TruDiagnostic™
The Epigenetic Company

Introduction to Epigenetic Methylation Biomarkers

About TruDiagnostic: Epigenetic Methylation Specialists

Over 35 institutional research board approved clinical trials

20,000 sq ft CLIA Lab 3 Custom Aging and Imprintome Arrays

Started in July of 2020. Have tested over 19,000 patients in that time with multi-omic covariates.

HARVARD MEDICAL SCHOOL
Cornell University
Duke

Collaborative Research with the top epigenetic research institutions

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What is Epigenetics?

What is Epigenetics?

Every cell in your body has the exact same DNA sequence.

So how do your cells act so differently?

It is due to what DNA expression is turned on and turn off!

What is Epigenetics?

Epigenetics is

"the branch of biology which studies the causal interactions between genes and their products which bring the phenotype into being."

- Conrad Waddington 1940s

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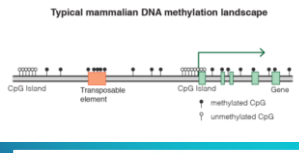
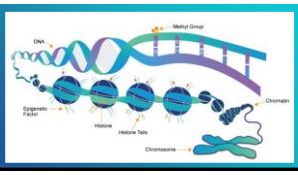
What is DNA Methylation?

DNA methylation is a biological process by which methyl groups are added to the DNA molecule. Methylation can change the activity of a DNA segment without changing the sequence.

When located in a gene promoter, DNA methylation typically acts to repress gene transcription

There are approximately 29 million locations in the genome where we can methylate and it is different in every cell.





What do we measure? How much is measured?

Definition of Beta-value and M-value

The Beta-value is the ratio of the methylated probe intensity and the overall intensity (sum of methylated and unmethylated probe intensities). Following the notation used by Illumina methylation assay [12], Beta-value for an i^{th} interrogated CpG site is defined as:

$$Beta_i = \frac{\max(y_{i,methy}, 0)}{\max(y_{i,unmethy}, 0) + \max(y_{i,methy}, 0) + \alpha} \quad (1)$$

where $y_{i,methy}$ and $y_{i,unmethy}$ are the intensities measured by the i^{th} methylated and unmethylated probes, respectively. To avoid negative values after background adjustment, any negative values will be reset to 0. Illu-

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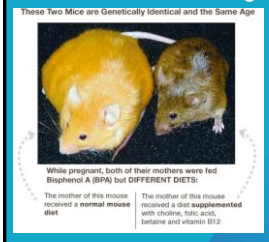
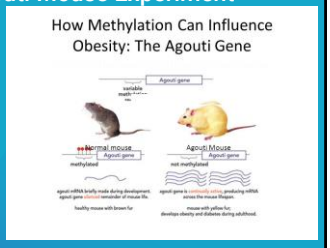
Why should we measure?
The Famous Agouti Mouse Experiment





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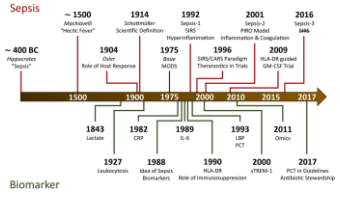
Methylation as a Biomarker and Treatment Target



Biomarkers have been defined as: "indicators of biological and pathogenic processes, or pharmacologic responses to a therapeutic intervention that defines what is normal while predicting or detecting what is abnormal."

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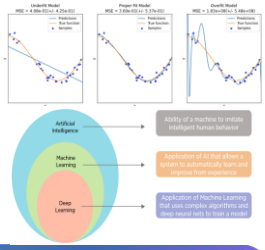
Methylation as a Biomarker

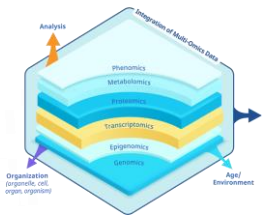
Measurement means nothing without interpretation of the data



What can Epigenetic Measurements Tell Us?

Tru's epigenetic methylation methodology can be trained to predict all other multi-omic values

The Multiome



Methylation is different in every cell and each cell has over 26 million CpG methylation locations.

Due to its large coverage and its place in the hierarchy of the multi-ome, it is able to be trained more accurately than most other multi-omic biomarkers to predict many different outcomes with a single test.

Beyond disease and phenotypes, methylation markers have been shown to predict prior exposures/lifestyle, future outcomes, and current behaviors

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Possibly, ONE test

One test to predict many chronic diseases, exposures and other omic biomarkers

TruDiagnostic becomes the best aging and disease predictor available on the market

... to replace MANY

Methylation trained to predict **health phenotypes (diagnostics, prognosis, status):**

- Efficacy and side effects response to medication
- Death prediction
- Biological aging rate
- Schizophrenia diagnosis
- Cancer (multiple types at as early as stage zero)
- Viral infections
- Immunodeficiencies

Methylation trained to predict **other omic biomarkers:**

- DNA epigenetic acetylation markers
- Serum albumin
- Adrenomedullin
- Beta-2-microglobulin
- CD56
- Cystatin C
- EGF ECM protein 1
- Leptin
- Myoglobin
- Plasminogen activator inhibitor
- Tissue inhibitor metalloproteinase
- Immune cell subsets
- Senescent T Cells
- SNP polymorphisms

Methylation trained to predict **exposures:**

- Aflatoxin B1
- Air pollution
- Arsenic
- Bisphenol A
- Cadmium
- Chromium
- Lead
- Mercury
- Polycyclic aromatic hydrocarbons
- Persistent organic pollutants
- Smoking status (active, former, current)
- Smoking pack years
- Current alcohol intake
- Radiation exposure
- Nutrient status

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Arthritis

Cognitive Decline

Cancer

Immune Decline

?

Kidney Disease

Type II Diabetes

Autoimmune Disorders

Cardiovascular Disease

What is the #1 risk factor for all of these conditions?

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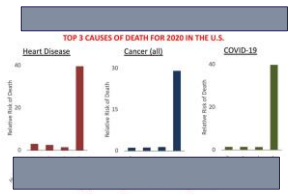

What is the #1 risk factor for all of these conditions?

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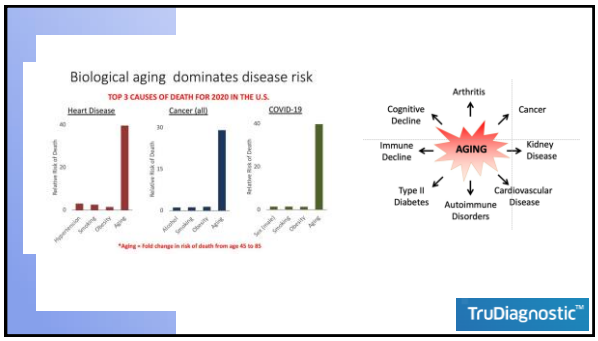

Aging As a Disease

Aging is the *#1 risk factor* for almost ALL chronic diseases.

However, delaying the aging rate by 7 years would *cut the incidence of disease in half!*

If everyone were to increase life expectancy by 1 year, we would save $256.7 billion and $8.8 trillion for +10 years life expectancy.

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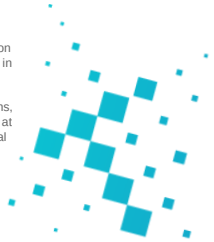
Since aging is the greatest risk factor for every disease, how can we appropriately measure it? Are there better methods than chronological age?

- Chronological age (CA) is a commonly used indicator for aging. However, life expectancy shows considerable variation among individuals with equal or similar CAs due to diversity in genotypes and in living habits and environments.
- A 50-year-old individual may have 60-year-old body functions, and many people look older or younger compared to others at the same CA (even in twins). Therefore, CA is not an optimal indicator for the aging progress.



The History Of Biological Age Measurements

During the past decades, extensive effort has been made to identify such aging biomarkers that, according to the stage-setting definition (Baker and Sprott, 1988), are "biological parameters of an organism that either alone or in some multivariate composite will, in the absence of disease, better predict functional capability at some late age, than will chronological age". Later on, the American Federation for Aging Research (AFAR) formulated the criteria for aging biomarkers as follows:

1. It must predict the rate of aging. In other words, it would tell exactly where a person is in their total life span. It must be a better predictor of life span than chronological age.

2. It must monitor a basic process that underlies the aging process, not the effects of disease.

3. It must be able to be tested repeatedly without harming the person. For example, a blood test or an imaging technique.

4. It must be something that works in humans and in laboratory animals, such as mice. This is so that it can be tested in lab animals before being validated in humans.

However, until 2013, no such marker or marker combination had emerged.

How does this compare to telomeres?

"Briefly, telomere length is extensively validated but has low predictive power."


EBioMedicine

Review

Biological Age Predictors

Jasilia Jylhävä, Nancy L. Pedersen, Sara Hägg *

We need a way to track age… Thankfully we finally have one. Epigenetics Clocks!

5 to 10 years from now, the health system that doesn't use this data to improve their medical delivery is going to be deemed archaic.

- Atul Butte,
Biomedical Informatics Researcher at Silicon Valley

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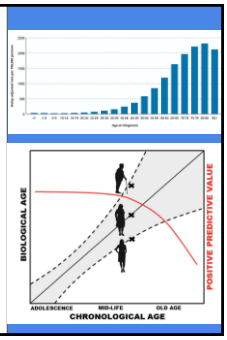
How should someone use Epigenetic Age Testing?

- Epigenetic Age = Biggest Risk Factor For Chronic Disease and Death
- Epigenetic Age = Major correlate of quality of life metrics like IQ, Balance, Muscle Mass, and Facial Aging
- Accelerated Aging = Shorter Lifespan and Healthspan
- Decelerated aging = Longer Lifespan and Healthspan
- Aging starts as soon as we are born. So aging optimization early can be a great preventative tool.

Why wouldn't everyone try and measure and treat their major risk factor for all disease? (especially if they are currently "healthy")



The Predictive Power of Epigenetic Aging: Cancer

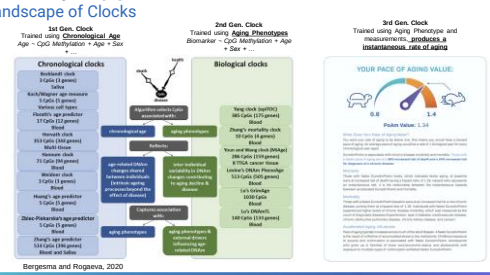
- Cancer is an age-related condition linked to epigenetic age acceleration
- *"For each one year increase in the difference between chronological and epigenetic age (the Δage), there was a 6% increased risk of developing cancer within three years and a 17% increased risk of dying of cancer in the next five years"* (Jylhävä et al 2017)
- *"Some cancers, tumours with fewer somatic mutations, indicative of more stable genomes, have greater epigenetic age acceleration"* (Horvath & Raj 2018)

TruDiagnostic's **Focus on Aging**

Patient Reports

The History of Epigenetic Clocks

Landscape of Clocks



Bergsma and Rogaeva, 2020

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We can also tell you your rate of aging!

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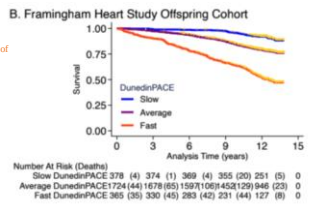
The DunedinPACE is the Most Predictive Clock

Dunedin Study members with methylation data at age 45, N = 817 had measured Pace of Aging (M = 0.99, SD = 0.30). This group formed the analysis sample to develop DunedinPACE.



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The DunedinPACE is the Most Predictive Clock

Time-to-Event Analysis of Mortality, Cardiovascular Disease (CVD) Diagnosis, and Stroke or Transient Ischemic Attack (TIA)

	Mortality			CVD			Stroke/TIA		
	HR	95% CI	p-value	HR	95% CI	p-value	HR	95% CI	p-value
DunedinPACE	1.54	[1.44–1.65]	6.44E-23	1.38	[1.23–1.54]	8.08E-10	1.42	[1.18–1.70]	1.57E-04
Horvath Clock	1.02	[0.95–1.10]	0.564	1.04	[0.95–1.14]	0.439	1.06	[0.90–1.27]	0.448
DunedinPACE	1.60	[1.43–1.78]	8.59E-17	1.34	[1.11–1.61]	1.73E-06	1.35	[1.13–1.61]	0.001
Hannum Clock	1.09	[1.01–1.17]	0.019	1.13	[1.02–1.25]	0.020	1.17	[1.00–1.36]	0.052
DunedinPACE	1.57	[1.48–1.75]	2.15E-15	1.35	[1.17–1.56]	9.15E-06	1.39	[1.17–1.65]	0.011
PhenoAge Clock	1.14	[1.03–1.25]	0.009	1.16	[1.00–1.35]	0.053	1.26	[1.03–1.60]	0.025
DunedinPACE	1.54	[1.12–1.98]	6.69E-05	1.18	[1.05–1.34]	0.007	1.32	[1.05–1.84]	0.020
GrimAge Clock	1.61	[1.49–1.74]	1.18E-33	1.33	[1.19–1.49]	5.74E-07	1.11	[0.91–1.35]	0.285

Repeated Measures Analysis of Incident Limitation in Activities of Daily Living (ADLs)

	Nagi ADLs			Katz ADLs			Rosow-Breslau ADLs		
	IRR	95% CI	p-value	IRR	95% CI	p-value	IRR	95% CI	p-value
DunedinPACE	1.58	[1.17–2.05]	1.49E-04	1.91	[1.14–3.78]	1.55E-03	1.40	[1.04–1.93]	2.96E-02
Horvath Clock	1.05	[0.88–1.26]	0.565	1.11	[0.98–1.26]	0.091	0.90	[0.80–1.01]	0.085
DunedinPACE	1.37	[1.14–1.64]	6.63E-04	1.90	[1.14–3.51]	0.07E-04	1.37	[1.20–1.57]	2.66E-06
Hannum Clock	1.08	[0.91–1.28]	0.381	1.08	[0.98–1.19]	0.167	1.04	[0.93–1.18]	0.562
DunedinPACE	1.48	[1.14–1.72]	0.001	1.26	[1.06–1.50]	0.007	1.43	[1.25–1.64]	1.14E-07
PhenoAge Clock	1.08	[0.77–1.38]	0.973	1.13	[0.95–1.41]	0.141	0.91	[0.81–1.07]	0.298
DunedinPACE	1.27	[1.03–1.58]	0.031	1.24	[1.02–1.54]	0.029	1.27	[1.08–1.50]	0.005
GrimAge Clock	1.18	[0.94–1.48]	0.158	1.18	[0.96–1.34]	0.157	1.15	[0.97–1.37]	0.098

DunedinPACE associations with health-span endpoints were little-changed by covariate adjustment for the Horvath, Hannum, or PhenoAge Clocks. In models adjusted for GrimAge, DunedinPACE associations with mortality, CVD, and disability were attenuated, but remained statistically different from zero (mortality HR = 1.24 [1.49–1.74], CVD HR = 1.18 [1.05–1.34], Nagi ADL IRR = 1.27 [1.02–1.58], Katz ADL IRR = 1.26 [1.02–1.54], Rosow-Breslau ADL IRR = 1.27 [1.08–1.50]); associations with stroke were similar to unadjusted models (HR = 1.33 [1.05–1.69]). Results for all models are reported in Supplementary file 1C. Thus, Dunedin PACE adds incremental prediction over and above all clocks studied here."

Nagi ADL Scale. Count of activities for which participants reported a lot of difficulty or inability to perform.

- Pulling or pushing large objects
- Stooping, crouching, or kneeling
- Reaching or extending arms below shoulder level
- Reaching or extending arms above shoulder level
- Writing, handling, or fingering small objects
- Standing in one place for long periods (15 minutes)
- Lifting for long periods (one hour)
- Lifting or carrying weights under 10 lbs
- Lifting or carrying weights over 10 lbs

Katz ADL Scale. Count of activities for which participants required assistance or could not do at all.

- Dressing
- Bathing
- Eating
- Transferring (getting in and out of a chair)
- Toileting

Rosow-Breslau ADL Scale. Count of activities participants were not able to do.

- Heavy work around the house
- Walk half a mile without assistance
- Walk up and down one flight of stairs

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The DunedinPACE is the Most Predictive Clock

Even just being slightly above an aging rate of 1 biological year/chronological year can increase your risk of death by 56% in the next 7 years and increase your risk of a chronic disease diagnosis by 54% over the next 7 years.

In fact, patients who were considered fast agers, were 16% more likely to die and 23% more likely to develop a chronic disease. That means they were 65% more likely to die in our cohorts than those at normal or slow aging.

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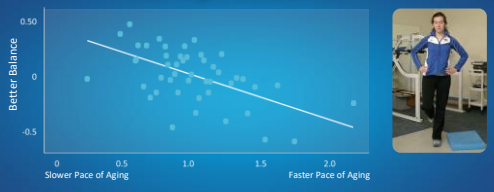
One-leg Balance Test



Pace of Aging 26 - 45 R = 0.36, p < 0.001

Each plotted point represents 20 study members

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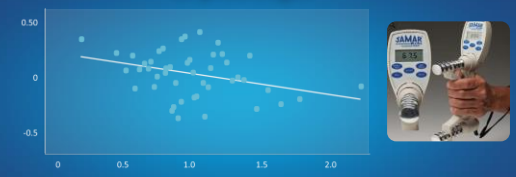
Grip Strength



Pace of Aging 26 - 45 R = 0.07, p = 0.033

Each plotted point represents 20 study members

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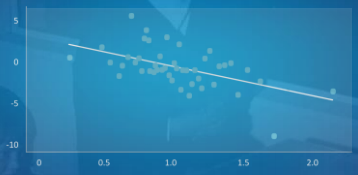
Cognitive Decline

(IQ Change from Childhood to Age 45)



Pace of Aging 26 - 45 R = 0.16, p < 0.001

Each plotted point represents 20 study members

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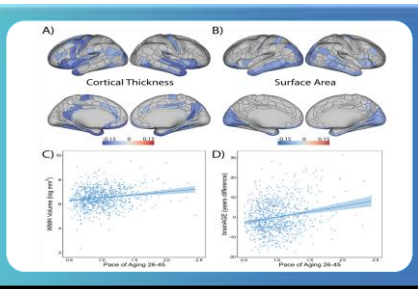
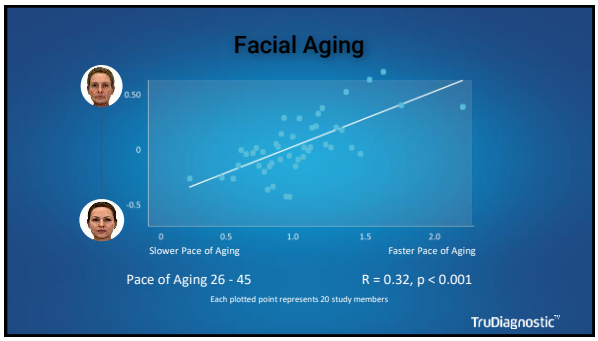
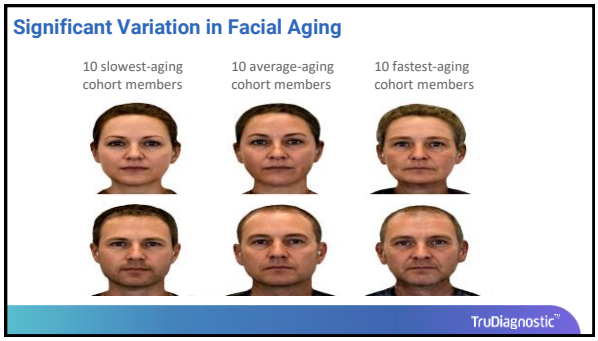
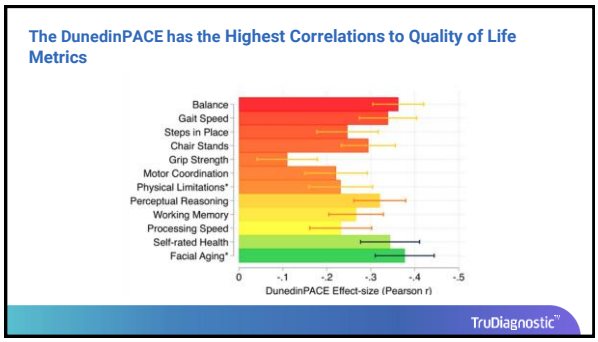
Cortical Thickness and Surface Area of the Brain




Facial Aging
0.50
0
-0.5
0
0.5
1.0
1.5
2.0
Slower Pace of Aging
Faster Pace of Aging
Pace of Aging 26 - 45
R = 0.32, p < 0.001
Each plotted point represents 20 study members
TruDiagnostic™


Significant Variation in Facial Aging
10 slowest-aging cohort members
10 average-aging cohort members
10 fastest-aging cohort members
TruDiagnostic™


The DunedinPACE has the Highest Correlations to Quality of Life Metrics
Balance
Gait Speed
Steps in Place
Chair Stands
Grip Strength
Motor Coordination
Physical Limitations*
Perceptual Reasoning
Working Memory
Processing Speed
Self-rated Health
Facial Aging*
0
-.1
-.2
-.3
-.4
-.5
DunedinPACE Effect-size (Pearson r)
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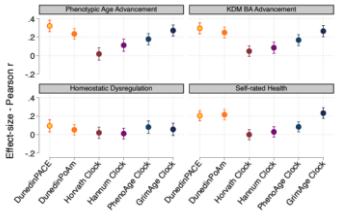
The DunedinPACE has the Highest Correlations to Quality of Life Metrics



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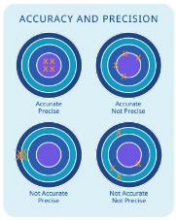
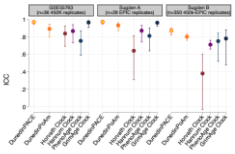
The DunedinPACE is the Most Precise Clock





ICC	Interpretation
<0.5	Poor agreement
0.5 to <0.75	Moderate agreement
0.75 to <0.9	Good agreement
0.9 - 1.0	Excellent agreement

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The DunedinPACE is modifiable by interventions which we already KNOW improve healthspan and lifespan

***This is EXTREMELY important

Biomarker Criteria	Horvath epigenetic age	Hannum epigenetic age	GrimAge	PhenoAge	DunedinPoAm
DNA Methylation Biomarker Calibrated to Detect:	Chronologic Age	Chronologic Age	Biomarkers, Smoking, Death	Phenotypic Age	Pace of Aging (change)
Feasible for use in a clinical trial in older adults?	✓	✓	✓	✓	✓
Robustly associated with chronological age across independent cohorts?	✓	✓	✓	✓	✓
Predict age-related change in function, chronic disease, or death?	✓	✓	✓	✓	✓
Responsive to interventions that beneficially affect the biology of aging?	--	--	--	--	--

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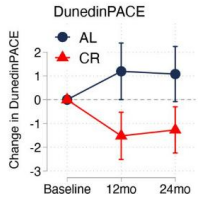
The DunedinPACE is modifiable by interventions which we already KNOW improve healthspan and lifespan



Change from baseline to 12- and 24-month follow-up in DunedinPACE (Pace of Aging) measures of aging in ad libitum (AL) and caloric restriction (CR) groups in CALERIE Trial (Waziry, R., et al.)

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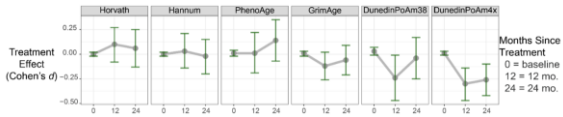
The DunedinPACE is modifiable by interventions which we already KNOW improve healthspan and lifespan

CALERIE RCT of caloric restriction (N=197)



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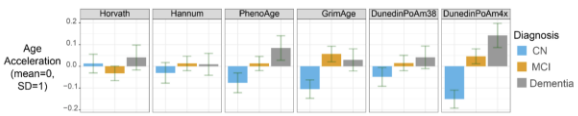
ADNI Study of ADRD (N=649)



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Leukocyte Telomere Length:
An Epigenetic Predictor
(accuracy/caveats)

Leukocyte DNAmTL (telomere length) is applicable across the entire age spectrum and is more strongly associated with age than measured leukocyte TL (LTL) **(*r* ~-0.75 for DNAmTL versus *r* ~ -0.35 for LTL).**

DNA methylation-based estimator of telomere length

Ake T. Lu,[1] Anne Seeboth,[2] Pei-Chien Tsai,[3,4,5] Dianjianyi Sun,[6,7] Austin Quach,[1] Alex P. Reiner,[8] Charles Kooperberg,[8] Luigi Ferrucci,[9] Lifang Hou,[10] Andrea A. Baccarelli,[11] Yun Li,[12] Sarah E. Harris,[13,14] Janie Corley,[13,14] Adele Taylor,[13,14] Ian J. Deary,[13,14] James D. Stewart,[15] Eric A. Whitsel,[15,16] Themistocles L. Assimes,[17,18] Wei Chen,[7] Shengxu Li,[19] Massimo Mangino,[3] Jordana T. Bell,[3] James G. Wilson,[20] Abraham Aviv,[21] Riccardo E. Marioni,[2,13] Kenneth Rai,[22,*] and Steve Horvath[23,23,*]

Epigenetic Leukocyte Telomere Length:
A More Accurate Predictor of Health Outcomes

Leukocyte DNAmTL outperforms LTL in predicting
- Time-to-death (p=2.5E-20)
- Time-to-coronary heart disease (p=6.6E-5)
- Time-to-congestive heart failure (p=3.5E-6)
- Association with smoking history (p=1.21E-17)

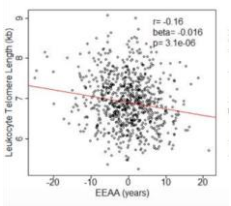
DNAmTL is not only an epigenetic biomarker of replicative history of cells, but a useful marker of age-related pathologies that are associated with it

Leukocyte Telomere Length:

Shorter LTL is associated with increased EEAA (r=-0.16, p=3.1x10-6). LTL is inversely related to proportions of memory CD8+ T cells (p=4.04x10-16) and positively related to proportions of naïve CD8+ T cells.

Blood that contains more memory CD8+ T cells and less naïve CD8+ T cells would display a relatively shorter LTL and older DNA methylation age.

EEAA is highly predictive of all-cause mortality. Epigenetic mortality risk is strongly associated with telomere length.



Better together!

From the previous slide we know that chronological clocks, phenotypic clocks, and telomere length don't really correlate well with each other meaning they represent different processes.

"Evidence that TL and epigenetic clock estimates are independent predictors of chronological age and mortality risk was obtained in the study by Marioni et al. (2018) performed in two Scottish cohorts aged from 70 to 90 years.

*In both cohorts studied, combined whole-blood TL and DNAm age explained more variance in age than each of them individually. **In a combined cohort analysis, TL and DNAm age explained 2.8 and 28.5% of the variance in age, respectively, and jointly they explained 29.5%.** Also in a combined cohort, one standard deviation increase in a baseline DNAm age was associated with a 25% increased mortality risk ($p < 0.001$) while in the same model, one standard deviation increase in a baseline TL was independently associated with an 11% reduced mortality risk only ($p = 0.05$)."*

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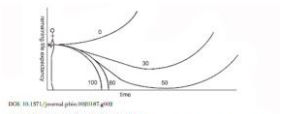
The Difficulty in Reversing Aging:



DOI: 10.1371/journal.pbio.1003187.g001

Figure 1. Physical and Actuarial Escape Velocities
Remaining life expectancy follows a similar trajectory whether one walks off a cliff or merely ages the time scales differ, but one's prognosis worsens with time. Mild mitigation of this (whether by jet propulsion or by rejuvenation therapies) merely postpones the outcome, but sufficiently aggressive intervention overcomes the force of gravity or frailty and increasingly distances the individual from a sticky end. Numbers denote plausible ages, at the time first-generation rejuvenation therapies arrive, of people following the respective trajectories.

At the moment, it is VERY difficult to make major differences in aging!

Aging is extremely complex and multi-factorial. While we would love to see these age markers decrease every time we test. This isn't likely. If it were, we would be immortal

We can SLOW this process for now.

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Case Studies

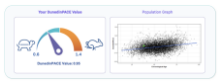
Epigenetic Age Reduction Case Study: Patient #1

Patient #1 first epigenetic age test was in September of 2020.

At a chronological age of 37.26, the patient had the following age metrics on the first test.

Patient implemented, Fisetin, Vitamin D, Caloric Restriction, and Stress maintenance exercises.

Patient Initials	Kit Number	Chronological Age	TL	PC Extrinsic	PC Intrinsic	DunedinPACE
ANON-1	#1	37.26	7.186274	36.65	51.746611	1.01



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The DunedinPACE is modifiable by interventions which we already KNOW improve healthspan and lifespan

***This is EXTREMELY important

Biomarker Criteria	Horvath epigenetic age	Hannum epigenetic age	GrimAge	PhenoAge	DunedinPoAm
DNA Methylation Biomarker Calibrated to Detect:	Chronologic Age	Chronologic Age	Biomarkers, Smoking, Death	Phenotypic Age	Pace of Aging (change)
Feasible for use in a clinical trial in older adults?	✓	✓	✓	✓	✓
Robustly associated with chronological age across independent cohorts?	✓	✓	✓	✓	✓
Predict age-related change in function, chronic disease, or death?	✓	✓	✓	✓	✓
Responsive to interventions that beneficially affect the biology of aging?	--	--	--	--	--

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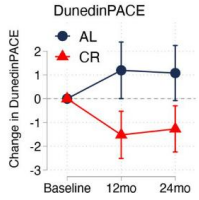
The DunedinPACE is modifiable by interventions which we already KNOW improve healthspan and lifespan



Change from baseline to 12- and 24-month follow-up in DunedinPACE (Pace of Aging) measures of aging in ad libitum (AL) and caloric restriction (CR) groups in CALERIE Trial (Waziry, R., et al.)

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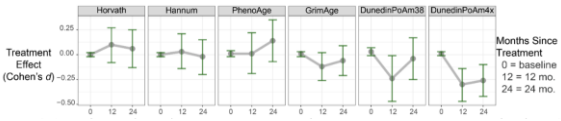
The DunedinPACE is modifiable by interventions which we already KNOW improve healthspan and lifespan

CALERIE RCT of caloric restriction (N=197)



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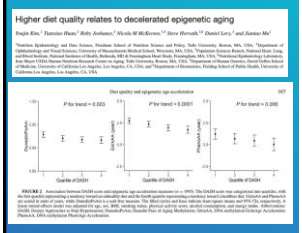

Higher diet quality relates to decelerated epigenetic aging

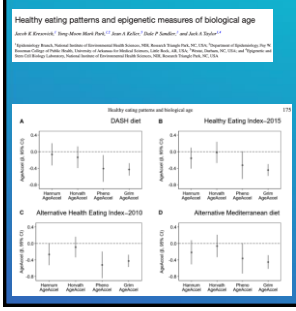
Major Diet Recommendations:
Diet Quality Matters

We calculated the DASH score based on 8 dietary components: higher intake of vegetables, fruits, nuts and legumes, whole grains, and low-fat dairy and lower intake of red and processed meat, sugar-sweetened beverages, and sodium.

Results: A higher DASH score was associated with lower levels of:

- DunedinPoAm (β = −0.05; SE = 0.02; P = 0.007),
- GrimAA (β = −0.09; SE = 0.02; P < 0.001),
- PhenoAA (β = −0.07; SE = 0.02; P = 0.001).

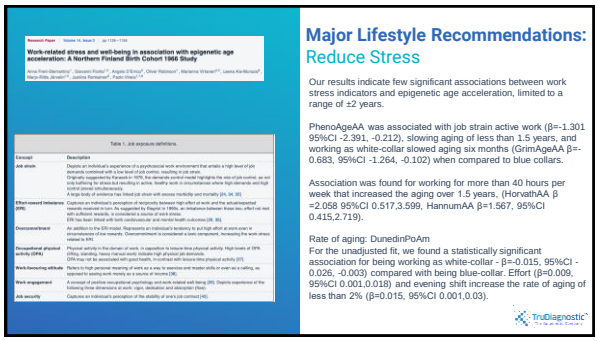
All 3 epigenetic measures mediated the association between the DASH score and all-cause mortality, with mean proportions of 22.1% for DunedinPoAm (Pmediation = 0.04), 45.1% for GrimAA (Pmediation = 0.001), and 22.9% for PhenoAA (Pmediation = 0.03).


Healthy eating patterns and epigenetic measures of biological age

Major Diet Recommendations:
Diet Quality Matters

Results: All 4 healthy eating indexes had inverse associations with epigenetic age acceleration, most notably with PhenoAgeAccel and GrimAgeAccel. Of these, the strongest associations were for HEI [per 1-SD increase in diet quality, PhenoAgeAccel β = −0.5 y (95% CI: −0.8 to −0.2 y) and GrimAgeAccel β = −0.4 y (95% CI: −0.6 to −0.3 y)]. Although effect modification was not observed for most lifestyle factors, in analyses stratified by physical activity, the benefits of a healthy diet on epigenetic age acceleration were more pronounced among women who did not meet physical activity guidelines (reporting <2.5 h/wk of exercise).

Conclusions: Higher diet quality is inversely associated with methylation-based measures of biological age. Improving diet could have the most benefits in lowering biological age among women with lower levels of physical activity



Major Lifestyle Recommendations:
Reduce Stress

Our results indicate few significant associations between work stress indicators and epigenetic age acceleration, limited to a range of ±2 years.

PhenoAgeAA was associated with job strain active work (β=-1.301 95%CI -2.391, -0.212), slowing aging of less than 1.5 years, and working as white-collar slowed aging six months (GrimAgeAA β=-0.683, 95%CI -1.264, -0.102) when compared to blue collars.

Association was found for working for more than 40 hours per week that increased the aging over 1.5 years, (HorvathAA β =2.058 95%CI 0.517,3.599, HannumAA β=1.567, 95%CI 0.415,2.719).

Rate of aging: DunedinPoAm
For the unadjusted fit, we found a statistically significant association for being working as white-collar - (β=-0.015, 95%CI -0.026, -0.003) compared with being blue-collar. Effort (β=0.009, 95%CI 0.001,0.018) and evening shift increase the rate of aging of less than 2% (β=0.015, 95%CI 0.001,0.03).

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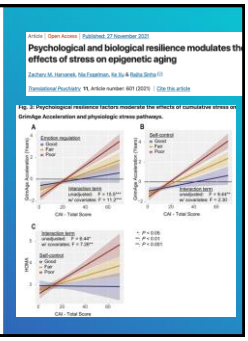
Epigenetic Age Acceleration:
Lifetime Stress

Cumulative stress was associated with accelerated GrimAge ($P = 0.0388$) and stress-related physiologic measures of adrenal sensitivity (Cortisol/ACTH ratio) and insulin resistance (HOMA). After controlling for demographic and behavioral factors, HOMA correlated with accelerated GrimAge ($P = 0.0186$).
Remarkably, psychological resilience factors of emotion regulation and self-control moderated these relationships. Emotion regulation moderated the association between stress and aging ($P = 8.82e{-}4$) such that with worse emotion regulation, there was greater stress-related age acceleration, while stronger emotion regulation prevented any significant effect of stress on GrimAge. Self-control moderated the relationship between stress and insulin resistance ($P = 0.00732$), with high self-control blunting this relationship. In the final model, in those with poor emotion regulation, cumulative stress continued to predict additional GrimAge Acceleration even while accounting for demographic, physiologic, and behavioral covariates.

These results demonstrate that **cumulative stress is associated with epigenetic aging in a healthy population, and these associations are modified by biobehavioral resilience factors.**



Epigenetic Age Acceleration:
Relaxing Treatment

"Moreover, we compare DNAmAge with another mechanism of biological age, leucocyte telomere length (LTL) and telomerase. DNAmAge is reduced after training in healthy subjects ($p = 0.053$), but not in patients. LTL is preserved after intervention in healthy subjects, while it continues to decrease in patients ($p = 0.051$).
The conventional negative correlation between LTL and chronological age becomes positive after training in both patients ($p < 0.01$) and healthy subjects ($p < 0.05$). In our subjects, DNAmAge is not associated with LTL.

Our findings would suggest that intensive relaxing practices influence different aging molecular mechanisms, i.e., DNAmAge and LTL, with a rejuvenating effect.

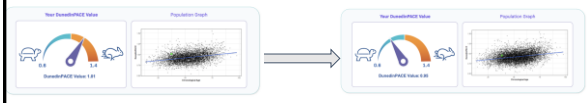
Our study reveals that DNAmAge may represent an accurate tool to measure the effectiveness of lifestyle-based interventions in the prevention of age-related diseases."

Epigenetic Age Reduction Case Study: Patient #1

Patient #1 first epigenetic age test was in September of 2020.

At a chronological age of 37.26, the patient had the following age metrics on the first test.

Patient implemented, Fisetin, Vitamin D, Caloric Restriction, and Stress maintenance exercises.



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Epigenetic Age Reduction Case Study: Patient #1

Patient #1 first epigenetic age test was in September of 2020.

At a chronological age of 37.26, the patient had the following age metrics on the first test.

The patient tested again .93 years later.

Patient implemented, Fisetin, Vitamin D, Caloric Restriction, and Stress maintenance exercises.

We saw Age reduction in each marker!

Patient Initials	Kit Number	Chronological Age	TL	PC Extrinsic	PC Intrinsic	DunedinPACE
ANON-1	#1	37.26	7.18	36.65	51.746611	1.01
ANON-2	#2	38.19	7.20	37.19	51.96	.95
Delta		**.93**	**.02**	**0.54**	**0.213389**	**-0.06**

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Diabetes has many markers in the Methylome

Loci Specific methylation can yield clinical insight.

> Epigenetics. 2016 Jul 2;11(7):482-8. doi: 10.1080/15592294.2016.1178418. Epub 2016 May 5.

DNA methylation of loci within ABCG1 and PHOSPHO1 in blood DNA is associated with future type 2 diabetes risk

Tasnim Dayeh [1], Tinamaja Tuomi [2, 3, 4], Petter Almgren [2], Alexander Perfilyev [1], Per-Anders Jansson [5], Vanessa D de Mello [6], Jussi Pihlajamäki [6, 7], Allan Vaag [8], Leif Groop [4], Emma Nilsson [1], Charlotte Ling [1]

> Epigenomics. 2019 Jun;11(8):885-897. doi: 10.2217/epi-2018-0159. Epub 2019 Jun 6.

Critical evaluation of the DNA-methylation markers ABCG1 and SREBF1 for Type 2 diabetes stratification

Christin Krause [1], Helen Sievert [1], Cathleen Geißler [1], Martina Grohs [1], Alexander T El Gammal [2], Stefan Wolter [2], Olena Ohlei [2], Fabian Kilpert [3], Ulrike M Krämer [4, 5], Meike Kasten [6, 7], Christina Klein [8], Georg E Brabant [1], Oliver Mann [2], Hendrik Lehnert [1, 9], Henriette Kirchner [1, 9]

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Diabetes Risk Report

DNA methylation at the ABCG1 locus cg06500161 in blood DNA was associated with a 9% increased risk for future T2D (OR = 1.09, 95% CI = 1.02-1.16, P-value = 0.007, Q-value = 0.018)

DNA methylation at the PHOSPHO1 locus cg02650017 in blood DNA was associated with a decreased risk for future T2D (OR = 0.85, 95% CI = 0.75–0.95, P-value = 0.006, Q-value = 0.018) after adjustment for age, gender, fasting glucose, and family relation.

A DNA methylation score of 5.0% or greater at the PHOSPHO1 locus cg02650017 in blood DNA was associated with a 15% decreased risk for future type 2 diabetes occurrence.

Your DNA methylation score at ABCG1 locus cg06500161 gives an indication of your level of risk for type 2 diabetes; if your score is 70.1% or greater it is associated with a 9% increased risk for future type 2 diabetes occurrence.

Response to Caloric Restricton

	CpG site	Gene	ß-value Responders	Your Score	Response Status
1	cg15500865	PON3	0.072	0.83	Hypomethylated
2	cg25161512	PON3	0.115	0.111	Hypomethylated
3	cg11435506	PON3	0.165	0.161	Hypomethylated
4	cg03301582	PON3	0.120	0.117	Hypomethylated
5	cg08898155	PON3	0.163	0.167	Hypermethylated
6	cg04080282	PON3	0.324	0.321	Hypomethylated
7	cg26457160	PON3	0.490	0.494	Hypermethylated
8	cg10329418	PON3	0.252	0.250	Hypomethylated
9	cg27188921	PON3	0.253	0.251	Hypomethylated
10	cg24750391	PON3	0.355	0.359	Hypermethylated
11	cg08461772	PON3	0.418	0.417	Hypomethylated

YOUR EPITYPE:

Based on your methylation, you are a **non-responder** for weight-loss interventions using caloric restriction.

Possible Outcomes:

Non-Responder: CpG loci around your PON3 gene are generally under-methylated. Your response to caloric restriction alone is low, so it is not likely to be the most effective form of weight loss.

Intermediate Responder: CpG loci around your PON3 gene are in the normal range. Using a calorie deficit diet for weight loss may work, but it may not be as successful as other therapies.

Full Responder: CpG loci around your PON3 gene have been hypermethylated, so calorie restriction as a method of weight loss should be very effective.

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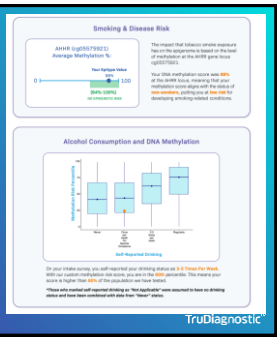
Smoking and Drinking Reports

This can be used as a good metric to measure patient adherence.



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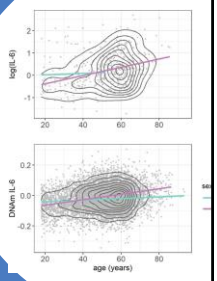
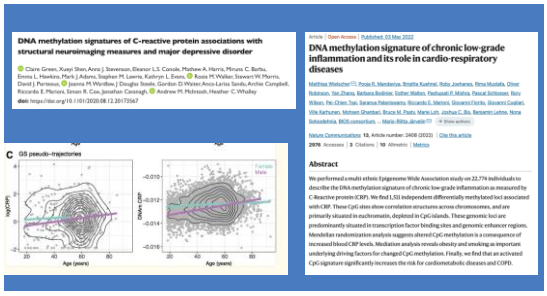
Inflammatory Marker Reports:

The utility of this proxy phenotype is particularly evident for studies in which DNAm is quantified but for which IL-6 is unavailable. Additionally, utilizing a composite methylation score that integrates information from multiple sites could potentially provide a more reliable estimate of chronic inflammation, allowing for clearer insight when assessing its relation to health outcomes.

DNAm is a proxy for the proinflammatory cytokine IL-6 that associates with pertinent health, lifestyle, and cognitive outcomes





DNA methylation signatures of C-reactive protein associations with structural neuroimaging measures and major depressive disorder

Clare Green, Xueyi Shen, Anna J. Stevenson, Eleanor L.S. Conole, Mathew A. Harris, Miruna C. Barbu, Emma L. Hawkins, Mark J. Adams, Stephen M. Lawrie, Kathryn L. Evans, Rosie M. Walker, Stewart W. Morris, David J. Porteous, Joanna M. Wardlaw, J. Douglas Steele, Gordon D. Waiter, Anca-Larisa Sandu, Archie Campbell, Riccardo E. Marioni, Simon R. Cox, Jonathan Cavanagh, Andrew M. McIntosh, Heather C. Whalley

doi: https://doi.org/10.1101/2020.08.12.20173567

C GS pseudo-trajectories

DNA methylation signature of chronic low-grade inflammation and its role in cardio-respiratory diseases

Abstract

We performed a multi-ethnic Epigenome-Wide Association study on 22,774 individuals to describe the DNA methylation signature of chronic low-grade inflammation as measured by C-Reactive protein (CRP). We find 1,511 independent differentially methylated loci associated with CRP. These CpG sites show correlation structures across chromosomes, and are primarily situated in euchromatin, depleted in CpG islands. These genomic loci are predominantly situated in transcription factor binding sites and genomic enhancer regions. Mendelian randomization analysis suggests altered CpG methylation is a consequence of increased blood CRP levels. Mediation analysis reveals obesity and smoking as important underlying driving factors for changed CpG methylation. Finally, we find that an activated CpG signature significantly increases the risk for cardiometabolic diseases and COPD.