TruDiagnostic™

Reveal Your TruAge™

Collection Results Report

Hi ,

Thank you for taking the TruAge™ test by TruDiagnostic™. TruDiagnostic™ is a company that has been built on *one premise*. We want to be able to read your DNA methylation patterns so that we can help you live a longer, better quality life. In the report below, we will explain everything about our test including why it is important and how you can use this metric to live a healthier life.

By purchasing TruAge™, you have now unlocked a lifetime of information about yourself. As we get better at reading each methylation spot on your DNA, and the outcomes that each spot is correlated to, we will continue to update you on this information and what it tells us about you. You are one of the first to have your DNA read and interpreted by our innovative algorithms. We are thankful to you for adding to the growing science and innovation around these areas.

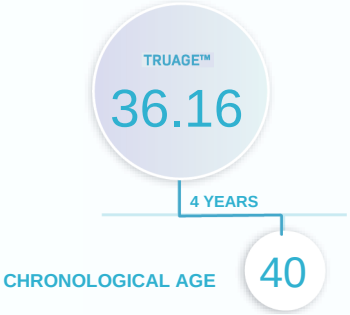
Hopefully this will be the first of many times we report our hard work to you and help you unlock a longer, healthier life.

Thanks,
The TruDiagnostic™ Team

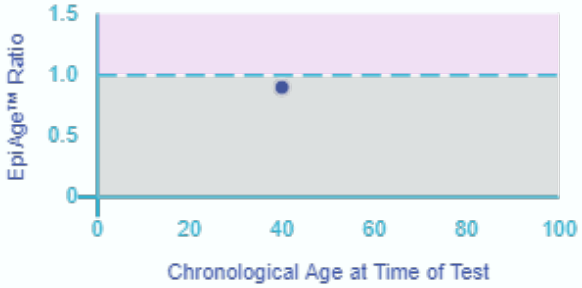
Mindy Williams, PhD, DABT
CLIA Lab Director

YOUR TRUAGE™

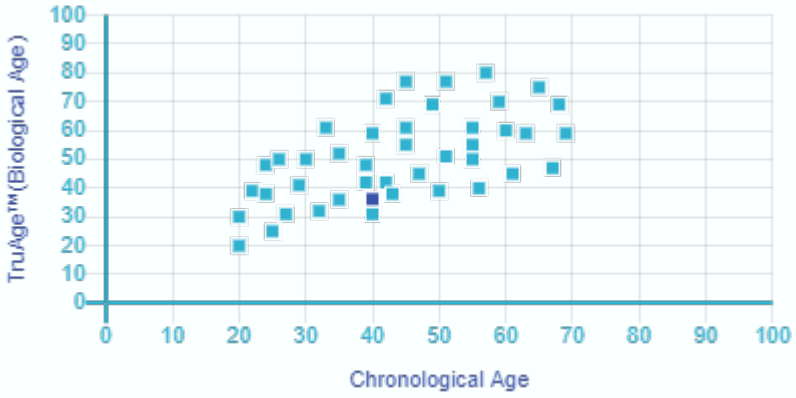
Summary



Your biological age vs chronological age



Your EpiAge™ Ratio

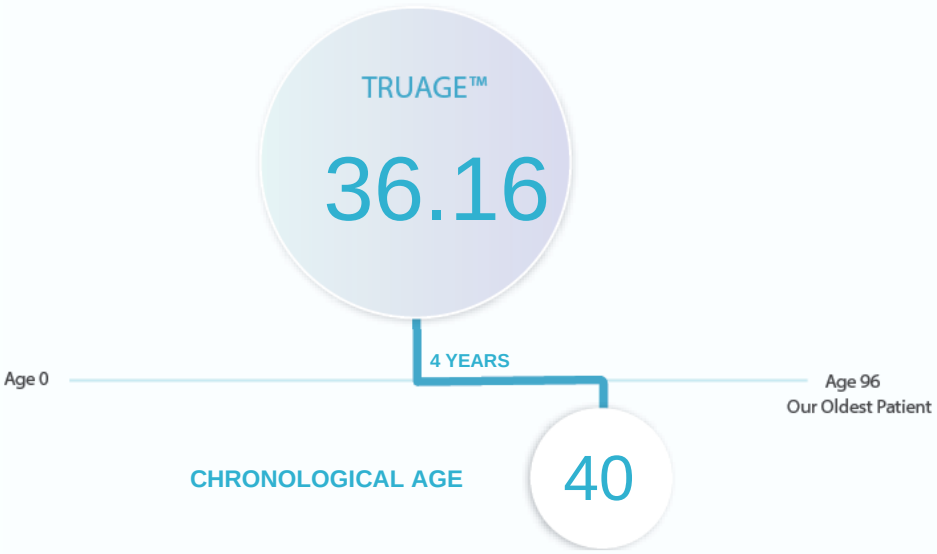


Your TruAge™ Compared to the General Population

Read further to see explanations for each of your results!
Also, see your TruAge™ Treatment Framework on pg 64.

YOUR TRUAGE™

Biological Age vs Chronological Age

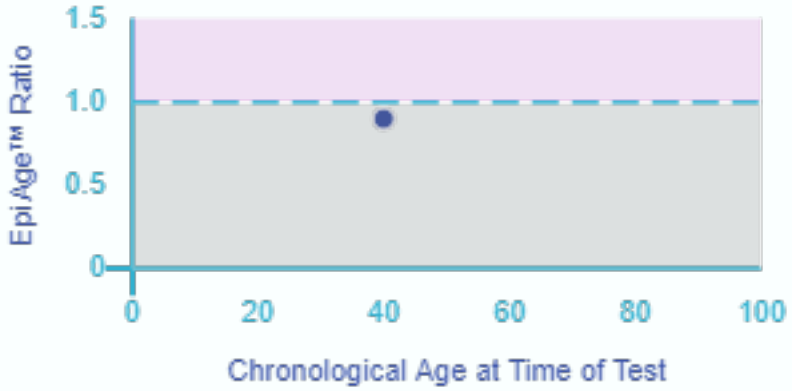


Your biological age is lower than your chronological age.

This is the first of hopefully many tests to measure the status of your DNA. You are more than your DNA. While tests like 23andMe might predict risk of certain diseases, TruAge™ can see how much your DNA can be changed through proper lifestyle changes.

If your TruAge™ is much higher than your chronological age, don't worry! There are plenty of things you can do to slow your aging. If your TruAge™ is under your chronological age, don't stop doing what you are doing, but maybe add things which could make it even better!

YOUR EPIAGE™ RATIO



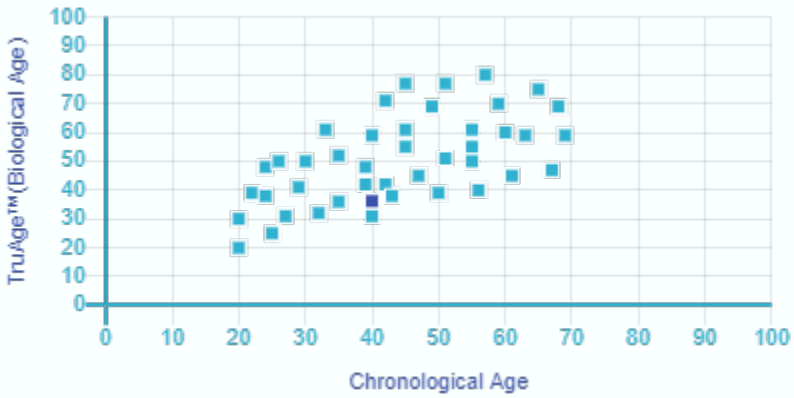
$$\frac{\text{Biological}}{\text{Chronological}} = \text{EpiAge™} \quad \frac{36.16}{40} = 0.90$$

You show decelerated aging.

Your EpiAge™ ratio is slower than your chronological age. Individuals whose epigenetic age is less than their chronological age (i.e., individuals exhibiting “epigenetic age deceleration”) are at a decreased risk for death from all causes, even after accounting for known risk factors.

Your EpiAge™ ratio is 0.90. This means you age 90% for every year. If you lived 20 more years at this rate, your age would be 54 when you are 60.

HOW DO YOU COMPARE
to the general population?



Your TruAge™ Compared to the General Population

This graph shows you where most people would fall on the graph when comparing their chronological age versus their TruAge™.

One thing to remember is that a majority of our patient population are receiving this test in a preventative, integrative, functional medical community. As a result, our population metrics might be slightly different than those of the general population. That is because often, the individuals who are being tested can afford the test and are most likely interested in aging in a healthy manner. In order to avoid this bias, TruDiagnostic™ actively recruits participants outside of this population to make sure we have a good snapshot of all variables such as socioeconomic status, race, gender, nationality and many others. If you have a connection to a under represented group who would like to be involved in this research please let us know!

OLIVIER'S TRUAGE™

Treatment Framework

Fitness

- You mentioned that you participate in Balance exercise. It is important to get a diverse type of exercise in order to change methylation epigenetic markers in association with aging. Consider alternating the types of exercise you do

- Epigenetic markers of exercise are more changeable and predictive as you age. If you are older, you should make sure you work out regularly

- You mentioned that you exercise 3-4 times per week. Epigenetic study data suggests that exercising 4 times per week is the target minimum to reduce epigenetic aging.

Nutrition

- Consider increasing your consumption of polyphenols such as trans-resveratol, sulforaphane, epigallocatechin-3-gallate (EGCG), quercetin, and genistein

- You mentioned that your diet mostly consists of both meat and vegetables. Fish and poultry have shown to lower epigenetic age.

Medications

- You stated that you do not take any supplements or medications like metformin or rapamycin.Consult with your doctor about taking these types of supplements and medications, as they have shown to slow the rate of aging.

- You said that you do not engage in anti-aging intervetions. Ask your doctor about these types of therapies.

Drugs/Alcohol

- You mentioned that you drink Once per week. Consider regular consumption of small quantities of wine and beer. Consult with your physician before making any major health changes.

- You mentioned that you have not taken illicit drugs. Unfortunately, illicit drugs have not been studied in relation to epigenetic aging. We are hoping to change this! By letting us know, we are able to collect this data and let our computer learning system help us connect the dots between illicit drugs and aging. While many of these drugs have been investigated, we hope to have more substantial data soon!.

Psychosocial

- You mentioned that your stress level is a 5. Physical and emotional stress have been shown to increase epigenetic aging. Consider what you need to do to keep your subjective level of stress to a 2-3

Comorbidities

- Continue to avoid behaviors that increase your risk of type 2 diabetes and obesity

- You mentioned that you sleep 6 to 8 hours hours a night. Insomnia and low amounts of sleep have been associated with age acceleration. Consider what you need to do to get at least 7 hours of sleep each night.

- Continue to avoid behaviors that increase your risk of viruses.

Exposures (Toxins, Pollution)

- Wear a mask in highly polluated areas

- PM2.5 particle matter data for your zip code can be found online. Create a treatment plan with your physician to avoid this type of pollution.

- Avoid exposures to pesticides and pesticide treated foods without washing them

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WHAT IS YOUR INTRINSIC AND EXTRINSIC EPIGENETIC AGE?

Immune Report

TruDiagnostic™

A Recap on TruAge™

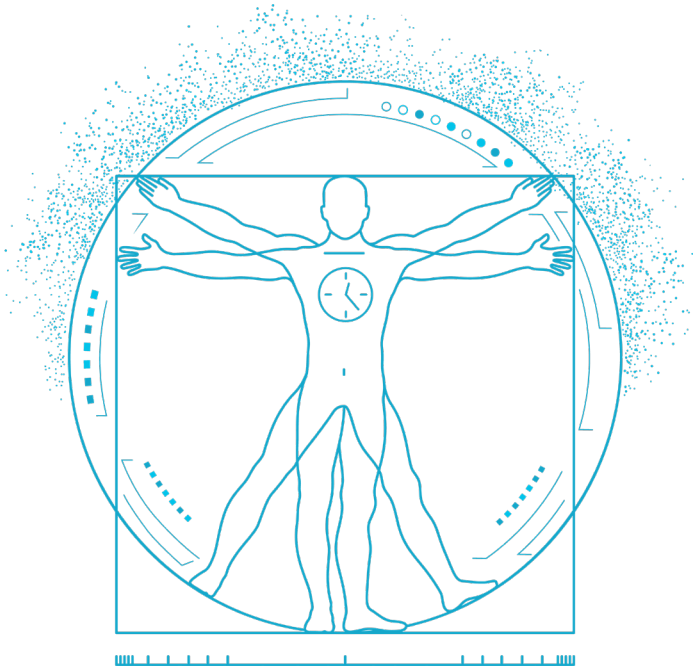
Most people know that their body has an internal clock which can be measured via methylation specific epigenetic testing. We have reported this age output to you in our TruAge™ test! However, did you also know that we can break this down even further?

While biological age clocks are a good measure of the age of 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 which give you more information above and beyond biological age. These are the intrinsic and extrinsic age 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 how a cell develops and functions by turning on and off certain genes. By selecting which genes are turned off and on, you can control your phenotype, or how your cells behave.



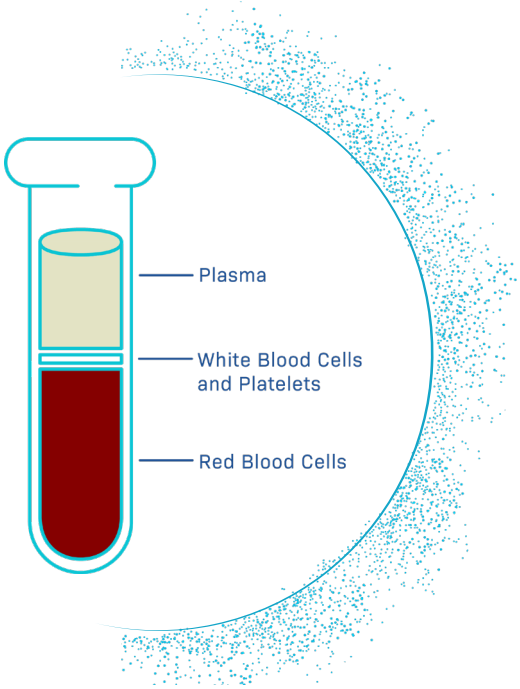
Thus, it makes sense that the epigenetic regulation of each cell would depend on what cell type it is. You wouldn't want your heart to make the proteins found in your bone and vice versa.

When we are measuring methylation (an epigenetic mechanism to turn off genes), we see that this is true and that all types of cells have different epigenetic signatures. Beyond that, if we measure biological aging of different tissues, we see them aging at different rates! In fact, our cerebellum and brain ages slower than the rest of the body. We also see that sometimes breast tissue in women can age faster than 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, 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 in a damaged blood vessel to prevent blood loss.



Thus, our blood has many different functions. In order to do a good job at a lot of different things, our blood contains many different parts:

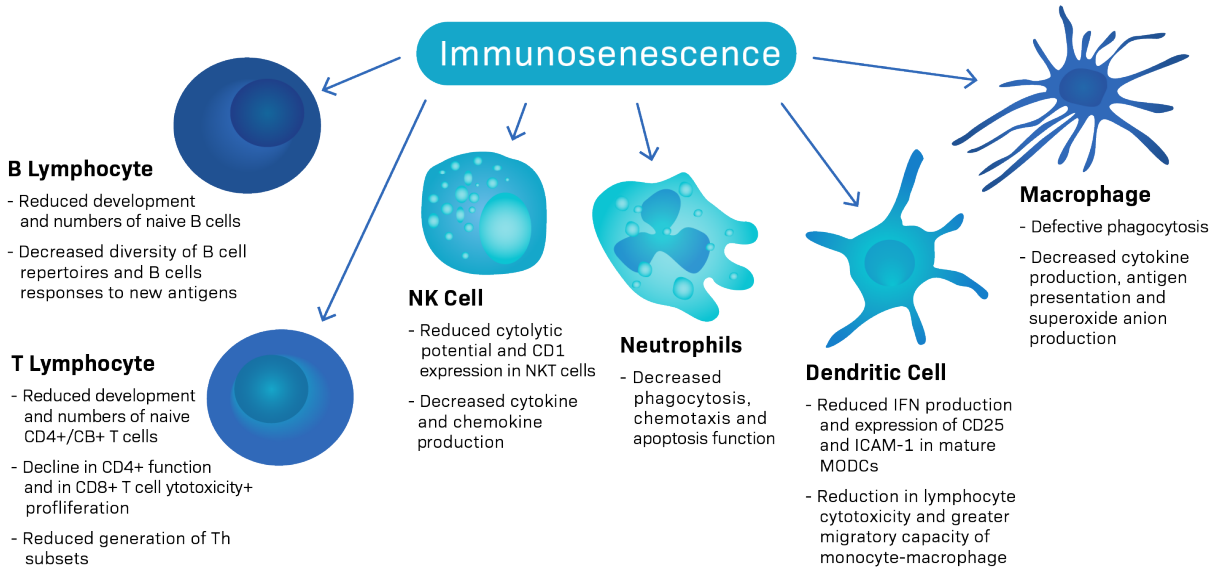
- One of the most important constituents of blood are the red blood cells. These cells contain hemoglobin and work to carry oxygen all throughout the body.

- The straw-colored fluid that forms the top layer is called plasma and forms 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 are the cells which help your blood clot. They 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. They are in the blood so that if we get a tiny cut we don't bleed out.

- White Blood Cells (WBCs) are the cells of your immune system which help fight infections. WBCs 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.

The interesting part about WBCs is that the amounts of these cells greatly change with age as shown in the graphic below!



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

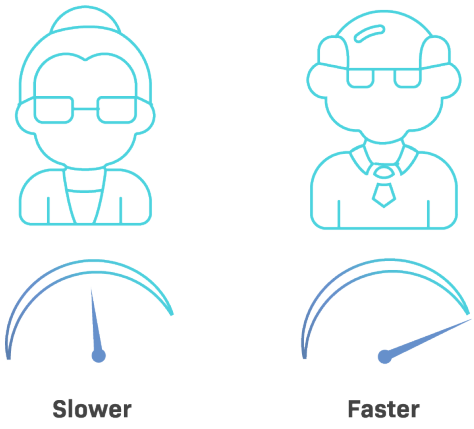
In humans, as well as in many other species, it is known that the immune system declines with age. This is known as immunosenescence. The process of immunosenescence leads to a higher incidence of infections, cancer and autoimmune diseases in the population. [Pawelec 1999]

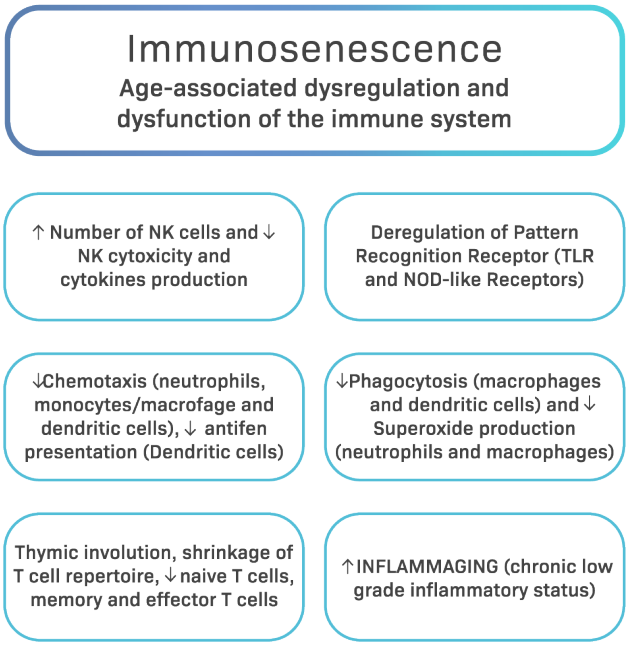
As you can see in the picture, it also changes the amount of immune cells in our blood. As we age we have fewer Naive T Cells, Natural Killer Cells, Macrophages, Dendritic cells and others.

This means, when we process the DNA of your blood as you age, the DNA markers also change. Thus, it can affect our reading of biological age!

We can control for this change though. If we don't control for it in our interpretation, we get a measurement of extrinsic epigenetic age. This is a surrogate marker for the age of our immune system.

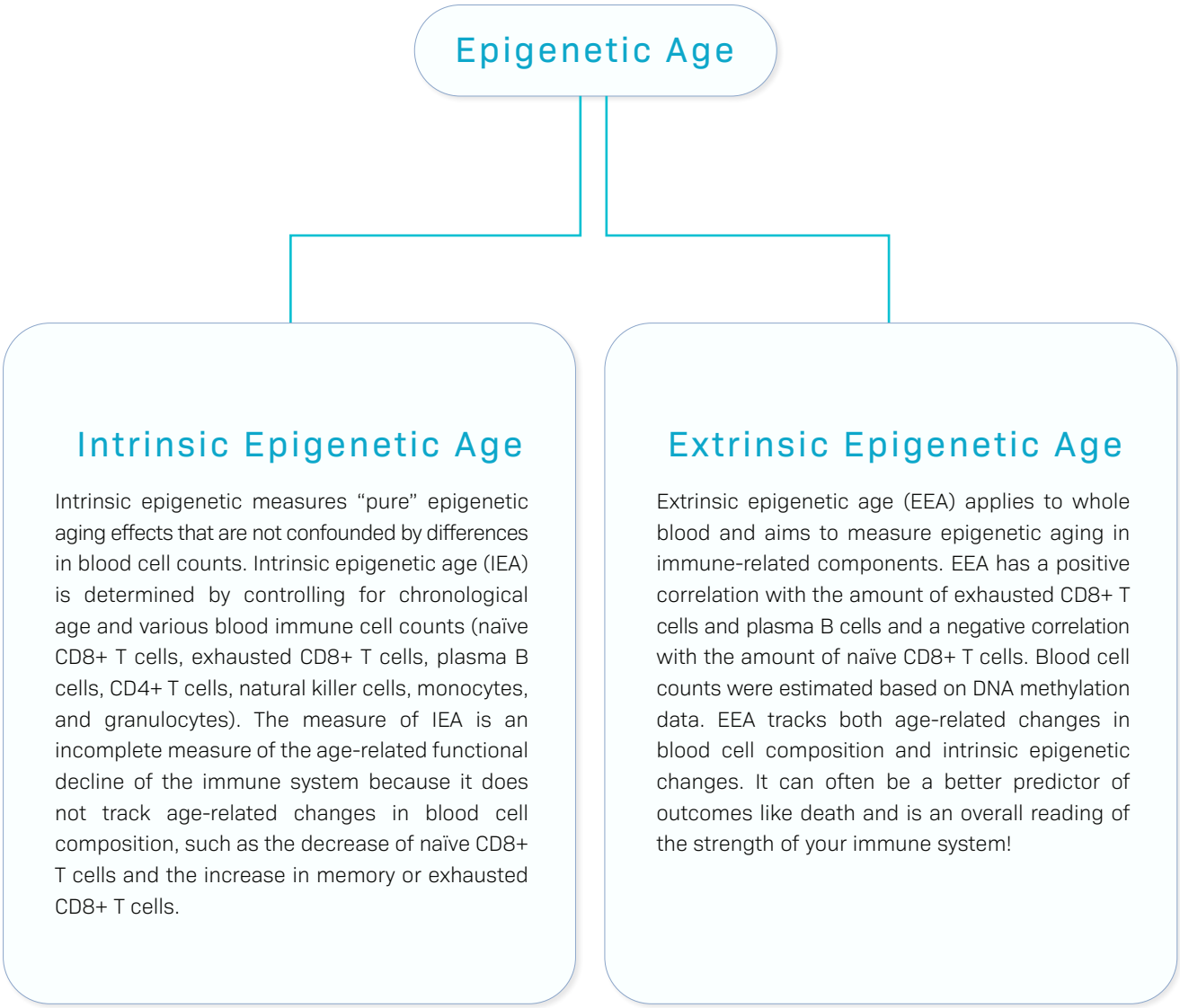
If we do control for this, we get the intrinsic epigenetic age. Which is the age of our cells without taking immune sets into account!





The Difference Between Intrinsic and Extrinsic Epigenetic Aging:

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

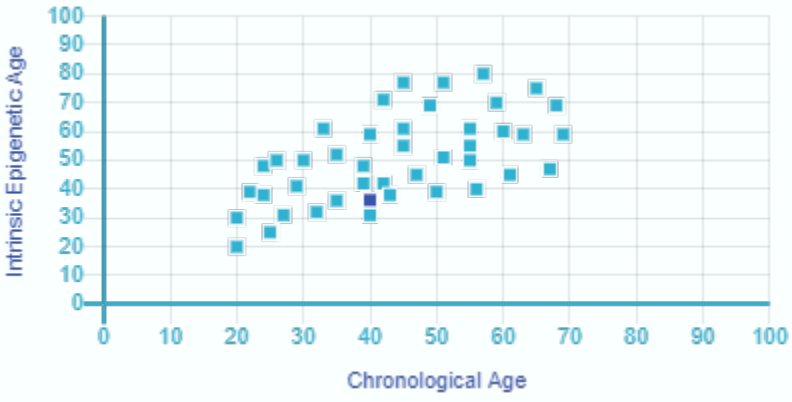


Therefore, we have two markers of epigenetic biological age which can tell us two very different things. One is aging with relationship to the immune system, and the other is the intrinsic basic fundamental process of cellular aging.

YOUR INTRINSIC
Epigenetic Age

Your measurement of Intrinsic Epigenetic Age is 36.16 years

You versus the population:



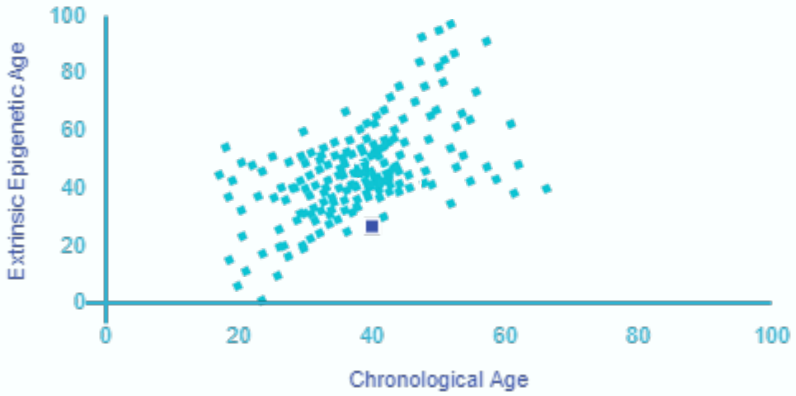
YOUR EXTRINSIC
Epigenetic Age

Your measurement of Extrinsic Epigenetic Age is 26.92 years

Test	Meaning	Normal Range Values	Your Percentage as Calculated using Lymphocyte Deconvolution
Lymphs %	Percentage of Lymphocytes:	20% to 40%	32.00%
	Bcell		0.00%
	CD4T		32.00%
	CD8T		0.00%
Neuts.%	Percentage of Neutrophils	40% to 60%	53.91%
Monos.%	Percentage of Monocytes	2% to 8%	17.79%
Eos.%	Percentage of Eosinophils	1% to 4%	0.00%
CD4/CD8 Ratio			0.00

This is a functional measurement of your immune system! This measures and predicts many of the cells which change as our immune system declines. If you have more of these cells, it means your immune system looks younger and can be more effective at protecting you from viruses, bacteria, cancer, and general inflammaging.

You versus the population:



Disclaimer: The 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 rates of aging. These are able to give us insights into the behaviors which are correlated with better extrinsic aging rates (and therefore less mortality and better immune systems).

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 below as well.

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	1.7×10^{-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)	1.6×10^{-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

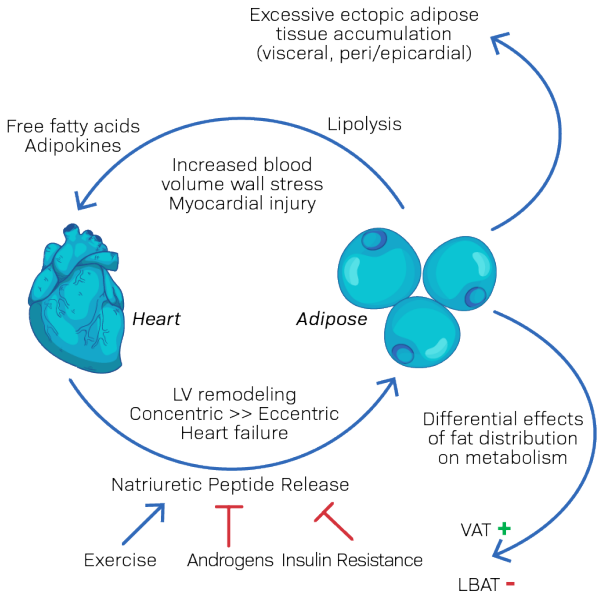
Cardiometabolic Disease, Metabolic Syndrome, and BMI:

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 affect your extrinsic aging rate! Epigenetic age acceleration (EAA) is linked more closely with risk factors for cardiometabolic disease than intrinsic aging according to one study.

Extrinsic epigenetic age acceleration (EEAA) was generally higher in individuals with higher triglyceride levels, higher C-Reactive protein, and higher creatinine.

Neither intrinsic nor extrinsic aging rates of blood tissue are predictive of incident coronary heart disease (CHD) in the Women's Health Initiative study (WHI) even though EEAA is weakly associated with several cardiometabolic risk factors of CHD (such as hypertension, triglycerides, and CRP). [Horvath 2016]



Dietary Intake

EEAA exhibits significant associations with fish intake, moderate alcohol consumption, and blood carotenoid levels (p=1x10-5), an indicator of fruit and vegetable consumption. [Quach 2017]

Race/Ethnicity

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

Hispanics and Tsimane have a higher EEAA than Caucasian:

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 EEAA than Caucasian:

African Americans have lower EEAA than Caucasians in the WHI and in the Bogalusa Study. In fact, one 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 EEAA: race/ethnicity, hypertension, and gender (p = 0.0093, Table 5). A marginal analysis in the Bogalusa study identifies a significant association between EEAA and hypertension (p = 8.0 × 10−5, Additional file 5G–I), type II diabetes status in Caucasians (p = 0.0085, Additional file 6H), but not in African Americans (Additional file 6I). [Horvath 2016]

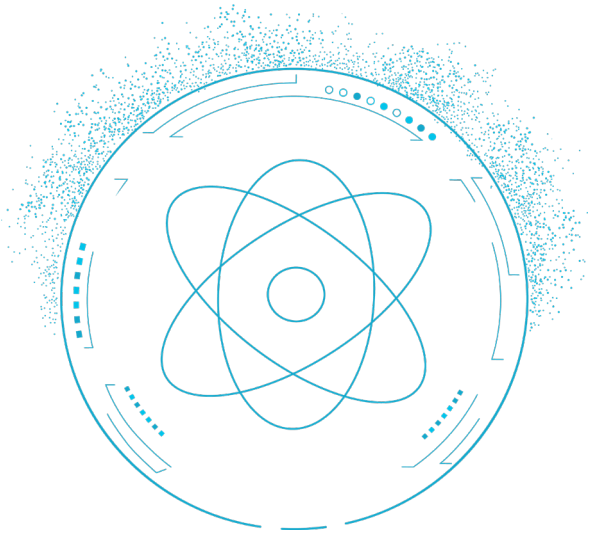
Contributing Factors

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 EEAA.

However, contrary to the findings in the WHI, no significant association can be observed between EEAA and educational level in other studies. It might be too early to tell how this is correlated to Extrinsic epigenetic aging.



Mood Stabilizers

Are you currently taking mood stabilizers? Compared with controls, there was a decrease in EEAA and IEAA in patients with Bipolar Disorder (BD). Further, there was a significant decrease in EEAA and IEAA in patients with BD taking medication combinations of mood stabilizers (including lithium carbonate, sodium valproate, and carbamazepine) than in those taking no medication/monotherapy. [Okazaki 2020]

Smoking

Nominally significant genetic correlations between EEAA and lifestyle factors including smoking behaviors and education support the hypothesis that Hannum-based epigenetic ageing is sensitive to variations in 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 some of your immune values, it is also considered a surrogate marker of the immune system. As a result, a low score here might mean that your immune system isn't doing the things that it should.

When the immune system isn't functioning correctly, your risk of some diseases and disease complications increase. 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 physician to learn more.

Your Longevity

Unfortunately, higher extrinsic epigenetic aging is also correlated with shorter lifespans. Chen et al. (18) included 2,734 deaths in a study and showed that higher extrinsic epigenetic age correlated to a higher hazard ratio for death.

Thus, the high predictive significance of EEAA 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 blood cell composition and cell-intrinsic epigenetic changes. It has been known for decades that poor T cell functioning is predictive of mortality. [Roberts 1974]

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

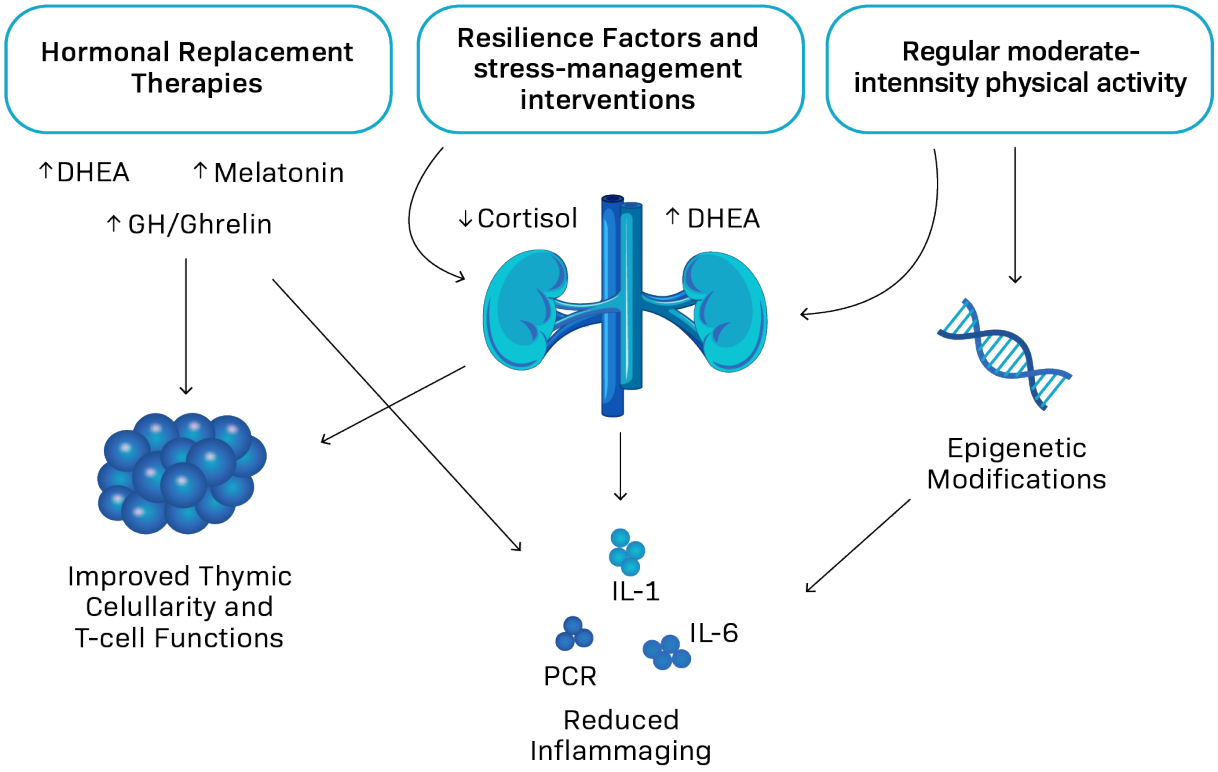
One of the best things about epigenetic measurements on aging is that they can be changed for better health. While research is still in its infancy on this topic. We are working to find the best interventions to change these metrics! So far, there is still some data on how to change this and many ways to speculate on these outcomes as well.

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). [Quach 2017]

This means that moderate consumption of alcohol (only validated at 1 drink per week) could help reduce this metric. Fish intake is also correlated to lower values. Increasing your diet of both of these could help reduce this metric! Additionally, increasing your consumption of fruits and vegetables is also correlated with improvement.

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

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



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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?

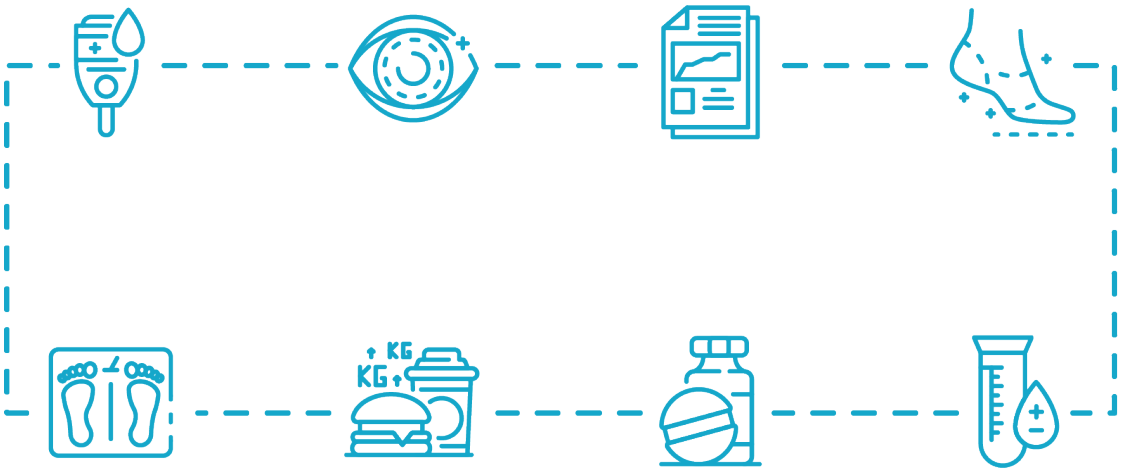
Epigenetic biomarkers for Type 2 Diabetes

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].

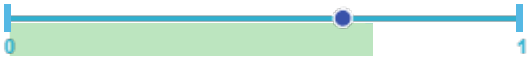
The ABCG1 marker has been replicated across different tissues in more than 10,000 individuals representing different ethnicities. Altered DNA methylation in ABCG1 is associated with the downregulation of mRNA levels from T2D individuals [2]. DNA methylation at this site in blood DNA has demonstrated 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.

ABCG1 (Cg06500161)
Average DNA methylation Score:

Your Epitype Value

0.65



[0 - 0.7]

NO INCREASED RISK

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.

PHOSPHO1 (Cg02650017)
Average DNA methylation Score:

Your Epitype Value

0.14

[.05 - 1]

NO INCREASED RISK

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 0.65 at the ABCG1 locus and 0.14 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.

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OBESITY RISK METHYLATION REPORT

FGFRL1 (cg25932599), NCAPH2 (cg25152348),
PNKD (cg22712983), SMAD3 (cg0757622) Genes

TruDiagnostic™

Are You at Greater Risk for Becoming Obese and Developing Obesity-Related Disorders?

Epigenetic Biomarkers for Obesity and Metabolic Disorders

The prevalence of obesity is increasing rapidly worldwide, the number of obese individuals has tripled between 1975 and 2016. With 650 million obese people all over the world, this condition is responsible for 3 million deaths each year [11]. The current status of obesity and its associated disorders have reached global pandemic status and are a major challenge for the healthcare system.

Metabolic alterations, such as obesity, are due to the interplay between environmental, lifestyle, and genetic factors [1]. Obesity originates from a failure of the body-weight control systems, which may be affected by changing environmental influences. Basically, obesity risk depends on two important mutually-interacting factors: genetic variants (inheritance, epigenetic mechanisms, etc.) and exposure to environmental risks (diet, physical activity, etc.). DNA methylation at specific gene loci for obesity may act as effect modifiers for environmental factors [5].

Fat tissue, frequently the largest organ in humans, is central to mechanisms involved in longevity, the origin of age-related disease, inflammation, and metabolic dysfunction. Fat distribution and function change dramatically throughout life. Obesity is associated with accelerated onset of diseases common in old age, including diabetes, hypertension, cancers, cognitive dysfunction, and atherosclerosis leading to heart attacks and strokes [7].

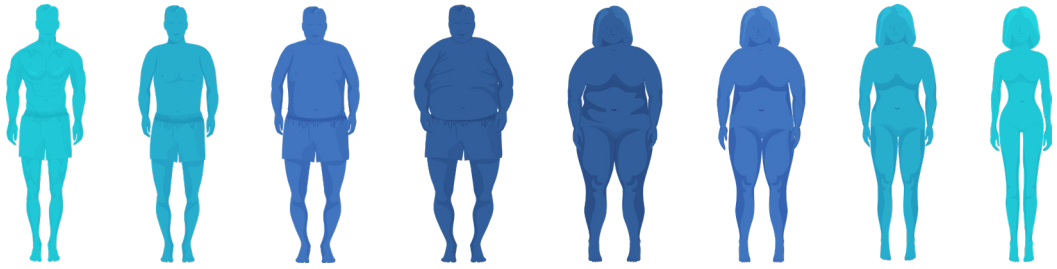
Epigenetic Mechanisms Influence the Process of Becoming Obese

It is well known that physical inactivity and unhealthy dietary patterns exert major influences on metabolic syndrome, diabetes, and obesity. However, despite intensive genetic research into these alterations, the basic mechanisms and pathogenesis of obesity are still poorly understood. In this regard, emerging evidence suggests that epigenetics represents one link between environmental factors and the greater predisposition to develop obesity and its associated conditions.

Therefore, there is great interest in the use of epigenetic biomarkers such as DNA methylation, which, unlike the DNA sequence, can be influenced by the environment, and has the potential to improve how healthcare officials treat those with obesity and those who are at higher risk for the onset of obesity. The characterization of the epigenetic changes within obesity-related adipose tissue provides new insights into understanding toward metabolic disorders.

Researchers discovered a test that uses blood cells to reflect whether an individual is more or less susceptible to becoming obese based on their DNA methylation status. This is huge because now we can measure your personal obesity risk by using blood as the one collection method to rule them all [1].

Analysis of DNA samples shows us what your specific level of risk is for developing obesity. ***Therefore your EpiType for the methylation levels of FGFRL1, NCAPH2, PNKD, and SMAD3 provides valuable insight regarding your overall susceptibility to becoming obese and your risk for diseases associated with obesity.***



What Is Your Epitype:

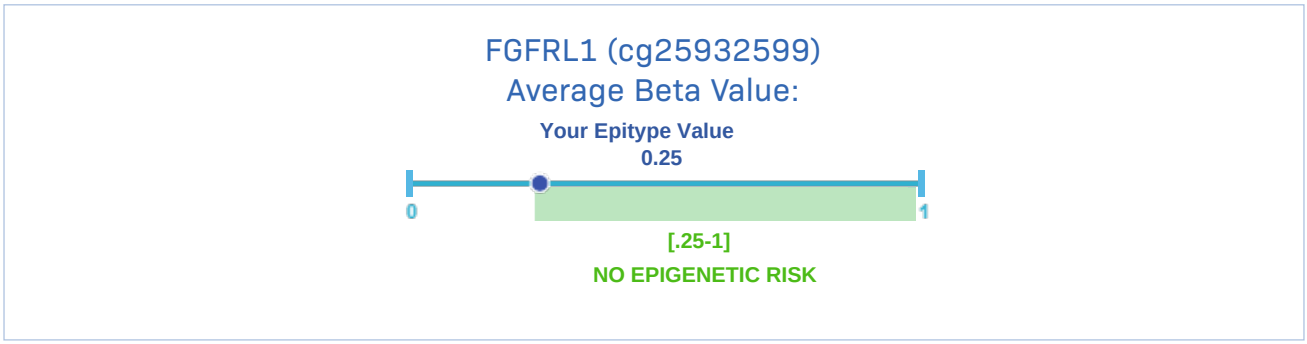
Your EpiType is your methylation status at these particular locations. The research mentioned on the first page shows that DNA methylation at 4 gene loci are linked to gene expression and your risk of becoming obese, we will define these particular sites and your ß-values for each of these.

What is FGFRL1?

FGFRL1 (fibroblast growth factor receptor-like 1) is a gene that is involved in metabolism signaling and insulin processing. This gene is an early indicator of adipogenesis which is known to increase fat tissue mass concerning obesity.

This gene was found to have increased methylation in fat tissue of twins that have different BMIs. FGFRL1 activity shows changes in glucose-stimulating and insulin secretion. FGFRL1 induced signaling regulates cells and is associated with the onset and progression of metabolic disorders such as diabetes [5].

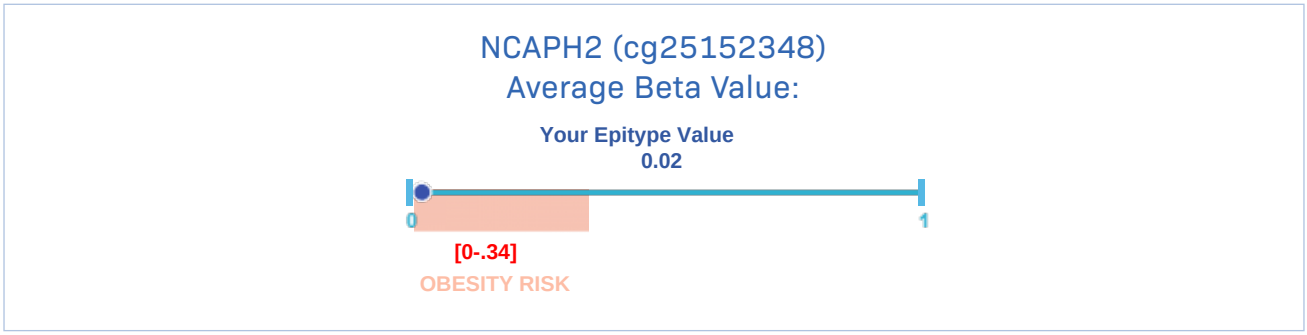
Having a beta value of 0.24 or less puts you at higher risk for disturbed metabolism signaling and insulin processing which increases the chance accumulating excess body fat.



What is NCAPH2?

The condensin II gene (NCAPH2) does non-structural maintenance of chromosomes by providing chromosomes with additional organization and rigidity. This chromosome-associated gene is involved in senescence by driving senescence through genomic reorganization. Cellular senescence is characterized by cell cycle arrest and is a process that is increased in dysfunctional obese fat tissue and is related to the development of metabolic disturbances [10].

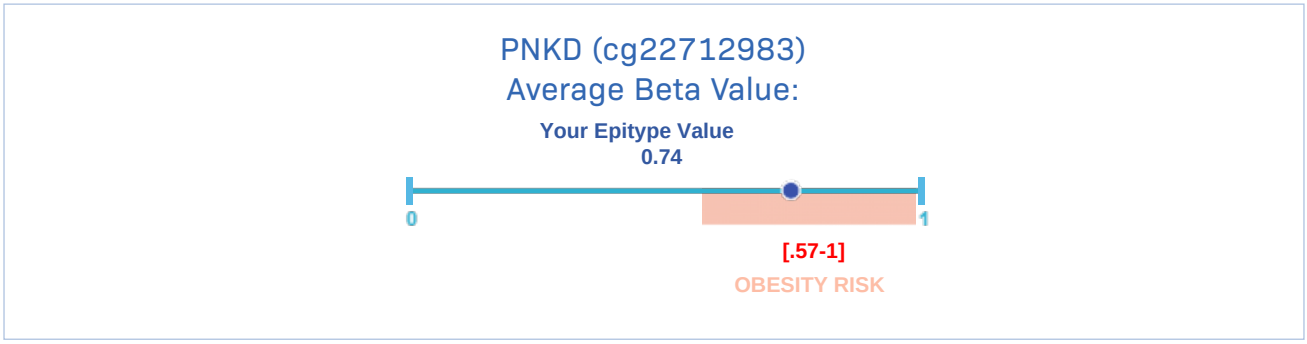
Having a beta value of 0.34 or less means that you are at higher risk for developing a metabolic disorder. This contributes to excess fat tissue accumulation that can lead to the development of obesity.



What is PNKD?

Regarding mitochondrial function, mutations of the identified paroxysmal nonkinesigenic dyskinesia (PNKD) gene are involved in the development of a rare motor disease induced by mitochondrial dysfunction. Research suggests that the PNKD gene mutations alter the structure of the PNKD protein and interfere with its ability to function. The methylation levels observed in the blood of the obese patients compared with the non-obese patients establishes this gene's loci role in the onset of accumulating too much fat tissue [2].

Having a beta value of 0.57 or greater suggests that you have an increased chance for developing the obese phenotype.

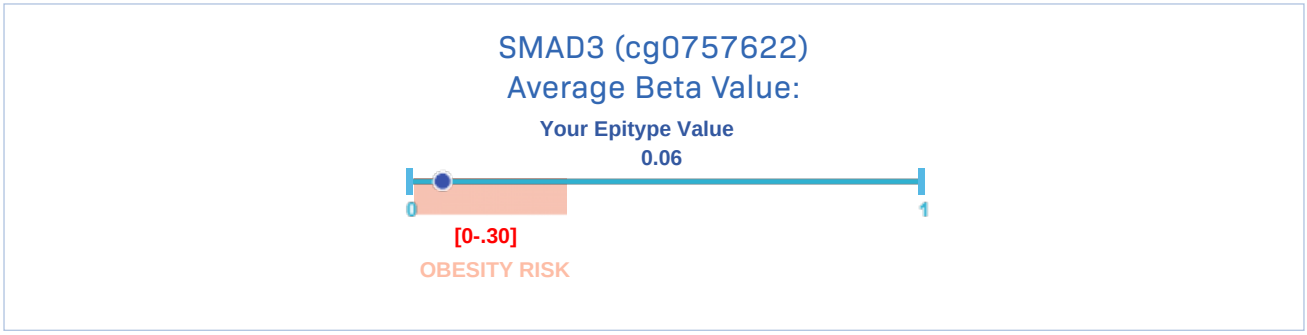


What is SMAD3?

This relevant, methylated gene identified was SMAD family member 3 (SMAD3). Our bodies' have an energy imbalance when energy intake exceeds energy expenditure, leading to the net storage of excess calories in the form of fat in the adipose tissue. The excess-accumulation of fat in adipose tissue is what leads to obesity. Mammalian adipose tissue is broadly classified as either white adipose tissue (WAT) or brown adipose tissue (BAT)

The loss of SMAD3 results in the transformation of white adipose tissue (WAT) to brown adipose tissue (BAT) which increases the minimal level of energy expenditure for the body to function properly. SMAD3 signaling protects against high-fat diet-induced obesity and Type 2 diabetes. Therefore, the blockage of SMAD3 contributes to a higher risk of obesity and type 2 diabetes mellitus [9].

Having a beta value of 0.30 or less means you are at greater risk for adipogenesis, this is the formation of adipocytes (fat cells) and is known to contribute to the excessive increase of fat tissue mass. These fat cells produce an accumulation of fat stores which can trigger cellular stress and cause the onset of obesity.



The Science

The area under the receiver operating characteristic (ROC) curves further analyzed the diagnostic power of this report's findings. Relevantly, the areas under the ROC curves (AUCs) measures how well the parameters of FGFRL1, NCAPH2, PNKD, and SMAD3 can be distinguished from obese and non-obese groups (figure 1). There is a great correlation in the DNA methylation between the subcutaneous adipose tissue and leukocytes for FGFRL1 ($r = 0.77$; $p < 0.001$), NCAPH2 ($r = 0.68$; $p < 0.001$), and SMAD3 ($r = 0.74$; $p < 0.001$) [1].

FGFRL1, NCAPH2, PNKD, and SMAD3 are target genes that could reflect the obesity state in leukocytes because they exhibited the greatest differences between the obese and non-obese samples. These genes are involved in important processes associated with adipose tissue balance. It is evident that the methylation levels of these gene loci suggest epigenetic regulation associated with obesity.

DNA methylation at the FGFRL1 locus cg25932599 in DNA is statistically correlated with metabolism signaling and insulin processing [6]. DNA methylation at the FGFRL1 locus cg25932599 in blood DNA displays statistical significance in the differentiation of having either obesity status or non-obesity status (AUC > 0.80; p < 0.05). Also, the level of DNA methylation at the FGFRL1 locus cg25932599 is statistically correlated with Body Mass Index indicating its role in the increase of excess fat [1].

Strong evidence that complex diseases, such as metabolic disorders, are under the influence of epigenetic modifications even in early life. This is opening up new avenues for the identification of DNA methylation biomarkers associated with these disorders and the estimation of future disease risk.

The characterization of the epigenetic changes associated with obesity will provide new insights about the pathophysiological processes and environmental influences (figure 2) involved with this metabolic disorder to help us develop better approaches for prevention and disease management [1].

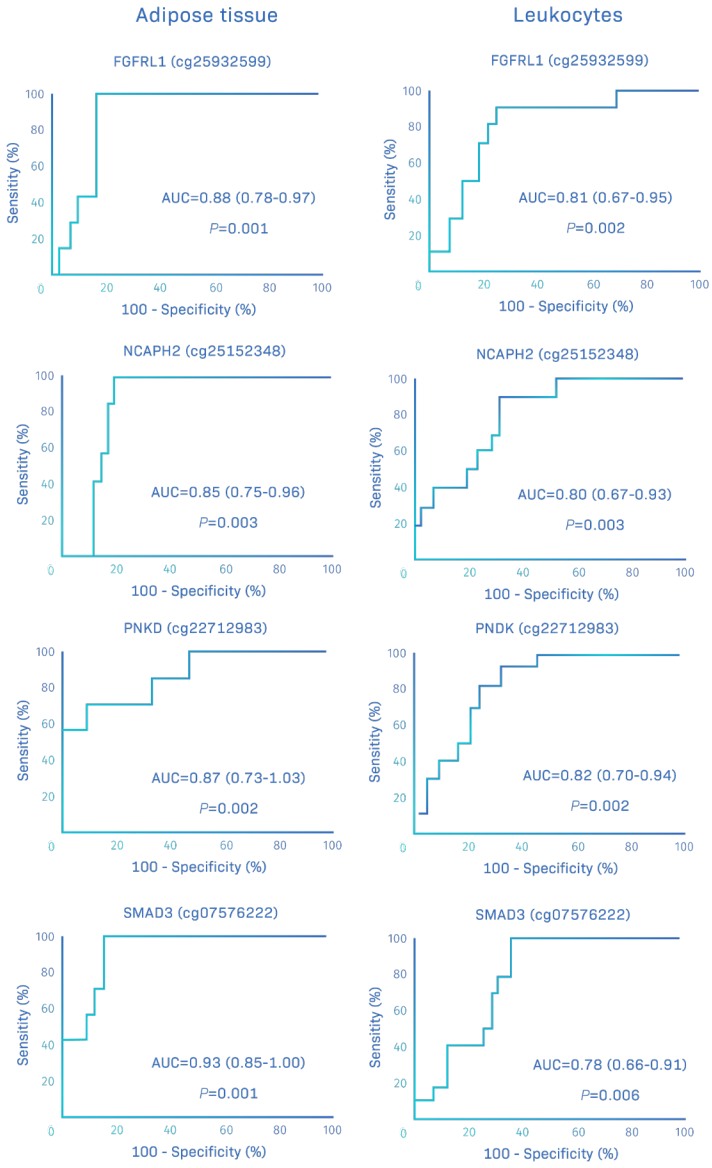


Fig 2 Receiver operating characteristic (ROC) curves for the methylation levels of the obesity-related differentially methylated CpGs. The abilities to discriminate the obese from the non-obese samples of adipose tissue (a) and leukocytes (b).

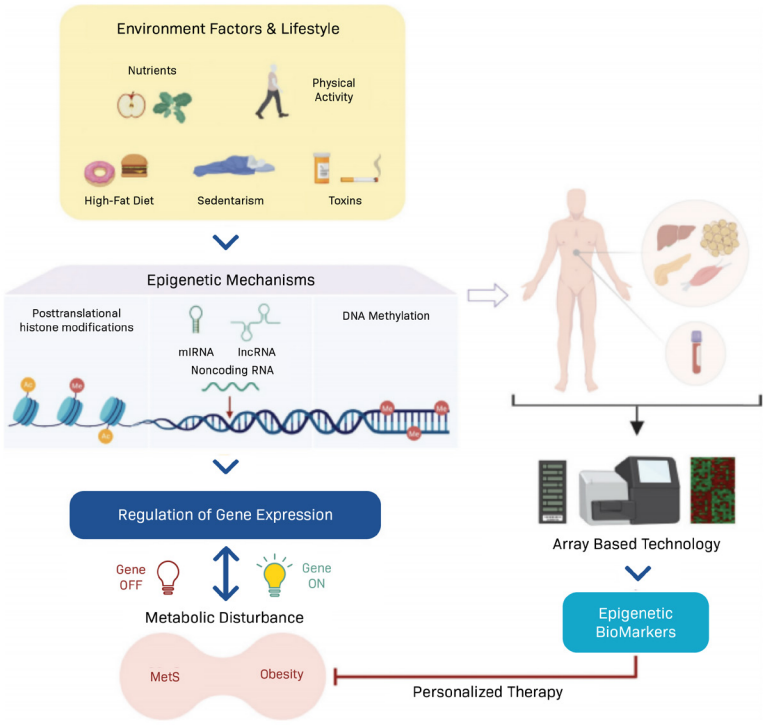


Fig. 2 The interplay between environment/lifestyle factors and metabolic diseases via epigenetic machinery and epigenetic biomarkers. Environment and lifestyle may contribute to metabolic disorders through the dysregulation of the epigenetic machinery. The alteration of the epigenetic mechanisms can modify the activity of genes related to the metabolism that trigger metabolic disorders such as insulin resistance and, consequently, diabetes mellitus and metabolic syndrome. These epigenetic modifications can be used as clinical biomarkers for metabolic diseases since these can be reversed by epigenetic drugs or functional foods and thus restore the right metabolic state of the gene in patients [3]

THE IMPACT TO YOU

The impact your genes' have on you is based on your level of methylation at these gene loci compared with the level of risk for the development of obesity.

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

Many metabolic alterations, such as obesity, are due to the interplay between environmental, lifestyle, and genetic factors. ***Recent studies have found 4 DNA methylation loci, FGFRL1, NCAPH2, PNKD, and SMAD3, that are able to predict your risk of becoming obese. By altering these loci's levels of methylation you have the power to change risk factors for obesity. TruDiagnostic™ is continuing to uncover interventions that will change these risk factors in order for you to make appropriate adjustments to reduce your risk.*** The alteration of epigenetic mechanisms can modify the activity of genes related to metabolism that triggers metabolic disorders. These genes' epigenetic modifications can be used as clinical biomarkers for obesity-related metabolic diseases, by predicting disease onset and determining a patient's response to therapy.

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