DNA methylation based biomarkers of aging and lifecourse

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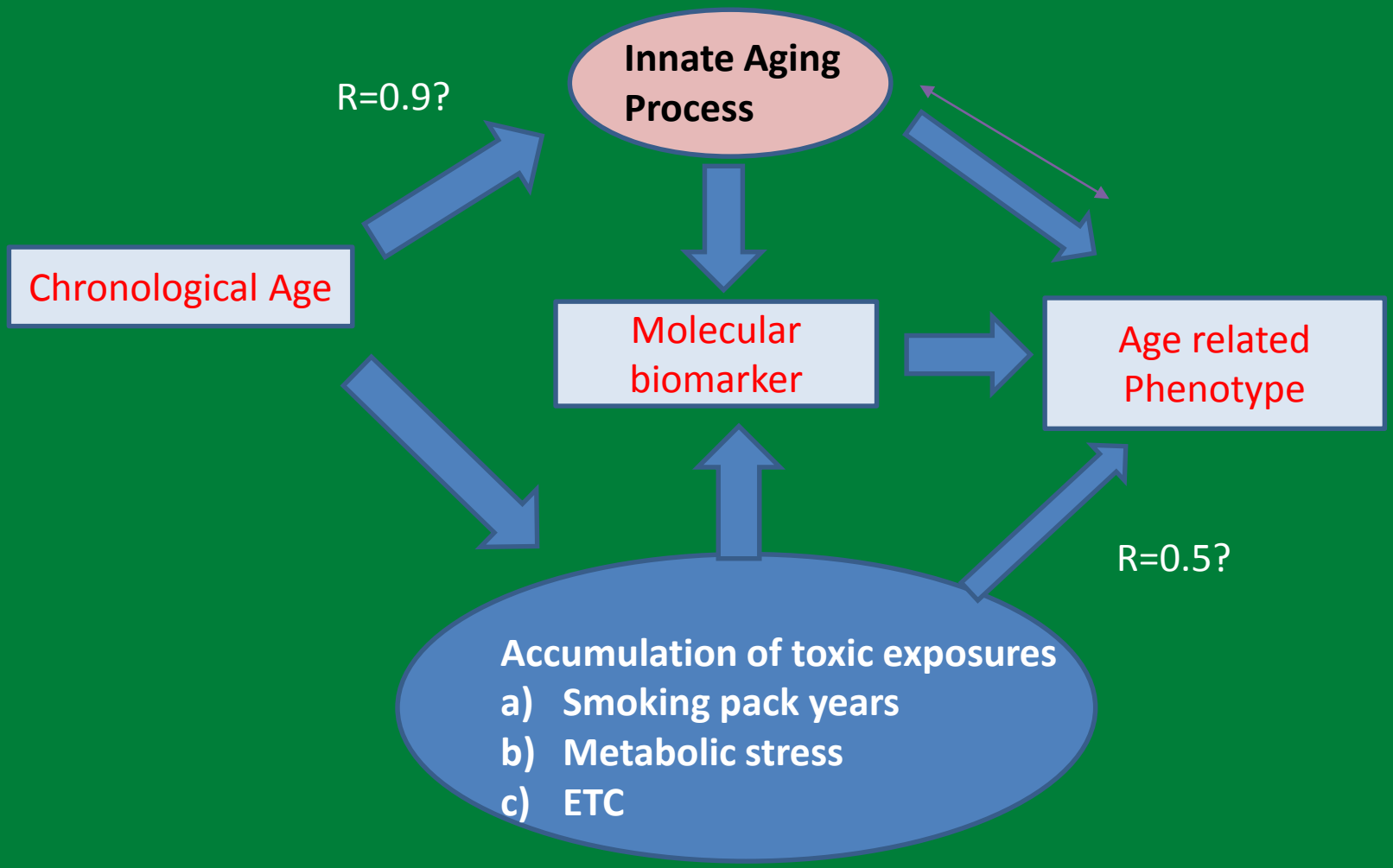
Credit: Nat Rev Genetics

What is the biological age of this 71 year old man?

- He is probably young according to most clinical biomarkers such as body mass index, grip strengths, blood pressure.
- He is probably old according to molecular biomarkers such as the epigenetic clock
- Message: Molecular markers will not be misled

Dion Friedland

Conceptual framework:
Molecular biomarkers relate to innate aging processes, confounders, and clinical phenotypes.



DNA methylation: epigenetic modification of DNA

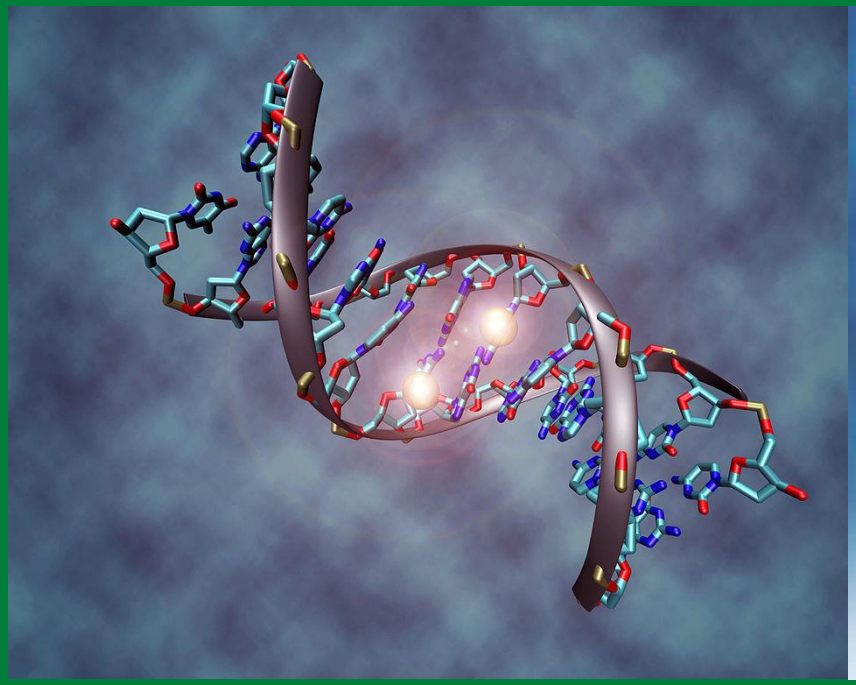
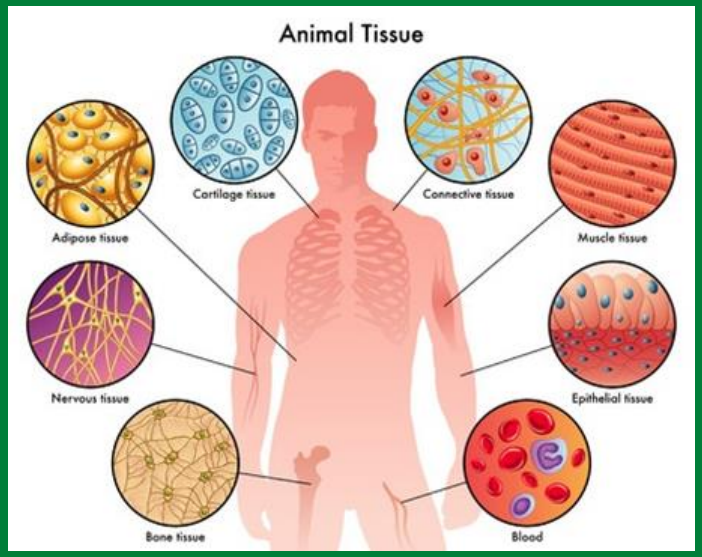
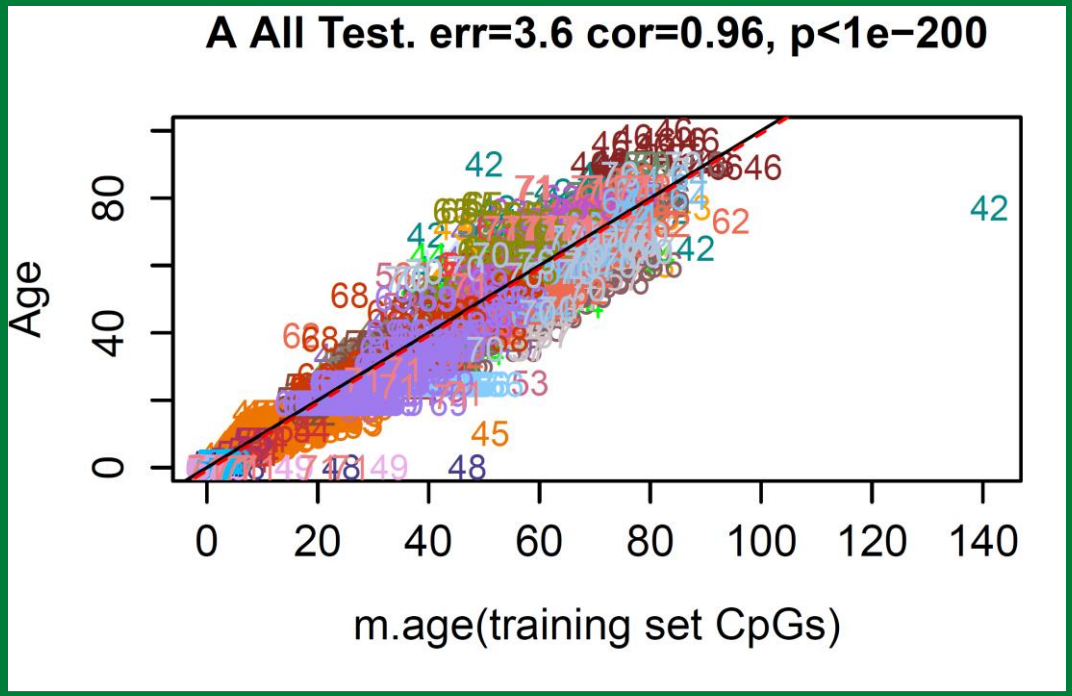


Illustration of a DNA molecule that is methylated at the two center cytosines.
DNA methylation plays an important role for epigenetic gene regulation
in development and disease.

The human pan-tissue DNAm age estimator is the most accurate molecular biomarker of age across tissues. Hence the name “epigenetic clock”.





Pros and cons of DNAm based biomarkers

- Pros
  - Exceptional correlation with age
  - Apply to all sources of DNA, all tissues and cell types
  - Apply to all vertebrates.
  - Predictive of a host of health outcomes
  - Relate to most age related conditions including healthspan
  - Lend themselves for in vivo studies
  - Are already being used in human clinical trials
  - Highly robust and relatively cheap assay (250 US dollars)
- Cons
  - In general, weak correlation with transcriptomic data
  - Incomplete understanding of underlying biology

Message: It is imperative that we conduct mechanistic studies for understanding the underlying biology.

  - Genetic studies in humans and model organisms
  - Knock out models
  - Human longitudinal cohort studies for causal modeling

Status of DNAm based biomarkers of aging

2013

- Development of epigenetic clock: very accurate age estimator that applies to all tissues and cell types

2015

Epigenetic age acceleration

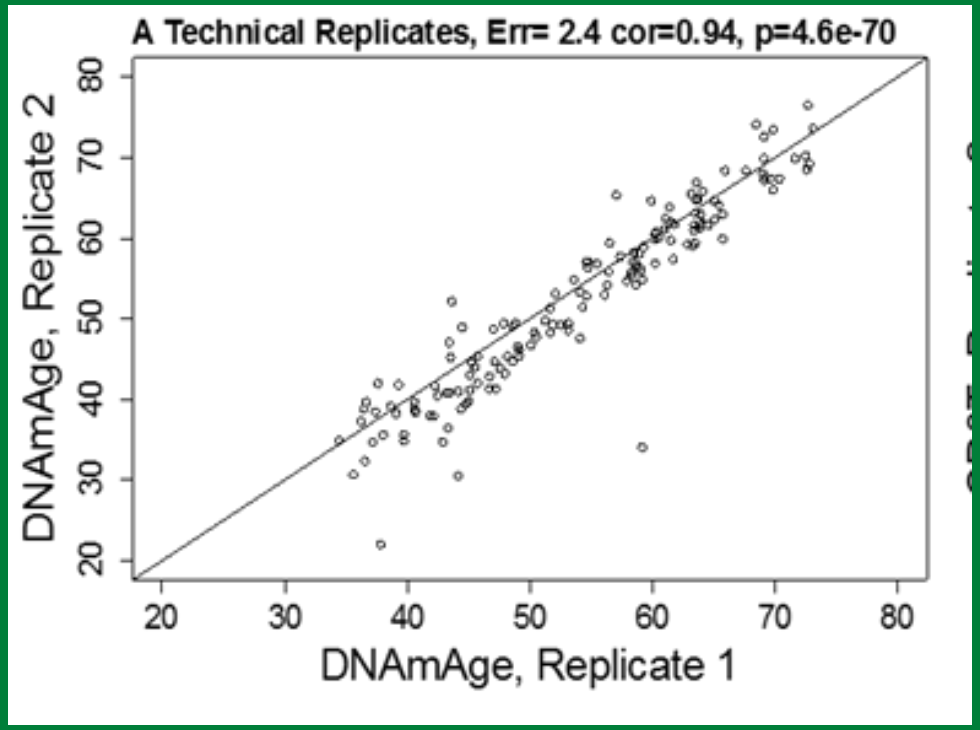
- predicts mortality
- Cognitive functioning
- Obesity
- Down syndrome

2018

- Novel clocks strongly predict healthspan
- Custom clocks for ex vivo studies
- Mouse clocks
- Biological insights

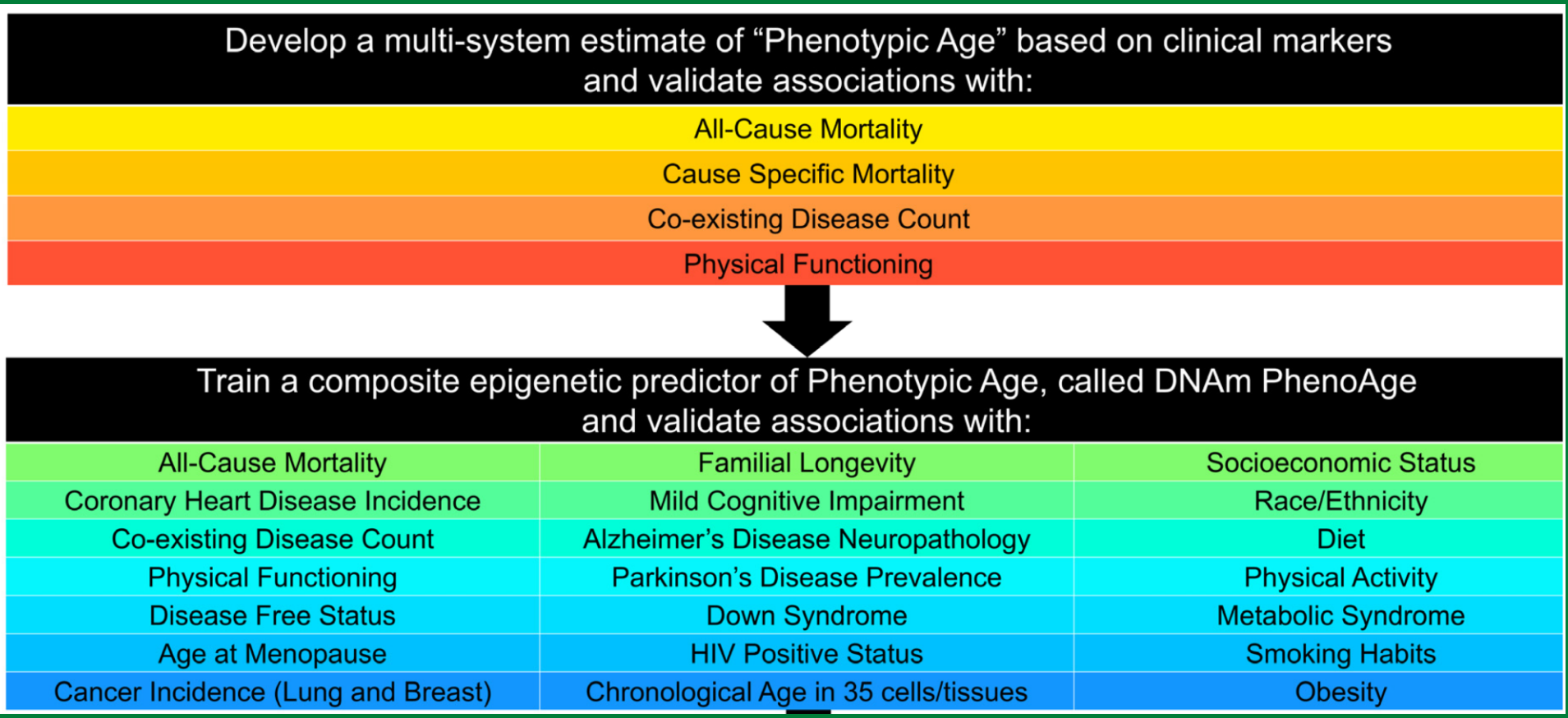
2021 (?) Universal epigenetic clocks that applies to most mammals

Reproducibility of DNAm age estimates based on technical replicates



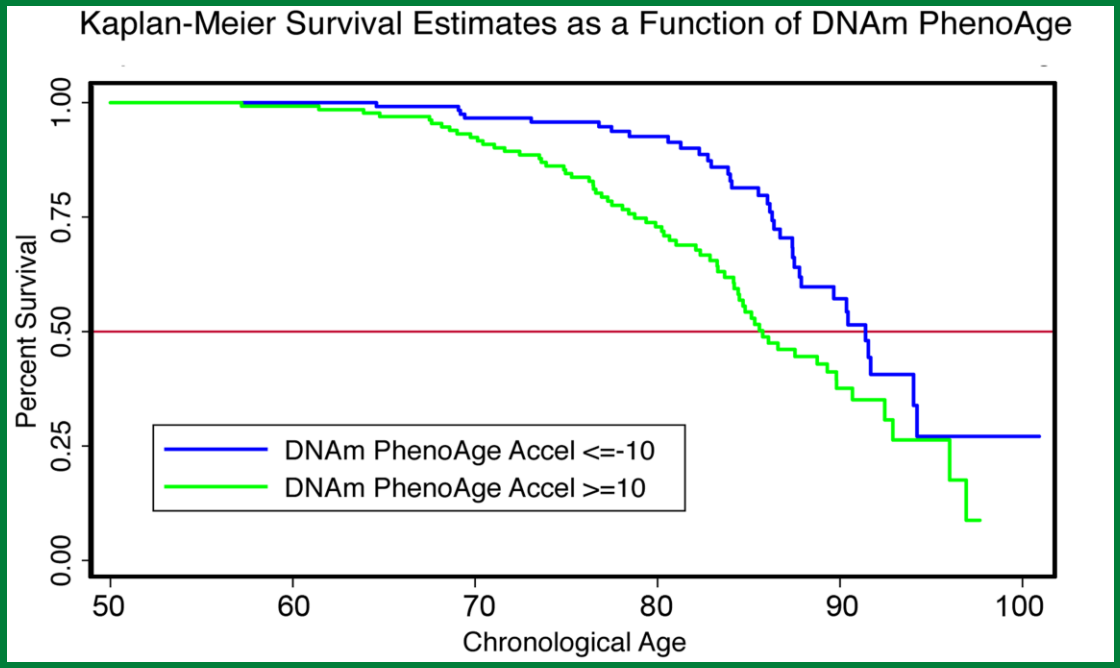
Two aliquots of blood (collected at the same time) were sent to two separate core facilities for generating Illumina EPIC array data.

Novel predictor of lifespan, DNAm PhenoAge, based on 513 CpGs.



Morgan E Levine et al 2018 Aging

Prediction of life span with DNAm PhenoAge



Validate Associations with:		
All-Cause Mortality	Familial Longevity	Socioeconomic Status
Coronary Heart Disease Risk	Dementia	Race/ethnicity
Coexisting Disease Count	Down Syndrome	Diet
Physical Functioning	Parkinson's Disease	Physical activity
Disease Free Status	HIV positive	Metabolic Syndrome
Age at Menopause	Chronological age in 35 tissues/cells	Smoking Status
Cancer (Lung, Breast)	Neuropathology (Brain DNAm)	Obesity (Liver DNAm)

Morbidity Validation for DNAm PhenoAge

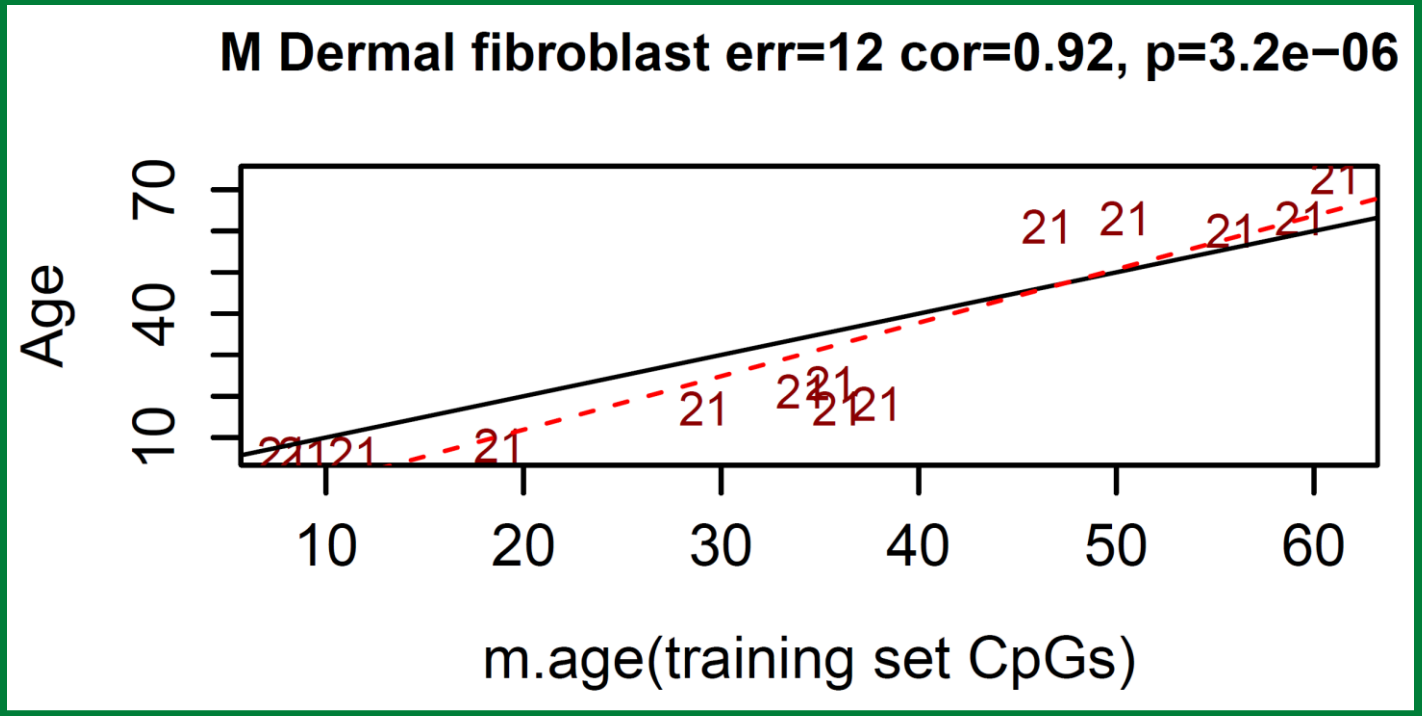
- Higher DNAm PhenoAge is associated with
  - Incident coronary heard disease (P-value=2.43E-10)
  - a decrease in likelihood of being disease-free (P=1.06E-7),
  - a person's number of coexisting morbidities (P=4.6E-15),
  - an increase in physical functioning problems (P=2.1E-13).

Epigenetic clocks are useful for identifying and validating anti-aging targets

- Reality check: epigenetic clocks do not stand out in terms of lifespan prediction.
  - Many alternatives: blood pressure, smoking, frailty indices, lipid levels, glucose levels
- Advantage of epigenetic clocks
  - Clocks relate to at least one root cause of aging
  - proximal to an innate aging process
  - they can be applied to cells in a dish (in vitro studies)
  - DNAm age can be used as phenotype in genetic studies

Novel epigenetic clock for skin and blood cells

Motivation: the original pan-tissue estimator led to a large errors in fibroblasts



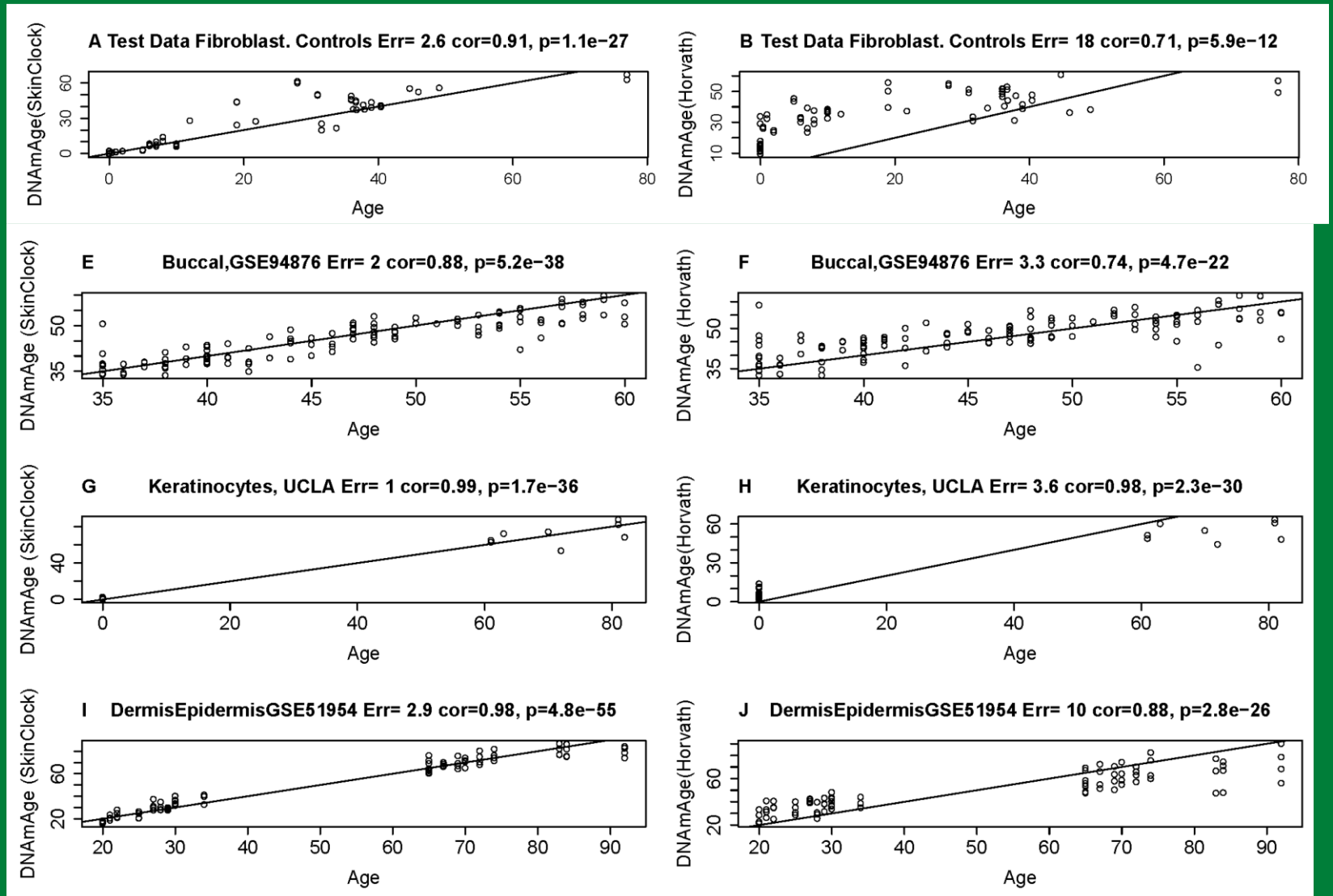
Highly accurate skin & blood clock

- Novel and highly robust DNAm age estimator (based on 391 CpGs) for human fibroblasts, keratinocytes, buccal cells, endothelial cells, lymphoblastoid cells, skin, blood, and saliva samples.
- High age correlations can also be observed in sorted neurons, glia, brain, liver, and even bone samples.

Comparison of DNAm age estimators

Skin & Blood Clock

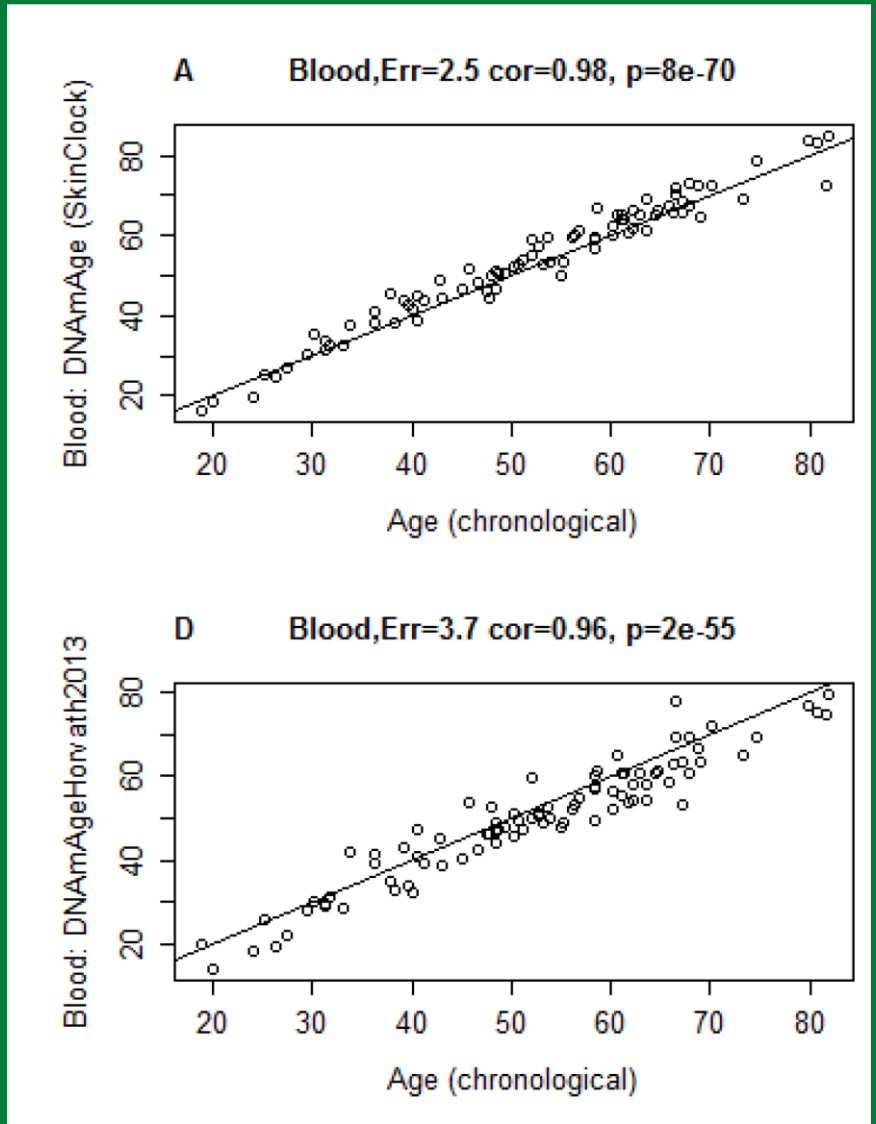
Original Pan Tissue Clock (Horvath 2013)



The skin & blood clock has superior accuracy in blood and saliva samples

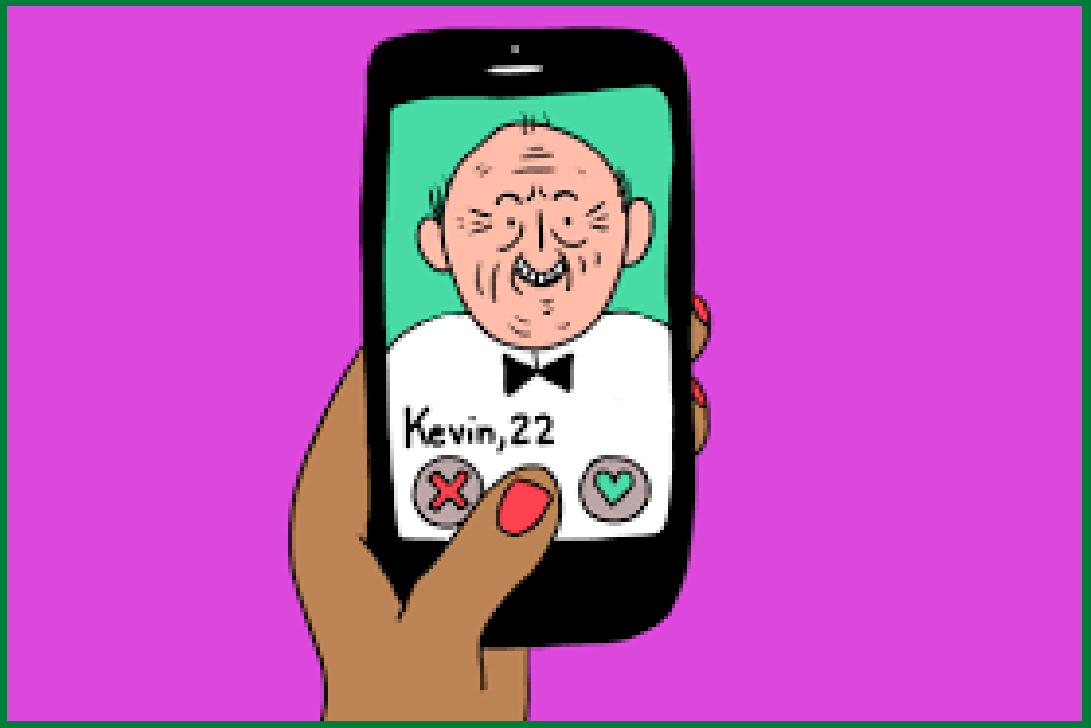
Skin & blood clock:
Median error=2.5 years

Original pan tissue clock (Horvath 2013)
Median error=3.7 years



Forensic applications

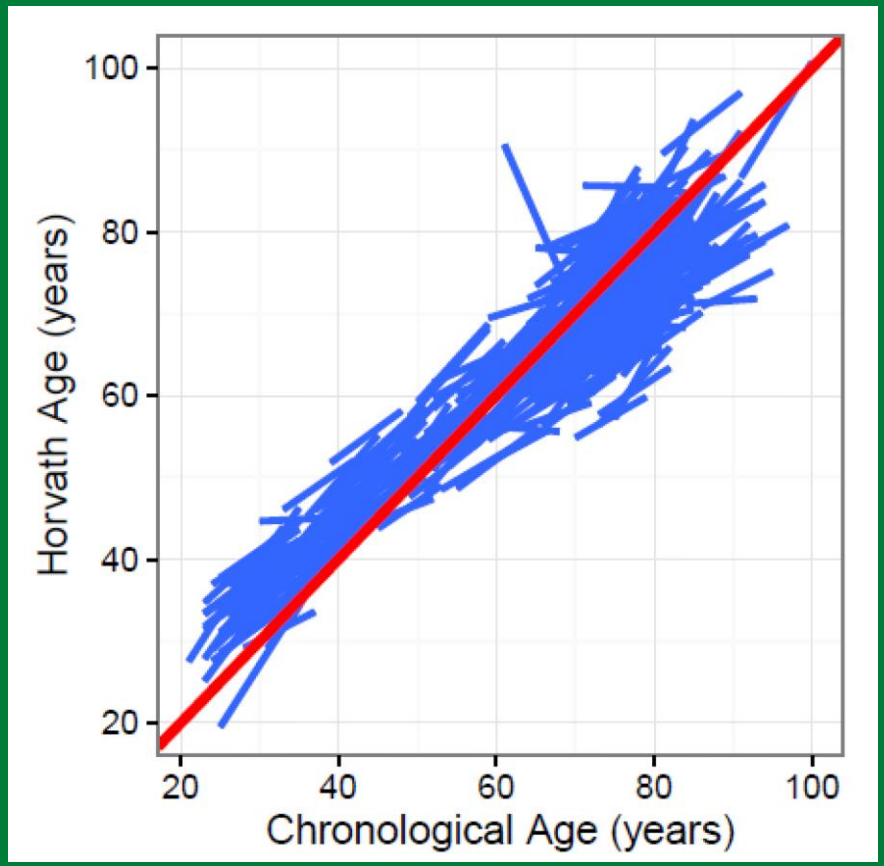
- The superior accuracy of the skin & blood clock in buccal cells, saliva cells, and blood cells entails that it lends itself for forensic applications.



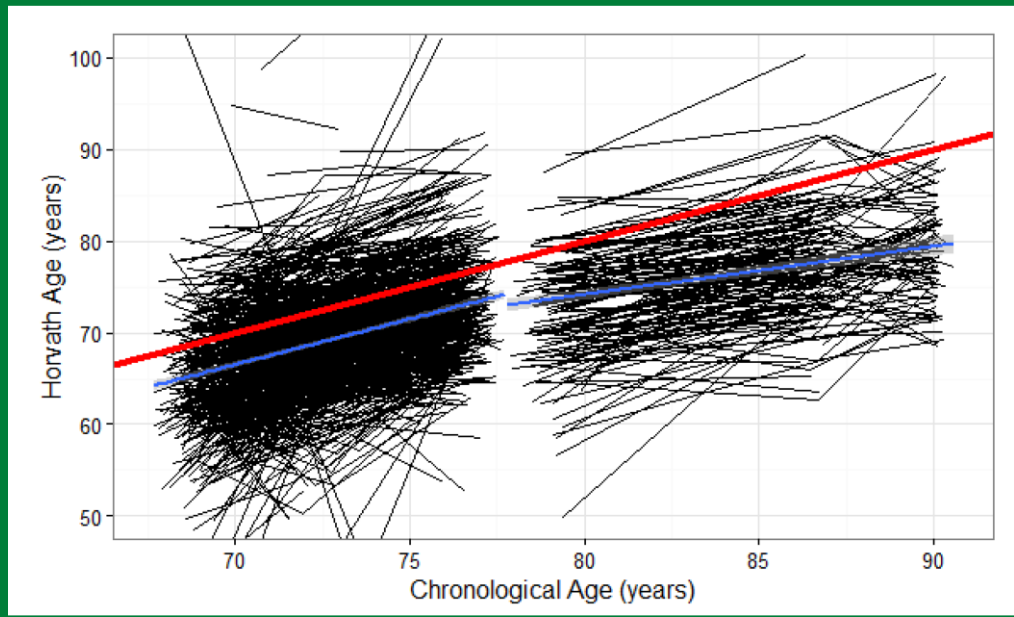
Tracking the epigenetic clock across the human life-course

R. Marioni, S. Hagg (2018)

InCHIANTI cohort



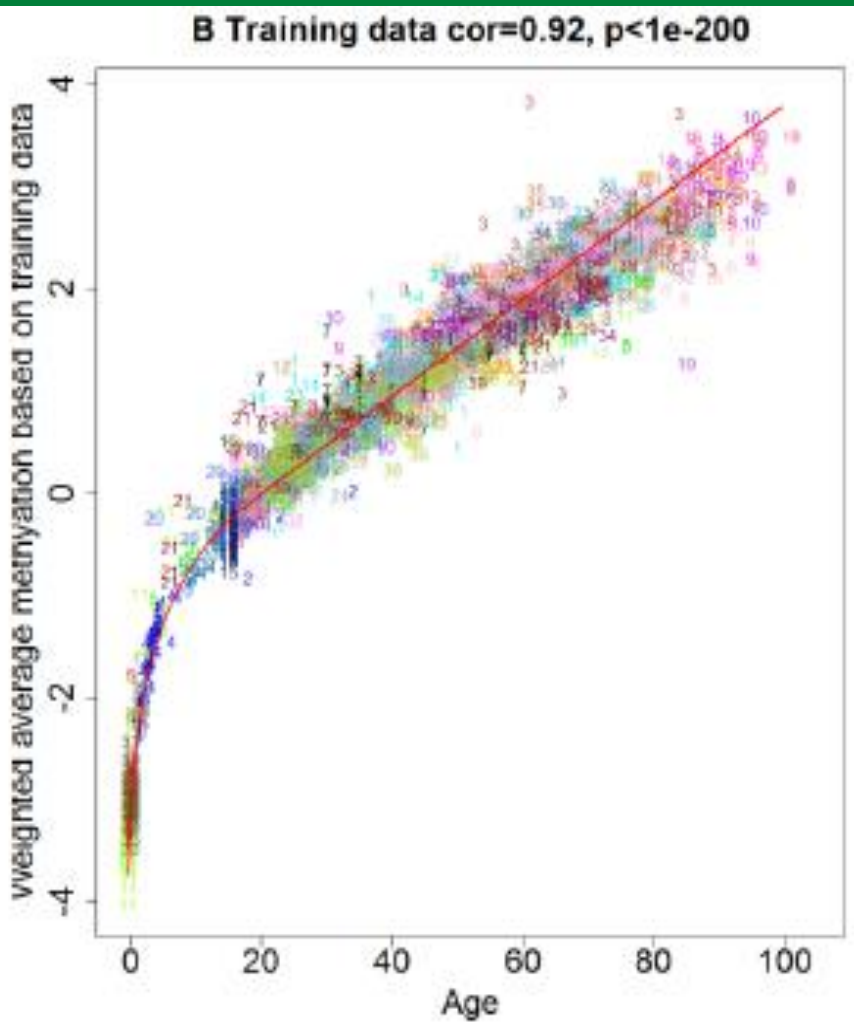
Lothian Birth Cohorts

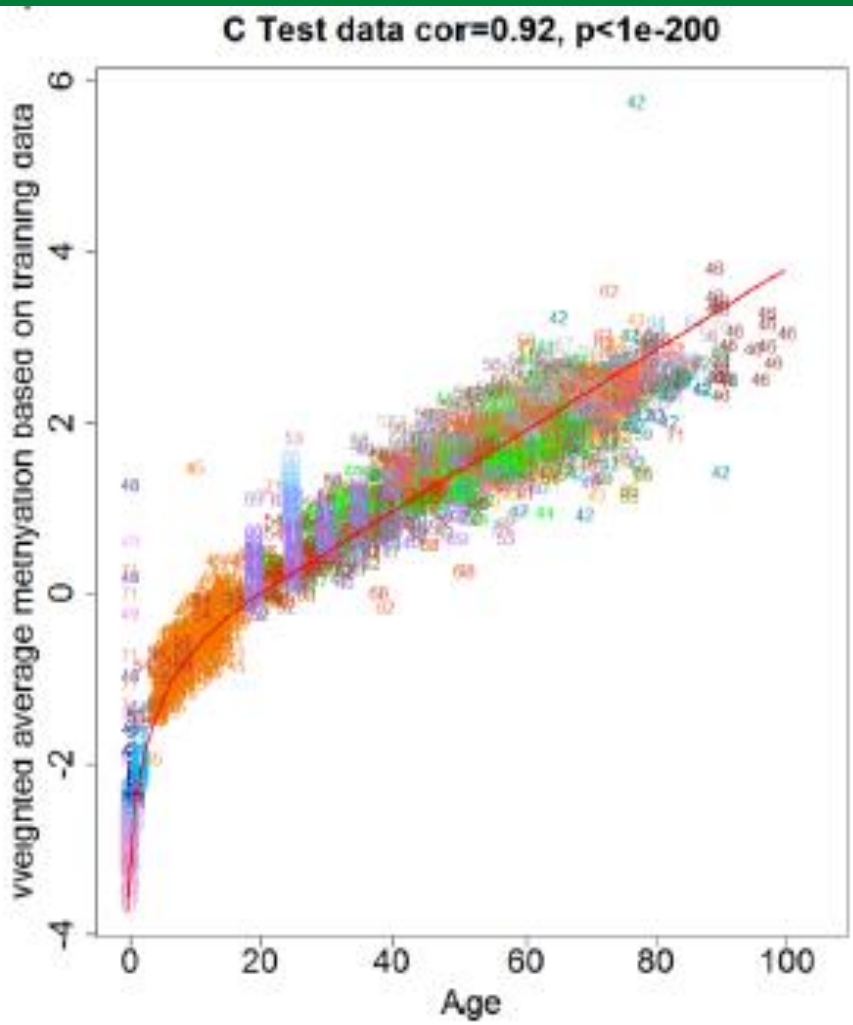


How do you call a biomarker of aging that applies to the entire life course?

"Lebensuhr"=Life Clock

Multi-tissue DNAm age estimator (353 CpGs) applies to all tissues/cell types across the entire life course





Future goal: epigenetic age estimator that applies to all mammals

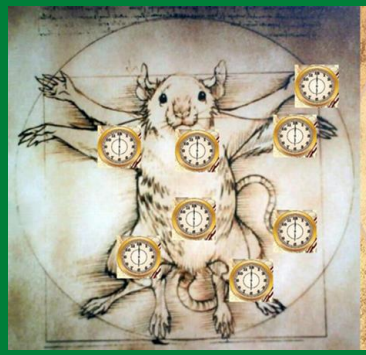
Step 1: Develop a mammalian DNA methylation chip for CpGs in highly conserved DNA sequences

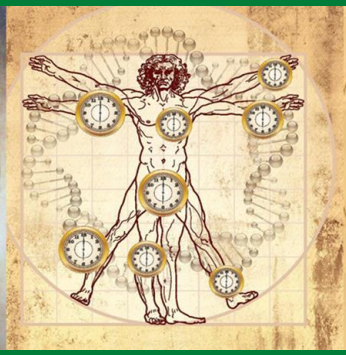
Status: Available August 2018

Step 2: Develop an age estimator for each of 50 species.

Step 3: Develop a universal cross-species age estimator

Max lifespan	3 years	122	40





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Challenge: Understand the molecular causes and consequences of epigenetic clocks

- Methylation clocks have different biological interpretations
  - Some clocks (e.g. DNAm PhenoAge) capture aspects of immunosenescence, inflammation, age related changes in cell composition, and relate weakly to telomere length
  - Other clocks relate more closely to stem cell properties (multi-tissue clock)

Conclusions

- Several epigenetic clocks
- As a class, epigenetic clocks satisfy all criteria for biomarkers.
- DNAm age applies to cell culture systems
- DNAm age will not replace standard clinical measures (blood pressure, lipid levels, etc)
- DNA methylation levels should be measured in many anti-aging clinical trials
  - Judicious choice of source of DNA: blood, saliva, buccal, urine, fat tissue

Acknowledgement

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- Down syndrome, centenarians: Claudio Franceschi, Paolo Garagnani
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NIH National Institute on Aging

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