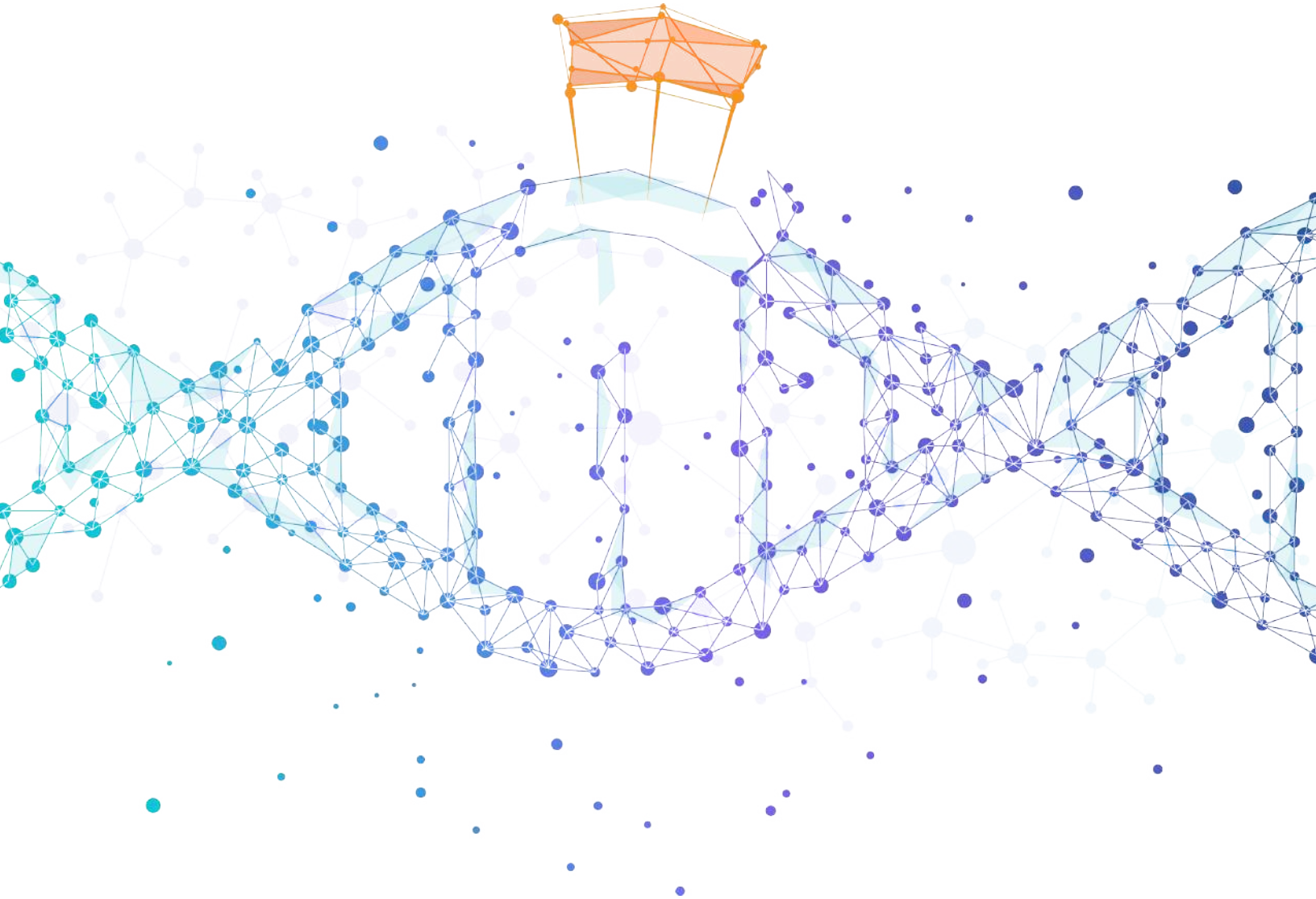
TruDiagnostic™
The Epigenetic Company

AIWO

Reduce Biological Age, Improve Health Span

SAMPLE REPORT



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PROVIDED BY: TruDiagnostic

OMICm Age

*Developed with Harvard**

38.59
YEARS OLD
OMIC*m* Age

Calendar Age 27.20

Biological Age 38.59

Your OMICm Age is

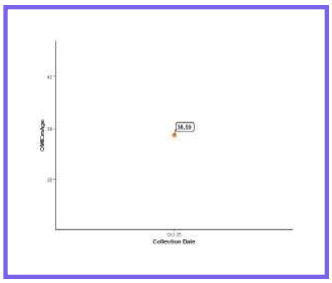
HIGHER THAN

your calendar age by **11.39** years.

YOUR OMIC*M* Age IS IN THE:

71.14st

PERCENTILE MEANING THAT YOUR OMIC*M* AGE IS HIGHER THAN **71.14%** OF THE POPULATION AT YOUR SAME CHRONOLOGICAL AGE.



Aging has been scientifically proven to be the number one risk factor for major chronic diseases world-wide. Accelerated aging (having an older biological age than your calendar age) increases your **risk of disease with each year of discrepancy,** and having a younger biological age decreases these risks. Based on age, we can predict the following increase or decreased risk of Death, Cancer, Heart Disease, Stroke, Type 2 Diabetes, COPD, and Depression.

YOUR RISK OF DISEASE

-28% Disease Risk — Reflects your current risk. A -28% score means that your risk is 28% lower compared to people of your same chronological age.

47% Disease Risk — Reflects your current risk. A 47% score means that your risk is 47% higher compared to people of your same chronological age.

31 — Reflects your **potential risk** score based on potential changes to your biological age.

DEATH

-24.02% | -1 YEAR | -12.99% Disease Risk | -0.35% | +1 YEAR

CANCER

-11.62% | -1 YEAR | -6.06% Disease Risk | -0.16% | +1 YEAR

HEART DISEASE

-13.79% | -1 YEAR | -7.24% Disease Risk | -0.19% | +1 YEAR

STROKE

-12.79% | -1 YEAR | -6.69% Disease Risk | -0.17% | +1 YEAR

TYPE 2 DIABETES

-11.79% | -1 YEAR | -6.15% Disease Risk | -0.16% | +1 YEAR

COPD

-7.91% | -1 YEAR | -4.08% Disease Risk | -0.1% | +1 YEAR

DEPRESSION

-7.67% | -1 YEAR | -3.96% Disease Risk | -0.1% | +1 YEAR

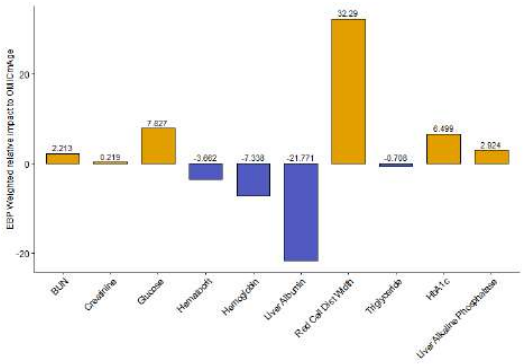
COLLECTED: 10/25/2023 | REPORTED: 11/12/2023

PROVIDED BY: TruDiagnostic

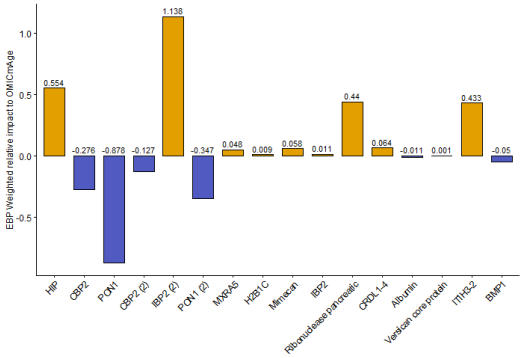
The Epigenetic Biomarker Proxies Driving Your Biological Age

We use epigenetic biomarker proxies to predict genomics, transcriptomics, proteomics, and metabolomics sum values that are positive for your aging, and some that are negative for your aging. In the graph below you will see the factors contributing to your aging the most. If a bar is above zero, its increasing your OMICm Age, if below zero, it is decreasing your OMICm Age.

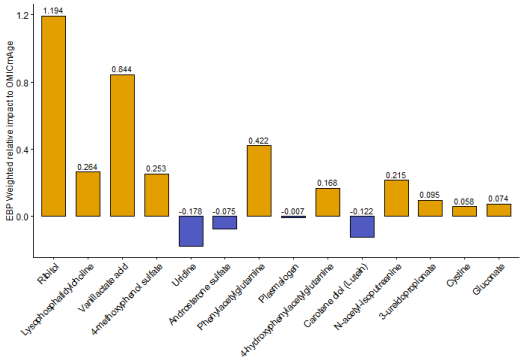
Your Clinical Epigenetic Biomarker Proxies (EBP)



Your Protein Epigenetic Biomarker Proxies (EBP)



Your Metabolites Epigenetic Biomarker Proxies (EBP)



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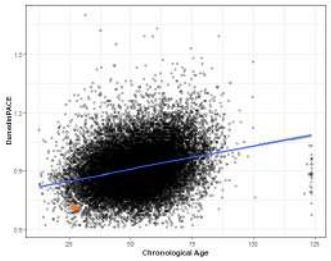
DunedinPACE of Aging

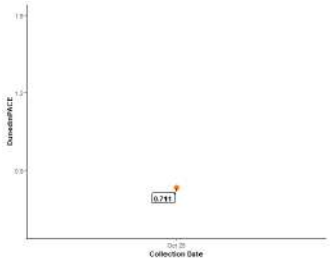
DunedinPACE Value

DunedinPACE Value
0.71

Population

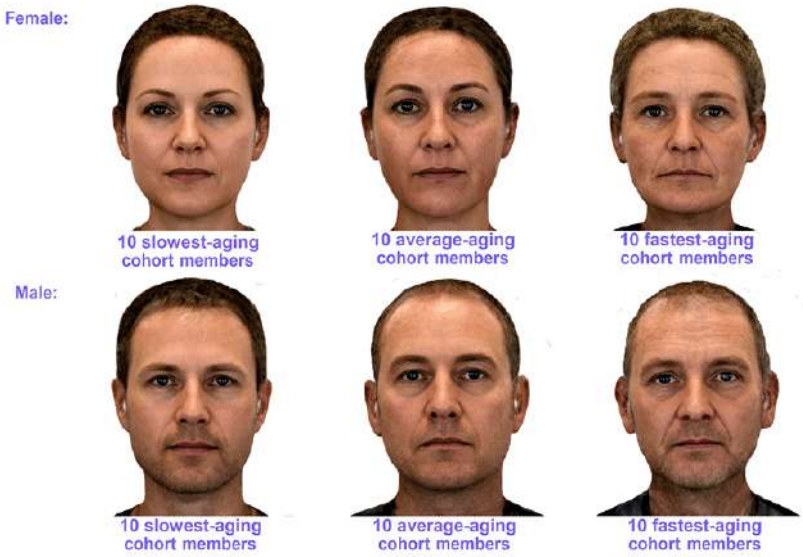


Changes Over Time



ALGORITHM	PATIENT DATA	MORBIDITY AND MORTALITY ASSOCIATIONS	RISK STATEMENT
DunedinPACE	0.71 Biological years per year	**All-Cause Mortality** (Beslsky et al., 2020)	If you are aging above a rate of 1.00, you would increase risk of death by 56% over the next 7 years.
		Chronic Disease (Beslsky et al., 2020)	If you are aging above a rate of 1.00, you would increase risk of chronic disease diagnosis by 54% over the next 7 years.

Significant Variation in Facial Aging



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Immune Health

IMMUNE CELL TYPE	95% CONFIDENCE INTERVAL RANGE	YOUR PERCENTAGE	MEAN	SD	# OF STANDARD DEVIATIONS ABOVE OR BELOW MEAN	IS THIS HIGHER OR LOWER THAN ANTICIPATED?
Naïve CD4T	7.196%-7.35%	9.27%	7.273	0.0383	0.52	**Higher**
Memory CD4T	5.14%-5.284%	0.00%	5.212	0.0361	-1.44	**Lower**
Memory CD8T	6.519%-6.691%	7.46%	6.605	0.0430	0.20	**Higher**
Naïve CD8T	1.09%-1.16%	1.25%	1.125	0.0175	0.07	**Higher**
Basophils	1.026%-1.056%	0.00%	1.041	0.0076	-1.37	**Lower**
B Memory	1.689%-1.785%	1.73%	1.737	0.0241	0.00	**Lower**
Naïve B	2.207%-2.311%	0.00%	2.259	0.0260	-0.87	**Lower**
Regulatory T	0.604%-6.408%	8.56%	3.506	1.4510	3.48	**Higher**
Esosinphils	0.376%-0.424%	0.00%	0.400	0.0121	-0.33	**Lower**
Natural Killer	3.353%-3.459%	3.19%	3.406	0.0264	-0.08	**Lower**
Neutrophils	62.899%-62.953%	67.62%	62.93	0.0136	0.35	**Higher**
Monocyte	4.453%-4.567%	0.92%	4.510	0.0285	-1.26	**Lower**

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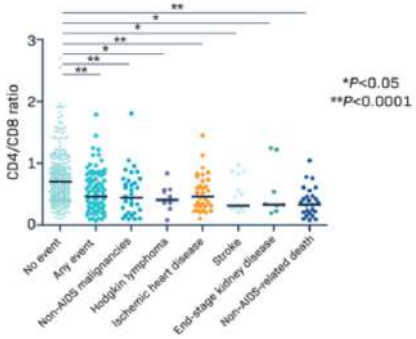
CD4/CD8 T Cell Ratio

CD4/CD8T cell ratio is incredibly informative on disease. A value between 1 and 4 is ideal. A value between 0 and 1 marks “inverted ratio”. A low or inverted CD4/CD8 ratio is an immune risk phenotype and is **associated with altered immune function, immune senescence, and chronic inflammation.**

The prevalence of an inverted CD4/CD8 ratio increases with age. An inverted ratio is seen in 8% of 20-59 year olds and in 16% of 60-94 year olds. Women across all age groups are less likely to have an inverted ratio than their male counterparts.

Age, and hormone-related atrophy of the thymus is theorized to explain the differences between populations. Hormonal influence on the ratio is supported by a correlation between low Plasma Estradiol levels, high circulating CD8, and low CD4/CD8 ratios in women with premature ovarian failure.



We have been able to refer patients for additional testing to diagnose HIV, Chronic Lymphocytic Leukemia, and even individuals taking their Rapamycin at too high of a dose. **If you see a low CD4/CD8 ratio, it is not an immediate cause for concern but we might recommend testing via traditional labs just in case.** A value of 4+ marks hyperactivity or possible infection, autoimmunity or additional immune risk phenotypes.

CELL TYPE	REFERENCE RANGE	YOUR RATIO	MEAN	SD	# OF STANDARD DEVIATIONS ABOVE OR BELOW MEAN	IS THIS HIGHER OR LOWER THAN ANTICIPATED?
CD4/CD8 T Cell Ratio	1.00-4.00	1.06	2.59	0.074	-0.21	**Lower**

RATIO	ABOUT THIS RATIO	YOUR VALUE
Regulatory T Cells to Total T Lymphocytes (RegT/all other T Cells)	There is evidence that Tregs exhibit atheroprotective properties by suppression of autoreactive T cell responses or by secretion of anti-inflammatory cytokines (Pastrana et al., 2012). Thus this might be a marker for cardiovascular disease. (www.sciencedirect.com)	0.48
Adaptive to Innate Immune (A/A Ratio)	The adaptive-to-innate immune ratio (A/I ratio) has been linked to response to several types of immunotherapy.	0.44

Other Immunosenescence Ratios

RATIO	ABOUT THIS RATIO	NORMATIVE RATIO	YOUR VALUE
Neutrophil to Lymphocyte	The NLR is simply the number of Neutrophils divided by the number of Lymphocytes. Under physiologic stress, the number of Neutrophils increases, while the number of Lymphocytes decreases. The NLR **combines** both of these changes, making it more sensitive than either alone: Effect of Physiologic Stress on the NLR: ↑↑ NLR= Neutrophils / Lymphocytes Endogenous cortisol and catecholamines may be major drivers of the NLR. Increased levels of cortisol are known to increase the neutrophil count while simultaneously decreasing the lymphocyte count. Thus, NLR is not solely an indication of infection or inflammation. Any cause of physiologic stress may increase the NLR (e.g. hypovolemic shock).	 Neutrophil-to-Lymphocyte ratio (NLR) reflects the amount of physiologic stress. The optimal cutoff value will vary depending on the specific patient population and disease state. The numbers provided above are. intended merely to provide a general concept of NLR interpretation.	2.39
Monocyte to Lymphocyte	MLR (Monocyte to Lymphocyte ratio) has demonstrated to be a novel hematological and inflammatory parameter. MLR is associated with various diseases, such as community-acquired pneumonia, axial spondylarthritis, and coronary angiography, as well as the systemic inflammatory response, which reflects the abnormal immune status of diseases.	The mean Neutrophil-toLymphocyte ratio in the whole population was 1.70±0.70 (Range: 8.38, Min: 0.23, Max: 8.61), mean lymphocyte-to-monocyte ratio was 11.15±3.14 (Range:23.21, Min:3.46, Max:26.67), and mean platelet-to-lymphocyte ratio was 117.05±47.73 (Range:93.60, Min:19.11, Max:1598.77).	30.57

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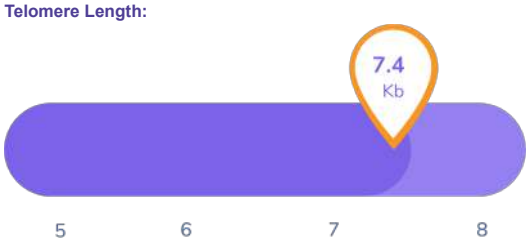
Telomere Length

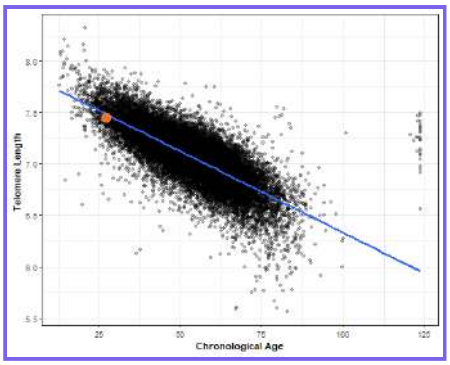
Telomere Length:



If we were to estimate your biological age **strictly from your telomere measurement,** we would anticipate your age to be:

29.10
YEARS OLD

Telomere Length Based on Biological Age Prediction:



Your Average telomere prediction length:

7.4 kb

This puts you in the:

37.40th Percentile

Changes Over Time

ALGORITHM	PATIENT DATA	MORBIDITY AND MORTALITY ASSOCIATIONS	RISK STATEMENT
Telomere	7.4 Kilobase Unit	At your chronological age of 27.20, your telomeres are longer than 37.40th% of people. who share the same chronological age as you.	Shorter telomeres are not only associated with age but with disease too. Shorter telomere length and low telomerase activity are correlated with several chronic preventable diseases.

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Type 2 Diabetes Risk

PHOSPHO1 (Cg02650017)
Average Beta Value:

Your Epitype Value:
0.11

[.05-1]
NO INCREASED RISK

ABCG1 (Cg06500161)
Average Beta Value:

Your Epitype Value:
0.57

[0-0.7]
NO INCREASED RISK

RISK REPORT	PATIENT OUTCOMES	SUMMARY	IMPACT	ADDITIONAL NOTE
Type 2 Diabetes	**Low Risk**	Your DNA methylation score was **0.57** at the ABCG1 locus and **0.11** at the PHOSPHO1 locus.	Your DNA methylation score at ABCG1 and PHOSPHO1 gives an indication of your level of risk for type 2 diabetes. If your score is 73% or greater at the ABCG1 locus, it is associated with a 9% increased risk for future type 2 diabetes occurrence. A DNA methylation score of 4% or greater at the PHOSPHO1 locus is associated with a 15% decreased risk for future type 2 diabetes occurrence.	DNA methylation at these loci is associated with cholesterol levels, triglyceride levels, ischemic stroke, and risk of T2D. Studies on these particular CpG loci have suggested that fasting and low carb diets can reduce methylation at these loci to lower your risk. Please consult your doctor to discuss this and more treatment options.

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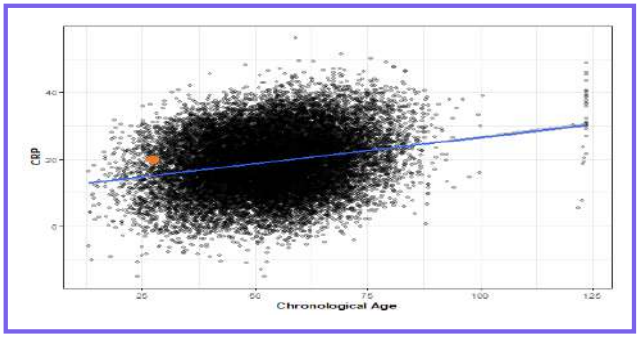
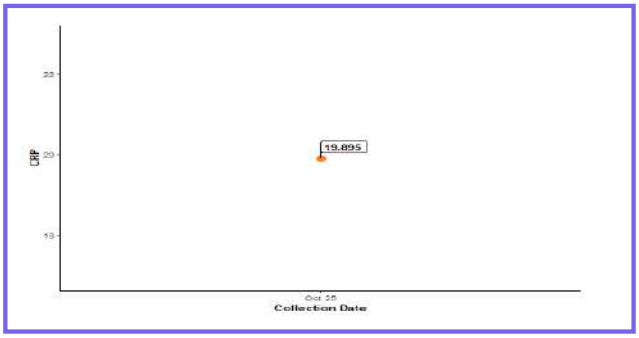
Inflammation

DNAm CRP

Changes Over Time



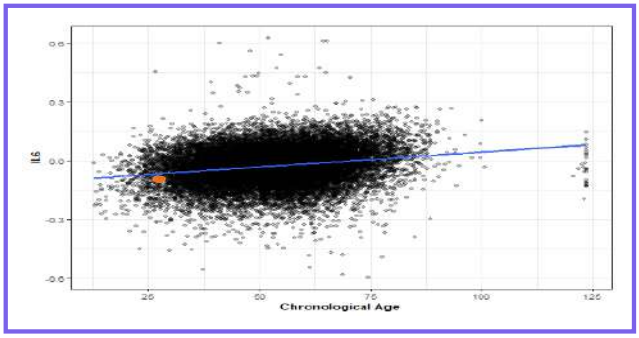


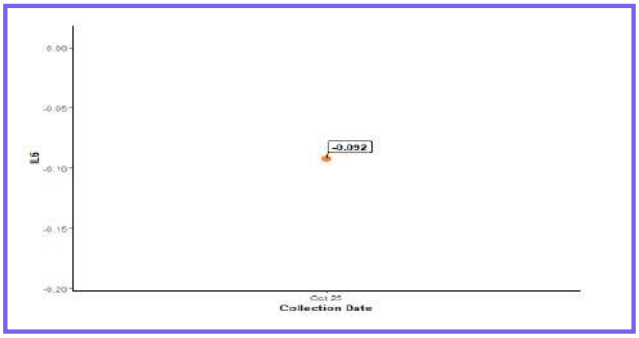
Your CRP methylation level is higher than 80.49% of the population at your same calendar age and sex.

CRP is produced by the liver in response to acute inflammation. DNAm CRP has an inverse relationship with cognitive functions such as memory, speed, and visuospatial functions.

DNAm IL-6

Changes Over Time





Your IL-6 methylation level is higher than 30.49% of the population at your same calendar age and sex.

IL-6 is a widely used marker of inflammation and circulating levels of the cytokine typically rise in older age. DNAm IL-6 is positively associated with BMI, self-reported smoking status, and alcohol intake.

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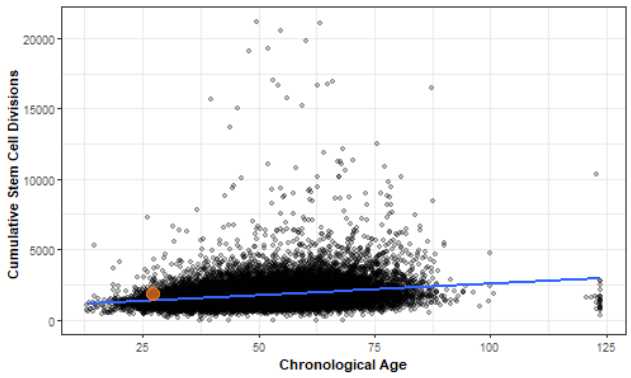
Mitotic Clock

Cumulative number of stem cell divisions per stem cell per year:



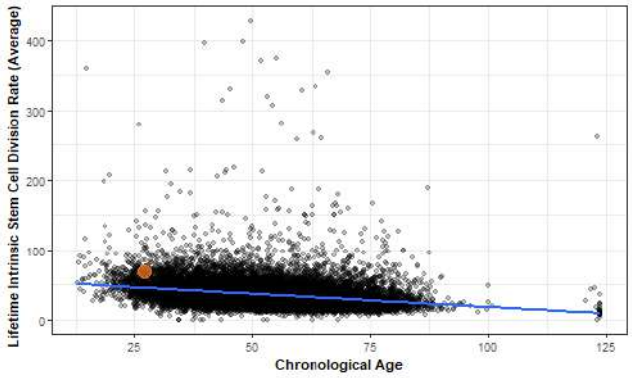
Stem cell divisions per stem cell per year:

1869.17

This puts you in the:

85.07th Percentile

Average estimate for the intrinsic rate of stem-cell division for the tissue:



Average estimate for intinsic rate of stem-cell division:

68.72

This puts you in the:

85.57th Percentile

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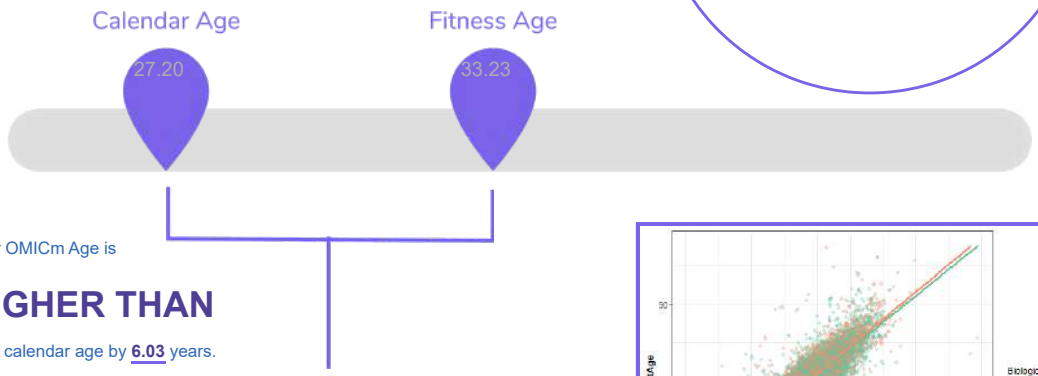
Fitness Age

OMICm FitAge

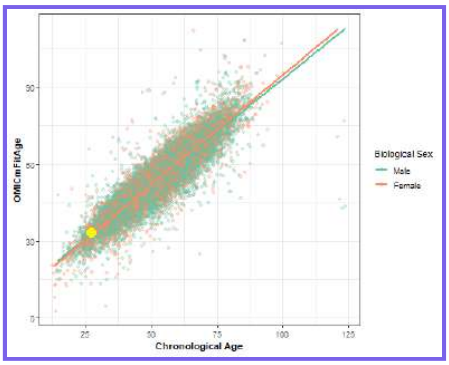
The incorporation of physical fitness measurements into epigenetic clocks **increases the measurable effects of lifestyle, medical, and environmental interventional changes** on the aging process. The DNAmFitAgeAccel algorithm, also simply known as FitAgeAcceleration, was developed by researchers at UCLA, and is an estimate of epigenetic age acceleration. We have created a version of this, however, we incorporated our **OMICm Age** algorithm (developed with Harvard) instead. We call this **OMICm FitAge,** which tells you how old you are according to your physical fitness and functionality.



Your OMICm Age is

HIGHER THAN

your calendar age by **6.03** years.



IFor every one year older OMICm FitAge is, there is an average **0.29 decrease in relative grip strength and 0.32 increase in BMI.** OMICm FitAge has estimated that high-fit individuals (classified through VO2max) have a **1.5 to 2.0 younger biological age** compared to low/medium fit individuals in females and males, respectively. Younger OMICm FitAge was associated with better memory test performance, emphasizing the beneficial role of physical exercise on cognitive health.

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OMICm FitAge is impacted by:

GRIP STRENGTH

Maximum hand grip strength (GripMax) a measurement of force taken in kg and is used to measure the age-associated decline in terms of muscle strength.

GAIT SPEED

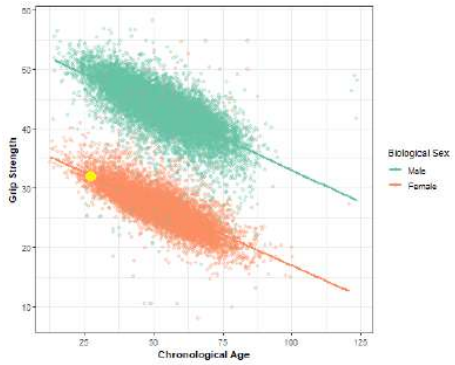
Gait speed, also known as walking speed, is measured in meters per second.

VO2MAX

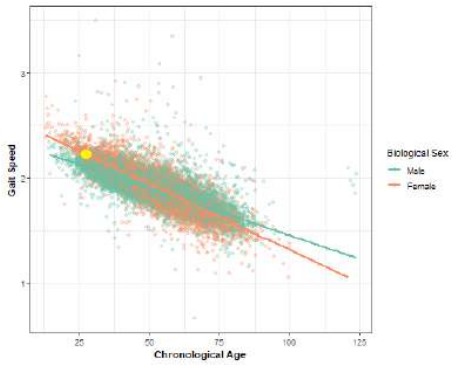
Maximal oxygen uptake, or VO2max, is a measure of cardiovascular health and aerobic endurance.

FEV1

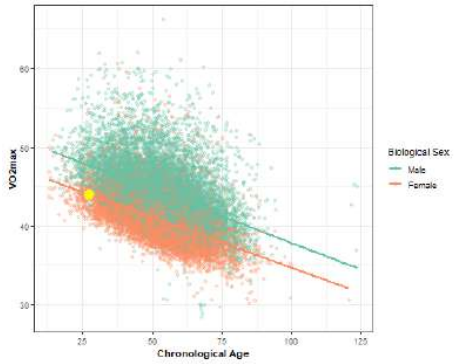
Forced expiratory volume, also known as FEV1, measures lung function by determining the amount of air forced from the lungs in one second.



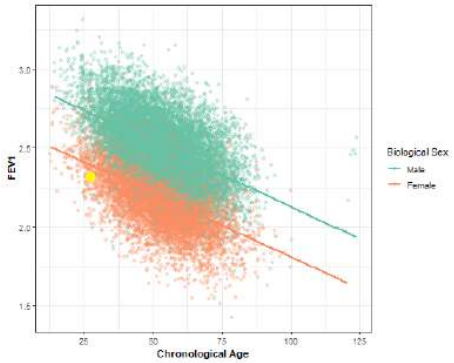
Your Grip Strength Epigenetic Biomarker Proxy is **32.05.** This puts you scoring higher than **46.95%** of the population with a similar reported age and sex.



Your Gait Speed Epigenetic Biomarker Proxy is **2.23.** This puts you scoring higher than **45.12%** of the population with a similar reported age and sex.



Your V02Max Epigenetic Biomarker Proxy is **44.08.** This puts you scoring higher than **50.61%** of the population with a similar reported age and sex.



Your FEV1 Epigenetic Biomarker Proxy is **2.32.** This puts you scoring higher than **27.44%** of the population with a similar reported age and sex.

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Smoking & Drinking

Smoking and Disease Risk

AHRR (cg05575921)
Average Beta Value %:

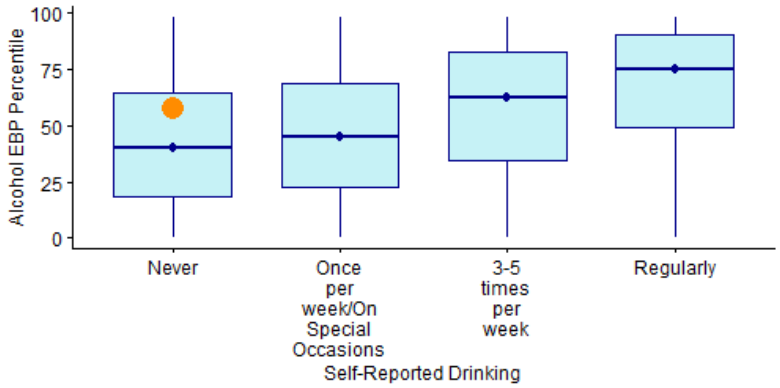
Your Epitype Value: 74%

[64%-100%]
No Epigenetic Risk

The impact that tobacco smoke exposure has on the epigenome is based on the level of methylation at the AHRR gene locus cg05575921.

Your DNA methylation score was **74%** at the AHRR locus, meaning that your methylation score aligns with the status of **non-smoker,** putting you at **Low risk** for developing smoking-related conditions.

Alcohol Consumption and DNA Methylation



On your intake survey, you self-reported your drinking status as **Never.** With our custom epigenetic biomarker proxy, you are in the **57.73th** percentile. This means your score is higher than **57.73%** of the population we have tested.

***Those who marked self-reported drinking as "Not Applicable" were assumed to have no drinking status and have been combined with data from "Never" status.**

PROVIDED BY: TruDiagnostic

Weight Loss Response

CPG SITE	GENE	β - VALUE RESPONDERS	YOUR SCORE	RESPONSE STATUS
cg15500865	PON3	0.072	0.08	Hypermethylated
cg25161512	PON3	0.115	0.09	Hypomethlyated
cg11435506	PON3	0.165	0.04	Hypomethlyated
cg03301582	PON3	0.120	0.09	Hypomethlyated
cg08898155	PON3	0.163	0.02	Hypomethlyated
cg04080282	PON3	0.324	0.18	Hypomethlyated
cg26457160	PON3	0.490	0.47	Hypomethlyated
cg10329418	PON3	0.252	0.20	Hypomethlyated
cg27166921	PON3	0.253	0.33	Hypermethylated
cg24750391	PON3	0.355	0.29	Hypomethlyated
cg08461772	PON3	0.418	0.24	Hypomethlyated

RISK REPORT	PATIENT OUTCOMES	SUMMARY	IMPACT	ADDITIONAL NOTE
Weight Loss Response	**Non Response**	Your DNA methylation scores at the above loci indicate you are a **Non Responder** for weight loss treatment utilizing a hypocaloric diet. This means a calorie deficit diet **passably works** as your weight loss strategy.	If your DNA methylation score puts you in the category of non-responder or intermediate responder then a hypocaloric diet might not be the best treatment option for you. If you are a responder, that means a hypocaloric diet has a greater chance of positively impacting your weight loss goals.	Studies on these particular CpG loci have concluded that some individuals have a better response to a calorie deficit diet than others. This may indicate why weight loss has been difficult to achieve and can provide insight into finding the best weight loss strategy.

COLLECTED: 10/25/2023 | REPORTED: 11/12/2023

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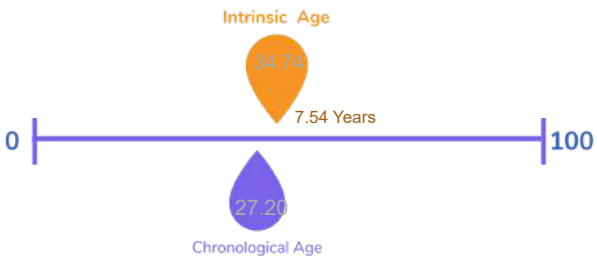
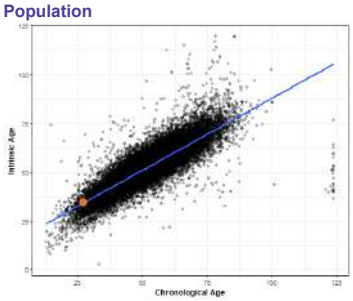
Intrinsic & Extrinsic Age

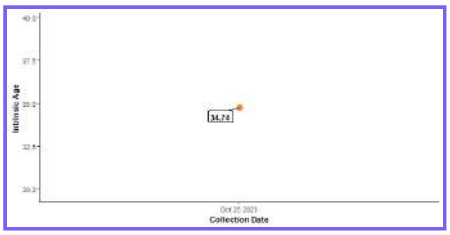
Intrinsic Epigenetic Age



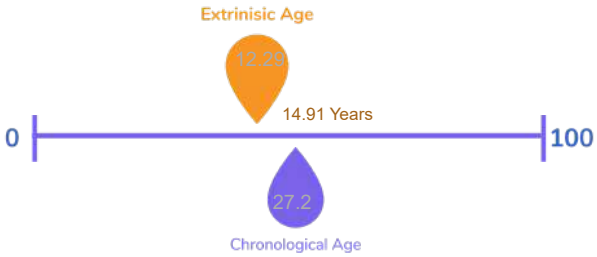
Population



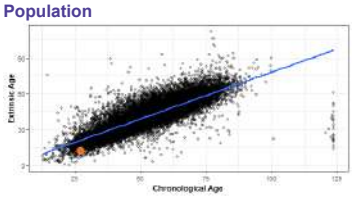
Changes Over Time



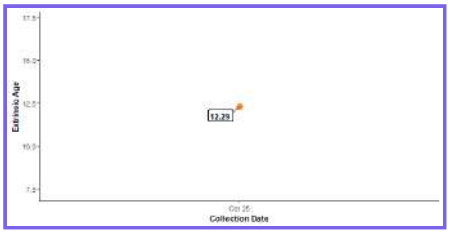
Extrinsic Epigenetic Age



Population



Changes Over Time



COLLECTED: 10/25/2023 | REPORTED: 11/12/2023

NIKITHA'S TRIGLYCERIDES AND DIABETES RISK METHYLATION REPORT

ABCG1 (cg06500161), PHOSPHO1 (cg02650017), SOCS3, SREBF1, and TXNIP Genes

TruDiagnostic™

Are You At Increased Risk For Developing Type 2 Diabetes?

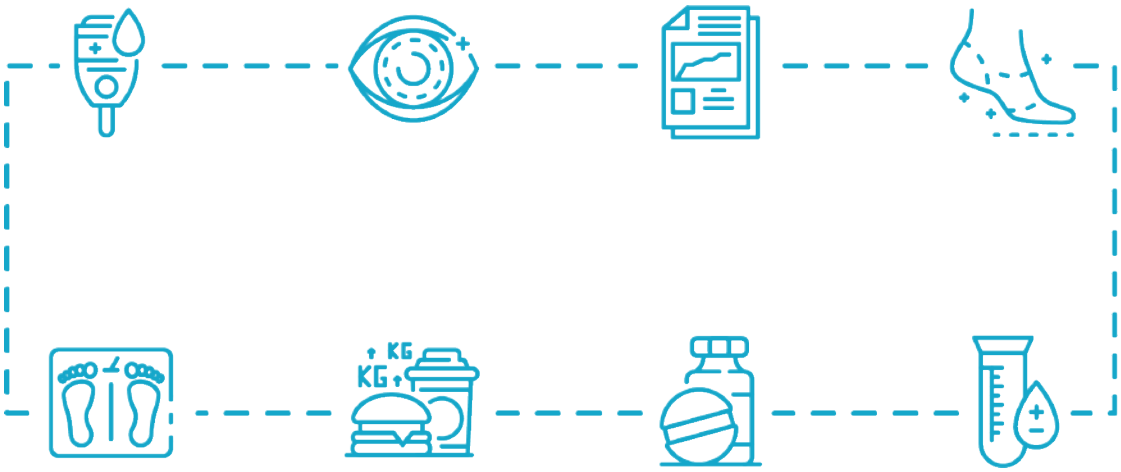
Epigenetic biomarkers for Type 2 Diabetes

Type 2 diabetes (T2D) is a complex disease that results from genetic and environmental interactions that can be modified and/or mediated by epigenetic changes. A number of genetic and non-genetic factors have been identified that increase the risk of T2D. However, a healthier lifestyle, including proper diet and exercise, can potentially reduce the risk of T2D by almost 50 percent in high-risk groups [3].

Therefore, there is great interest and need to identify individuals that have a high risk of developing T2D. By postponing and/or preventing T2D and its complications, it may be possible to reduce T2D-associated mortality and the financial cost of treating the disease and its complications.

To date, more than 65 genetic variants have been identified that increase the risk of T2D by almost 10 percent [8]. However, genetic screening for T2D risk variants has not been implemented in clinics. Despite the potential value of such screening tests, a number of limitations have hindered their use. These limitations include small effect size, their low discriminative ability, a small added value compared with the clinical risk factors, and a lack of models that take into account gene-gene and gene-environment interactions [3]. Failure to understand the pathophysiology of T2D hinders the efforts to develop improved therapeutic strategies [7].

There is great interest in epigenetic biomarkers such as DNA methylation, which, unlike the DNA sequence, can be influenced by the environment, and has the potential to improve T2D prediction [3]. Recently, an epigenome-wide association study identified 5 DNA methylation loci ***(ABCG1, PHOSPHO1, SOCS3, SREBF1, and TXNIP)*** in the blood that were associated with T2D. Furthermore, the study showed that a methylation score that combined the results from these 5 methylation loci found an association with prospective T2D occurrence [1].

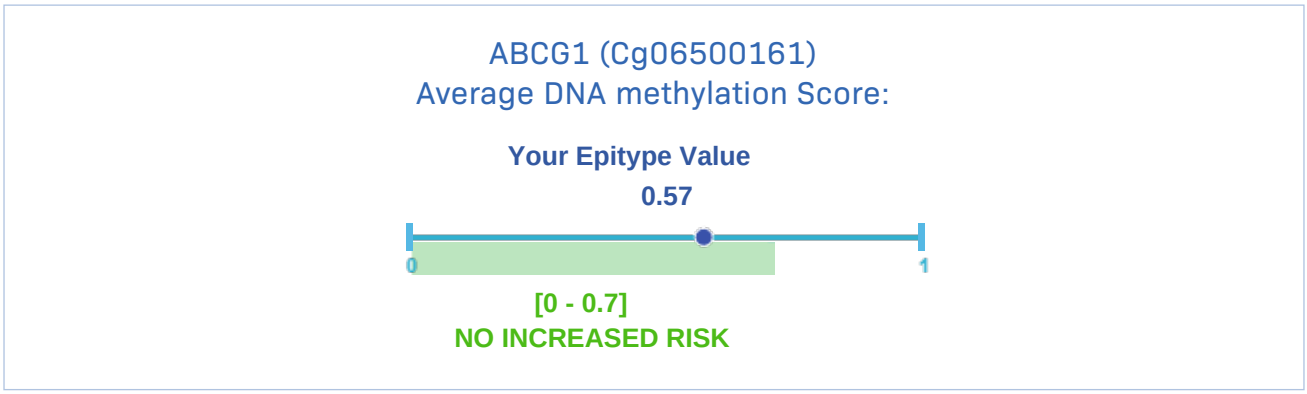


What is ABCG1?

ABCG1 is a gene that encodes a member of the ATP-binding cassette (ABC) protein family, which plays a role in the homeostasis of glucose and lipids. These proteins do so by removing excess cholesterol from peripheral tissues and transporting it to the liver. The HDL-mediated increase in insulin secretion is dependent on ABCG1 [2]. Loss of both the ABCA1 and ABCG1 genes results in sterol accumulation, impaired glucose-stimulated insulin secretion, and inflammation of pancreatic ß-cells which can all lead to diabetes [6].

The ABCG1 marker has been replicated across different tissues in more than 10,000 individuals representing different ethnicities. Altered DNA methylation in ABCG1 is associated with the downregulation of mRNA levels from T2D individuals [2]. DNA methylation at this site in blood DNA has demonstrated to be functionally correlated with a number of T2D risk factors, such as BMI, triglycerides, and HbA1c [3].

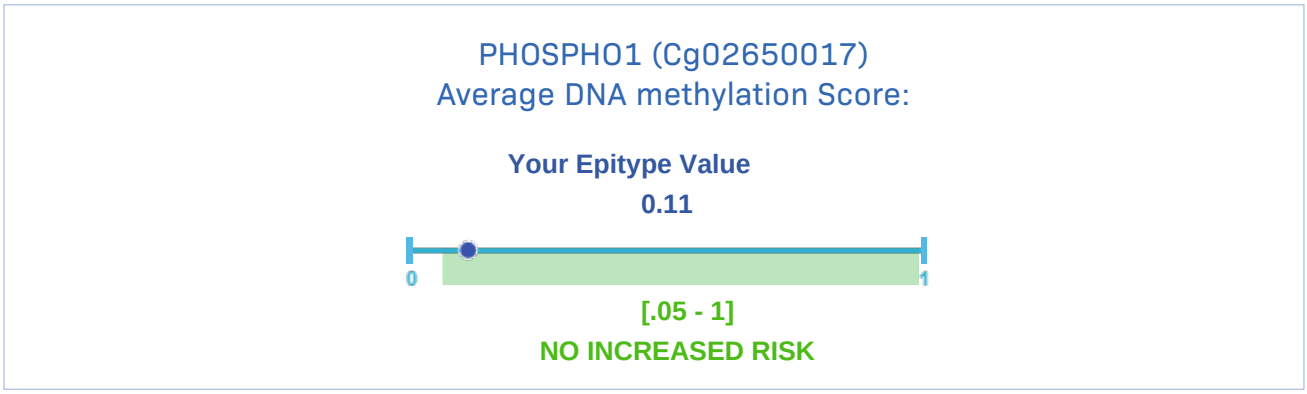
Your DNA methylation score at ABCG1 locus cg06500161 gives an indication of your level of risk for type 2 diabetes; if your score is 70.1% or greater it is associated with a 9% increased risk for future type 2 diabetes occurrence.



What is PHOSPHO1?

PHOSPHO1 encodes a phosphatase that is highly expressed in skeletal muscle and plays a role in skeletal mineralization. Under certain circumstances, it may also cause vascular mineralization. Cardiovascular calcification is a common consequence of aging, diabetes, and hypercholesterolemia. PHOSPHO1, is also considered to be an attractive target for cardiovascular therapy. Interestingly, it has been found that DNA methylation at the PHOSPHO1 locus cg02650017 in blood correlated positively with HDL levels. DNA methylation at the PHOSPHO1 locus cg02650017 is associated with future T2D risk [3].

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.



The Science

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), while 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.

Furthermore, the level of DNA methylation at the ABCG1 locus cg06500161 in blood DNA correlated positively with BMI, HbA1c, fasting insulin, and triglyceride levels, and was increased in adipose tissue and blood from the diabetic twin among monozygotic twin pairs discordant for T2D. DNA methylation at the PHOSPHO1 locus cg02650017 in blood correlated positively with HDL levels [3].

THE IMPACT TO YOU

The impact to you is based on your level of methylation at these gene loci compared with the risk categories determined and assessed in the cited papers in regards to T2D risk.

Your DNA methylation score was 0.57 at the ABCG1 locus and 0.11 at the PHOSPHO1 locus.

Your DNA methylation scores at these gene loci would reflect a 15% decreased risk for future T2D based on your DNA methylation at the PHOSPHO1 locus cg02650017 according to the referenced study. [3]

Some studies on this particular CpG loci have suggested that fasting and low carb diets can reduce methylation at these loci to lower your risk. Please consult your doctor to discuss this and more treatment options.

Summary

Type 2 diabetes can be modified and/or mediated by epigenetic changes, and a number of genetic and non-genetic factors have been identified that increase the risk of T2D. Recent studies have found 5 DNA methylation loci associated with T2D occurrence. ABCG1 is a gene that insulin secretion is dependent on. Altered DNA methylation in ABCG1 is associated with the locus downregulation of mRNA levels from T2D individuals. DNA methylation at the ABCG1 locus cg06500161 in blood DNA was associated with an increased risk for future T2D and DNA methylation at the PHOSPHO1 locus cg02650017 in blood DNA was associated with a decreased risk for future T2D. DNA methylation at these loci is associated with cholesterol levels, triglyceride levels, ischemic stroke, and risk of T2D. Identifying T2D risk factors is fundamental for the prevention of disease.

REFERENCES

1. Chambers, J. C., Loh, M., Lehne, B., Drong, A., Kriebel, J., Motta, V., Wahl, S., Elliott, H. R., Rota, F., Scott, W. R., Zhang, W., Tan, S.-T., Campanella, G., Chadeau-Hyam, M., Yengo, L., Richmond, R. C., Adamowicz-Brice, M., Afzal, U., Bozaoglu, K., … Kooner, J. S. (2015). Epigenome-wide association of DNA methylation markers in peripheral blood from Indian Asians and Europeans with incident type 2 diabetes: a nested case-control study. The Lancet. Diabetes & Endocrinology, 3(7), 526–534. https://doi.org/10.1016/s2213-8587(15)00127-8

2. Cheng, Z., Zheng, L., & Almeida, F. A. (2018). Epigenetic reprogramming in metabolic disorders: nutritional factors and beyond. The Journal of nutritional biochemistry, 54, 1–10. https://doi.org/10.1016/j.jnutbio.2017.10.004

3. Dayeh, T., Tuomi, T., Almgren, P., Perfilyev, A., Jansson, P.-A., de Mello, V. D., Pihlajamäki, J., Vaag, A., Groop, L., Nilsson, E., & Ling, C. (2016). DNA methylation of loci within ABCG1 and PHOSPHO1 in blood DNA is associated with future type 2 diabetes risk. Epigenetics, 11(7), 482–488. https://doi.org/10.1080/15592294.2016.1178418

4. Dedeurwaerder, S., Defrance, M., Bizet, M., Calonne, E., Bontempi, G., & Fuks, F. (2014). A comprehensive overview of Infinium Human-Methylation450 data processing. Briefings in bioinformatics, 15(6), 929–941. https://doi.org/10.1093/bib/bbt054

5. Dolzhenko, E., & Smith, A. D. (2014). Using beta-binomial regression for high-precision differential methylation analysis in multifactor whole-genome bisulfite sequencing experiments. BMC bioinformatics, 15, 215. https://doi.org/10.1186/1471-2105-15-215

6. Kruit, J. K., Wijesekara, N., Westwell-Roper, C., Vanmierlo, T., de Haan, W., Bhattacharjee, A., Tang, R., Wellington, C. L., LütJohann, D., Johnson, J. D., Brunham, L. R., Verchere, C. B., & Hayden, M. R. (2012). Loss of both ABCA1 and ABCG1 results in increased disturbances in islet sterol homeostasis, inflammation, and impaired t-cell function. Diabetes, 61(3), 659–664. https://doi.org/10.2337/db11-1341

7. Lyssenko, V., & Laakso, M. (2013). Genetic Screening for the Risk of Type 2 Diabetes. Diabetes Care, 36(Supplement 2), S120 LP-S126. https://doi.org/10.2337/dcS13-2009

8. McCarthy, M. I. (2010). Genomics, Type 2 Diabetes, and Obesity. New England Journal of Medicine, 363(24), 2339–2350. https://doi.org/10.1056/NEJMra0906948

9. Pfeiffer, L., Wahl, S., Pilling, L. C., Reischl, E., Sandling, J. K., Kunze, S., Holdt, L. M., Kretschmer, A., Schramm, K., Adamski, J., Klopp, N., Illig, T., Hedman, Å. K., Roden, M., Hernandez, D. G., Singleton, A. B., Thasler, W. E., Grallert, H., Gieger, C., Herder, C., … Waldenberger, M. (2015). DNA methylation of lipid-related genes affects blood lipid levels. Circulation. Cardiovascular genetics, 8(2), 334–342. https://doi.org/10.1161/CIRCGENETICS.114.000804

10. Sun, Z., Chai, H. S., Wu, Y., White, W. M., Donkena, K. V, Klein, C. J., Garovic, V. D., Therneau, T. M., & Kocher, J.-P. A. (2011). Batch effect correction for genome-wide methylation data with Illumina Infinium platform. BMC Medical Genomics, 4(1), 84. https://doi.org/10.1186/1755-8794-4-84

11. Qin, X., Li, J., Wu, T., Wu, Y., Tang, X., Gao, P., Li, L., Wang, M., Wu, Y., Wang, X., Chen, D., & Hu, Y. (2019). Overall and sex-specific associations between methylation of the ABCG1 and APOE genes and ischemic stroke or other atherosclerosis-related traits in a sibling study of Chinese population. Clinical epigenetics, 11(1), 189. https://doi.org/10.1186/s13148-019-0784-0

12. Weinhold, L., Wahl, S., Pechlivanis, S., Hoffmann, P., & Schmid, M. (2016). A statistical model for the analysis of beta values in DNA methylation studies. BMC bioinformatics, 17(1), 480. https://doi.org/10.1186/s12859-016-1347-4

TRUAGE BY TRUDIAGNOSTIC

Immune

This report explores the impacts of various types of immune cells and their concentrations on biological age by examining associated methylation patterns at various locations of your DNA.

Developed By TruDiagnostic's Bioinformatics & Research Department

UNDERSTANDING

biological aging & the immune system.

Do you ever wonder why many older adults experience a harder time battling diseases like COVID-19 or the common flu, compared to younger people who typically have an **easier time recovering** from the same illnesses?

It all boils down to the capabilities of one's immune cells to effectively respond to internal and foreign health threats; capabilities which tend to decrease with age. **This age-related decline of the immune response in our blood is called immunosenescence.**

As we get older, and immunosenescence occurs, higher incidences of infections, cancer, and autoimmune disease emerge. As research indicates, the progression of one's immune system decline, to the point of immunosenescence, **occurs faster in men than in women.** It is also characterized by age-related changes in immune cells and inflammatory mediators.

Immunosenescence also **changes the number of immune cells** in our blood. The average adult has more than five liters of blood in their body that carries oxygen and nutrients to living cells and disposes of cellular waste. Blood also delivers various types of **immune cells, which are types of white blood cells** (WBC), to fight infections throughout the body. These WBCs come in many different shapes and sizes, and the concentration of each immune cell type has varying associations with age-related DNA methylation patterns.

COLLECTED: 10/25/2023 | REPORTED: 11/12/2023

THE REASON WHY

immune cells are important to all epigenetic algorithms.

As we age, we have **overall fewer** Naïve T Cells, Natural Killer Cells, Macrophages, Dendritic Cells, and other immune cell types throughout our body. However, concentrations of immune cell types also change based on what kind of sample or tissue you are analyzing, such as blood or saliva, regardless of age.

Each immune cell type, and its respective concentration, can indicate vastly different aging implications from other types of immune cells when isolated; forcing algorithm developers to ask, **'Is this pattern actually caused by aging, or is this pattern caused by the type of cell and the type of tissue we are examining?'**

Further potential for data pollution rests in whether or not someone's immune system was actively or recently fighting an illness at the time of sample collection; which can cause temporary changes in immune cell concentrations.

Say we were to isolate Naïve CD8 immune cells from Memory CD8 immune cells (both of which are found in different concentrations in your blood sample) to determine your biological age based on those cells alone. **The Naïve CD8 cells might say you're 40 years old,** for example, **while the Memory CD8 cells would say you're 55 to 60 years old.**

Instead of addressing this challenge by completely excluding immune biomarkers, all of our algorithms were trained and developed with a weighted and controlled representation of each immune cell type and its concentration in blood tissue.

In doing so, we can ensure accurate results, free of potentially corrupting biodata. This includes our novel OMICm Age algorithm, as well as our other custom algorithms that have been developed to, as well as our other custom algorithms that have been developed to **ensure changes in immune cells don't give us false information about your biological age.**

THE IMPACTS OF

immunosenescence on different immune cell types.

T Lymphocyte

- Reduced development and numbers of Naïve CD4+/CB+ T cells
- Decline in CD4+ function and in CD8+ T cell totoxicity+ proliferation
- Reduced generation of Th subsets

Dendritic Cell

- Reduced IFN production and expression CD25 and ICAM-1 in mature MODCs
- Reduction in lymphocyte cytotoxicity and greater of monocyte-macrophage derived APCs

B Lymphocyte

- Reduced development and numbers of Naïve B cells
- Decreased diversity of B cell repertoires and B cells responses to new antigens

Macrophage

- Defective phagocytosis
- Decreased cytokine production, antigen presentation, and superoxide anion production

Neutrophils

- Decreased phagocytosis, chemotaxis, and apoptosis function

Natural Killer

- Reduced cytolytic potential and CD1 expression in NKT cells
- Decreased cytokine and chemokine production

PROVIDED BY: TruDiagnostic™ The Epigenetic Company

Your Results.

Your immune cell results are below, outlining your percentage of each cell type, as well as the standard deviation from the average concentration.

IMMUNE CELL TYPE	95% CONFIDENCE INTERVAL RANGE	YOUR PERCENTAGE	MEAN	SD	# OF STANDARD DEVIATIONS ABOVE OR BELOW MEAN	IS THIS HIGHER OR LOWER THAN ANTICIPATED?
Naïve CD4T	7.196%-7.35%	9.27%	7.273	0.0383	0.52	**Higher**
Memory CD4T	5.14%-5.284%	0.00%	5.212	0.0361	-1.44	**Lower**
Memory CD8T	6.519%-6.691%	7.46%	6.605	0.0430	0.20	**Higher**
Naïve CD8T	1.09%-1.16%	1.25%	1.125	0.0175	0.07	**Higher**
Basophils	1.026%-1.056%	0.00%	1.041	0.0076	-1.37	**Lower**
B Memory	1.689%-1.785%	1.73%	1.737	0.0241	0.00	**Lower**
Naïve B	2.207%-2.311%	0.00%	2.259	0.0260	-0.87	**Lower**
Regulatory T	0.604%-6.408%	8.56%	3.506	1.4510	3.48	**Higher**
Esosinphils	0.376%-0.424%	0.00%	0.400	0.0121	-0.33	**Lower**
Natural Killer	3.353%-3.459%	3.19%	3.406	0.0264	-0.08	**Lower**
Neutrophils	62.899%-62.953%	67.62%	62.93	0.0136	0.35	**Higher**
Monocyte	4.453%-4.567%	0.92%	4.510	0.0285	-1.26	**Lower**

COLLECTED: 10/25/2023 | REPORTED: 11/12/2023

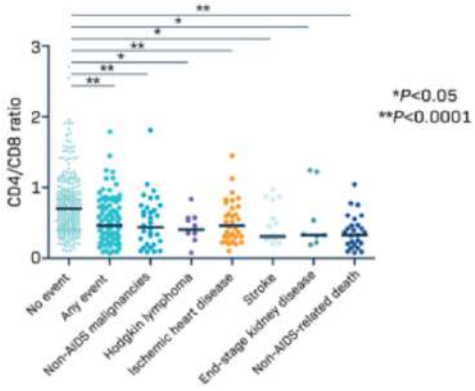
UNDERSTANDING

CD4/CD8 T Cell Ratio.

CD4/CD8T cell ratio is incredibly informative on disease. A value between 1 and 4 is ideal. A value between 0 and 1 marks an “inverted ratio”. A low or inverted CD4/CD8 ratio is an immune risk phenotype and is **associated with altered immune function, immune senescence, and chronic inflammation.**

The prevalence of an inverted CD4/CD8 ratio increases with age. An inverted ratio is seen in 8% of 20-59 year olds and in 16% of 60-94 year olds. Women across all age groups are less likely to have an inverted ratio than their male counterparts.

Age and hormone-related atrophy of the thymus are theorized to explain the differences between populations. Hormonal influence on the ratio is supported by a correlation between low Plasma Estradiol levels, high circulating CD8, and low CD4/CD8 ratios in women with premature ovarian failure.



We have been able to refer patients for additional testing to diagnose HIV, Chronic Lymphocytic Leukemia, and even individuals taking their Rapamycin at too high of a dose. **If you see a low CD4/CD8 ratio, it is not an immediate cause for concern but we might recommend testing via traditional labs just in case.** A value of 4+ marks hyperactivity or possible infection, autoimmunity, or additional immune risk phenotypes.

CELL TYPE	REFERENCE RANGE	YOUR RATIO	MEAN	SD	# OF STANDARD DEVIATIONS ABOVE OR BELOW MEAN	IS THIS HIGHER OR LOWER THAN ANTICIPATED?
CD4/CD8 T Cell Ratio	1.00-4.00	1.06	2.59	0.074	-0.21	**Lower**

RATIO	ABOUT THIS RATIO	YOUR VALUE
Regulatory T Cells to Total T Lymphocytes (RegT/all other T Cells)	There is evidence that Tregs exhibit atheroprotective properties by suppression of autoreactive T cell responses or by secretion of anti-inflammatory cytokines (Pastrana et al., 2012) . Thus this might be a marker for cardiovascular disease. (www.sciencedirect.com)	0.48
Adaptive to Innate Immune (A/A Ratio)	The adaptive-to-innate immune ratio (A/I ratio) has been linked to response to several types of immunotherapy.	0.44

Other Ratios to Prioritize.

RATIO	ABOUT THIS RATIO	NORMATIVE RATIO	YOUR VALUE
Neutrophil to Lymphocyte	The NLR is simply the number of Neutrophils divided by the number of Lymphocytes. Under physiologic stress, the number of Neutrophils increases, while the number of Lymphocytes decreases. The NLR **combines** both of these changes, making it more sensitive than either alone: **Effect of Physiologic Stress on the NLR:** ↑ ↑ NLR= Neutrophils / Lymphocytes Endogenous cortisol and catecholamines may be major drivers of the NLR. Increased levels of cortisol are known to increase the neutrophil count while simultaneously decreasing the lymphocyte count. Thus, NLR is not solely an indication of infection or inflammation. Any cause of physiologic stress may increase the NLR (e.g. hypovolemic shock).	 Neutrophil-to-Lymphocyte ratio (NLR) reflects the amount of physiologic stress. The optimal cutoff value will vary depending on the specific patient population and disease state. The numbers provided above are. intended merely to provide a general concept of NLR interpretation.	2.39
Monocyte to Lymphocyte	MLR (Monocyte to Lymphocyte ratio) has been demonstrated to be a novel hematological and inflammatory parameter. MLR is associated with various diseases, such as community-acquired pneumonia, axial spondylarthritis, and coronary angiography, as well as the systemic inflammatory response, which reflects the abnormal immune status of diseases.	The mean Neutrophil-toLymphocyte ratio in the whole population was 1.70±0.70 (Range: 8.38, Min: 0.23, Max: 8.61), mean lymphocyte-to-monocyte ratio was 11.15±3.14 (Range:23.21, Min:3.46, Max:26.67), and mean platelet-to-lymphocyte ratio was 117.05±47.73 (Range:93.60, Min:19.11, Max:1598.77).	30.57

THE PERCENTAGE OF

immune cells in our blood can be highly informative to health.

'Health outcomes' is a term used to encompass an interconnected set of attributes, that cumulatively describe the consequences of disease for an individual; aka, **how extensively does an illness impact your life and overall health?**

These attributes include impairments, symptoms, functioning capabilities, participation in activities and social roles, and overall health-related quality of life. Health outcomes also tell us how long, on average, people live within a given community, and how much physical and mental health they experience within their lifetime.

There are **many factors that impact health,** such as education, environment, lifestyle habits, access to healthcare, and socioeconomic stability.

There are also **many immune cell types** that are influenced by these factors, and that have direct associations with health risks and outcomes. Some of these include **Naïve CD4** and **Naïve B** T-cells, which help protect the body from infection and cancer, **Natural Killer** immune cells that use enzymes to kill infected and cancerous cells, as well as **Memory CD4** and **Memory B**T-cells which help the immune system coordinate and adapt its response.

Naïve CD4

Decreased concentrations have been associated with an increased risk of **COPD** and **Type 2 Diabetes,** but decreased risk **of all-cause mortality.**

Naïve B

Decreased concentrations have been associated with a decreased risk of **all-cause mortality.**

Memory CD4

Decreased concentrations have been associated with a decreased risk of **all-cause mortality.**

Memory B

Increased concentrations have been associated with an increased risk of **cancer** but decreased risk of **Type 2 Diabetes**

Natural Killer

Decreased concentrations have been associated with a decreased risk of **all-cause mortality.**

Immune cells impact health outcomes regardless of age, sex, race, smoking habits, obesity, and alcohol consumption. However, **lifestyle habits and environmental factors can impact the quantity of, and health of, different immune cells.** For example, research shows a decrease in Natural Killer cell counts associated with smoking, obesity, and stress levels.

DNA methylation patterns show that certain lifestyle and environmental factors are **associated with increases and decreases in specific types of immune cells.** As the concentration of these cells changes, so does the risk for diseases such as stroke, Type 2 Diabetes, COPD, depression, cancer, and more.

ASSOCIATED WITH higher levels of	Memory CD4T B Memory Natural Killer (NK) Neutrophils Monocyte	Memory CD4T	Memory CD4T Basophils Monocyte	Natural Killer (NK) Monocyte
ASSOCIATED WITH lower levels of	Naïve CD4T Naïve CD8T Regulatory	Naïve CD4T Naïve CD8T Regulatory Natural Killer (NK)		Naïve B

Natural Killer (NK)

Natural Killer (NK)

Naïve CD4T

Natural Killer (NK)

Memory CD8T

COLLECTED: 10/25/2023 | REPORTED: 11/12/2023

CELL TYPE	FACTOR WHICH ARE ASSOCIATED WITH HIGHER LEVELS	FACTORS WHICH ARE ASSOCIATED WITH LOWER LEVELS
Naïve CD4		
Memory CD4		
Memory CD8		
Naïve CD8		
Basophils		
B Memory		
Naïve B		
Regulatory		
Esosinphils		
Natural Killer		
Neutrophils		
Monocyte		

Notably, Naïve CD4+ T-cell, Naïve B-cell, and Natural Killer cell fractions are all associated with a reduced risk of allcause mortality, even after adjustment for all major disease risk factors. Interestingly, whilst the Naïve CD4+ T-cell fraction also displayed negative associations with many health outcomes, notably with COPD and Type 2 Diabetes (T2D), the Memory CD4+ T-cell fraction was only negatively associated with all-cause mortality. An increased Memory B-cell fraction was specifically associated with an increased risk of cancer but a reduced risk for T2D, whilst no associations were observed for the other outcomes.

A SCIENTIFIC DEEP DIVE

Methods & Applications

You may be wondering how it's possible that every cell in your body has the same DNA, but a heart cell behaves like a heart cell, and a hair cell behaves like a hair cell, etc. The answer is epigenetics! Epigenetics controls cell development and function by **switching certain genes on and off, which determines phenotype and how your cells behave.** It makes sense that the epigenetic regulation of each cell would depend on its cell type. You wouldn't want your heart to make the proteins found in your hair and vice versa. Thus, each cell has a different **epigenetic signature.**

This means that in order to create an accurate, predictive algorithm from DNA methylation data, one must know **what cell types** are being tested. Otherwise, the information from these **algorithms can give false information.**

For example, if you were to test your brain cells, you would see lower biological ages than if testing blood. We also see that breast tissue can age faster than other tissues across the rest of our body. The same is true with blood if someone is sick and they have increased B cells (cells that produce antibodies), that could alter results in a way that is not consistent with the actual health of an individual.

Therefore, the **rate of aging we calculate is dependent on what cell types we measure.** Using blood as the sample type, we determine what cells are we looking at, and more importantly, we control for different cell representations so our algorithm is accurate and predictive.

Immune Deconvolution.

As cells differentiate from pluripotent stem cells to the tissue type they become, they start to **form unique DNA methylation** patterns that can tell us which cell type they belong to.

By analyzing DNA methylation patterns in a tissue sample, we can infer the relative abundance of different cell types present in that sample. This is because different cell types have distinct DNA methylation profiles. With that information, we create algorithms that use DNA methylation to estimate the relative proportions of different cell types within a tissue sample.

Overall, this technique allows TruDiagnostic to gain a **better understanding of the cellular composition of a complex tissue sample, which can be useful for understanding disease processes and monitoring the effects of interventions.**

Research & Partnerships.

The algorithms we've used to generate your results in this report are a product of academic processing and analysis from our private epigenetic database, along with research partnerships with Harvard University, Johns Hopkins University, and the Chinese Academy of Sciences

Additionally, research into senescence has grown exponentially over the last few years. However, there are still very few tools to easily quantify this process. With Ohio State, we have created senescence predictors of t-cells through DNA methylation.

We are **now developing methods**to apply this to all tissues with additional datasets and believe this will be a valuable tool to quantify this hallmark of aging.

Accuracy of Results.

We've successfully demonstrated that our testing is comparable with the gold standard of Flow Cytometry with **less than 3% error.** We believe this is a needed algorithm to improve all methylation analysis algorithms in the future, and we have also developed a saliva deconvolution method for this very reason.

This immune deconvolution tool has been used in large biobank datasets to look at associations and trends. We believe that this might be a great tool to quantify the immune system and to find novel associations to disease conditions without having to use Flow Cytometry; which can be expensive, require high volumes of blood, and requires refrigerated sample processing.

A meta-analysis of immune cell fractions at high resolution reveals novel associations with common phenotypes and health outcomes

Qi Luo, Varun B. Dwaraka, Qingwen Chen, Huige Tong, Tianyu Zhu, Kirsten Seale, Joseph M Raffaele, Shijie C. Zheng, Tavis L. Mendez, Yulu Chen, Sofina Begum, Kevin Mendez, Sarah Voisin, Nir Eynon, Jessica A. Lasky-Su, Ryan Smith, Andrew E. Teschendorff

WHAT IS YOUR INTRINSIC AND EXTRINSIC EPIGENETIC AGE?

Immune Report

TruDiagnostic™
The Epigenetic Company

A Recap on TruAge

Methylation is an epigenetic mechanism responsible for turning genes on and off. This phenomenon gives your body an internal clock that can be measured via methylation-specific epigenetic testing. We have reported this age output to you in our TruAge test. However, did you also know that we can break this down even further?

While biological age clocks are a good measure of age for your body, we can look at the age of particular systems in your body as well.

In this expanded report, we will discuss two metrics that give you more information beyond biological age. These metrics are the intrinsic and extrinsic ages of your body.

Our Clocks Tick in Different Ways, Thus Cell Type is Important

If every cell in your body has the same DNA, how do your heart cells become heart cells and your hair cells become hair cells?

The answer is epigenetics. Epigenetics controls cell development and function by switching certain genes on and off, which determines your phenotype and how your cells behave.

It makes sense that the epigenetic regulation of each cell would depend on its cell type. You wouldn't want your heart to make the proteins found in your hair and vice versa; thus, each cell has a different epigenetic signature.

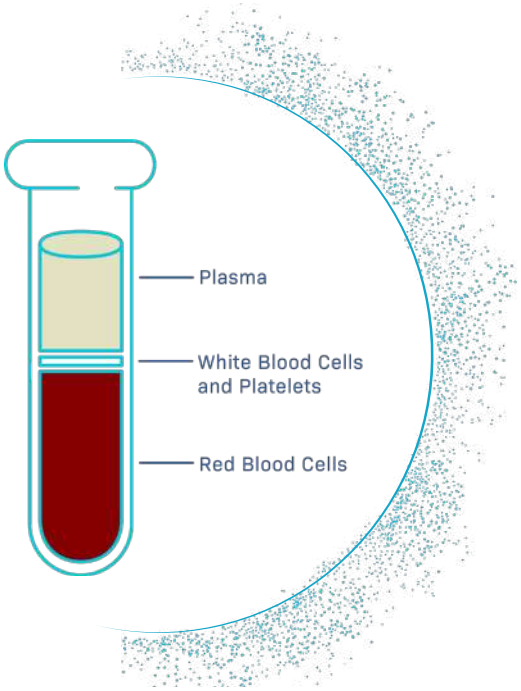
When measuring methylation, moreover, different tissues biologically age at different rates. For example, our cerebellum and brain age slower than the rest of the body. We also see that breast tissue in women can age faster than other tissues across the rest of our body.

Therefore, the rate of aging we calculate is dependent on what cell types we measure. So if we are using blood a the sample type, what cells are we looking at?

What Cells are Found in Your Blood?

The average human adult has more than 5 liters (6 quarts) of blood in his or her body. Blood carries oxygen and nutrients to living cells and takes away their waste products. It also delivers immune cells to fight infections and contains platelets that can form a plug within damaged blood vessels to prevent blood loss.

Thus, our blood has many different functions. For our circulatory system to function properly, our blood must contain these parts:



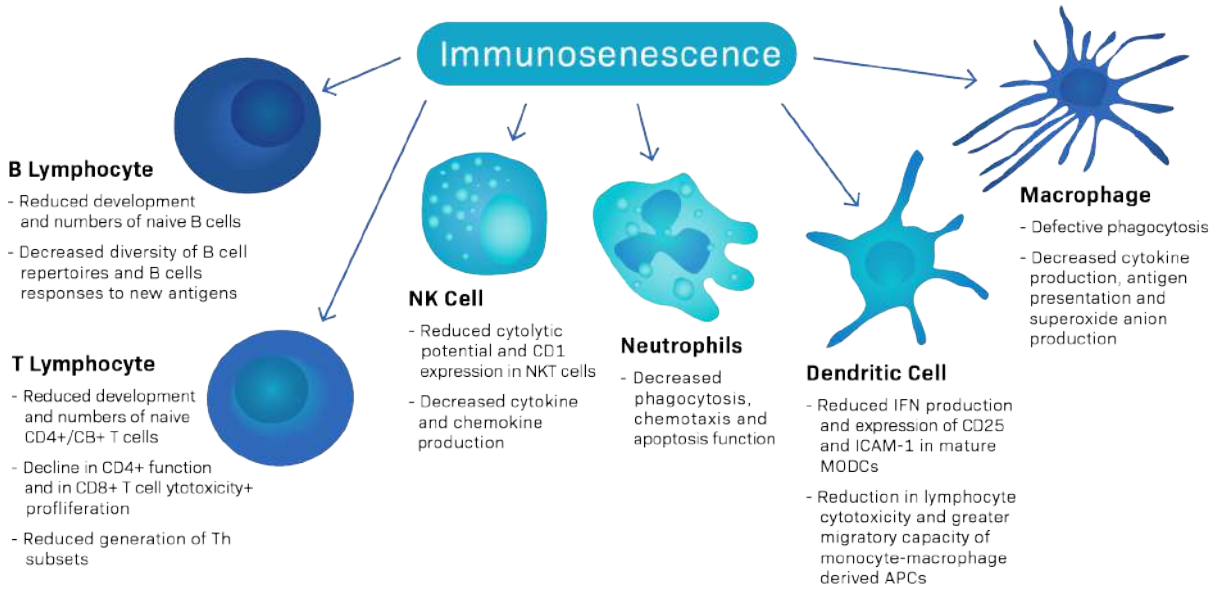
- **Red Blood Cells:** These cells contain hemoglobin and work to carry oxygen throughout the body.

- **Plasma:** The straw-colored fluid that forms the top layer and makes up about 60% of blood. Plasma is mainly water, but it also contains many important substances such as proteins (albumin, clotting factors, antibodies, enzymes, and hormones), sugars (glucose), and fat particles.

- **Platelets:** These are irregularly shaped fragments of cells that circulate in the blood until they are either activated to form a blood clot or are removed by the spleen. Platelets are in the blood so that if we get a tiny cut, we don't bleed out.

- **White Blood Cells (WBCs):** WBCs are an essential part of your immune system for fighting infections. They come in many different shapes and sizes. Some cells have nuclei with multiple lobes, whereas others contain one large, round nucleus. Some contain packets of granules in their cytoplasm, known as granulocytes.

It is important to note that the amounts of white blood cells greatly change with age as shown in the graphic below.



Do you ever wonder why older people are more likely to have negative outcomes with things like COVID-19 and the regular flu? It is because the cells needed to mount an effective response tend to decrease in the blood as we age. **This is called Immunosenescence.**

Immunosenescence: How it relates to Health, Aging, and Biological Age

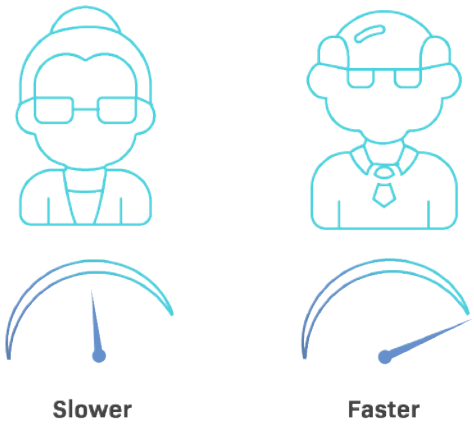
In humans, as well as in many other species, the immune system declines with age. This is known as immunosenescence. The process of immunosenescence leads to a higher incidence of infections, cancer, and autoimmune diseases in the population (6).

As you can see on the right, the figure displays that the progression to immunosenescence is faster in men than in women and is characterized by age-related changes in immune cells and inflammatory mediators. Immunosenescence also changes the number of immune cells in our blood. As we age we have fewer Naive T Cells, Natural Killer Cells, Macrophages, Dendritic cells, and others.

Since your immune cells change concentrations as we age, this means that our reading of biological age can be affected.

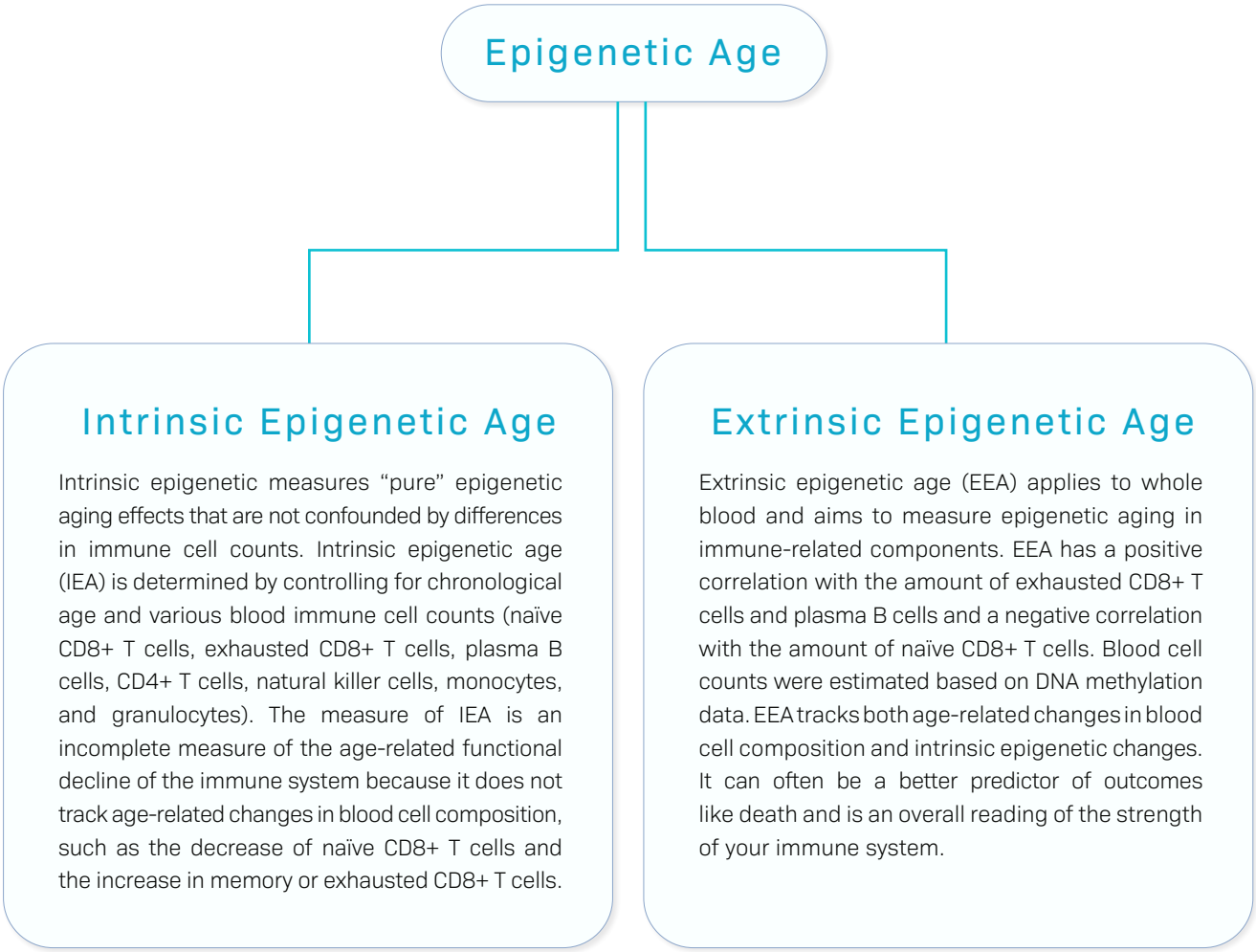
For Intrinsic Epigenetic Age, we control those immune cell changes so that your immune cell subsets are not affecting your reading of age.

We also report out an Extrinsic Epigenetic age measurement, which does not control for those immune cell subsets. Hence, your extrinsic epigenetic age is a surrogate marker for the age of your immune system.



The Difference Between Intrinsic and Extrinsic Epigenetic Aging

If we break down epigenetic age, it can be split into two important categories: ***intrinsic*** and ***extrinsic*** epigenetic age.



Definitions:

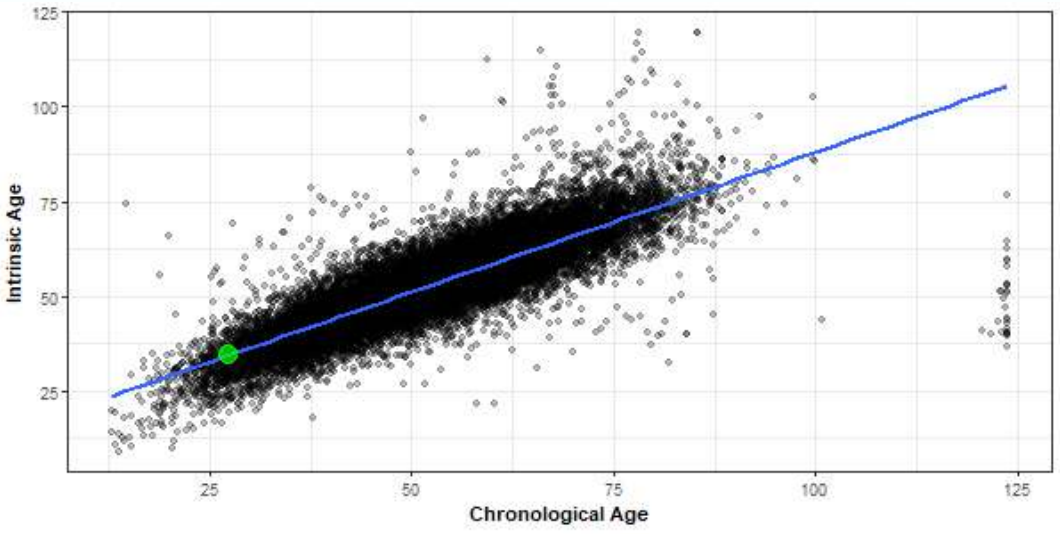
IEAA (Intrinsic Epigenetic Age Acceleration):
A measure that describes age acceleration of the body, independent of age-related changes associated with immune aging.

EEAA (Extrinsic Epigenetic Age Acceleration):
Calculates age acceleration and includes the immune system, thus capturing immune aging.

YOUR INTRINSIC
Epigenetic Age

TruAge IEAA=-5.94

You versus the Population



30.99

This is your Intrinsic Epigenetic Age using our previous (not Principle Component Analysis corrected) algorithm.

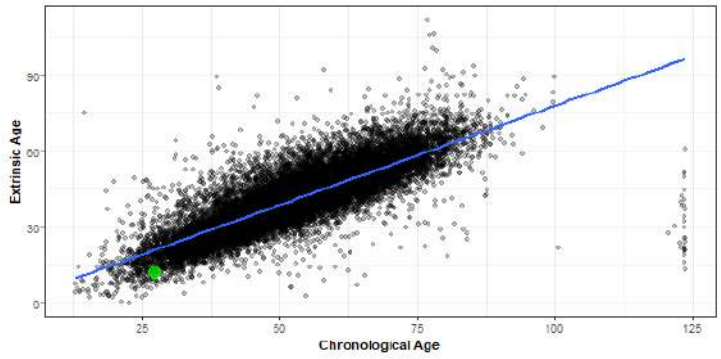
YOUR EXTRINSIC
Epigenetic Age

TruAge IEAA=-3.88 | TruAge EEAA=-0.11

Parameters	Reference Range	Percentage Values (%)
Bcell	20% to 40%	3.93%
CD4T		0.36%
CD8T		25.83%
NK		0.00%
Lymphocyte Total		30.12%
Neutrophils	40% to 60%	63.97%
Monocytes	2% to 8%	5.91%
Eosinophils	1% to 4%	0.00%
CD4T/CD8T Cell Ratio	1 to 4	0.01

This is a functional measurement of your immune system. This measures and predicts many of the cells which change in concentration as we age.

You versus the Population



Disclaimer: The immune cell estimation algorithm is based on a reference-free protocol that uses only your epigenetic profile to calculate the values. This makes the algorithm extremely sensitive to any changes in the methylation values of your DNA. Therefore, the number generated in this report may not be a true reflection of your immune cell counts and you might see some abnormal values as this continues to improve.

What Might have Played into my Score?

The Two Main Studies

Two studies have been done which have looked at correlations between these biological ages. One is the ***Bogalusa study*** and the other is the ***Women's Health Initiative Study*** (WHI). We have included tables of their associations below and summarized many results in the text.

Bogalusa Study Multivariate model that regresses epigenetic age acceleration on participant characteristics in the Bogalusa Study. Coefficients and p-values from regressing measures of intrinsic and extrinsic epigenetic age acceleration on participant characteristics from dataset 1.

Multivariate linear regression		Intrinsic EAA			Extrinsic EAA		
		Estimate (SE)	Z	*p*	Estimate (SE)	Z	*p*
Race	Caucasian vs. African American	-0.013 (0.316)	-0.04	0.97	0.843 (0.316)	2.67	0.0076
Gender	Female vs. Male	-0.622 (0.278)	-2.24	0.025	-0.718 (0.277)	-2.60	0.0093
Education	Grade 8-9 vs. < Grade 8	1.583 (1.468)	1.08	0.28	2.177 (1.465)	1.49	0.14
	Grade 10-12 vs. < Grade 8	1.285 (1.27)	1.01	0.31	2.267 (1.267)	1.79	0.074
	Vocat/Tech vs. < Grade 8	0.307 (1.299)	0.24	0.81	1.921 (1.295)	1.48	0.14
	College vs. < Grade 8	0.85 (1.281)	0.66	0.51	2.375 (1.277)	1.86	0.062
	Graduate vs. < Grade 8	0.147 (1.336)	0.11	0.91	1.53 (1.332)	1.15	0.25
Diabetes (II)		0.173 (0.485)	0.36	0.72	0.012 (0.483)	0.03	0.98
Hypertension		0.539 (0.291)	1.86	0.064	1.247 (0.29)	4.30	$1.7x10^{-5}$
R-squared		0.025			0.043		

WHI Study Multivariate model that regresses epigenetic age acceleration on participant characteristics in the WHI Study. Coefficients and p-values from regressing measures of intrinsic and extrinsic epigenetic age acceleration on participant characteristics from dataset 2.

Multivariate linear regression		Intrinsic EAA		Extrinsic EAA	
		Estimate (SE)	*p*	Estimate (SE)	*p*
Race/Ethnicity	Hispanic vs. African American	-0.94 (0.35)	0.007	3.363 (0.439)	$<10^{-15}$
	White vs. African American	0.71 (0.295)	0.016	1.94 (0.37)	$1.6x10^{-7}$
HDL-cholesterol		0.006 (0.01)	0.558	-0.003 (0.013)	0.799
Triglyceride		0.003 (0.002)	0.059	0.004 (0.002)	0.04
Insulin		0 (0.001)	0.664	0.001 (0.001)	0.337
Glucose		0.003 (0.004)	0.486	0.007 (0.005)	0.112
CRP		0.023 (0.018)	0.215	0.052 (0.023)	0.023
Creatinine		0.703 (0.594)	0.237	1.985 (0.745)	0.008
BMI		0.035 (0.021)	0.103	0.045 (0.027)	.093
Education	High School (HS) vs. no HS	0.357 (0.426)	0.403	-0.784 (0.534)	0.142
	Some College vs. no HS	0.469 (0.381)	0.219	-1.172 (0.478)	0.014
	College vs. no HS	0.486 (0.519)	0.349	-2.253 (0.65)	0.001
	Grad School vs. no HS	0.36 (0.424)	0.396	-1.648 (0.531)	0.002
Alcohol	Past Drinker vs. Never	1.668 (1.1)	0.13	-0.598 (1.379)	0.665
	Light Drinker vs. Never	-0.101 (0.536)	0.85	-0.751 (0.672)	0.264
	Moderate vs. Never	-0.416 (0.748)	0.578	-0.401 (0.937)	0.669
	Heavy vs. Never	-0.354 (0.88)	0.687	-0.833 (1.103)	0.45
Smoking	Former vs. Current	-0.573 (1.039)	0.581	-0.104 (1.302)	0.936
	Never vs. Current	-0.376 (1.039)	0.718	-0.122 (1.303)	0.925
Diabetes		0.216 (0.43)	0.616	-0.061 (0.539)	0.909
Hypertension		0.364 (0.241)	0.131	0.262 (0.302)	0.386
R-squared		0.029		0.069	

Contributing Factors

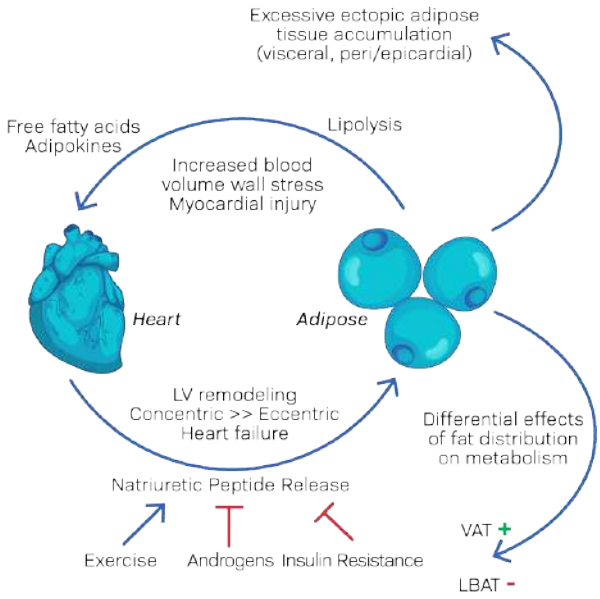
Cardiometabolic Disease, Metabolic Syndrome, and BMI

The health of your metabolic system and your cardiovascular system are intimately related. In fact, because these account for a large proportion of all disease risk, it is no wonder that these metrics can have effects on aging. Cardiometabolic disease can also have an affect on your extrinsic age.

EEA is linked more closely with risk factors for cardiometabolic disease than intrinsic aging according to one study.

EEA is also generally higher in individuals with higher triglyceride levels, higher C-Reactive protein, and higher creatinine.

Neither the intrinsic nor extrinsic epigenetic age of blood tissue are predictive of coronary heart disease (CHD) in the Women's Health Initiative study (WHI) even though EEA is weakly associated with several cardiometabolic risk factors of CHD (such as hypertension, triglycerides, and CRP) (1).



Dietary Intake

Extrinsic epigenetic age acceleration (EEAA) exhibits significant associations with fish intake, moderate alcohol consumption, and blood carotenoid levels (p=1x10-5), an indicator of fruit and vegetable consumption (7).

Race/Ethnicity

Race, ethnicity, and other underlying genetic features also have a significant effect on extrinsic epigenetic age. One study looked at race and found the correlations below.

Hispanics and Tsimane have a higher EEA than Caucasians

Hispanics have a significantly older extrinsic epigenetic age than Caucasians and fewer naïve CD4+ T cells, based on cytometric data from several studies. This pattern of fewer naïve CD4+ T cells is even more pronounced for Tsimane, who experience repeated acute infections and elevated, often chronic, inflammatory loads.

African Americans have lower EEA than Caucasians

African Americans have lower EEA than Caucasians in the WHI and the Bogalusa Study. A study found that African Americans have indications of a significantly younger immune system age than Caucasians ($p = 0.0076$) after controlling for gender, educational level, diabetes status, and hypertension.

In the Bogalusa study, we find three significant predictors of EEA: race/ethnicity, hypertension, and gender ($p = 0.0093$). A marginal analysis in the Bogalusa study identifies a significant association between EEA and hypertension ($p = 8.0 \times 10–5$), type II diabetes status in Caucasians ($p = 0.0085$), but not in African Americans (1).

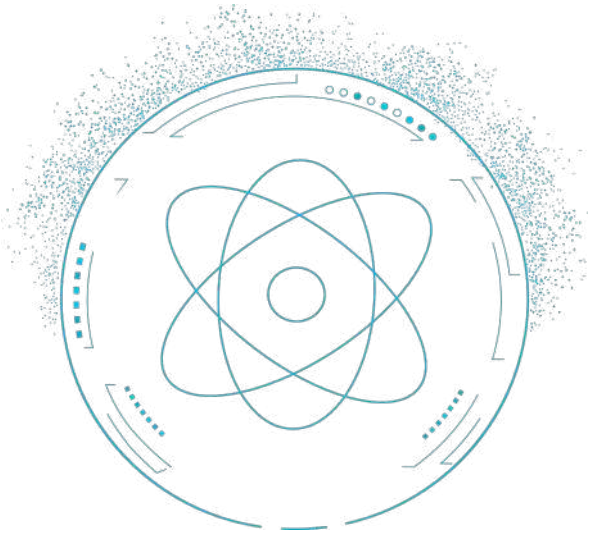
Contributing Factors

Education

Often, education is linked to changes in aging because it is correlated to other lifestyle metrics.

In the WHI study, extrinsic epigenetic age was lower with higher levels of education in all ethnic groups. For each racial/ethnic group, we find that women who did not finish high school exhibit the highest levels of EEA.

However, contrary to the findings in the WHI, no significant association can be observed between EEA and educational level in other studies. More studies are needed to correlate education with extrinsic epigenetic aging.



Mood Stabilizers

Are you currently taking mood stabilizers? Compared with controls, there was a decrease in EEA and IEA in patients with Bipolar Disorder (BD). Further, there was a significant decrease in EEA and IEA in patients with BD taking medication combinations of mood stabilizers (including lithium carbonate, sodium valproate, and carbamazepine) than in those taking no medication/monotherapy (5).

Smoking

Nominally, significant genetic correlations between EEA and lifestyle factors (including smoking behaviors and education) support the hypothesis that the extrinsic epigenetic age is sensitive to variations in the environment.

What are my concerns if my reading is high?

Your Immune System

Since extrinsic epigenetic age is also able to predict the amount of several of your immune values, it is also considered a surrogate marker of the immune system. As a result, a high score may signify that your immune system is not functioning at its highest potential.

When the immune system isn't functioning correctly, your risk of some diseases and disease complications increases. Some of these things include higher cancer risk, higher inflammation (often called inflammaging), higher burdens of senescence, higher risk of autoimmune disease, and much more. If you are worried about your score in this regard, please contact your healthcare provider to learn more.

Your Longevity

Unfortunately, a higher extrinsic epigenetic age is also correlated with shorter lifespans (1). 2,734 deaths were included in a study and it showed that higher extrinsic epigenetic age correlated to a higher hazard ratio for death.

Thus, the high predictive significance of EEA for all-cause mortality probably reflects the fact that it assesses multiple aspects of the biological age of the immune system including both changes in immune cell composition and cell-intrinsic epigenetic changes. It has been known for decades that poor T cell functioning is predictive of mortality (8).

How to Positively Affect this Metric and What Could have Affected your Metric

One of the best things about epigenetic measurements on aging is that proper interventions can lead to better health. While interventional trails on this topic are still in their infancy, we are working to find the best methods to change these metrics.

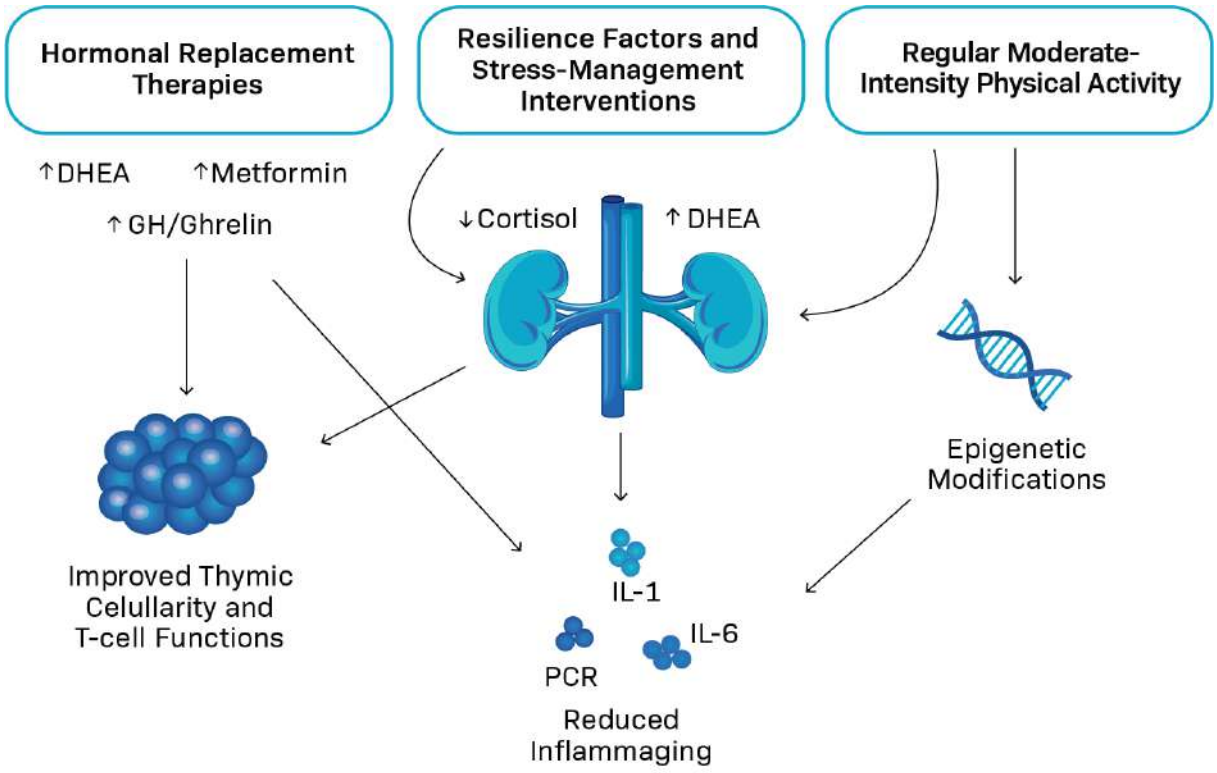
Currently, there is data on how to change this and ways to increase healthier outcomes furthermore.

First, extrinsic epigenetic age acceleration (EEAA) exhibits significant associations with fish intake (p=0.02), moderate alcohol consumption (p=0.01), BMI (p=0.01), and blood carotenoid levels (p=1x10-5), (an indicator of fruit and vegetable consumption) whereas intrinsic epigenetic age acceleration (IEAA) is associated with poultry intake (p=0.03) and BMI (p=0.05) (7).

This means that moderate consumption of alcohol (only validated at 1 drink per week) could help reduce this metric. The consumption of fish, fruits, and vegetables is correlated with an improved EEA.

Other interventions like reducing your BMI and body weight are also correlated with improved metrics.

It is plausible that therapies which prevent or delay the immune systems decline over time might be helpful as well. One validated intervention in this space revolves around the regeneration of the thymus. The thymus is one of our most important immune organs and gets smaller as we age. DHEA, Metformin, and GH-related therapies have all shown improvement in regenerating the thymus and changing the immune cells in our body. Please talk to your healthcare provider about therapies that can help benefit the immune system.



REFERENCES

1. Chen, B. H., Marioni, R. E., Colicino, E., Peters, M. J., Ward-Caviness, C. K., Tsai, P. C., Roetker, N. S., Just, A. C., Demerath, E. W., Guan, W., Bressler, J., Fornage, M., Studenski, S., Vandiver, A. R., Moore, A. Z., Tanaka, T., Kiel, D. P., Liang, L., Vokonas, P., Schwartz, J., … Horvath, S. (2016). DNA methylation-based measures of biological age: meta-analysis predicting time to death. Aging, 8(9), 1844–1865. https://doi.org/10.18632/aging.101020

2. Gibson, J., Russ, T. C., Clarke, T.-K., Howard, D. M., Hillary, R. F., Evans, K. L., Walker, R. M., Bermingham, M. L., Morris, S. W., Campbell, A., Hayward, C., Murray, A. D., Porteous, D. J., Horvath, S., Lu, A. T., McIntosh, A. M., Whalley, H. C., & Marioni, R. E. (2019). A meta-analysis of genome-wide association studies of epigenetic age acceleration. PLOS Genetics, 15(11), e1008104. https://doi.org/10.1371/journal.pgen.1008104

3. Horvath, S., Gurven, M., Levine, M. E., Trumble, B. C., Kaplan, H., Allayee, H., Ritz, B. R., Chen, B., Lu, A. T., Rickabaugh, T. M., Jamieson, B. D., Sun, D., Li, S., Chen, W., Quintana-Murci, L., Fagny, M., Kobor, M. S., Tsao, P. S., Reiner, A. P., Edlefsen, K. L., … Assimes, T. L. (2016). An epigenetic clock analysis of race/ethnicity, sex, and coronary heart disease. Genome biology, 17(1), 171. https://doi.org/10.1186/s13059-016-1030-0

4. Neeland, I. J., Poirier, P., & Després, J. P. (2018). Cardiovascular and Metabolic Heterogeneity of Obesity: Clinical Challenges and Implications for Management. Circulation, 137(13), 1391–1406. https://doi.org/10.1161/CIRCULATIONAHA.117.029617

5. Okazaki, S., Numata, S., Otsuka, I., Horai, T., Kinoshita, M., Sora, I., Ohmori, T., & Hishimoto, A. (2020). Decelerated epigenetic aging associated with mood stabilizers in the blood of patients with bipolar disorder. Translational Psychiatry, 10(1), 129. https://doi.org/10.1038/s41398-020-0813-y

6. Pawelec G. (1999). Immunosenescence: impact in the young as well as the old?. Mechanisms of ageing and development, 108(1), 1–7. https://doi.org/10.1016/s0047-6374(99)00010-x

7. Quach, A., Levine, M. E., Tanaka, T., Lu, A. T., Chen, B. H., Ferrucci, L., Ritz, B., Bandinelli, S., Neuhouser, M. L., Beasley, J. M., Snetselaar, L., Wallace, R. B., Tsao, P. S., Absher, D., Assimes, T. L., Stewart, J. D., Li, Y., Hou, L., Baccarelli, A. A., Whitsel, E. A., … Horvath, S. (2017). Epigenetic clock analysis of diet, exercise, education, and lifestyle factors. Aging, 9(2), 419–446. https://doi.org/10.18632/aging.101168

8. Roberts-Thomson, I. C., Whittingham, S., Youngchaiyud, U., & Mackay, I. R. (1974). Ageing, immune response, and mortality. Lancet (London, England), 2(7877), 368–370. https://doi.org/10.1016/s0140-6736(74)91755-3

9. Smith, J. A., Raisky, J., Ratliff, S. M., Liu, J., Kardia, S. L. R., Turner, S. T., Mosley, T. H., & Zhao, W. (2019). Intrinsic and extrinsic epigenetic age acceleration are associated with hypertensive target organ damage in older African Americans. BMC Medical Genomics, 12(1), 141. https://doi.org/10.1186/s12920-019-0585-5

DUNEDINPACE REPORT

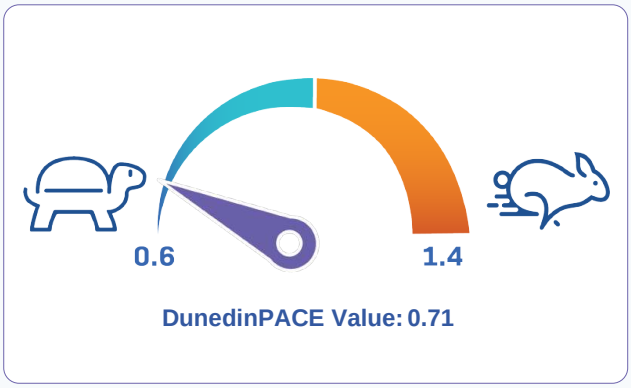
The Study Explained & Where You Land

TruDiagnostic™ The Epigenetic Company

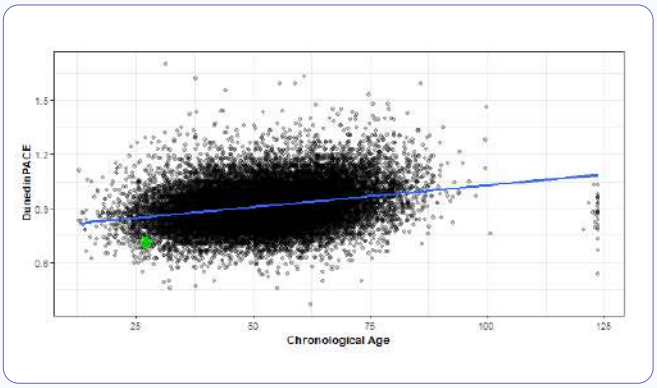
Summary of this Report:

- This report is able to tell you how many biological years you are aging per year at the precise moment.
- It separates what you are doing now from markers you've accumulated from your past, or inherited from your ancestry.
- You want your rate of aging to be below 1.
- Fastest rate of aging has been 1.4 biological years/1.0 year of chronological aging.
- Slowest rate of aging has been 0.6 biological years/1.0 year of chronological aging.
- The average person will age at a rate of 1.0 biological years/1.0 year of chronological aging
- Dietary interventions like fasting have been shown to decrease the aging rate.
- This algorithm was created by Duke and Columbia via a longitudinal study. This means the researchers followed the same individuals over time which is different from other algorithms of aging.

Your DunedinPACE Value



Population Graph

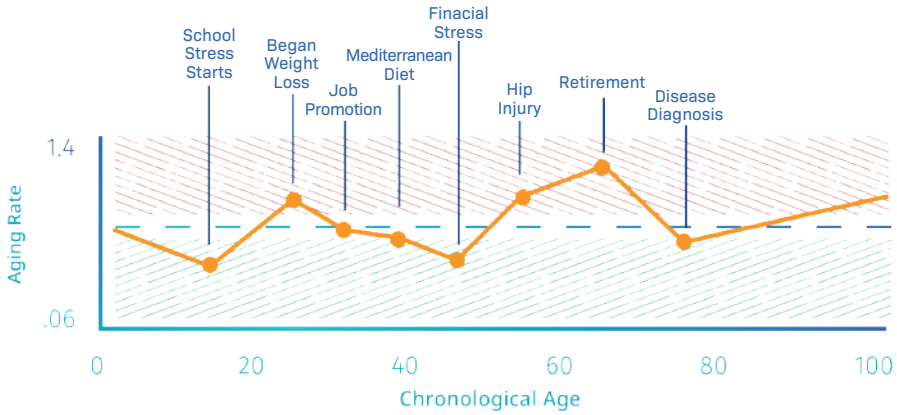


Methylation based biological aging clocks changed the way we look at aging and preventive medicine!
Aging is the number one risk factor for most chronic diseases. Unfortunately, traditional determinants of age (the number of years since birth) don't always match up with how each individual ages. Some people in their 70s look and feel like they are 50, and then there are some 70-year-olds that look like they could be 90. This is called ***phenotypic variation***, and as a result, people have been searching for objective markers to measure the aging process. Thankfully, a highly accurate one was created by measuring epigenetic biomarkers.

Having an objective biological age measurement has massive implications for preventative health and future investigations. However, if we can combine this with an instantaneous rate of aging, we can learn even more about our aging process, our individual aging biology, and the interventions for better preventative health when we combine these two metrics.

Your Aging Rate *Versus* Your Body's Biological Age:

Quantifying one's rate of aging versus biological age is like having a speedometer of aging instead of determining age at a fixed moment in time. Biological age is a great metric, but it doesn't compare past history from current influences on the methylome.



There are many external factors that influence one's pace of aging. The above image is a graphical representation of potential influences on your pace of aging.

There are several cases where knowing both of these metrics can be useful. The best example to illustrate this might be the theoretical case of two identical twins; Twin 1 and Twin 2.

Twin 1 (40 years old chronologically) has lived a very healthy life by implementing proper nutrition, exercise, medications, and lifestyle patterns. On the other hand, Twin B (40 years old chronologically) hasn't lived a life full of similar, healthy habits. For instance, Twin B had a very stressful life in their twenties and early thirties and recently turned their life around. Now, both twins have the exact same lifestyle, nutrition, and exercise regimens along with having the same baseline DNA sequence.

If we only looked at their biological age, we would most likely see that Twin A has a lower biological age due to their consistent history of healthy habits. The same logic would lead us to expect that Twin B might have a worse biological age due to their health history. This might lead us to believe that Twin B is currently doing things in their life to lead to faster aging when in fact the lifestyles of each individual are exactly the same.

However, if we had a way to look at the instantaneous aging rate, we would be able to distinguish advanced aging, which occurred in the past, from the current rate of aging, which is regulated by ongoing lifestyle factors. Distinguishing these two points can also help us decide which lifestyle traits we should keep and which we should change.

Thankfully, due to researchers from Duke and Columbia, an algorithm that measures the pace of aging is already available for us to use.

YOUR PACE OF AGING VALUE:

DunedinPACE Value: 0.71

What Does Your Rate of Aging Mean?

You want your rate of aging to be below one; this means you would have a slowed pace of aging. An average pace of aging would be a rate of 1 biological year for every chronological year aged.

DunedinPACE is associated with chronic disease morbidity and mortality. *Within 7 years from testing those with a faster pace of aging are at a* ***56% increased risk of death and a 54% increased risk for diagnosis of a chronic disease.***

Mortality

Those with faster DunedinPACE levels, which indicates faster aging, at baseline were at increased risk of death having a hazard ratio of 1.29. Hazard ratio represents an instantaneous risk, it is the relationship between the instantaneous hazards between accelerated DunedinPACE and mortality.

Morbidity

Those with a faster DunedinPACE baseline were at an increased risk for a new chronic disease, putting them at a hazard ratio of 1.19. Individuals with faster DunedinPACE experienced higher levels of chronic disease morbidity, which was measured as the count of diagnosed diseases (hypertension, type-2 diabetes, cardiovascular disease, chronic obstructive pulmonary disease, chronic kidney disease, and cancer).

Accelerated Aging Influences

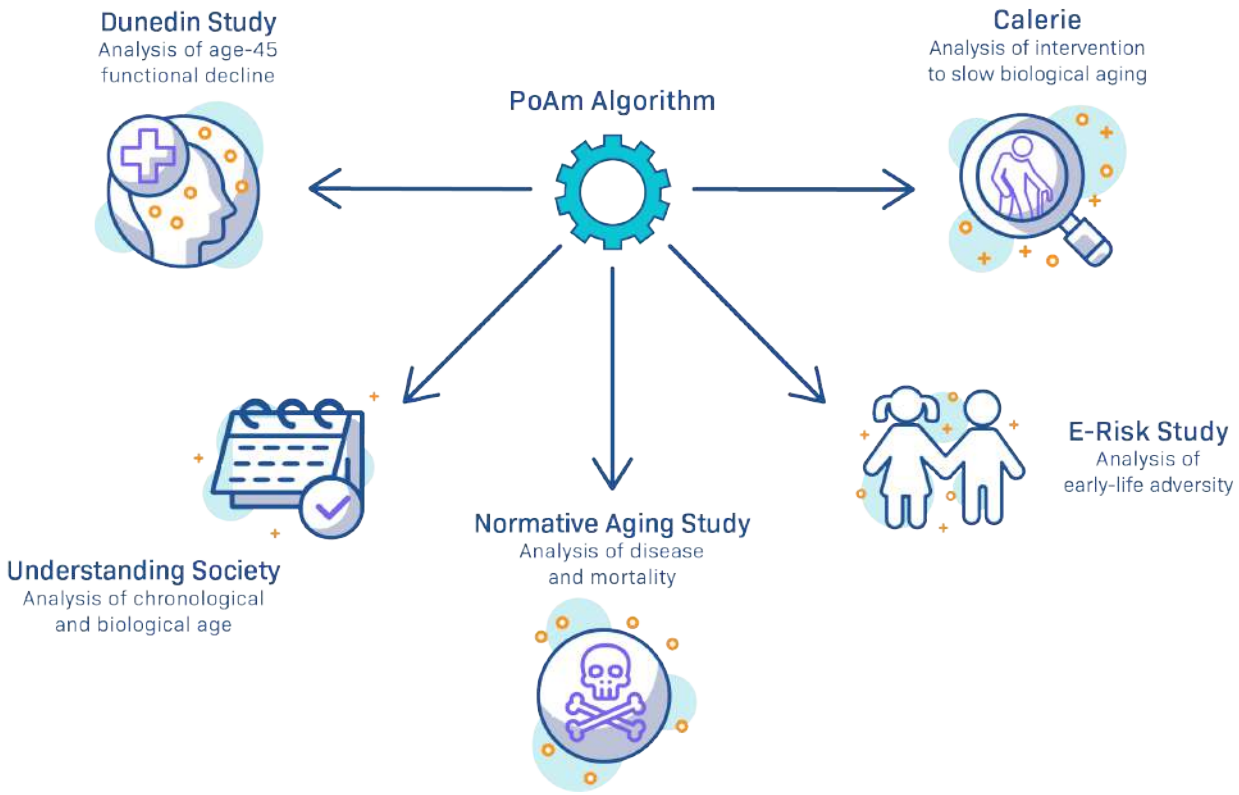
Pace of aging typically increases across much of the adult lifespan. A faster DunedinPACE is the result of a lifetime of accumulated stress to the methylome. Childhood exposure to poverty and victimization is associated with faster DunedinPACE. Adolescents who grew up in families of lower socioeconomic-status and adolescents with exposure to multiple types of victimization exhibited faster DunedinPACE.

The DunedinPACE Algorithm (for Dunedin (P)ace (o)f (A)ging in (m)ethylation) Overview

A team of researchers from Duke and Columbia were able to help create a test that could use blood samples to measure the pace of aging. This test is called the DunedinPACE and it can predict which people are at an increased risk of poor health, chronic disease, and more immediate death.

In order to develop this test, data on chemical changes to an individual's DNA, called DNA methylation, was collected from white blood cell samples from approximately 1,000 participants in a long-term health study known as "The Dunedin Study". Using the data obtained from this cohort the team developed an algorithm – named "DunedinPACE" – that identified people with an accelerated or slowed pace of aging based on a single blood test. [3]

The researchers used a machine-learning technique called elastic-net regression to sort through data on more than 400,000 different DNA methylation marks to find the ones that related to the physiological changes which were captured in their Pace of Aging measure. The analysis pulled out a set of 173 methylation marks that, together, measured the pace of aging for individuals at one point in time.



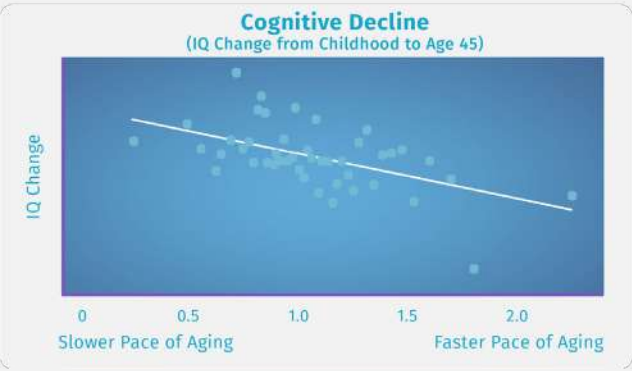
These 173 methylation marks are combined together in an algorithm the researchers named "**DunedinPACE**" for Dunedin (P)ace (o)f (A)ging in (m)ethylation. The average person has a DunedinPACE value of 1, which indicates a single year of biological aging per chronological year. Among Dunedin Study participants, the range of values extends from just above 0.6 (indicating an aging rate nearly 40 percent slower than the norm) to nearly 1.4 (indicating an aging rate 40 percent faster than the norm). [3]

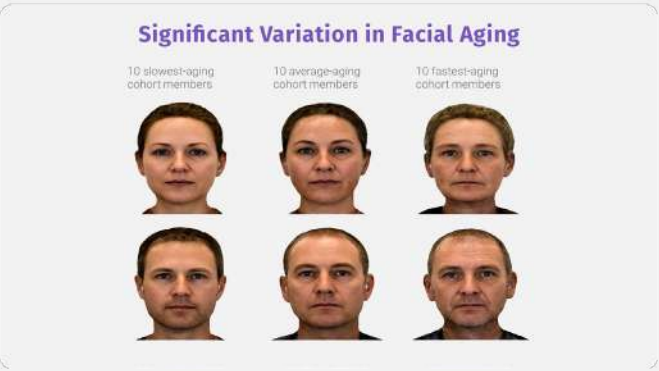
In order to validate the algorithm, the researchers used samples from participants in three other long-term studies. This analysis verified that the individuals whom the algorithm identified as aging faster; had a greater risk of poor health, developing chronic disease, or dying earlier. Similarly, those identified as aging more slowly performed better on tests of balance, strength, walking speed, and mental ability, and additionally they appeared physically younger to trained raters for physical signs of aging.

Additionally, the DunedinPACE researchers used the test on participants in a randomized trial testing whether restricting calories had the potential to extend a healthy lifespan. [9] The results suggested that the calorie restriction could counter the effects of an accelerated pace of aging.

Thanks to this studys promising findings, the test developed by the Dunedin Study team will provides an alternate way of measuring whether age slowing treatments work. This algorithm has the potential to allow faster testing of therapies able to extend the healthy lifespan of humans.

The following graphs are ***NOT*** your personal data. These graphs are showing how the increased rate of aging affects performance from the Dunedin cohort.





The Value of Pace of Aging

The pace of aging in methylation tells how many years you are aging per year at a precise moment. With a single blood test, we are able to identify this. Acquiring your pace of aging gives insight into your current health and disease state. The goal is to have your rate of aging below 1.

The pace of aging algorithm not only provides benefits to the individual tested but it also has application for clinical studies. The DunedinPACE test provides an alternative way of measuring whether age-slowing treatments may work. It is sensitive to health interventions and will allow faster testing of treatment intended to extend healthspan in humans. The more data collected on individuals with accelerated or slowed aging can potentially help reveal the mechanisms of aging and how some individuals are more adversely affected by their lifestyle and environment than others. DunedinPACE will help public health officials test whether policies of programs have the power to help people lead a longer, healthier life.

How Is This Algorithm Game-Changing?

This is a report about an individuals rate of aging. Most epigenetic tests take a snapshot of biological age at the moment in time when the test was taken, but because DunedinPACE determines pace of aging, it is able to differentiate prior biological age factors and the rate of aging at that given time. The pace of aging in a methylation algorithm outperforms a number of other methylation-based biological clock algorithms because its data is unmatched, making DunedinPACE one of the BEST predictors of health outcomes.

The algorithm is noteworthy because it considers the details of one's life and by doing so it interprets your epigenetic alterations to determine the best reading of how you age. Other biological age clock outcomes are dampened by the influences across one's lifetime and will compound the negative outcomes instead of predicting how fast a person is aging at the time of testing. DunedinPACE can interpret small adjustments to your lifestyle while still taking into consideration methylation patterns from earlier years to produce a robust measurement of how one biologically ages.

The algorithm was developed from data collected from the Dunedin study group. The significance of this study was minimizing variables. The Dunedin cohort stands out by having its subjects all born within the same year. All current methylation-clock algorithms have been developed to identify the methylation patterns that characterize individuals of different chronological ages. The limitations of these other algorithms is that the study group consists of individuals born in different years that also grew up in different historical conditions.

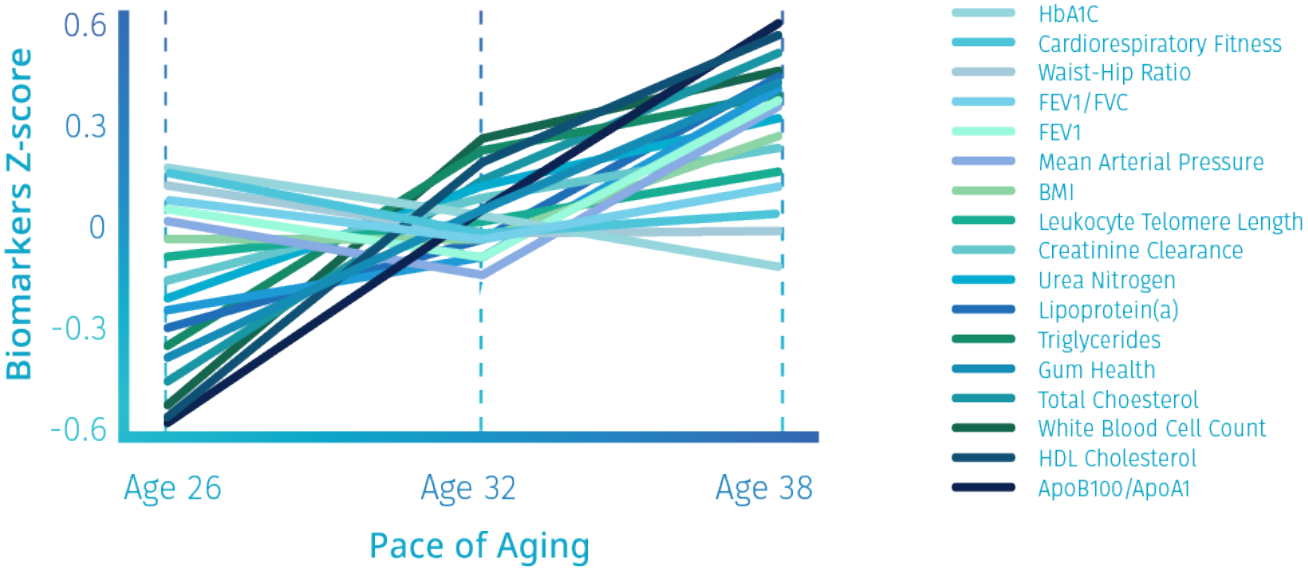
People the algorithm identified as having a faster pace of aging had a greater risk of poor health, chronic disease, and premature death. Other methylation-clock algorithms have been developed to identify methylation patterns that characterize individuals of different chronological ages which imposes a series of limitations on the outcomes being provided by. These other methylation-clock algorithms display their outcomes as an unwavering point instead of where your aging is currently.

How It Compares Against Other Methylation Clocks

Unlike any other biological test out there, the DunedinPACE Algorithm doesn't let us see your biological age, but instead it looks at how fast you are aging. There are a number of benefits of knowing your pace of aging versus your age at a set point in time. By 2050, the world population aged 80 years old and above will more than triple, approaching more than 400 million individuals. This useful measure is non-invasive, inexpensive, reliable, and highly sensitive to biological change; making it an easy tool for health professionals to use to combat the challenges we will soon face with the growing aging population based on real-time measurements of interventions.

The Dunedin researchers tested if higher DunedinPACE levels, which indicate faster aging, were correlated with older chronological age. Mortality rates increase with advancing chronological age, although there may be some slowing at older ages. This suggests the hypothesis that the rate of aging increases across much of the adult lifespan. Consistent with this hypothesis, understanding society participants with older chronological age tended to have faster DunedinPACE value.

Dunedin Longitudinal Study



The above chart shows the Dunedin Longitudinal Study. Dunedin researchers collected a blood panel of 19 markers (shown above) and organ-system-function biomarkers at four successive waves of the Dunedin Study. By using repeated measures of data the study members were aged 26, 32, 38, and 45 years old.

They calculated the rate of change in each biomarker and how each individual's rate of change differed from the cohort's norm. Then they combined the individual's 18 personal rates of change across the panel of biomarkers to compute a composite for each study member, which is how they determine the pace of aging.

More About The DunedinPACE Study
And Its Development

Dunedin: a small city located in the South Island of New Zealand and home to the Dunedin School of Medicine at the University of Otago.

PACE: the pace of aging measure.

The Dunedin Multidisciplinary Health and Development Study is a detailed study of human health, development, and behavior. The Dunedin Study has followed the lives of 1037 babies born between April 1, 1972, and March 31, 1973, at Queen Mary Maternity Hospital, Dunedin, New Zealand, since their birth. The study is now in its fifth decade and has produced a considerable amount of data that shapes what we know about the pace at which humans age. [1] This algorithm was created by looking at over 1,000 individuals' data at 173 CpG sites.

The Dunedin Study

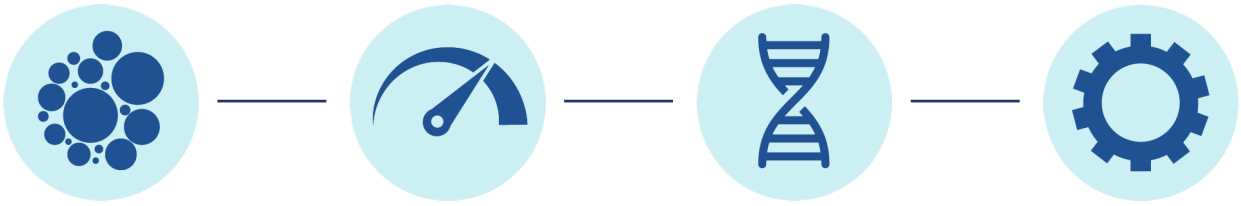
The Dunedin cohort is one of the most remarkable resources for studying human biology. This is not the biggest nor the longest longitudinal study conducted, but it is special because it has a very high retention rate of participants. With 95% of the original cohort remaining in the study since its launch, the Dunedin cohort is THE MOST closely examined group on earth. To put in perspective a good retention rate for longitudinal studies is between 60 to 80 percent of the original cohort population. [11]

Previous studies have attempted to measure the pace of aging by analyzing DNA methylation differences between people of different chronological ages. However, the "limitation of this approach is that individuals born in different years have grown up under different historical conditions, with a possibility of more exposure to childhood diseases, tobacco smoke, airborne lead, and less exposure to antibiotics and other medications, as well as lower quality nutrition -- all of which affect DNA methylation. An alternative approach is to study individuals who were all born the same year, and find methylation patterns that differentiate those who have been aging biologically faster or slower than their same-age peers." [3] The Dunedin study focuses on a one-year age cohort makes it more effective at tracking its participants, which contributes to the low number of extraneous variability in the results.

Following the one-year birth cohort, the repeated measures of data were collected via blood when the study members were 26, 32, 38, and 45 years old to quantify their rates of biological aging. The gathered data represents a personal rate of multi-organ system decline over a dozen years which determines the algorithm for pace of aging.

Four-Step Approach

The researchers took a ***four-step*** approach toward developing a blood DNA methylation metric that represents individual variation in the pace of biological aging.



Step 1
Dunedin Longitudinal Study

Step 2
Pace of Aging

Step 3
Elastic Net Regression

Step 4
DundedinPACE Algorithm

Step 1: In the initial step, the Dunedin researchers collected a blood panel of 18 and organ-system-function biomarkers at three successive waves of the Dunedin Study. By using repeated measures of data the study members were aged 26, 32, 38, and 45 years old. They calculated the rate of change in each biomarker and how each individual's rate of change differed from the cohort's norm. Then they combined the individual's 18 personal rates of change across the panel of biomarkers to compute a composite for each study member, which is how they determine the pace of aging. [7]

Step 2: In the second step they validated the pace of aging from known criteria. Members of the studys cohort who had faster paces of aging performed more poorly on tests of physical function, by showing signs of cognitive decline on a panel of dementia-relevant neuropsychological tests from an early-life baseline for the individuals. These individuals were also rated, using an impartial system, as looking older based on their facial photographs. They also reported themselves to be in worse health.

They also found that a faster pace of aging is associated with early-life factors important for aging: familial longevity, low childhood social class, and adverse childhood experiences. [6] Notably, the pace of aging was not well-correlated with published epigenetic clocks, which were designed to measure how old a person is rather than how fast they are biologically aging. [5]

Step 3: In step three, they refined the pace of aging into a measurement that is obtained from a single blood sample. Here we focused on blood DNA methylation as an accessible molecular measurement that is sensitive to changes in physiology occurring in multiple organ systems.

Researchers used the data from a previous study published by the same authors (Belsky et al., 2018b) to apply an algorithm that captured the DNA methylation patterns linked with variation among individuals in their pace of aging. This algorithm is what they termed "DunedinPACE."

Step 4: Step four is the validation step of the algorithm. They validated it in five ways

First, using the Dunedin Study, they tested if study member's DunedinPACE measured when they were aged 45 years could predict deficits in physical and cognitive functioning seven years later.

Second, researchers applied the DunedinPACE algorithm to DNA methylation data from a second, cross-sectional, study of adults to evaluate patterning of DunedinPACE by chronological age and sex and to test correlations of DunedinPACE with self-reported health and proposed measures of biological age, including three epigenetic clocks.

Third, the DunedinPACE algorithm was applied to DNA methylation data from a third, longitudinal study of older men to test associations with chronic-disease morbidity and mortality.

Fourth, the DunedinPACE algorithm was then applied to DNA methylation data from a fourth, longitudinal, study of young people to test if DunedinPACE was accelerated by exposure to poverty and victimization, factors which are known to shorten healthy lifespan.

Fifth, to ascertain the potential usefulness of DunedinPACE as a measure for trials of geroprotector treatments, the algorithm was applied to DNA methylation data from a randomized trial of caloric restriction, CALERIE [Ravussin et al., 2015]. Earlier we reported from this trial that the intervention (two years of prescribed 25% caloric restriction) slowed the rate of biological aging as measured by a blood-chemistry biological-age composite measure [Belsky et al., 2018a]. Here, using newly generated methylation data from blood drawn at the CALERIE baseline assessment, it was tested if (a) DunedinPACE from blood drawn before caloric restriction could predict the future rate of biological aging of participants during the two-year trial, and (b) if this prediction was disrupted in participants who underwent caloric restriction, but not among control participants. Promising results from this four-step research program was reported, while appreciating that additional measurement development will be needed to support the applied use of DunedinPACE.

REFERENCES

1. About Us: The Dunedin Study - Dunedin Multidisciplinary Health & Development Research Unit. The Dunedin Study - DMHDRU. https://dunedinstudy.otago.ac.nz/about-us.

2. Bell, C. G., Lowe, R., Adams, P. D., Baccarelli, A. A., Beck, S., Bell, J. T., Christensen, B. C., Gladyshev, V. N., Heijmans, B. T., Horvath, S., Ideker, T., Issa, J.-P. J., Kelsey, K. T., Marioni, R. E., Reik, W., Relton, C. L., Schalkwyk, L. C., Teschendorff, A. E., Wagner, W., … Rakyan, V. K. (2019). DNA methylation aging clocks: challenges and recommendations. Genome Biology, 20(1), 249. https://doi.org/10.1186/s13059-019-1824-y

3. Belsky, D. W., Caspi, A., Arseneault, L., Baccarelli, A., Corcoran, D. L., Gao, X., Hannon, E., Harrington, H. L., Rasmussen, L. J. H., Houts, R., Huffman, K., Kraus, W. E., Kwon, D., Mill, J., Pieper, C. F., Prinz, J. A., Poulton, R., Schwartz, J., Sugden, K., … Moffitt, T. E. (2020). Quantification of the pace of biological aging in humans through a blood test, the DunedinPACE DNA methylation algorithm. ELife, 9, e54870. https://doi.org/10.7554/eLife.54870

4. Belsky, D. W., Huffman, K. M., Pieper, C. F., Shalev, I., & Kraus, W. E. (2018a). Change in the Rate of Biological Aging in Response to Caloric Restriction: CALERIE Biobank Analysis. The Journals of Gerontology: Series A, 73(1), 4–10. https://doi.org/10.1093/gerona/glx096

5. Belsky, D. W., Moffitt, T. E., Cohen, A. A., Corcoran, D. L., Levine, M. E., Prinz, J. A., Schaefer, J., Sugden, K., Williams, B., Poulton, R., & Caspi, A. (2018b). Eleven Telomere, Epigenetic Clock, and Biomarker-Composite Quantifications of Biological Aging: Do They Measure the Same Thing? American Journal of Epidemiology, 187(6), 1220–1230. https://doi.org/10.1093/aje/kwx346

6. Belsky, D. W., Caspi, A., Cohen, H. J., Kraus, W. E., Ramrakha, S., Poulton, R., & Moffitt, T. E. (2017). Impact of early personal-history characteristics on the Pace of Aging: implications for clinical trials of therapies to slow aging and extend healthspan. Aging Cell, 16(4), 644–651. https://doi.org/10.1111/acel.12591

7. Belsky, D. W., Caspi, A., Houts, R., Cohen, H. J., Corcoran, D. L., Danese, A., Harrington, H., Israel, S., Levine, M. E., Schaefer, J. D., Sugden, K., Williams, B., Yashin, A. I., Poulton, R., & Moffitt, T. E. (2015). Quantification of biological aging in young adults. Proceedings of the National Academy of Sciences, 112(30), E4104 LP-E4110. https://doi.org/10.1073/pnas.1506264112

8. Campisi, J., Kapahi, P., Lithgow, G. J., Melov, S., Newman, J. C., & Verdin, E. (2019). From discoveries in ageing research to therapeutics for healthy ageing. Nature, 571(7764), 183–192. https://doi.org/10.1038/s41586-019-1365-2

9. Racette, S. B., Das, S. K., Bhapkar, M., Hadley, E. C., Roberts, S. B., Ravussin, E., Pieper, C., DeLany, J. P., Kraus, W. E., Rochon, J., & Redman, L. M. (2011). Approaches for quantifying energy intake and %calorie restriction during calorie restriction interventions in humans: the multicenter CALERIE study. American Journal of Physiology-Endocrinology and Metabolism, 302(4), E441–E448. https://doi.org/10.1152/ajpendo.00290.2011

10. Ravussin, E., Redman, L. M., Rochon, J., Das, S. K., Fontana, L., Kraus, W. E., Romashkan, S., Williamson, D. A., Meydani, S. N., Villareal, D. T., Smith, S. R., Stein, R. I., Scott, T. M., Stewart, T. M., Saltzman, E., Klein, S., Bhapkar, M., Martin, C. K., Gilhooly, C. H., … Kritchevsky, S. (2015). A 2-Year Randomized Controlled Trial of Human Caloric Restriction: Feasibility and Effects on Predictors of Health Span and Longevity. The Journals of Gerontology: Series A, 70(9), 1097–1104. https://doi.org/10.1093/gerona/glv057

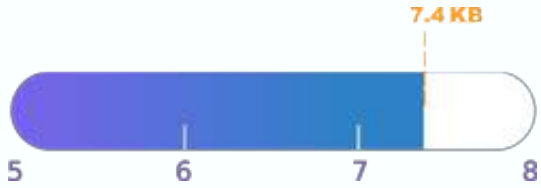
11. Teague, S., Youssef, G. J., Macdonald, J. A., Sciberras, E., Shatte, A., Fuller-Tyszkiewicz, M., Greenwood, C., McIntosh, J., Olsson, C. A., Hutchinson, D., & SEED Lifecourse Sciences Theme (2018). Retention strategies in longitudinal cohort studies: a systematic review and meta-analysis. BMC medical research methodology, 18(1), 151. https://doi.org/10.1186/s12874-018-0586-7

NIKITHA R
TELOMERE LENGTH REPORT
And How Their Length Affects You

TruDiagnostic™
The Epigenetic Company

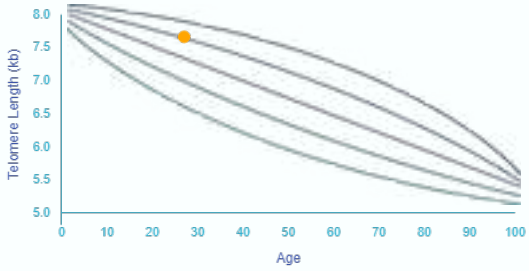
YOUR RESULTS:

Average Telomere Length



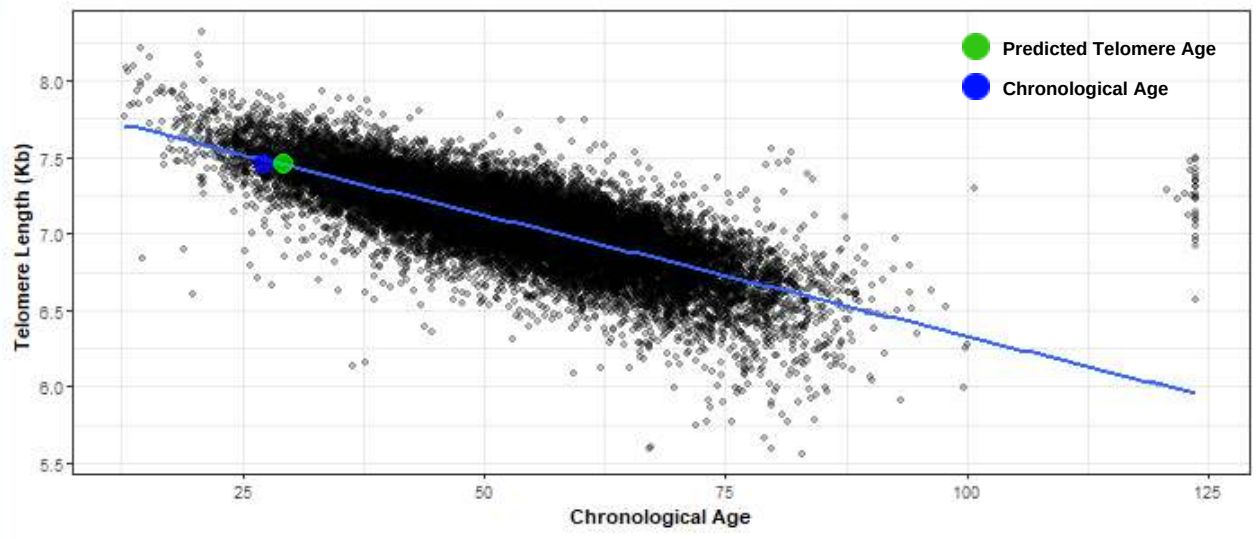
Your average telomere length is:
7.45 kilobases (Kb).

Your Percentile



At your chronological age of **27.20**, you would be in the **37.40th** percentile of telomere length compared to others of your same chronological age. This means that your telomeres are longer than **37.40%** of people your age

Your Telomere Length Based Biological Age Prediction



Estimated Telomere Age: 29.11

If we were to use the data from our sample subjects to predict your biological age from your telomere measurement we would anticipate your age to be **29.11.**

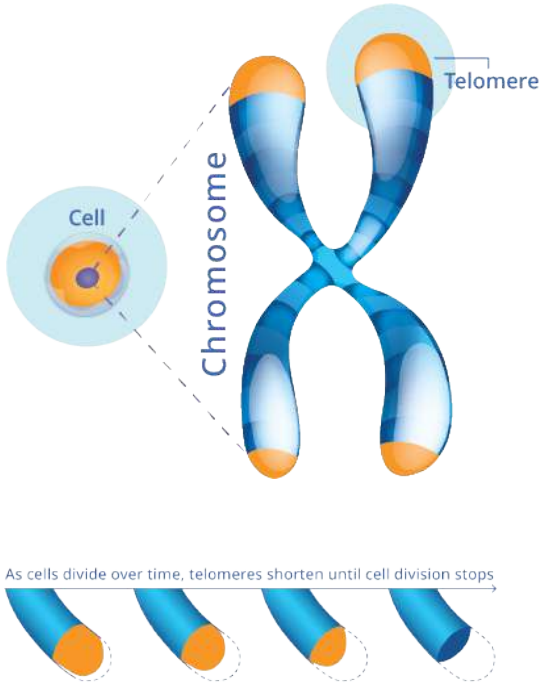
Introduction to Telomeres

Telomeres are repeating sequences of nucleotide sequences (TTAGGG) that tag the ends of all chromosomes. They are designed to prevent unpredictable changes in the DNA strand, keeping the genome stable [3].

Their primary function is to prevent chromosomal "fraying" when a cell replicates, much like the plastic tips on the end of shoelaces [5]. As a cell ages, its telomeres become shorter.

This shortening is thought to be one of several factors that causes cells to age. In actively dividing cells, such as those in the bone marrow, the stem cells of the embryo, and germ cells in the adult, telomere length (TL) is kept constant by the enzyme telomerase.

As the organism grows, this enzyme becomes less active over time. This leads to a slow decrease in telomere length, until a point is reached at which the cell is no longer capable of replication ('replicative senescence'). A cell can no longer divide when telomeres are too short—once they reach a critical point, the cell becomes inactive (or 'senescent'), slowly accumulating damage that it can't repair, or it dies [6].



Why are Telomeres Important?

Telomere length is affected by both genetic and epigenetic contributions. A new study found that DNA methylation is closely linked to TL. The study by researchers at the University of California Los Angeles shows a very significant linkage between two different markers that indicate aging [2].

Telomeres are an essential part of human cells that affect how our cells age [1]. Telomere length has emerged as an important determinant of replicative senescence and cell fate - an important indicator of the aging process and a wide range of disease states, including cancers, cardiovascular disease, and age-related disorders.

Shorter telomeres are not only associated with age but with disease too. In fact, shorter telomere length and low telomerase activity are associated with several chronic preventable diseases. These include hypertension, cardiovascular disease, insulin resistance, type 2 diabetes, depression, osteoporosis, and obesity.

Shorter telomeres have also been implicated in genomic instability and oncogenesis. Older people with shorter telomeres have three and eight times increased risk to die from heart and infectious diseases, respectively [4].

The rate of telomere shortening and telomere length is therefore critical to an individual's health and pace of aging.

REFERENCES

1. Jaskelioff, M., Muller, F. L., Paik, J.-H., Thomas, E., Jiang, S., Adams, A. C., Sahin, E., Kost-Alimova, M., Protopopov, A., Cadiñanos, J., Horner, J. W., Maratos-Flier, E., & DePinho, R. A. (2011). Telomerase reactivation reverses tissue degeneration in aged telomerase-deficient mice. Nature, 469(7328), 102–106. https://www.researchgate.net/publication/49640982_Telomerase_reactivation_reverses_tissue_degeneration_in_aged_telomerase-deficient_mice

2. Lee, Y., Sun, D., Ori, A. P. S., Lu, A. T., Seeboth, A., Harris, S. E., Deary, I. J., Marioni, R. E., Soerensen, M., Mengel-From, J., Hjelmborg, J., Christensen, K., Wilson, J. G., Levy, D., Reiner, A. P., Chen, W., Li, S., Harris, J. R., Magnus, P., … Horvath, S. (2019). Epigenome-wide association study of leukocyte telomere length. Aging, 11(16), 5876–5894. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6738430/

3. Lu, A. T., Seeboth, A., Tsai, P.-C., Sun, D., Quach, A., Reiner, A. P., Kooperberg, C., Ferrucci, L., Hou, L., Baccarelli, A. A., Li, Y., Harris, S. E., Corley, J., Taylor, A., Deary, I. J., Stewart, J. D., Whitsel, E. A., Assimes, T. L., Chen, W., … Horvath, S. (2019). DNA methylation-based estimator of telomere length. Aging, 11(16), 5895–5923. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6738410/

4. Shammas M. A. (2011). Telomeres, lifestyle, cancer, and aging. Current opinion in clinical nutrition and metabolic care, 14(1), 28–34. https://doi.org/10.1097/MCO.0b013e32834121b1

5. Songyang, Z. (2017). Introduction to Telomeres and Telomerase. Methods in Molecular Biology (Clifton, N.J.), 1587, 1–13. https://pubmed.ncbi.nlm.nih.gov/21461806/

6. Zvereva, M., Shcherbakova, D., & Dontsova, O. (2010). Telomerase: Structure, functions, and activity regulation. Biochemistry (00062979), 75(13), 1563–1583. https://www.researchgate.net/publication/50591672_Telomerase_Structure_functions_and_activity_regulation

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IMPACT: Weight loss response to caloric restriction

GENES: PON3

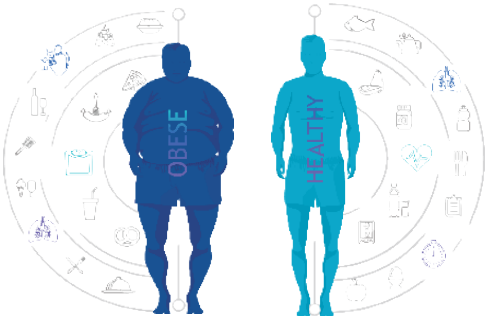
Weight Loss

Weight loss can be difficult, especially for individuals who have been overweight for years. There is not a one-size-fits-all approach to weight loss therapy. A hypocaloric (calorie-deficient) diet is typically considered one of the best approaches for weight loss, but not everyone responds to calorie deficit in the same way. Even with a strict regimen and no deviations, there are molecular and epigenetic components to how a body will respond to calorie deficit. Sometimes weight loss isn't a sure thing.

This report aims to help individuals identify their personal weight-loss response to caloric restriction.

YOUR EPITYPE:

Based on your methylation, you are a **Non-Responder** for weightloss interventions using caloric restriction



What is Obesity?

Obesity can be defined as a disease in which excess body fat has accumulated such that health may be adversely affected.
The prevalence of obesity has increased dramatically over the past few decades. It presents major health obstacles because of its substantial increase in risk for diseases such as type 2 diabetes, hypertension, myocardial infarction, stroke, dementia, osteoarthritis, and many types of cancer.

The rising incidence of obesity has caused the condition to be so common within the world's population that it is beginning to replace undernutrition and infectious diseases as the most significant contributor to ill health.

The global epidemic of obesity is fueled by a combination of genetic susceptibility, increased availability of high-calorie foods and decreased requirement for

Analyzing DNA Methylation

Our laboratory uses array-based DNA methylation testing to identify sites where methylation has occurred and measure the degree to which that location has been methylated. Advances in the field of epigenetics have found that we can use these variations in methylation can identify changes in specific biological responses.
For each gene, there is a range of methylation statuses that are considered normal, and methylation that falls outside that range.

Hypomethylation is when the methylation status is below a specific threshold. This threshold depends on the CpG loci it's on. Hypomethylation is also what we call the process of a gene losing methylation.

Hypermethylation, is when a gene's methylation status is over a specific threshold. It can also be the process of gaining more methylation.
A hypermethylated gene is usually being repressed or silenced.

What are CpG Loci?

CpG loci are specific locations on your DNA where methylation can occur. They are often near sites that begin transcribing a gene's instructions so those instructions can be carried out by the body.
When methylation occurs on the loci around a gene, the instructions can be changed from their original meaning or silenced entirely. A single gene can be influenced by the methylation on many nearby CpG loci.
In this report, we examine how much methylation has occurred on CpG loci around specific genes, and offer insights into how those changes affect you.

What affects DNA Methylation?

We have not yet uncovered the full list of everything that can influence DNA Methylation development. Known factors include nutrition, chronic or acute stress, sleep habits, activity levels, inflammation, oxidative stress, hypoxia, and much more.

Many patterns of methylation are not permanant, and studies have found they can be reversed by interventions like reducing stress, improving nutrition, and reducing exposure to pollutants or biological stressors. Neither of these lists are comprehensive.

What are Beta Values?

A beta value is essentially the percent-value that a specific CpG loci has been methylated. Depending on how many methyl molocules attached to that CpG, the loci could be anywhere from 0% to 100% methylated.
The more methylation a CpG has, the more it's working to silence nearby genes.
While a single CpG that is 100% methylated generally cannot silence an entire gene, groups of highly methylated CpG can work together to do so.

Your CpG Beta Values

	CpG site	Gene	ß-value Responders	Your Score	Response Status
1	cg15500865	PON3	0.072	0.08	Hypermethylated
2	cg25161512	PON3	0.115	0.09	Hypomethlyated
3	cg11435506	PON3	0.165	0.04	Hypomethlyated
4	cg03301582	PON3	0.120	0.09	Hypomethlyated
5	cg08898155	PON3	0.163	0.02	Hypomethlyated
6	cg04080282	PON3	0.324	0.18	Hypomethlyated
7	cg26457160	PON3	0.490	0.47	Hypomethlyated
8	cg10329418	PON3	0.252	0.20	Hypomethlyated
9	cg27166921	PON3	0.253	0.33	Hypermethylated
10	cg24750391	PON3	0.355	0.29	Hypomethlyated
11	cg08461772	PON3	0.418	0.24	Hypomethlyated

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 successsful 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.

Learn About Your Genes

PON3

PON3 creates a protein that circulates in the blood stream. This protein binds to lipoproteins, which transport fat molecules through the blood.

The PON3 protein protects lipoproteins like HDL and LDL (also known as cholesterols) against oxidation. When LDL is oxidized, it can cause inflammation which leads to plaque in the arteries and possible damage to arterial walls.

Oxidized LDL is also believed to play a role in increasing the amount of fat your body deposits. It increases the production of triglycerides, which is the most common type of fat produced when your body has extra calories available.

Studies have found that the pattern of methylation on PON3 can predict how a person's weight and body fat will respond to caloric restriction.



THE IMPACT ON YOU

CpG loci around your PON3 gene are generally under-methylated. Based on your methylation, you are a **Non-Responder** for weight-loss interventions. This means your response to caloric restriction alone is low, so it is not likely to be an effective form of weight loss

Knowing the impact your gene expression has on your ability to treat weight management can help you and your healthcare provider determine the best interventions for weight loss in the future.



REFERENCES

1. Blüher, M. (2019) Obesity: global epidemiology and pathogenesis. Nat Rev Endocrinol 15, 288–298. https://doi.org/10.1038/s41574-019-0176-8

2. Cawley, J., & Meyerhoefer, C. (2012). The medical care costs of obesity: an instrumental variables approach. Journal of health economics, 31(1), 219–230. https://doi.org/10.1016/j.jhealeco.2011.10.003

3. Edillor, C. R., Parks, B. W., Mehrabian, M., Lusis, A. J., & Pellegrini, M. (2019). DNA Methylation Changes More Slowly Than Physiological States in Response to Weight Loss in Genetically Diverse Mouse S https://doi.org/10.3389/fendo.2019.00882

4. Garcia-Lacarte, M., Milagro, F. I., Zulet, M. A., Martinez, J. A., & Mansego, M. L. (2016). LINE-1 methylation levels, a biomarker of weight loss in obese subjects, are influenced by dietary antioxidant capacity. Redox report : communications in free radical research, 21(2), 67–74. https://doi.org/10.1179/1351000215Y.0000000029

5. Hruby, A., & Hu, F. B. (2015). The Epidemiology of Obesity: A Big Picture. PharmacoEconomics, 33(7), 673–689. https://doi.org/10.1007/s40273-014-0243-x

6. Kopelman P. G. (2000). Obesity as a medical problem. Nature, 404(6778), 635–643. https://doi.org/10.1038/35007508

7. Milagro, F. I., Mansego, M. L., De Miguel, C., & Martínez, J. A. (2013). Dietary factors, epigenetic modifications and obesity outcomes: progresses and perspectives. Molecular aspects of medicine, 34(4), 782–812. https://doi.org/10.1016/j.mam.2012.06.010

8. Moleres, A., Campión, J., Milagro, F. I., Marcos, A., Campoy, C., Garagorri, J. M., Gómez-Martínez, S., Martínez, J. A., Azcona-Sanjulián, M. C., Martí, A., & EVASYON Study Group (2013). Differential DNA methylation patterns between high and low responders to a weight loss intervention in overweight or obese adolescents: the EVASYON study. FASEB journal : official publication of the Federation of American Societies for Experimental Biology, 27(6), 2504–2512. https://doi.org/10.1096/fj.12-215566

9. Salas-Pérez, F., Cuevas-Sierra, A., Cuervo, M., Goni, L., Milagro, F. I., Martínez, J. A., & Riezu-Boj, J. I. (2021). Differentially methylated regions (DMRs) in PON3 gene between responders and non-responders to a weight loss dietary intervention: a new tool for precision management of obesity. Epigenetics, 1–12. https://doi.org/10.1080/15592294.2021.1873629

10. van Dijk, S.J., Tellam, R.L., Morrison, J.L. et al. Recent developments on the role of epigenetics in obesity and metabolic disease. Clin Epigenet 7, 66 (2015). https://doi.org/10.1186/s13148-015-0101-5

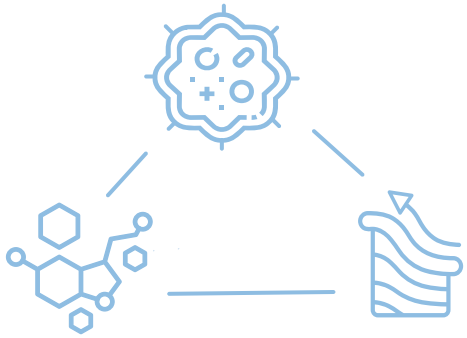
11. Young, S., McEneny J., (2001) Lipoprotein oxidation and atherosclerosis.Biochem Soc Trans. 29 (2): 358–362. https://doi.org/10.1042/bst0290358

YOUR MITOTIC CLOCK REPORT

And The Epigenetic Timer Of Cancer

TruDiagnostic™
The Epigenetic Company

The link between cellular replication and cancer: **The "Bad Luck" Hypothesis**

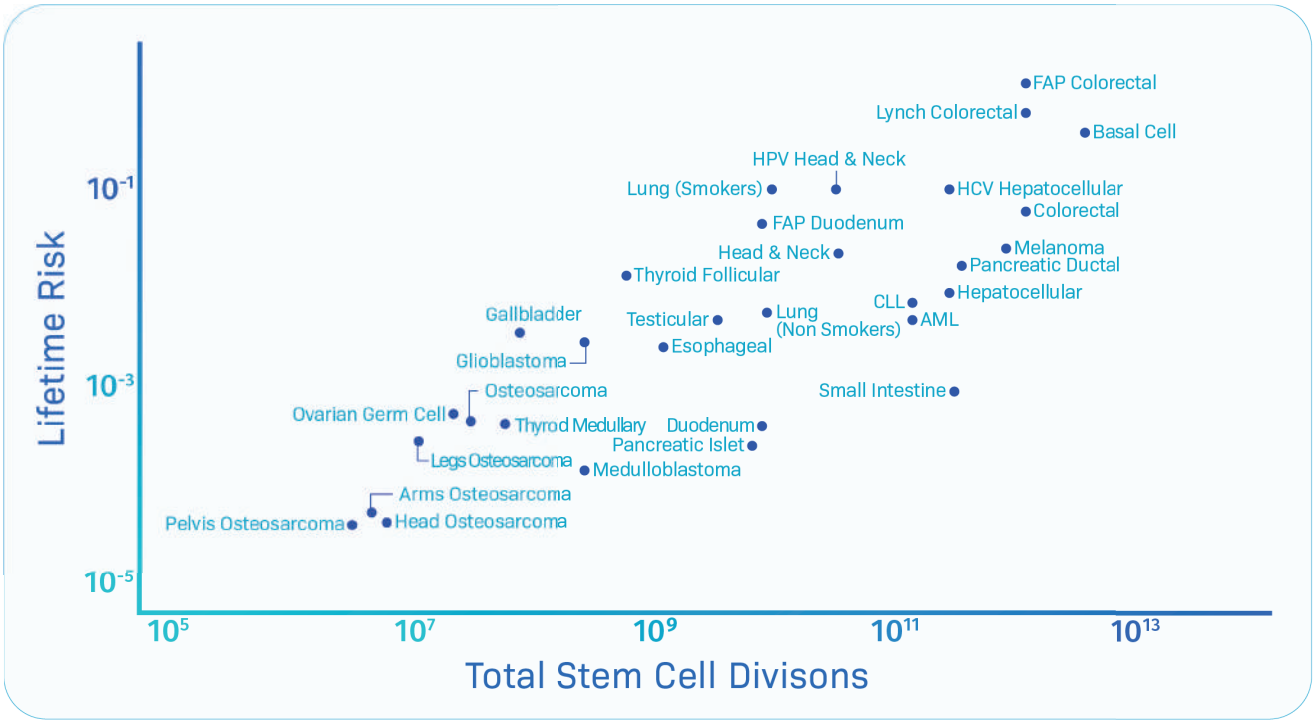


Some tissue types give rise to human cancers millions of times more often than other tissue types. For example, the lifetime risk of being diagnosed with cancer is 6.9% for lung, 1.08% for thyroid, 0.6% for brain and the rest of the nervous system, and 0.003% for pelvic bone.

Some of these differences are associated with well-known risk factors such as smoking, alcohol use, ultraviolet light, or human papilloma virus (HPV). However, such exposures cannot explain why cancer risk is so vastly different in different tissues. Cancers of the small intestinal epithelium are three times less common than brain tumors, even though small intestinal epithelial cells are exposed to much higher levels of environmental mutagens than are cells within the brain, which are protected by the blood-brain barrier. Therefore, the main driver is probably not environmental exposures.

Another well-studied contributor to cancer is inherited genetic variation. However, only 5 to 10% of cancers have a heritable component, and even when hereditary factors in predisposed individuals can be identified, the way in which these factors contribute to differences in cancer incidences among different organs is difficult to determine. Therefore, genetics are probably not the main driver.

A study by Andrew Teschendorff, PhD found that inflammatory conditions also increased mitotic rates in tissue (Teschendorff 2020). ***Increased mitotic rates measured with this algorithm could also indicate stem cell depletion.***

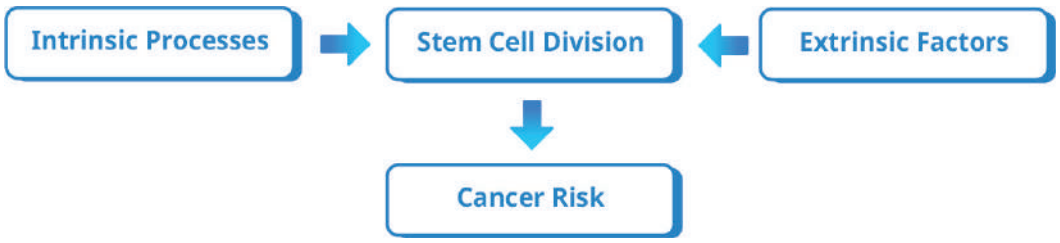


If hereditary and environmental factors cannot fully explain the differences in organ-specific cancer risk, how else can these differences be explained?

In 2016, a paper tried to explain why cancer risk is so different in some tissues than others. The research done by a group at Johns Hopkins (Tomasetti, Et. al), showed that the lifetime risk of cancers of many different types is strongly correlated (r2=0.81) with the total number of divisions of the normal self-renewing cells maintaining that tissue's homeostasis.

These results suggest that only a third of the variation in cancer risk among tissues is attributable to environmental factors or inherited predispositions. The rest is due to the "Bad Luck Hypothesis". This hypothesis points out that your cells are statistically more likely to make mistakes while copying DNA when they are replicating more often. As these mistakes accumulate, they can lead to cancer. Unfortunately, some of us just have bad luck and have more of these (intrinsic) errors than others.

"Intrinsic processes" include those that result in mutations due to random errors in DNA replication. "Extrinsic factors" are environmental factors that affect mutagenesis rates (such as UV radiation, ionizing radiation, and carcinogens).



How do I know how many stem cell divisions I have?

Cells replicate in a process called mitosis, where the DNA is fully copied during cell division. This process is fundamentally important to our survival, since cell replication is necessary for growing, healing and repairing our tissues. As time passes we end up creating trillions of cells, each with a tiny risk of making a mistake during cell division. With each small mistake our risk of cancer also slowly increases.

So how do we measure how much our cells are turning over? With epigenetic based mitotic clocks.

The Mitotic Clock score is estimated by looking at 385 locations (PCGT/PRC2-marked promoter CpGs) that are unmethylated at birth, but gain DNAm as chronological age increases. The algorithm to sort through this data was trained on a large cohort of healthy individuals, as assessed in one tissue type (blood). You can read the algorithm itself below.

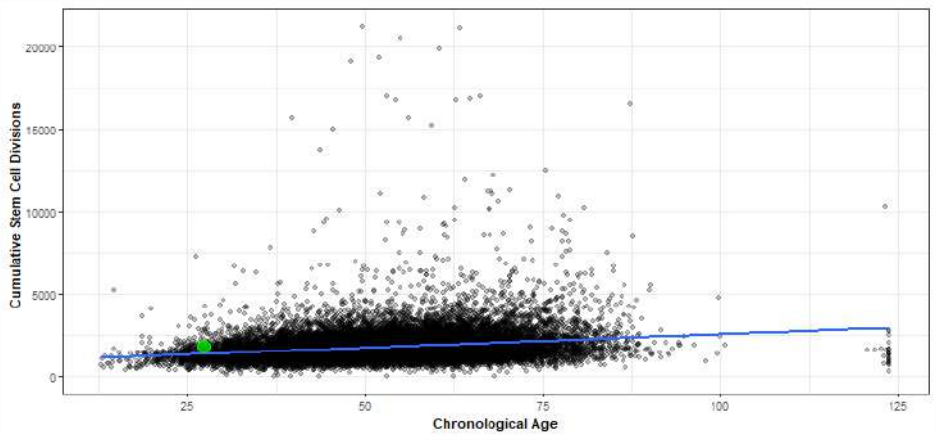
$$TNSC(s) = \frac{1}{n}\sum_{i=1}^{n} w_i \beta_{is} = \frac{1}{n}\sum_{i=1}^{n} \frac{2\,\beta_{is}}{\delta_i}$$

YOUR RESULTS:

Your ***cumulative number of stem cell divisions*** per stem cell per year:

1869.17

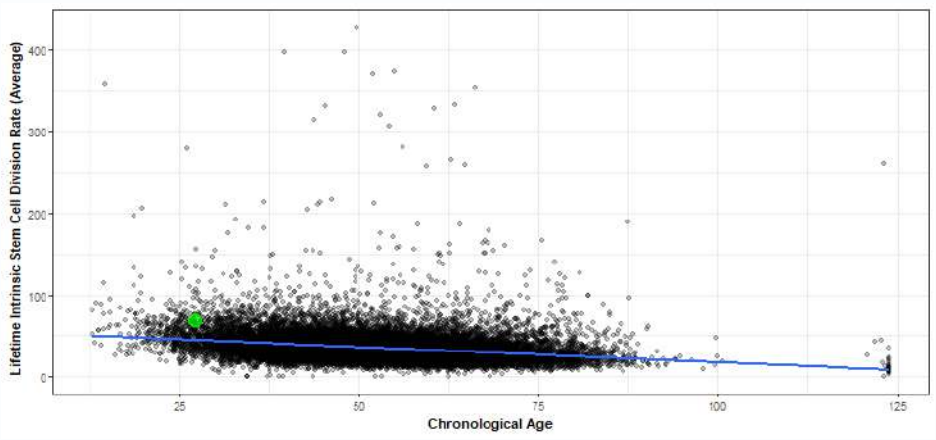
Based on your results, you would be in the **85.07th** percentile of Cell Division compared to others of your same chronological age.



Your ***average estimate for the intrinsic rate of stem-cell division*** for the tissue:

68.72

Based on your results, you would be in the **85.57th** percentile of lifetime intrinsic rate of stem - cell division compared to others of your same chronological age.



WHAT DOES THIS MEAN FOR ME?

The Impact To You

It is important to note that this report **does not detect cancer.** This algorithm has been trained to predict the amount of stem cell division in tissue samples. While tissue turnover is generally correlated with increased risk of cancer, It is NOT a definitive diagnostic.

A study by Andrew Teschendorff found that inflammatory conditions also increased mitotic rates in tissue. Increased mitotic rates measured with this algorithm could also indicate stem cell depletion.

Still, it does highly correlate to cancer development **risk.** The mitotic clock exhibits age acceleration in normal buccal tissue from smokers compared with nonsmokers, and in normal breast tissue from patients with cancer compared with healthy women, making it aunique biological clock for estimating cancer risk.

If you are reading **exceptionally high** in this category, we would encourage you to pay special attention to getting regularly examined for any health issues with your physician.

TRUAGE BY TRUDIAGNOSTIC

Inflammation

This report explores the impacts of inflammation on biological age and accelerated biological aging by examining associated methylation patterns at various locations of your DNA.

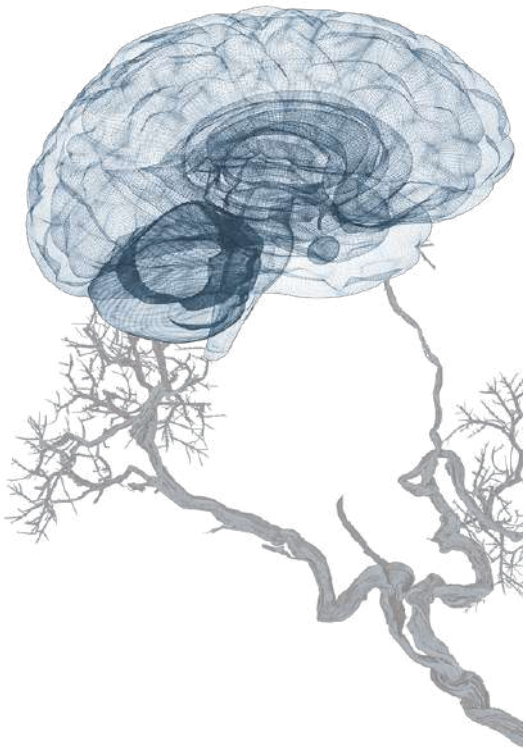
Developed By TruDiagnostic's Bioinformatics & Research Department

UNDERSTANDING

inflammation's impact on cognitive health.

As we age, baseline levels of inflammatory biomarkers increase, leading to a decline in cognition. The cognitive processes that are negatively impacted by inflammation and age-related advanced inflammation can include things like memory, speed of processing, and overall **cognitive function**. Additionally, inflammation has been linked to the beginning stages of **dementia and neurodegenerative diseases.**

When aging occurs, epigenetic changes occur that promote inflammation. This causes a decrease in the global genome methylation, which than causes an increase in methylation to specific regions (including a notable impact on CD8+ and CD4+ T cells). Additionally, several studies indicate that DNA methylation is better associated with chronic inflammation than traditional measures; highlighting how epigenetic mechanisms play a major role in inflammatory imbalance. These mechanisms have also been linked to an **accumulation of cellular damage that can induce a constant inflammatory response.**



Acute inflammation is a biological response to harmful stimuli. However, chronic and elevated levels of inflammation can mark the development of **age-related diseases such as cancer, atherosclerosis, and Alzheimer's.** Inflammaging, defined as an agerelated increase in the levels of pro-inflammatory markers in blood and tissues, is a strong risk factor for multiple diseases that are highly prevalent and frequent causes of disability in elderly individuals

Through DNA methylation (DNAm) we have the ability to estimate the total extent of inflammation in your body. This is able to provide more in-depth insights into inflammation-related health information than traditional, inflammation-based bio-measurements can.

PROVIDED BY: TruDiagnostic™ The Epigenetic Company

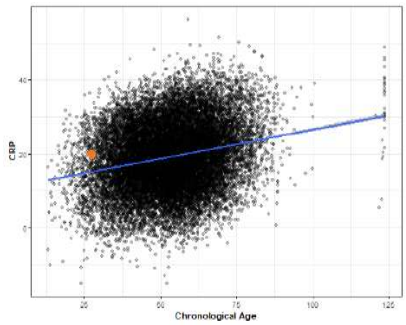
Your Results.

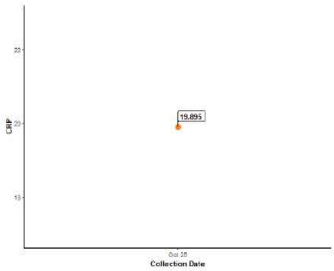
DISCLAIMER: All population graphs included in this report are based off of data from thousands of research participants and TruAge test takers.

Your **DNAm CRP** lavel is higher than

80.49%

of the population at your same calendar age and sex





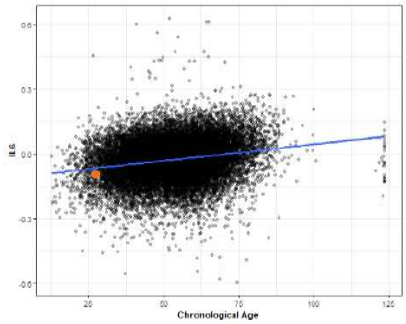
RESULTS OVER TIME

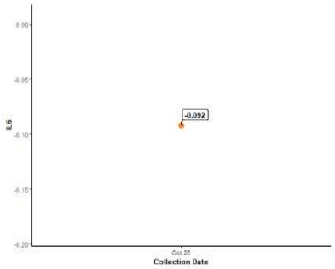
DNAm CRP is produced by the liver in response to acute inflammation. DNAm CRP has an inverse relationship with cognitive functions such as memory, speed, and visuospatial functions.

Your **DNAm IL-6** lavel is higher than

30.49%

of the population at your same calendar age and sex





RESULTS OVER TIME

DNAm IL-6 is a widely used marker of inflammation, and circulating levels of the cytokine typically rise in older age. DNAm IL-6 is positively associated with BMI, self-reported smoking status, and alcohol intake.

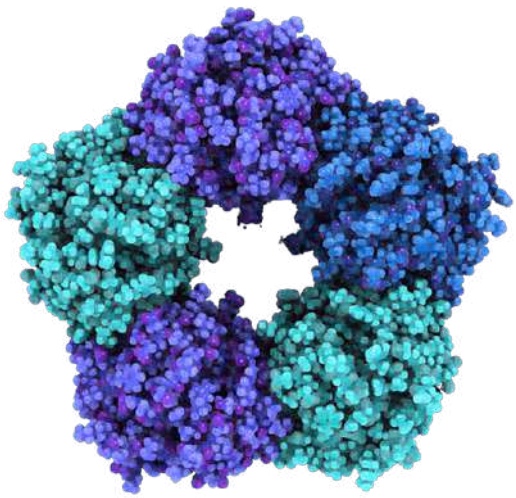
UNDERSTANDING

CRP's impact on cognitive health.

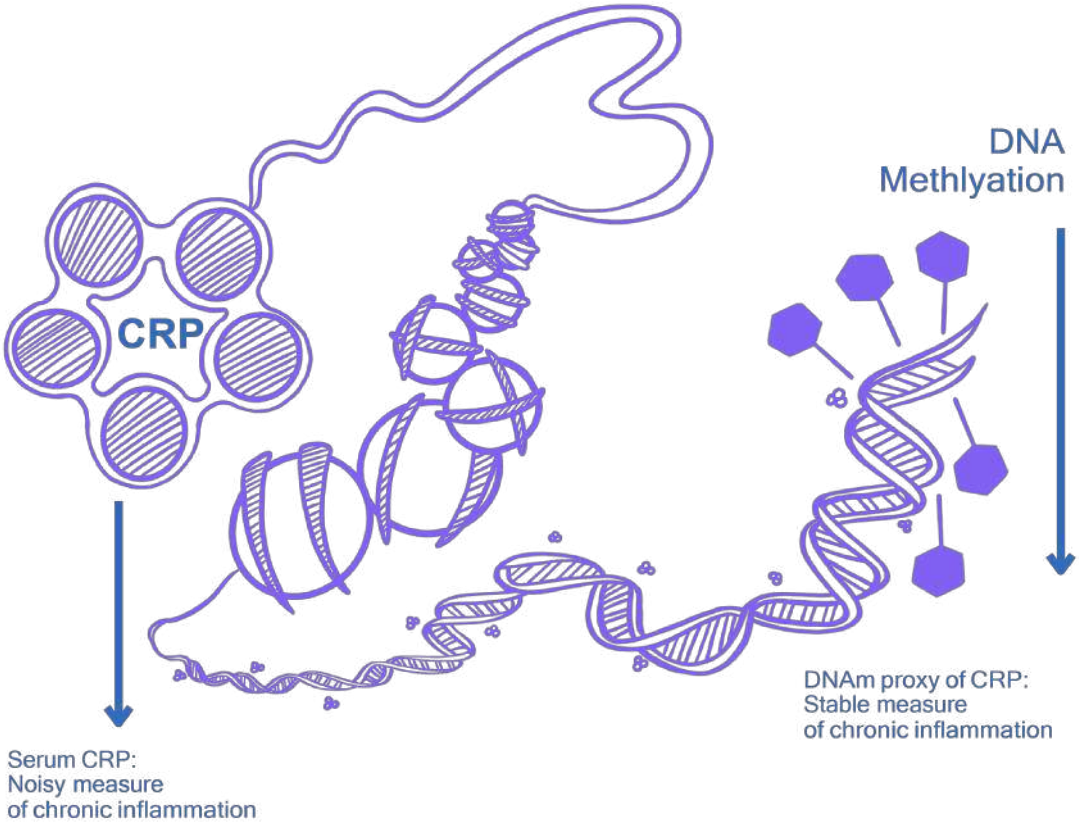
The liver produces CRP in response to acute inflammation. Healthcare providers use CRP measurements to indicate inflammation for various health conditions.

Elevated CRP levels are found to be associated with **low initial memory and verbal fluency** scores. Baseline inflammatory status is quantifiable by both peripheral inflammation-serum Creactive protein (CRP) and an epigenetic measure methylation signature of CRP (DNAm CRP). However, DNAm has benefits over traditional measures

One study conducted by Conole et al found that DNAm CRP is associated with total **brain volume,** (β = -0.197, 95% confidence interval [CI] -0.28 to -0.12, p FDR = 8.42 × 10-6), **gray matter volume** (β = -0.200, 95% CI -0.28 to -0.12, p FDR = 1.66 × 10-5), and **white matter volume** (β = -0.150, 95% CI -0.23 to -0.07, p FDR = 0.001).



Visualization of the CRP protein



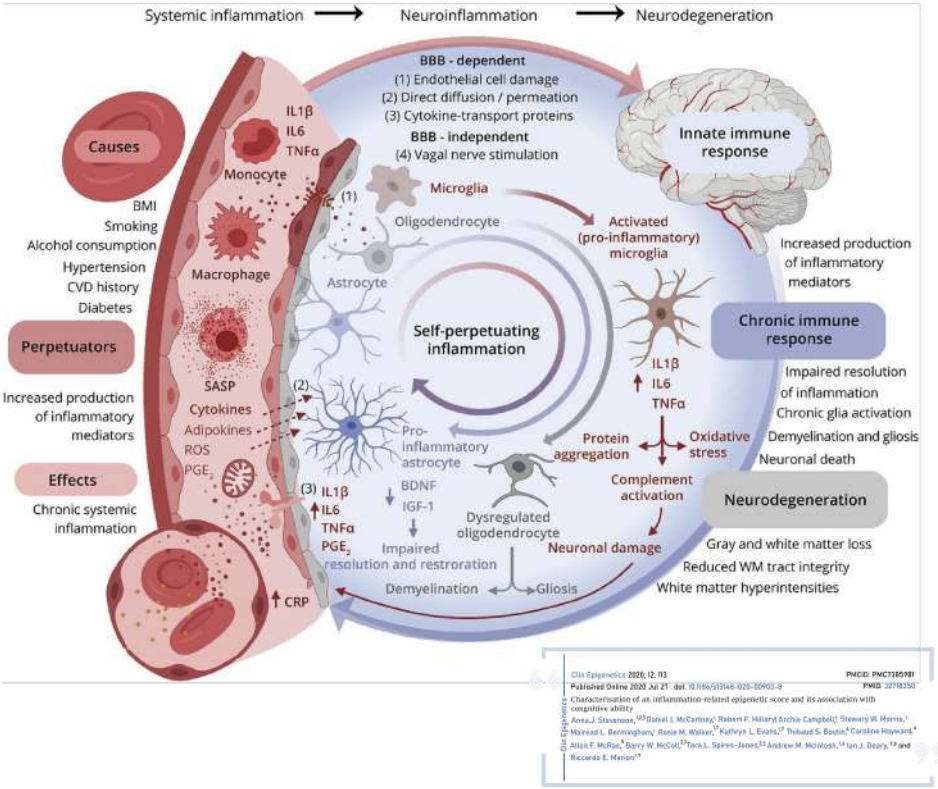
PRECISE & INFORMATIVE

Measuring CRP through DNAm

DNAm CRP has emerged as a more precise bio-measurement than traditional CRP quantification and shows significantly **stronger associations with brain health outcomes** like chronic inflammation, brain structure, and cognitive functioning, compared to serum CRP measurements (on average **6.4-fold**). DNAm CRP has an inverse relationship with cognitive functions such as memory, speed, and visuospatial functions.

Only recently has there been a push for integrated multi-omics approaches to better characterize chronic inflammation. DNAm profiles may act as promising peripheral biomarkers for cognitive–aging differences at the population level, given their relative stability in the short term, and their **joint modulation by both genetic and lifestyle traits.** Elsewhere, DNAm markers of inflammation have proved informative in predicting a range of agerelated health outcomes, from cardiovascular disease to depression, however, few studies have applied this same approach to cognitive aging differences in healthy cohorts.

As chronic inflammation is considered to be an insidious, cumulative, and often undetected contributor to cognitive aging, the importance of such epigenetic markers may be their **utility to index inflammatory load with greater reliability than phasic protein measures.**



UNDERSTANDING

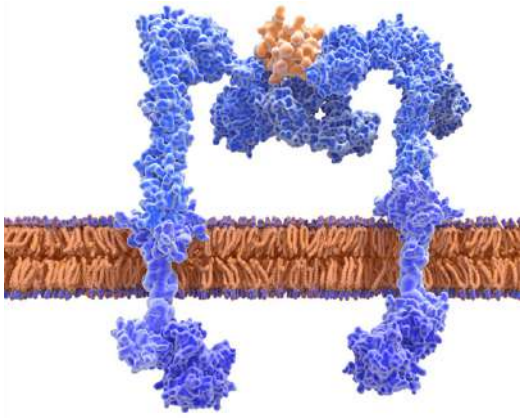
IL-6's impact on cognitive health.

Interleukin-6, also known as IL-6, is a pleiotropic, pro-inflammatory cytokine and is a principal stimulator of various acute-phase inflammatory proteins, such as CRP. IL-6 is a widely used marker of inflammation and circulating **circulating levels of the cytokine typically rise with age.** On average, males show higher scores compared to women.

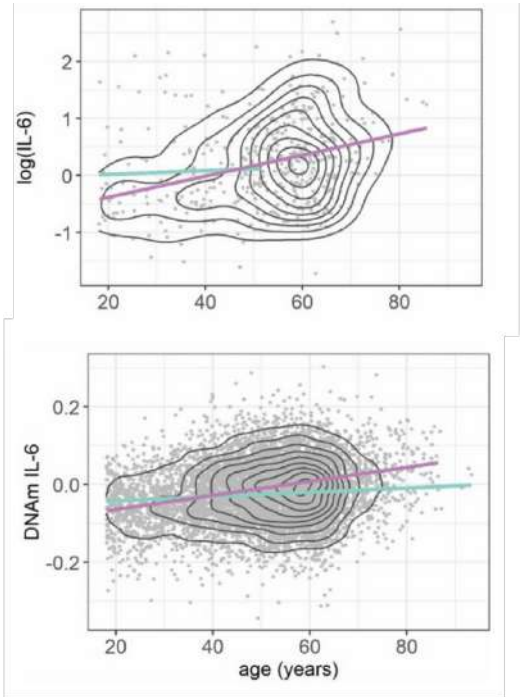
IL-6 is thought to be the transitionary biomarker, swinging from an acute, beneficial response to a chronic, deleterious state of inflammation. The DNAm IL-6 score created by Stevenson et al. was found to increase with age and is **is negatively associated with cognitive function** ($\beta = -0.19$, SE = 0.07, pFDR = .014).

Research indicates that traditional IL-6 measures may be an unreliable predictor of chronic inflammation when focusing on temporal variability. Additionally, **DNAm IL-6 is able to track alterations in cell proportions** more directly than serum IL-6.

DNAm IL-6 measures have associations with sex, BMI, social deprivation, alcohol intake, and smoking status, while traditional IL-6 is typically just associated with increasing age. Of these known associations, DNAm IL-6 is positively associated with BMI, self-reported smoking status, and alcohol intake.



Visualization of the IL-6 protein



As noted in the graphs to the left by Stevenson et al., both serum IL-6 and DNAm IL-6 were found to increase with age(**serum IL-6**: $\beta = 0.022$, SE = 0.004, $p = 1.3 \times 10^{-7}$; **DNAm IL-6 score:** $\beta = 0.015$, SE = 0.0009, $p < 2 \times 10^{-16}$).

Interestingly enough, males were found to have higher DNAm IL-6 scores compared to females ($\beta = 0.25$, SE = 0.02, $p < 2 \times 10^{-16}$).

TRUAGE BY TRUDIAGNOSTIC

OMICm Age

This report calculates biological age by examining age-associated methylation patterns at approximately one million locations on your DNA, using the novel OMICm Age algorithm.

Developed By TruDiagnostic's Bioinformatics & Research Department

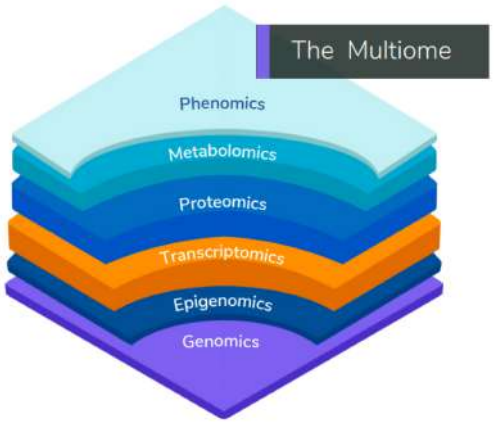
A NEW AGING ALGORITHM

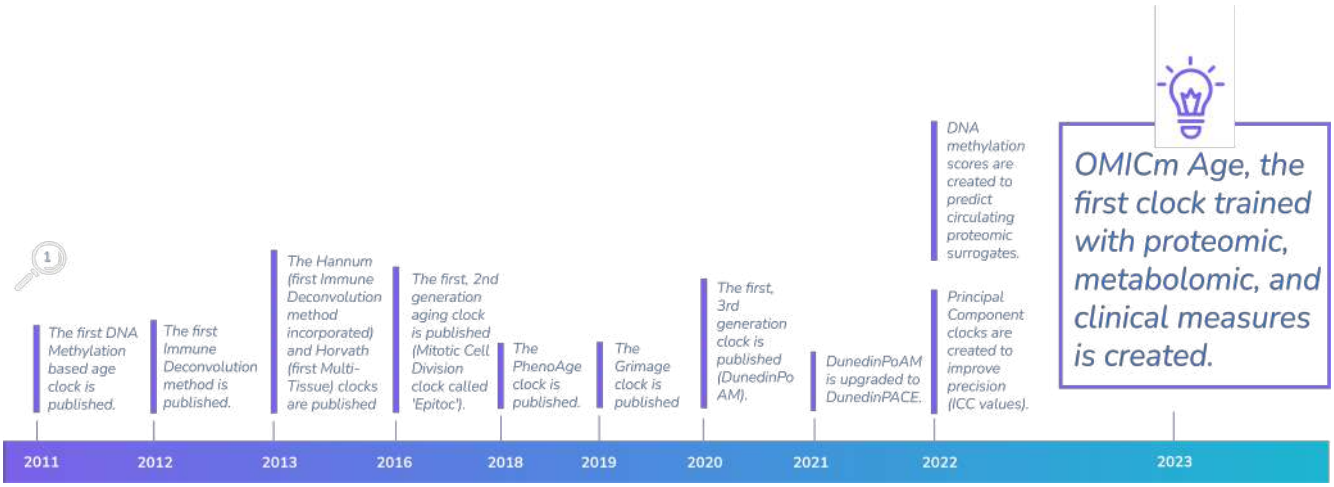
Raising the bar on measuring aging.

When TruDiagnostic was founded in 2020, we set out on a mission to create the best scientific algorithm (clock) that analyzes epigenetic patterns to accurately quantify biological age. To do this, we needed an extensive amount of data, which is why we partnered with researchers from Harvard University and Partners Biobank.

This biobank included thousands of samples saved from over the last 50 years. With these samples, **we were able to collect the extensive amount of interconnected biodata needed to create the most accurate predictors of biological aging.**

This process has taken us almost three years to finalize, but we are proud to announce the completion of the best biological age clock ever created; the **OMICm Age** algorithm.





COLLECTED: 10/25/2023 | REPORTED: 11/12/2023

OUR APPROACH

Multi Omics & Biological Aging.

When the Human Genome Project (an initiative to map the entire human genome) was first announced decades ago, many people thought the results would inform us about everything related to human biology. While it was a great project, the actionable health information gained from its efforts left many people disappointed. One reason why is that genetic composition is only one small piece of the puzzle.

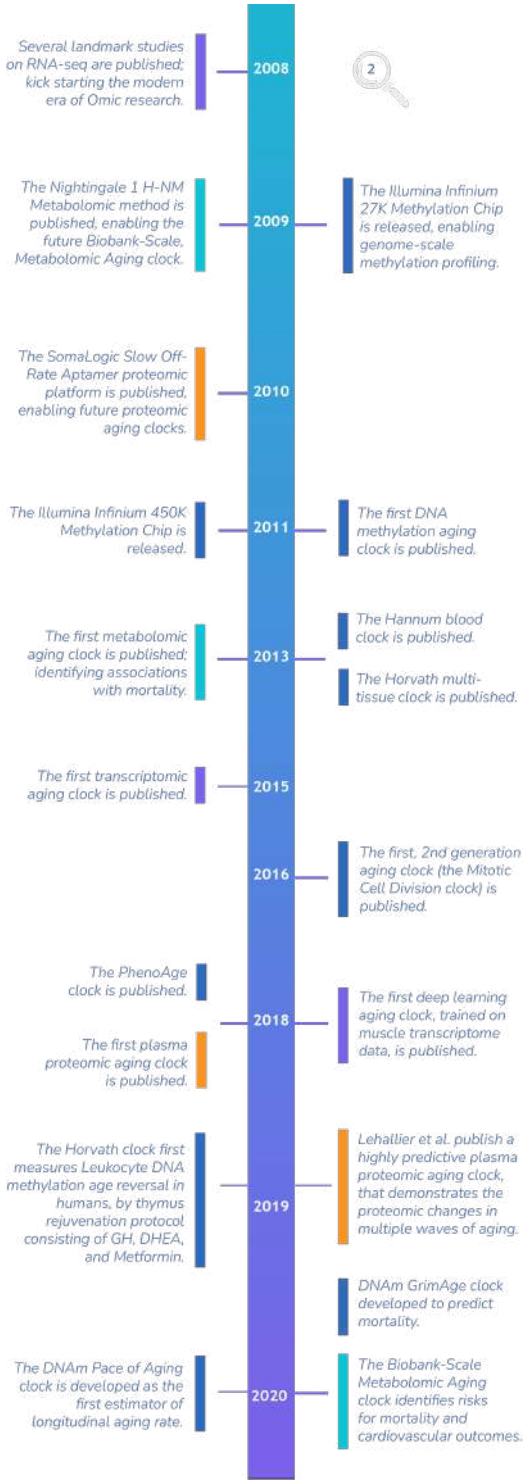
We now know that **the functionality of your body, as well as your health outcomes (phenotypes), are a result of much more than just your DNA.** Your epigenetics and transcriptome, the peptides and proteins in your body (proteome), and the metabolites from your body's processes and environmental exposures are all crucial factors in how your biology operates. This large picture of interconnected cellular processes is often called the multiome (Multi Omics) and it is a combination of all the different measurements we can perform on the body.

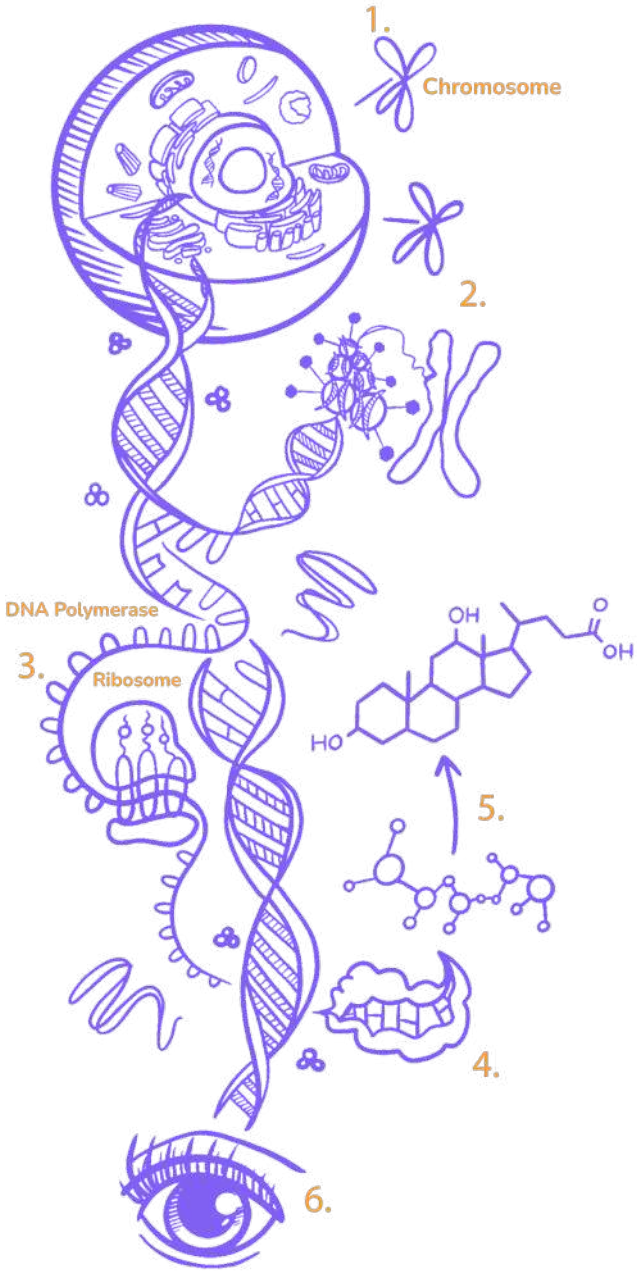
Thus, to create the best biological age clock, we didn't want to just measure epigenetics. We wanted to measure the entire multiome. So, we did! **In 5,000 people, we used advanced analysis techniques to quantify all biomarkers that make up the multiome.' Proteins, metabolites, and DNA methylation altogether were measured in only 1500 subjects. We used these individuals to train the epigenetic biomarker proxies (EBPs) for proteins and metabolites and, later on, we quantified these EBP in the 5000 subjects with DNA methylation.** We used Whole Exome Sequencing. Untargeted Plasma Proteomics, Plasma Metabolomics, as well as Clinical Data and Outcome Data for our large group (cohort). Together, this novel data allows for an unmatched resolution in quantifying the whole body's aging process. It also allows us to view aging throughout the multiome, through the lens of DNA methylation.

In our initial publication regarding the research and findings used to develop our OMICm Age algorithm, we **showed that this clock is better at predicting health and aging outcomes** than any other methylation age clock to date.

Epigenomics Transcriptomics

Proteomics Metabolomics





1. Genomics

The study of the genes housed in our DNA. Our DNA, located in the nucleus of our cells, contains sections of instructions (genes) that tell a cell how to behave. Your genetics stay the same from conception to death.

2. Epigenomics

The study of how our genes are modified. Epigenetic molecules interact with our DNA, either amplifying or silencing certain instructions. These interactions change throughout your lifetime.

3. Transcriptomics

The study of how our genes turn into actionable RNA. During transcription, molecules called RNA copy the instructions of our DNA; skipping over or boosting sections based on the epigenetic patterns at that location.

4. Proteomics

The study of how proteins function. Proteins are created by RNA, and perform most of the work within a cell. Antibodies, enzymes, and hormones are all types of protein functions.

5. Metabolomics

The study of the chemical processes produced by protein interactions. Metabolites are a by-product of proteins hard at work, and are used to help break down food, drugs, chemicals, or the body's own tissue.

6. Phenomics

The study of observable traits such as eye, skin, and hair color. Epigenetics can curate those instructions, and the resulting proteins and metabolites impact your biology to result in a physical expression.

PROVIDED BY: TruDiagnostic

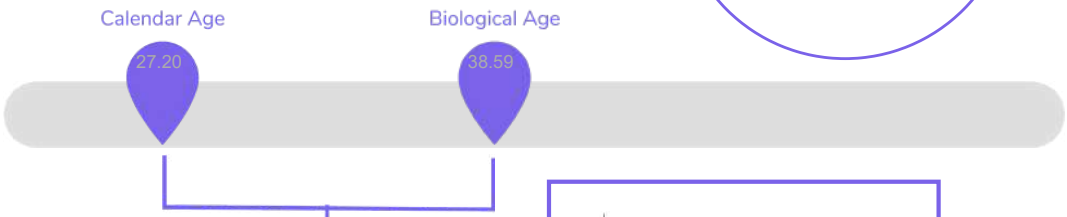
Your Results.

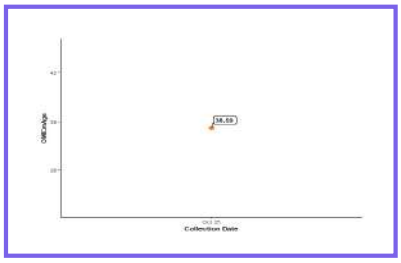
DISCLAIMER: All population graphs included in this report are based off of data from thousands of research participants and TruAge test takers. Unless otherwise specified, population graphs are included to provide context to your results, but are not necessarily reflections of individual scores.

38.59

YEARS OLD

OMICm Age

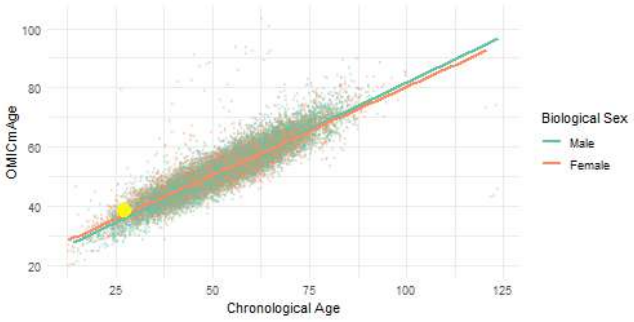




Your OMICm Age is

HIGHER THAN

your calendar age by **11.39** years.



YOUR OMICm Age IS IN THE:

71.14st

PERCENTILE MEANING THAT YOUR OMICm AGE IS HIGHER THAN **71.14%** OF THE POPULATION AT YOUR SAME CHRONOLOGICAL AGE .

YOUR RISK OF DISEASE

DISCLAIMER: The following, personalized risk scores were calculated based off of observed and validated patterns in data, from thousands of research participants involved in our Harvard University and TruDiagnostic partnered study. This cohort is believed to be a strong sample representation of larger population data.

Aging has been scientifically proven to be the number one risk factor for major chronic diseases world-wide. Accelerated aging (having an older biological age than your calendar age) increases your risk of disease with each year, and having a younger biological age decreases these risks.

Your OMICm Biological Age can represent an increase or decrease risk of Death, Cancer, Heart Disease, Stroke, Type 2 Diabetes, COPD, and Depression.

-28% Disease Risk

Reflects your current risk. A -28% score means that your risk is 28% lower compared to people of your same chronological age.

47% Disease Risk

Reflects your current risk. A 47% score means that your risk is 47% higher compared to people of your same chronological age.

31%

Reflects your potential risk score based on potential changes to your biological age.

DEATH

-3 YEARS	-1 YEAR		+1 YEAR	+3 YEARS
-42.07%	-24.02%	-12.99% Disease Risk	-0.35%	30.68%

CANCER

-3 YEARS	-1 YEAR		+1 YEAR	+3 YEARS
-21.76%	-11.62%	-6.06% Disease Risk	-0.16%	12.78%

STROKE

-3 YEARS	-1 YEAR		+1 YEAR	+3 YEARS
-23.81%	-12.79%	-6.69% Disease Risk	-0.17%	14.26%

COLLECTED: 10/25/2023 | REPORTED: 11/12/2023

HEART DISEASE

-3 YEARS	-1 YEAR	-7.24% Disease Risk	+1 YEAR	+3 YEARS
-25.54%	-13.79%		-0.19%	15.55%

TYPE 2 DIABETES

-3 YEARS	-1 YEAR	-6.15% Disease Risk	+1 YEAR	+3 YEARS
-22.06%	-11.79%		-0.16%	12.99%

COPD

-3 YEARS	-1 YEAR	-4.08% Disease Risk	+1 YEAR	+3 YEARS
-15.11%	-7.91%		-0.1%	8.36%

DEPRESSION

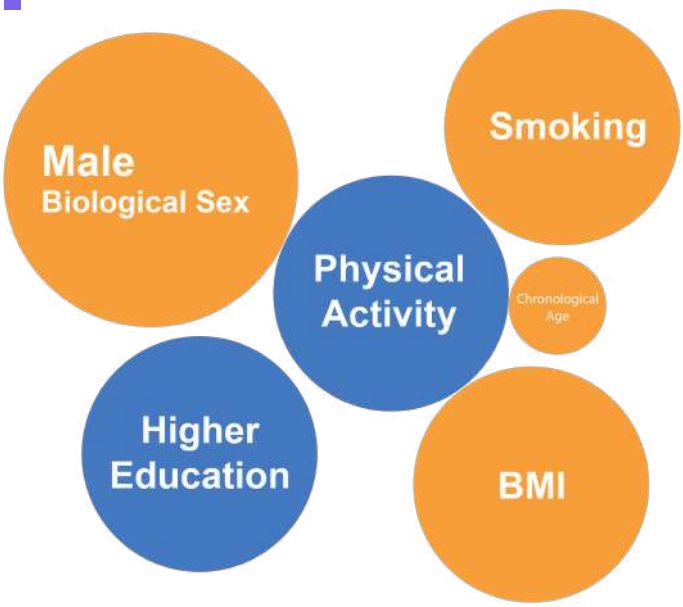
-3 YEARS	-1 YEAR	-3.96% Disease Risk	+1 YEAR	+3 YEARS
-14.66%	-7.67%		-0.1%	8.08%

- END OF DISEASE RISK RESULTS -

In the chart below, you can see some of the top factors that contribute to an increase (yellow) or decrease (blue) of OMICm Age.

While some influences like sex and chronological age are innate and unchangeable, **mostt contributing factors like smoking and physical activity can be modified.** It is important to note that an influence, or association, is not necessarily a cause. The chart below shows **research-backed associations with a higher or lower biological age.** These factors may or may not be direct causes, however, strong age-related trends have been distinguished.

HARVARD BIOLOGICAL AGING COHORT



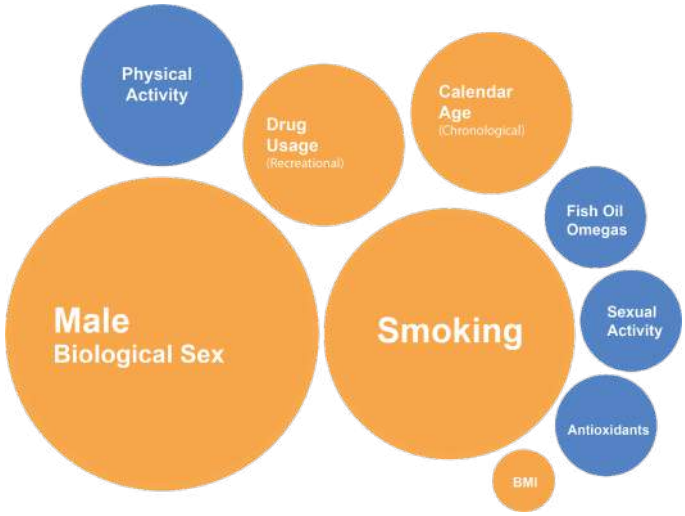
Associated with
lower
OMICm Age

Associated with
higher
OMICm Age

TRU COHORT

Associated with
lower
OMICm Age

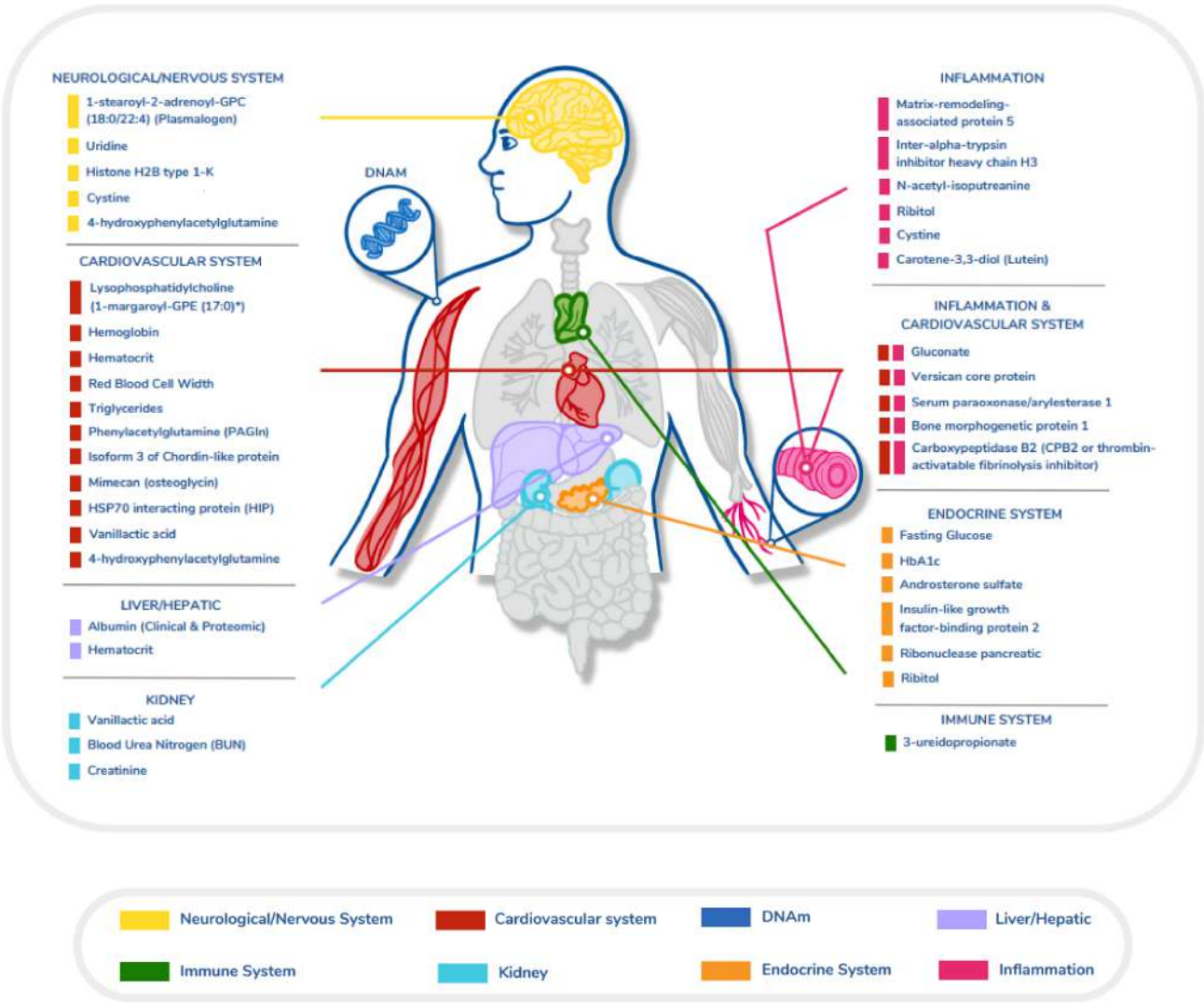
Associated with
higher
OMICm Age



THE EPIGENETIC BIOMARKER PROXIES DRIVING YOUR BIOLOGICAL AGE

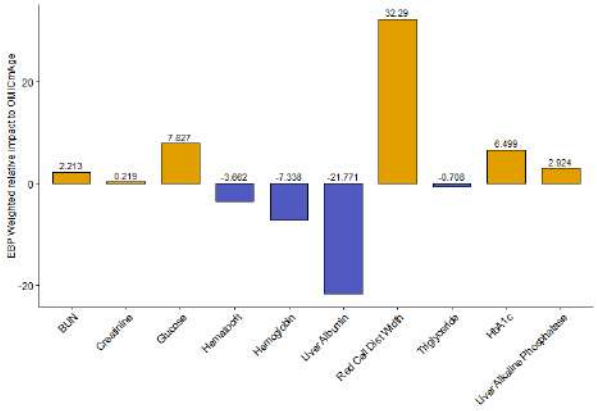
We use epigenetic biomarker proxies (EBPs) scores to predict genomics, transcriptomics, proteomics, and metabolomics sum values that are positive for your aging, and some that are negative for your aging. In the graph below you will see the factors contributing to your aging the most. If a bar is above zero, it's increasing your OMICm Age, if below zero, it is decreasing your OMICm Age.

BODY SYSTEMS CONTRIBUTING TO THE DEVELOPMENT OF OMICAGE THROUGH OMICS



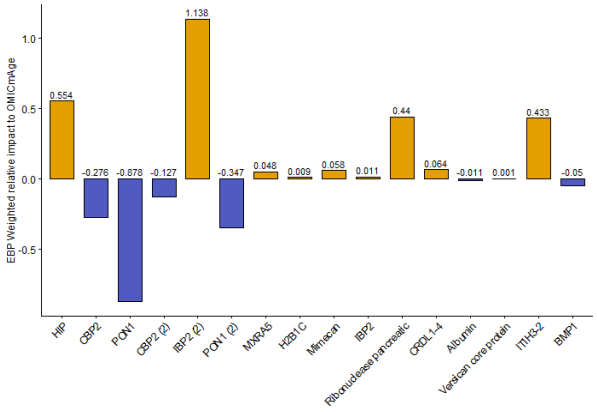
CLINICAL FACTORS

Your Clinical Epigenetic Biomarker Proxies (EBP)



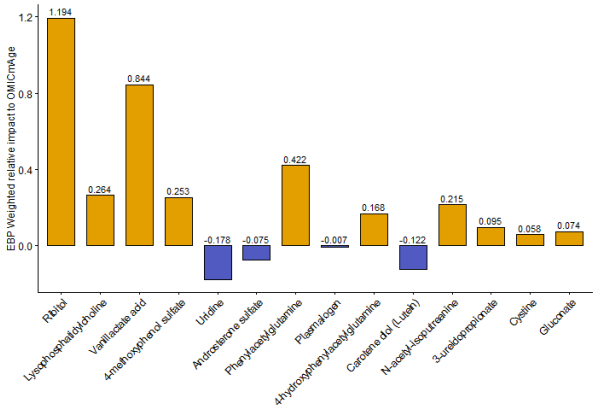
PROTEINS

Your Protein Epigenetic Biomarker Proxies (EBP)



METABOLITES

Your Metabolites Epigenetic Biomarker Proxies (EBP)



CLINICAL RESULTS

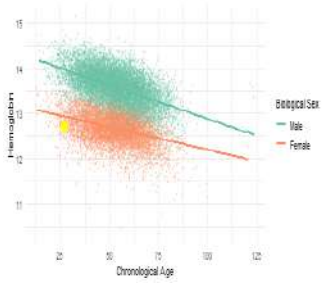
EXPANDED

Hemoglobin

HIGHER RATES ASSOCIATED WITH IMPROVED OMIC AGE

12.7 g/dl

Your **Hemoglobin** is higher than **27.43%** of the population at your same calendar age and sex.

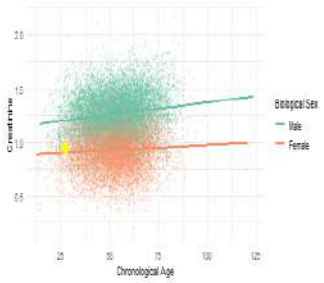


Creatinine

LOWER RATES ASSOCIATED WITH IMPROVED OMIC AGE

0.94 mg/dL

Your **Creatinine** is higher than **57.31%** of the population at your same calendar age and sex.

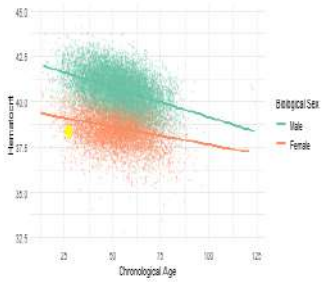


Hematocrit

HIGHER RATES ASSOCIATED WITH IMPROVED OMIC AGE

38.37 L/L

Your **Hematocrit** is higher than **28.04%** of the population at your same calendar age and sex.

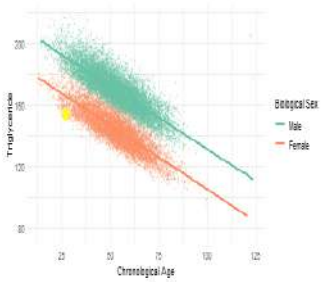


Triglycerides

HIGHER RATES ASSOCIATED WITH IMPROVED OMIC AGE

154.36 mmol/L

Your **Triglycerides** is higher than **4.26%** of the population at your same calendar age and sex.

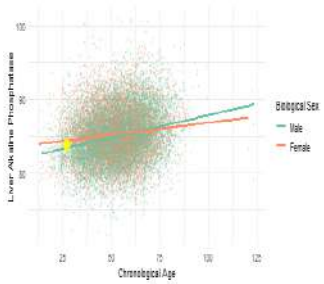


Alkaline Phosphatase (ALP)

LOWER RATES ASSOCIATED WITH IMPROVED OMIC AGE

83.83 U/L

Your **Alkaline Phosphatase (ALP)** is higher than **42.07%** of the population at your same calendar age and sex.

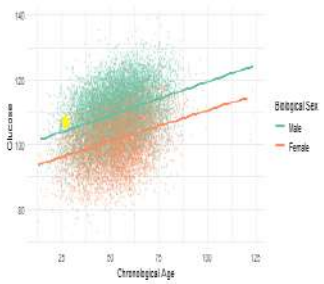


Fasting Glucose

LOWER RATES ASSOCIATED WITH IMPROVED OMIC AGE

107.15 mmol/L or in mg/dL

Your **Fasting Glucose** is higher than **95.12%** of the population at your same calendar age and sex.

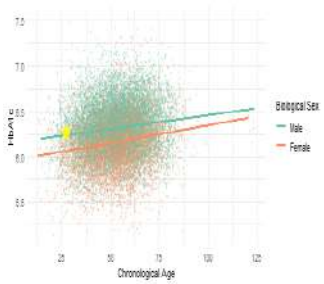


HbA1c

LOWER RATES ASSOCIATED WITH IMPROVED OMIC AGE

6.26 mmol/mol

Your **HbA1c** is higher than **78.04%** of the population at your same calendar age and sex.

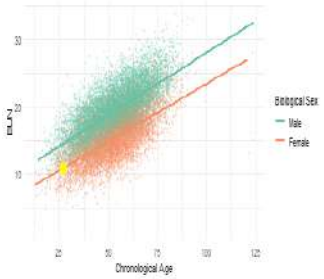


Blood Urea Nitrogen (BUN)

LOWER RATES ASSOCIATED WITH IMPROVED OMIC AGE

10.97 mg/dLor in mmol/L

Your **Blood Urea Nitrogen (BUN)** is higher than **43.29%** of the population at your same calendar age and sex.

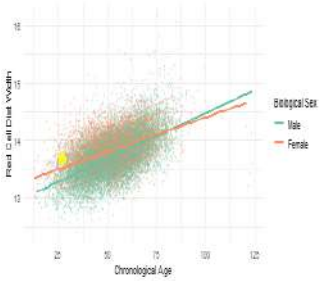


Red Blood Cell Width

LOWER RATES ASSOCIATED WITH
IMPROVED OMIC AGE

13.67 fL

Your **Red Blood Cell Width** is higher than **64.63%** of the population at your same calendar age and sex.

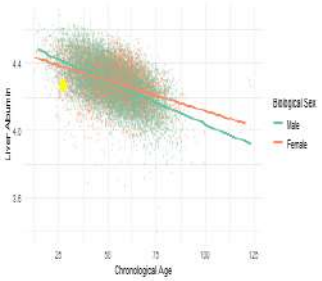


Albumin (Clinical)

HIGHER RATES ASSOCIATED WITH
IMPROVED OMIC AGE

4.26 g/dL

Your **Albumin (Clinical) Cell Width** is higher than **19.51%** of the population at your same calendar age and sex.



METABOLITE RESULTS

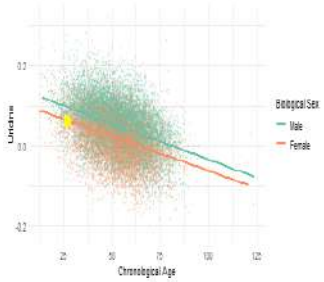
EXPANDED

Uridine

HIGHER RATES ASSOCIATED WITH IMPROVED OMIC AGE

0.06

Your **Uridine** is higher than **51.21%** of the population at your same calendar age and sex.

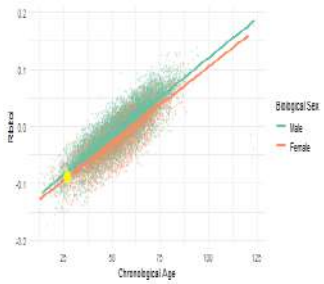


Ribitol

HIGHER RATES ASSOCIATED WITH IMPROVED OMIC AGE

-0.08

Your **Ribitol** is higher than **53.65%** of the population at your same calendar age and sex.

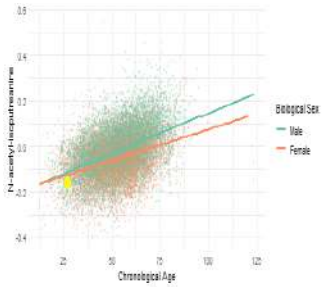


N-acetyl-isoputreanine

LOWER RATES ASSOCIATED WITH IMPROVED OMIC AGE

-0.16

Your **N-acetyl-isoputreanine** is higher than **25%** of the population at your same calendar age and sex.

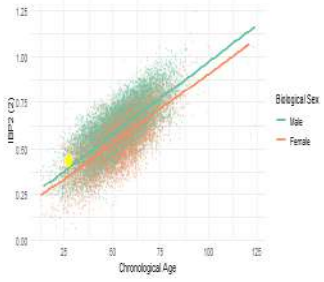


Vanillactate acid

LOWER RATES ASSOCIATED WITH IMPROVED OMIC AGE

-0.37

Your **Vanillactate acid** is higher than **54.26%** of the population at your same calendar age and sex.

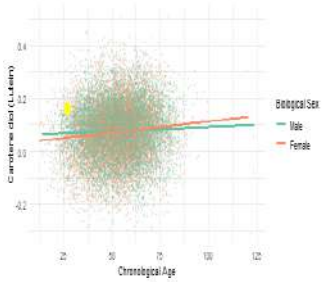


Carotene-3,3-diol (Lutein)

HIGHER RATES ASSOCIATED WITH IMPROVED OMIC AGE

0.16

Your **Carotene-3,3-diol (Lutein)** is higher than **87.8%** of the population at your same calendar age and sex.

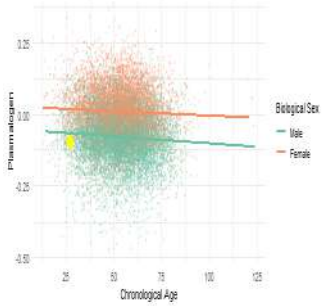


1-stearoyl-2-adrenoyl-GPC (18:0/23:4) (Plasmalogen)

HIGHER RATES ASSOCIATED WITH IMPROVED OMIC AGE

-0.09

Your **1-stearoyl-2-adrenoyl-GPC (18:0/23:4) (Plasmalogen)** is higher than **9.75%** of the population at your same calendar age and sex.

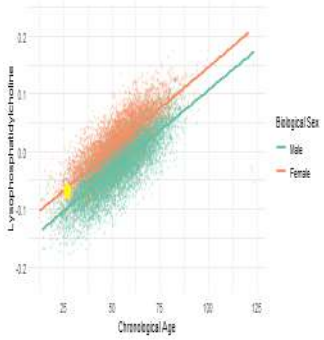


Lysophosphatidylcholine (1-margaroyl-GPE (17:0)*)

LOWER RATES ASSOCIATED WITH IMPROVED OMIC AGE

-0.06

Your **Lysophosphatidylcholine (1-margaroyl-GPE (17:0)*)** is higher than **35.97%** of the population at your same calendar age and sex.

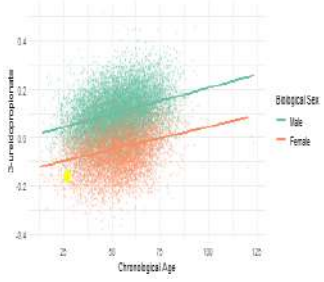


3-ureidopropionate

LOWER RATES ASSOCIATED WITH IMPROVED OMIC AGE

-0.15

Your **3-ureidopropionate** is higher than **20.12%** of the population at your same calendar age and sex.



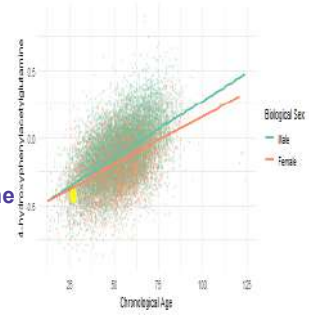
COLLECTED: 10/25/2023 | REPORTED: 11/12/2023

4-hydroxyphenylacetylglutamine

LOWER RATES ASSOCIATED WITH
IMPROVED OMIC AGE

-0.42

Your **4-hydroxyphenylacetylglutamine** is higher than **29.87%** of the population at your same calendar age and sex.

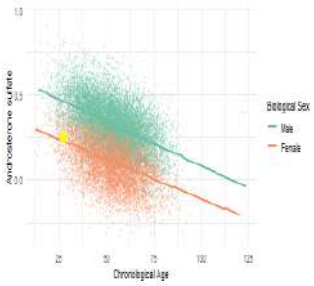


Androsterone Sulfate

HIGHER RATES ASSOCIATED WITH
IMPROVED OMIC AGE

0.25

Your **Androsterone Sulfate** is higher than **51.21%** of the population at your same calendar age and sex.

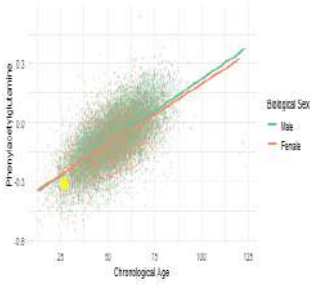


Phenylacetylglutamine (PAGln)

LOWER RATES ASSOCIATED WITH
IMPROVED OMIC AGE

-0.3

Your **Phenylacetylglutamine (PAGln)** is higher than **31.7%** of the population at your same calendar age and sex.

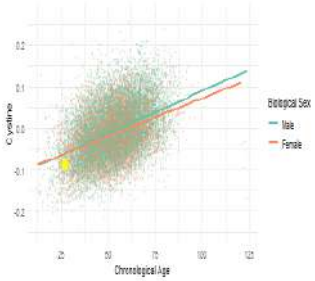


Cystine

LOWER RATES ASSOCIATED WITH
IMPROVED OMIC AGE

-0.08

Your **Cystine** is higher than **40.24%** of the population at your same calendar age and sex.

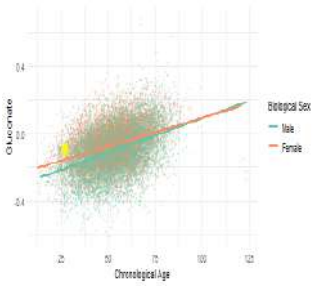


Gluconate

LOWER RATES ASSOCIATED WITH
IMPROVED OMIC AGE

-0.09

Your **Gluconate** is higher than **65.24%** of the population at your same calendar age and sex.



PROTEIN RESULTS

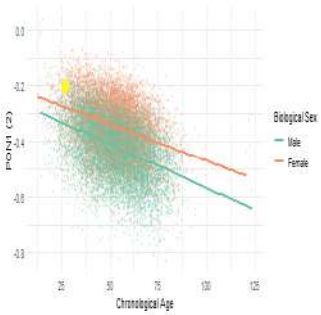
EXPANDED

Serum paraoxonase/ arylesterase

HIGHER RATES ASSOCIATED WITH IMPROVED OMIC AGE

-0.2

Your **Serum paraoxonase/ arylesterase** is higher than **73.78%** of the population at your same calendar age and sex.

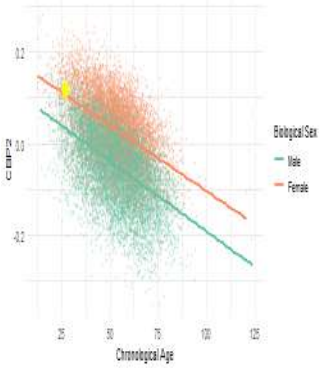


Carboxypeptidase B2 (CPB2 or thrombin-activatable fibrinolysis inhibitor)

HIGHER RATES ASSOCIATED WITH IMPROVED OMIC AGE

-0.01

Your **Carboxypeptidase B2 (CPB2 or thrombin-activatable fibrinolysis inhibitor)** is higher than **49.39%** of the population at your same calendar age and sex.

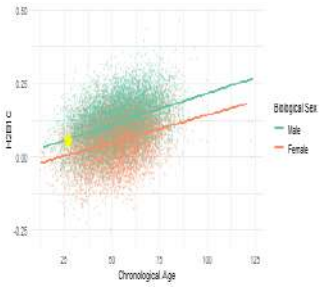


Histone H2B type 1-K

LOWER RATES ASSOCIATED WITH IMPROVED OMIC AGE

0.05

Your **Histone H2B type 1-K** is higher than **71.95%** of the population at your same calendar age and sex.

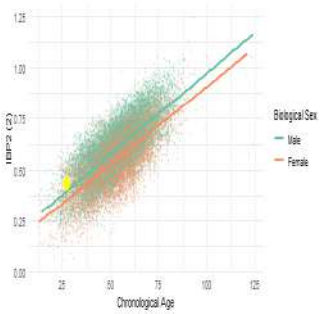


Insulin-like growth factor-binding protein 2

LOWER RATES ASSOCIATED WITH IMPROVED OMIC AGE

0.43

Your **Your Insulin-like growth factor-binding protein 2** is higher than **71.34%** of the population at your same calendar age and sex.

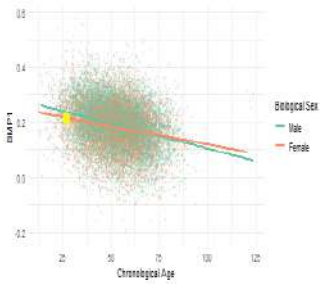


Bone morphogenetic protein 1

HIGHER RATES ASSOCIATED WITH IMPROVED OMIC AGE

0.21

Your **Bone morphogenetic protein 1** is higher than **45.12%** of the population at your same calendar age and sex.

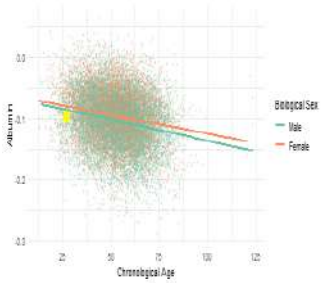


Albumin

HIGHER RATES ASSOCIATED WITH IMPROVED OMIC AGE

4.26

Your **Albumin** is higher than **19.51%** of the population at your same calendar age and sex.

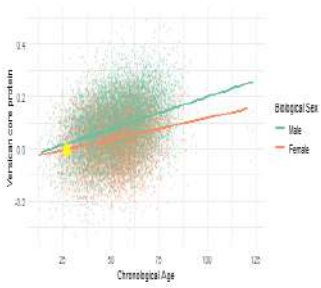


Versican core protein

LOWER RATES ASSOCIATED WITH IMPROVED OMIC AGE

-0.003

Your **Versican core protein** is higher than **49.39%** of the population at your same calendar age and sex.

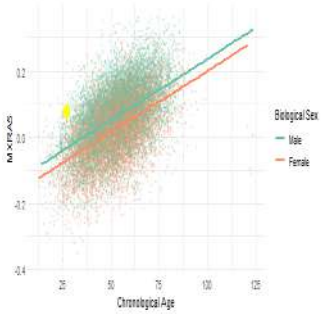


Matrix-remodeling -associated protein 5

LOWER RATES ASSOCIATED WITH IMPROVED OMIC AGE

0.07

Your **Matrix-remodelingassociated protein 5** is higher than **98.78%** of the population at your same calendar age and sex.



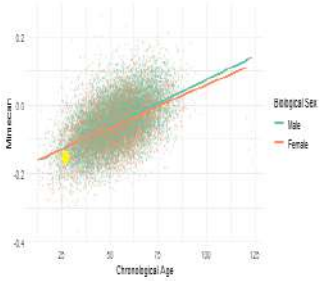
COLLECTED: 10/25/2023 | REPORTED: 11/12/2023

Mimecan

LOWER RATES ASSOCIATED WITH
IMPROVED OMIC AGE

-0.15

Your **Mimecan** is higher than **34.75%** of the population at your same calendar age and sex.

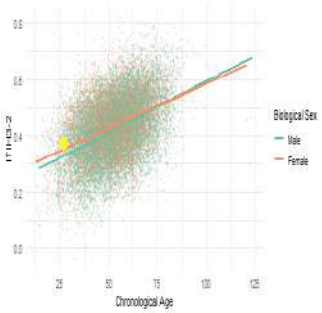


Inter-alpha-trypsin inhibitor heavy chain H3

LOWER RATES ASSOCIATED WITH
IMPROVED OMIC AGE

0.37

Your **Inter-alpha-trypsin inhibitor heavy chain H3** is higher than **56.7%** of the population at your same calendar age and sex.

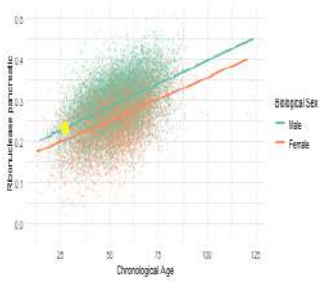


Ribonuclease pancreatic

LOWER RATES ASSOCIATED WITH
IMPROVED OMIC AGE

0.23

Your **Ribonuclease pancreatic** is higher than **65.85%** of the population at your same calendar age and sex.

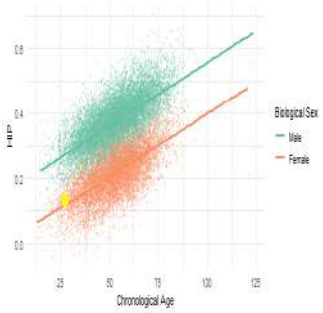


HSP70 interacting protein (HIP)

LOWER RATES ASSOCIATED WITH
IMPROVED OMIC AGE

0.13

Your **HSP70 interacting protein (HIP)** is higher than **57.31%** of the population at your same calendar age and sex.



VALUES FACTORED IN OMICm AGE

Biomarker definitions.

Hemoglobin

Red blood cells contain the protein hemoglobin, which transports oxygen. How much hemoglobin is in your blood is determined by the hemoglobin test. The most significant part of red blood cells is hemoglobin. It is made up of heme, a protein that binds oxygen.

Hematocrit

The volume percentage of red blood cells in blood is assessed as part of a blood test and is referred to by a number of other names. Red blood cell quantity and size determine this measurement.

Creatinine

Creatinine is a waste product that comes from the normal wear and tear on muscles of the body. Everyone has creatinine in their bloodstream. However, amounts vary based on age, body size, race, and gender.

Triglycerides

Triglycerides are a type of fat, called lipid, that circulate in your blood. They are the most common type of fat in your body. Triglycerides come from foods, especially butter, oils, and other fats. Unused calories are stored as triglycerides in fat cells. When your body needs energy, it releases the triglycerides. High triglyceride levels in your blood can raise your risk of heart disease and stroke.

Alkaline Phosphatase (ALP)

Your body contains an enzyme called alkaline phosphatase (ALP). One of the tests in a full metabolic panel, ALP blood tests evaluate the amount of ALP produced by your liver and bones in your blood. High blood levels of ALP may be a sign of liver disease or specific bone problems.

Fasting Glucose

The primary sugar present in your blood is glucose. It serves as the main energy source for your body. It originates in the food you consume. The majority of that meal is converted by your body into glucose, which is then released into your bloodstream. Your pancreas releases insulin when your blood glucose levels rise.

HbA1c

The A1C test, sometimes called a HbA1c test or a hemoglobin A1C test, is a quick blood test that gauges your average blood sugar levels over the previous three months. The primary test to assist you and your healthcare team in managing your diabetes, it is one of the often utilized tests to diagnose prediabetes and diabetes.

Blood Urea Nitrogen (BUN)

The amount of urea nitrogen in your blood is determined by a blood urea nitrogen (BUN) test. When your liver breaks down protein, urea nitrogen is produced as a waste product. Your blood carries it, your kidneys filter it out, and your urine excretes it from your body.

Red Blood Cell Width

The measure of the difference in the volume and size of your red blood cells (erythrocytes). The volume of red blood cells varies even in healthy blood, with an average volume of 80–100 femtoliters. However, some illnesses result in a markedly greater fluctuation in cell size. Greater size variation is indicated by higher RDW values. RDW-CV in human red blood cells typically falls between 11.5 and 15.4%.

Albumin (Clinical)

The protein albumin is produced by your liver. Albumin enters your bloodstream and aids in preventing fluid from seeping into other tissues from your blood vessels. It also transports vitamins, enzymes, and hormones throughout the body. If your blood doesn't contain enough albumin, fluid may leak out and accumulate in your lungs, abdomen, or other areas of your body. Low albumin levels may indicate liver, renal, or other types of illness. Dehydration may be indicated by high levels.

Uridine

Uridine is an important building block used in the creation of RNA. It may support brain health, synaptic connections, and cholinergic function. A 2018 study identified it as one of 12 metabolites predictive of living over the age of 85 in women. Other studies have also shown that it is linked to all-cause mortality. Lower uridine levels in Alzheimer's disease (AD) were associated with clinical progression. In some studies, it has been identified as a factor that promotes human stem cell activity and enhanced regeneration in multiple tissues across multiple mammal species.

Carotene-3,3-diol (Lutein)

Carotene-3,3-diol is one of 600 known naturally occurring carotenoids. It is synthesized only by plants and is found in high quantities in green leafy vegetables such as spinach, kale, and yellow carrots. Some studies have shown that supplementation can help improve cognitive function and eye health. A large meta-analysis involving 71 published papers and representing more than 387,000 individuals showed that people with higher lutein intake, or higher blood concentrations of lutein, have a reduced risk of coronary heart disease, stroke, and metabolic syndrome. Lutein provides such wide-reaching effects because it protects tissues from oxidative stress and inflammation—two factors that play a significant role in cardiovascular and metabolic diseases.

Ribitol

Ribitol is a pentose alcohol formed by the reduction of ribose. Ribitol forms part of the chemical structure of riboflavin and flavin mononucleotide (FMN). It is also a metabolic end product formed by reducing ribose in human fibroblasts and erythrocytes. It has been a blood-based biomarker of diabetic retinopathy and biological process clustering studies have shown it to be associated with insulin secretion and diabetes pathways which are highly related to mortality. Higher concentrations of similar metabolites like ribonic acid have also been linked to CKD.

1-stearoyl-2-adrenoyl-GPC (18:0/23:4) (Plasmalogen)

This is a choline ether phospholipid (ePC) that is present in human serum or plasma. Decreases in ether phospholipids (plasmalogens) in serum (plasma) have been reported in several diseases such as Alzheimer's disease, Parkinson's disease, metabolic syndrome, and schizophrenia.

N-acetyl-isoputreanine

Isoputreanine belongs to the class of organic compounds known as gamma amino acids and derivatives.

Gluconate

Gluconic acid occurs naturally in fruit,honey, and wine . It has been identified as a lifestyle-related biomarker that may be a target to reduce stroke risk in Black adults. Higher levels of gluconic acid in the blood were associated with high blood pressure and increased risk of ischemic stroke among Black adults when compared to white adults. It also may be considered as a dietary-related oxidative stress marker due to its availability in food, potentially produced by the gut microbiome, and related to diseases with oxidative stress. Of the 162 metabolites measured in one study, elevated levels of gluconic acid were found in Black adults who had high blood pressure but not their white peers with high blood pressure. Black adults with the highest gluconic acid levels were 86% more likely to have high blood pressure. Black adults with the highest gluconic acid levels had a 53% increased risk of ischemic stroke. No such association was found for white participants. Gluconic acid accounted for 25% of the association between high blood pressure and stroke among Black adults. After adjusting for multiple factors, a higher level of gluconic acid was associated with a Southern diet (foods high in added fats, fried foods, processed meats, and sugary drinks), and a lack of exercise.

Phenylacetylglutamine (PAGln)

Phenylacetylglutamine (PAGln) is a gut microbiota-derived metabolite that may induce cardiovascular events by activating platelets and increasing the risk of thrombosis. The highly-nitrogenous compound is most commonly encountered in human subjects with urea cycle disorders. These conditions, such as uremia or hyperammonemia, tend to cause high levels of nitrogen in the form of ammonia in the blood. It also has been used as a biomarker of acute stroke. High levels of phenylacetylglutamine in the urine following metabolism by the gut microbiota may also indicate early renal decline associated with kidney dysfunction and chronic kidney disease (CKD). In CKD, phenylacetylglutamine is considered a uremic toxin which is taken up, circulated, and retained in the blood after microbial fermentation of certain proteins and amino acids in the gut. Blood serum levels of phenylacetylglutamine in CKD are used as a mortality determinant. Blood plasma levels of phenylacetylglutamine increase with exposure to cigarette smoke, in patients with ischemic heart failure, with cardiovascular risk or hypertension, in patients with disease, and in patients with type 2 diabetes.

Serum paraoxonase/arylesterase

Serum paraoxonase and arylesterase 1 (PON1) is an enzyme encoded by the PON1 gene. Serum PON1 is secreted mainly by the liver, although local synthesis occurs in several tissues and PON1 protein is found in almost all tissues. PON1 is also a major anti-atherosclerotic component of HDL Cholesterol (good cholesterol). The PON1 gene is activated by PPAR-γ, which increases synthesis and release of paraoxonase 1 enzyme from the liver, reducing atherosclerosis. In addition to protecting against exposure to some organophosphorus (OP) pesticides by hydrolyzing their toxic oxon metabolites, PON1 is important in protecting against vascular disease by metabolizing oxidized lipids. Circulating plasma levels of leptin, hs-CRP, and IL-6 were significantly non-linearly associated with arylesterase activity. Leptin levels were also significantly associated with paraoxonase activity independently from confounding factors, including high-density lipoprotein (HDL) cholesterol. With increasing levels of inflammatory parameters, arylesterase, and paraoxonase activities increased; This suggests that in persons with very high levels of inflammation, PON1 activity may be impaired, a fact that might subsequently be accompanied by a higher risk for cardiometabolic diseases.

Lysophosphatidylcholine (1-margaroyl-GPE (17:0)*)

Lysophosphatidylcholine (LPC) is increasingly recognized as a key marker/factor positively associated with cardiovascular and neurodegenerative diseases. LPC is mainly derived from the turnover of phosphatidylcholine (PC) in circulation by phospholipase A2 (PLA2). In the presence of Acyl-CoA, lysophosphatidylcholine acyltransferase (LPCAT) converts LPC to PC. However, overexpression or enhanced activity of PLA2 increases the LPC content in modified low-density lipoprotein (LDL) and oxidized LDL, which play significant roles in the development of atherosclerotic plaques and endothelial dysfunction. Hydrolysis of LPC by autotaxin, an enzyme with lysophospholipase D activity, generates lysophosphatidic acid, which is highly associated with cancers.

Vanillactic acid

Vanillactic acid, also referred to as vanillactate or VLA falls within the category of organic substances termed phenylpropanoic acids. Phenylpropanoic acids are compounds characterized by a structure that incorporates a benzene ring connected to propanoic acid. Vanillactic acid possesses potential toxicity and has been associated with inborn metabolic disorders, including aromatic l-amino acid decarboxylase deficiency.

3-ureidopropionate

Ureidopropionic acid is essentially a urea derivative of beta-alanine. High levels of ureidopropionic acid are found in individuals with beta-ureidopropionase (UP) deficiency. It has been identified as one of the major metabolites Metabolites Associated With the Risk of Developing Mobility Disability This can also be present in Albuminuria. Albuminuria is an indicator of sub-clinical organ damage and a marker of cardiovascular risk and renal disease.

4-hydroxyphenylacetylglutamine

4-Hydroxyphenylacetylglutamic acid belongs to the class of organic compounds known as glutamic acid and derivatives. This is a metabolite which is upregulated in cystic fibrosis. It also has been suggested to be a novel biomarker of type 2 diabetes with polyneuropathy and also has shown a link to systolic blood pressure in women.

Cystine

Cysteine (Cys) the primary sulfur-containing amino acid (SAA) is a semiessential amino acid (AA) because it can be obtained from the diet or produced from methionine degradation via the transsulfuration pathway. Cystine is common in many foods such as eggs, meat, dairy products, and whole grains as well as skin, horns, and hair. Within the body, cysteine catabolic pathways are sources of the synthesis of coenzyme A, glutathione, taurine, and oxidized and reduced inorganic sulfur. Cysteine is more easily absorbed by the body than cystine, so most supplements contain cysteine rather than cysteine.

Androsterone Sulfate

Androsterone sulfate (Andros-S) is the most abundant 5-alpha-reduced androgen metabolite in serum. This means higher testosterone levels generally yield higher versions of this metabolite.

Bone morphogenetic protein 1

Bone morphogenetic protein 1, also known as BMP1, is a protein that in humans is encoded by the BMP1 gene. It induces bone and cartilage development. BMP-1 stimulates the conversion of newly secreted proapo A1 to its phospholipid- (PL-) binding form. In this way, it promotes the formation of functional HDL and reverse cholesterol transport. Higher levels of inflammation have been shown to be associated with a decrease in BMP1 and therefore APOA1 and thus it has been suggested as a marker for inflammation and cardiovascular disease risk.

Carboxypeptidase B2 (CPB2 or thrombin-activatable fibrinolysis inhibitor)

CPB2 is synthesized by the liver and circulates in the plasma as a plasminogen-bound zymogen. When it is activated by the thrombin/thrombomodulin complex, CPB2 exhibits carboxypeptidase activity. Activated CPB2 reduces fibrinolysis by removing the fibrin C-terminal residues that are important for the binding and activation of plasminogen. Lower CPB2 has been suggested as a biomarker of peripheral artery disease. This could be a biomarker of chronic hepatitis and thrombotic risk. Profound hypercoagulability seems to be mediated by the overexpression of plasminogen activator inhibitor 1 (PAI-1) and CBP2.

Albumin

The protein albumin is produced by your liver. Albumin enters your bloodstream and aids in preventing fluid from seeping into other tissues from your blood vessels. It also transports vitamins, enzymes, and hormones throughout the body. If your blood doesn't contain enough albumin, fluid may leak out and accumulate in your lungs, abdomen, or other areas of your body. Low albumin levels may indicate liver, renal, or other types of illness. Dehydration may be indicated by high levels.

Histone H2B type 1-K

Histone H2B type 1-K is a core component of the nucleosome or the proteins which wrap and control the expression of DNA. Nucleosomes wrap and compact DNA into chromatin, limiting DNA accessibility to the cellular machinery which requires DNA as a template. Histones thereby play a central role in transcription regulation, DNA repair, DNA replication, and chromosomal stability. DNA accessibility is regulated via a complex set of post-translational modifications of histones, also called histone code, and nucleosome remodeling. H2B Type 1-K has been shown to accumulate in senescent Fibroblasts with Persistent DNA Damage.

Versican core protein

Versican is an extracellular matrix protein that has been shown to increase during inflammation in a number of different diseases such as cardiovascular and lung disease, autoimmune diseases, and several different cancers. Versican interacts with inflammatory cells either indirectly via hyaluronan or directly via receptors such as CD44, P-selectin glycoprotein ligand-1 (PSGL-1), and toll-like receptors (TLRs) present on the surface of immune and non-immune cells. These interactions activate signaling pathways that promote the synthesis and secretion of inflammatory cytokines such as TNFα, IL-6, and NFκB.

Insulin-like growth factorbinding protein 2

IGFBP-2 is an insulin-like growth factor (IGF) binding protein (IGFBPs) that modulates IGF-I's actions. It plays an important role in the regulation of several cellular processes. IGFBP-2 is the second most abundant IGFBP and is expressed in several tissues, including blood vessels and the skeleton. IGFBP-2 can prevent IGF-I binding to its receptor, but it also modulates cellular functions independently of IGF-I binding It has been suggested to be a biomarker of metabolic disease and diabetes.

Matrix-remodeling-associated protein 5

This gene encodes one of the matrix-remodeling associated proteins. MMPs are capable of degrading all kinds of extracellular matrix proteins but also can process a number of bioactive molecules. They are known to be involved in the cleavage of cell surface receptors, the release of apoptotic ligands, and chemokine/ cytokine inactivation. MMPs are also thought to play a major role in cell behaviors such as cell proliferation, migration (adhesion/dispersion), differentiation, angiogenesis, apoptosis, and host defense.

Mimecan

Mimecan, also known as osteoglycin, is an ECM component. Mimecan affects several biological processes including the regulation of collagen fibrillogenesis and angiogenesis. Mimecan is expressed in atherosclerotic tissue and Human coronary arteries and is downregulated in intimal vascular smooth muscle cells (VSCMs). Studies have shown mimecan is associated with a vulnerable plaque phenotype, possibly regulated by plaque inflammation, and thus might predict future cardiovascular death and arterial stiffness.

Ribonuclease pancreatic

Pancreatic ribonuclease also known as ribonuclease A (RNase A) or ribonuclease 1 (RNase1) is an enzyme that catalyzes the breakdown of RNA and plays a role in the digestion of RNA in vertebrate species. RNase is present in much lower amounts in humans than in other species and may account for only 0.5 to 1% of pancreatic enzymes. Although only a few studies exist, pancreatic RNase in all species appears to break down dietary nucleic acid in the gut lumen to nucleotides. Not much is described about this protein as a biomarker, however, highway levels have been linked to more aggressive cancers.

Inter-alpha-trypsin inhibitor heavy chain H3

Inter-alpha (globulin) inhibitor 3 (ITIH3), one of the constituents of plasma serine protease inhibitors, has been shown to be related to the proinflammatory process (Fries and Kaczmarczyk 2003). This complex, named pre-alpha trypsin inhibitor (PαI) is synthesized by hepatocytes and released to the blood vessel upon stimulation of the proinflammatory cytokines (tumor necrosis factor or interleukin-1). Then, ITIH3 makes a complex with the locally synthesized hyaluronan (HA) and interacts with inflammatory cells (Fries and Kaczmarczyk 2003). - ITIH3-HA complex has been reported to be involved in inflammatory diseases, including rheumatoid arthritis and inflammatory bowel diseases (Zhuo et al. 2004). Variants with this protein have also been shown to be associated with psychiatric diseases.

HSP70 interacting protein (HIP)

HSP90 interacting protein is a co-chaperone heat shock protein that helps with appropriate protein folding. One aspect of this protein, C terminus of Hsc70-interacting protein (CHIP), frequently promotes ubiquitination and degradation of several proteins. The impact of upregulated CHIP has not been well studied. CHIP has been reported to play an important role in preventing cell apoptosis. CHIP also displays a critical cardioprotective effect in response to ischemia/reperfusion injury. CHIP is a negative regulator of FoxO1 activity through ubiquitin-mediated degradation, and inhibition of CHIP has been postulated to serve as a potential therapeutic target for reducing proliferative arterial diseases.

EDUCATIONAL GRAPHS

Report references.

1 Timeline Data Sourced From

Bocklandt, S. et al. Epigenetic predictor of age. PLoS One 6, e14821 (2011)

Houseman, E.A., Accomando, W.P., Koestler, D.C. et al. DNA methylation arrays as surrogate measures of cell mixture distribution. BMC Bioinformatics 13, 86 (2012)

Hannum, G. et al. Genome-wide methylation profiles reveal quantitative views of human aging rates. Mol. Cell 49, 359–367 (2013). Horvath, S. DNA methylation age of human tissues and cell types. Genome Biol. 14, 3156 (2013).

Yang, Z. et al. Correlation of an epigenetic mitotic clock with cancer risk. Genome Biol. 17, 205 (2016).

Levine, M. E. et al. An epigenetic biomarker of aging for lifespan and healthspan. Aging 10, 573–591 (2018).

Lu, A. T. et al. DNA methylation GrimAge strongly predicts lifespan and healthspan. Aging 11, 303–32.

Belsky, D. W. et al. Quantification of the pace of biological aging in humans through a blood test, the DunedinPoAm DNA methylation algorithm. eLife 9, e54870 (2020).

2 Timeline By

Rutledge, J., Oh, H. & Wyss-Coray, T. Measuring biological age using omics data. Nat Rev Genet 23, 715– 727 (2022). https://doi.org/10.1038/s41576-022-00511-7

3 Sources for Biomarker Definitions

“Association between inflammatory markers and serum paraoxonase and arylesterase activities in the general population: a cross-sectional study. (2021, July 31). NCBI. Retrieved July 18, 2023, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8325814/

Carboxyl Terminus of HSC70-interacting Protein (CHIP) Down-regulates NF-κB-inducing Kinase (NIK) and Suppresses NIK-induced Liver Injury. (n.d.). NCBI. Retrieved July 18, 2023, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4416871/

Circulating autoantibodies against neuroblastoma suppressor of tumorigenicity 1 (NBL1): A potential biomarker for coronary artery disease in patients with obstructive sleep apnea. (2018, March 29). NCBI. Retrieved July 18, 2023, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5875805/

Cross-species metabolomic analysis identifies uridine as a potent regeneration promoting factor. (2022, February 1). NCBI. Retrieved July 18, 2023, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8803930/

The effects of lutein on cardiometabolic health across the life course: a systematic review and metaanalysis. (2016, January 13). PubMed. Retrieved July 18, 2023, from https://pubmed.ncbi.nlm.nih.gov/26762372/

Emerging Biomarkers in Vascular Cognitive Impairment and Dementia: From Pathophysiological Pathways to Clinical Application. (2019, June 8). Semantic Scholar. Retrieved July 18, 2023, from https://pdfs.semanticscholar.org/dce6/374794efafcea8278ab4231b449b70e92d63.pdf

Furlong, C. E. (2010, March 23). Human PON1, a biomarker of risk of disease and exposure. NCBI. Retrieved July 18, 2023, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3035622/

H2B Type 1-K Accumulates in Senescent Fibroblasts with Persistent DNA Damage along with Methylated and Phosphorylated Forms of HMGA1. (2021, June 21). NCBI. Retrieved July 18, 2023, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8293446/

Insulin-Like Growth Factor (IGF) Binding Protein 2 Functions Coordinately with Receptor Protein Tyrosine Phosphatase β and the IGF-I Receptor To Regulate IGF-I-Stimulated Signaling. (n.d.). NCBI. Retrieved July 18, 2023, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3457336/

Lutein and Zeaxanthin and Their Roles in Age-Related Macular Degeneration—Neurodegenerative Disease. (n.d.). MDPI. Retrieved July 18, 2023, from https://www.mdpi.com/2072-6643/14/4/827

Lutein Has a Positive Impact on Brain Health in Healthy Older Adults: A Systematic Review of Randomized Controlled Trials and Cohort Studies. (2021, May 21). NCBI. Retrieved July 18, 2023, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8223987/

A Metabolomics study of metabolites associated with the glomerular filtration rate - BMC Nephrology. (2023, April 21). BMC Nephrology. Retrieved July 18, 2023, from https://bmcnephrol.biomedcentral.com/articles/10.1186/s12882-023-03147-9

PON1 and Mediterranean Diet - PMC. (2015, May 27). NCBI. Retrieved July 18, 2023, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4488773/

The Search for Dietary Supplements to Elevate or Activate Circulating Paraoxonases. (2017, February 15). NCBI. Retrieved July 18, 2023, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5343950

Upregulation of C Terminus of Hsc70-Interacting Protein Attenuates Apoptosis and Procoagulant Activity and Facilitates Brain Repair After Traumatic Brain Injury. (2020, September 8). Frontiers. Retrieved July 18, 2023, from https://www.frontiersin.org/articles/10.3389/fnins.2020.00925/full

Versican—A Critical Extracellular Matrix Regulator of Immunity and Inflammation. (2020, March 6). Frontiers. Retrieved July 18, 2023, from https://www.frontiersin.org/articles/10.3389/fimmu.2020.00512/full

Yang, J., Griffiths, M., & Everett, A. D. (2020, October 6). Insulin-like growth factor binding protein-2: a new circulating indicator of pulmonary arterial hypertension severity and survival - BMC Medicine. BMC Medicine. Retrieved July 18, 2023, from https://bmcmedicine.biomedcentral.com/articles/10.1186/s12916- 020-01734-3

Zhou, L. (2022, November 3). Integrated proteomic and metabolomic modules identified as biomarkers of mortality in the Atherosclerosis Risk in Communities study and the African American Study of Kidney Disease and Hypertension - Human Genomics. Human Genomics. Retrieved July 18, 2023, from https://humgenomics.biomedcentral.com/articles/10.1186/s40246-022-00425-9

TRUAGE BY TRUDIAGNOSTIC

Fitness

This report measures how physical fitness impacts biological age and accelerated biological aging, by examining associated methylation patterns at various locations of your DNA.

Developed By TruDiagnostic's Bioinformatics & Research Department

A NEW AGING ALGORITHM

How your physical fitness impacts age.

It is a visible and well-known fact that physical fitness declines as we age. This functionality and performance loss is well- correlated with health, and can be measured indirectly through reduced function in specific organs (such as the lungs), as well performance tests of strength.

The rate and extent of this decline varies between individuals, however, **those who maintain physical fitness as they age are at lower risk for a range of diseases**. These people also tend to live longer lives.

The use of DNA methylation (DNAm) has allowed for the development of fitness biomarkers, as well as biomarkers of age- related changes in physical fitness. Physiological data can be incorporated into algorithms in order to **predict aging-related morbidity, disability, and mortality** through DNAm biomarkers; indicating that individual differences in various fitness parameters can be reflected in DNAm data.

The incorporation of physical fitness measurements into epigenetic clocks **increases the measurable effects of lifestyle, medical, and environmental interventional changes** on the aging process. The DNAmFitAgeAccel algorithm, also simply known as FitAgeAcceleration, was developed by researchers at UCLA, and is an estimate of epigenetic age acceleration. We have created a version of this, however, we incorporated our OMICm Age algorithm (developed with Harvard) instead. We call this **OMICm FitAge**, which tells you how old you are according to your physical fitness and functionality.

COLLECTED: 10/25/2023 | REPORTED: 11/12/2023

VO2MAX

Maximal oxygen uptake, or VO2Max, is a measure of cardiovascular health and aerobic endurance. It measures **the volume of oxygen the body processes** during incremental exercise, in milliliters used in one minute of exercise per kilogram of body weight (mL/kg/min). DNAmVO2Max can be measured by blood to provide an epigenetic calculation of one's physical fitness. Highly fit individuals, as classified by VO2Max scores, are correlated with having a lower BMI and a higher GripMax (grip strength).

GRIPSTRENGTH

Maximum hand grip strength (GripMax) is a measurement of force (taken in kg), and is used to calculate the age-associated decline in terms of **muscle strength.** Evidence suggests that grip strength may be a predictor of all-cause and disease-specific mortality, future function, bone mineral density, fractures, cognition and depression, and problems associated with hospitalization.

FEV1

Forced Expiratory Volume, also known as FEV1, measures **lung function** by determining the amount of air that is forced from the lungs in one second. DNAmFEV1 is a strong predictor of mortality and comorbidities.

GAITSPEED

Gait speed, also known as **walking speed,**is measured in meters-per-second, and can fluctuate based on ones fitness level, the type of terrain, and how much effort is used. Muscle strength, especially in your lower body and hip flexors, also affects gait speed. Gait speed significantly and cumulatively decreases as your age increases, however, smaller declines are often associated with each year that age increases. This averages out to a difference of 1.2 minutes slower for every kilometer at age 60, than at age 20. Both men and women have a walking speed that stays fairly consistent until reaching their 60s, which is when it starts to decline considerably.

PROVIDED BY: TruDiagnostic™ The Epigenetic Company

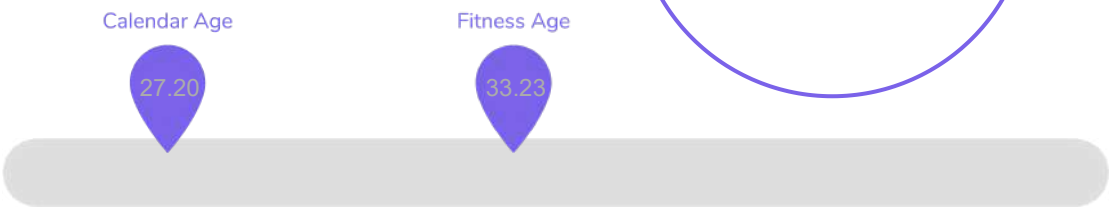
Your Results.

DISCLAIMER: All population graphs included in this report are based off of data from thousands of research participants and TruAge test takers. Population graphs are included to provide context to your results, with your individual scores being indicated by a neon green dot that is plotted amongst population trends.

33.23

YEARS OLD

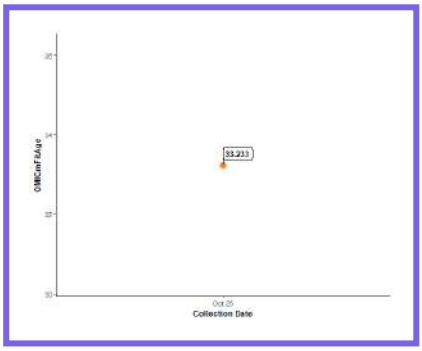
OMICm FitAge

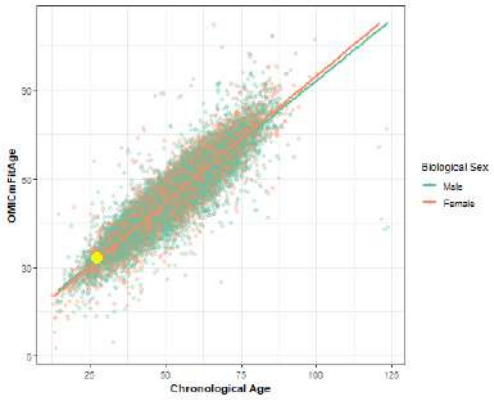


Your OMICm FitAge is

HIGHER THAN

your calendar age by **6.03** years.





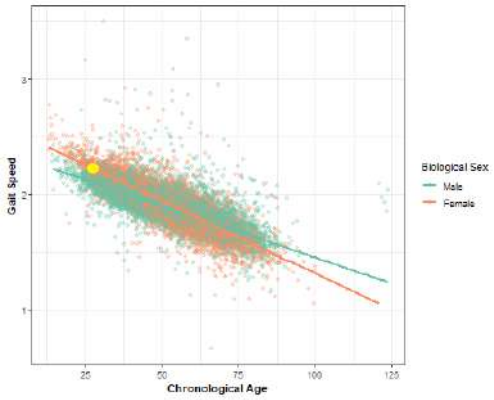
For every one year older that your OMICm FitAge is over your calendar age, there is an average **0.29 decrease in relative grip** strength and a 0.32 increase in BMI. OMICm FitAge has estimated that highly-fit individuals (classified through VO2max) have a **1.5 to 2.0 younger biological age** compared to low/medium fit individuals in females and males, respectively.

Younger OMICm FitAge was associated with better memory test performance, emphasizing the **beneficial role of physical exercise on cognitive health.**

Your **Gait Speed** epigenetic biomarker proxy is higher than

45.12%

of the population at your same calendar age and sex



Your Gait Speed epigenetic biomarker proxy score is 2.23.

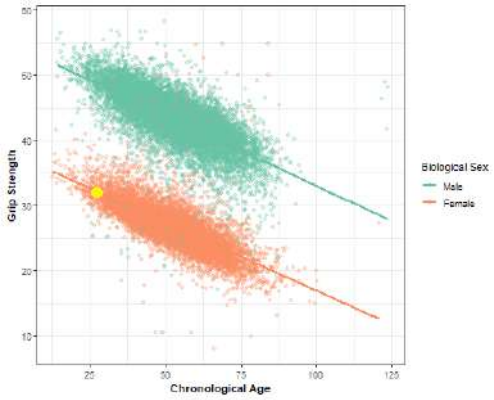
Lower gait speed is associated with impairment of daily activities, physical inactivity, and cardiovascular disease.

Faster gait speeds indicate greater mobility- which helps to prevent disability, disease, and loss of autonomy.

Your **Grip Strength** epigenetic biomarker proxy is higher than

46.95%

of the population at your same calendar age and sex



Your Grip Strength epigenetic biomarker proxy score is 32.05.

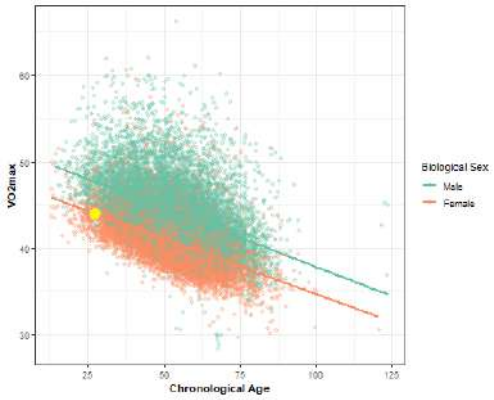
Higher levels of Gripmax (DNA methylated Grip Strength) are associated with better verbal short-term memory; which is further associated with decelerated aging.

However, traditional grip strength measurements are correlated with overall strength, upper limb function, bone mineral density, fractures, falls, malnutrition, cognitive impairment, depression, sleep problems, diabetes, multimorbidity, and **quality of life.**

Your **VO2Max** epigenetic biomarker proxy is higher than

50.61%

of the population at your same calendar age and sex

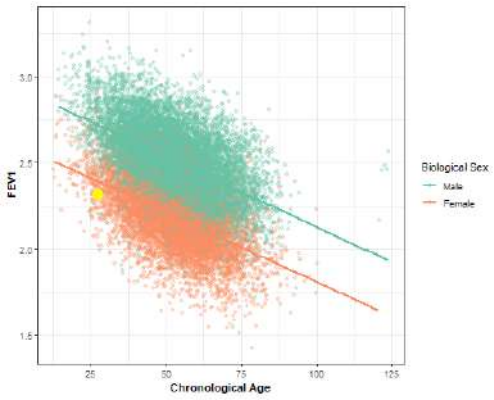


Your VO2Max epigenetic biomarker proxy score is 44.08

Your **FEV1** epigenetic biomarker proxy is higher than

27.44%

of the population at your same calendar age and sex



Your FEV1 epigenetic biomarker proxy score is 2.32

Higher levels of VO2Max is associated with better, verbal short term memory. Highly fit individuals, as considered by VO2Max levels, are associated with younger OMICm FitAge and lower BMI.

FEV1 specifically measures lung function. Collectively, these parameters make-up spirometry testing, which is beneficial in diagnosing chronic obstructive pulmonary disease (COPD), asthma, restrictive lung disease, and other disorders that affect lung function. In addition, **VO2Max and FEV1 are predictive of mortality.**

EDUCATIONALCONTENT

Report references.

Bohannon RW. Grip Strength: An Indispensable Biomarker For Older Adults. Clin Interv Aging. 2019;14:1681- 1691. Published 2019 Oct 1. doi:10.2147/CIA.S194543 Cirino, E. (2021, October 20). Spirometry: Procedure, "normal" values, and test results. Healthline. Retrieved December 22, 2022, from https://www.healthline.com/health/spirometry

Busch, T.d.A., Duarte, Y.A., Pires Nunes, D. et al. Factors associated with lower gait speed among the elderly living in a developing country: a cross-sectional population-based study. BMC Geriatr 15, 35 (2015). https://doi.org/10.1186/s12877-015-0031-2

Cronkleton, E. (2019, March 14). Average walking speed: Pace, and comparisons by age and sex. Health-line. Retrieved January 4, 2023, from https://www.healthline.com/health/exercise-fitness/average-walking-speed#lifetime-walking-stats

Jokai M, Torma F, McGreevy KM, et al. DNA methylation clock DNAmFitAge shows regular exercise is associated with slower aging and systemic adaptation. medRxiv. https://www.medrxiv.org/content/10.1101/2022.07 22.22277842v1.full. Published July 24, 2022. Accessed September 9, 2022.

McGreevy KM, Radak Z, Torma F, et al. DNAmFitAge: Biological age indicator incorporating physical fitness. medRxiv. https://www.medrxiv.org/content/10.1101/2022.03.21.22272043v1.full. Published March 22, 2022. Accessed September 9, 2022.