Epigenetics of aging and frailty in twin studies

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Part I: Epigenetics of aging using twins

1. Modeling the overall aging-associated changes (epigenetic drift) in twins

→ similarities/divergence in twins with age will shed light to familial and environmental influences

2. Twin models for the epigenetic clocks: Horvath and Levine clocks

→ gaining insights to the variance components (A, D, C, E)

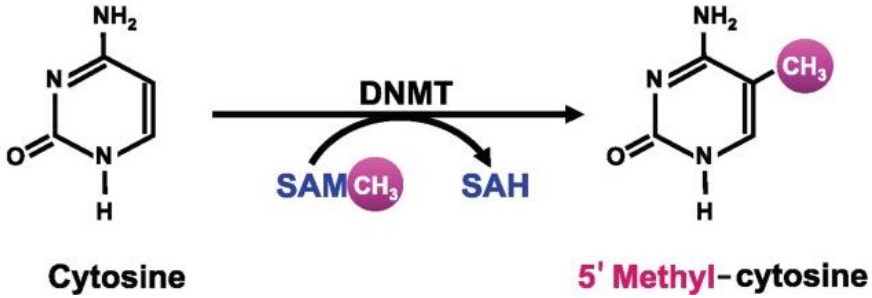
Part II: Aging-associated frailty syndrome

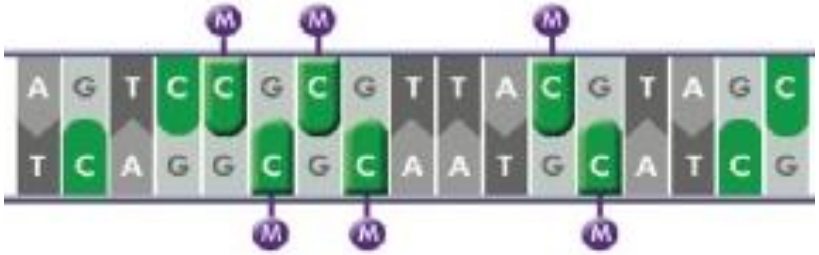
- *a shared frailty model* (between-within) in twins to account for unmeasured familial confounding in relation to frailty syndrome and mortality

- twin models (using latent growth curves) and epigenetics of frailty

Types of epigenetic modifications

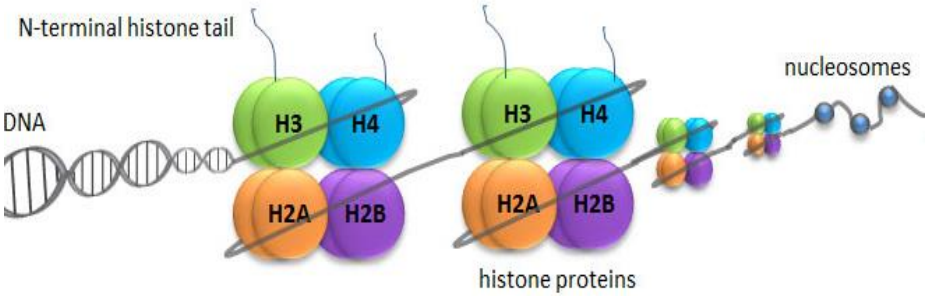
- DNA methylation → **CpG dinucleotide=CpG site**



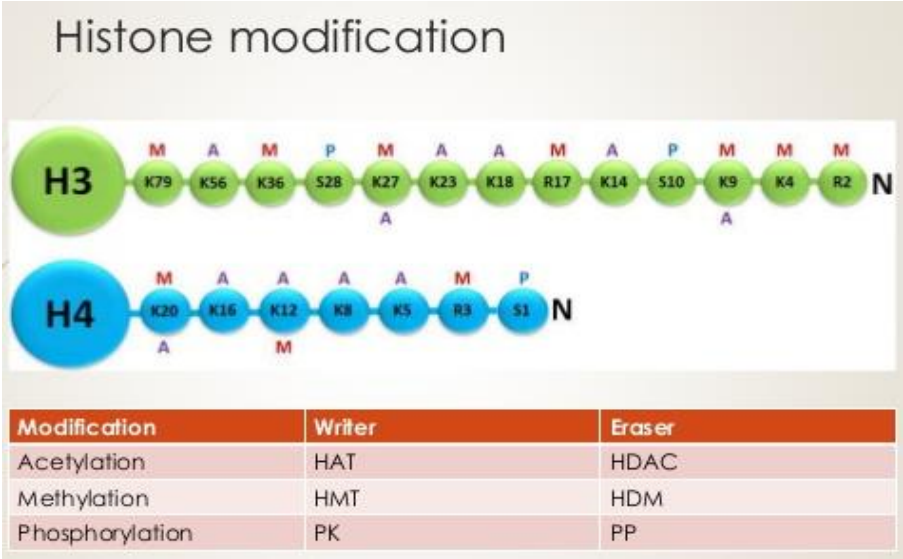


Alcohol Res. 2013; 35(1): 6–16.

- Histone modifications



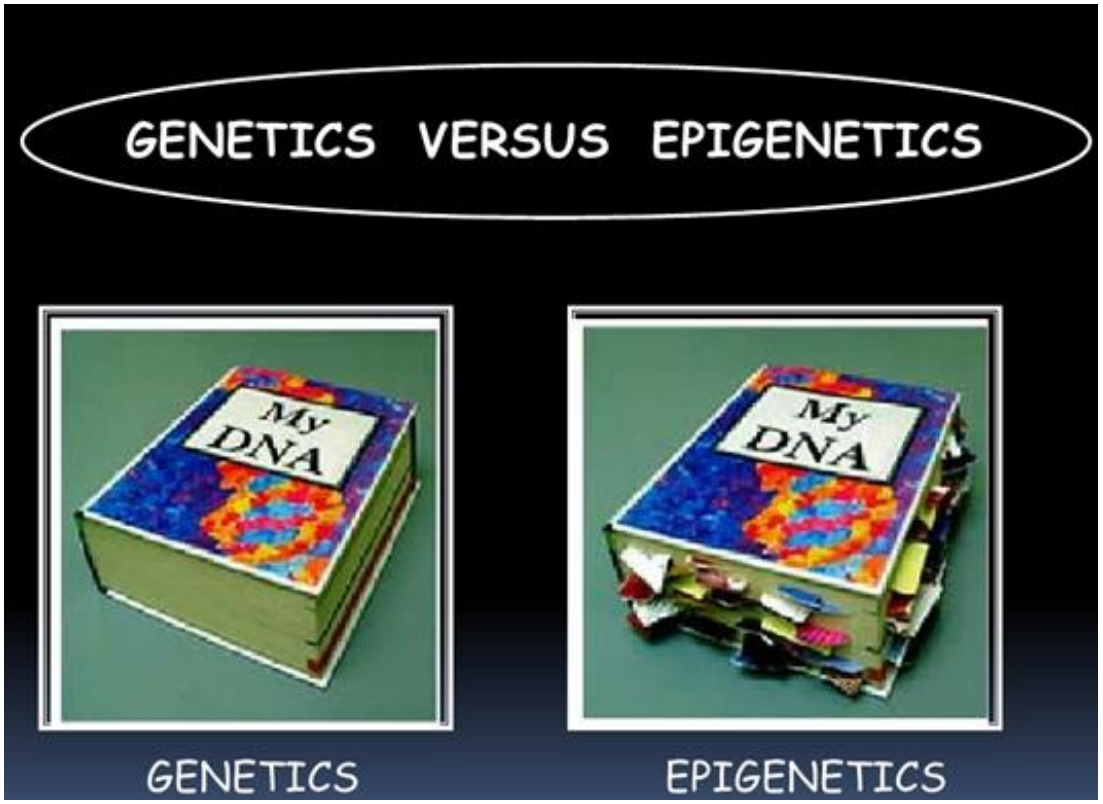
http://www.whatisepigenetics.com/histone-modifications/



Modification	Writer	Eraser
Acetylation	HAT	HDAC
Methylation	HMT	HDM
Phosphorylation	PK	PP

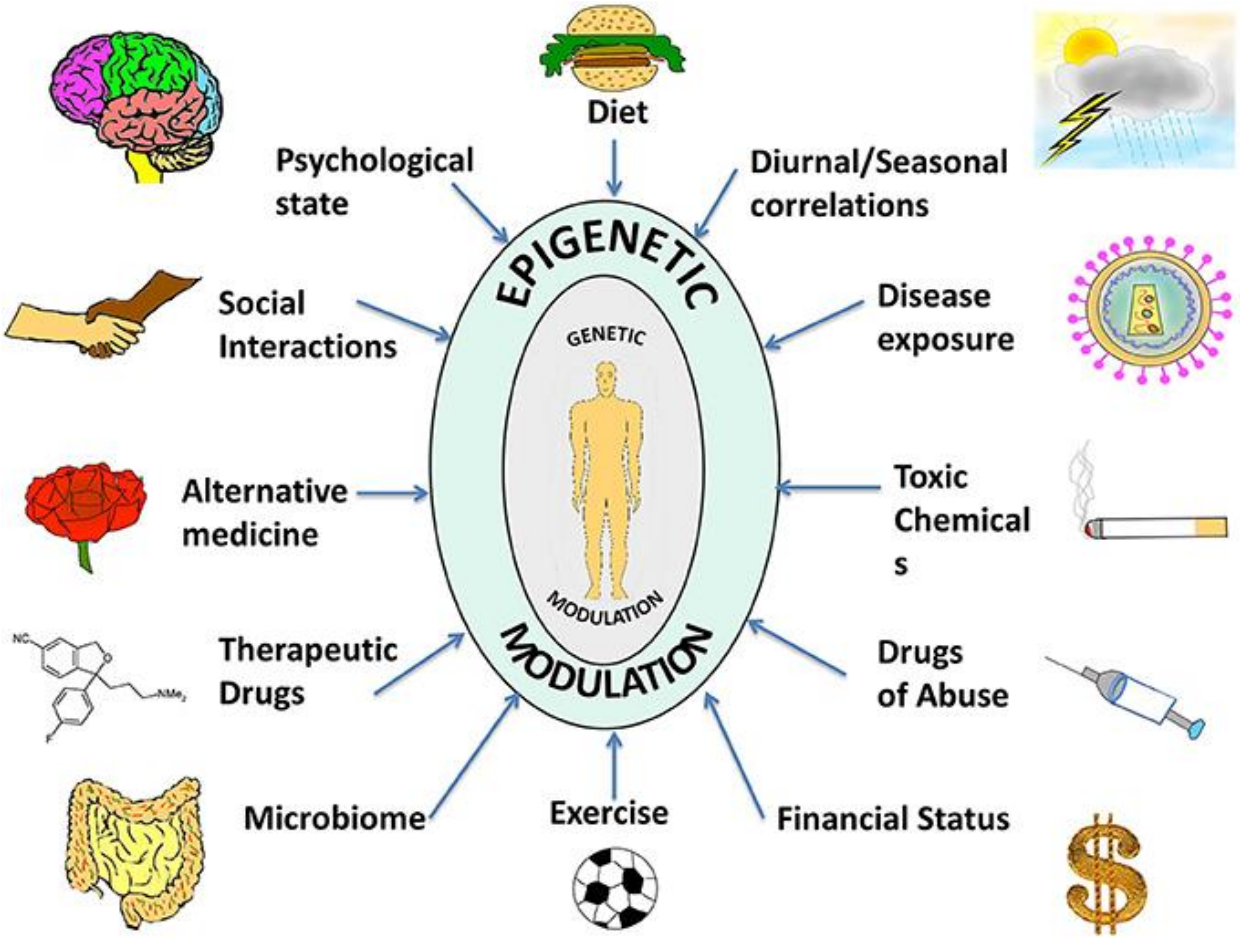
- Small RNA species

EPIGENETICS = mechanisms that have an effect on gene expression that are not based on the nucleotide sequence and can be inherited by cell division and even from parent to progeny



http://www.slideshare.net/SushmaMarla/dna-methylation

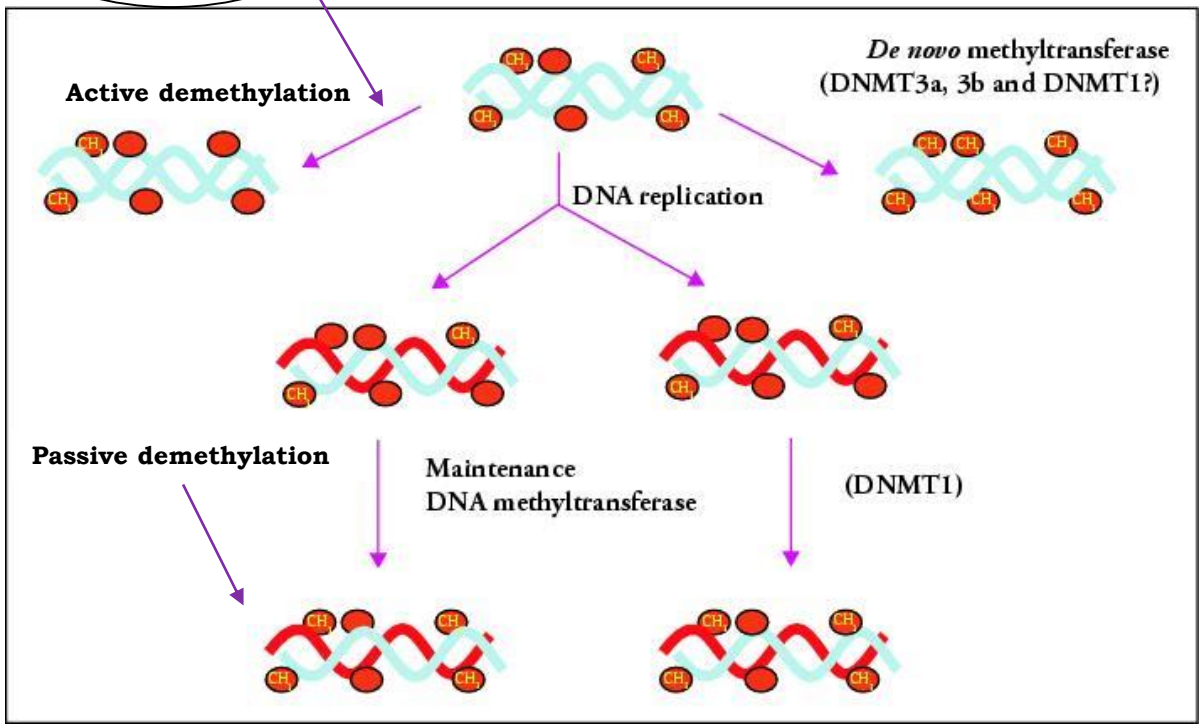
Modifiable and reversible patterns - but to what extent?



DNA methylation - how does it work?

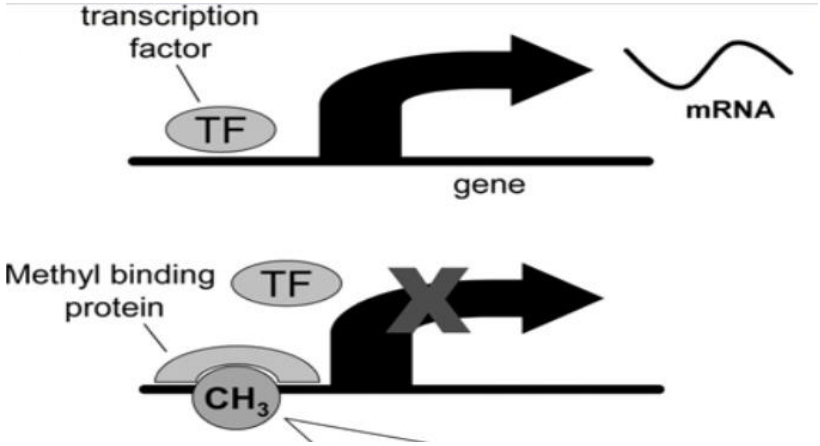
-oxidation
-TET1, TET2, TET3
-deamination and base excision repair
-TDG, AID, APOBEC

- active processes, excl. passive demethylation



DNA methylation - what does it do?

- direct and indirect regulation of gene expression



- cell differentiation, cell-type specific expression patterns
- repression of viral segments and repetitive elements, e.g. transposons
- female X chromosome inactivation
- imprinting of genes
- chromatin stabilization, interactions with nucleosomes

DNA methylation - facts

- More than 28 million CpG sites in the human genome
- 80% are methylated

- Form CpG islands (CGI) that have a high CG content
- 25 000 CGIs in the human genome, 1kb in length
- Usually locate within or close to gene promoters
- CGIs co-localize with promoters of **all constitutively** expressed genes and with 40% of all promoters

EWAS (epigenome-wide association study) arrays

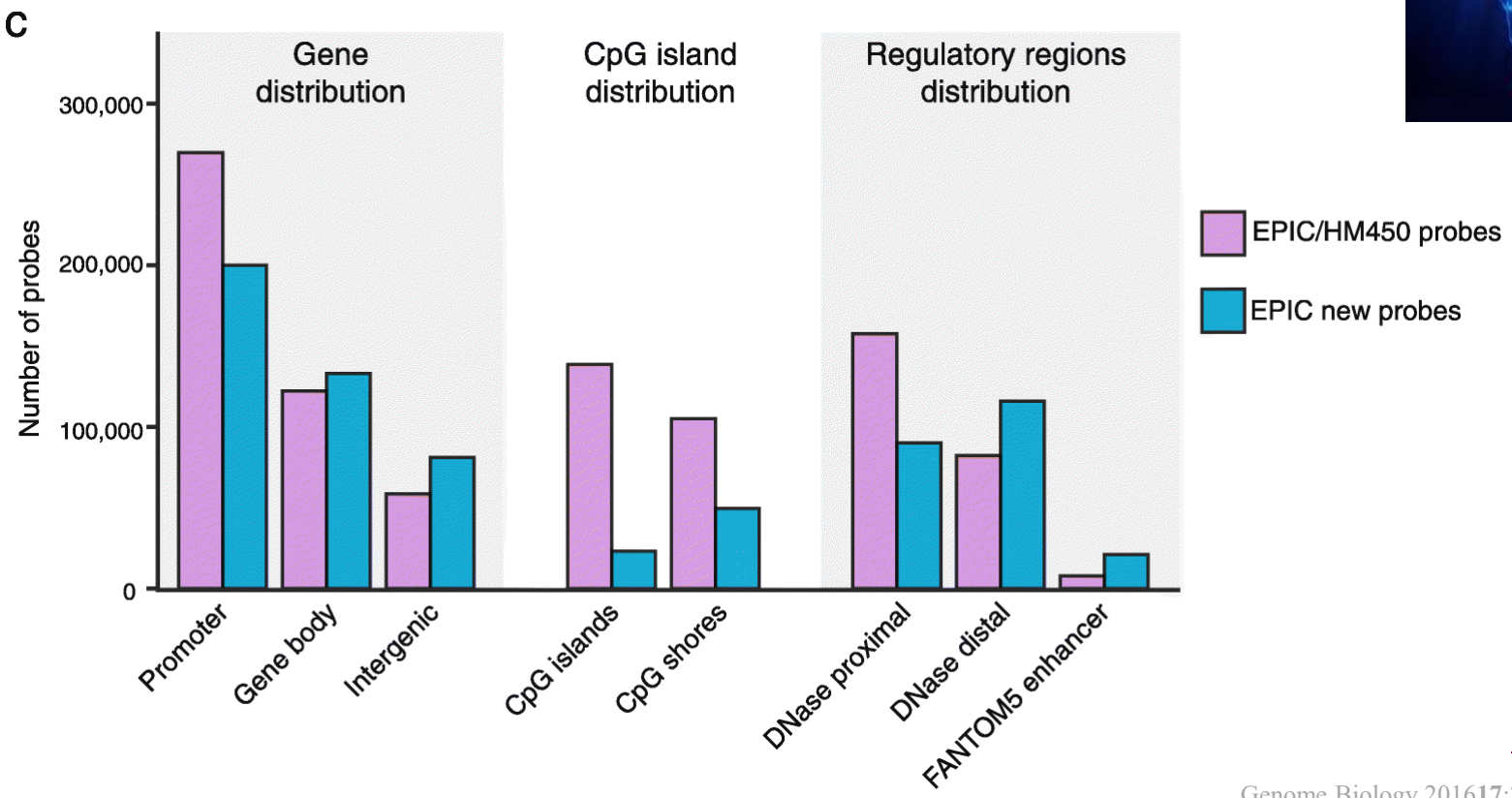
- Illumina 27k array
- Illumina 450k array
- **Illumina EPIC array (”850k array”)**

- whole-genome sequencing

From DNA to EWAS – what does it take?

- The Illumina Infinium bead arrays:

27k (27k promoter sites), 450k (~470 000 sites) and the EPIC array (~850 000 sites, incl. 95% of the 450k content)



Genome Biology 2016**17**:208

http://blog.oup.com/2012/03/epidemiology-epigenetics-journal/

EWAS = epigenome-wide association study

Many diseases have an ”epigenetic signature”

- cancer
- autoimmune diseases, inflammatory diseases e.g. asthma
- aging

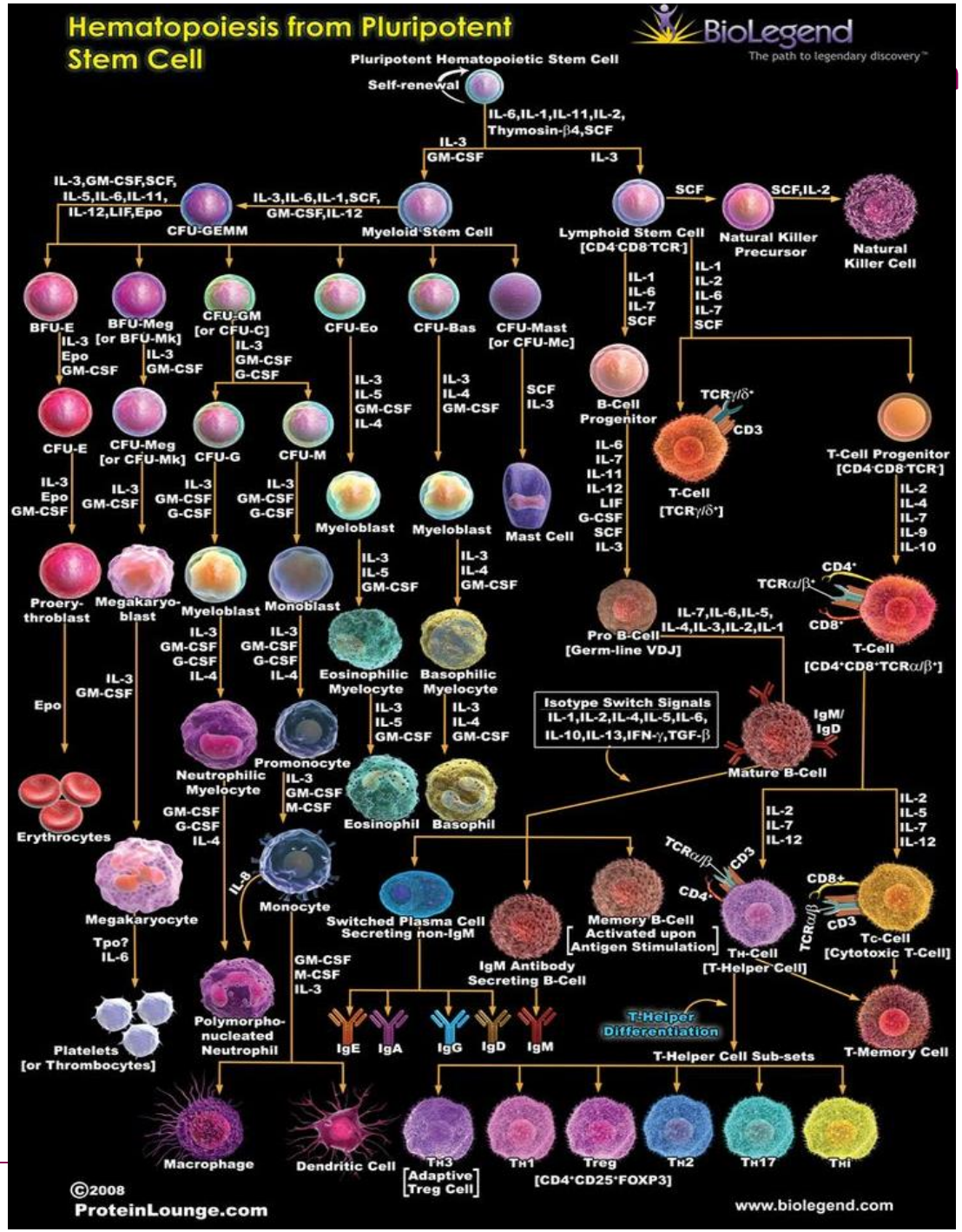
When using blood, correct for cell types!!

The Houseman algorithm: estimateCellCounts
CD8T, CD4T, NK, Bcell, Mono, Gran

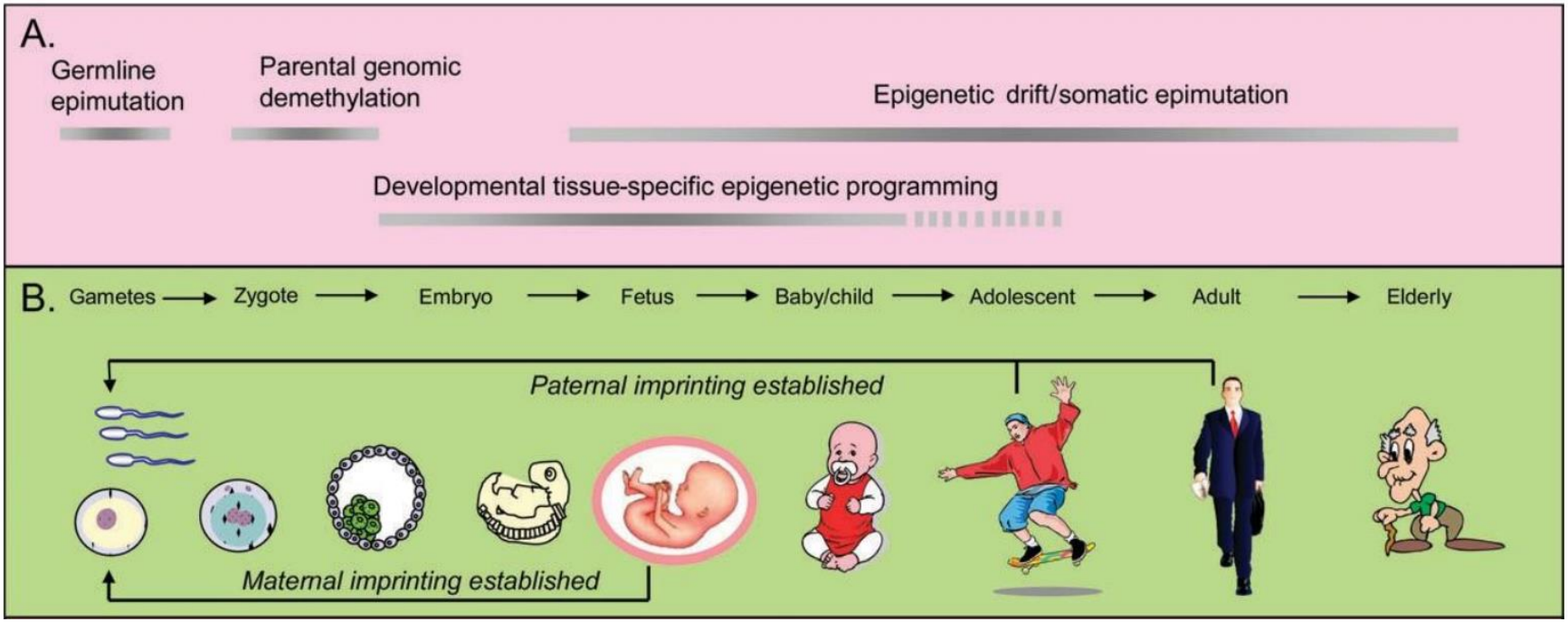
FACS

Also new methods available

QUIZ: why is adjustment for cell type proportions of utmost importance?



Aging & methylation

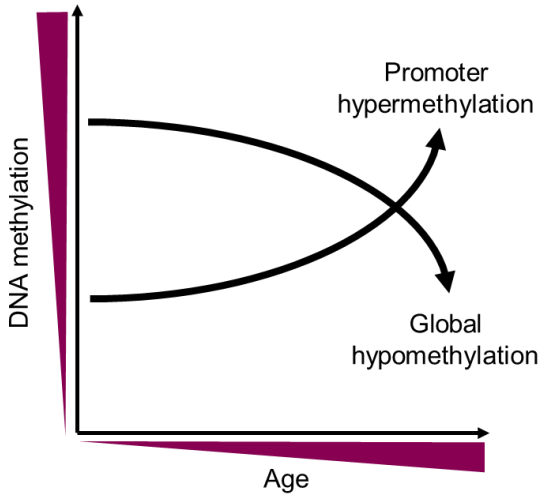


Aging Dis. 2014 Oct 1;5(5):346-55.

Different types of methylomic alterations with age

1. Aging-associated epigenetic drift

- profound changes throughout the epigenome: global hypomethylation and site-specific hypermethylation



Similarities
with cancer

- reproducible across different cohorts, “programmed pattern”?

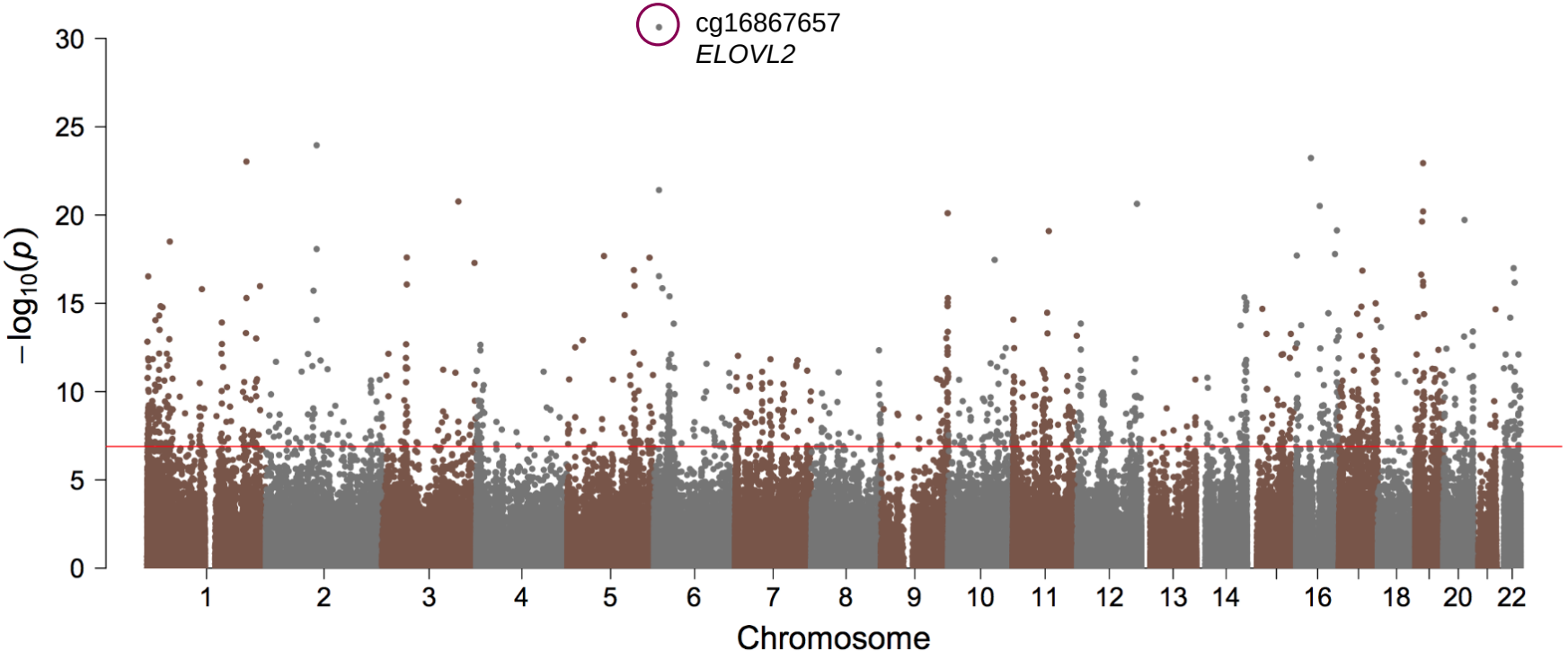
→ maladaptive or even adaptive? Purpose? Not predictive of mortality anyway

2. Epigenetic clock(s)

The Swedish Adoption/Twin Study of Aging (SATSA)

The epigenetic drift in SATSA

- Longitudinal epigenome-wide association study on age
- Fitted a mixed effect model with
  - → Fixed effects: Age, sex and zygosity
  - → Random effects: Twin pair
- Identified 1316 CpGs associated with age, with p-value < 1.3×10^{-7}



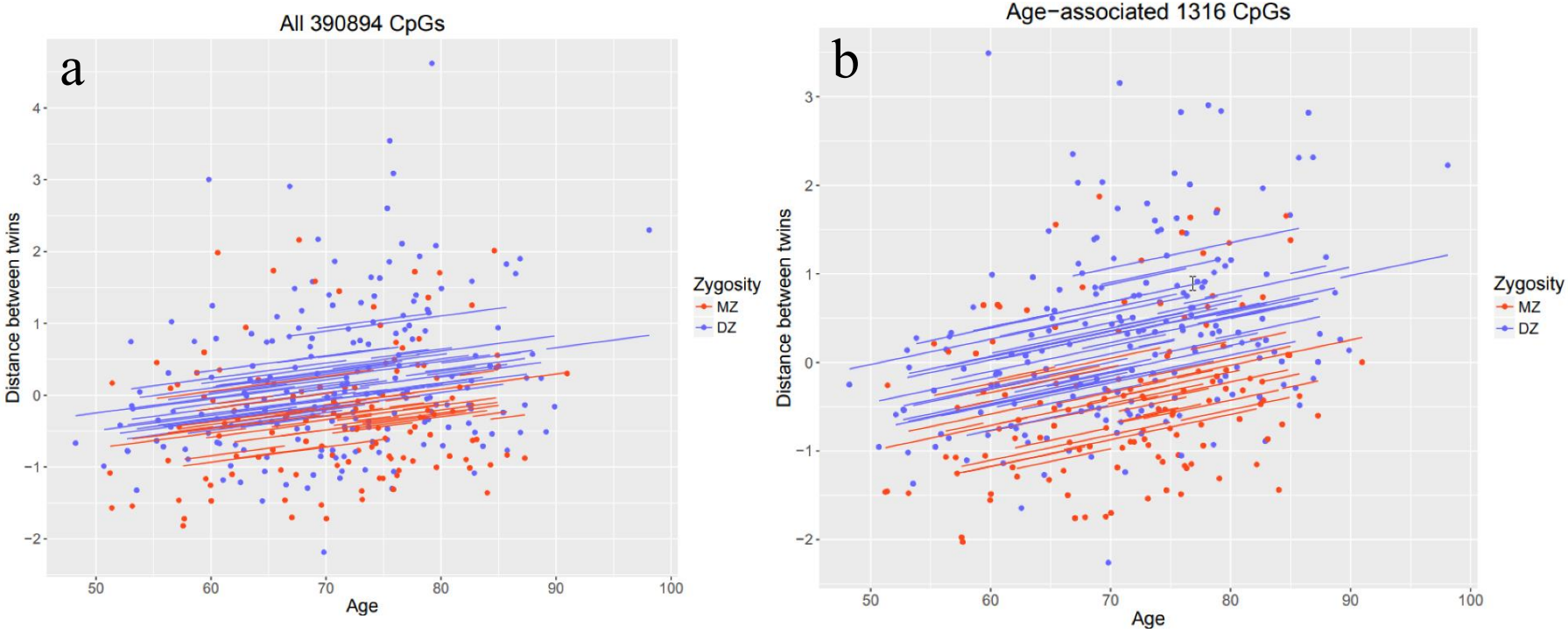
The Swedish Adoption/Twin Study of Aging (SATSA)

(Wang et al., bioRxiv https://doi.org/10.1101/226266)

Euclidean distances between twins, on average in all sites (a) and in age-associated sites (b)

- Differences within twin pairs increase with age
- DZs differ more than MZs
- Age-assoicated sites display a steeper slope than all CpGs

→ unique environmental factors come into play even more with age

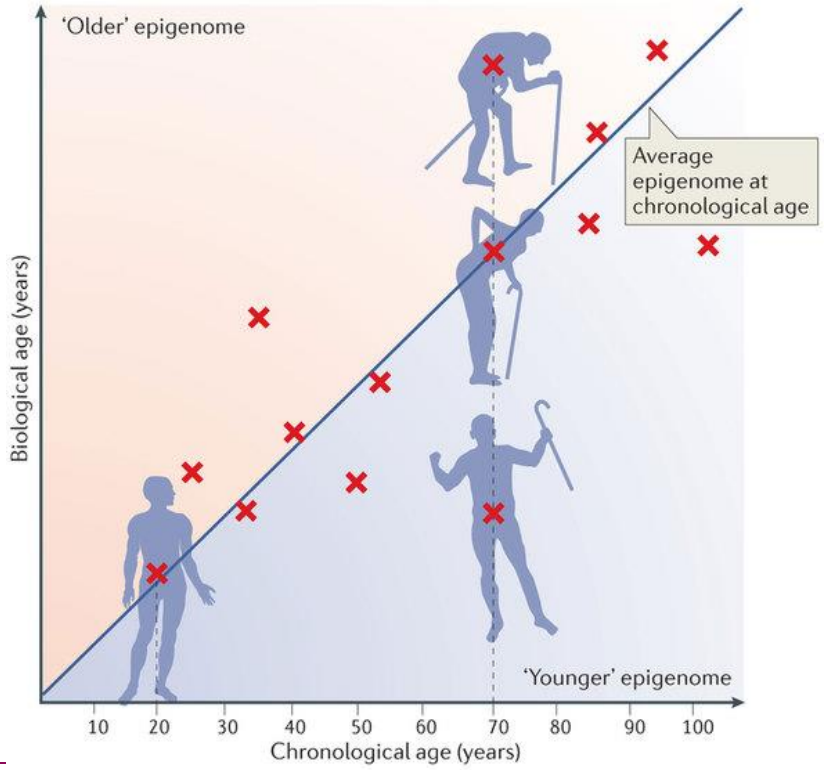


Epigenetic clock, epigenetic age

- Horvath clock = an epigenetic biomarker of aging based on DNA methylation levels

- a selection of CpG sites whose sum score of methylation levels is supposed to work as a biomarker reflecting biological aging

- chronological age ≠ biological age
- chronological age as a reference



Nature Reviews | Molecular Cell Biology

Steve Horvath

Brad Swonetz/Redux/Eyevine

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DNA methylation age of human tissues and cell types

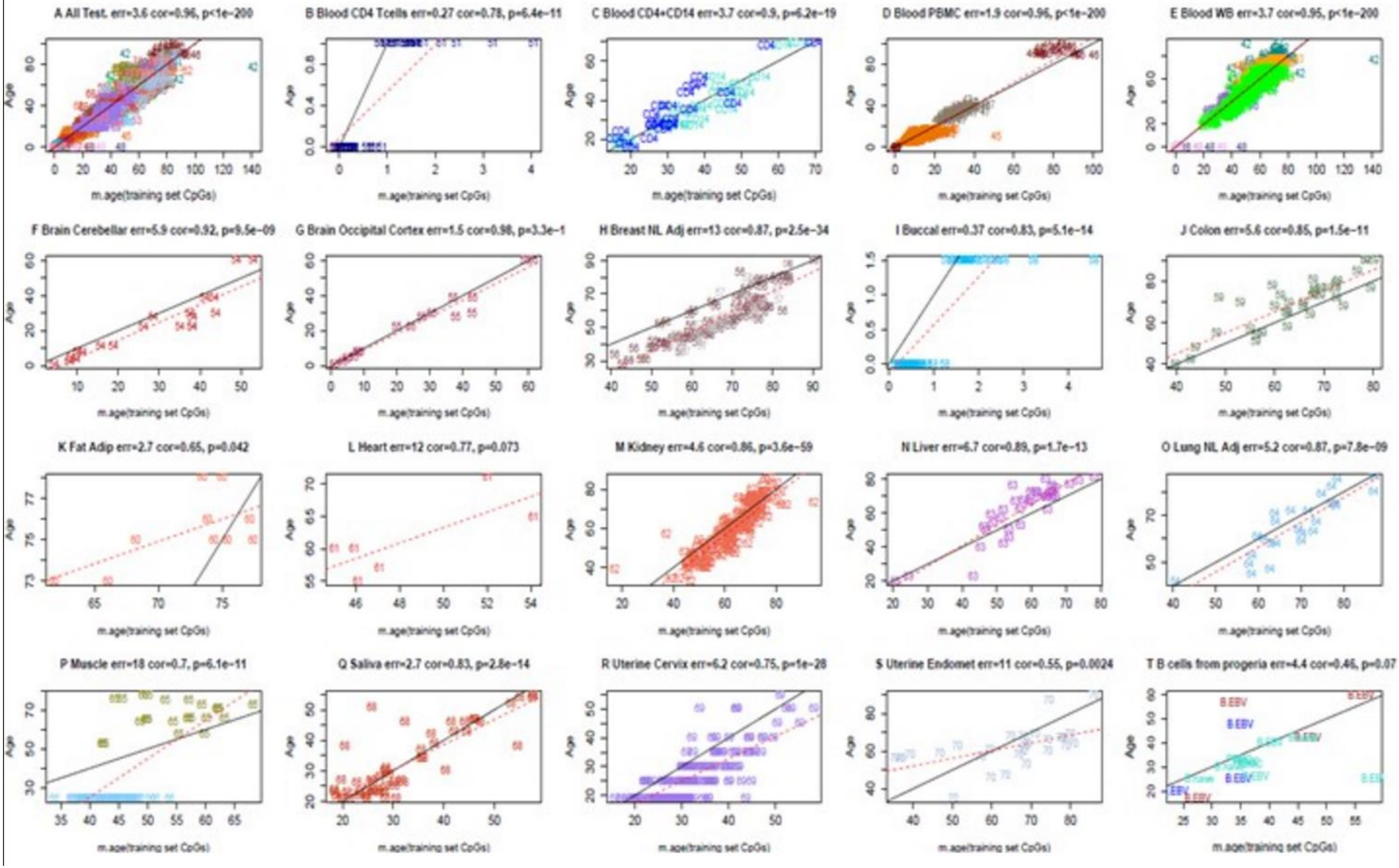
Steve Horvath

Genome Biology 2013 **14**:3156 | **DOI:** 10.1186/gb-2013-14-10-r115 |

Received: 10 June 2013 | **Accepted:** 4 October 2013 | **Published:** 10 December 2013

The Erratum to this article has been published in *Genome Biology* 2015 **16**:96

Accuracy across test data



The Horvath clock

- **Online calculator:**

https://labs.genetics.ucla.edu/horvath/dnamage/

- **FAQ:**

https://labs.genetics.ucla.edu/horvath/dnamage/faq.htm

- **Wikipedia**

https://en.wikipedia.org/wiki/Epigenetic_clock

”The other” epigenetic clocks

- The Hannum predictor (Genome-wide methylation profiles reveal quantitative views of human aging rates. Moll Cell. 2013 Jan 24;49(2))
- 71 CpG sites
- Designed in blood, needs adjustment to work in other tissues

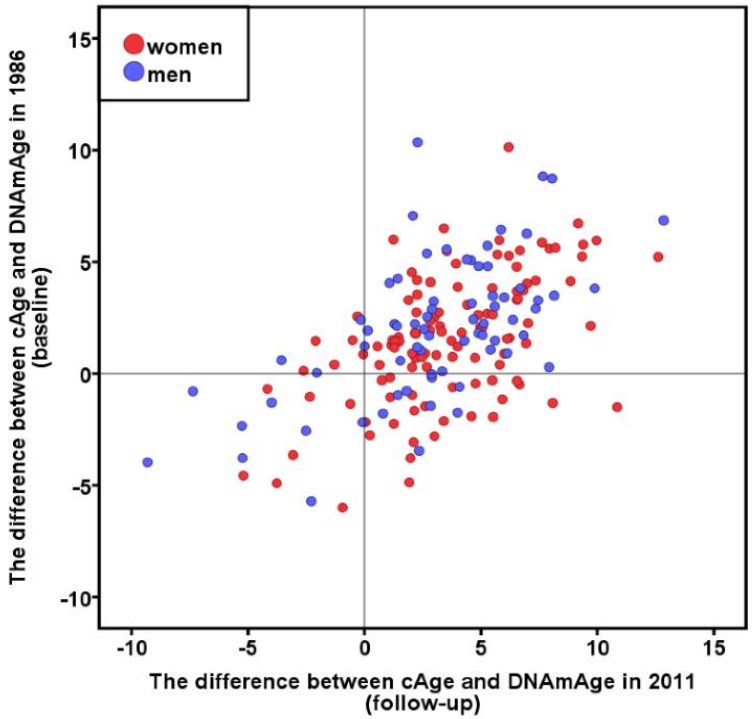
- The Weidener predictor (Aging of blood can be tracked by DNA methylation changes at just three CpG sites. Genome Biol. 2014 Feb 3;15(2))
- Uses only 3 CpG sites
- Works only in blood

Findings in two Finnish cohorts: Vitality 90+ and Young Finns study

Kananen L, Marttila S, Nevalainen T, Kummola L, Junttila I, Mononen N, Kähönen M, Raitakari OT, Hervonen A, Jylhä M, Lehtimäki T, Hurme M, Jylhävä J. *The trajectory of the blood DNA methylome ageing rate is largely set before adulthood: evidence from two longitudinal studies*. Age (Dordr). 2016 Jun;38(3):65

25-year follow-up

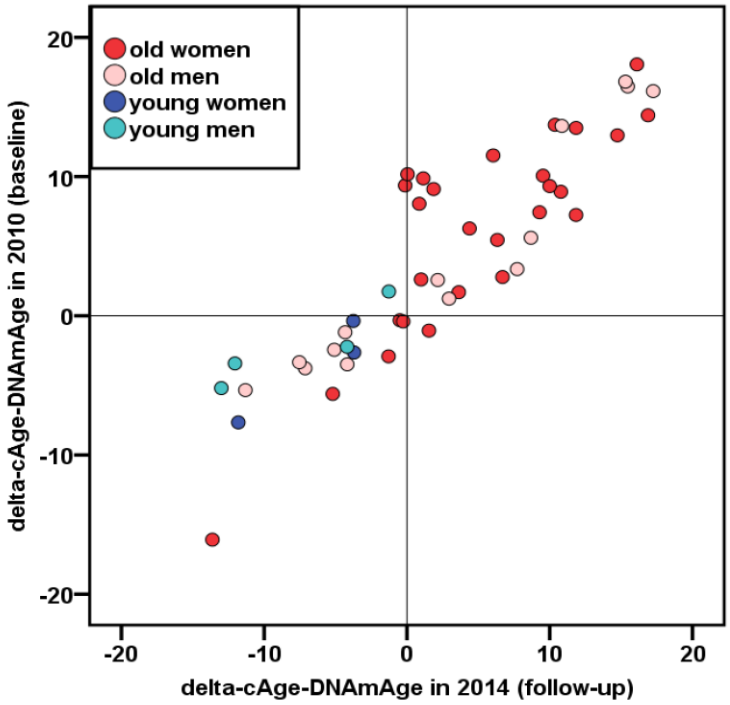
15-24 yrs



40-49 yrs

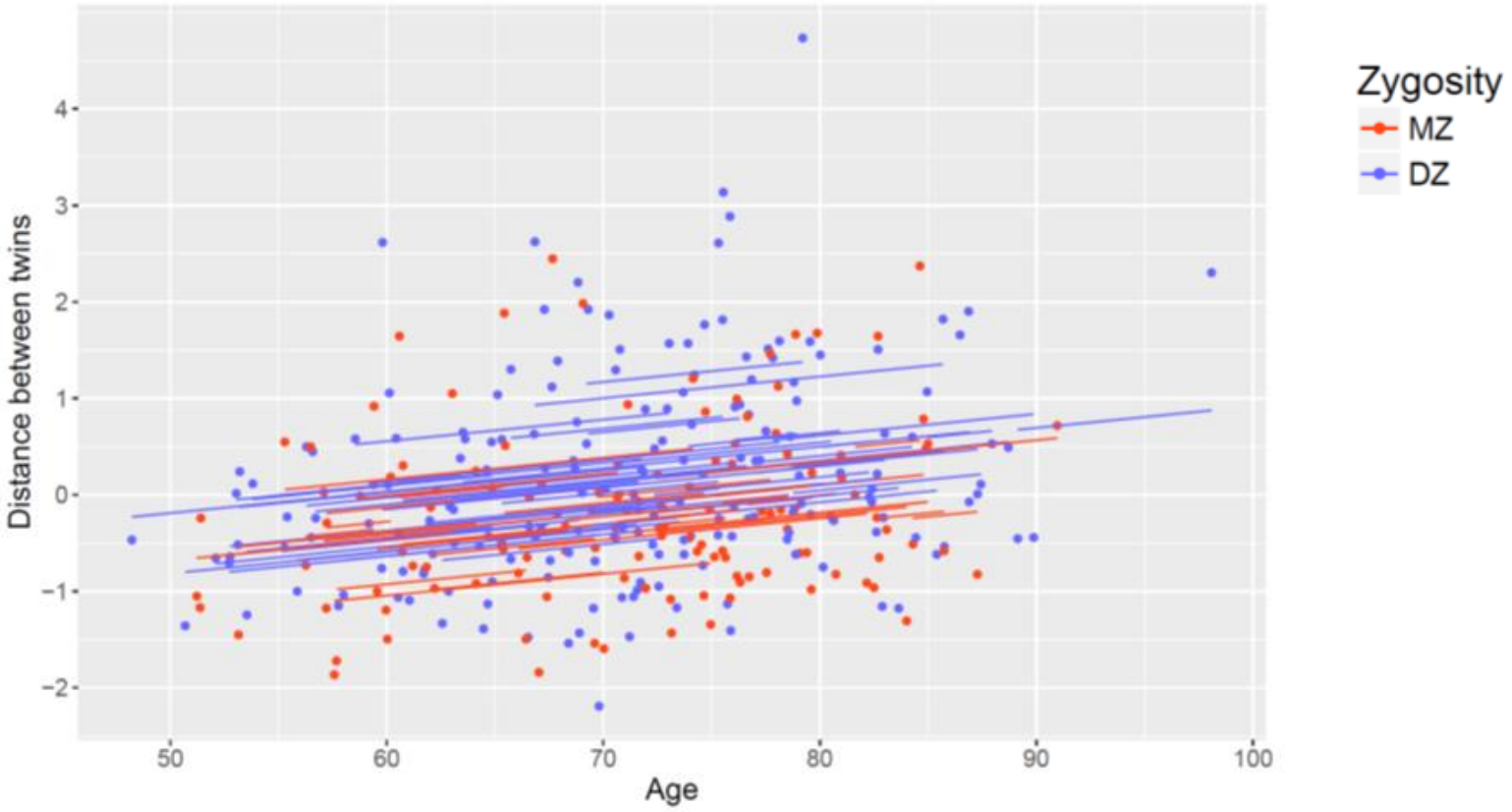
4year follow-up

old: 90 yrs
young : 19-29 yrs



old: 94 yrs
young : 23-33 yrs

Euclidean distances between twins in the Horvath epigenetic clock CpGs (SATSA, Wang et al., bioRxiv https://doi.org/10.1101/226266)



The Horvath clock

Quiz

- Outside academia/research, for what other purposes could the Horvath clock or any other age-predicting clocks be used for?

"The big question is whether the clock measures a biochemical process that serves a purpose"

www.aging-us.com AGING 2016, Vol. 8, No. 9

Priority Research Paper

DNA methylation-based measures of biological age: meta-analysis predicting time to death

-both Horvath and Hannum clocks predict all-cause mortality independent of other risk factors

Correlation with aging phenotypes

- Relatively few observations with aging traits
- Increased epigenetic aging reported in a rather small number of diseases, such as Parkinson's disease, progeroid diseases, Alzheimer, Down syndrome, HIV-1, osteoarthritis..

- tissue-specificity?

The DNAm PhenoAge = Levine clock

www.aging-us.com AGING 2018, Vol. 10, No. 4

Research Paper

An epigenetic biomarker of aging for lifespan and healthspan

Morgan E. Levine[1], Ake T. Lu[1], Austin Quach[1], Brian H. Chen[2], Themistocles L. Assimes[3], Stefania Bandinelli[4], Lifang Hou[5], Andrea A. Baccarelli[6], James D. Stewart[7], Yun Li[8], Eric A. Whitsel[7,9], James G Wilson[10], Alex P Reiner[11], Abraham Aviv[12], Kurt Lohman[13], Yongmei Liu[14], Luigi Ferrucci[2,*], Steve Horvath[1,15,*]

- 513 CpGs
- better predictor for all-cause mortality, cancers, health span, physical functioning, and Alzheimer's disease than the Horvath clock

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 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

Twin modeling for the Horvath and Levine epigenetic clocks

The Longitudinal Study of Aging Danish Twins (LSADT)

- 43 pairs
  - 18 MZ, 25 DZ
- 10 years between waves
  - Time 1 = 76.2 years (SD=1.8)
  - Time 2 = 86.1 years (SD=1.8)
- 72% female

The Swedish Adoption/Twin Study of Aging (SATSA)

- 53 pairs
  - 22 MZ, 31 DZ
- Av. 9.6 years between waves
  - Time 1 = 62.9 years (SD=7.2)
  - Time 2 = 72.5 years (SD=7.2)
- 53% female

- all like-sex twin pairs
- bivarate Cholesky model using two measurement occasions as the outcomes

- For the Levine clock, only the Swedish sample was used

Horvath clock

Twin correlations and phenotypic correlation

	Horvath clock Time1 (95% CI)	Horvath clock Time2 (95% CI)	Phenotypic correlation (95% CI)
MZ	**0.17** (-0.14-0.45)	**0.50** (0.22-0.70)	**0.54** (0.35-0.64)
DZ	**0.44** (0.21-0.62)	**0.23** (-0.02-0.45)	

Cross-twin cross-trait correlations (CTCT)
MZr: 0.38
DZr: 0.30

Quiz

What do the CTCT correlations tell you?

Levine clock

Twin correlations and phenotypic correlation

	Levine clock Time1 (95% CI)	Levine clock Time2 (95% CI)	Phenotypic correlation (95% CI)
MZ	**0.56** (0.15-0.80)	**0.41** (-0.03-0.71)	**0.20** (0.00-0.39)
DZ	**0.09** (-0.27-0.43)	**0.29** (-0.07-0.58)	

- Phenotypic correlation of 0.2 would suggest that the individuals change more in their Levine clock (DNAm PhenoAge) with age that in the Horvath clock

Cross-twin cross-trait correlations (CTCT)
MZr 0.24
DZr 0.10

Variance components for the Horvath clock: bivariate ADE model

Parameter estimates (% of variance explained + SEs) form the bivariate ADE model

- increase in genetic influences with age

	a^2	d^2	e^2	rA	rD	rE
Time 1	**0.35** (0.13)	**0.01** (0.05)	**0.65** (0.12)	**1.00** (0.00)	**1.00** (0.00)	**0.31** (0.13)
Time 2	**0.29** (0.24)	**0.21** (0.27)	**0.50** (0.12)			

Quiz

What do the correlations (rA, rD, rD) tell you?

Variance components for the Levine clock: bivariate ADE model

Parameter estimates (% of variance explained + SEs) form the bivariate ADE model

	a^2	d^2	e^2	rA	rD	rE
Time 1	**0.06** (0.26)	**0.41** (0.33)	**0.52** (0.21)	**1.00** (0.00)	**0.99** (0.00)	**-0.12** (0.22)
Time 2	**0.40** (0.20)	**0.01** (0.11)	**0.59** (0.16)			

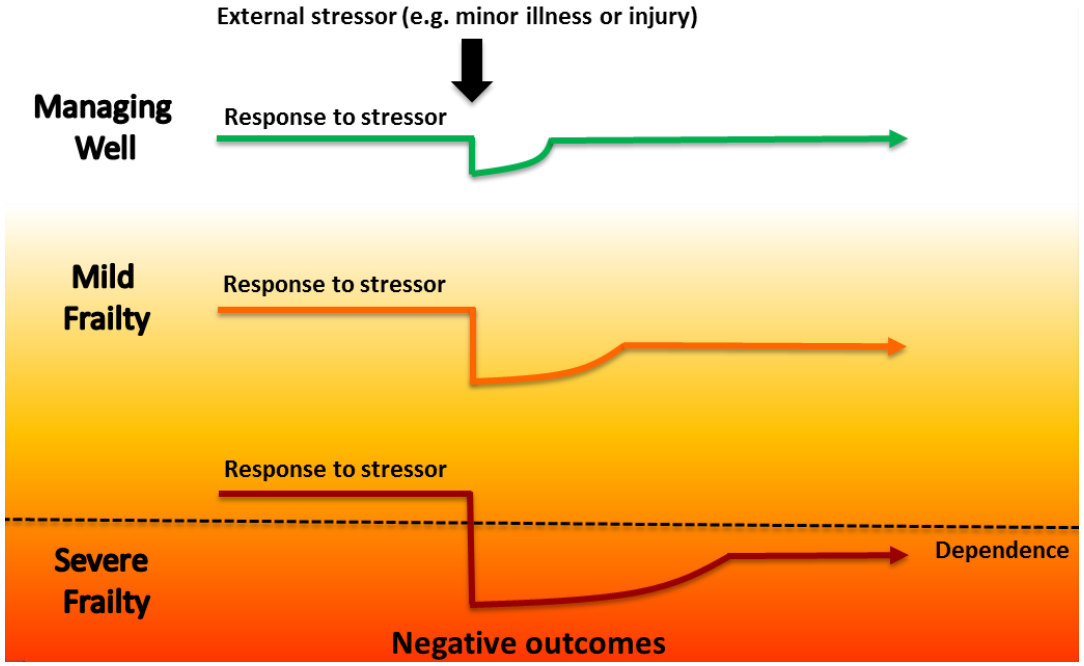
- small decrease in genetic influences with age, still the same genetic factors that act upon the Levine clock throughout aging
- however, a great amount of new unique environmental influences
- QUIZ: how would you interpret the negative correlation rE?

Sumary from the bivariate models - what purpose do the results serve?

- Moderate heritability for both clocks
- Small increase in genetic influences for the Horvath clock with age
- Small decrease in genetic influences for the Levine clock with age
- No new genetic influences for both clocks with age
- New environmental influences come into play with aging for both clocks!

Aging-associated frailty
What is it and why does it matter?

- a state of increased vulnerability and loss of capacity to maintain homeostasis after a stressor event
- significant risk factor for mortality and other adverse outcomes
- marker of biological age



Frailty and the epigenetick clock

Breitling *et al. Clinical Epigenetics* (2016) 8:21
DOI 10.1186/s13148-016-0186-5

Clinical Epigenetics

RESEARCH Open Access

Frailty is associated with the epigenetic clock but not with telomere length in a German cohort

Lutz Philipp Breitling[1*], Kai-Uwe Saum[1], Laura Perna[1], Ben Schöttker[1,3], Bernd Holleczek[2] and Hermann Brenner[1,3]

GeroScience (2017) 39:83–92
DOI 10.1007/s11357-017-9960-3

ORIGINAL ARTICLE

The frailty index outperforms DNA methylation age and its derivatives as an indicator of biological age

Sangkyu Kim · Leann Myers · Jennifer Wyckoff · Katie E. Cherry · S. Michal Jazwinski

How to assess frailty

- various ways (20+ scales!)
- the Rockwood frailty index (FI) and the Fried frailty phenotype most commonly used
- the FI is a continuous scale measure that provides good sensitivity and resolution also at the lower and middle ends of the frailty continuum

→ FI = frailty

Frailty (FI) and mortality
Between-within *frailty model* (gamma BW) for mortality

Screening Across the Lifespan Twin study (SALT)

N=43,000; MZs, DZs same and opposite sex

- 32,146 twins in complete pairs available for analysis

Research Article

Between–within models for survival analysis

Arvid Sjölander, Paul Lichtenstein, Henrik Larsson, Yudi Pawitan

First published: 03 March 2013 | https://doi.org/10.1002/sim.5767 | Cited b

Article

Regression standardization and attributable fraction estimation with between-within frailty models for clustered survival data

Elisabeth Dahlqwist, Yudi Pawitan and Arvid Sjölander

Statistical Methods in Medical Research
0(0) 1–24

DOI: 10.1177/0962280217727558
journals.sagepub.com/home/smm
SAGE

The between-within (BW) model

- Control for the familiar unmeasured confounding between *frailty syndrome* and mortality
- genetics and early life exposures contribute to the associations?

Gamma BW model:

$$h(t_{ij}|\mathrm{X}_i, \text{cluster } i) = h_0(t_{ij})\tilde{u}_i \exp(\beta_B \bar{x}_i + \beta_W x_{ij})$$

$h_0(t_{ij})$: baseline hazard
i: twin pair
j: individual twin
u: shared frailty (cluster specific), following gamma distribution
x: frailty index
$\bar{x}$: mean frailty index within each twin pair
β_B: between-cluster effect, quantifying the degree of shared confounding
β_W: within-cluster effect, quantifying the exposure-survival association within twin pairs

Time-varying effects under the generalized survival model framework

$$\log\left(-\log\left(S\left(t_{ij}|x_{ij},u_i\right)\right)\right) = s_0\left(t_{ij};\gamma\right) + \log(u_i) + \beta_B\bar{x}_i + x_{ij}s_1\left(t_{ij};\beta_W\right) + \delta_{B_m}\bar{C}_m + \delta_{W_m}C_{i_m}$$

$s1(tij;\beta W)$: time-dependent within twin-pair effect (smooth function)
$s0(tij;\gamma)$: baseline survival (smooth function)
C: covariates (sex, BMI, education, smoking)

- detailed dissection of the age-varying HRs using splines
- also testing for sex-interaction with the time-varying HRs
- both all-cause and cause-specific mortality as outcomes

Assessing the public health relevance of the exposure (FI): the attributable fraction (AF)

$$\mathrm{AF}(t) = 1 - \frac{\Pr(T_0 \leq t)}{\Pr(T \leq t)}$$

where

Pr($T \leq t$) =1-$S(t)$ is the factual probability of an event at or before time $T=t$,

Pr($T_0 \leq t$) is the counterfactual probability of an event at or before time t

had the exposure been eliminated from the population at baseline

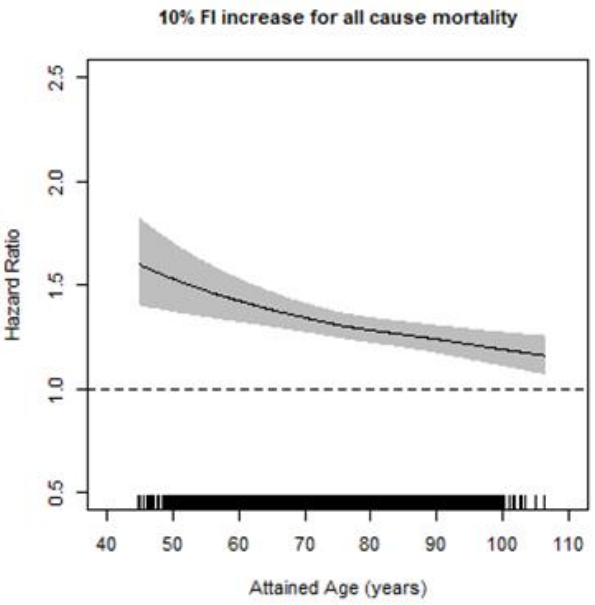
- Analyzed under the BW model and extended for time-varying AFs (fraction at any given age)
- The FI was categorized to ”low FI” and ”high FI” using the medians as cut-off
- To recap: AF(t) measures the proportion of events that would have been prevented before time $T=t$, had the whole population been unexposed

All-cause mortality

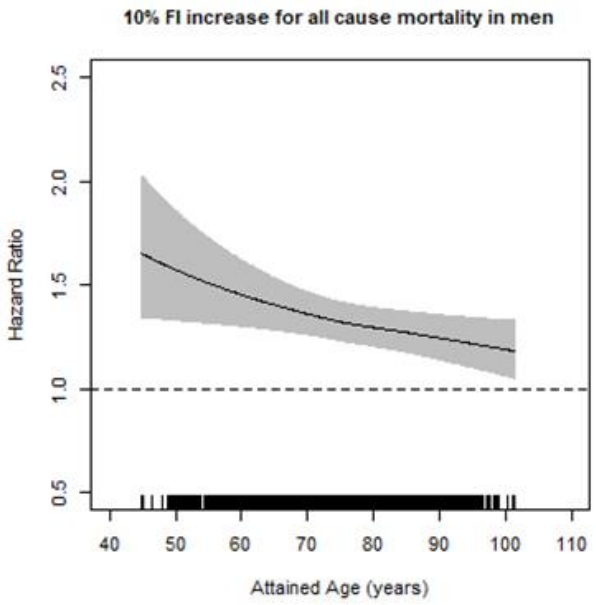
- MZs and DZs (same and opposite sex) tested for the frailty term
→ analyzed together
- however, sex differences were observed → models stratified for sex

On average, HR for 10% increase in FI is associated with 50% increased mortality risk at midlife, the risk declines towards the old ages

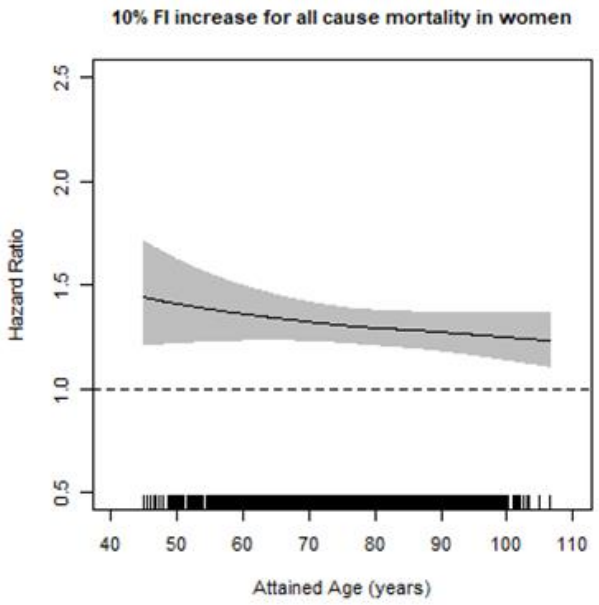
All



Men



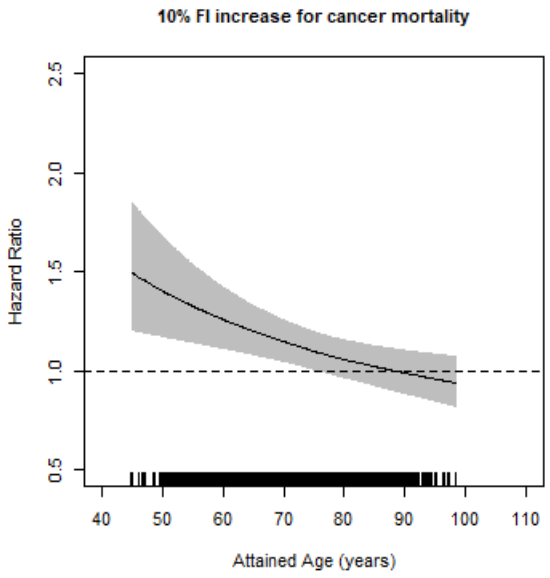
Women



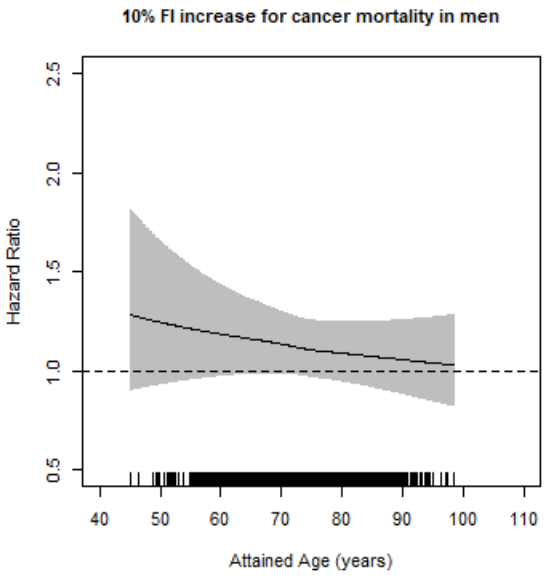
Cause-specific mortality: CANCER
- adjusted for baseline cancer diagnosis

Overall, a significant risk at midlife in the whole population and in women, but the HRs become non-significant towards the old ages

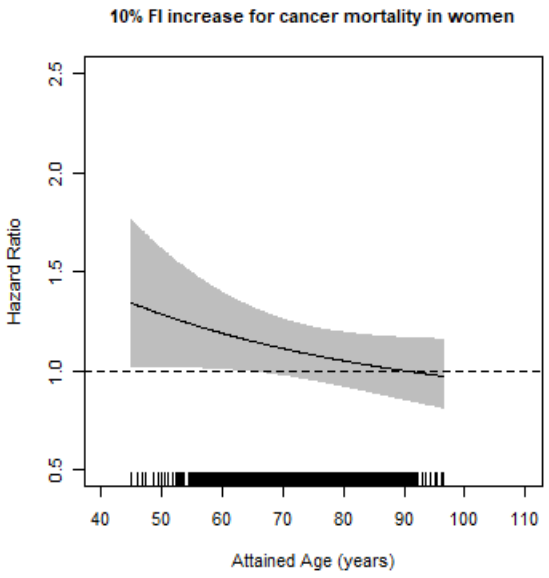
All



Men



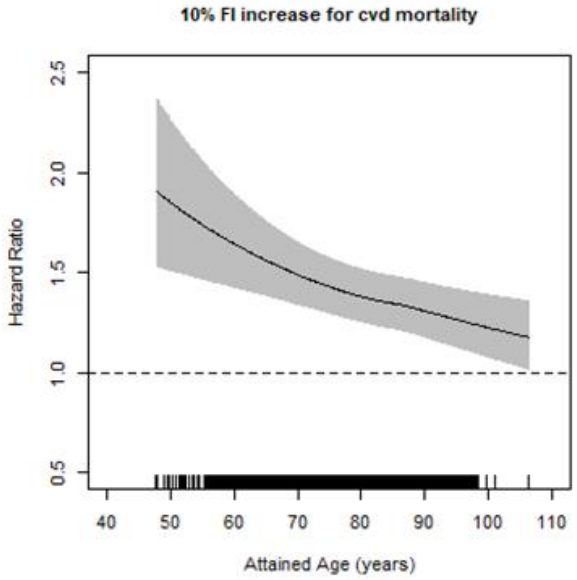
Women



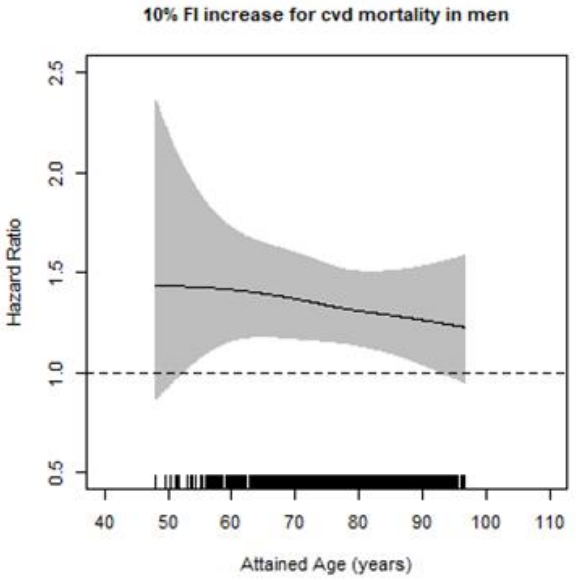
Cause-specific mortality: cardiovascular disease (CVD)
- adjusted for baseline CVD status

FI strognly predictive of CVD-mortality, especially in women
- 10% increase in FI is associated with 50-80% increase in CVD mortality

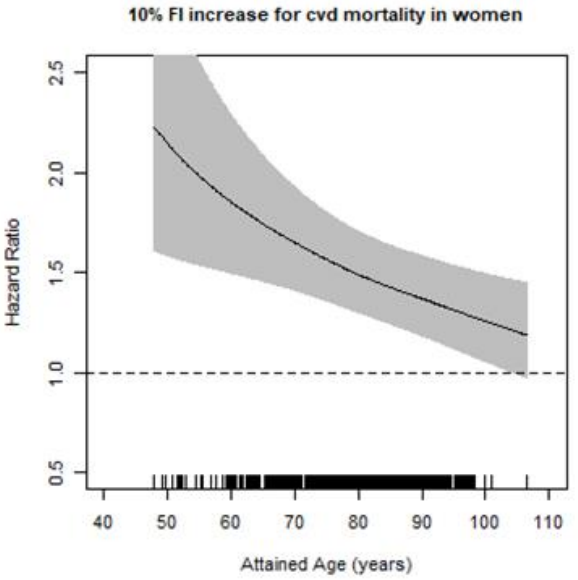
All



Men

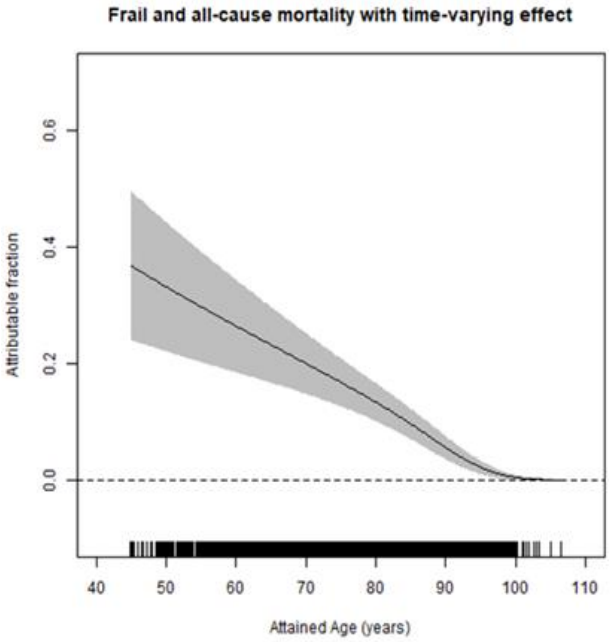


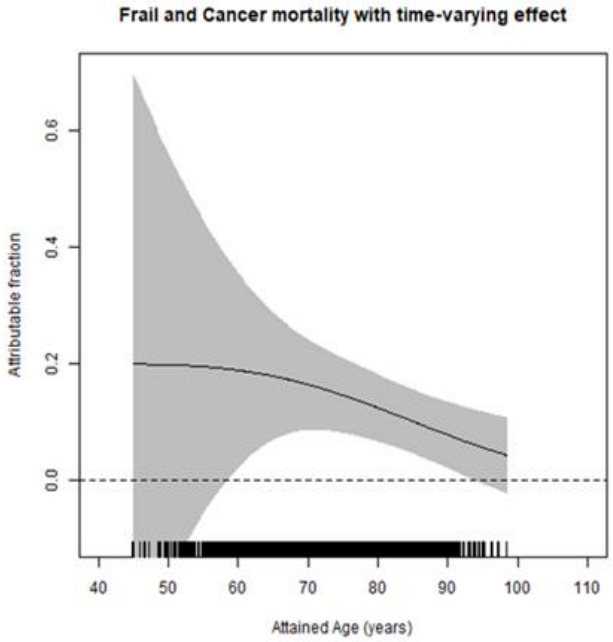
Women

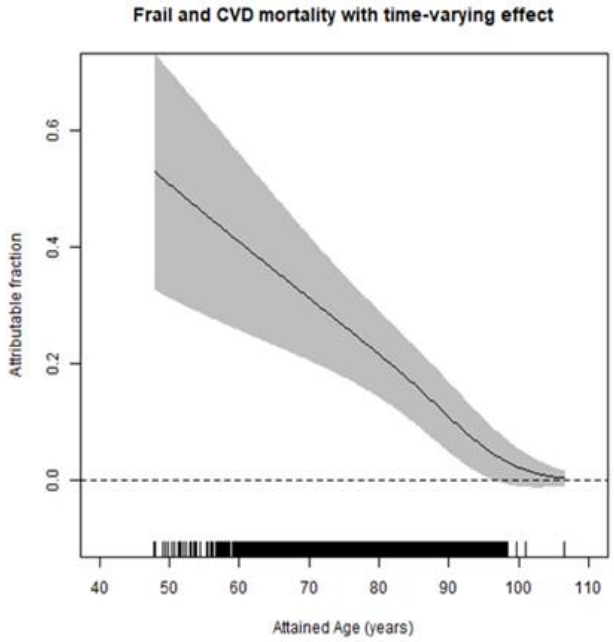


AFs for all-cause, cancer and CVD mortality

A very simple quiz: how would you intrepret the results?







We also tested deaths due to

- dementia (N.S)
- diabetes (N.S, low power)
- respiratory causes (significnat, but low power)

Ongoing & upcoming

- Heritability of frailty
- Two previous studies have demonstrated moderate heritability, depending on the measure used to assess frailty

Dato et al. Age (Dordr). 2012 Jun; 34(3): 571–582 and

Young et al. Twin Res Hum Genet. 2016 Dec;19(6):600-609.

- What we can do: longitudinal analysis using LGM across 30 years of serial assessment for FI in SATSA (N=1,831)

- Epigenetics of frailty

- EWAS hits and their heritability

Thank you!

- Questions?