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La méthylation de l'ADN, un nouveau biomarqueur de santé

genknowme 
Knowledge, actionable.

Identical twins, different ...



Identical twins, different health status

Difference in diet and exposure
to air pollution



Difference in sun exposure

Difference in
diet



BMI = 37 vs. BMI = 23

Smoker | Non-smoker



Identical twins, different health status

Difference in diet and exposure
to air pollution



Difference in
diet



Smoker

Non-
smoker



BMI = 37 vs. BMI = 23

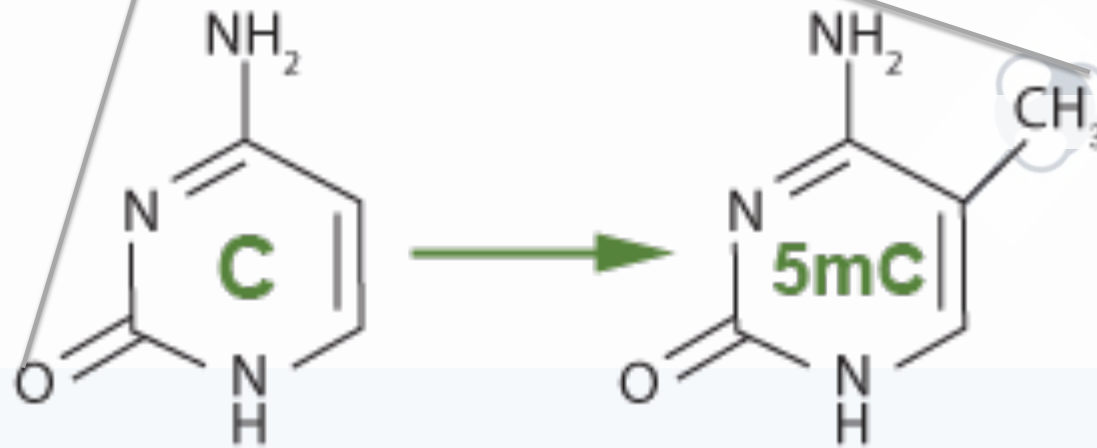


Difference in sun exposure

EPIGENETICS

Epigenetic Processes: DNA methylation

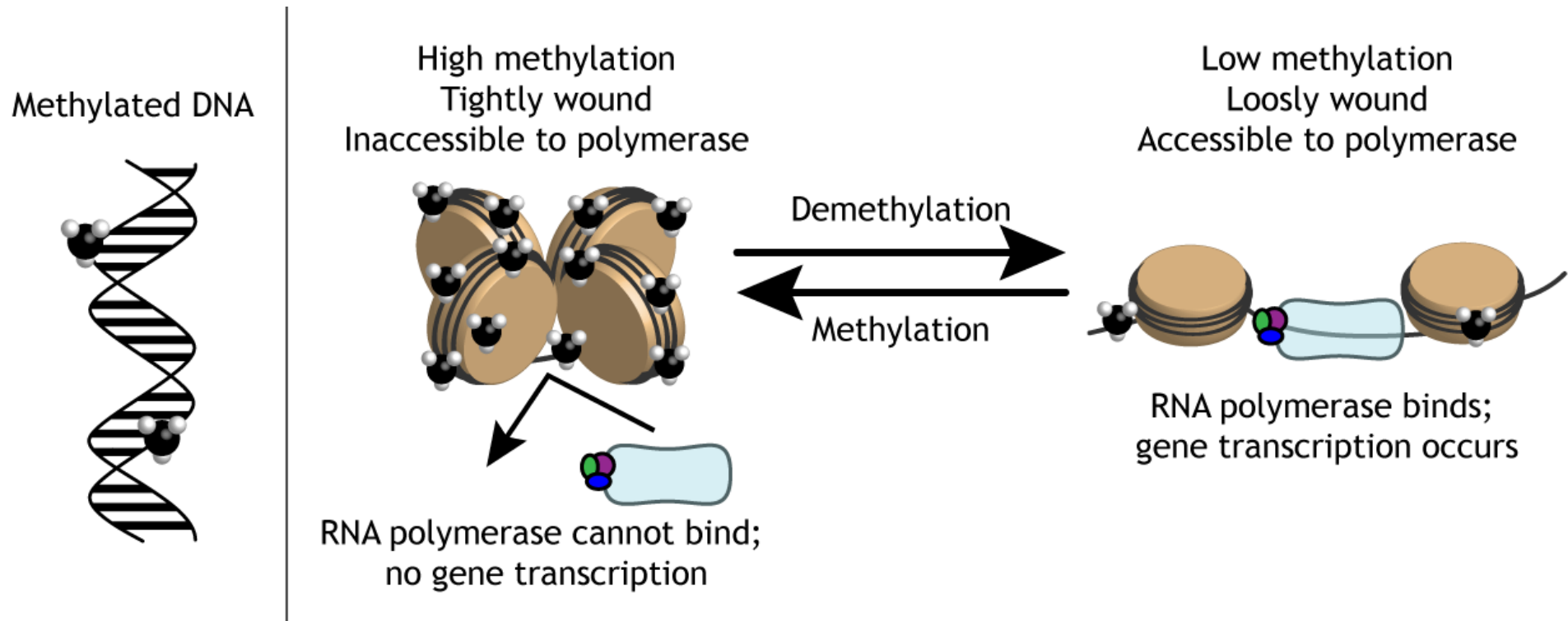
...AT**C**GTATCCTAGACGATC...
...TAGCATAGGATCTGCTAG...



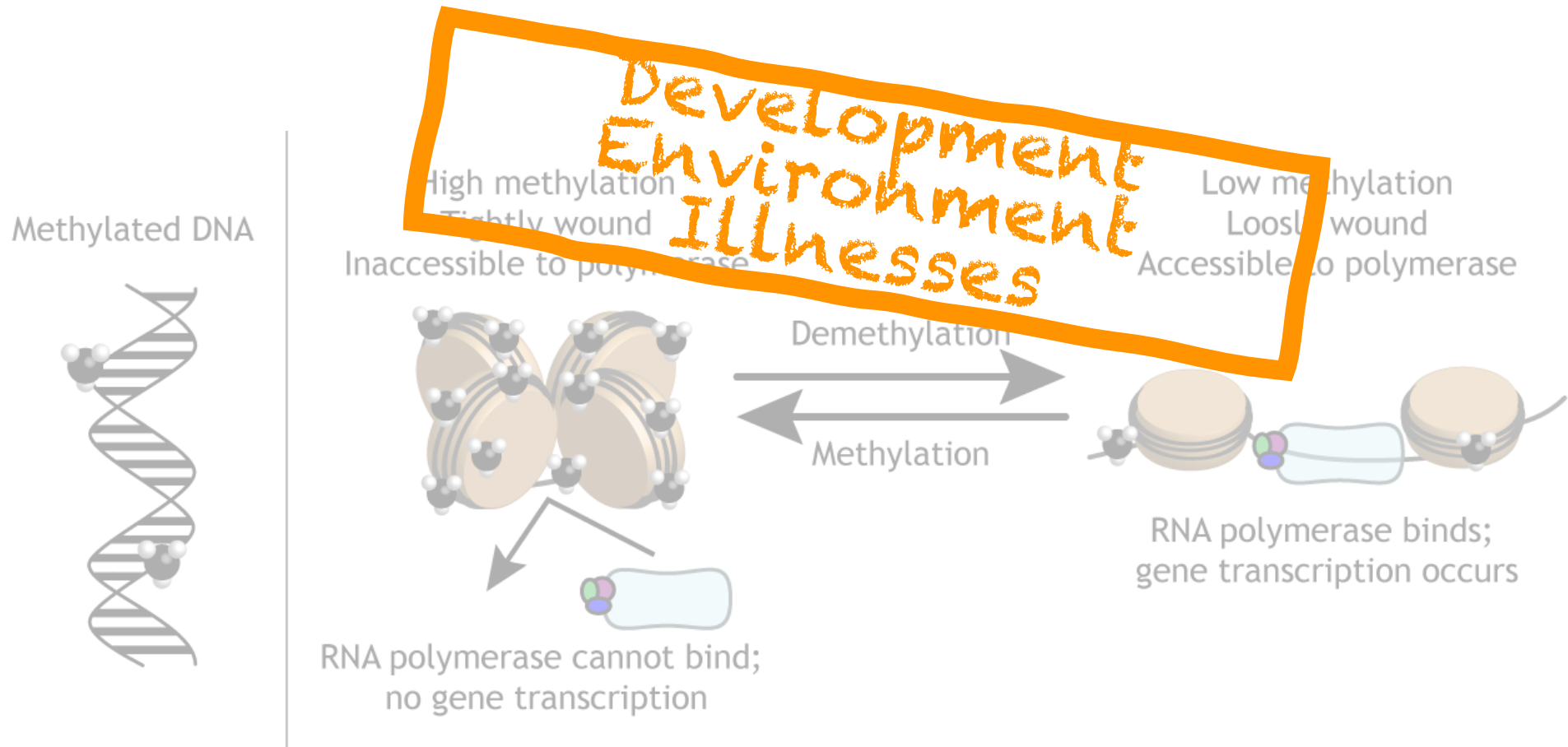
Unmethylated
cytosine

5-methylated
cytosine

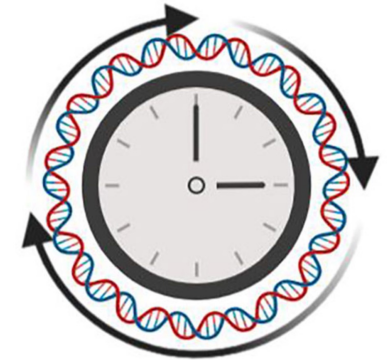
Epigenetic Processes



Epigenetic Processes



Epigenetic clocks of ageing and disease



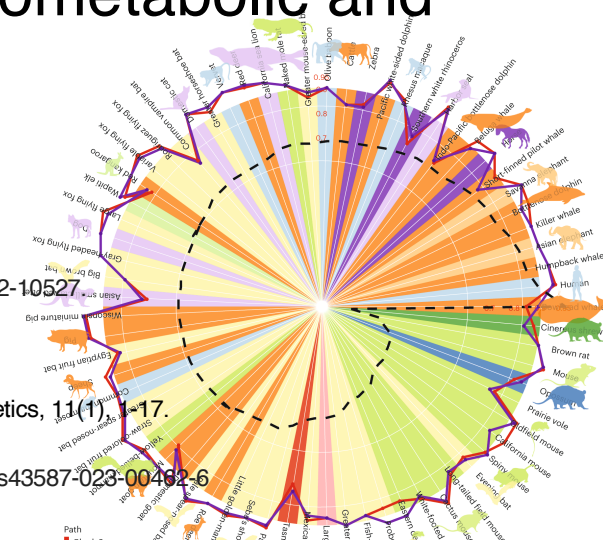
- Global DNA methylation levels decrease with age
- Specific markers of the time that passes → epigenetic clocks of ageing
- Meta-analyses on 40'000 participants: association with mortality, time-to-death, cancers incidence, cardiometabolic and neuro-degenerative diseases
- Pan-mammalian clock

Heyn, Holger, et al. "Distinct DNA methylomes of newborns and centenarians." *Proceedings of the National Academy of Sciences* 109.26 (2012): 10522–10527.

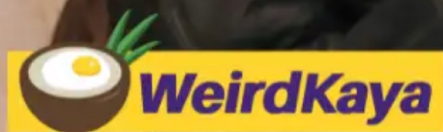
Horvath, S., Raj, K. DNA methylation-based biomarkers and the epigenetic clock theory of ageing. *Nat Rev Genet* **19**, 371–384 (2018)

Fransquet, P. D., et al. (2019). The epigenetic clock as a predictor of disease and mortality risk: a systematic review and meta-analysis. *Clinical epigenetics*, 11(1), 1–17.

Lu, A.T., Fei, Z., Haghani, A. *et al.* Universal DNA methylation age across mammalian tissues. *Nat Aging* **3**, 1144–1166 (2023). <https://doi.org/10.1038/s43587-023-00462-6>



Breaking The Barriers Of Ageing: M'Sia's First
Biological Age Testing Collaboration



DNA Methylation as Biomarkers of Lifestyle & Environment

- Mice and human studies → **Transgenerational** implications of **1-carbon metabolism cofactors** in maternal diet during offspring conception and pregnancy, lasting up to 60 years
Cooney C.A. et al. Journal of nutrition (2002); Dominguez-Salas, P., et al.. Nat Commun (2014); Heijmans, BT., et al. PNAS (2008); Tobi, E., et al. Nat Commun (2014; Tobi, EW., et al. Science advances (2018); Cheng M, et al. Innov Aging (2023)
- **Diet quality** is linked to hundreds of DNA methylation modifications in genes → adiposity, glucose control and chronic inflammation
Do, Whitney L., et al. "Epigenome-wide association study of diet quality in the Women's Health Initiative and TwinsUK cohort." International journal of epidemiology 50.2 (2021): 675-684.
- Individual **foods** items (cruciferous, leafy veg., garlic, ...) → direct DNA methylation modifications
Hellbach, F., et al (2022). Association between usual dietary intake of food groups and DNA methylation and effect modification by metabotype in the KORA FF4 cohort. *Life*, 12(7), 1064.
- **Mediterranean diet, supplements** of VitD, VitA, calcium → positive impact on biological age
Garcia-Garcia, I., et al. Frontiers in Aging (2024)
- **Alcohol, tobacco** smoke → hundreds of direct DNA methylation modifications
Liu et al. (2018) A DNA methylation biomarker of alcohol consumption. Molecular Psychiatry 23: 422-433
Joehanes, Roby, et al. "Epigenetic signatures of cigarette smoking." Circulation: cardiovascular genetics 9.5 (2016): 436-447.

A top-down view of a wooden surface covered with a variety of fresh and healthy ingredients. In the center, two large pieces of raw salmon are laid out. Surrounding them are several other items: a head of broccoli, a bunch of red cherry tomatoes on a vine, a whole avocado, a lemon half, a head of artichoke, a bowl of quinoa, a bowl of mixed grains, a bag of almonds, a head of lettuce, a red apple, and some walnuts. The overall composition is vibrant and emphasizes a diet rich in nutrients.

Interventions to improve epigenetic biomarkers

Reversibility of epigenetic age

8-weeks program
 + diet *guidance*
 + diet supplementation
 + exercise
 + sleep
 + stress management

N = 43

Fitzgerald KN et al. (2021). Potential reversal of epigenetic age using a diet and lifestyle intervention: a pilot randomized clinical trial. Aging (Albany NY), 13(7), 9419.

Table 2. Summary of dietary and lifestyle interventions*.

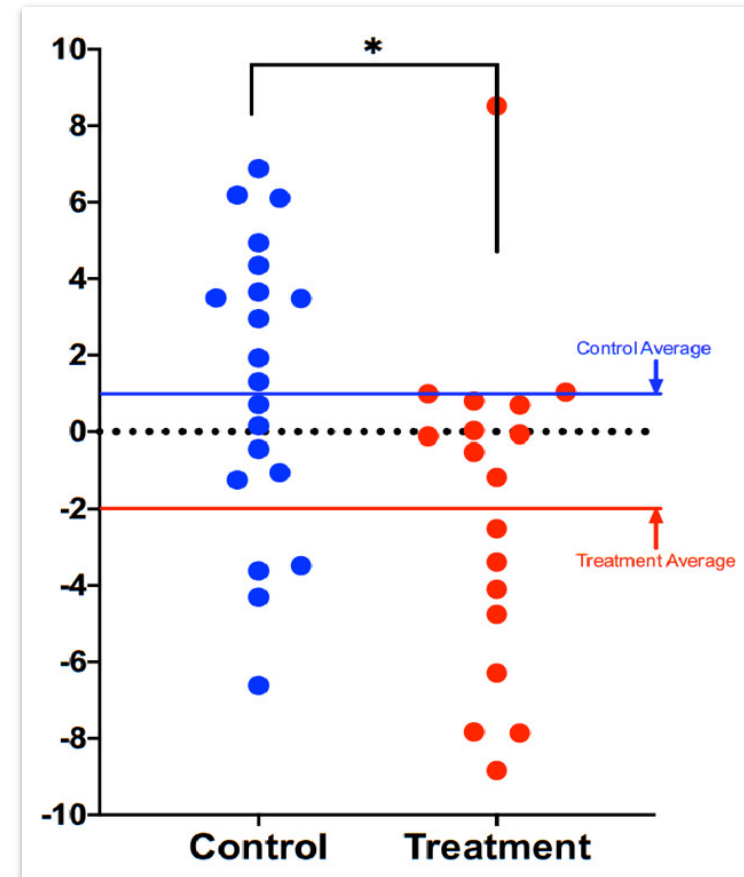
Intervention category	Details
Dietary Prescription	<p><i>Guidance per week:</i></p> <p>3 servings of liver</p> <ul style="list-style-type: none"> • (1 serving = 3 oz) • Preferably organic <p>5-10 eggs</p> <ul style="list-style-type: none"> • Ideally free-range, organic, omega-3 enriched <p><i>Guidance per day:</i></p> <p>2 cups of dark leafy greens</p> <ul style="list-style-type: none"> • Measured raw, chopped, and packed • Including kale, Swiss chard, collards, spinach, dandelion, mustard greens • Does not include salad greens such as romaine, iceberg, Spring mix <p>2 cups cruciferous vegetables</p> <ul style="list-style-type: none"> • Measured raw, chopped, and packed • Includes broccoli, cabbage, cauliflower, Brussels sprouts, bok choy, arugula, kale, mustard greens, watercress, rutabaga, kohlrabi, radish, Swiss chard, turnip <p>3 additional cups colorful vegetables of your choosing (excluding white potatoes, sweetcorn)</p> <p>1-2 medium beet</p> <p>4 tbsp (1/4 cup) pumpkin seeds (or pumpkin seed butter)</p> <p>4 tbsp (1/4 cup) sunflower seeds (or sunflower seed butter)</p> <p>1+ serving methylation adaptogens, choose from:</p> <ul style="list-style-type: none"> • 1/2 cup berries (wild preferred) • 1/2 tsp rosemary • 1/2 tsp turmeric • 2 medium cloves garlic • 2 cups green tea (brewed 10 minutes) • 3 cups oolong tea (brewed 10 minutes) <p>6 oz animal protein</p> <ul style="list-style-type: none"> • Grass-fed, pastured, organic and hormone/antibiotic-free <p>2 servings of low glycemic fruit</p> <p><i>General guidance:</i></p> <p>Organic preferred over conventional</p> <p>Stay hydrated</p> <p>Don't eat between 7pm and 7am</p> <p>Include “healthy” oils</p> <ul style="list-style-type: none"> • Balance types of fat • E.g. coconut, olive, flaxseed and pumpkin seed oil <p>Avoid added sugar/candy, dairy, grains, legumes/beans</p> <p>Minimize plastic food containers</p>
Supplement Prescription	<p>PhytoGanix®, a combination of organic vegetables, fruits, seeds, herbs, plant enzymes, prebiotics and probiotics at a dose of 2 servings daily, divided</p> <p>UltraFlora® Intensive Care, containing <i>Lactobacillus plantarum 299v</i> at a dose of 2 capsules daily, divided</p>
Exercise Prescription	<p>Minimum of 30 minutes of exercise per day for at least 5 days per week, at an intensity of 60-80% of maximum perceived exertion</p>
Sleep Prescription	<p>Average a minimum of 7 hours of sleep per night</p>
Stress Management Prescription ^A	<p>Breathing exercise <i>Steps to Elicit the Relaxation Response</i> developed by Herbert Benson MD, twice daily</p>

Reversibility of epigenetic age

8-weeks diet
+ diet *guidance*
+ diet supplementation
+ sleep
+ exercise
+ stress management

-> **-3.3 years DNAmAge**

N = 43

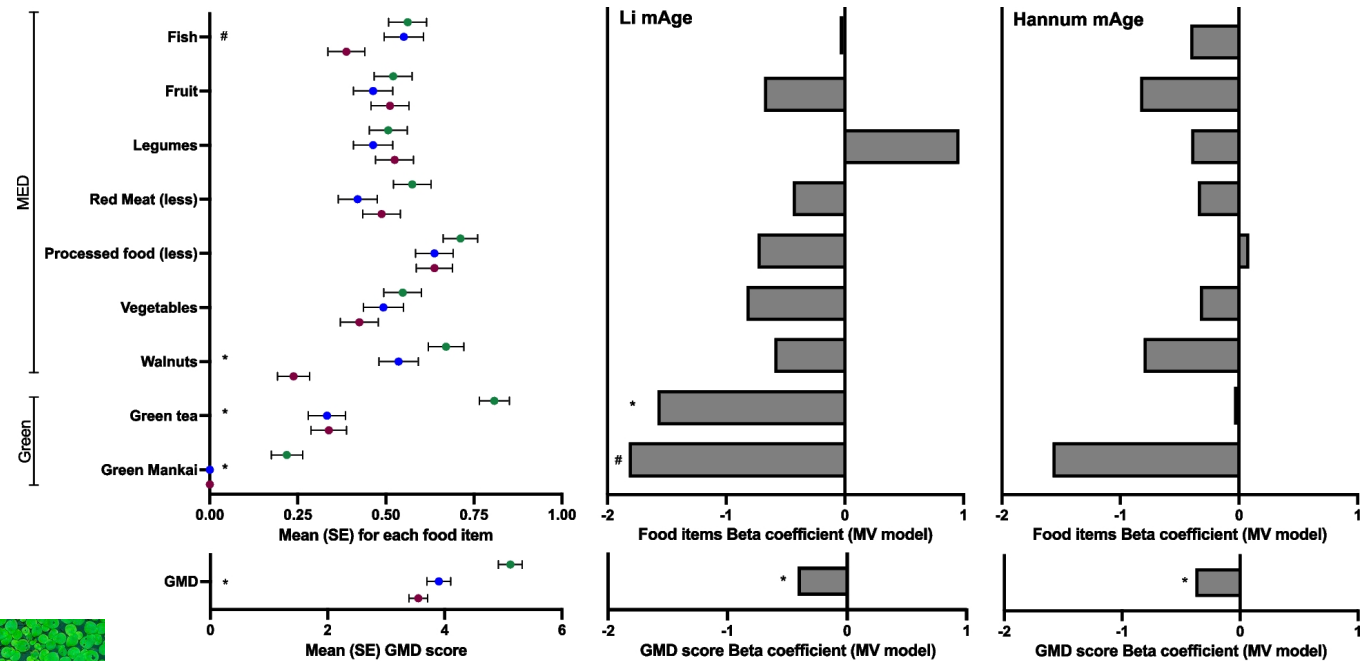
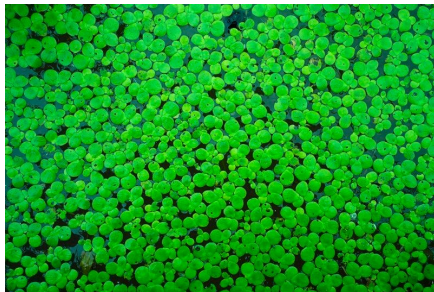


Comparison of DNAmAge change between treatment and control groups.

Fitzgerald KN et al. (2021). Potential reversal of epigenetic age using a diet and lifestyle intervention: a pilot randomized clinical trial. *Aging (Albany NY)*, 13(7), 9419.

Reversibility of epigenetic age

- DIRECT PLUS: RCT polyphenol-rich diet
- 18 months follow-up
- 230 participants
- Green tea & green mankai → max effect on epigenetic age



Yaskolka Meir, A., Keller, M., Hoffmann, A. *et al.* The effect of polyphenols on DNA methylation-assessed biological age attenuation: the DIRECT PLUS randomized controlled trial. *BMC Med* 21, 364 (2023).

Epigenetic age is decreased in vegan diet

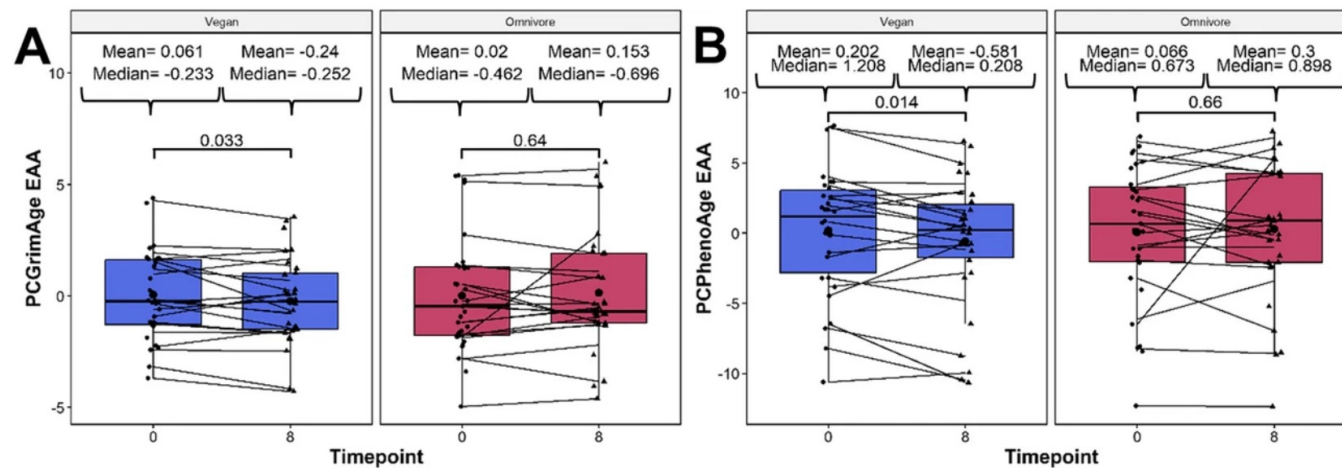


N = 42

Twins randomly assigned

8-week vegan or healthy omnivorous diet

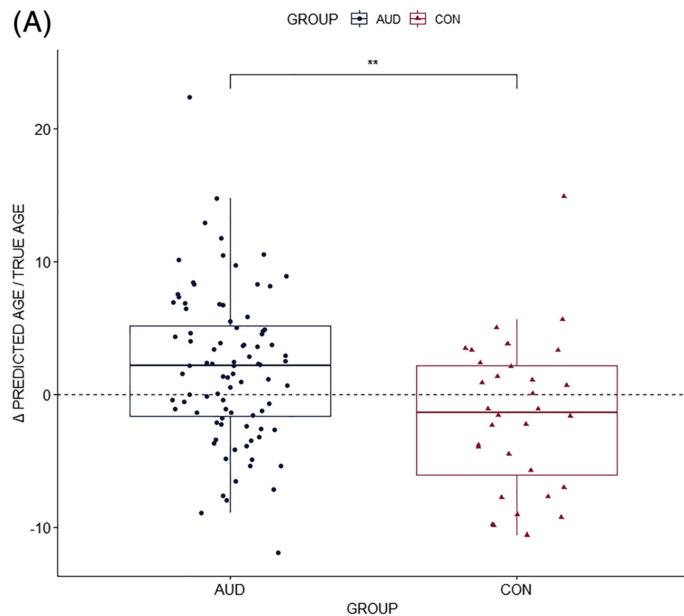
Reduction in epigenetic age* in the vegan group



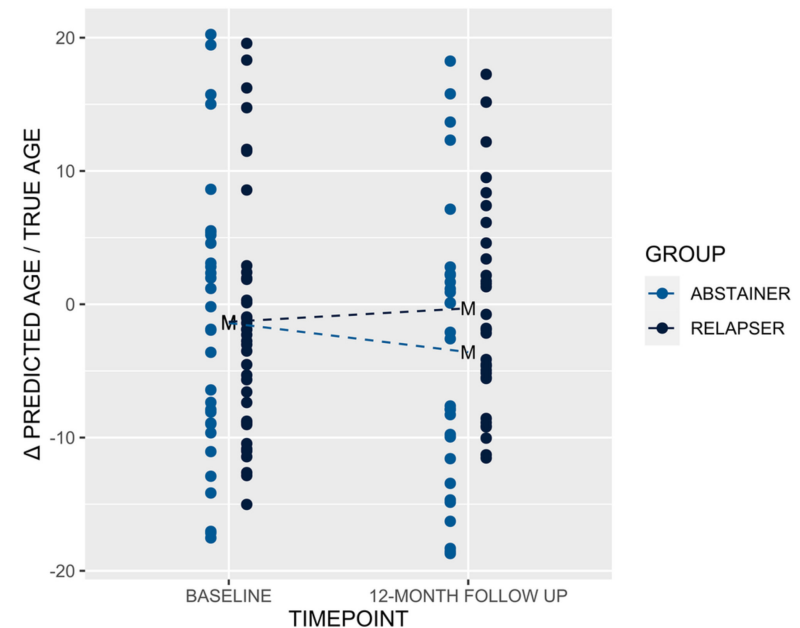
*The *amount* of change in time equivalent was not reported

Dwaraka, V.B., *et al.* Unveiling the epigenetic impact of vegan vs. omnivorous diets on aging: insights from the Twins Nutrition Study (TwiNS). *BMC Med* (2024).

Reversibility of epigenetic age: alcohol use



- Ageing was accelerated by **3.64 years** in epigenetic age of patients with alcohol use disorder compared with the control group



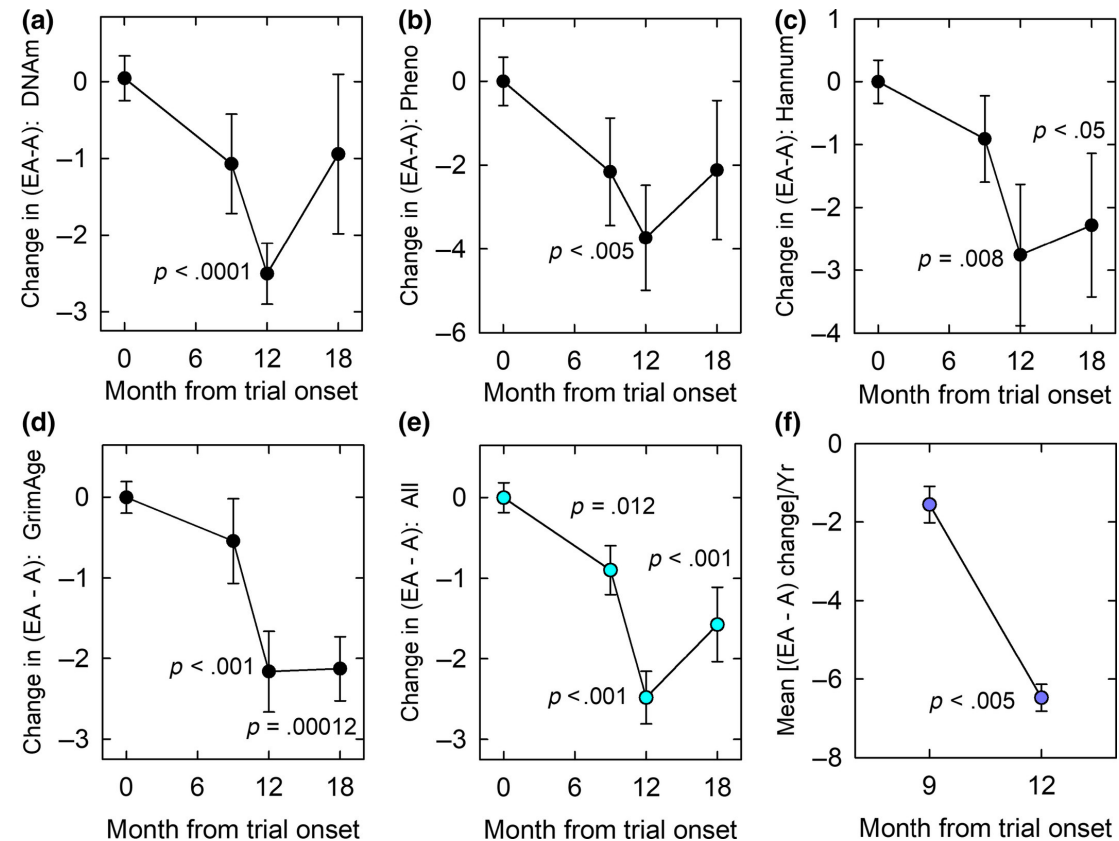
- Abstaining patients displayed a decrease in epigenetic clock by **3.1 years** after 12 months
- Increase by **2.7 years** in those who relapsed

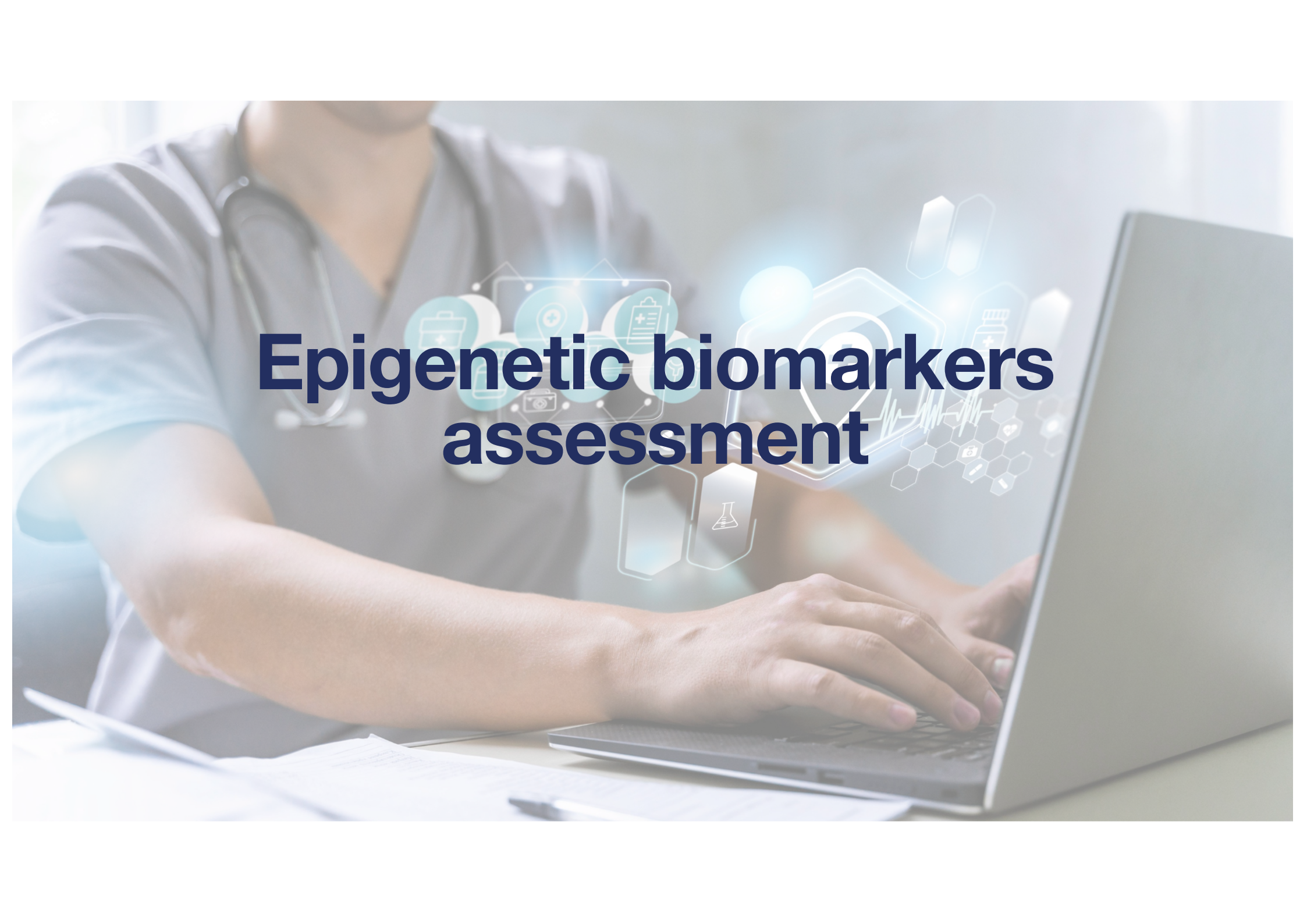
Reversibility of epigenetic age with metformin

1-year recombinant human growth hormone
+ **metformin** (antidiabetic drug)
+ DHEA

-> -2.5 years DNAmAge

N = 10



A doctor in grey scrubs is seated at a desk, typing on a laptop. The image is overlaid with various futuristic medical and technological icons, including a stethoscope, a location pin, a clipboard, a pill bottle, a heart rate line, and a hexagonal molecular structure. The text "Epigenetic biomarkers assessment" is centered in a bold, dark blue font.

Epigenetic biomarkers assessment

Epigenetic biomarkers



Biological Age



Equivalent of
years of age



Diet



Equivalent of fruits &
vegetable consumption



Physical Activity



Equivalent of active
minutes per week



Alcohol



Equivalent of standardized
units drunk per week



Tobacco & Pollution



Equivalent of cigarettes
smoked per day



Global Stress Overload



Global impact of
chronic stress



Cardiovascular Stress



Impact of stress on the
cardiovascular system



Metabolic Stress



Impact of stress on the
metabolic system



Immune Stress



Impact of stress on the
immune system



Neuroendocrine Stress



Impact of stress on the
neuroendocrine system

What is measured?

Biological Age and Lifestyle Impacts

31 ageing and lifestyle-associated markers* of DNA expression

- Biological Age (*The Swiss Clock*)

11 markers linked to the following genes : GPR62, EIF1, KLF14, PTPRN, ELOVL2, IPO8, CALHM2, KCNQ4, ABHD14, GRM2, VGF, and PCBP4.

- Physical activity

5 markers linked to the genes MAPKK, FOXK1, RGS6, and TLCD5.

- Diet

4 markers linked to the genes DYNC1H1 and MAPKK.

- Alcohol

6 markers linked to the genes PTP4A3, SLC7A11, CHI3L2, ANKS3, BBS10, JDP2, and DENND2D.

- Tobacco smoke

5 markers linked to the genes AHHR, ZNF385D, NDE1, CACNA1D.

**Patent-pending*

Chronic Stress and Systems Impacts

32 chronic stress-associated markers* of DNA expression

- Genes

ABCG1 CPT1A, APBA1, ATOX1, C14orf43, CYP7B1, DIAPH3, DPYSL2, ELMSAN1, ETF1, FAU, GALNT14, HEMK1, HSD17B12, ITPR1, JDP2, LOC100132354, MRPL49, ODF1, PHGDH, PTPRN2, RPS6KA2, SLC7A11, SREBF1, SYT13, TXNIP, WBP1L

Specific system's genes:

- Metabolic system

16 markers linked to the genes ABCG1, CPT1A, ITPR1, JDP2, LOC100132354, PHGDH, SARS, SLC1A5, SLC7A11, SREBF1, TANK, TXNIP

- Immune system

12 markers linked to the genes AIM2, DKFZp761E198, DPYSL2, FNBP1, LOC101929095, MIR21, MIR665, NTAN1, PARD3, PDE1C, RFX3, SBNO2, SYT13, THBS1, TMEM49, TPK1

- Cardiovascular system

14 markers linked to the genes ATAD2B, CELSR1, F2RL3, HPCAL4, KCNJ3, MASP1, OR56A3, PEX14, RPTOR, SPRED2, TENC1, TFAP2B, TPP1

- Neuroendocrine system

9 markers linked to the genes APBA1, DCBLD2, DEFB129, DIAPH3, ETF1, FAU, GALNT14, LRRFIP2, MRPL49, ODF1, RARB, ZSCAN10

Epigenetic clock and signatures development

25+ years of combined experience in academic and industry research to develop epigenetic tests measuring epigenetic accelerated ageing and the epigenetic impacts of lifestyle and stress

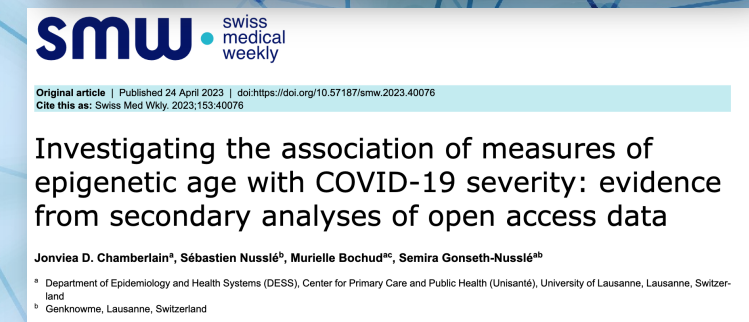
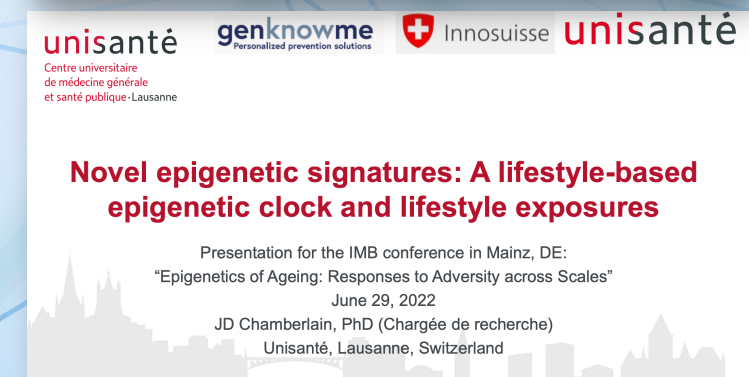
Filed patents :

Method Determining The Difference Between The Biological Age And The Chronological Age Of A Subject, filing number: EP 22 162 216.0 (15.03.2022)
Method determining a value of allostatic load: EP 23 179 588.1 (15.06.2023)

Scientific publications and contributions:

- A novel, lifestyle-based epigenetic clock, Chamberlain, (...), & Nusslé. Presented on June, 29, 2022, IMB Conference Mainz
- Chamberlain JD, Nusslé S, ... & Nusslé SG (2022). Blood DNA methylation signatures of lifestyle exposures: Tobacco and alcohol consumption, Clinical Epigenetics
- Chamberlain JD, Nusslé S, ... & Nusslé SG (2023). Investigating the association of measures of epigenetic age with COVID-19 severity, Swiss Medical Weekly
- Chamberlain JD, Nusslé S, ... & Nusslé SG (submitted). MethAL: An epigenetic signature of allostatic load

Two *Innosuisse* research and developments grants with the University center for primary care and public health, Lausanne, and the Lausanne University Hospital.



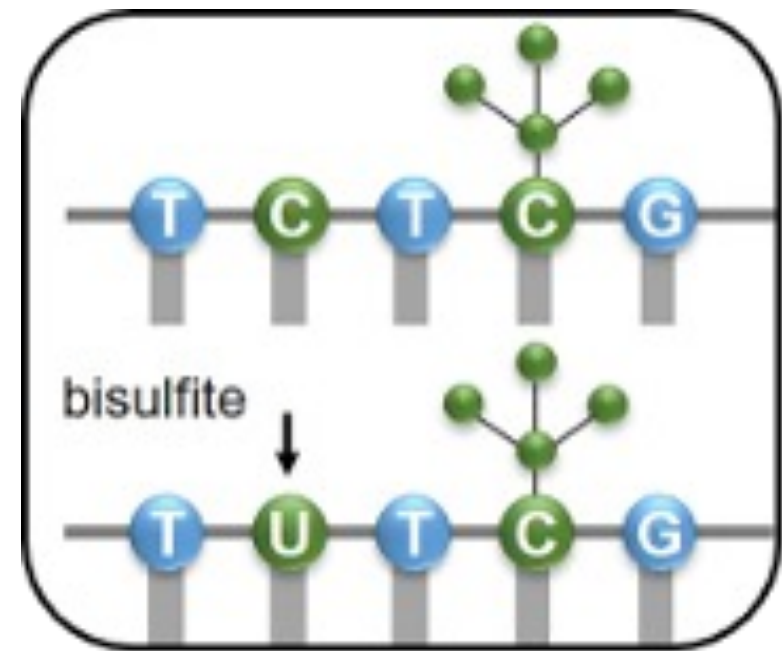
Bisulfite conversion

DNA methylation cannot be directly investigated using conventional DNA amplification

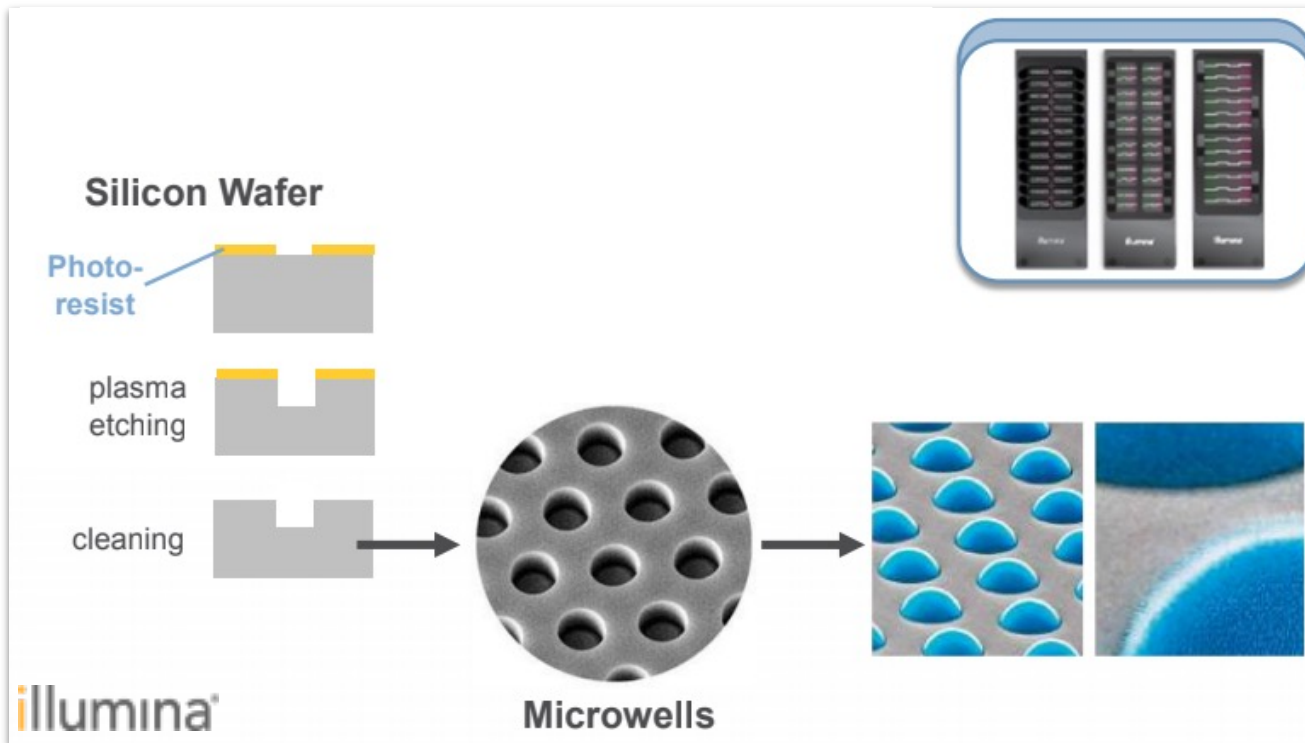
—> Bisulfite conversion

Principle:

bisulfite conversion is based on the chemical conversion of un-methylated cytosine to uracil, while methylated cytosine remains unchanged



Illumina bead chip technology



In each hole, a thousand beads coated with probes targeting the selected sequence



DNA PREPARATION

DAY 1

DNA
extracti
on

Start
Bisulfite
conversion

Incubate
O/N (16h30)

converted
DNA
purification

DAY 2



1 Make amplified DNA

2 Incubate amplification

Incubation O/N 18h30

INFINIUM PROTOCOL



GENOMIC DNA

DAY 3



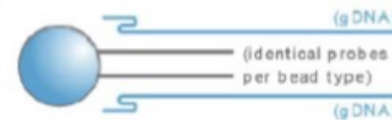
3 Fragment amplified DNA



4 Precipitate & resuspend

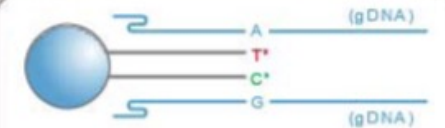


5 Load BeadChip



6 Hybridize samples on
BeadChip Incubation O/N 18h

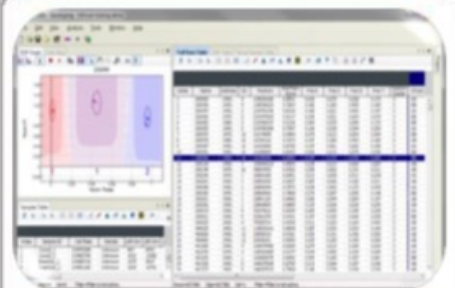
DAY 4-5



7 Extend/Stain samples on
BeadChip



8 Image BeadChip



9 GenomeStudio

Our products

1. Biological age estimation + lifestyle factors affecting health and longevity
2. Chronic stress impact affecting health

Used to define a care pathway for longevity and predictive medicine

- Identification of preventive care needs
- Personalized care recommendations
- Validation of preventive care pathway



Thank you for your attention!

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sebastien@genknowme.com, 079 460 20 69

