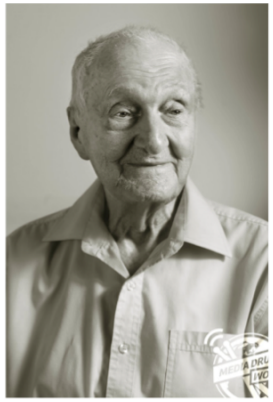
An New Epigenetic Clock for Aging and Life Expectancy

Morgan Levine

Yale Center for Research on Aging

Department of Pathology

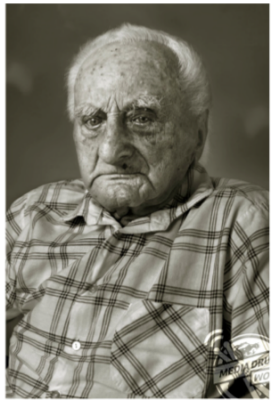
Yale School of Medicine

Department of Chronic Disease Epidemiology

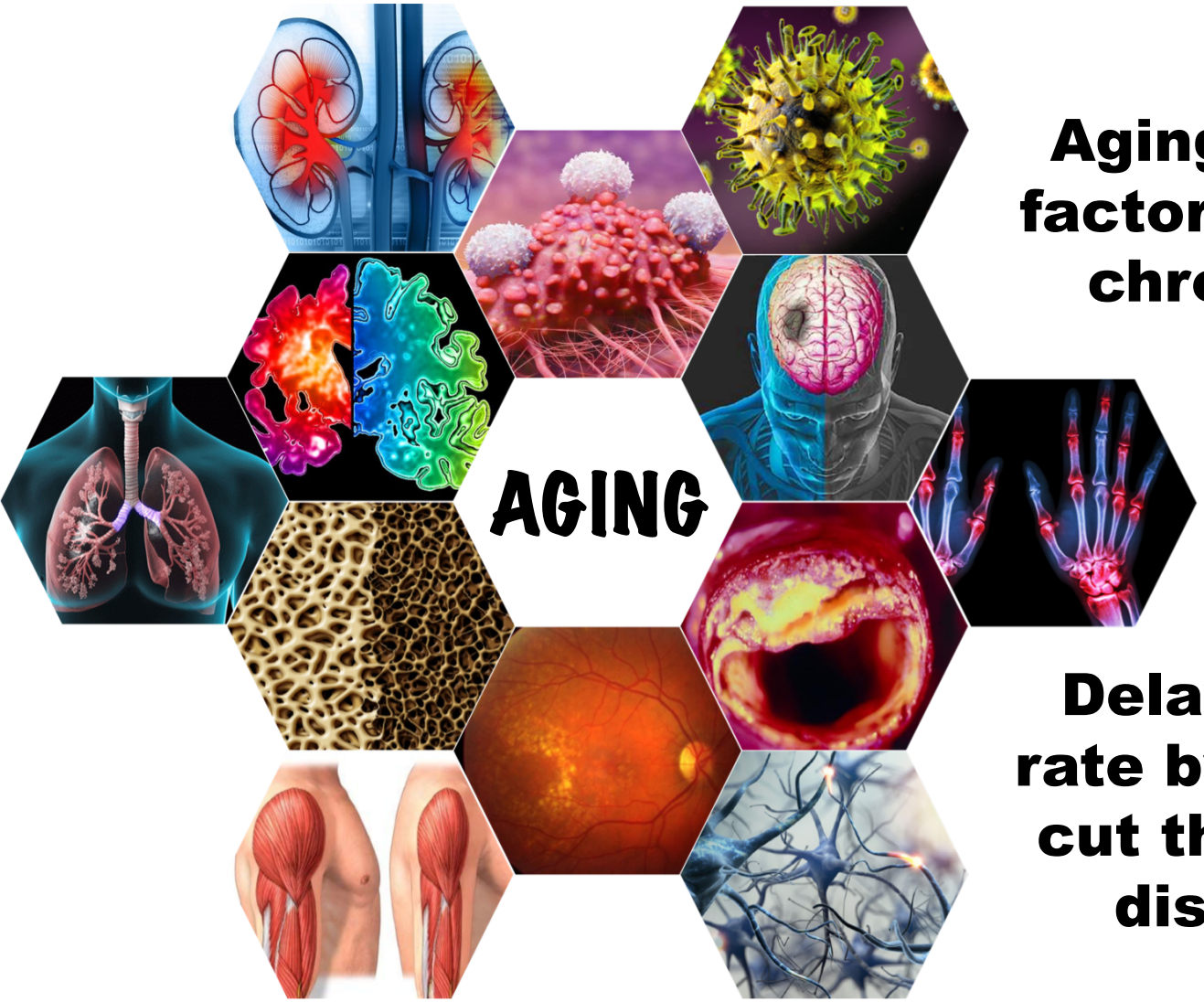
Yale School of Public Health







MORE THAN DEATH



Aging is the #1 risk factor for most major chronic diseases

Delaying the aging rate by 7 years would cut the incidence of disease in half!

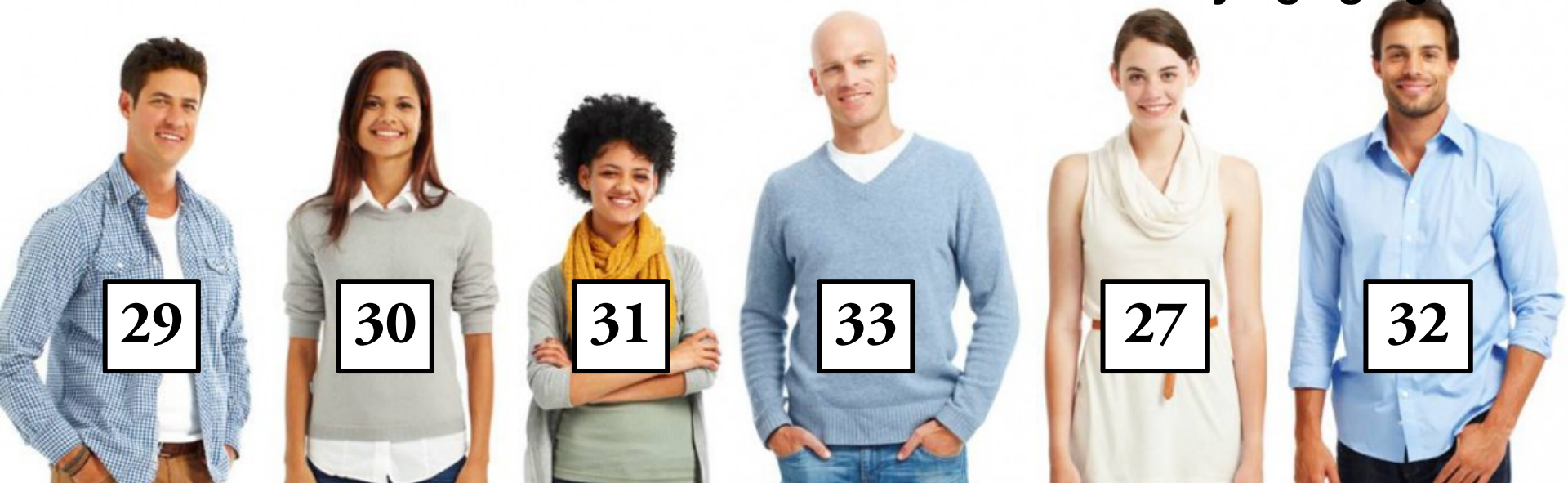
AGING HETEROGENEITY

We don’t all age in the same way or at the same rate.

Chronological age is an imperfect estimate of the latent concept, “biological aging”.

Quantifying “biological age” may:

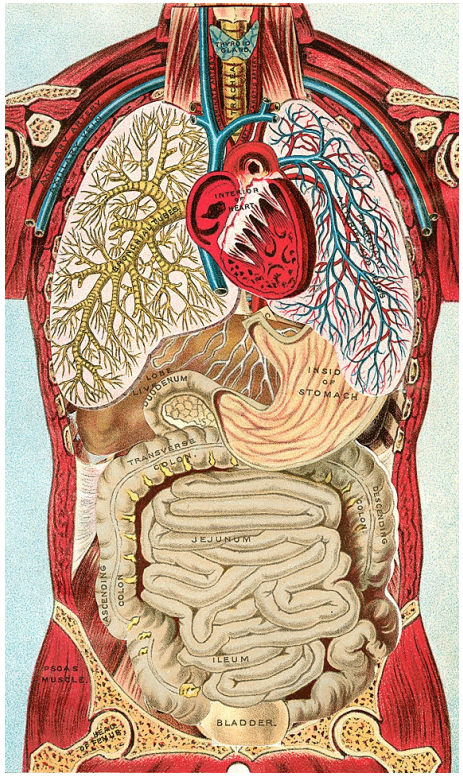
1. **Provide an endophenotype from which to identify genetic and environmental contributors to differences in lifespan and healthspan.**
2. **Facilitate evaluation of interventions aimed at delaying aging.**

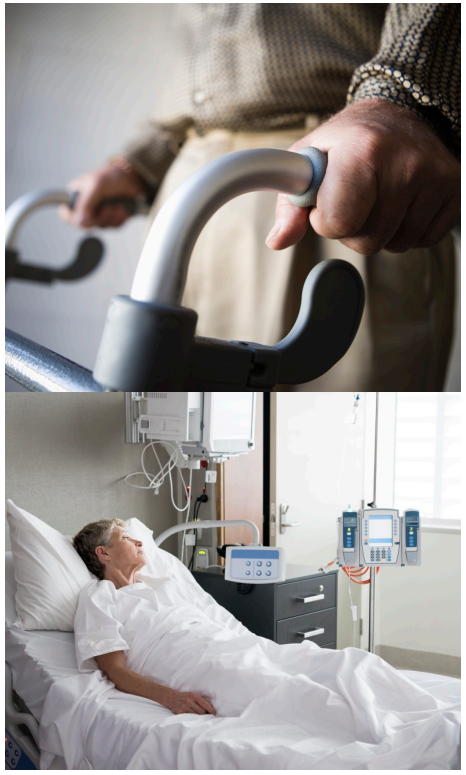


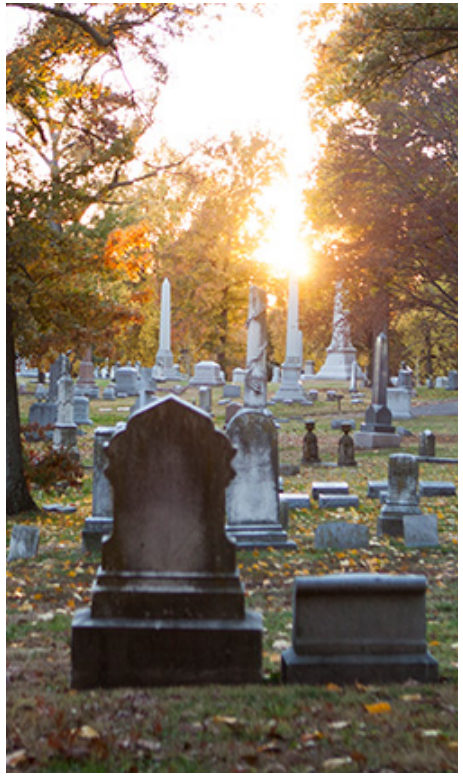
AGING TRAJECTORY

At what level should we estimate "aging?

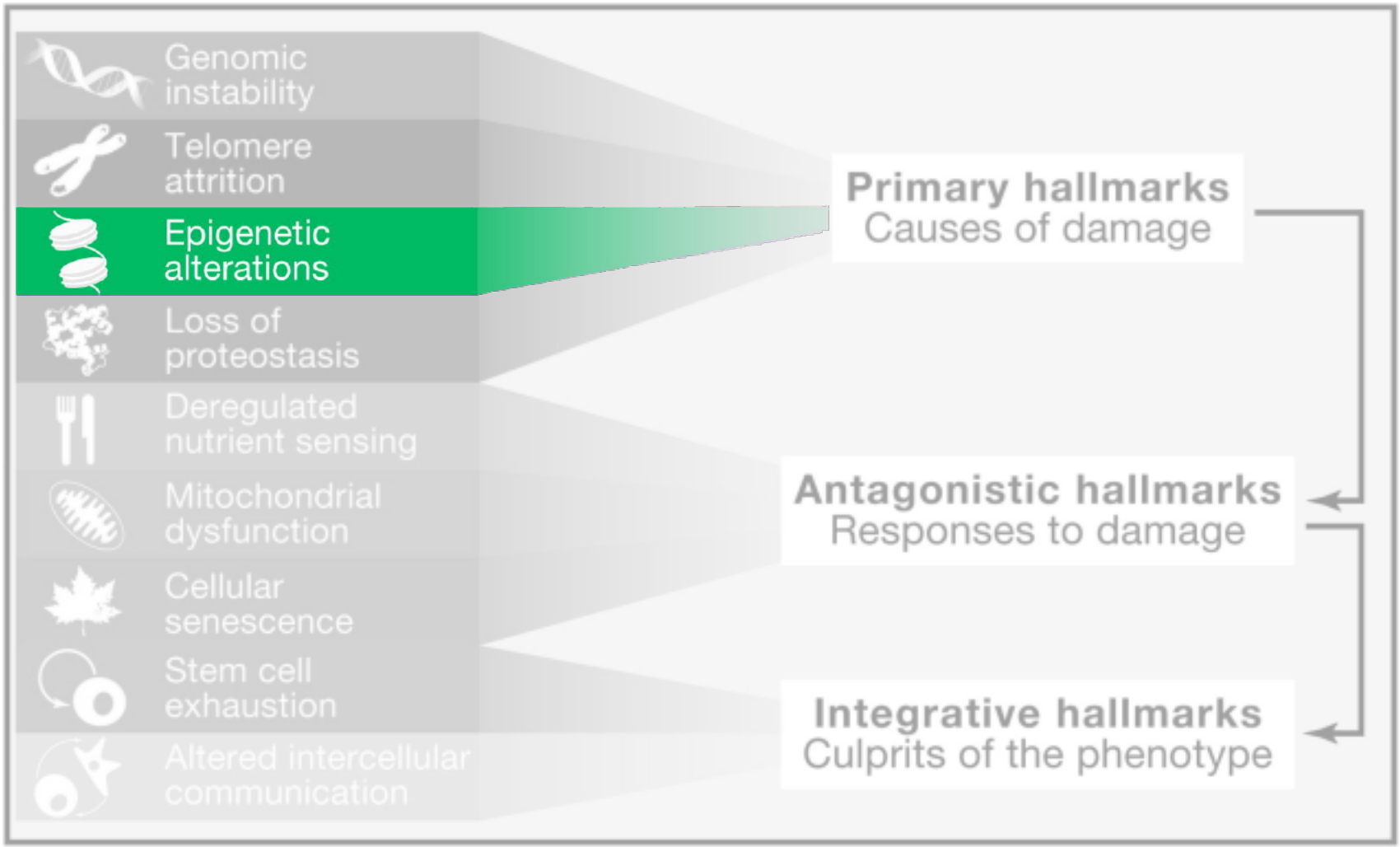
Proximal to Mechanisms	Proximal to Outcomes	Healthspan (Geroscience Goal)	Demographic Aging
Molecular Alterations	**Physiological Dysregulation**	**Disease/ Disability**	**DEATH**







WHAT IS AGING?

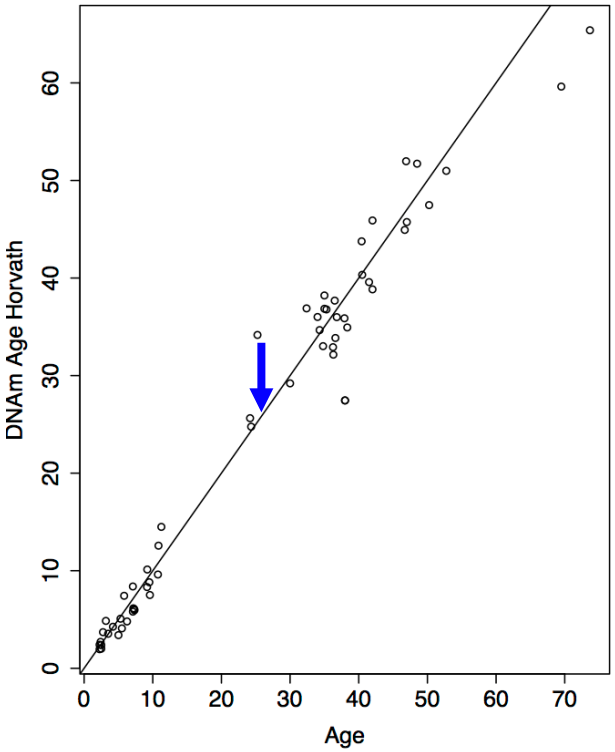


EPIGENETIC CLOCKS

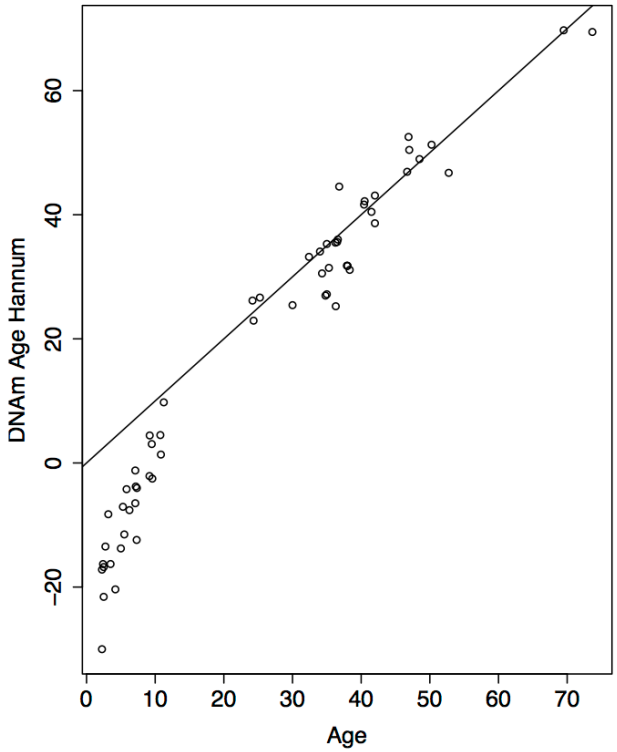
Chronological age has been shown correspond with distinct changes in DNA methylation (DNAm) at specific CpG sites.

Very accurate epigenetic age predictors have been developed

Horvath, r=0.98

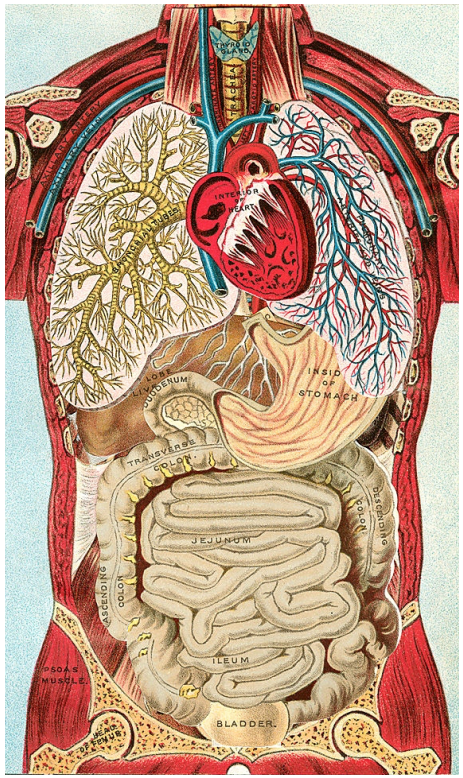


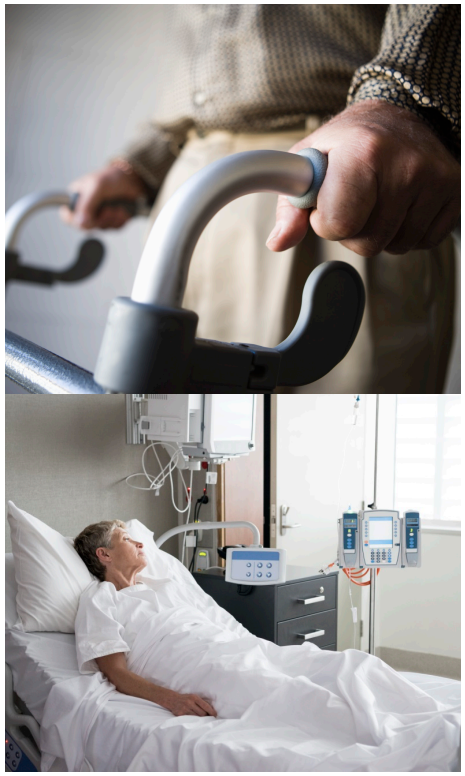
Hannum, r=0.97

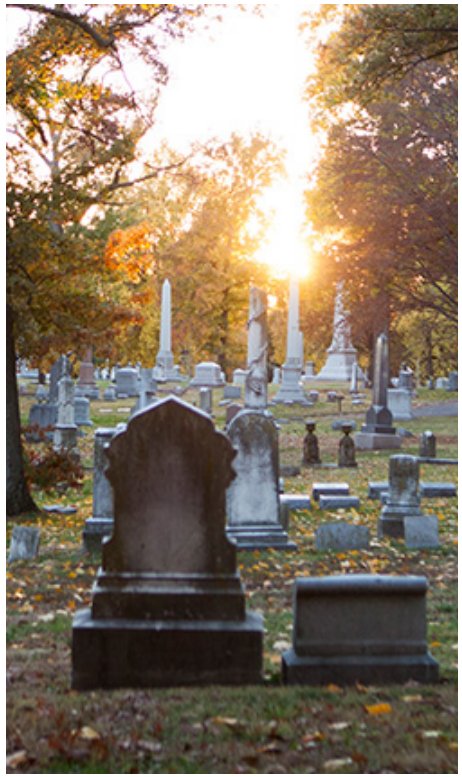


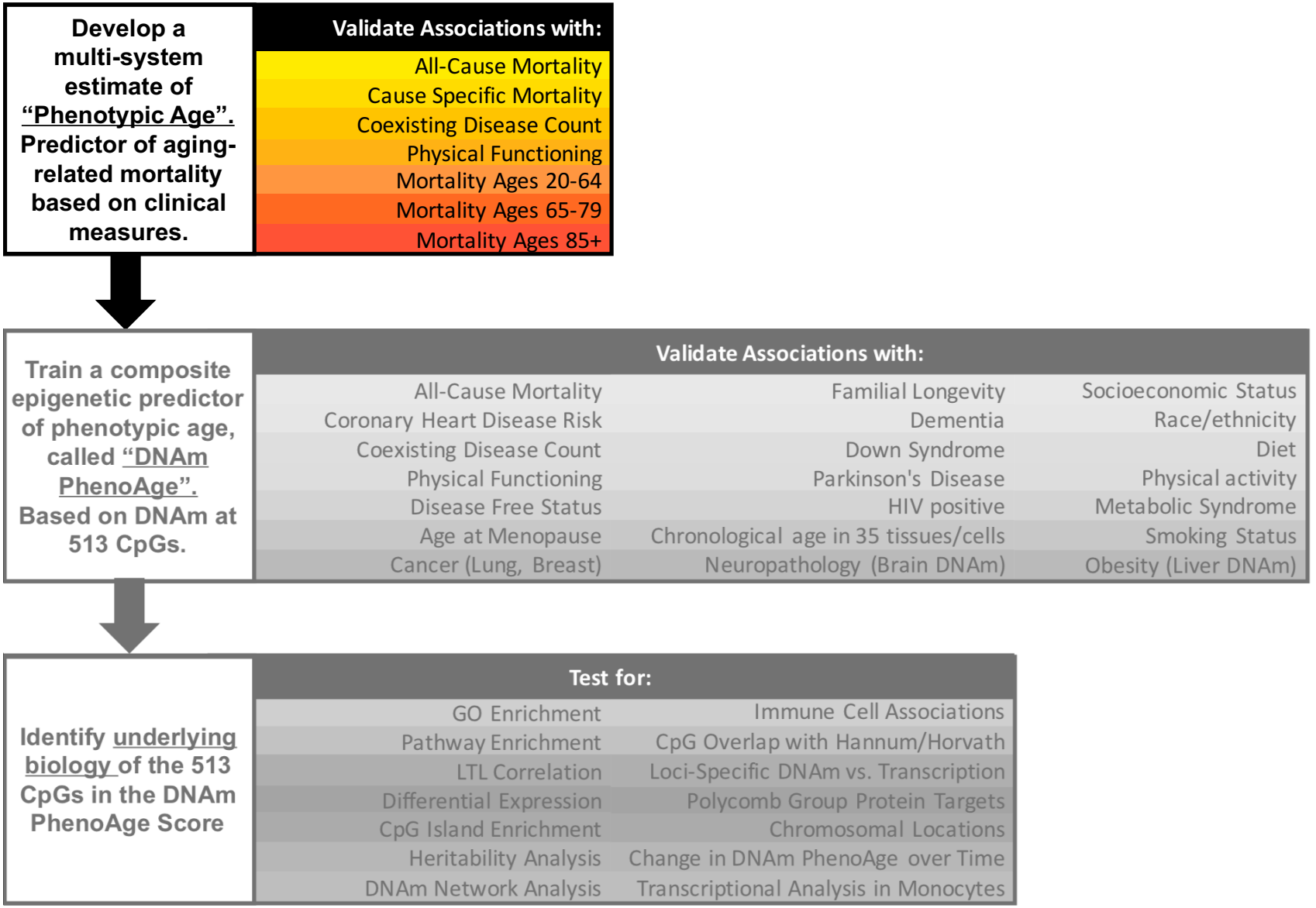
Instead of minimizing the residual, the goal should be to capture the "true residual". (i.e. decouple chronological time from biological aging)

AIM: Train a clock to predict a variable that already captures differences in physiological dysregulation; susceptibility to disease/disability; and risk of death among same aged individuals.








Develop a multi-system estimate of "Phenotypic Age". Predictor of aging-related mortality based on clinical measures.
Validate Associations with:
All-Cause Mortality
Cause Specific Mortality
Coexisting Disease Count
Physical Functioning
Mortality Ages 20-64
Mortality Ages 65-79
Mortality Ages 85+
Train a composite epigenetic predictor of phenotypic age, called "DNAm PhenoAge". Based on DNAm at 513 CpGs.
Validate Associations with:
All-Cause Mortality
Coronary Heart Disease Risk
Coexisting Disease Count
Physical Functioning
Disease Free Status
Age at Menopause
Cancer (Lung, Breast)
Familial Longevity
Dementia
Down Syndrome
Parkinson's Disease
HIV positive
Chronological age in 35 tissues/cells
Neuropathology (Brain DNAm)
Socioeconomic Status
Race/ethnicity
Diet
Physical activity
Metabolic Syndrome
Smoking Status
Obesity (Liver DNAm)
Identify underlying biology of the 513 CpGs in the DNAm PhenoAge Score
Test for:
GO Enrichment
Pathway Enrichment
LTL Correlation
Differential Expression
CpG Island Enrichment
Heritability Analysis
DNAm Network Analysis
Immune Cell Associations
CpG Overlap with Hannum/Horvath
Loci-Specific DNAm vs. Transcription
Polycomb Group Protein Targets
Chromosomal Locations
Change in DNAm PhenoAge over Time
Transcriptional Analysis in Monocytes

DEVELOPING A NEW EPIGENETIC CLOCK

Develop a Multisystem Phenotypic Age Estimate and Validate Predictions	Develop a New Epigenetic Age Estimate and Validate Predictions/Associations	Underlying Biology of the Clock and the 513 CpGs

Training Sample: (N=9,926), Ages 20+, up to 23 years of mortality follow-up

Input Variables: 42 clinical biomarkers and age.

Model: Proportional Hazard Elastic Net (Outcome=Mortality from major age-related diseases)

Variables
Albumin
Creatinine
Glucose
C-reactive protein
Lymphocyte percent
Mean cell volume
Red cell distribution width
Alkaline phosphatase
White blood cell count
Age

$$Linear\ Prediction = Albumin \times \beta_{Albumin} + CRP \times \beta_{CRP} + \cdots Age \times \beta_{Age} + constant$$

Converted to an age (units of years) using parameters from a Gompertz proportional hazard model.

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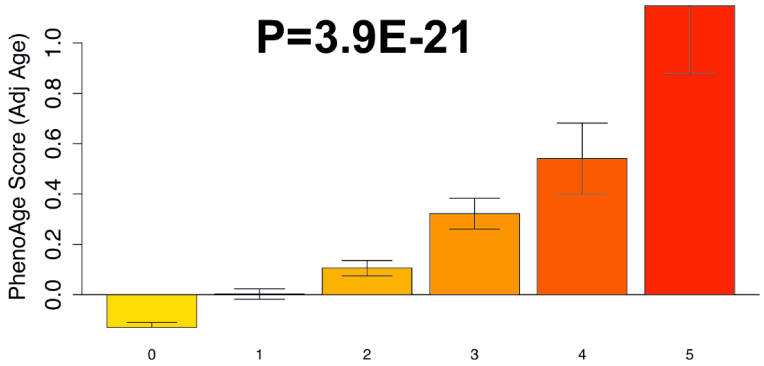
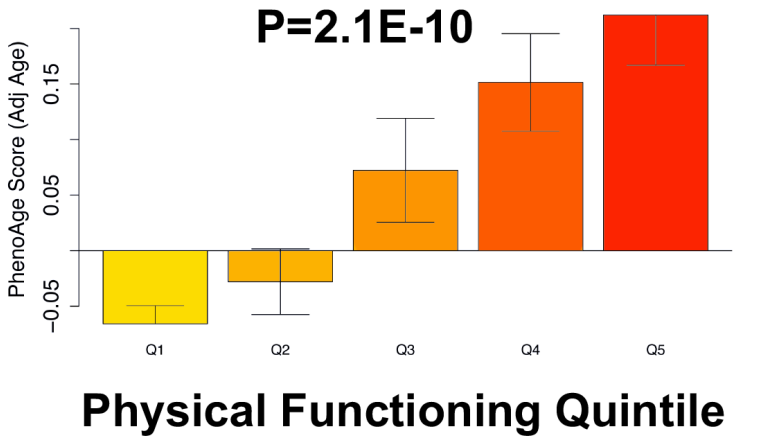
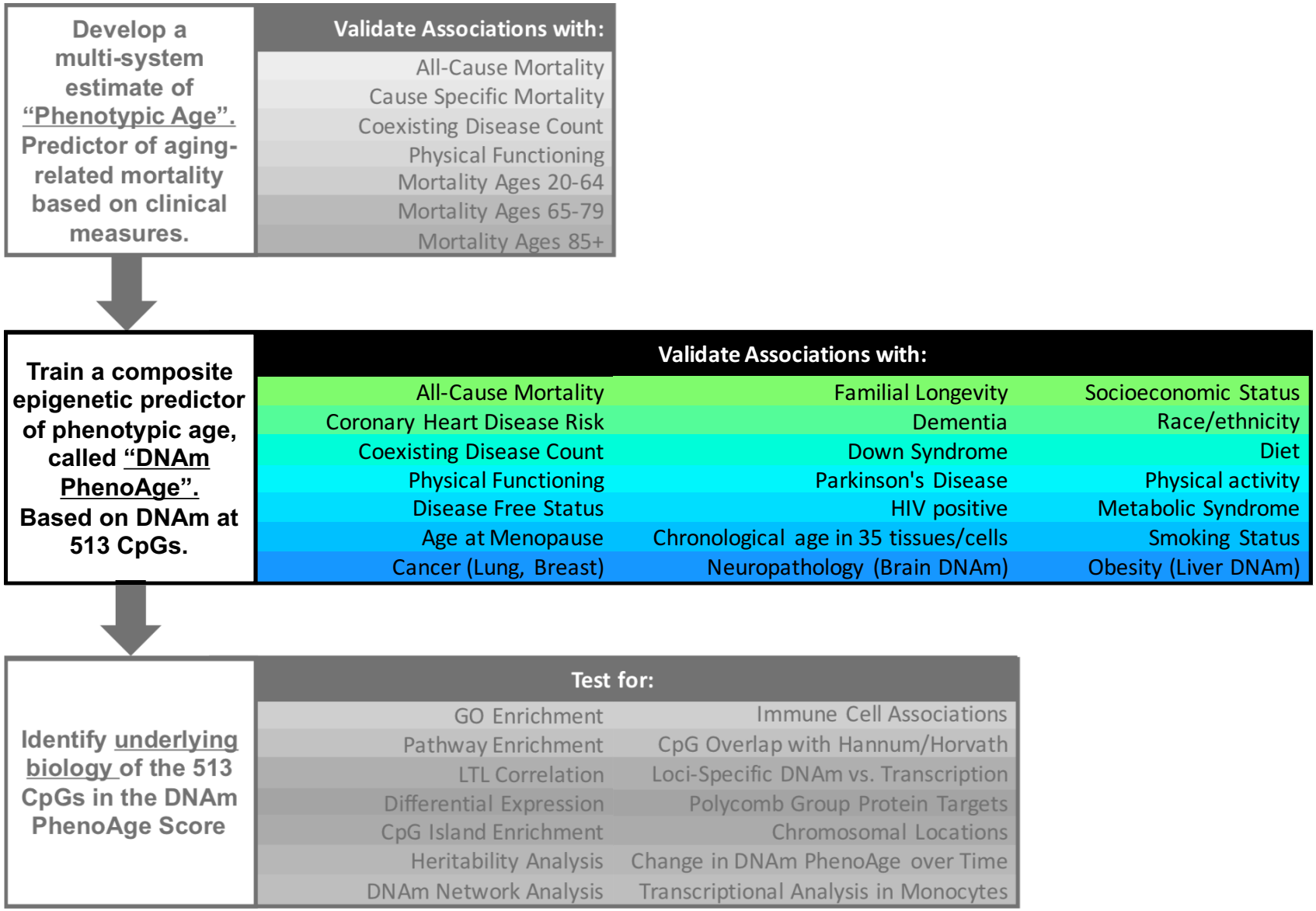
Mortality Prediction in Independent Sample

Cause	Cases	HR	P-Value
All-Cause	1052	1.09	3.8E-49
Aging-Related	661	1.09	4.5E-34
CVD	272	1.10	5.1E-17
Cancer	265	1.07	7.9E-10
Alzheimer's	30	1.04	2.6E-01
Diabetes	41	1.20	1.9E-11
Lung	53	1.09	6.3E-04



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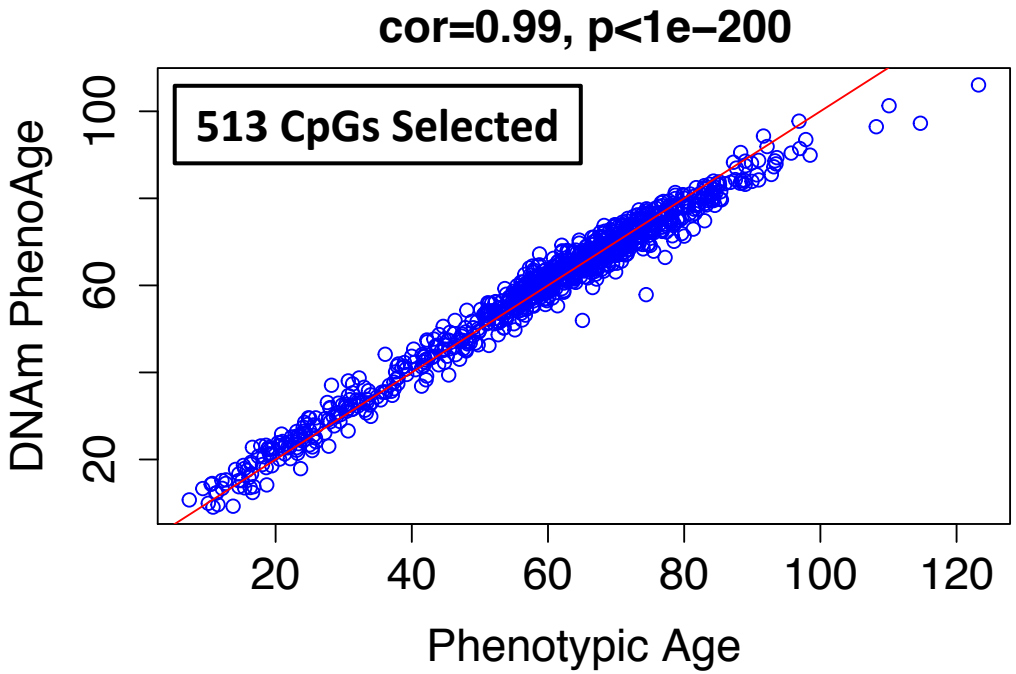
Training Sample: InCHIANTI—N=456 at two time-points (1998 & 2007).

Input Variables: DNAm from whole blood for about 20,000 CpGs (those on the 27k, 450k, and EPIC chips)

Model: Elastic Net (Outcome=Phenotypic Age)

$$DNAmPhenoAge = CpG1 \times \beta_{CpG1} + \cdots + CpG513 \times \beta_{CpG513} + constant$$



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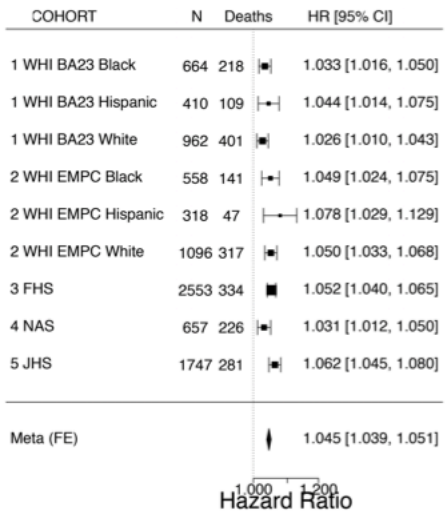
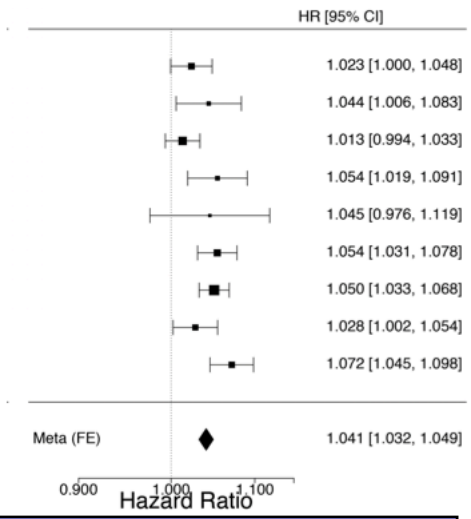
Levine



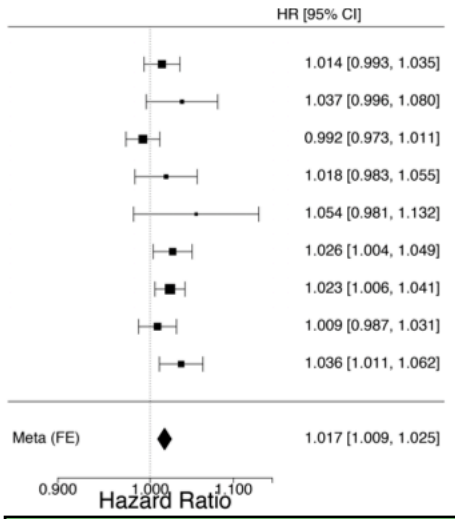
HR = 1.045 (1.039, 1.051)
***Meta-p* = 7.9E-47**

Hannum



HR = 1.041 (1.032, 1.049)
***Meta-p* = 1.7E-21**

Horvath



HR = 1.017 (1.009, 1.025)
***Meta-p* = 4.5E-05**

	Disease Count	Disease Free	CHD Risk	Physical Functioning
Levine	4.56E-15	1.06E-07	2.43E-10	2.05E-13
Horvath	6.76E-06	2.03E-03	1.10E-03	2.03E-05
Hannum	4.54E-02	1.31E-03	7.51E-01	4.66E-04

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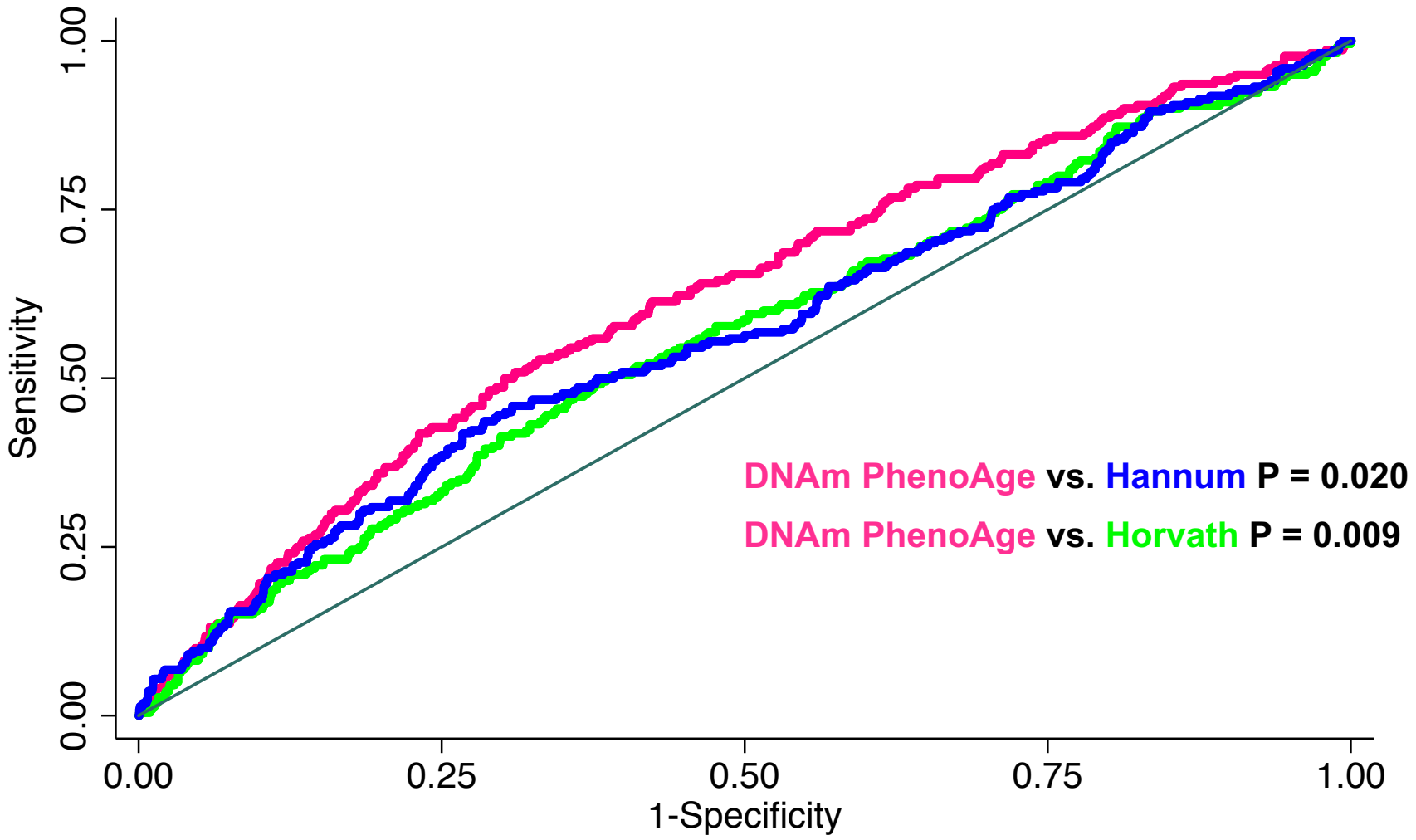
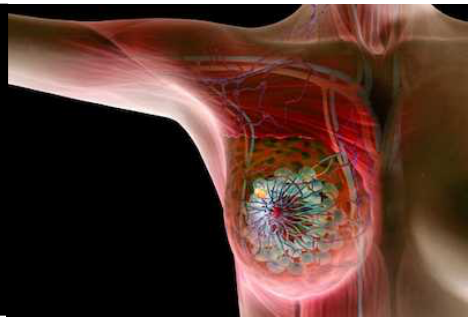
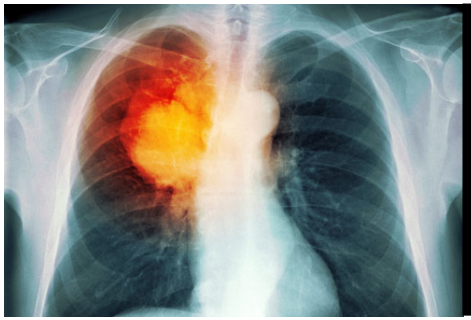


MORTALITY & MORBIDTY PREDICTIONS

Breast Cancer Incidence

(4% increased risk)





Lung Cancer Incidence

(10% increased risk)

Centenarian Offspring

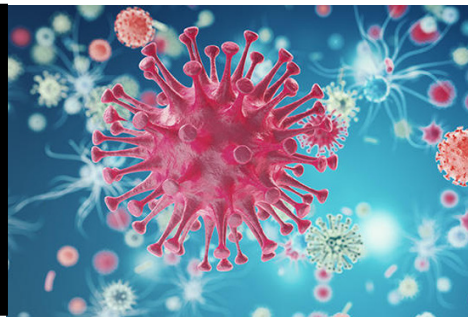
(2.4 years younger)

Down Syndrome

(5-12 years older)

HIV infection

(8 years older)



MCI

(2.4 years older)

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Underlying Biology of the Clock and the 513 CpGs

PRECIPITATING FACTORS

⇩ **Exercise**

⇩ **Females**

⇧ **Meat Consumption**

⇩ **Income**

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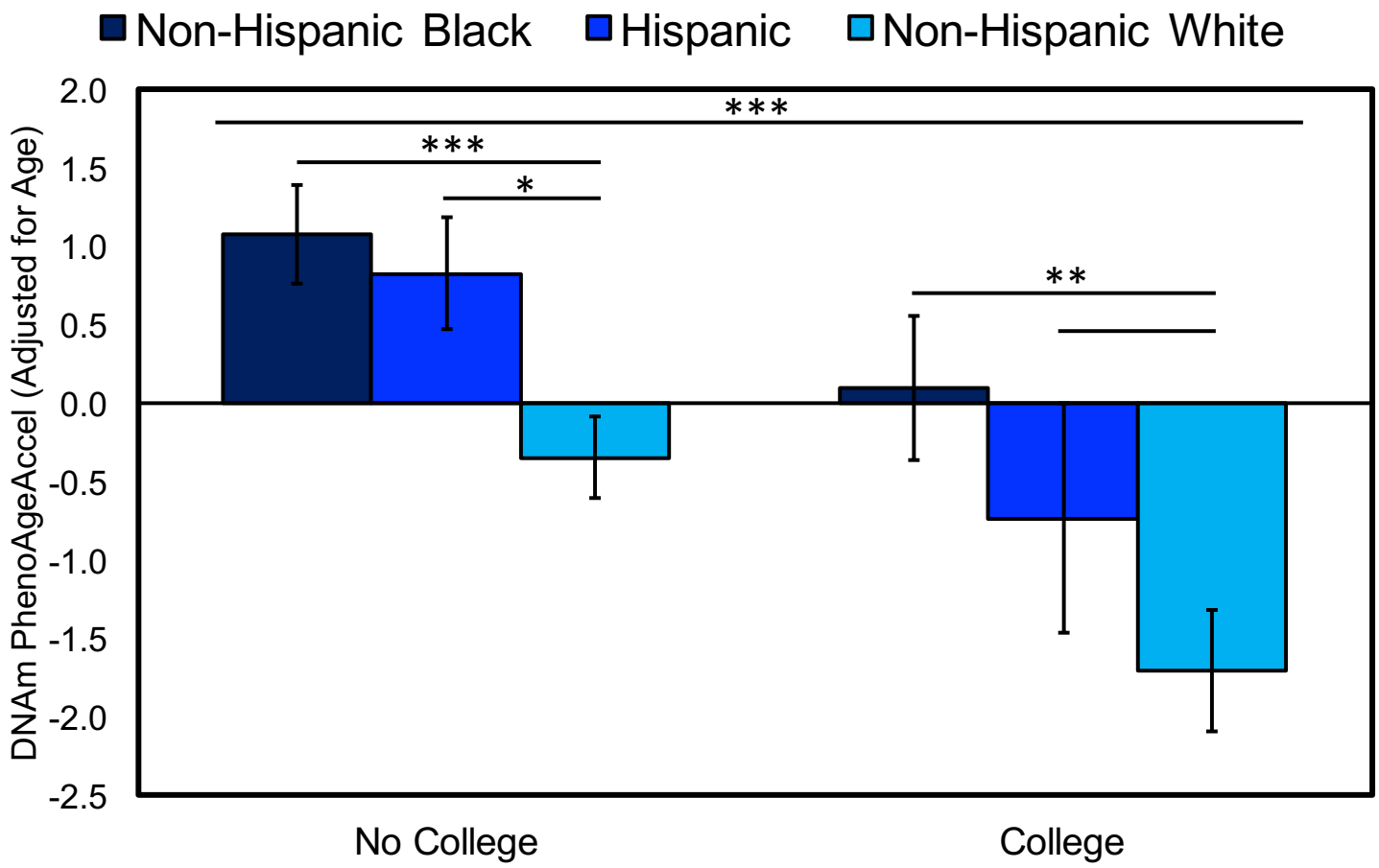
Race/Ethnicity and SES Relate to Differences in Epigenetic Age



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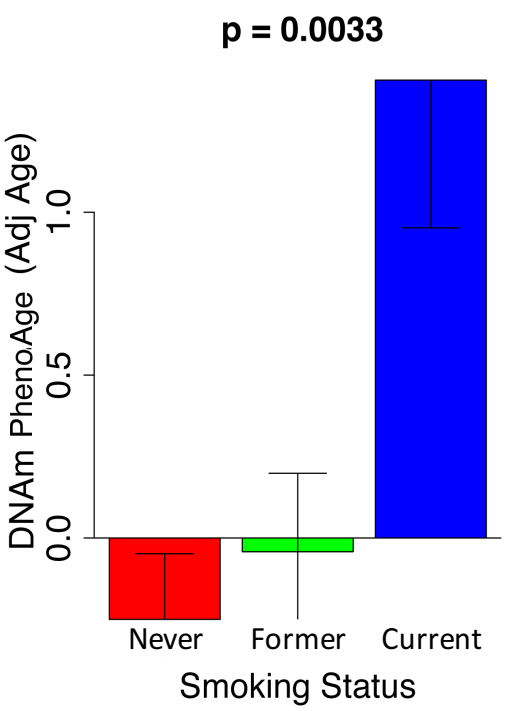
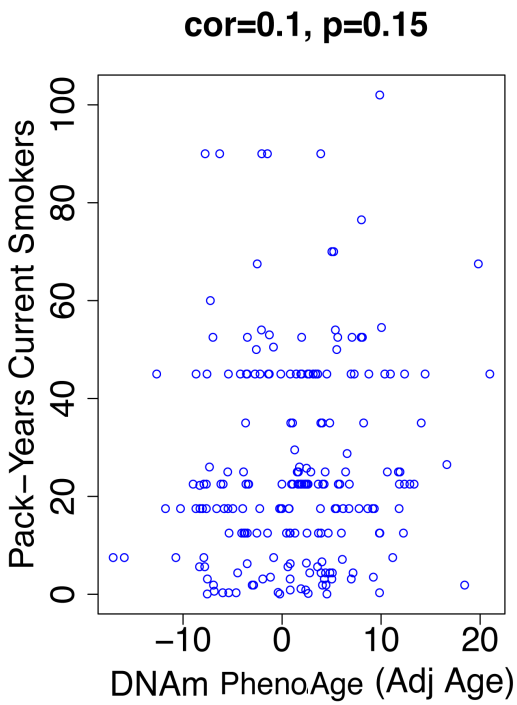
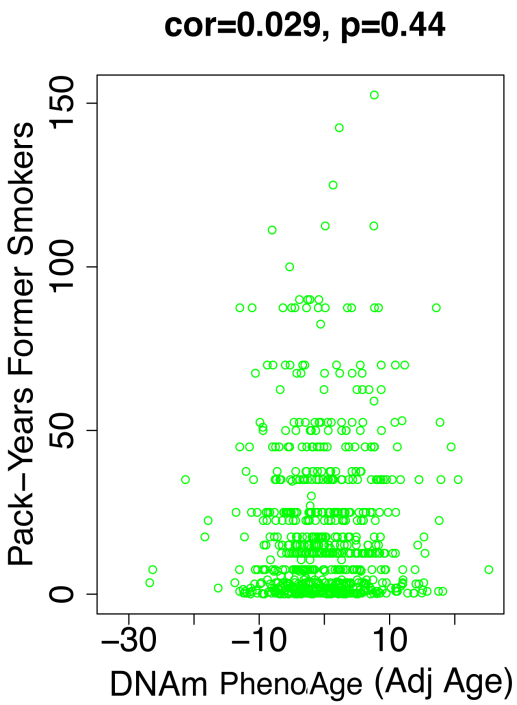
Smoking, but not pack-years is associated with higher DNAm PhenoAge.







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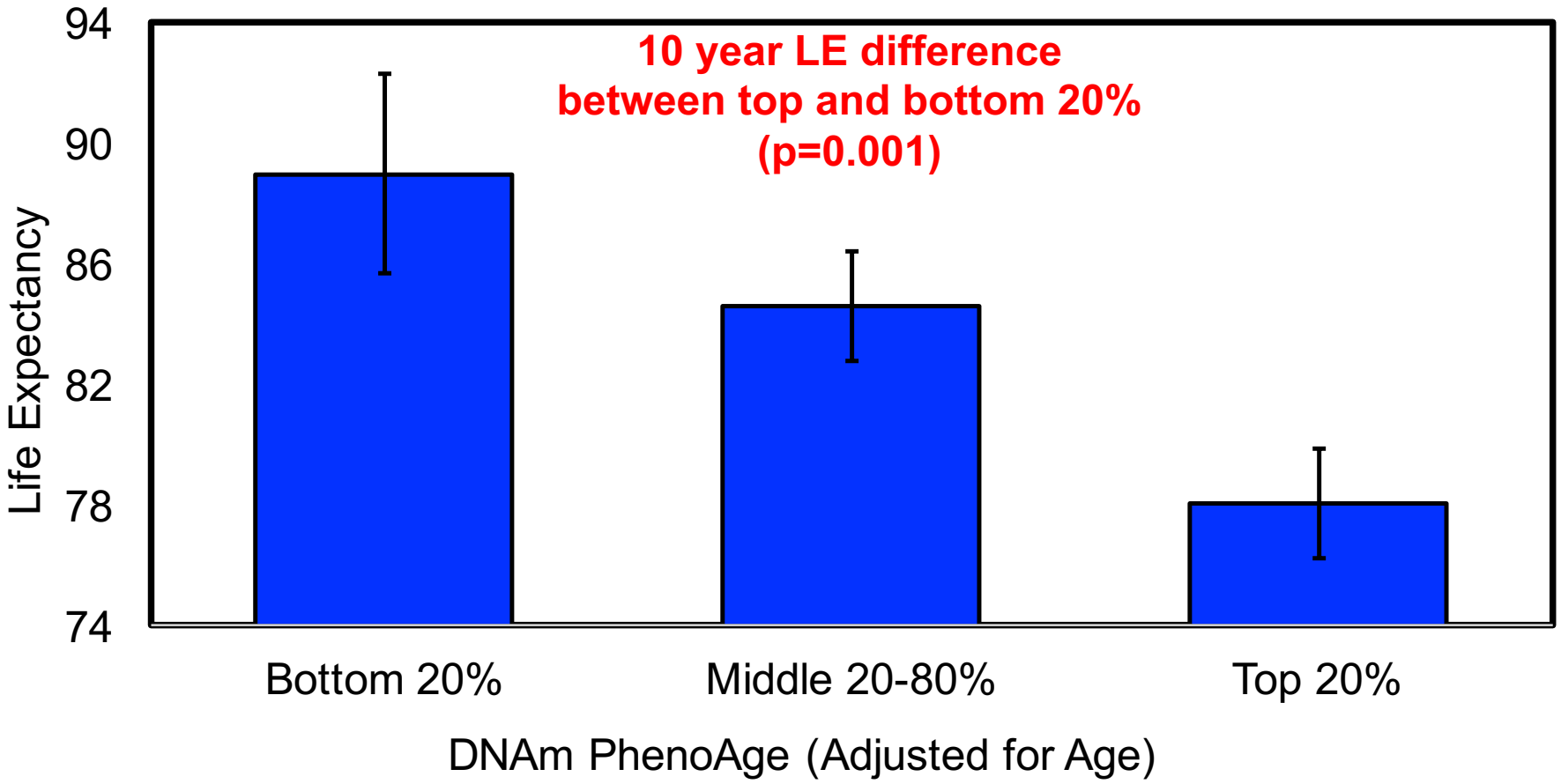
Does DNAm PhenoAge Capture Resilience?



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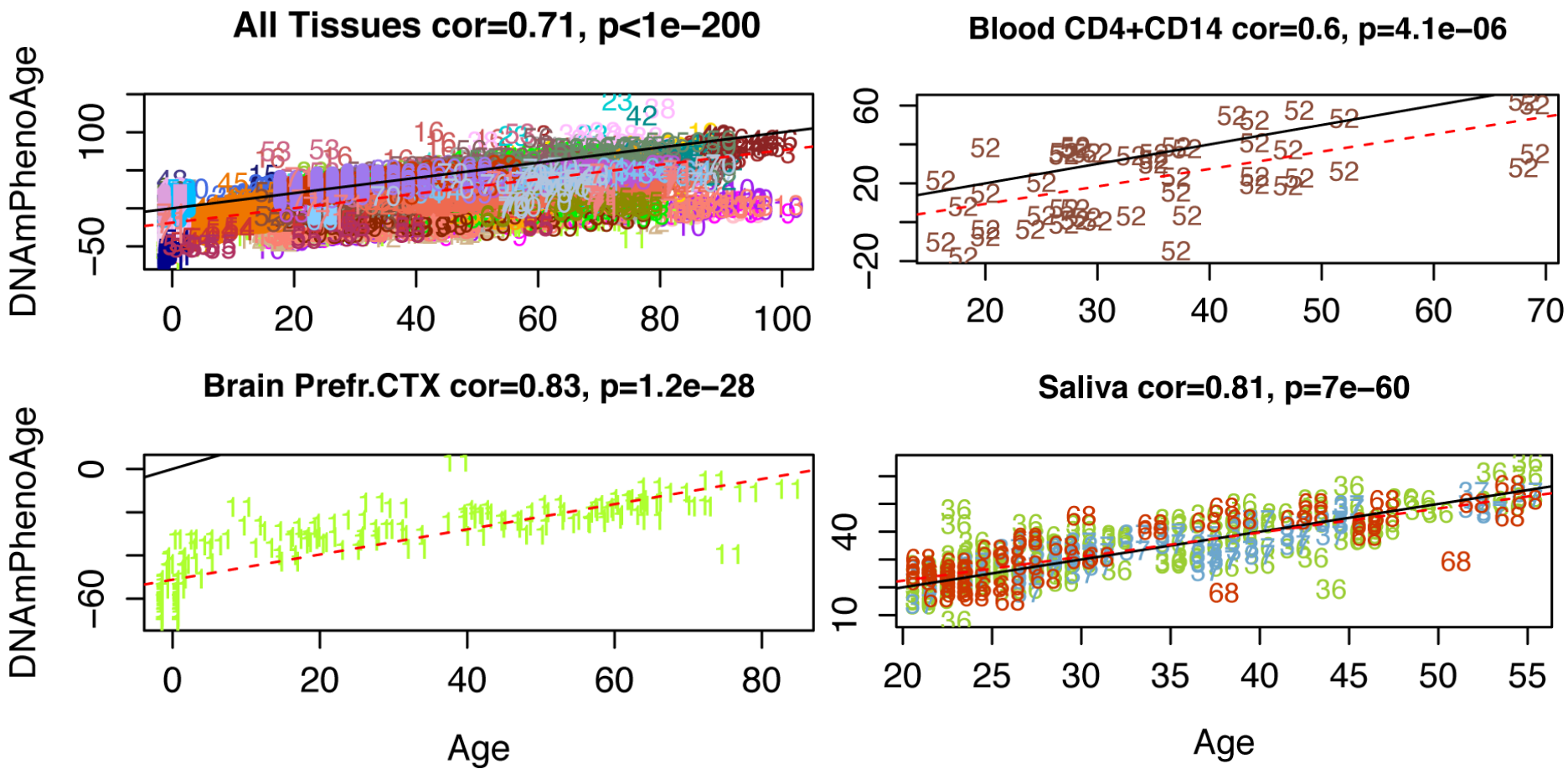

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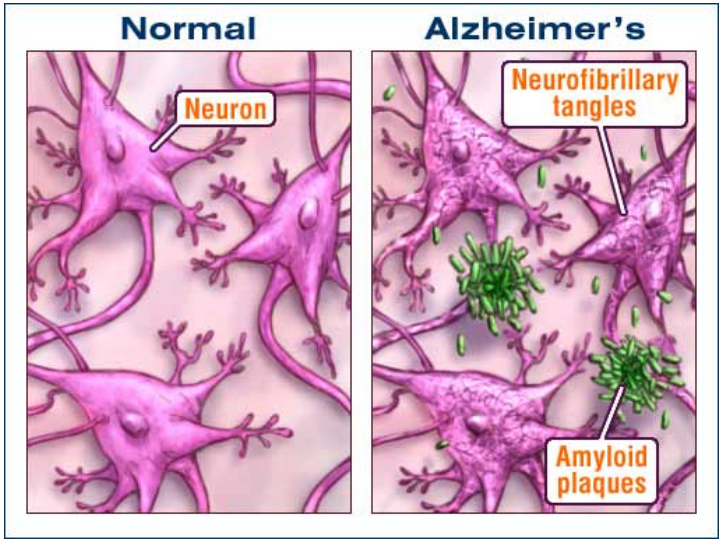

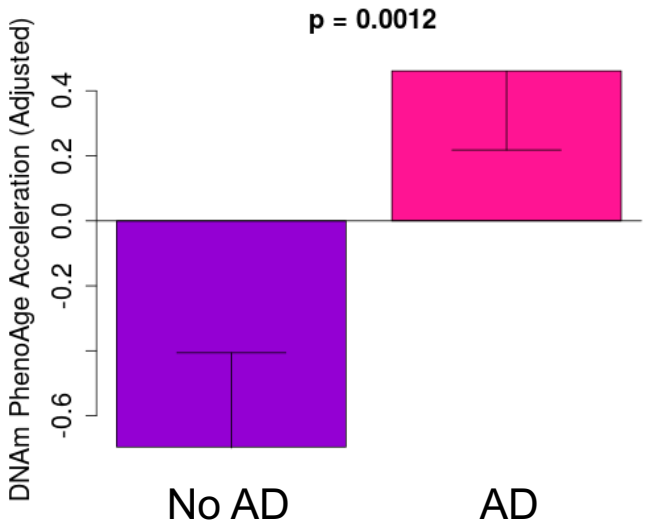

Multivariate Associations with DNAm PhenoAge	
	Beta (P-Value)
Amyloid Load	0.451 (0.004)
Neuritic Plaques	0.468 (0.004)
Diffuse Plaques	0.377 (0.021)
Neurofibrillary Tangles	0.100 (0.006)

Results are from independent multivariate models that adjust for age at death, study, and sex

Develop a multi-system estimate of "Phenotypic Age". Predictor of aging-related mortality based on clinical measures.	Validate Associations with:
	All-Cause Mortality
	Cause Specific Mortality
	Coexisting Disease Count
	Physical Functioning
	Mortality Ages 20-64
	Mortality Ages 65-79
	Mortality Ages 85+

Train a composite epigenetic predictor of phenotypic age, called "DNAm PhenoAge". Based on DNAm at 513 CpGs.	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)

Identify underlying biology of the 513 CpGs in the DNAm PhenoAge Score	Test for:	
	GO Enrichment	Immune Cell Associations
	Pathway Enrichment	CpG Overlap with Hannum/Horvath
	LTL Correlation	Loci-Specific DNAm vs. Transcription
	Differential Expression	Polycomb Group Protein Targets
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SNP HERITABILITY (h^2)

Defined as the total proportion of phenotypic variance attributable to genetic variation

h^2=0.38 to 0.54

CONCLUSIONS

1) Developed an aging biomarker that is predictive/relates to numerous multifactorial aging conditions and outcomes.
    - Better predictor than the Horvath & Hannum clocks
    - Predicts after adjusting for confounders (smoking, cell counts).
2) Variation in the residual relates to genetic, social, behavioral, and demographic factors.
3) Reliable age correlations in 35 different tissues.
4) Variations is non-blood tissues predict outcomes that are pathologically/physiologically related to that tissues.

NEXT STEPS

- Tissue Consensus WGCNA (group CpGs)
- Identify genetic determinants

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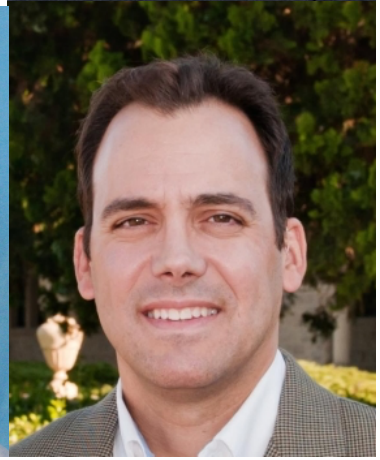
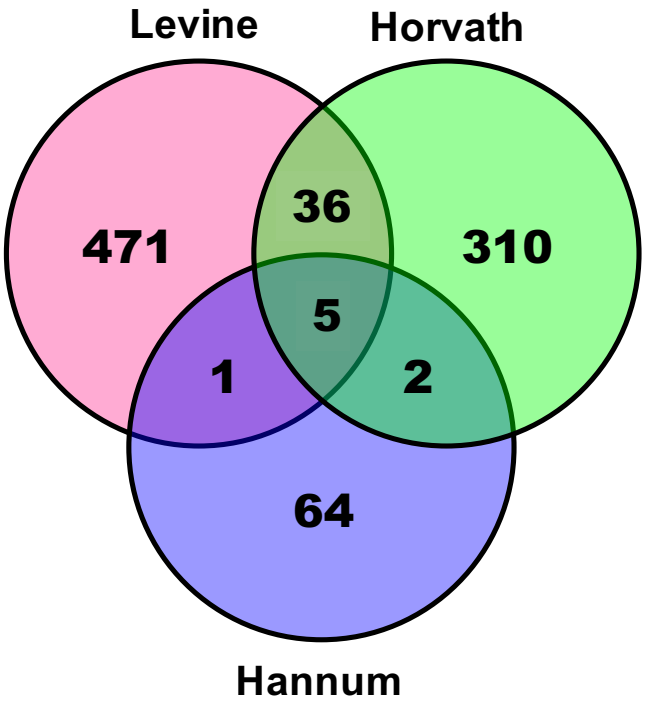


DEVELOPING A NEW EPIGENETIC CLOCK

Develop a Multisystem Phenotypic Age Estimate and Validate Predictions | **Develop a New Epigenetic Age Estimate and Validate Predictions/Associations** | Underlying Biology of the Clock and the 513 CpGs

	Levine DNAm Age	Horvath DNAm Age	Hannum DNAm Age
Levine DNAm Age	1	0.460	0.482
Horvath DNAm Age	0.460	1	0.511
Hannum DNAM Age	0.482	0.511	1



Only moderate correlations between the three clocks after adjusting for chronological age.

The clocks are not using the same CpGs.

They appear to be capture different phenomena.