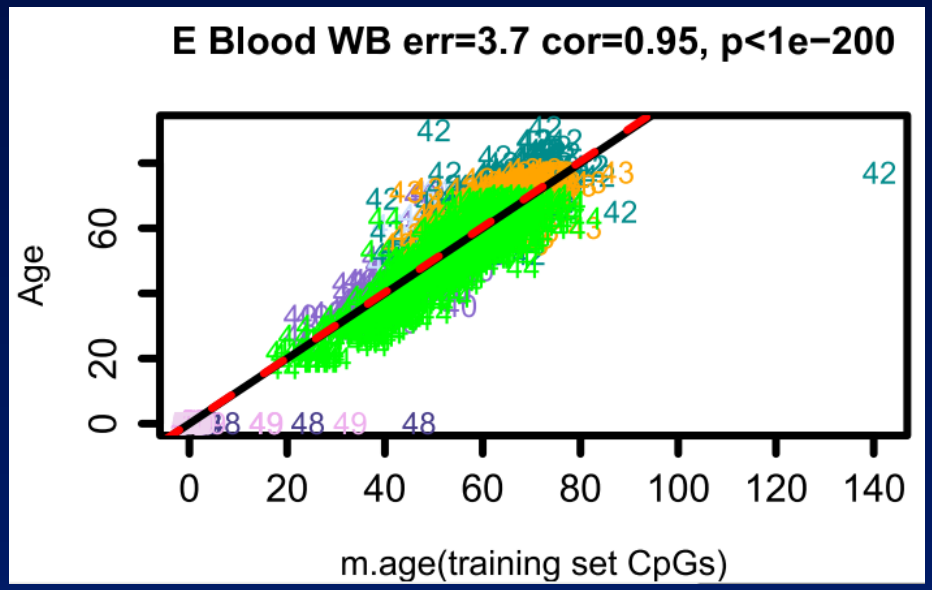
Multiple Organs

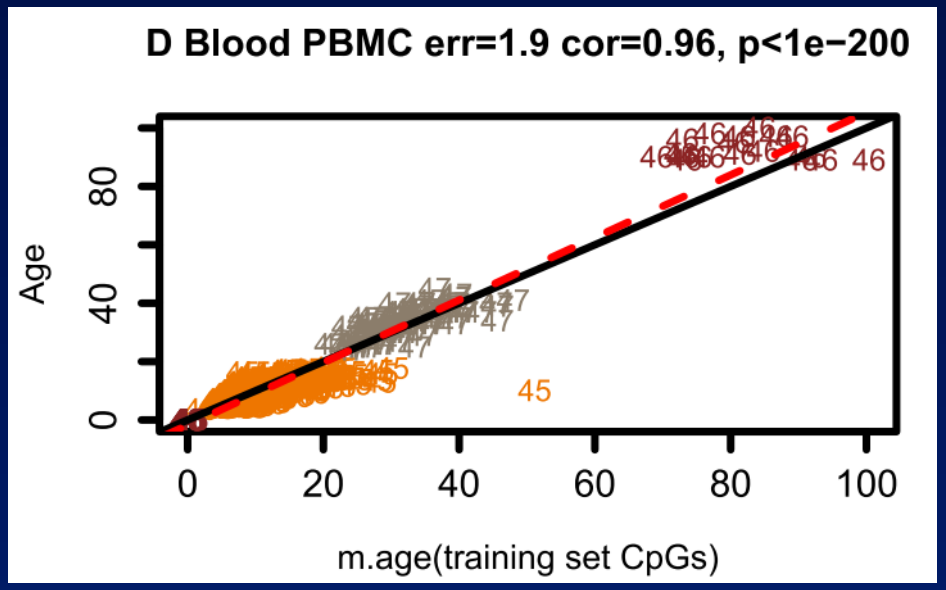


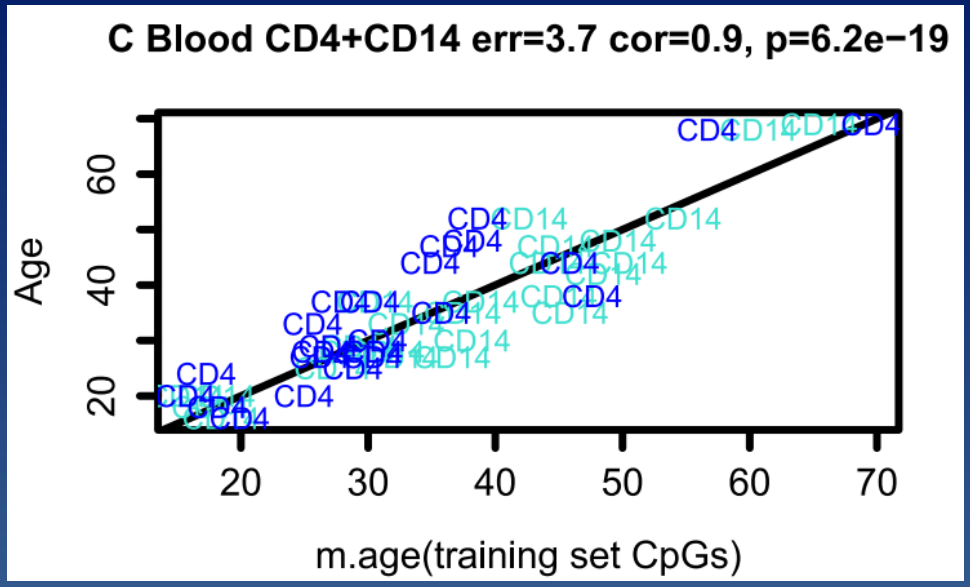
Multi-tissue age predictor (Horvath Clock)



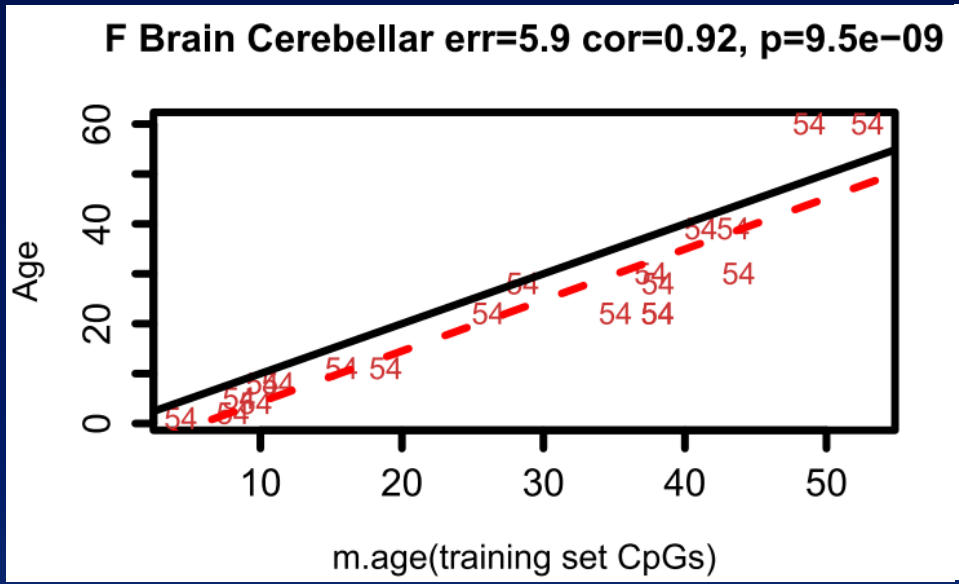
Multi-tissue age predictor on Blood

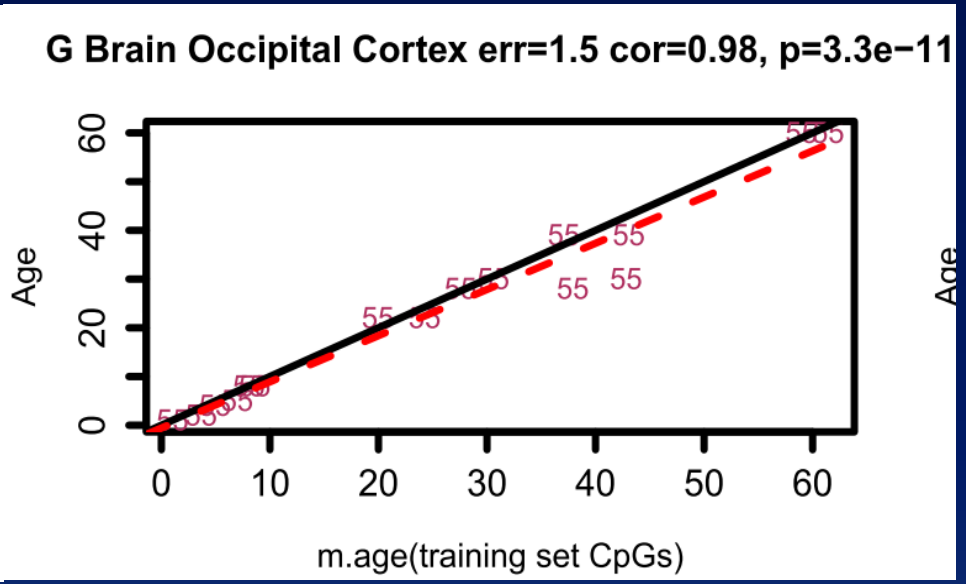




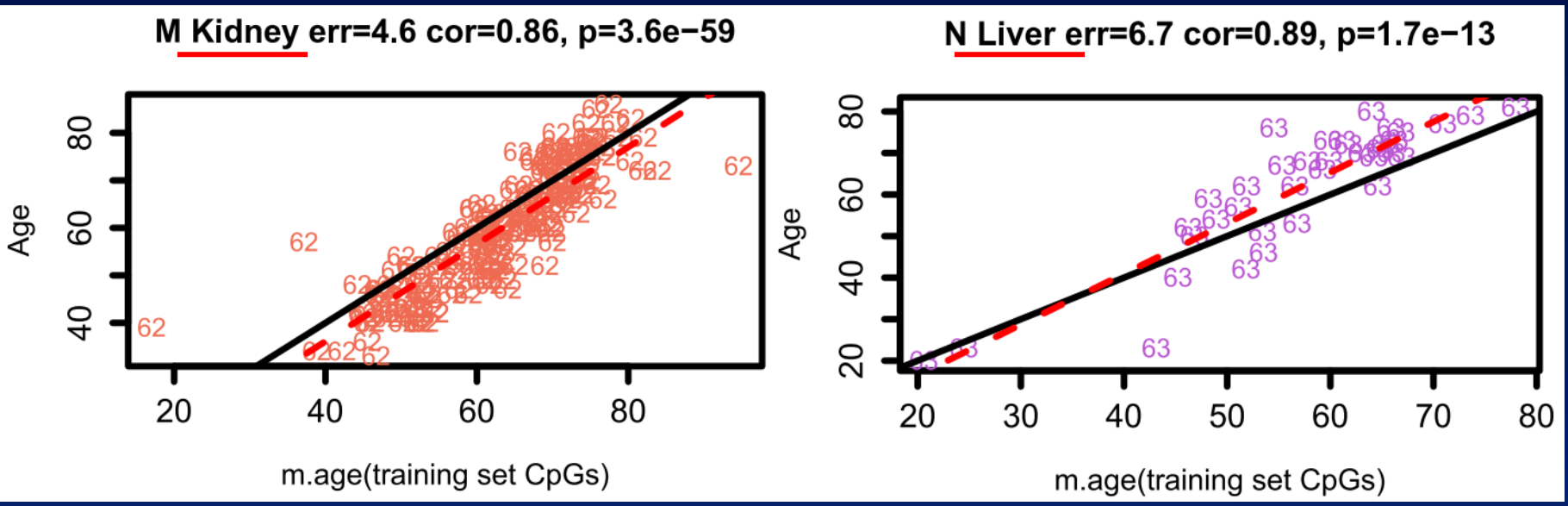


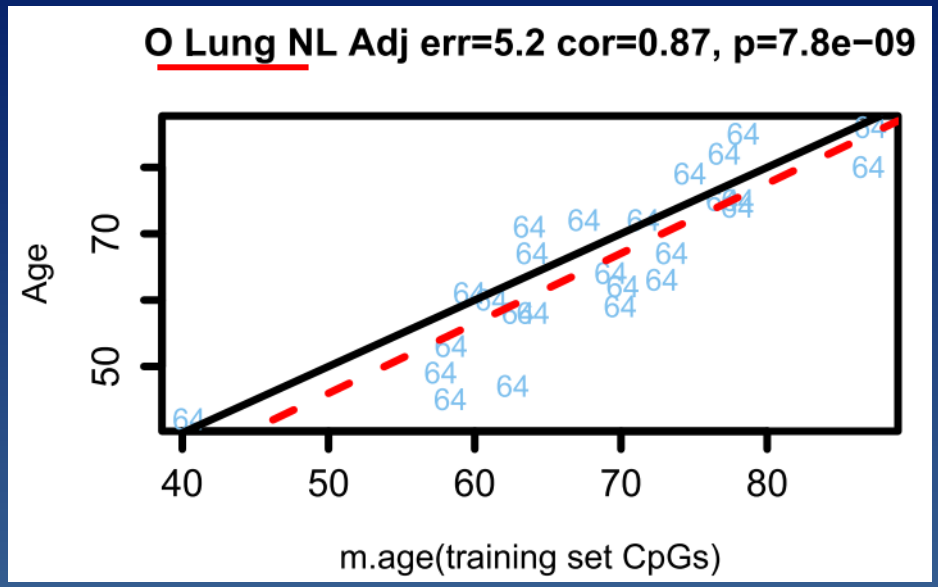
Multi-tissue age predictor on Brain



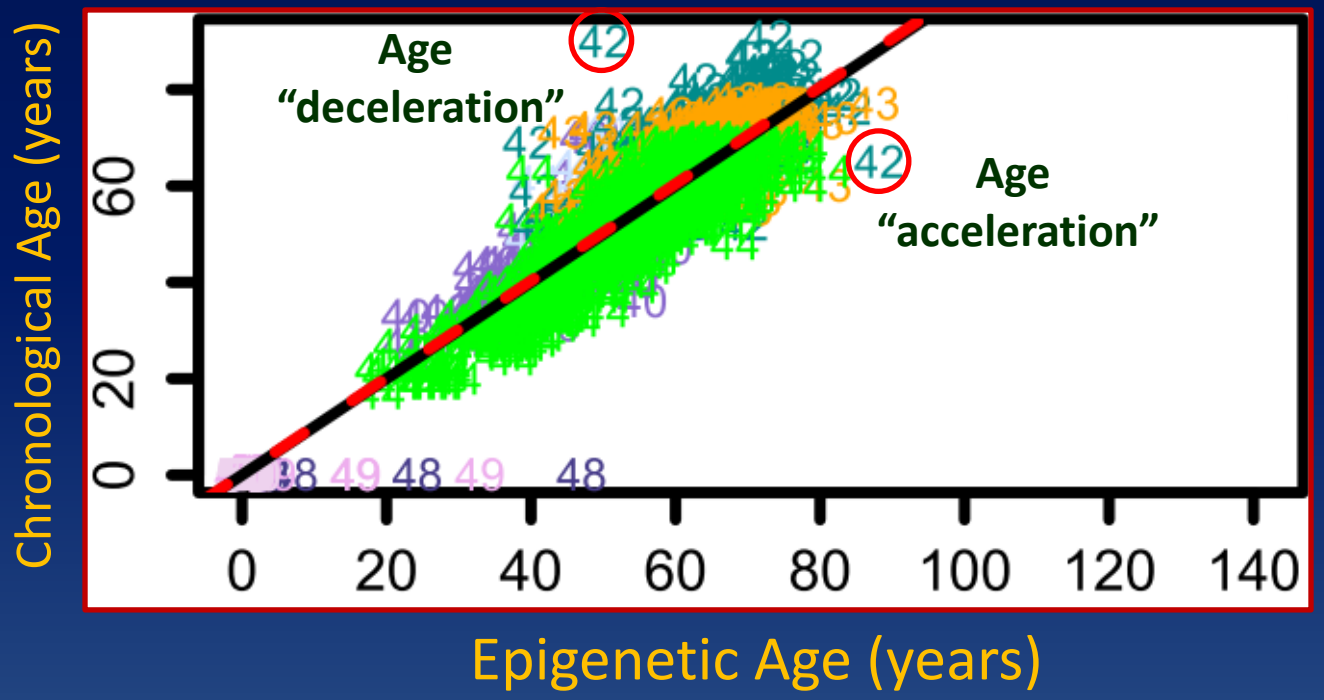


Multi-tissue age predictor on vital organs

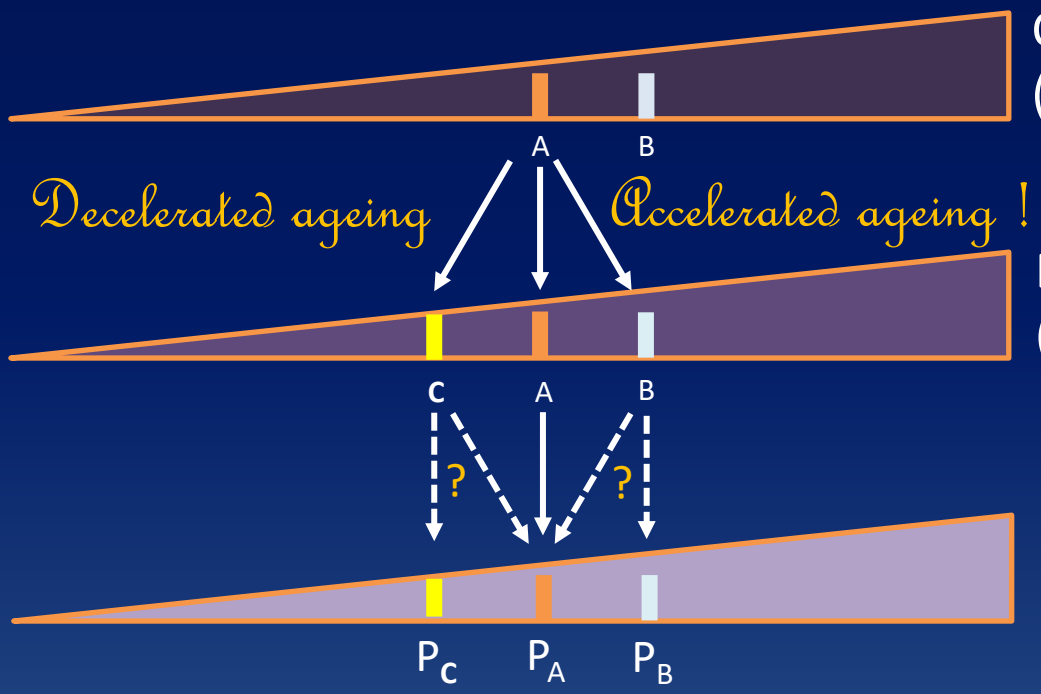




The Outliers



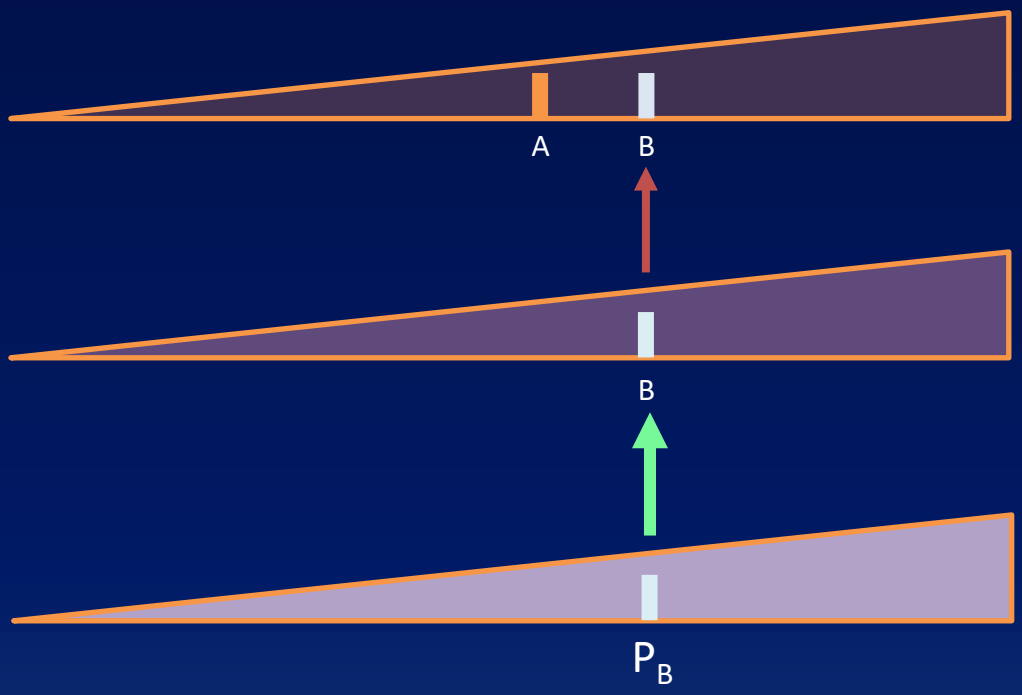
What could epigenetic age accel./decel. mean?



Chronological Age
(Passing of time)

Epigenetic Age
(Changes in DNA methylation)

Biological Age
(Probability of pathology)

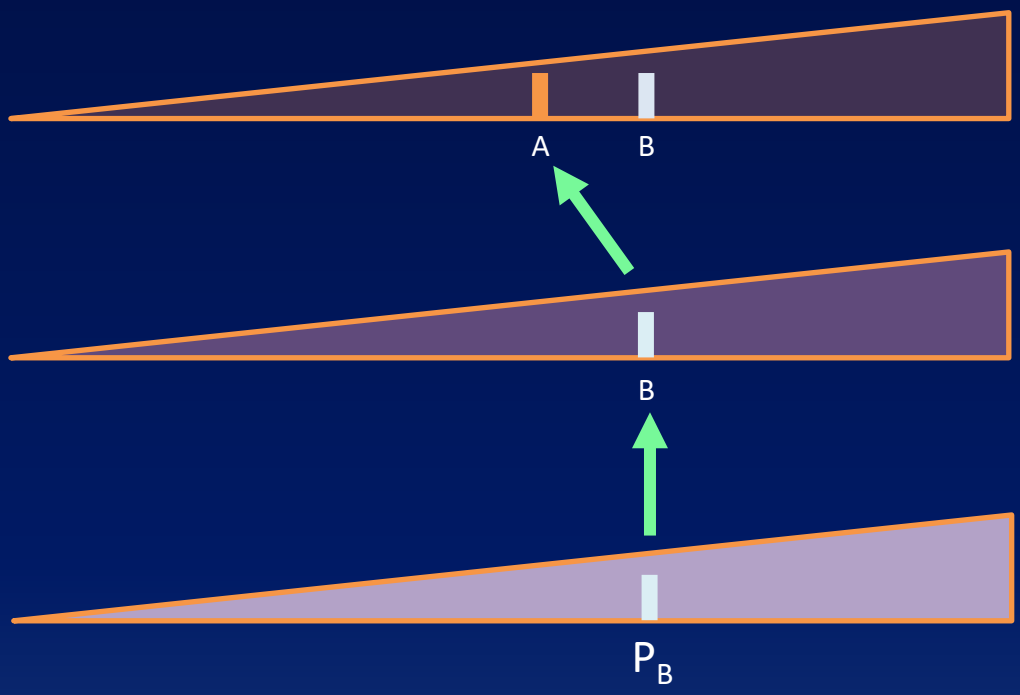


Alzheimer's Disease

Cardiovascular Disease

Parkinson's Disease

Epigenetic Age Tracks Biological Age



Chronological Age
(Passing of time)

Epigenetic Age
(Changes in DNA methylation)

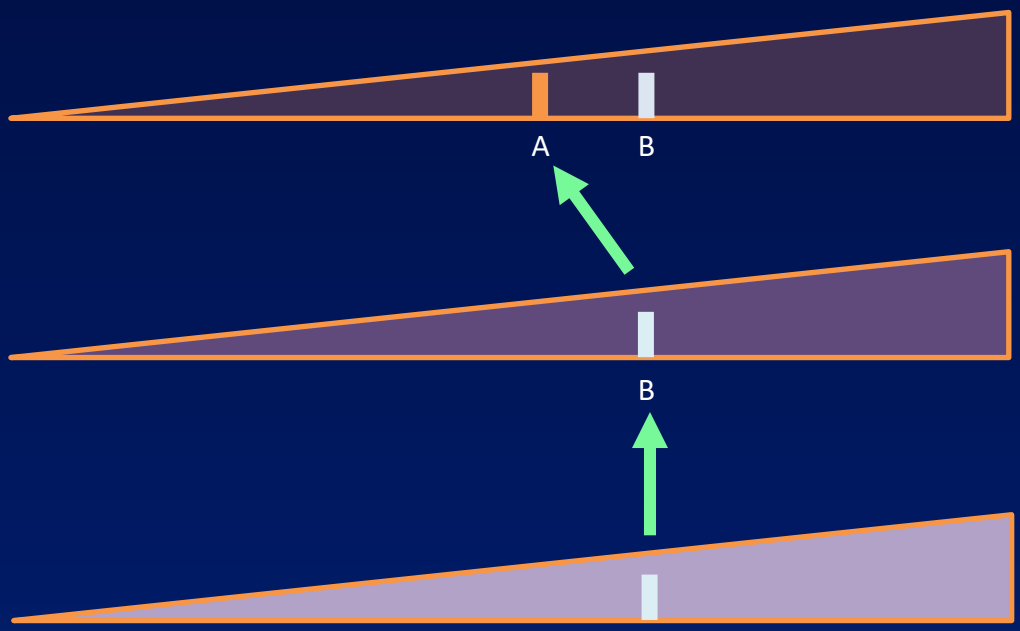
Biological Age
(Incidence of pathology)

Alzheimer's Disease

Cardiovascular Disease

Parkinson's Disease

Epigenetic Age Tracks Biological Age



Chronological Age
(Passing of time)

Epigenetic Age
(Changes in DNA methylation)

Biological Age
(Incidence of pathology)

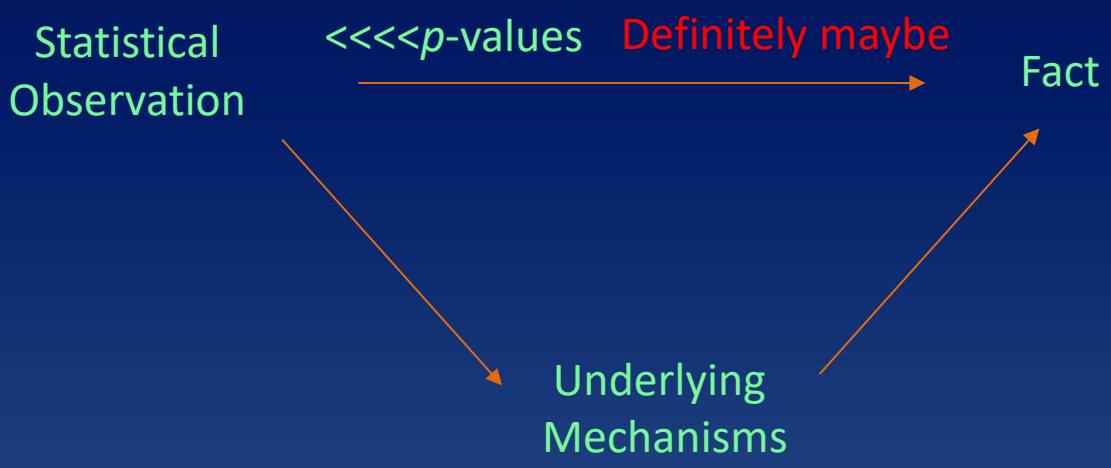
Congenital	Non-congenital	Conditions	Primary traits	Life-style
Down Syndrome	Alzheimer's Disease	Lipid Levels	Gender	Diet
Huntingdon Disease	Cardiovascular Disease	Blood Pressure	Menarche	Exercise
Werner Syndrome	Parkinson's Disease	Amyloid Load	Menopause	BMI
	Osteoarthritis	C-reactive Protein	Centenarian status	Obesity
	HIV infection	Frailty	Mortality (Time to death)	
		Cognitive Performance		

Epigenetic Age / DNA Methylation Age captures biological age

but

What is epigenetic ageing in cellular and molecular terms?

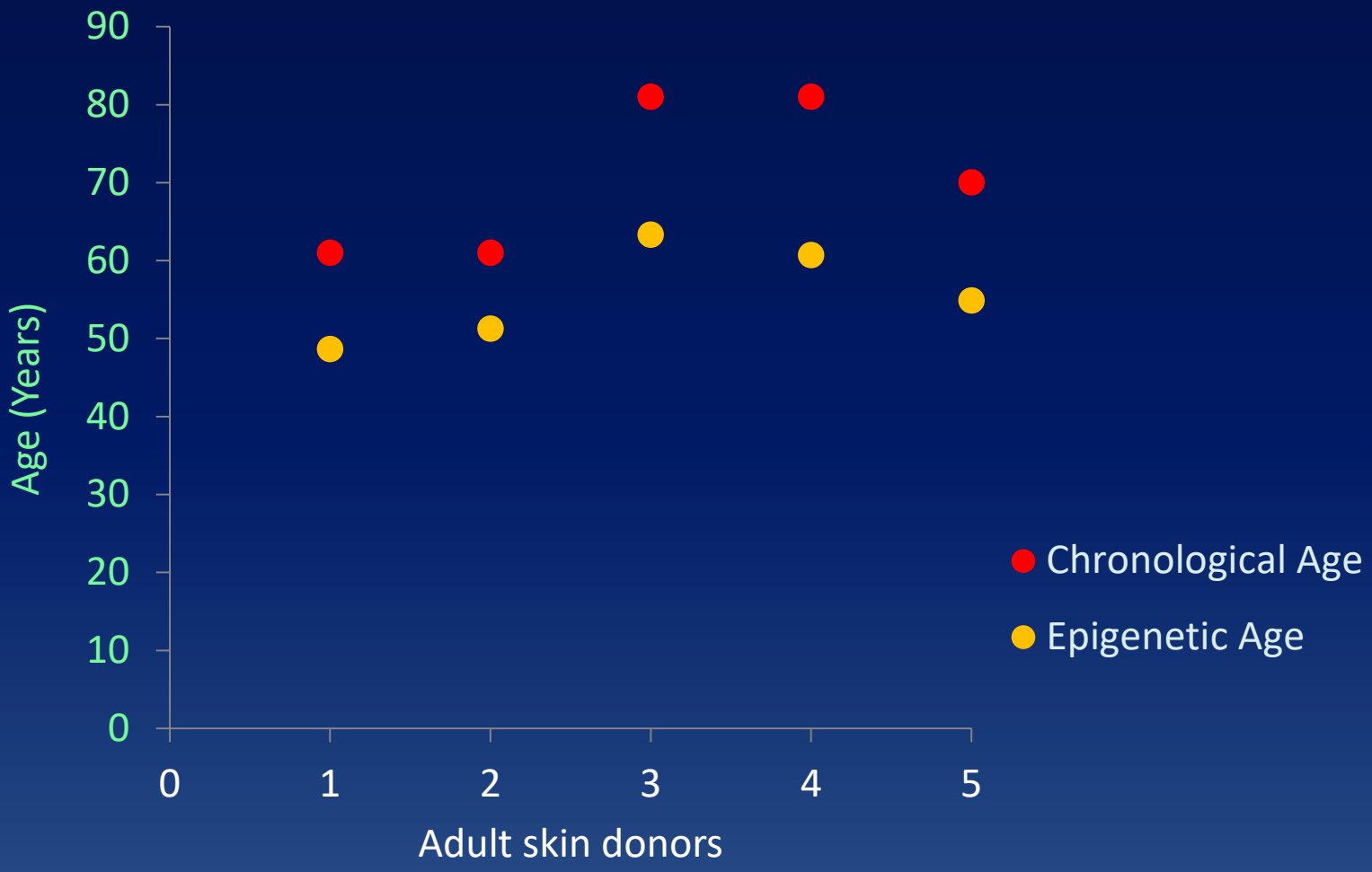
(What is the mechanism ?)



Rarely acquired through human studies

Almost always through in vitro (cells in culture) studies

Performance of Multi-tissue age estimator on adult **Keratinocytes in culture**



Multi-tissue age estimator
(Horvath clock)

Excellent for *in vivo* cells but no so for *ex vivo*

Need to develop a new age estimator compatible with *in vivo* and *ex vivo* cells

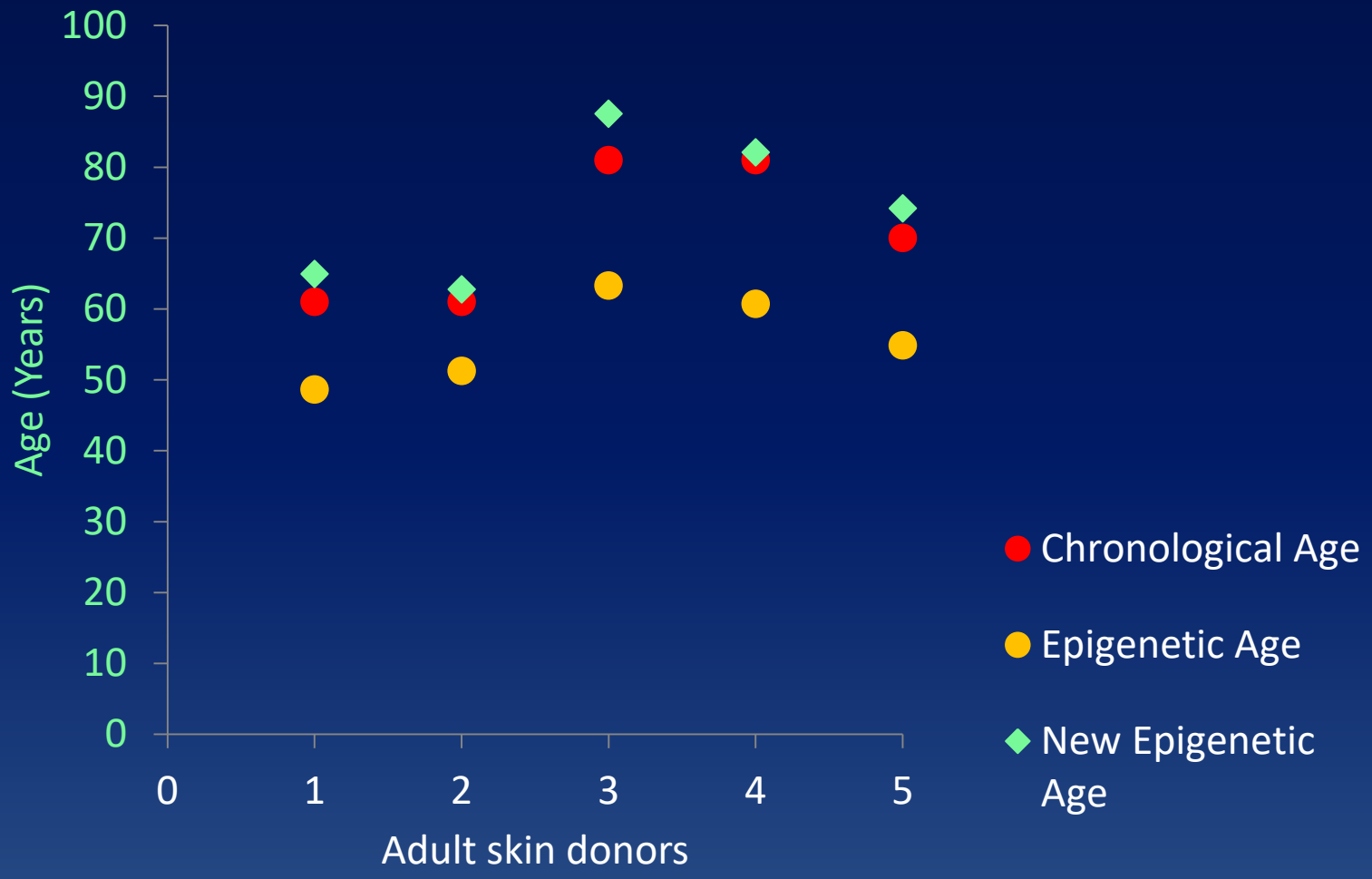
Regressed chronological age with DNA methylation profiles from skin cells

Skin clock (391 CpGs)

Aging (2018) Jul 26;10(7):1758-1775.

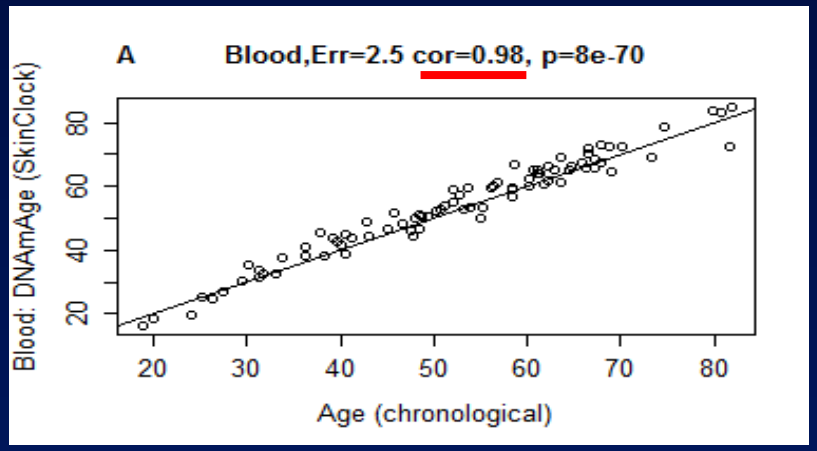
60 CpGs similar to Horvath's clock (353 CpGs)
331 CpGs are new

Performance of Skin age estimator on adult **Keratinocytes**

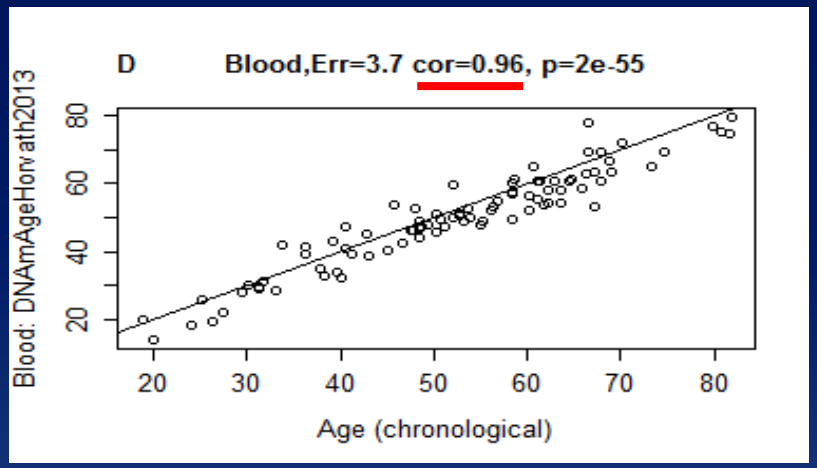


Blood

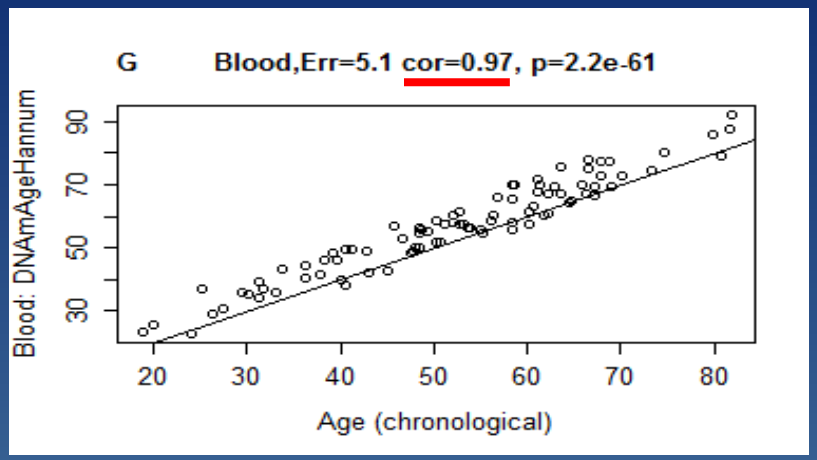
Skin Clock
2018



Pan-Tissue Clock
(Horvath)
2013

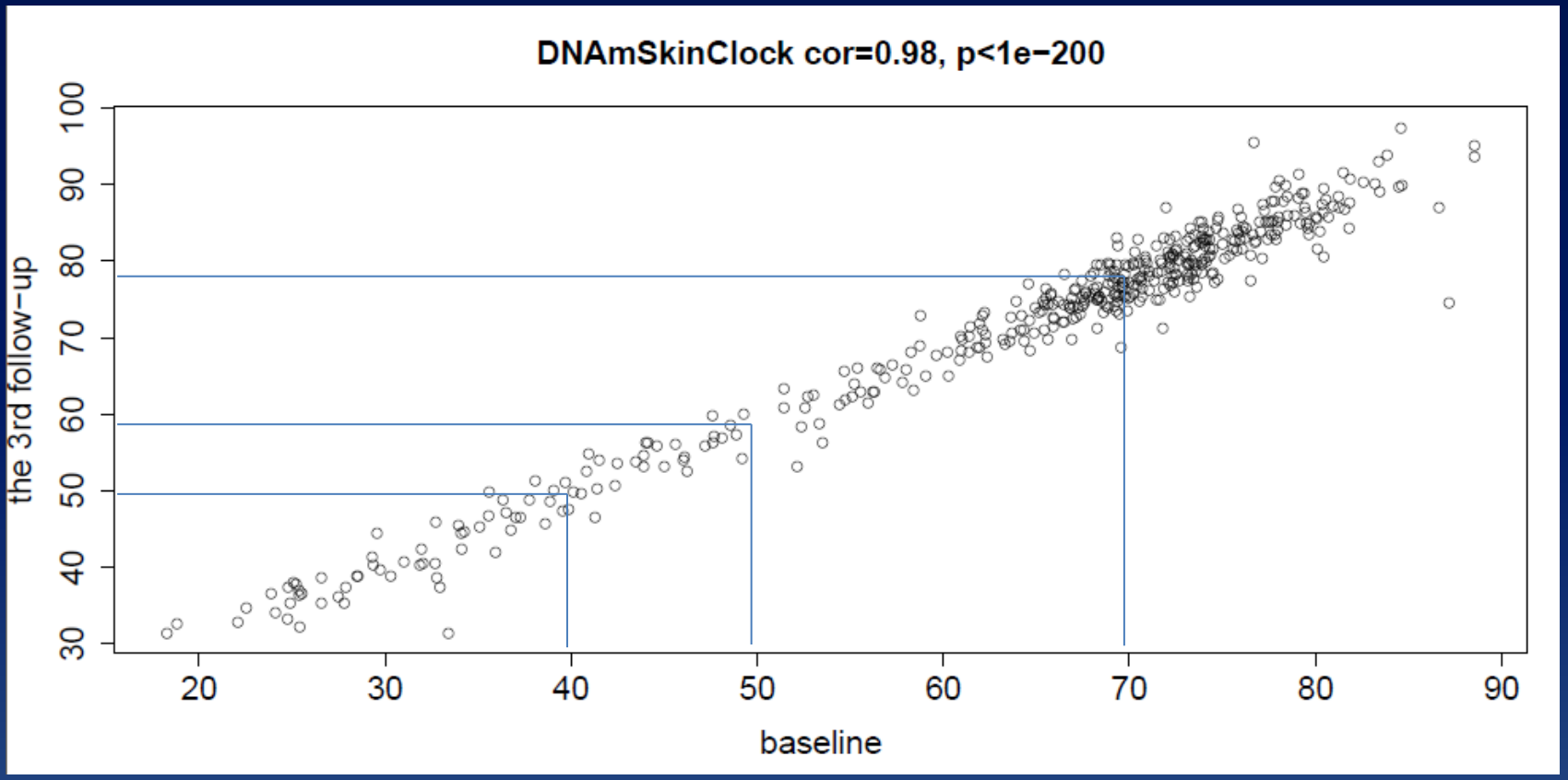


Blood Clock
(Hannum)
2013

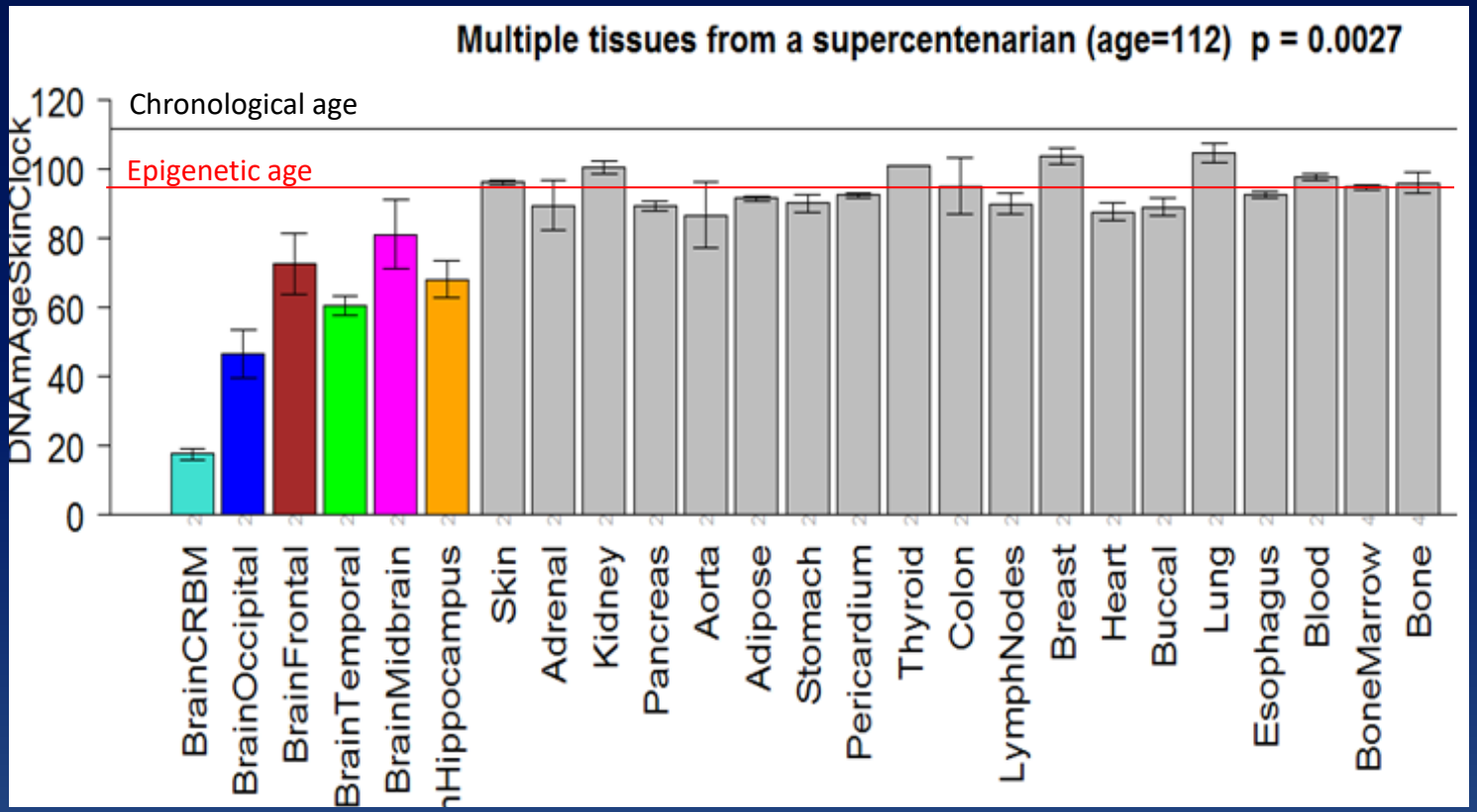


Hannum
Clock
(71 CpGs)
2013

Consistency of age analyses from 1st to 3rd follow-up (8-year gap)



Skin clock → Skin & blood clock → ~~Multi-tissue Age estimator II~~



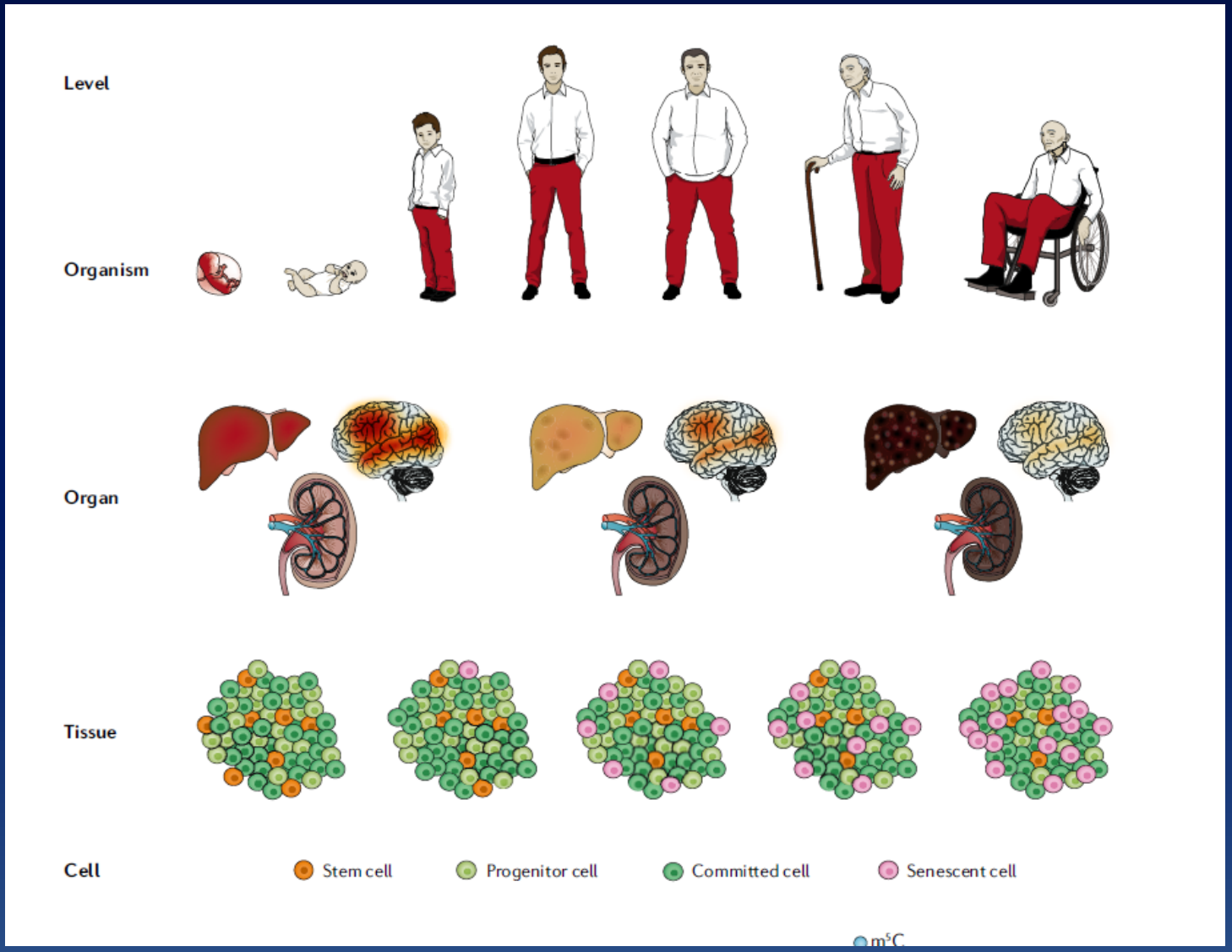
Epigenetic Ageing

(~5 years old)

Prime mover of ageing → DNA methylation changes → Epigenetic clock → Epigenetic Ageing

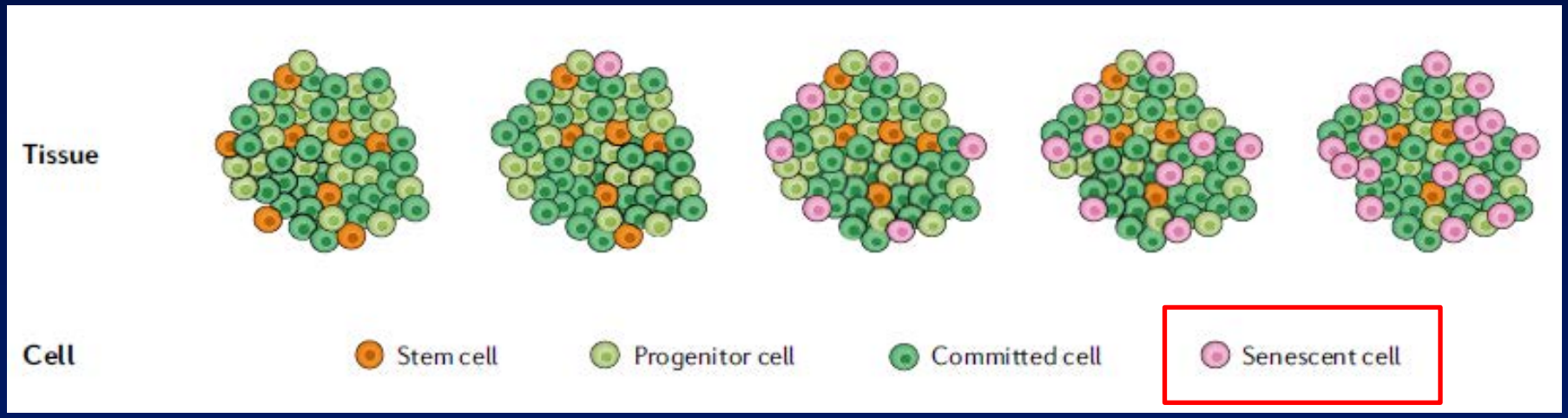
Epigenetic clock → Gene expression changes

Human Ageing

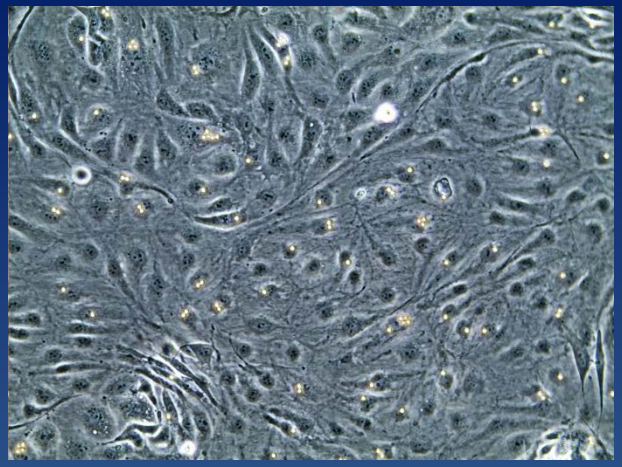


Sub-Cellular source of human ageing ? DNA

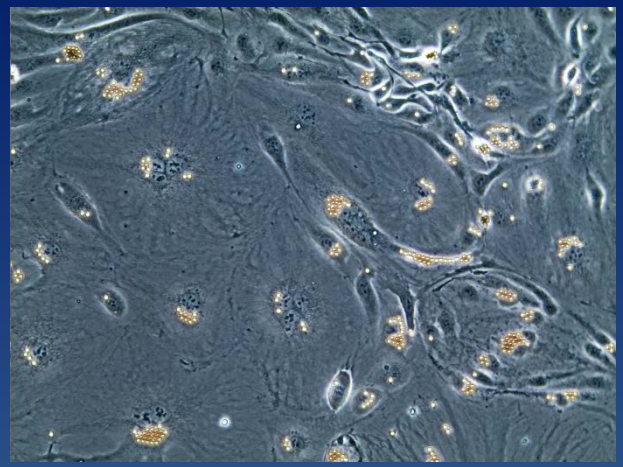
Increasing age



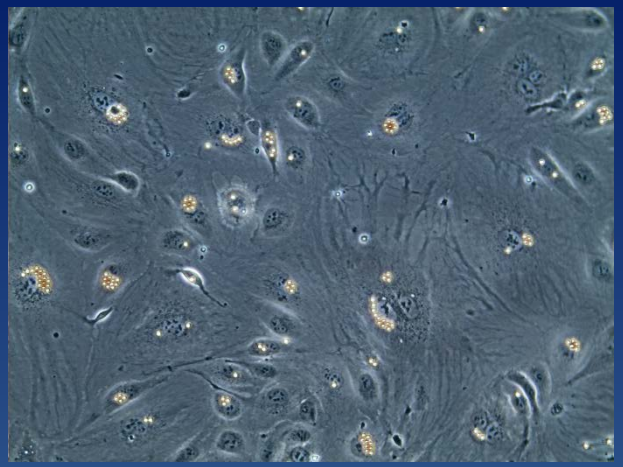
0 Years-old



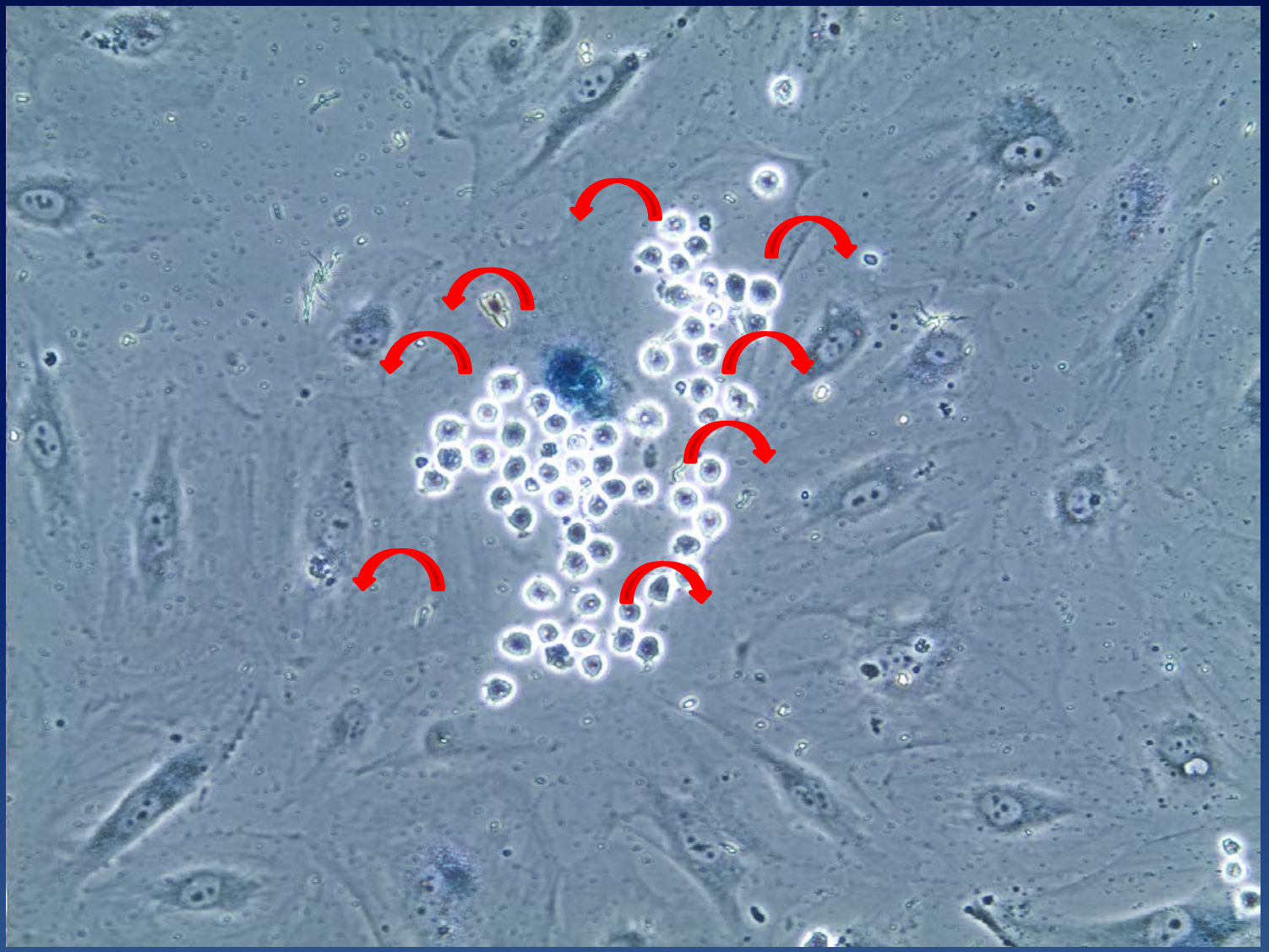
61 Years-old



81 Years-old



Senescent endothelial cells are adhesive



Epigenetic Ageing

(~5 years old)

Prime mover of ageing → DNA methylation changes → Epigenetic clock → Epigenetic Ageing

Epigenetic clock → Gene expression changes

Is Epigenetic Age a measure of Senescent cells ?

Cellular Senescence Ageing

(~35 years old)

Healthy Proliferating Cell

Age

Senescent cells

Epigenetic ageing is distinct from senescent-cell mediated ageing !

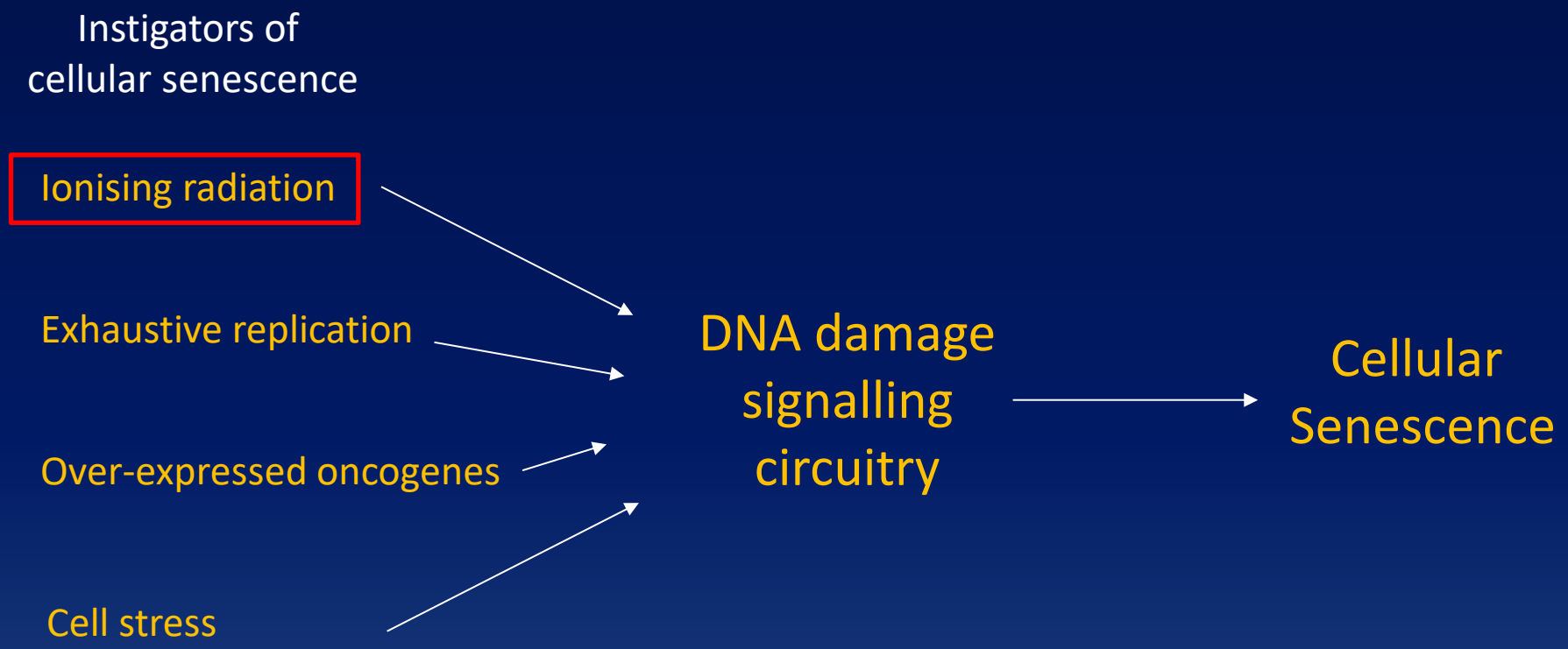
Prime mover 1 - - - - ▶ DNA methylation Changes → Epigenetic clock → Epigenetic Ageing

Epigenetic clock → Gene expression changes

Prime mover 2 - - - - ▶ DNA methylation Changes ? → Senescence clock ? → Cellular Senescence

Senescence clock ? → Gene expression changes

Is it possible to generate a composite biological age clock by combining epigenetic ageing and senescence?


Instigators of cellular senescence
Ionising radiation
Exhaustive replication
Over-expressed oncogenes
Cell stress
DNA damage signalling circuitry
Cellular Senescence

Neonatal foreskin Fibroblasts

Control X-irradiated

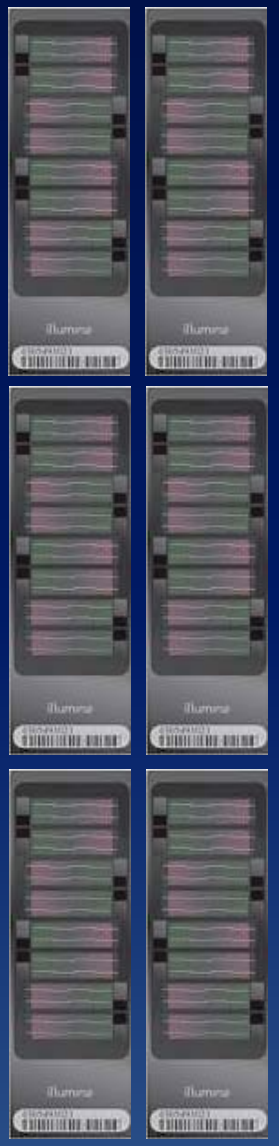
Donor 1

Donor 24

2 weeks

Extracted DNA

24 controls
24 senescent



£17,000

Data received

48 columns (24 controls and 24 senescent cells)

850,000 rows (each representing a specific CpG site on human genome)

Values between 0 and 1

Challenge

Identify DNA methylation markers of senescent cells

If epigenetic clocks measure biological health….

Health-affecting features should increase biological age, and be manifested as increased epigenetic age.

but

Associations of epigenetic age with most clinical measures of health are modest

Maybe these clocks capture “Inherited Health”
But not “Life-Affected Health”

Training data was from healthy subjects of 0 to 100 years. Potential effects of “Life” on Health may have been muted

Can the clocks be improved to provide a better measure of Life-affected Health?

Measurable "Life-affected" Features that correlate with health

Life-Affected Features

↓

Measurable Manifestations

Glucose
C-reactive Protein
Albumin
Creatinine
Lymphocyte percentage
Mean Cell volume
Red blood cell distribution width
Alkaline Phosphatase
White blood cell count
Chronological Age

Weighted average score

Regress on DNA methylation values in blood

DNAm PhenoAge

Levine Clock (513 CpGs)

Aging 2018 Apr; 10(4): 573–591

Chronological Age of multi human tissues →

Chronological Age of blood →

Regress on DNA methylation values in blood/ other tissues

Horvath Clock (353 CpGs)

Hannum Clock (71 CpGs)

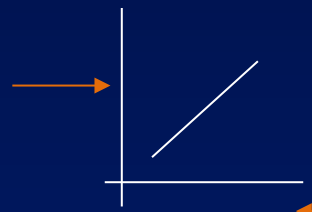
Measurable (1) biomolecular and (2) smoking features that correlate with health

(1) Biomolecular features: 88 proteins in blood directly measured by immunoassays
12 had correlations greater than 0.35 with CpG methylation
Cystatin C, Leptin, GDF-15, B2M, ADM, PAI-1, TIMP-1, CD56, ceruloplasmin, EGF fibulin-like ECM protein 1, myoglobin, serum paraoxonase / arylestarase I

(2) Smoking pack years: DNAm-based marker (172CpG) of smoking pack-years based on self-reported smoking pack-years

Developed predictor of mortality

(1) DNAm-based estimator of smoking pack-years
(2) Chronological age at time of blood draw
(3) Sex
(4) DNAm-based biomarkers of 12 blood proteins



Time-to-death from
All-cause mortality

Elastic net Cox regression model automatically selected:
(1), (2), (3) and *seven* of twelve blood protein levels as covariates

Each covariant is based on fewer than 200 CpGs, totalling 1,030 unique CpGs

Ake Lu

DNAm GrimAge

Aging 2019 Jan 31; 11(2): 303–327

The “One Chip Challenge”

Multi-tissue Age estimator Horvath Clock (2013)

Skin & Blood Clock (2018)

DNAPhenoAge Clock (2018)

DNAGrimAge Clock (2019)

DNAmMito Estimator (Soon)

“DNAmSen Estimator” (?)

“DNAmDam Estimator” (?)

Innate Ageing

State of Health

Risk of Pathology

Time-to-Death

State of Cells

Cell proliferation frequency

Cell senescence index

State of DNA

Thank you

Public Health England

Cellular Biology (PHE - UK)

Donna Lowe

Sylwia Kabacik

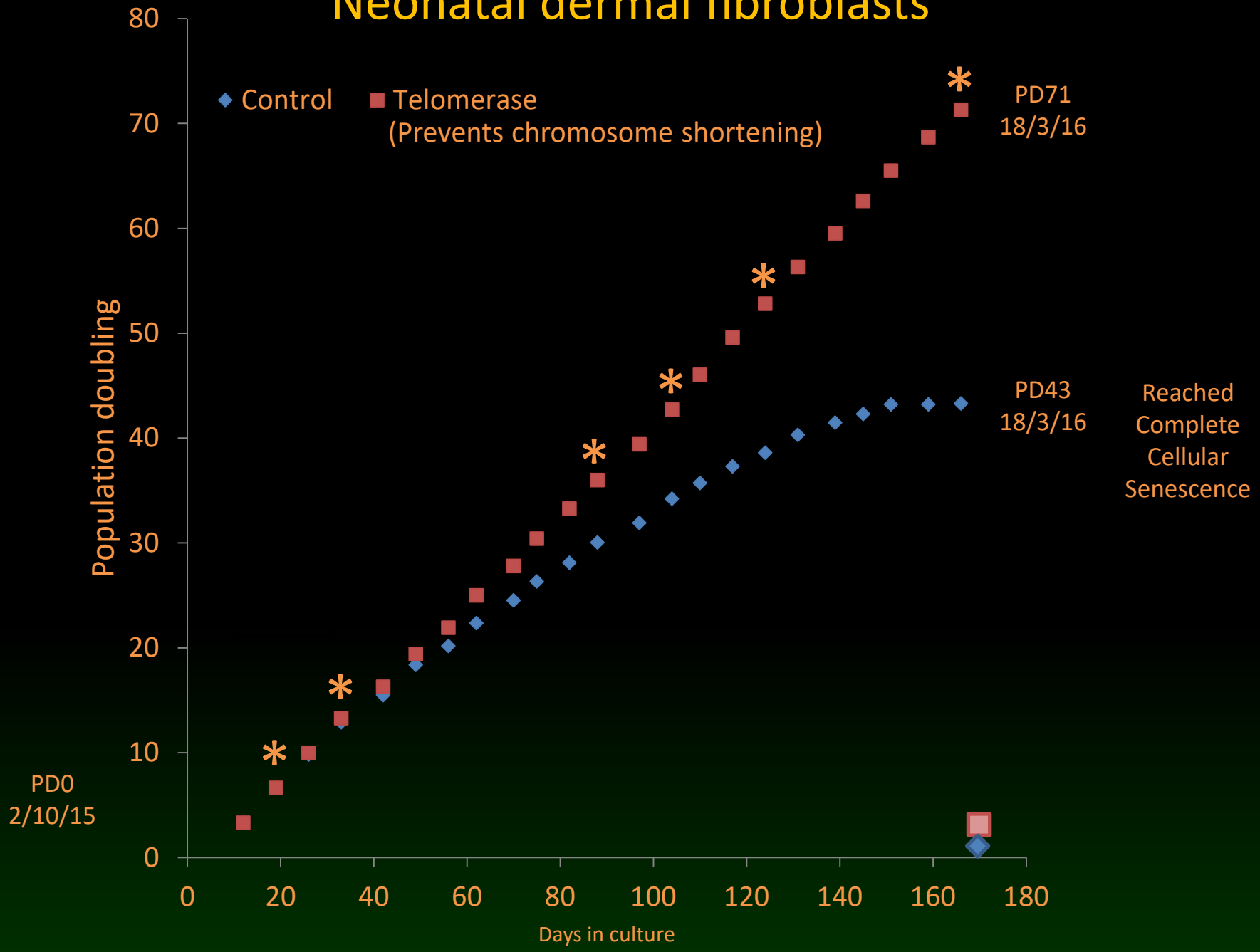
THE UNIVERSITY OF CALIFORNIA · LET THERE BE LIGHT · UCLA

Mathematics (UCLA – USA)

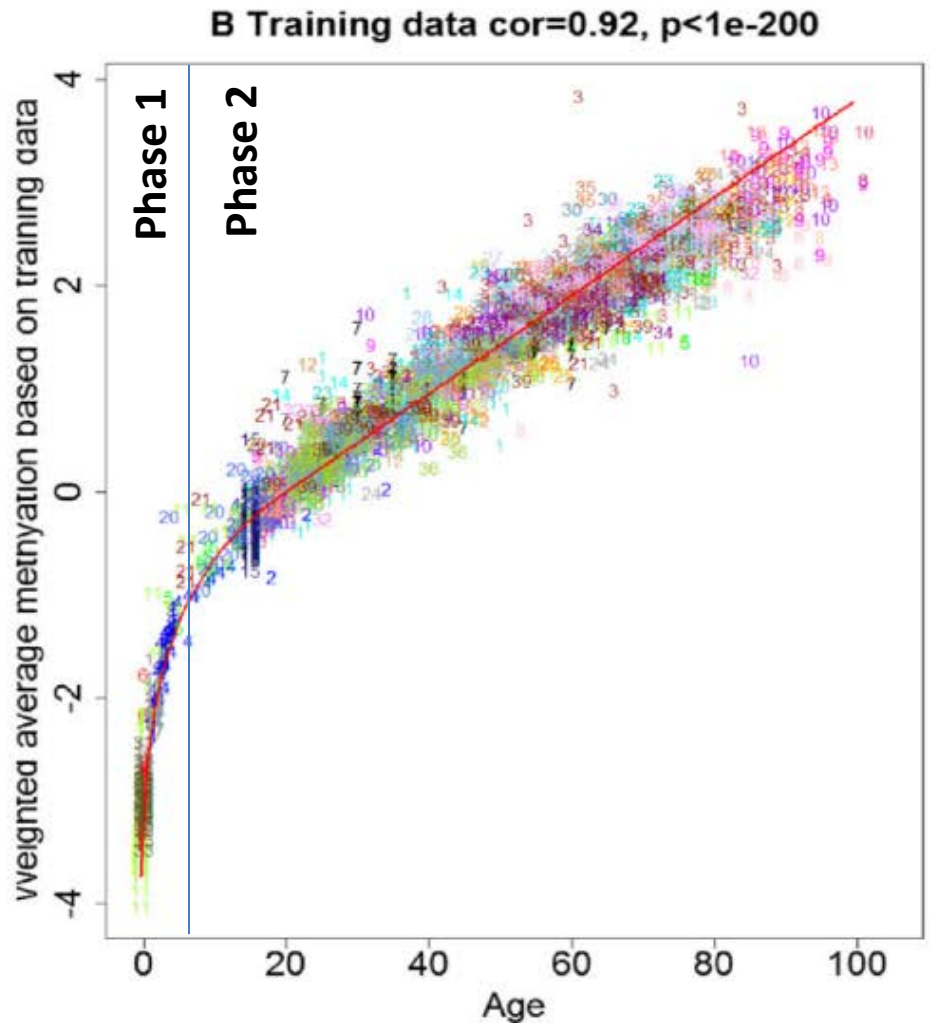
Ake Lu

Steve Horvath

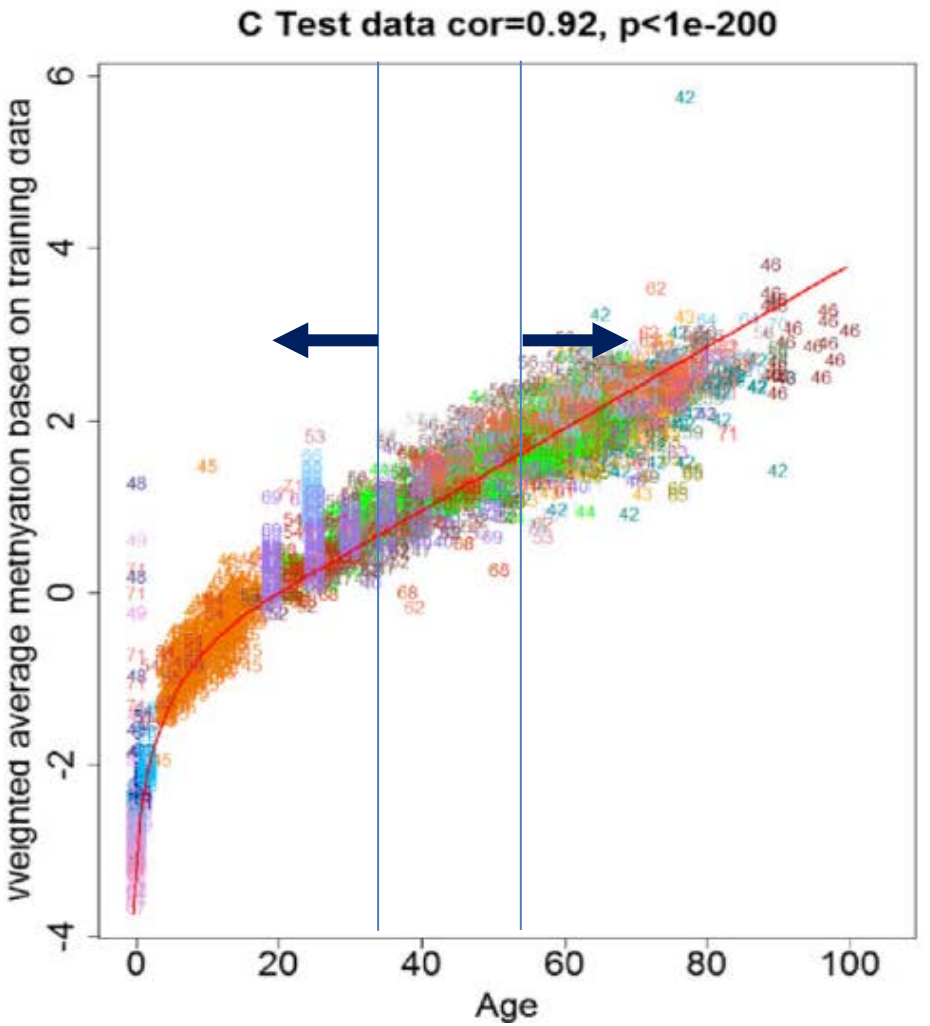
Neonatal dermal fibroblasts



Rate of 353 CpG methylation change in function of time (age)

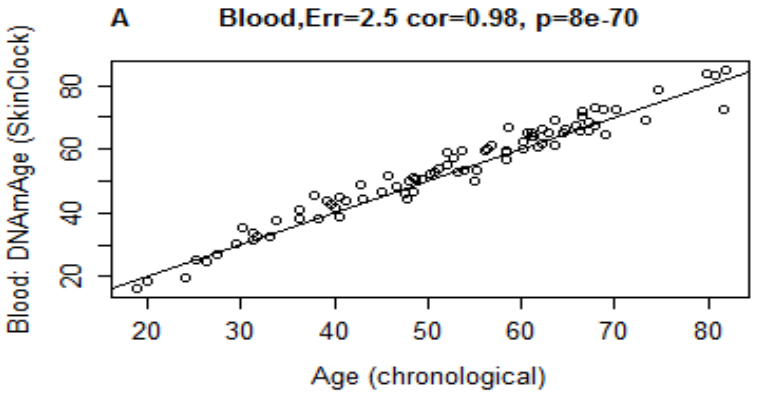


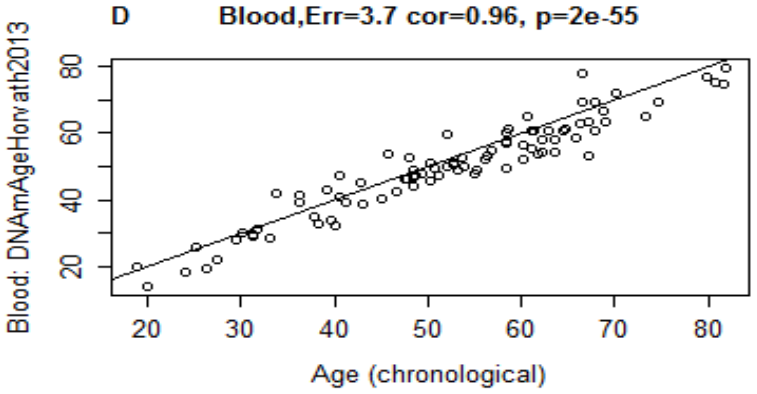
Methylation change of the 353CpG occurs at two very different rates in function of age. Rate of **Phase 1 is 24 X > Phase 2**

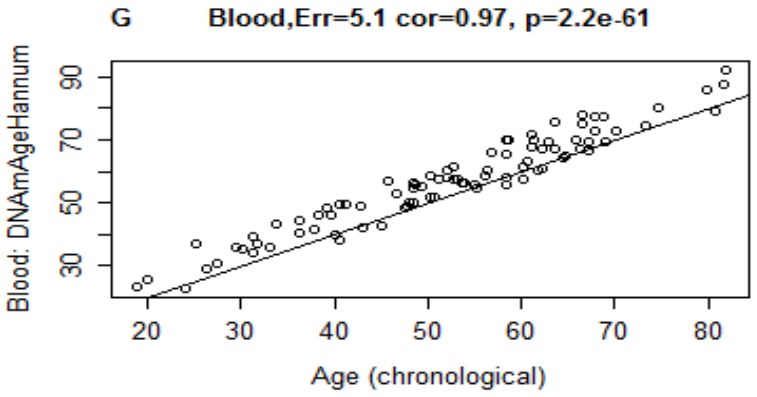


Average difference of methylation of the 353 CpGs between DNA from people younger than 35 years old and those above 55 years old is only **0.032 (3.2%)**

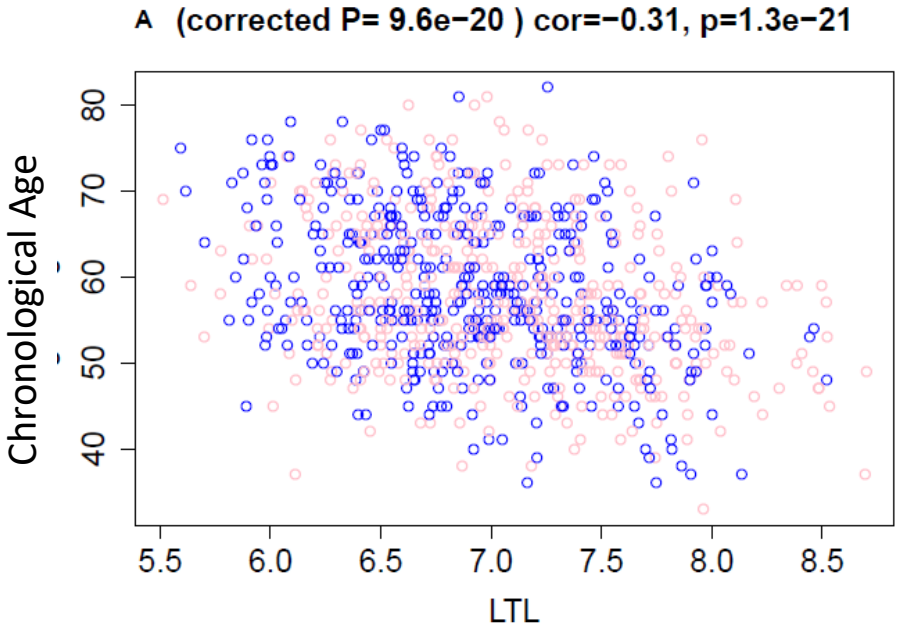
Performance of new estimator with Blood

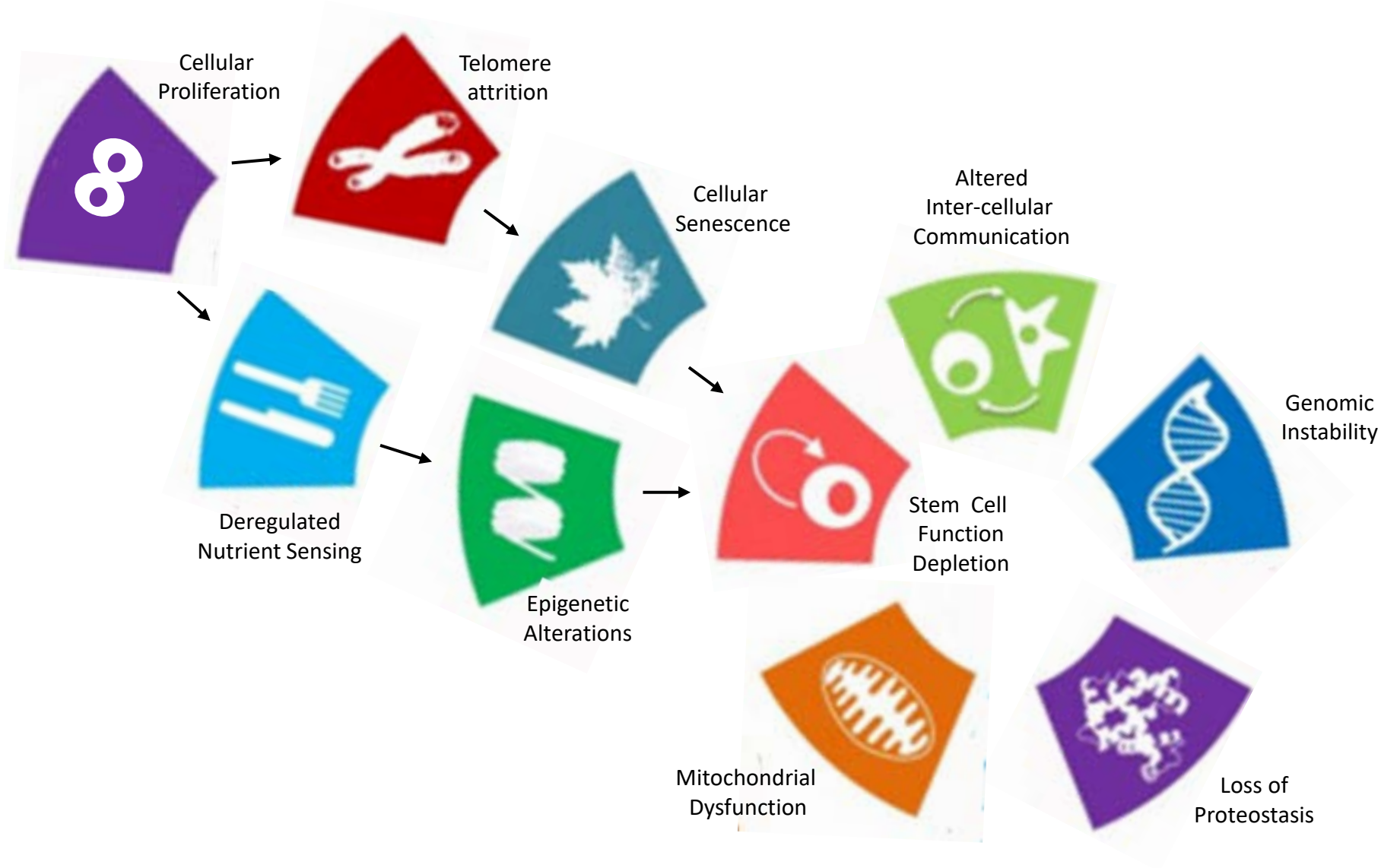






Performance of Leukocyte telomere length as age estimator



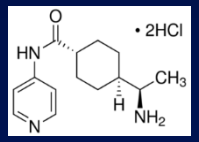

Cellular Proliferation
Telomere attrition
Cellular Senescence
Altered Inter-cellular Communication
Genomic Instability
Deregulated Nutrient Sensing
Epigenetic Alterations
Stem Cell Function Depletion
Mitochondrial Dysfunction
Loss of Proteostasis

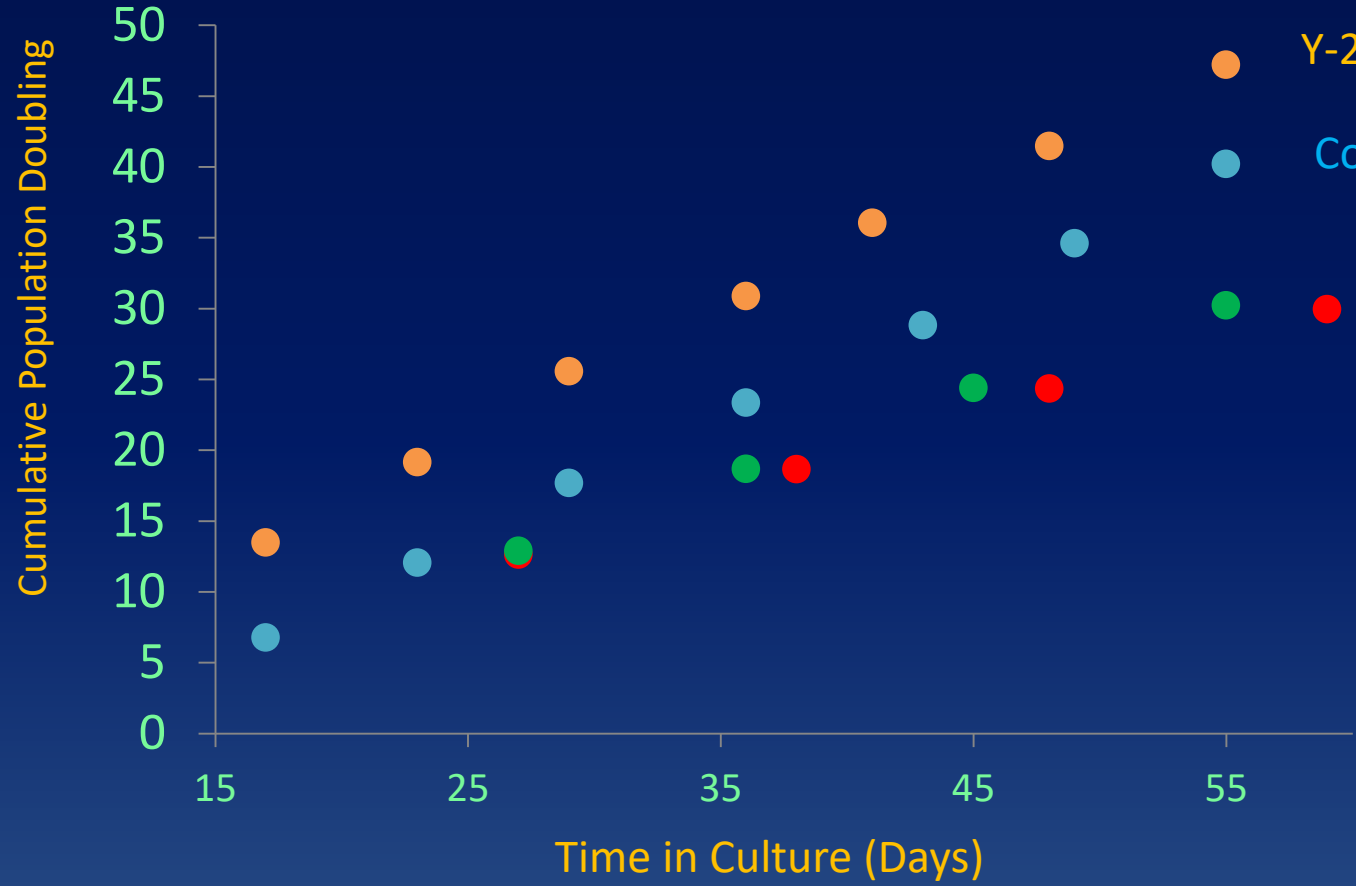
“Hallmarks” of Ageing

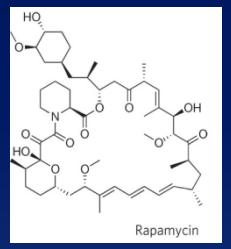
hTERT (telomerase) can prevent telomere attrition.
Would this prevent Epigenetic Ageing?

Senescent cells are responsible for ageing
Would avoidance of cellular senescence prevent Epigenetic ageing?

Effects of Rapamycin and Y-27632 on proliferation of primary human keratinocytes

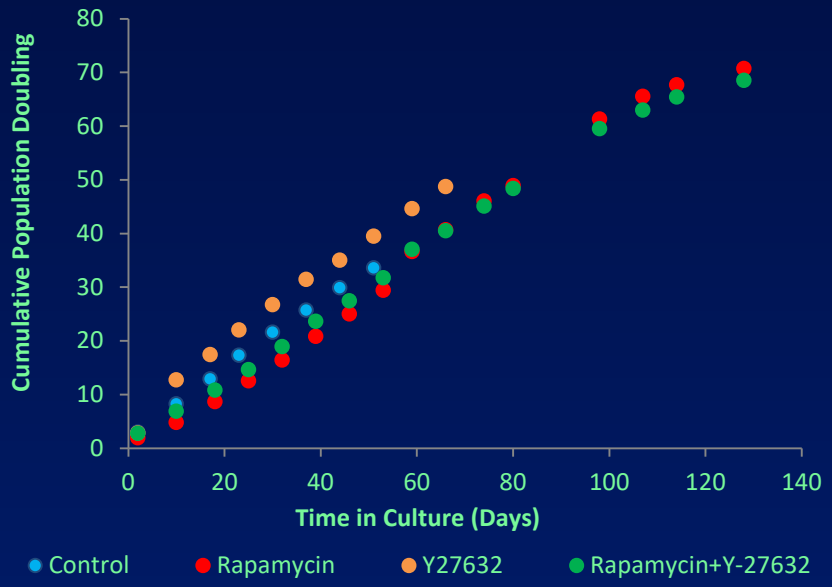




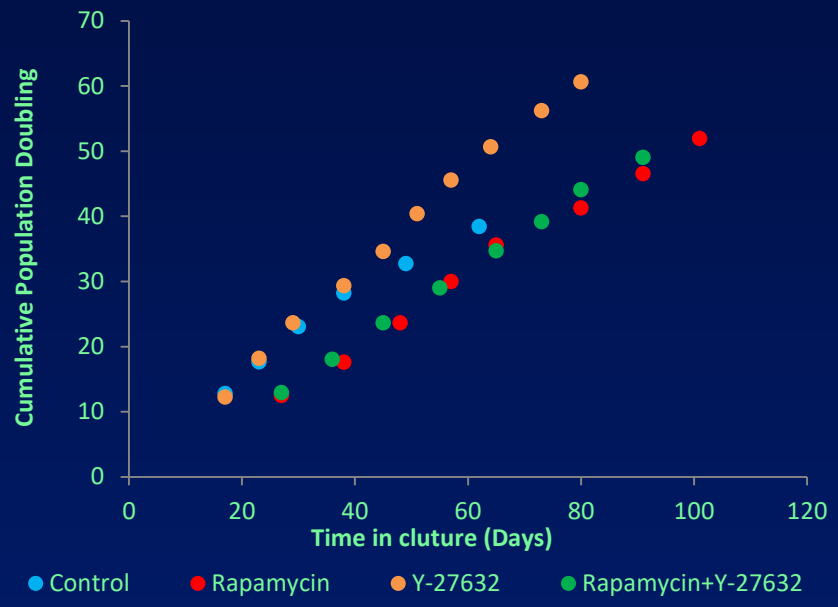


Control Rapamycin Y-27632 Rapamycin+Y-27632

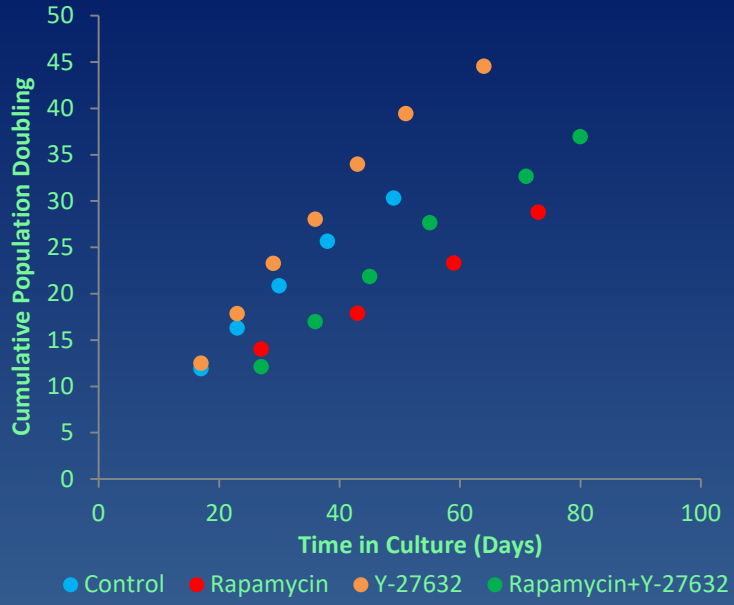
Donor A



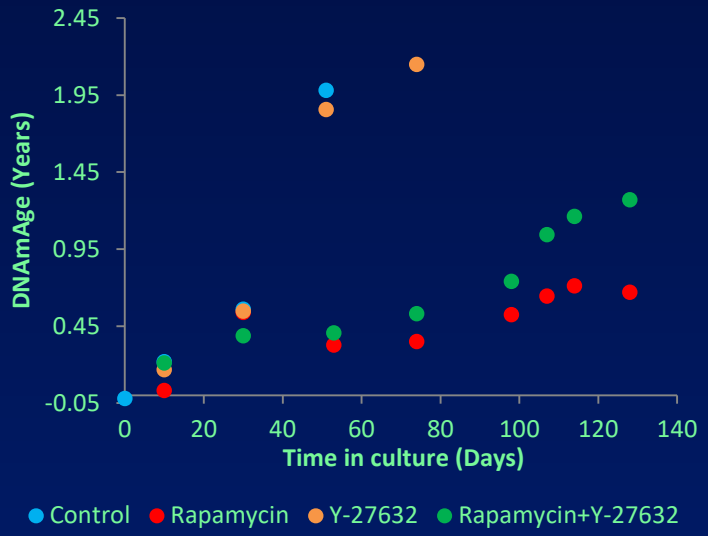
Donor B



Donor C



Donor A



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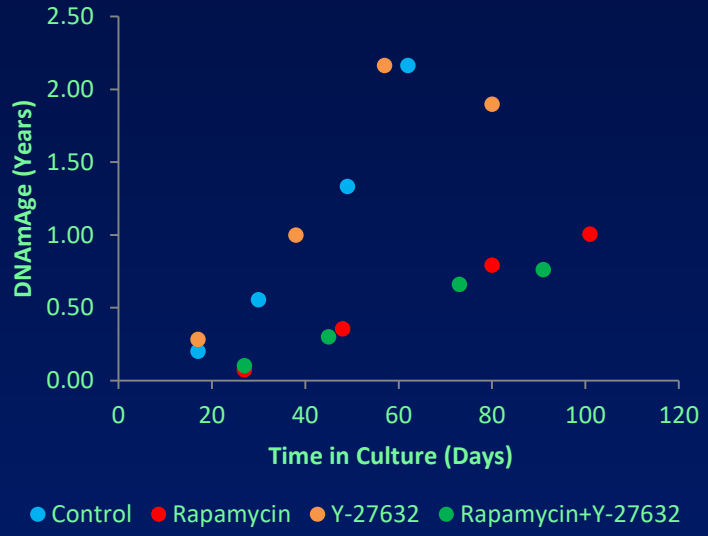
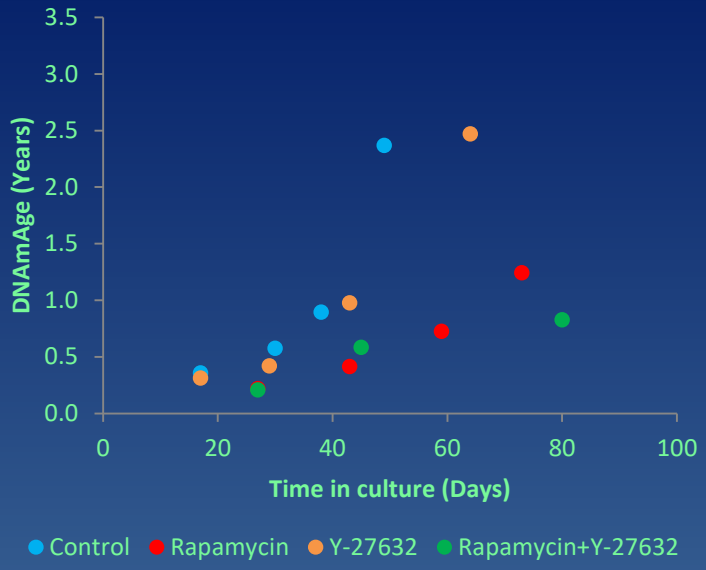


Donor C



Epigenetic Ageing

Not a measure of replicative senescence

Not a measure of replication frequency

Not a measure of replication rate

Not impeded by telomere maintenance

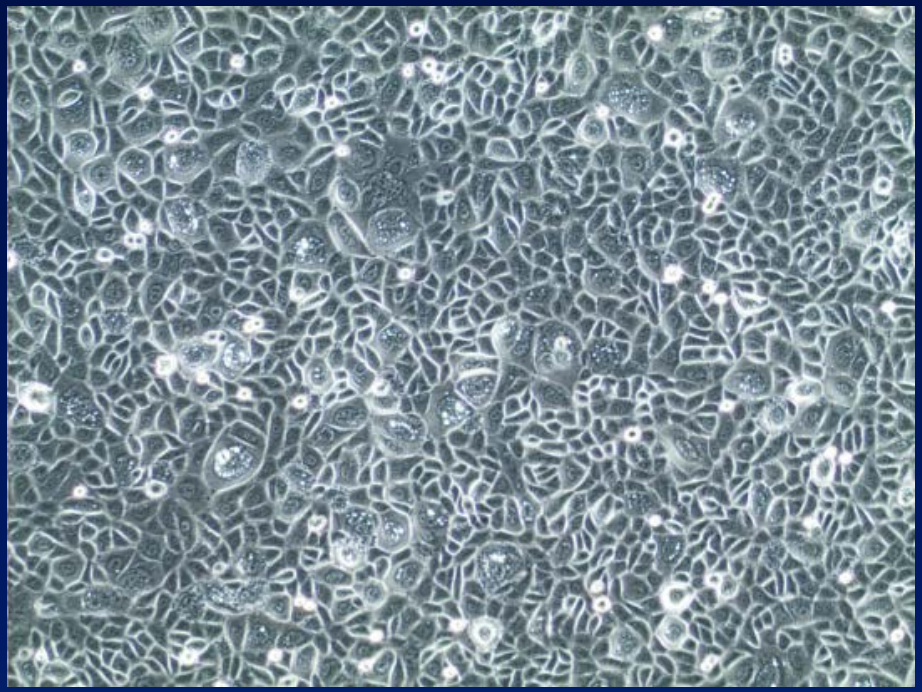
Not prevented by immortality

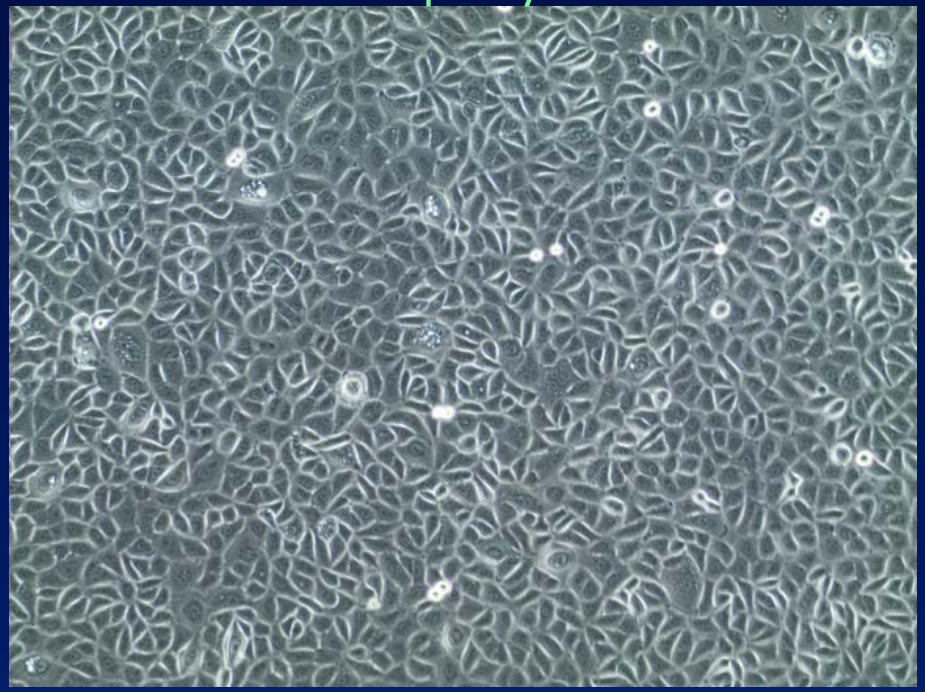
Retarded by Rapamycin!!!

What is Rapamycin doing to the cells?

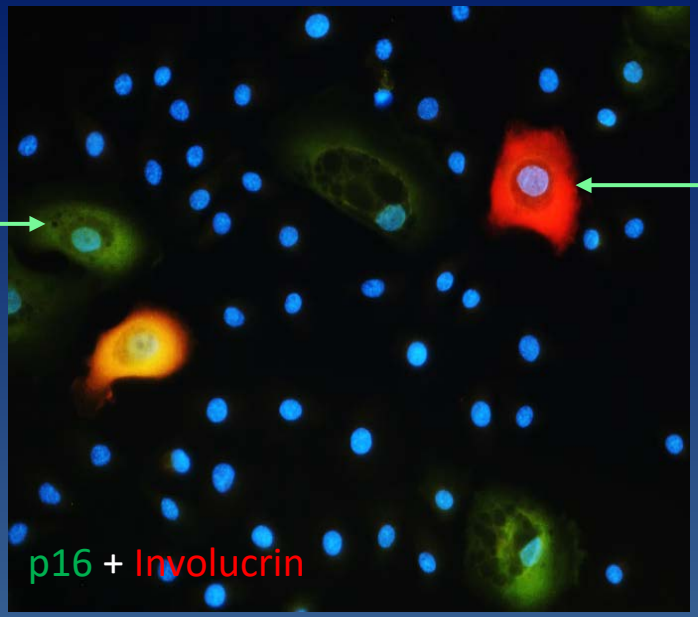
Control

Rapamycin



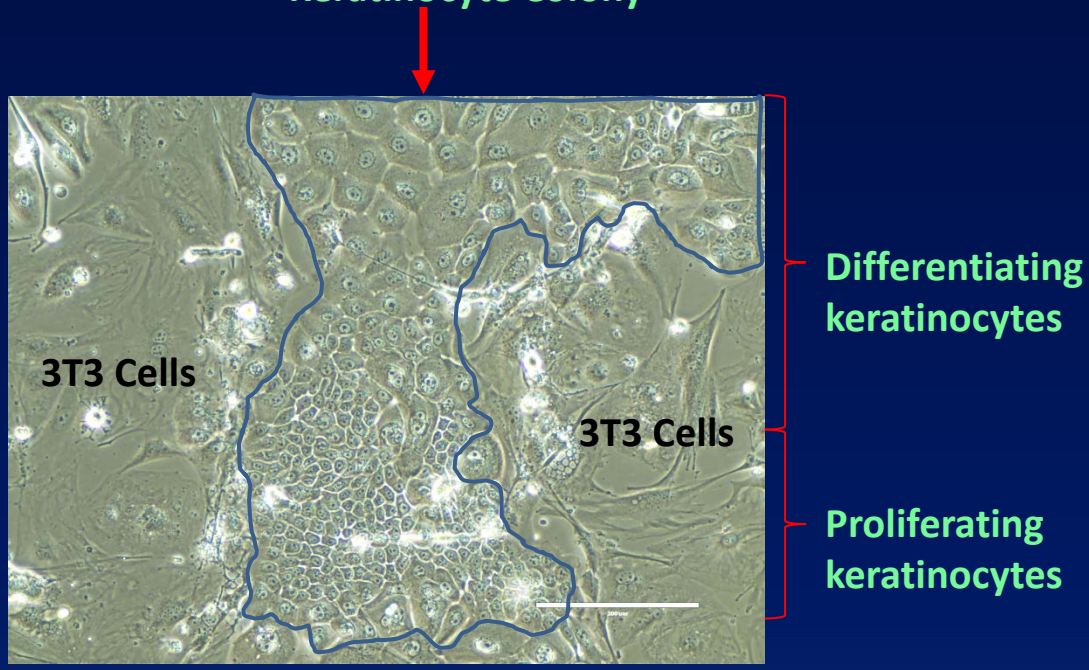


Senescent Keratinocytes

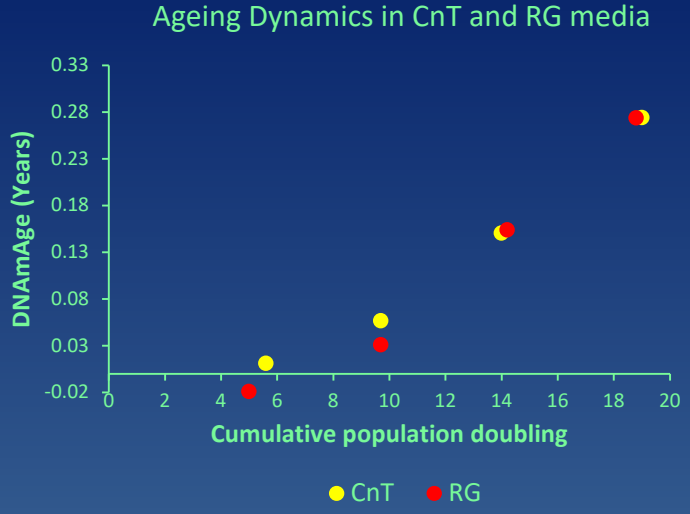


Differentiating Keratinocytes

(A)


Keratinocyte Colony
3T3 Cells
3T3 Cells
Differentiating keratinocytes
Proliferating keratinocytes

(B)


Ageing Dynamics in CnT and RG media
DNAmAge (Years)
0.33
0.28
0.23
0.18
0.13
0.08
0.03
-0.02
0
2
4
6
8
10
12
14
16
18
20
Cumulative population doubling
CnT
RG

Epigenetic Ageing

Not a measure of replicative senescence

Not a measure of replication frequency

Not a measure of replication rate

→ Not a measure of somatic cell differentiation

Not impeded by telomere maintenance

Not prevented by immortality

Retarded by Rapamycin!!!

What is Rapamycin doing to the cells?

Epigenetic Ageing

Not a measure of replicative senescence

Not a measure of replication frequency

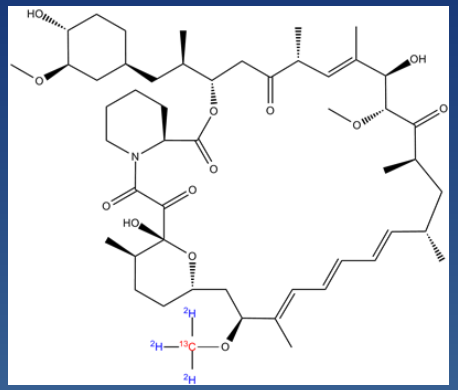
Not a measure of replication rate

Not a measure of somatic cell differentiation

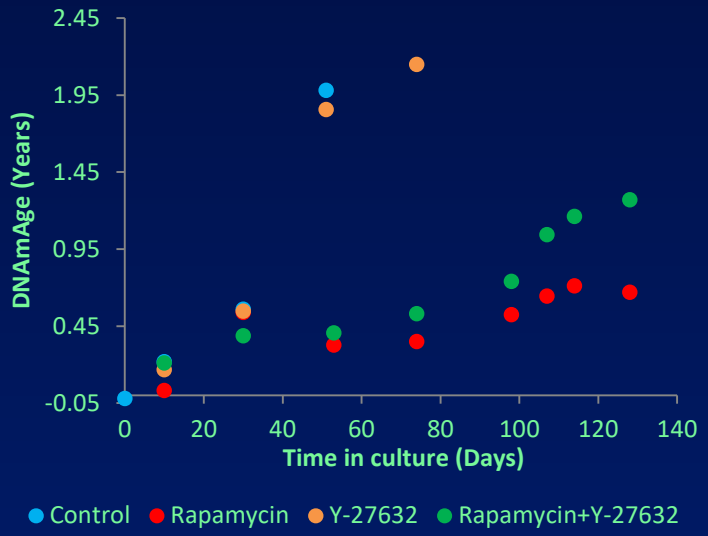
Not impeded by telomere maintenance

Not prevented by immortality

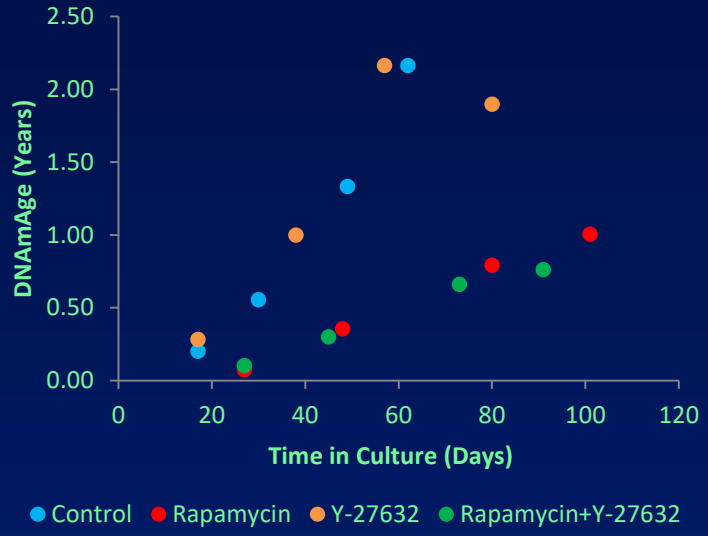
Retarded by Rapamycin!!!



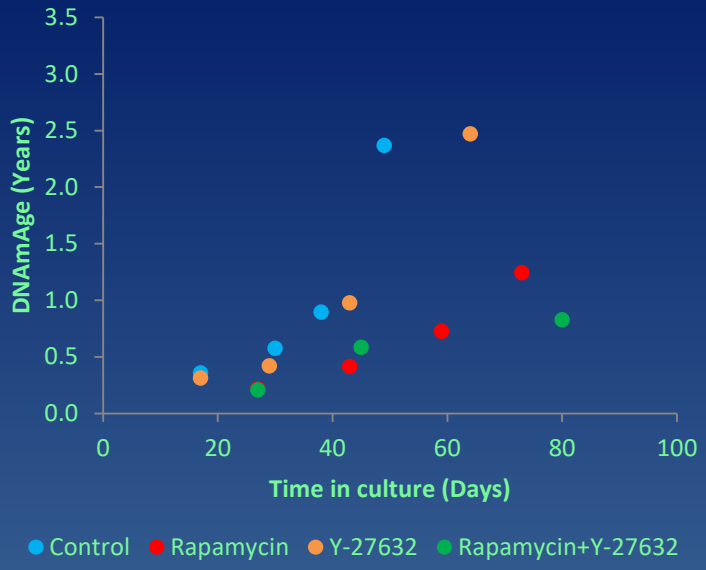
Donor A



Donor B



Donor C



Epigenetic Ageing

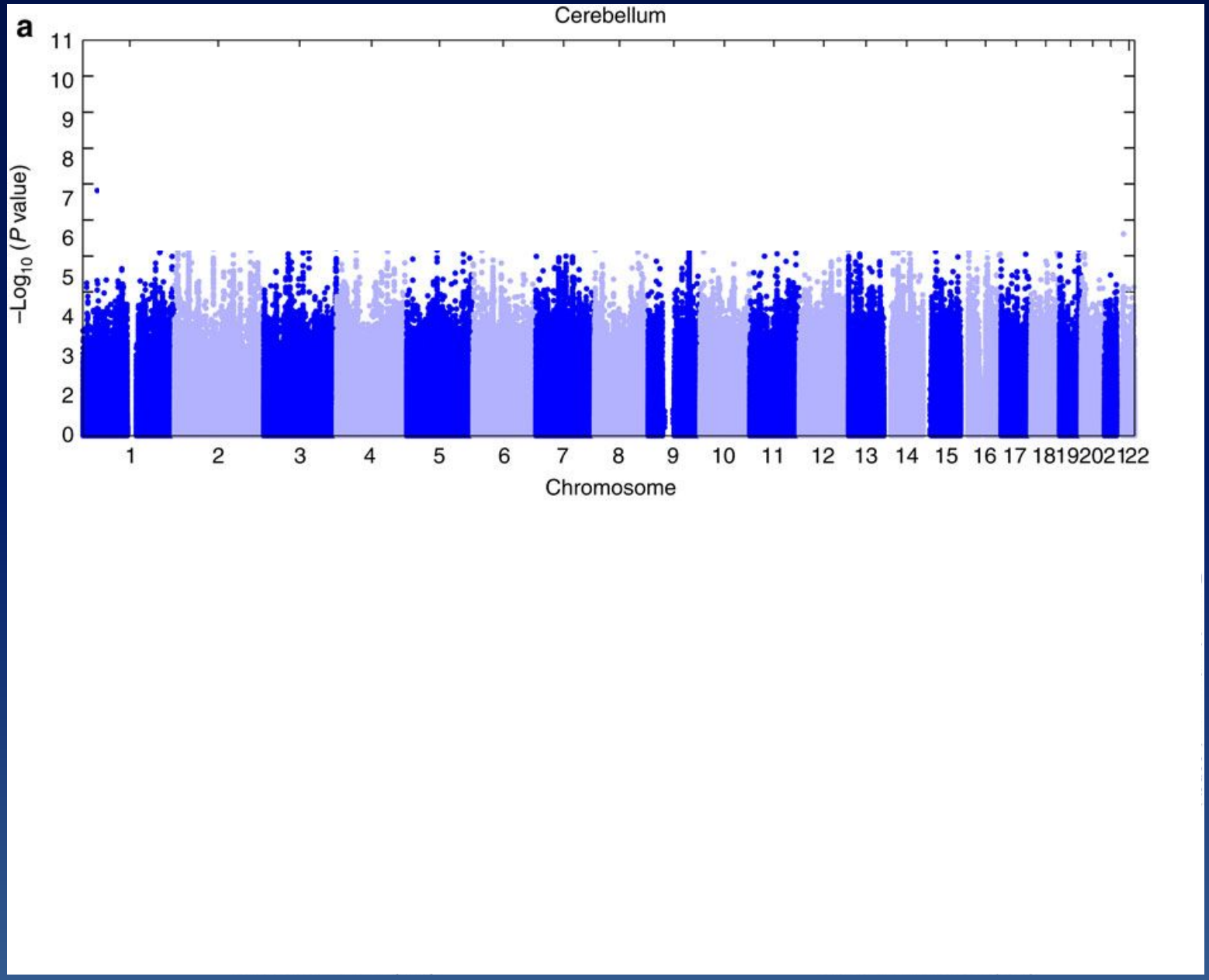
Study of the Nature of Epigenetic Ageing

Retarded by Rapamycin!!!

Study of Accelerated Epigenetic Ageing

GWAS:
Association between SNPs
and
Accelerated Ageing

Genome-wide search for SNPs that correlate with accelerated ageing in cerebellum

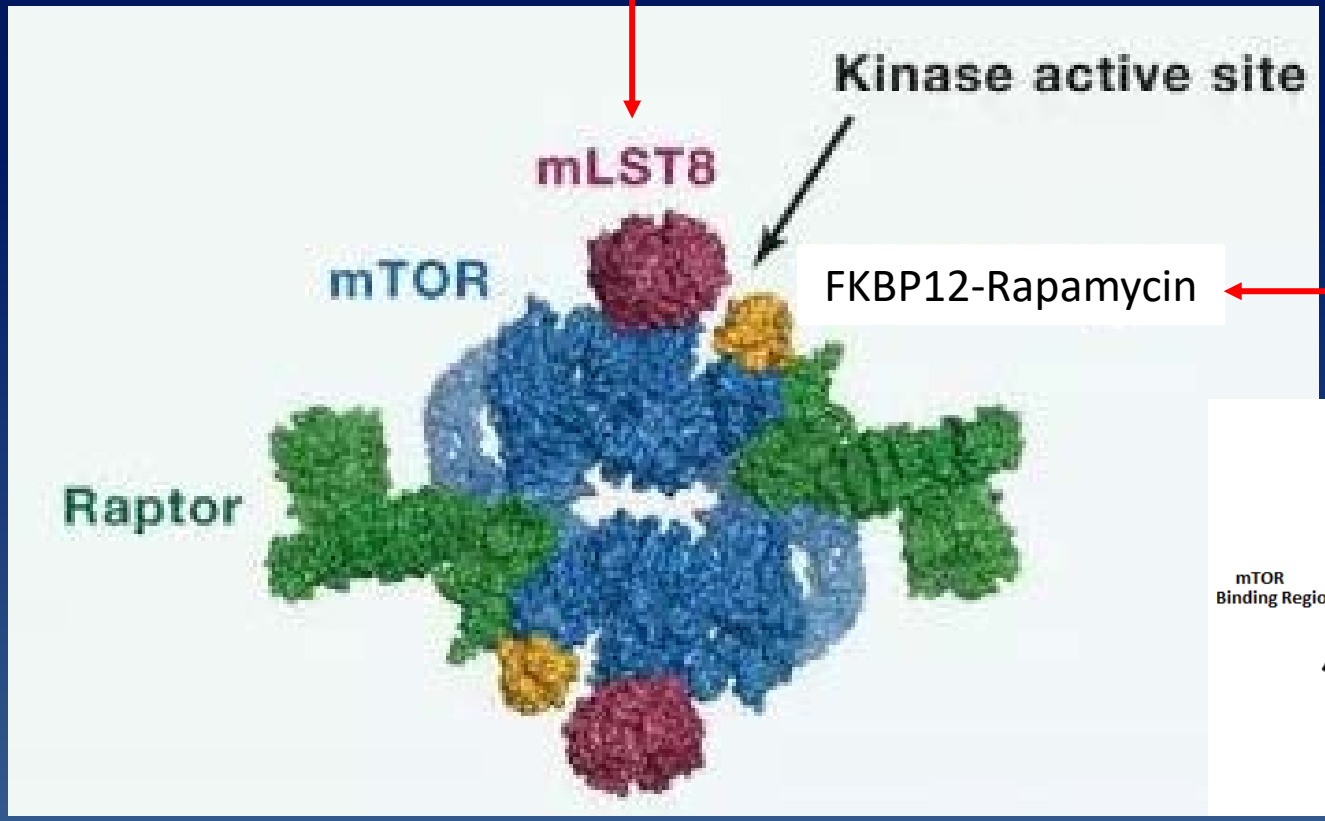


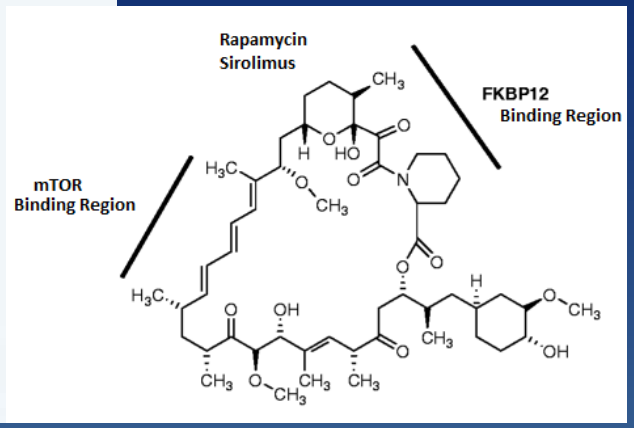
Human-derived
Epigenetic clock

Age-acceleration *in vivo* SNPs
from GWAS

Age-deceleration
effect from *In vitro*
Ageing assay

FKBP12-Rapamycin

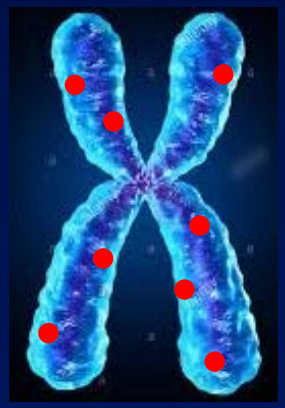




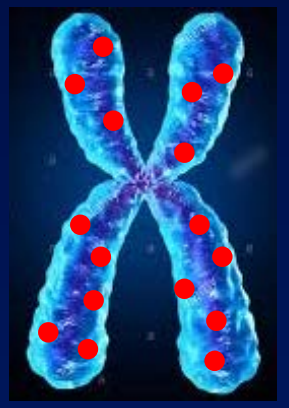
Young

Middle Age

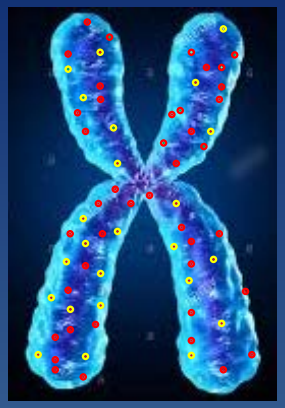
Old Age



Damaged DNA Accumulation



Shortening of Chromosome Ends



Changes in DNA Methylation