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Epigenetic Clock Testing

An accurate, low cost biomarker of aging

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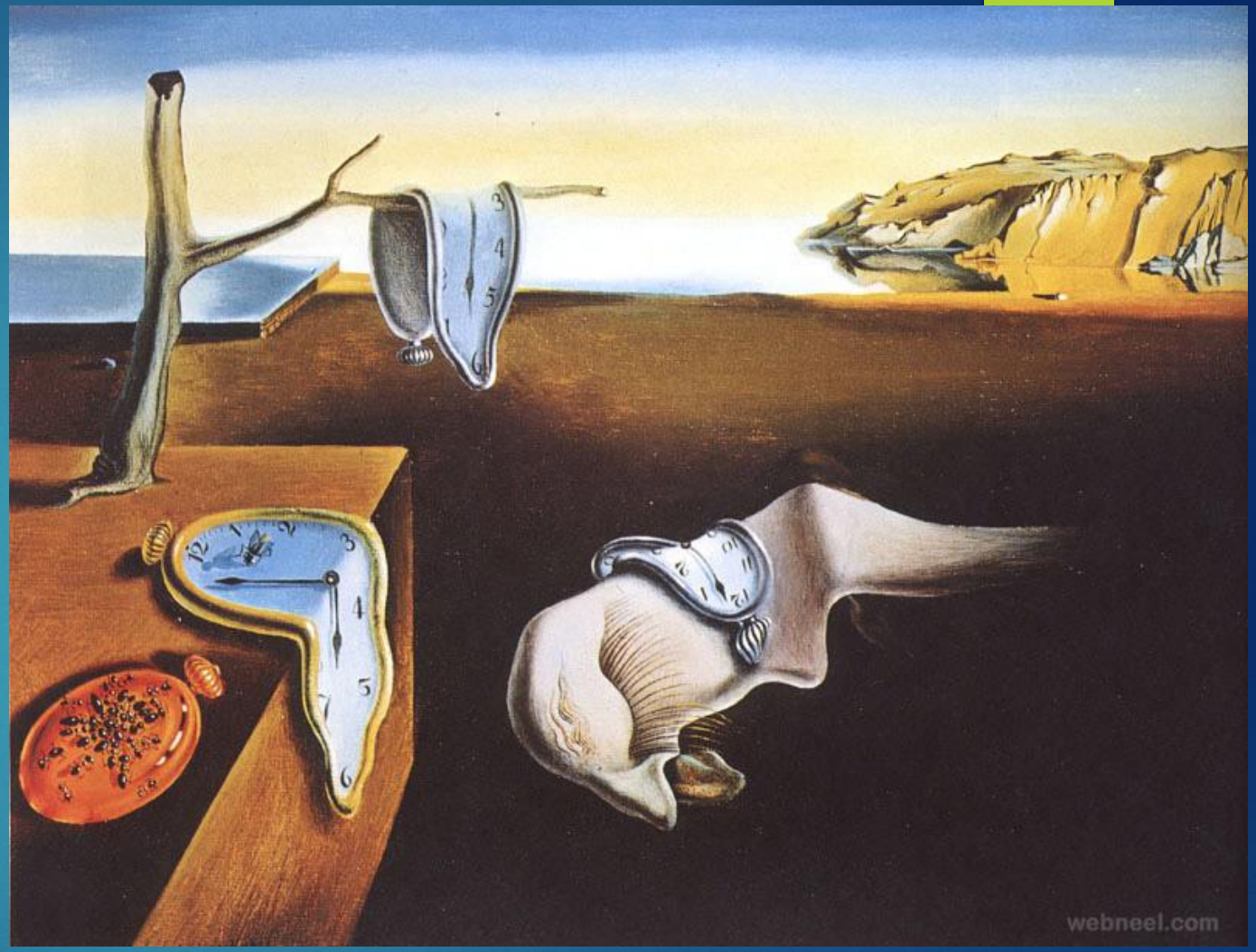
Aging "Clocks"

Q: Why do we need a new "Clock" for measuring aging **?**

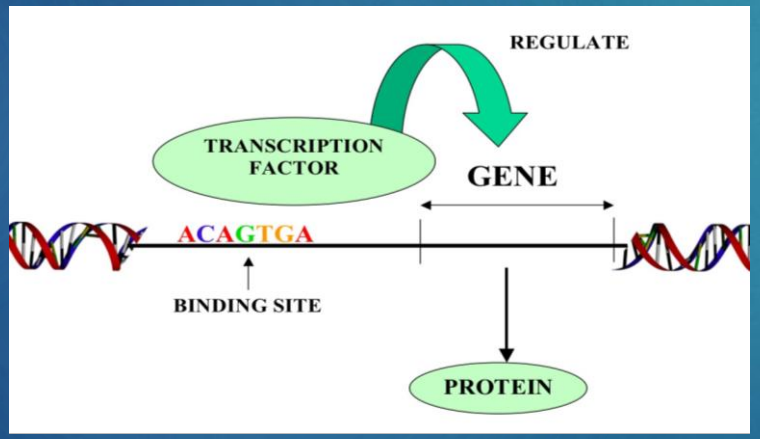
A: The old Clocks * are not very accurate "age time keepers"



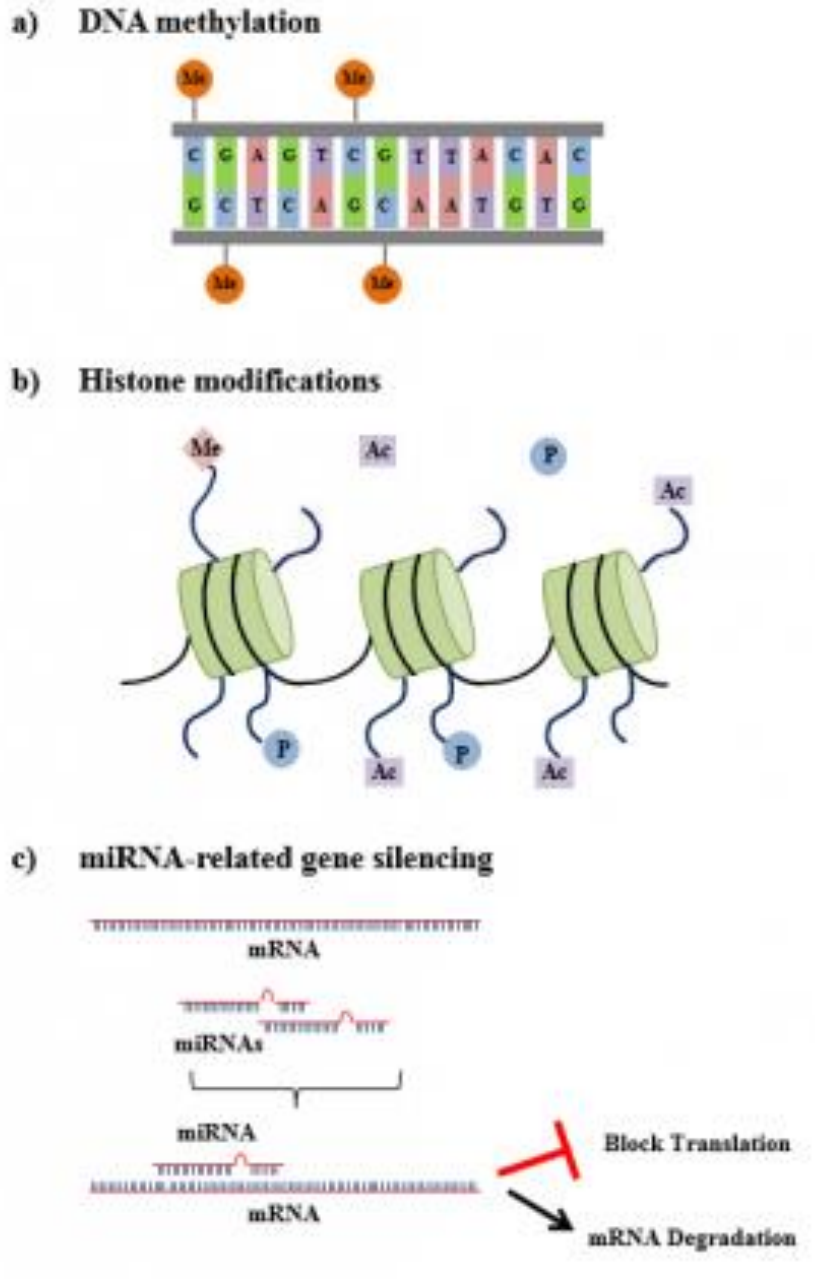
Examples of other "clocks": DNA damage markers, Leukocyte telomere length testing (both average LTLT and % short LTLT tests), biomarkers of Cellular senescence (Ex: H2AX staining, β-galactosidase staining, p16INK4A staining)

What is Epigenetics ?

Simple Definition: Molecular ways that regulate gene expression besides the classical Watson-Crick transcription factor method of gene regulation



Classic method of gene regulation – transcription factors



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This is "Epigenetics"

Q: What is DNA Methylation ?

A: It is part of "Epigenetics"

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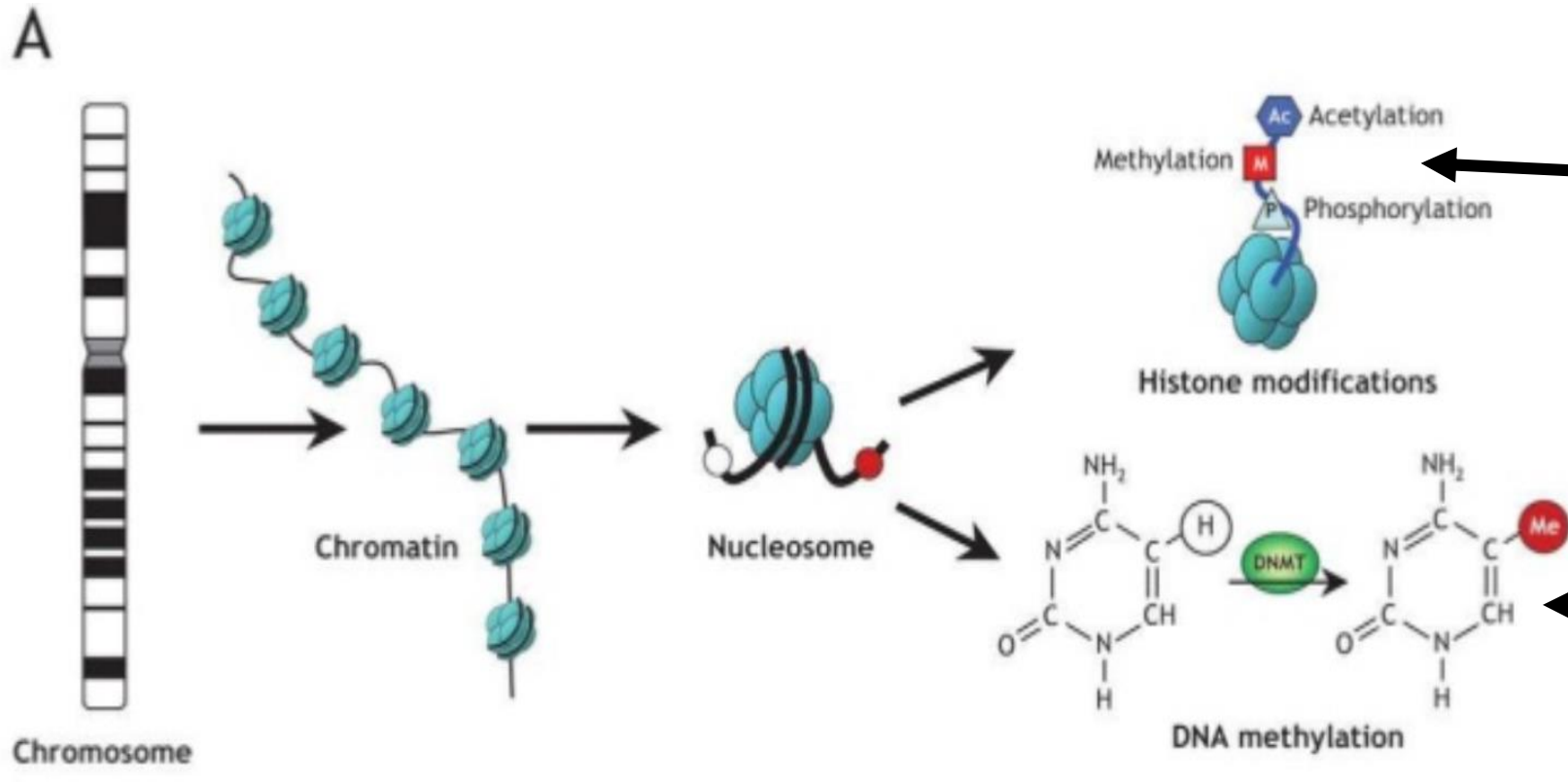

Histone protein Modification (aka Histone acetylation, Methylation, phos-Phorylation, etc.

DNA methylation of cytosine DNA bases

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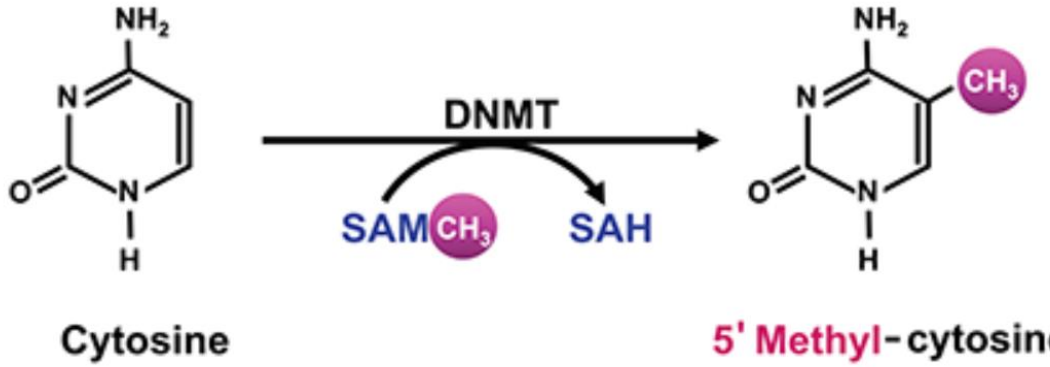
Q: How many DNA methylation (CpG) sites are there in the Human Genome ?

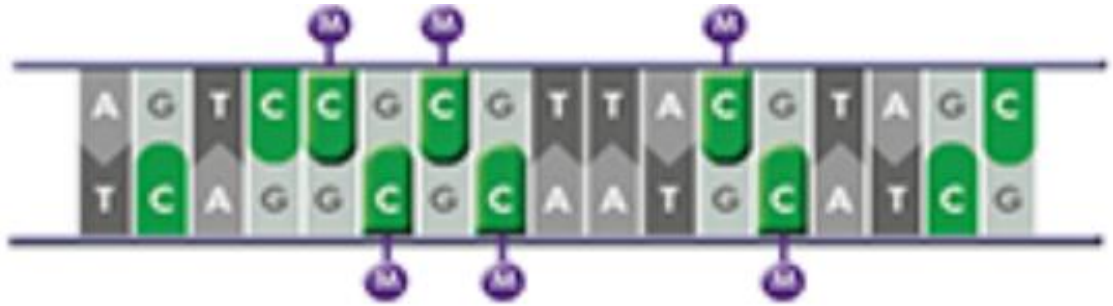
Answer: About 28 million CpG sites in the human genome. Only a fraction of these undergo age-related increases in DNA methylation or demethylation

Age-related Differential DNA methylation (definition): The DNA methylation sites that loose or gain a methyl group with aging. These sites can be found with computer programs that "data mine" existing databases of human methylomes

Which DNA Bases get Methylated?

- Only Cytosine (C) DNA bases are methylated
- 3 main methylating enzymes in humans – DNMT1, DNMT3A, and DNMT3B (2 minor ones)
- Methyl groups come from folate cycle (SAM), which activates DNMT enzymes
- SAH is the byproduct of DNA methylation, which inhibits DNMT enzymes





Clinical Correlation – Folate and B12 deficiencies prevent normal DNA methylation from occurring, but excess folate and B12 does not "stop" aging (DNA methylation is a tightly controlled, "site-specific" process)

What is a "**CpG**" ?

- A "**CpG**" is a cytosine, next to a guanine on the 5' DNA strand. CpG also called a "Cytosine-phosphate-Guanine dinucleotide"
- Only 1-1.5% of the human genome is made up of CpGs
- Certain areas of the human genome have more than 1.5% CpG content, such as promoters in CpG islands (18%) and 5'UTRs, and repetitive DNA (Ex: *Alu repeats* – 3%)

Q: What is the difference between DNA Methylation and DNA Demethylation?

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DNA Methylation *

DNA Demethylation *

Both DNA Methylation and Demethylation occur with aging at specific locations in all genomes

* DNA methylation/demethylation only occurs on Cytosine DNA bases next to an adjacent Guanine DNA base

How DNA Methylation and DNA Demethylation alters gene expression

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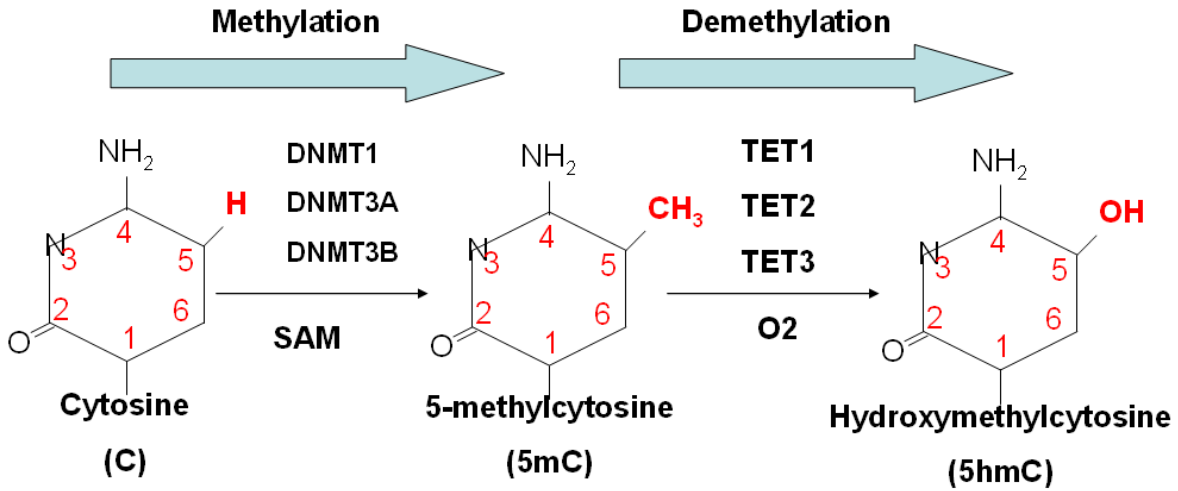
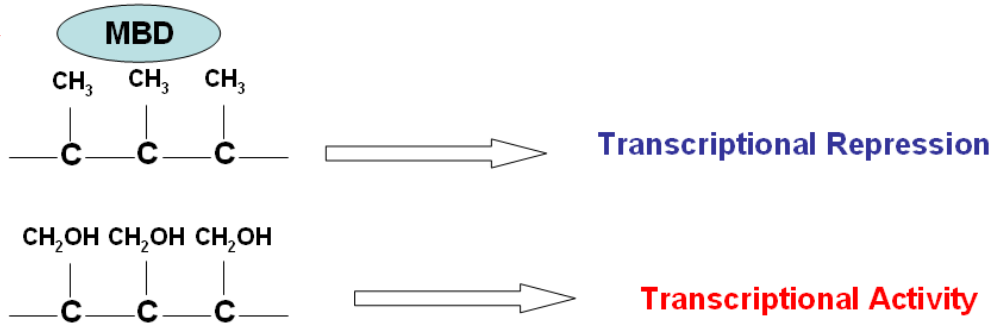


MBD: Methyl Binding Proteins



How DNA Methylation Silences Genes

DNA Methylation "silences" genes by creating a binding site for methyl binding proteins to attach to the methylated cytosines at promoters. This prevents transcription factors from "turning on" gene expression

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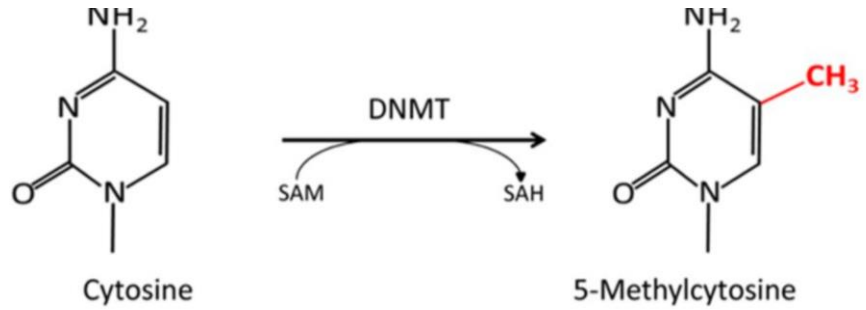

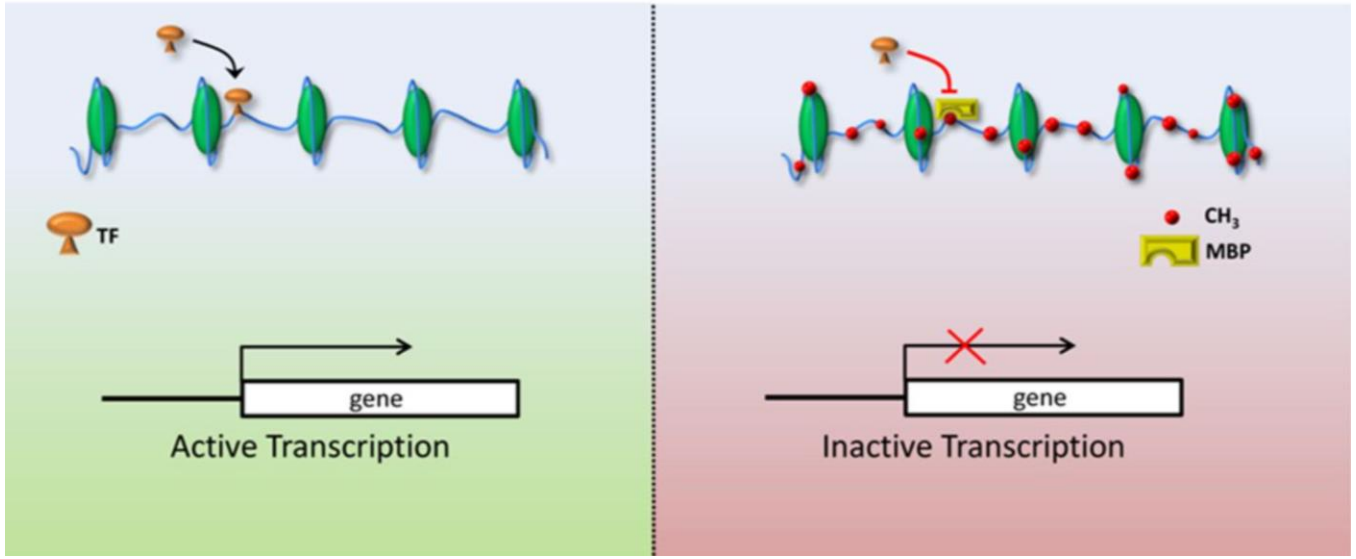

DNMT - DNA methyltransferase
SAM – S-adenosylmethionine
SAH – S-adenosylhomocysteine
TF – Transcription factor
MBP – Methyl binding protein

How DNA Methylation Silences Genes

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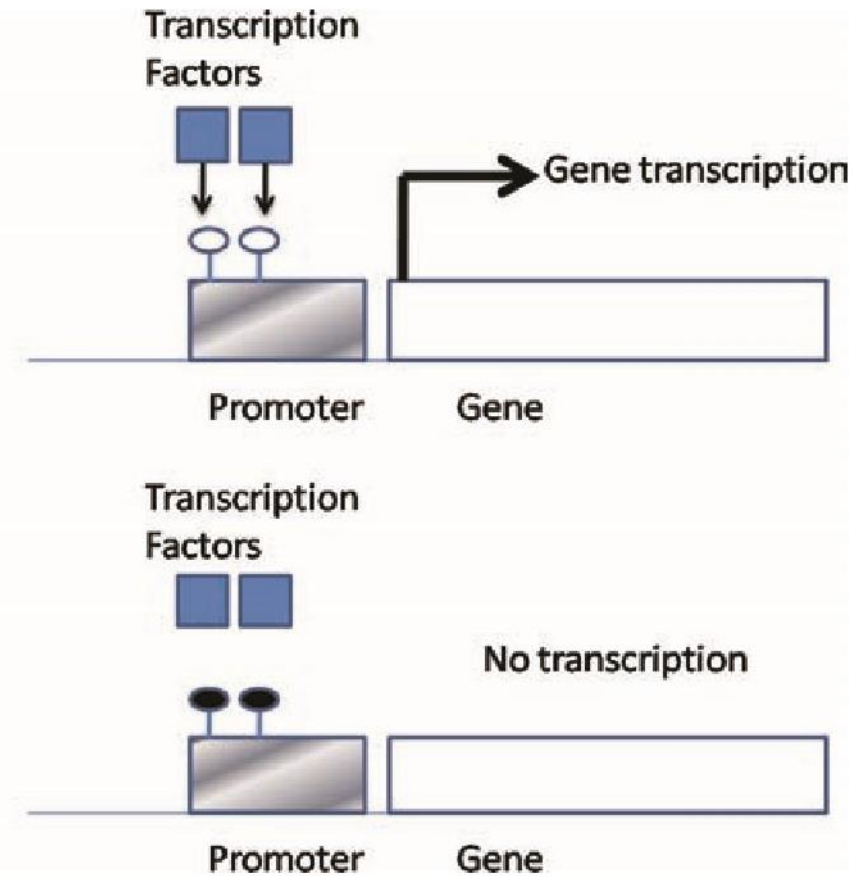
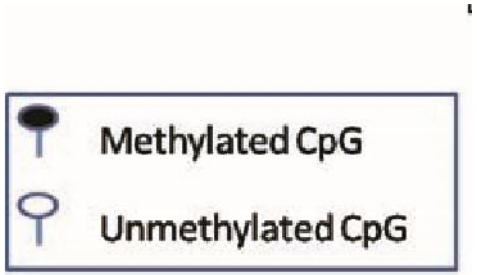
Transcription factor – a protein that binds to DNA to "turn on" gene expression

Promoter – the beginning of a gene DNA sequence where the transcription factor binds

DNA Methylation of CpG sites at promoter prevents the gene from being transcribed





Q: What DNA is normally hypermethylated?
A: Repetitive DNA (aka junk DNA)

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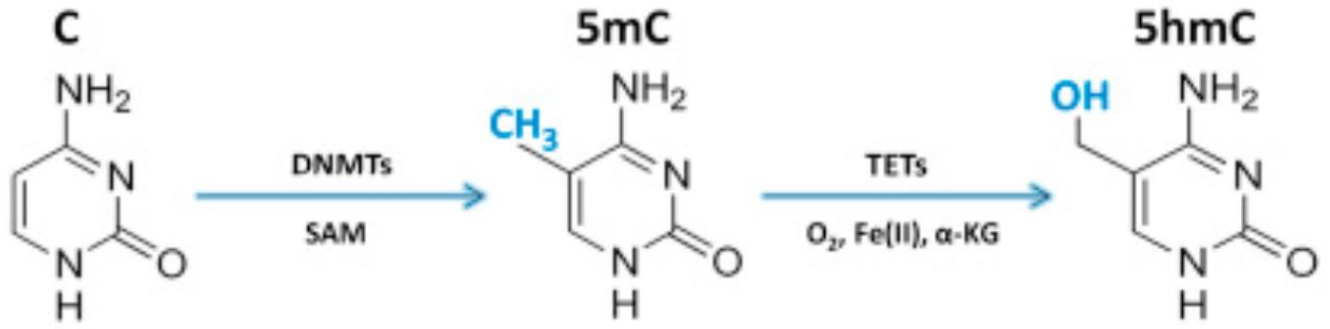

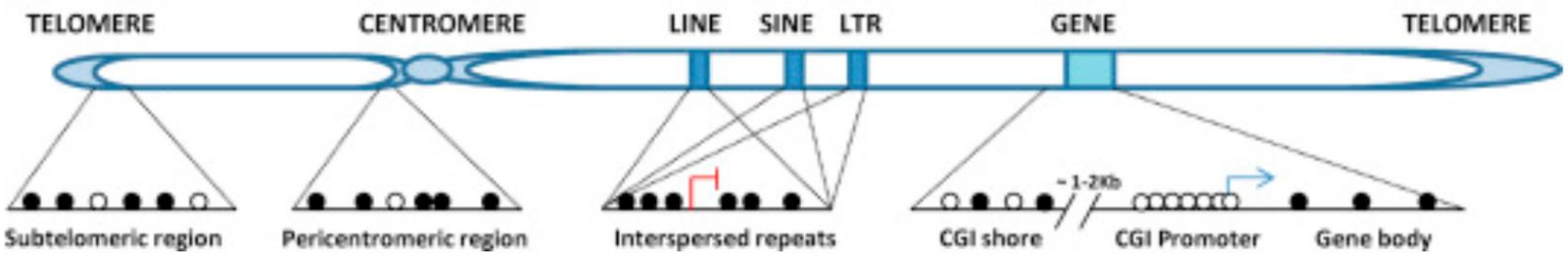

Types of Repetitive DNA
(aka Interspersed repeats, Transposable elements, TEs retrotransposons, etc.)

1. **LINE** – long interspersed nuclear elements
2. **SINE** – short intersperse nuclear elements
3. **LTR** – Long terminal repeats

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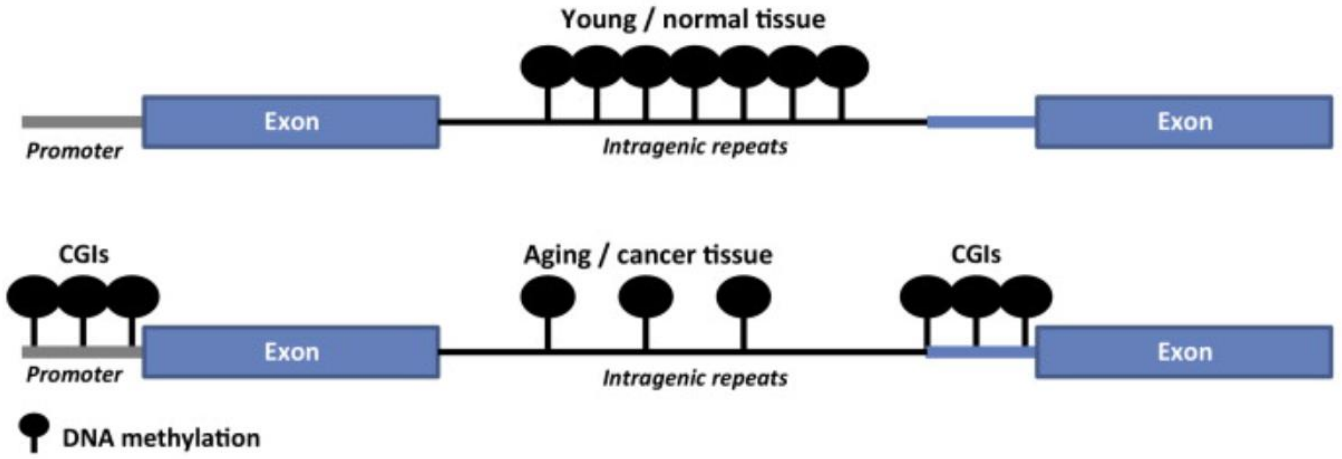
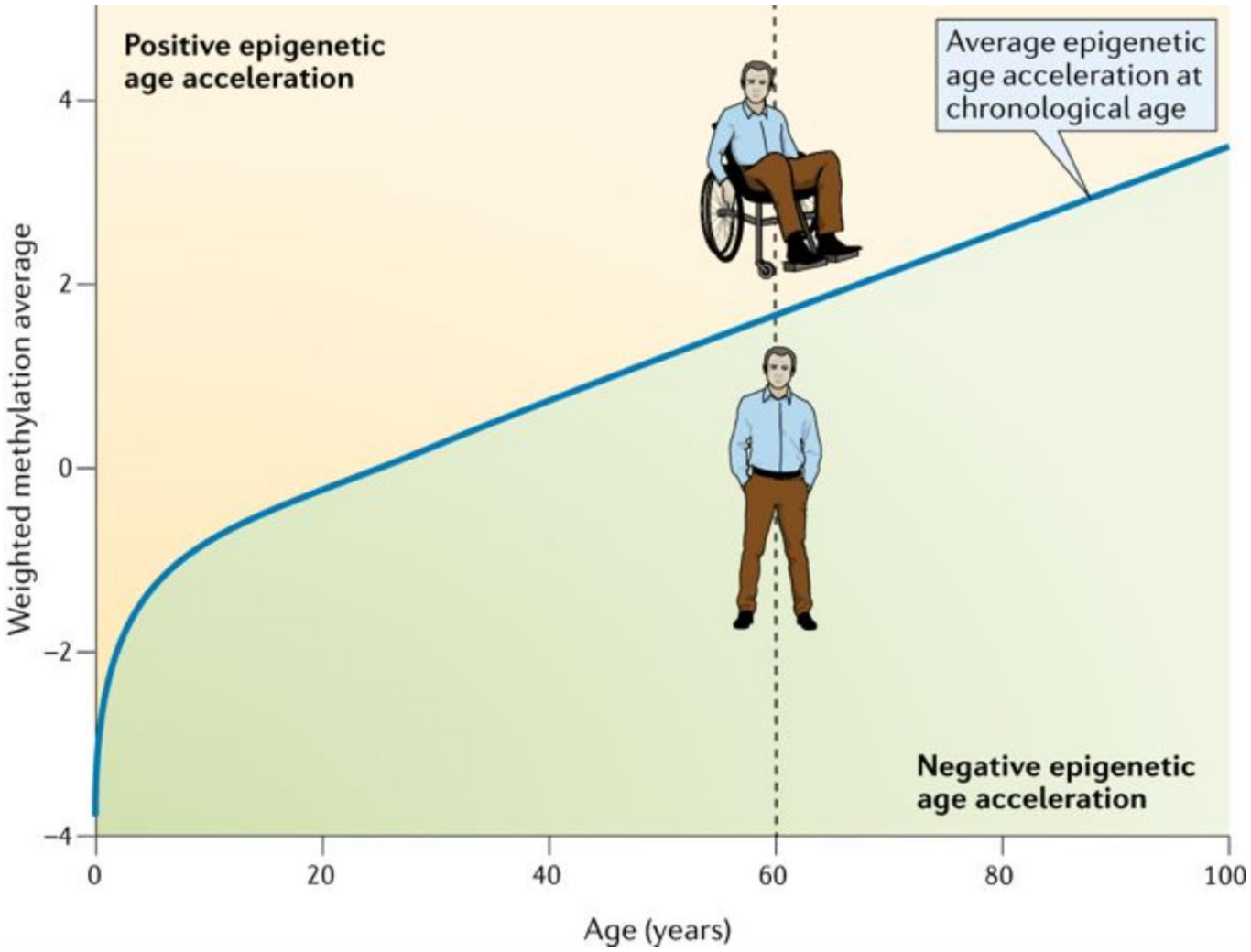


- DNA Methylation **increases** with aging a certain sites (millions of CpG sites)
- DNA Methylation **decreases** with aging at other sites (millions of CpG sites)

Clinical Significance of DNA Methylation in Age Management (aka DNAm Clock Testing)

Using computer algorithms and database mining of DNA methylomes, scientists have Identified found specific CpGs that either increase or decrease as a function of aging. Making a so called "clock" of these site-specific CpGs can predict age very accurately



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Epigenetic Clock Testing

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Reference: S Horvath, K Raj, *Nature Reviews Genetics*, Vol 19, April, 2018, pp 371- 384

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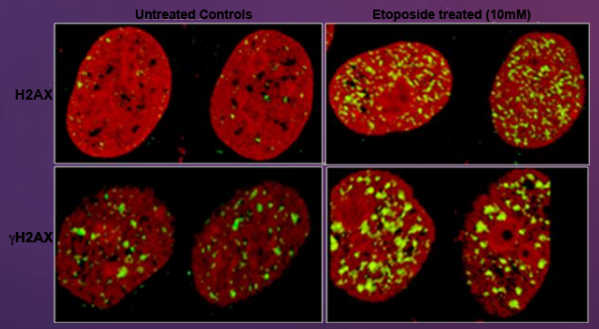
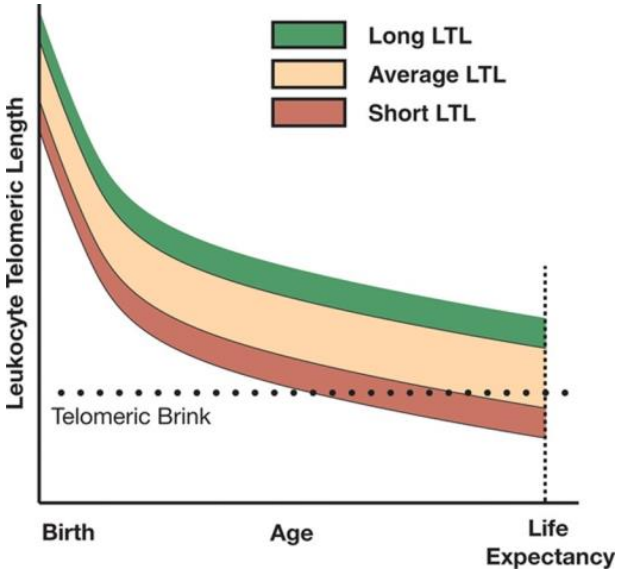
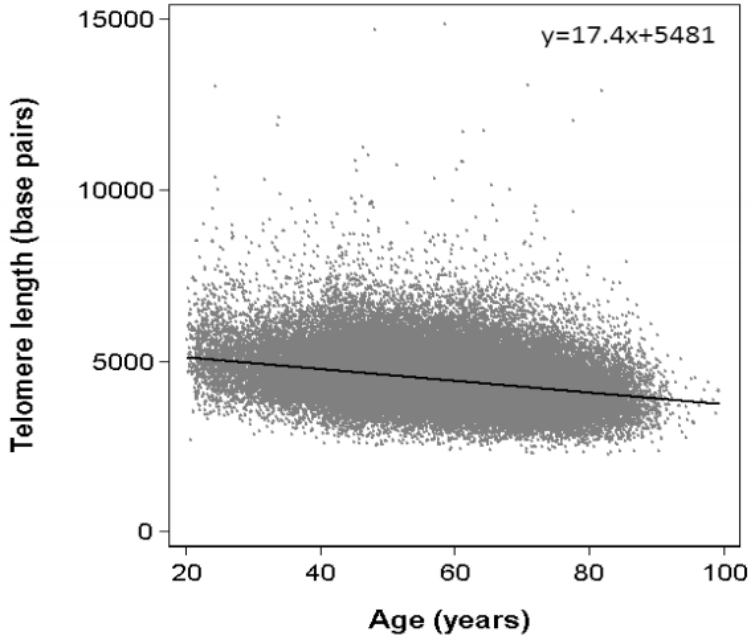
Older Less Accurate "*Aging Clocks*"

- **Blood Leukocyte Telomere Length** (LTL)
  - Measures Ave. LTL or % short LTLs
  - Correlation w/age is poor:
    r = -51 in females
    r = - 55 in males

- **DNA Damage Bioarkers**
  - H2AX antibody staining
  - DNA metabolites – 8-OH-dG
  - Free radical damage biomarkers
    - HNE – lipid peroxidation product
    - MDA – malandialdehyde
    - Isoprostanes
    - Reactive aldehydes
  - None correlate with chronological age







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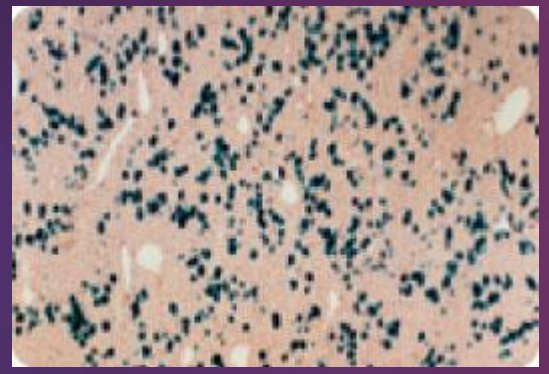
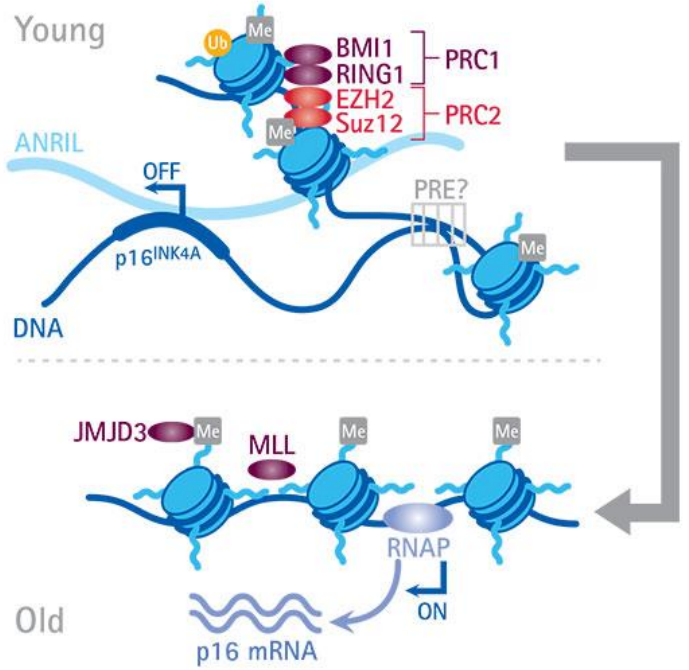
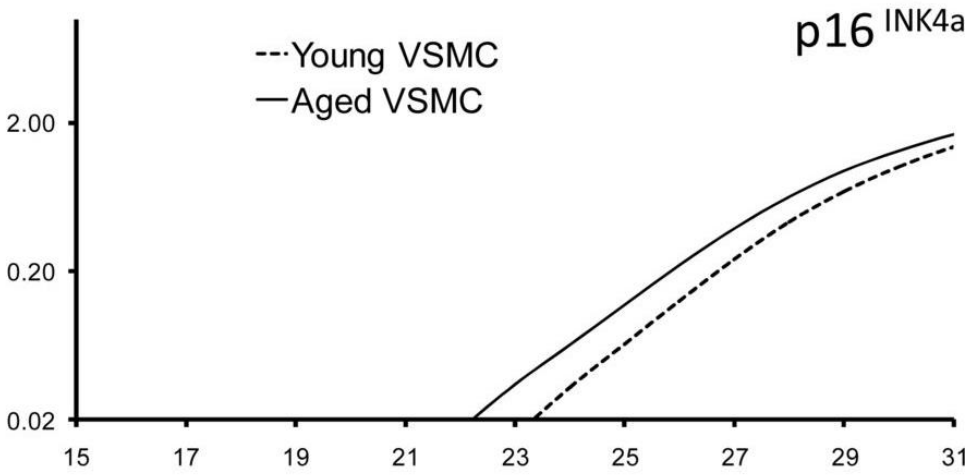
Older Less Accurate "*Aging Clocks*"

- **Cellular Scenescence Biomarkers**
  - β-Galactosidase or P16INK4 antibody staining
  - Correlation w/chronological age is poor
    r = 0.56 only in T cells
    No correlation w/age in other WBCS



- **Microsatellite mutations**
  - Correlates with # of cell divisions
  - Does NOT correlate with chrnological age





Key Point of Lecture: DNA Methylation Testing is 3X more accurate than WBC Telomere Length Testing (average and % short)

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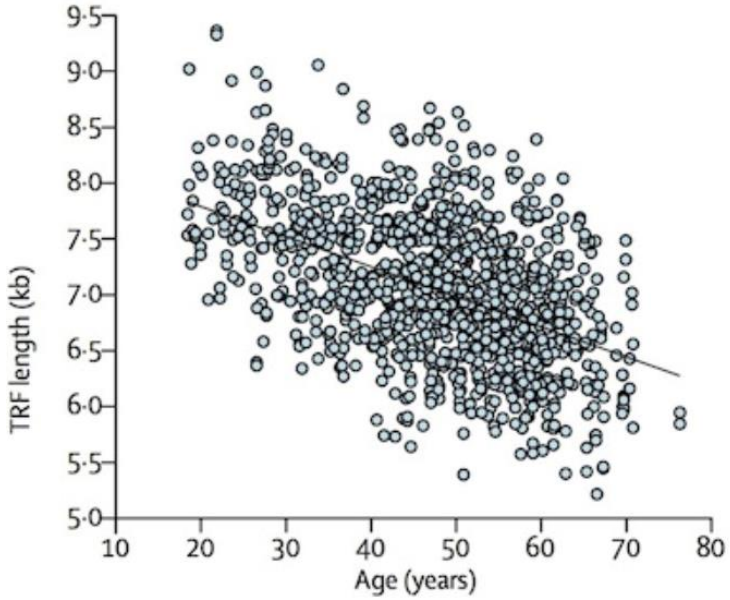
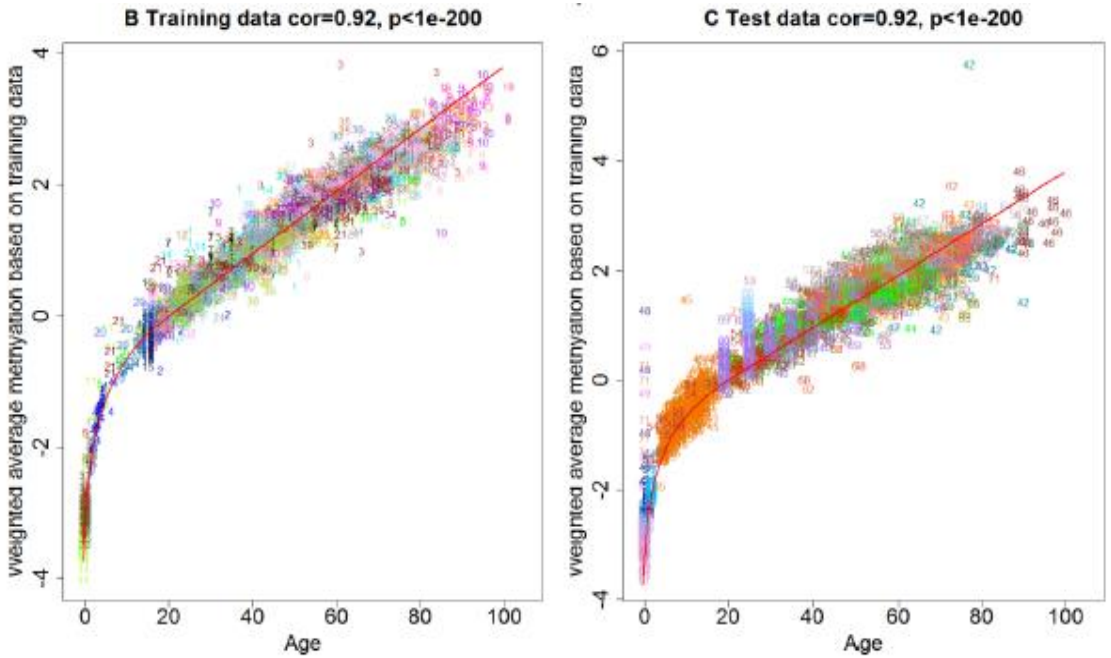
Telomere Length vs Age



DNA Methylation Clock vs Age



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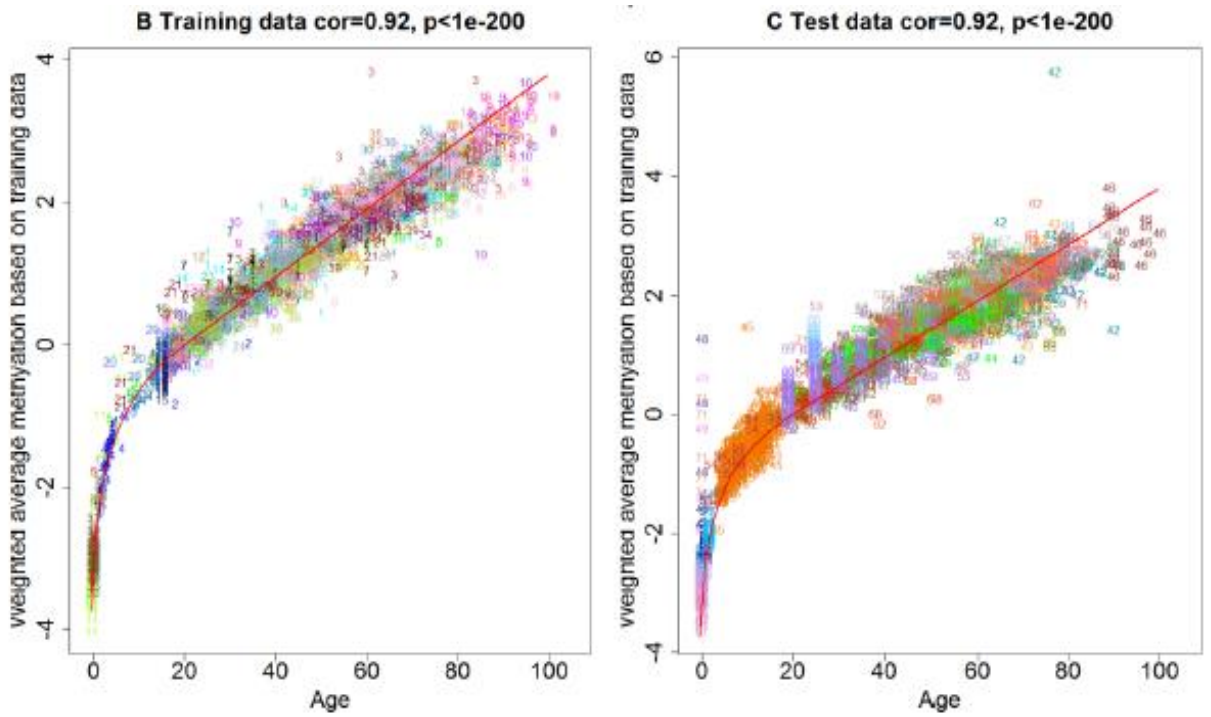
1st DNA Methylation Clock:

The Horvath *DNAm* Clock

- 2012: Horvath - described 1st "DNA methylation clock" in 2012 from 14,000 human methylome datasets
- CpG sites selection - via computer algorithm based on "elastic net regularization"
- 353 CpG sites – "selected" to make a "DNAm Clock"
  - 193 CpGs were hypermethylated with aging
  - 160 were hypomethylated with aging
- Conclusion: Differential DNA methylation at these 353 CpG specific sites predicted chronological age with extremely high correlation:
  - Training data: R = 0.97
  - Test data: R = 0.96
  - 3rd party testing: R = 0.98 *



* 3rd Party Independent Testing: Zymogen Research, Inc.

DNA Methylation Clockmakers:

- **110 CpG Clock** – Horvath, 2013

  Developed using blood & tissue samples (> 30 sites)
  Originally used the Illumina 29,369 CpG chip
  Chronological age prediction: ?

- **353 CpG Clock** – Horvath, 2013

  Developed using same algorithm as 110 CpG clock
  Validated using the Illumina 450K CpG chip data
  Chronological age prediction: +/- 3.6 yrs

- **3 CpG Clock** – Weidner, etc, 2014

  Developed using only blood samples
  Originally used Illumina 485K CpG chip data
  Chronological age prediction: +/- 4.5 years

- **73 CpG Clock**– Hannum, 2013

  Developed using only whole blood samples
  Originally Used Illumina 485K CpG chip
  Chronological age prediction: +/- 3.6 years

- **102 CpG Clock** – Hannum, 2014

  Developed using only whole blood samples
  Used Illumina 485K CpG chip
  Chronological age prediction:

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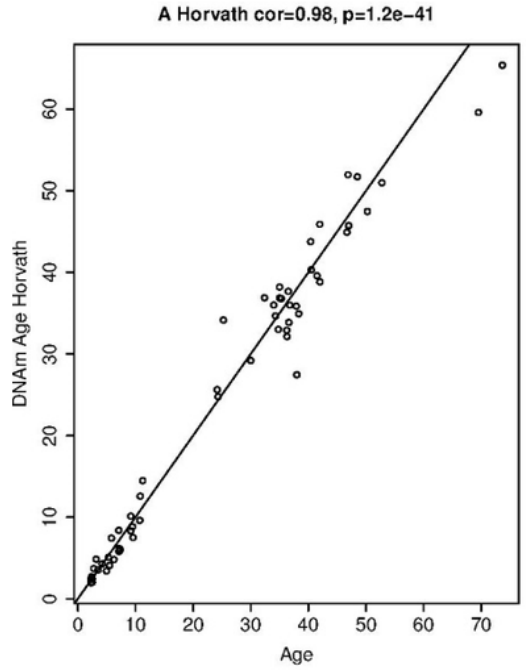

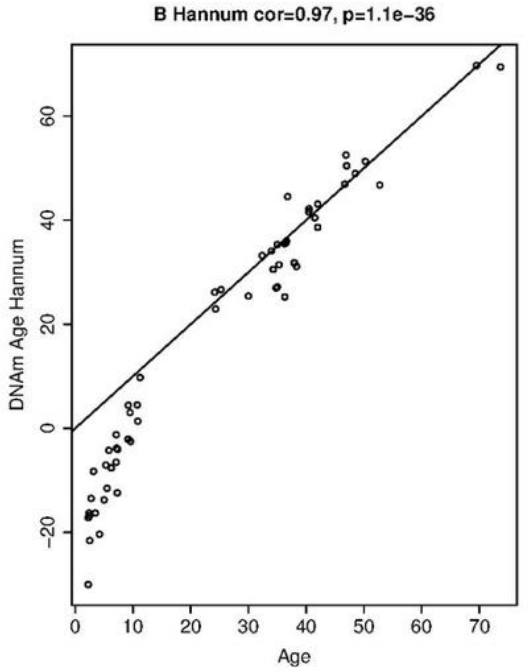

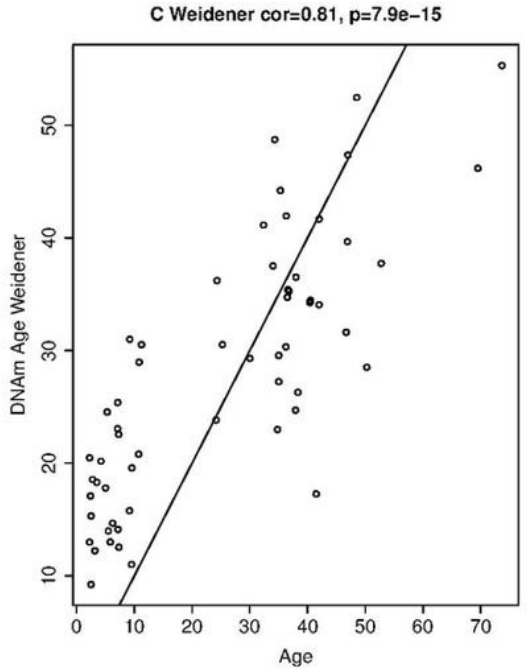

DNAm Clock Comparisons:

Hovath (353 CpG) vs Hannum (102 CpG)

Part II

- Soriano-Tarraga, et al, *Aging*, 2016
- Finding #3: Horvath and Hannum clocks showed the same age acceleration in stroke patients
- Finding #4: Horvath and Hannum clocks showed equal correlation: chronological vs *DNAm* age and "Inter-clock correlation"

DNAm Clock	Correlation
Horvath	*r* = 0.93
Hannum	*r* = 0.93
Horvath vs Hannum	*r* = 0.94

- Finding #5: △age was greater with Hannum *DNAm* clock in all 4 models

Clock	Odds Ratio (OR)
Hannum	1.13-1.14
Horvath	1.05-1.07

Horvath vs Hannum ***DNAm* Clock** Data in CVA patients

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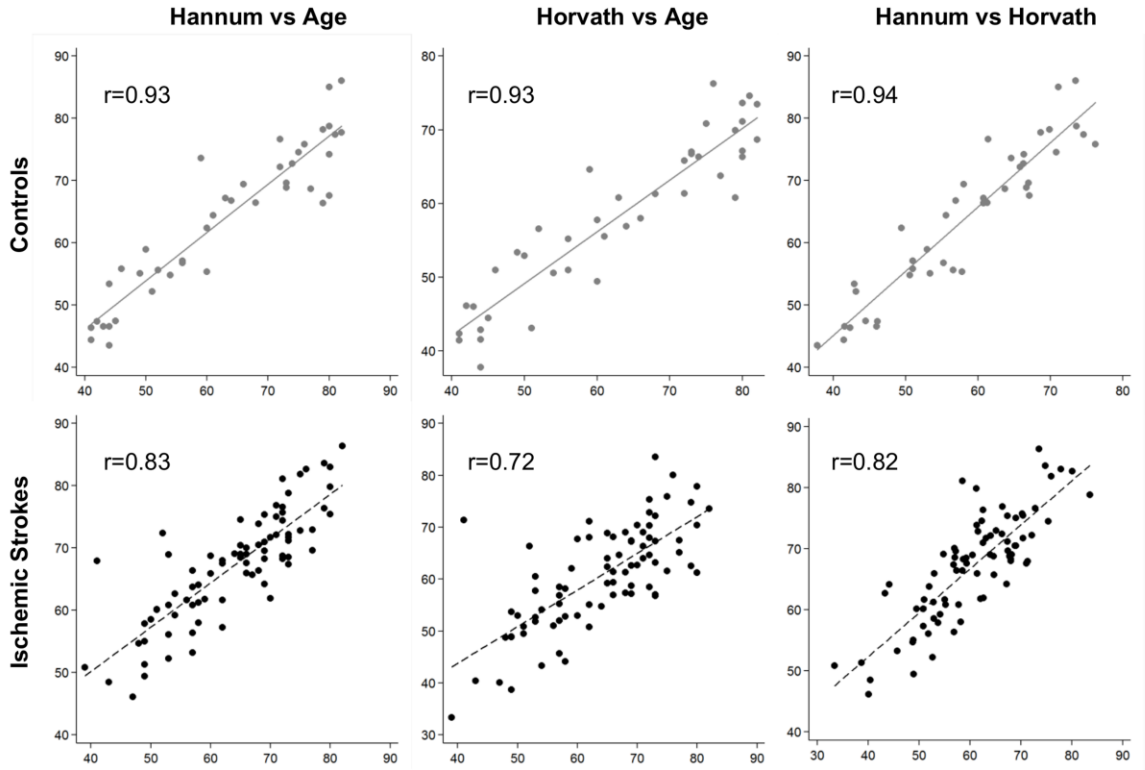
------ Ischemic stroke survivors (CVA)
—— Healthy age-matched controls



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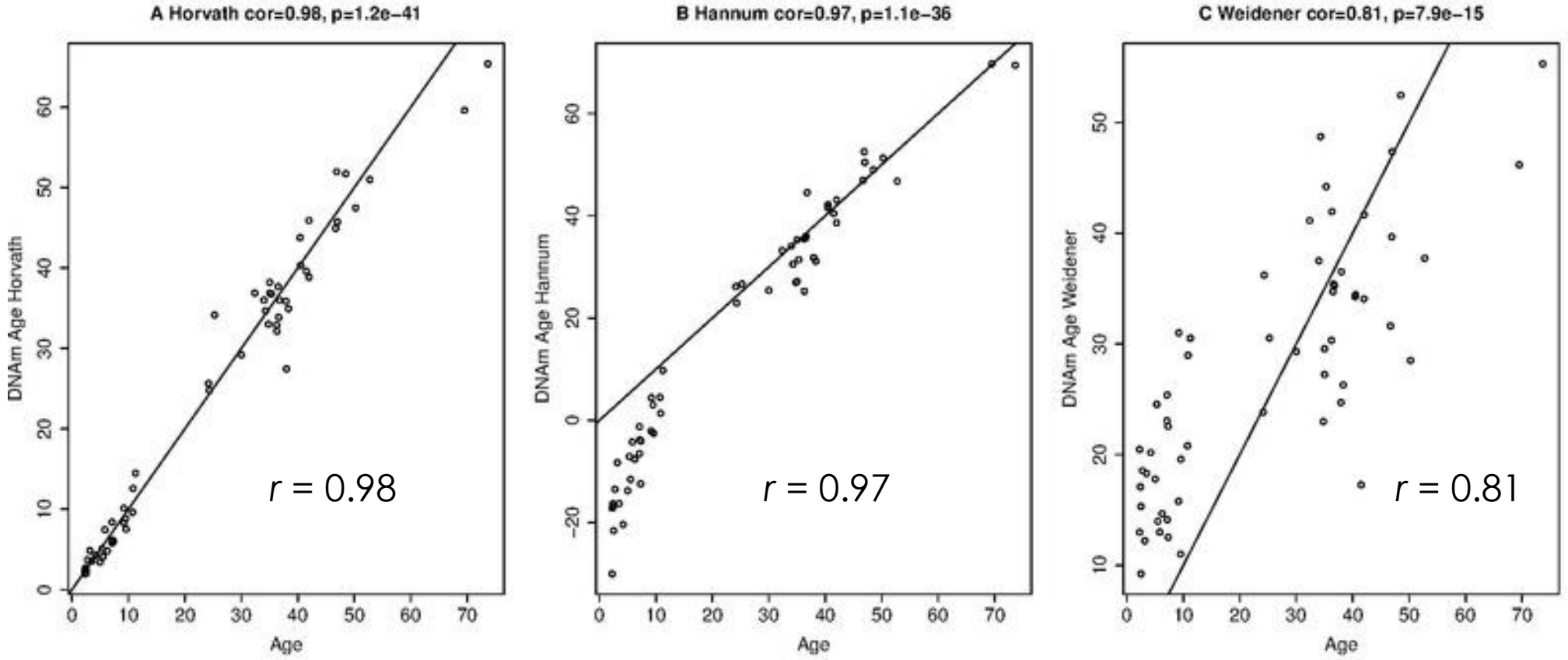
DNA Methylation Clock Comparisons

Hovath (353 CpGs) vs Hannum (73 CpGs) vs Weidner (3 CpGs)



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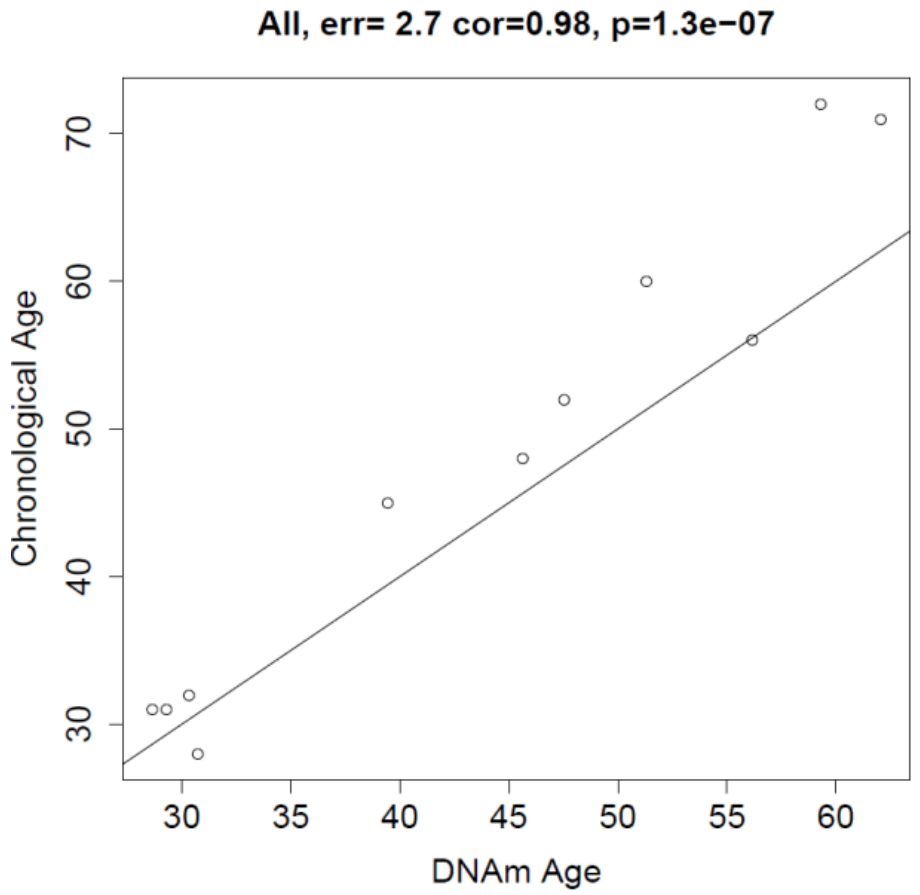
Discoveries made with Horvath's 353 CpG Methylation Clock

- Human tissue mostly ages at the same *△DNAm* rate
  exceptions: female breast – *△ DNAm* is faster
  cerebellum – *△ DNAm is* slower
- Aging rate accelerates in old age ~ 40%
  - Horvath's clock shows this age acceleration
  - Falkner's formula – age acceleration = 40% in old age
- Non-human primates – Horvath's clock is accurate in non-human primates – Ex: Rhesus monkey,
- Obesity - accelerates DNA methylation aging ONLY in the liver (not other tissue). Surgery-induced weight loss doesn't reverse this epigenetic age acceleration
- Cancer – some cancers show age acceleration
  - Cancers with silenced TSGs – no change in *△ DNAm*
  - ER/PR+ breast cancer – acceleration in *△ DNAm*



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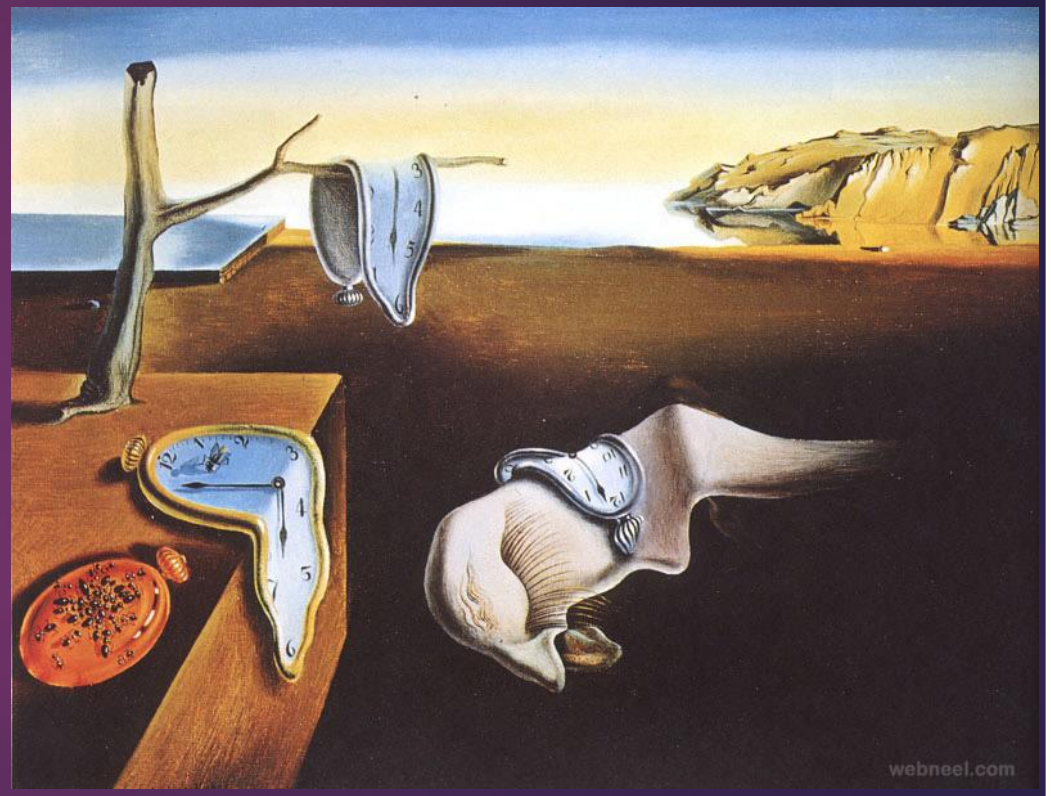
POSSIBLE CAUSES OF DNAm "*CLOCK TICKING*"

- Stochastic (random) ?
- Oxidative Stress (i.e. free radicals) ?
- DNA damge ?
- Time-dependent biological events ?
  - Day-night cycles (circaidian)
  - Molecular "drivers" of circaidian cycles within the cell?
- Cellular Inflamation or Senescence ?
  - NF-kB activated pathways?
  - JAK-STAT pathways?



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Q: Is DNA Methylation "*CLOCK TICKING*" at the DNAm Clock CpG sites a random event ?

A: Absolutely NOT!

Reason: Stochastic events follow a bell-shaped curve. CpG Clocks are non-stochastic – they undergo the same differential DNA methylation in all 7.2 billion humans

Conclusion: The consistent, reproducible pattern of DNA Differential methylation at specific CpG sites suggests that aging is a "*programmed event*", just like the consistent, reproducible pattern of DNA differential methylation that occurs during embryogenesis and fetal life

How Folic acid, Vit B12 and the SAM/SAH ratio affects DNMT activity

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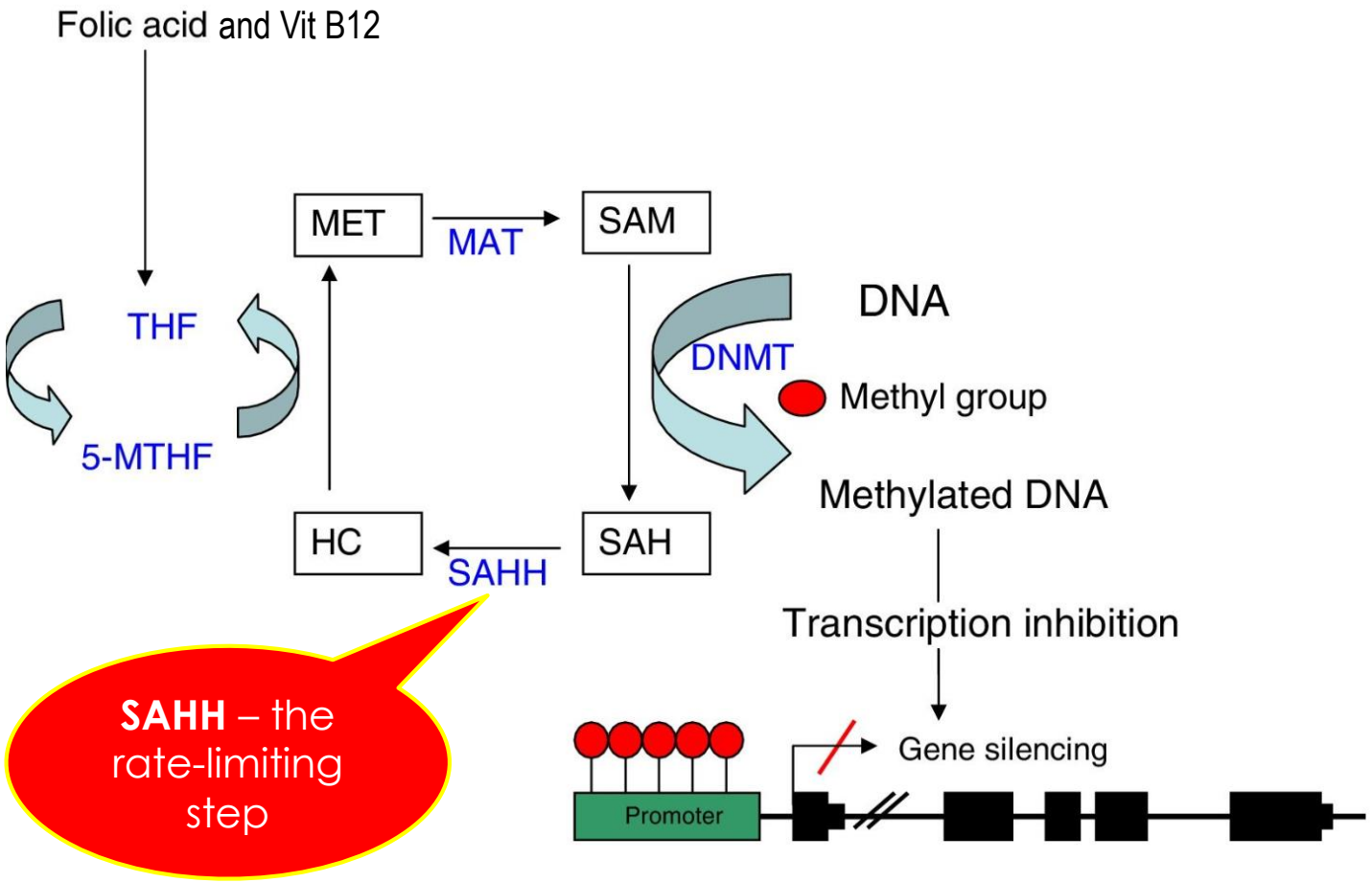

S-Adenosyl-l-methionine (SAM) is the necessary methyl donor (substrate) all of the DNMT enzymes

S-Adenosyl-l-homocystein (SAH) is the by product of DNA methylation and inhibits DNMTs by "feedback inhibition"

- **SAM/SAH ratio** determines DNMT enzyme activity rate
- **SAHH** is a "rate limiting enzyme" in the methionine cycle of DNA methylation

Specific Factors that alter the SAM/SAH ratio and DNMT activity

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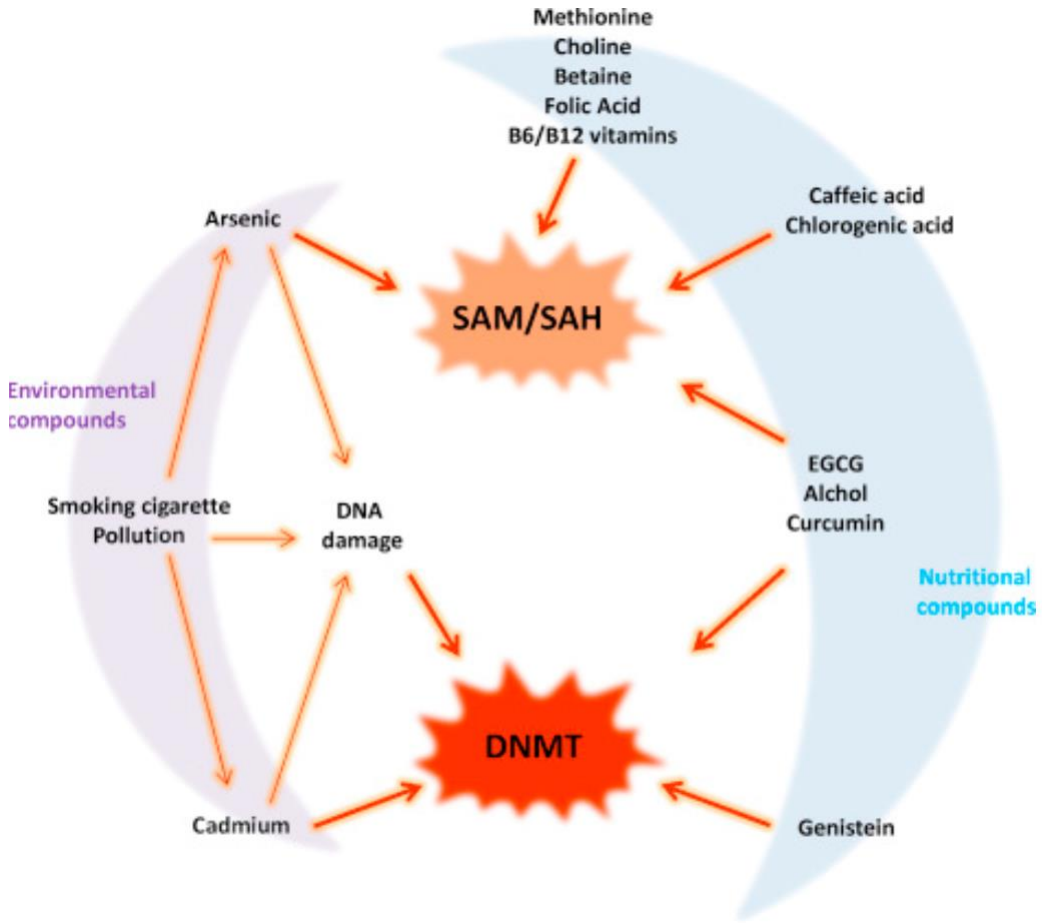

Factors that Accelerate Epigenetic Aging

- Folic acid and Vit B12 deficiency
- Smoking and air pollution
- Heavy metals – Arsenic, Cadmium
- Alcohol, malnutrition
- Chronic stress, sleep deprivation

Factors that Slow Down Epigenetic Aging

- Caloric restriction
- Methionine restriction
- Caffeic acid, Chlorogenic acid
- EGCG, Curcumin, Genistein
- Fish Oil, Vit D
- Metformin

How **Metformin** Slows Epigenetic Aging and Increases Longevity

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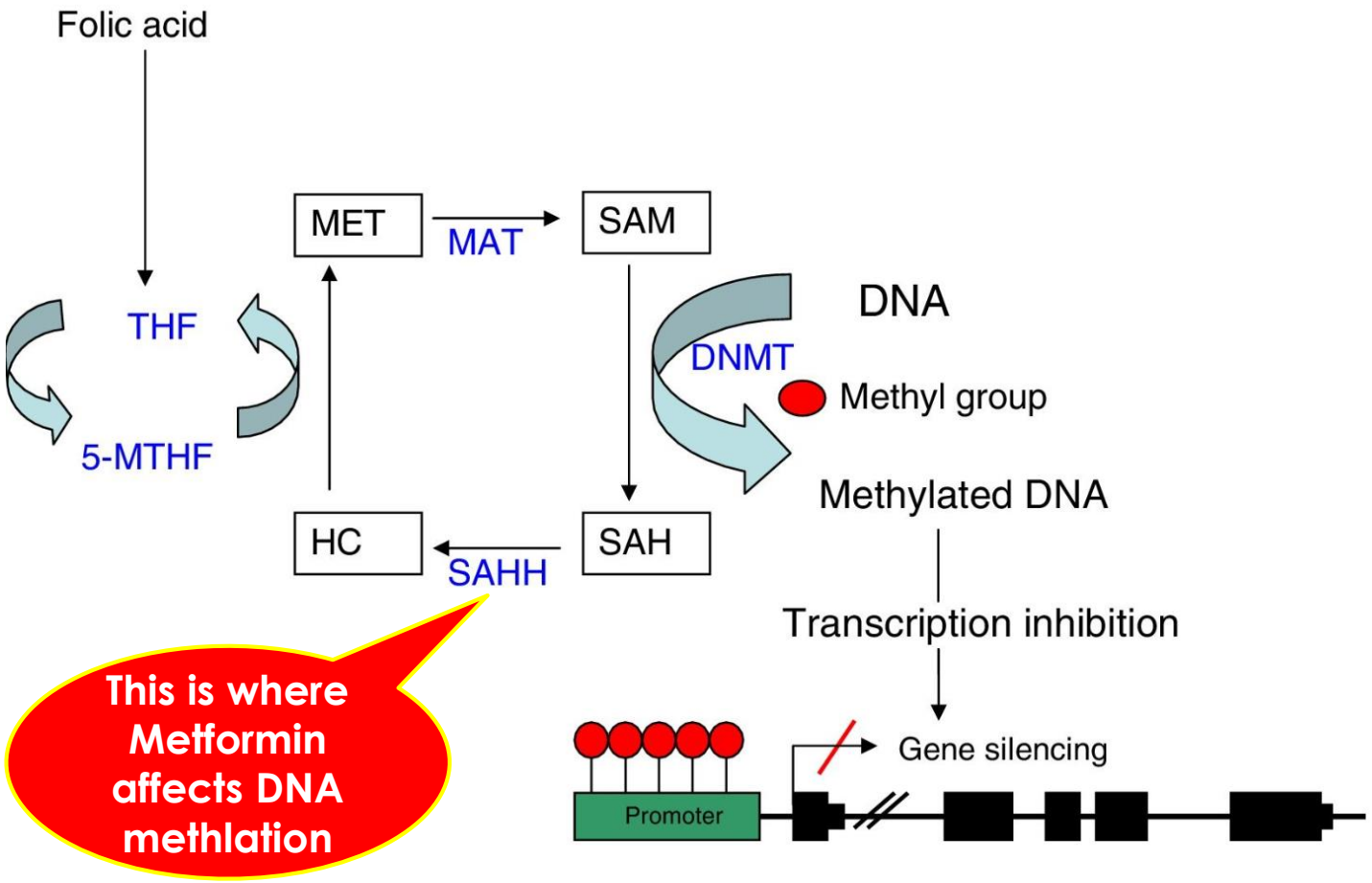
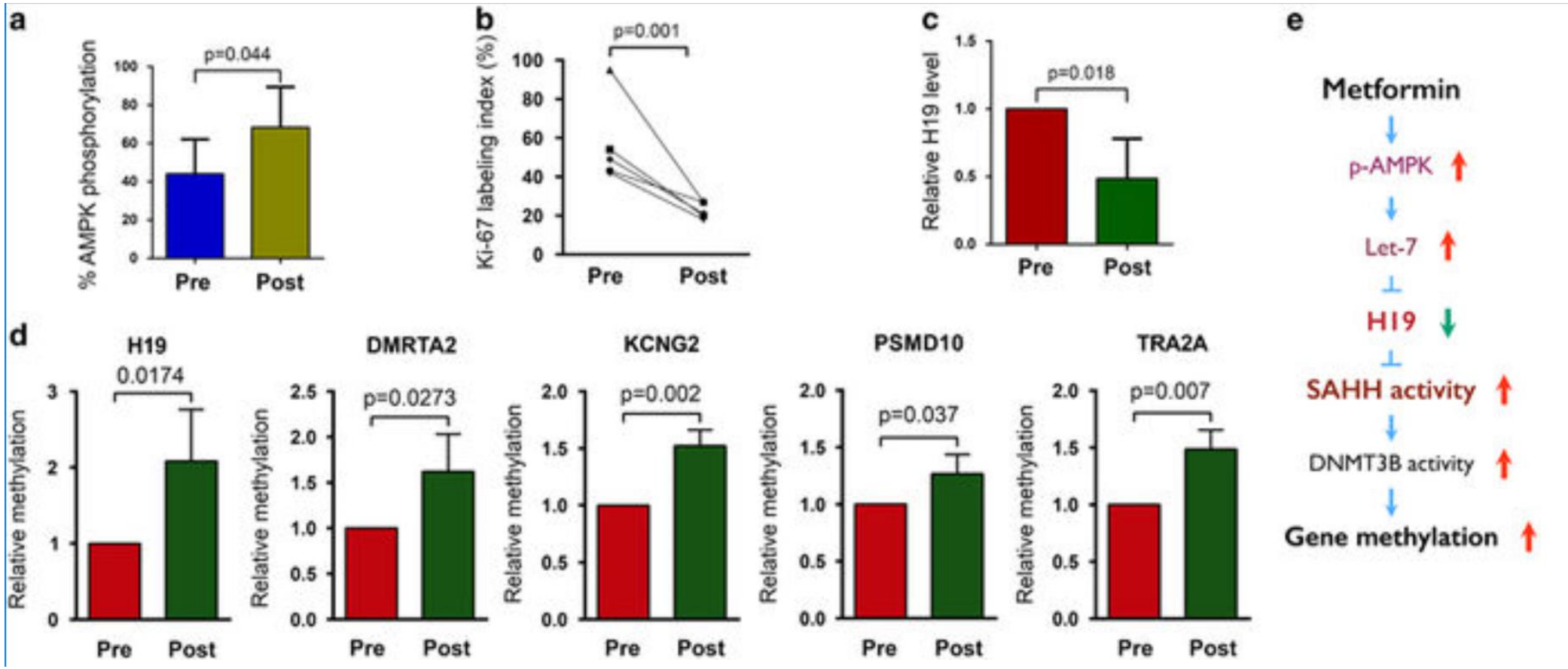


- **SAHH** is a "rate limiting enzyme" in the methionine cycle of DNA methylation
- **Metformin –** activates SAHH enzyme via an AMPK/Let-7/H19 pathway
- **Net Effect of Metformin** – less "feedback inhibition" of DNMTs due to build-up of SAH

Ref: Zhong, et.al, Metformin alters DNA methylation genome-wide via the H19/SAHH axis, *Oncogene*, April, 2017

How **Metformin** Slows Epigenetic Aging and Increases Longevity

"***Metformin is an FDA-approved drug that could be used off label as an anti-aging pill"***

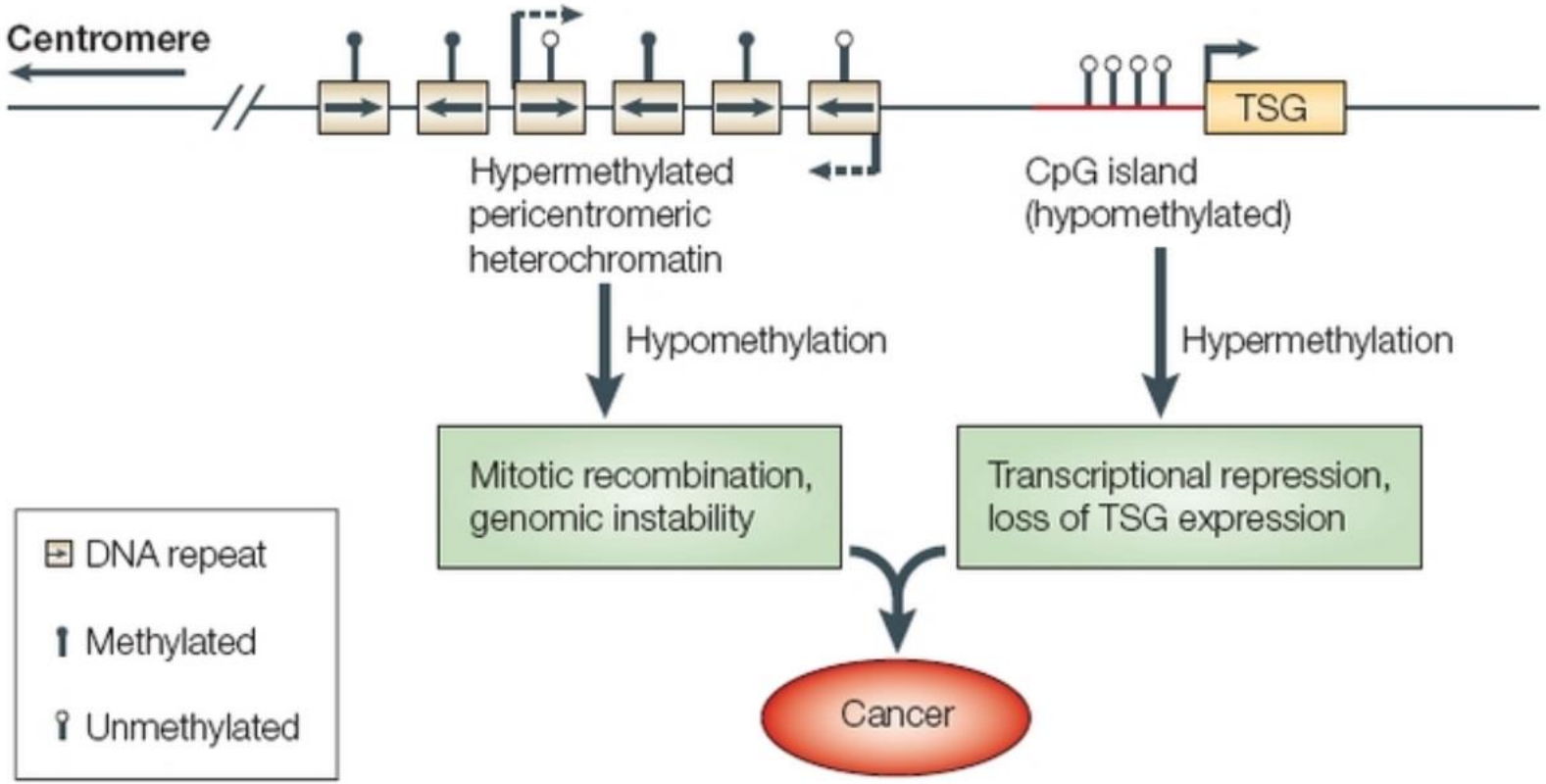


Zhong, et.al, Metformin alters DNA methylation genome-wide via the H19/SAHH axis, *Oncogene*, April, 2017

DNA Methylation Dysregulation in Cancer

"Epigenetic changes in DNA methylation are early events in cancer tumorigenesis and often precede DNA mutations"

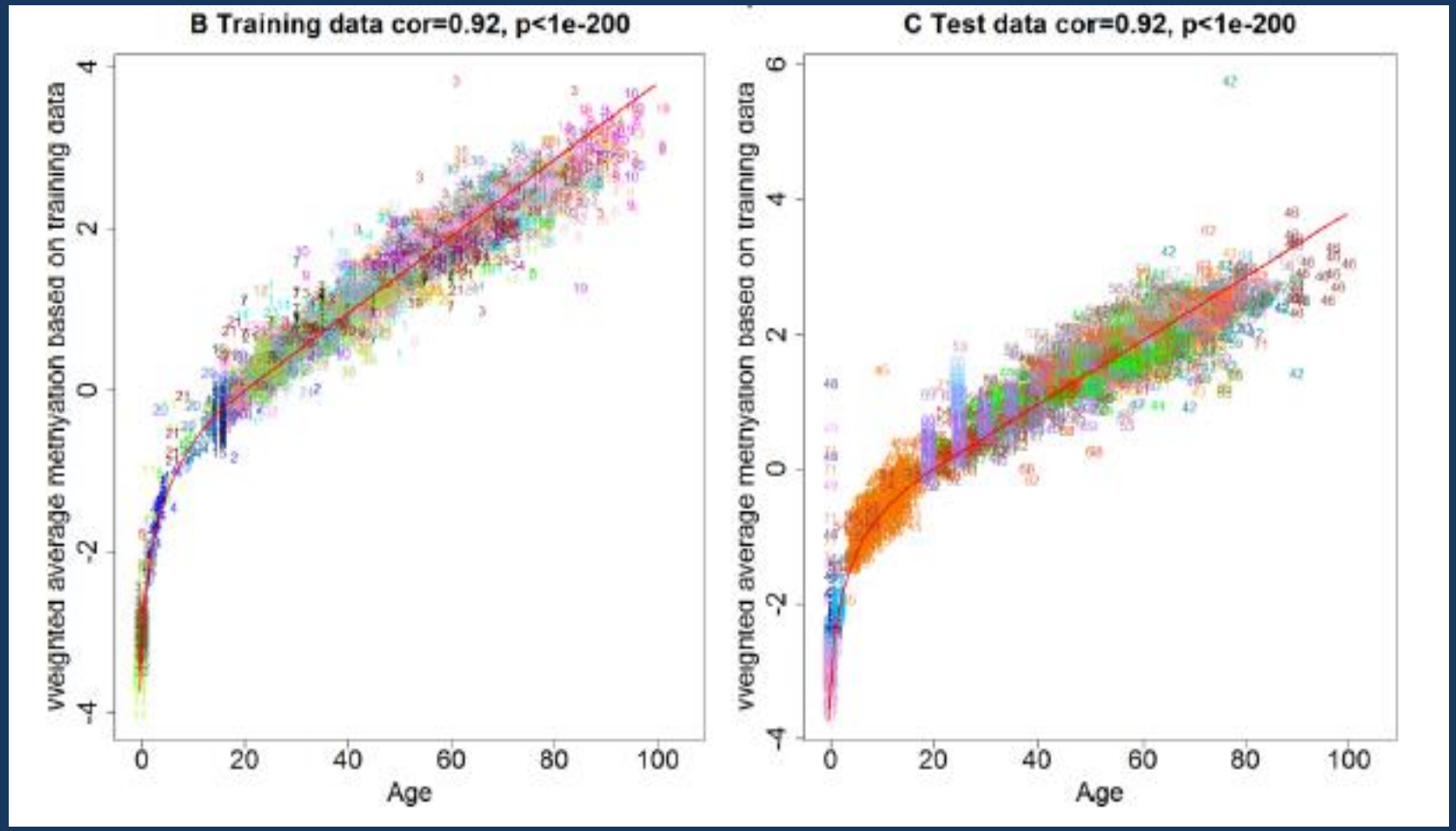
Robertson, K. DNA methylation and human disease.
Nature Reviews Genetics, 2005, Vol 6, p 598



Clinical Significance of DNA Methylation in Age Management (aka DNAm Clock Testing)

What's New and What's the Future Direction of DNA Methylation Clocks and DNAm testing?

DNAm Clocks Can Measure Age in Almost All Human Tissues



The Horvath DNAm Clock (353 CpG clock) applies to almost all human tissue types across the entire life course

Exceptions to the Rule: The Cerebellum

DNAm Clocks suggest that the cerebellum ages slower than most other tissues => DNAm Age Clock not accurate "time keeper"

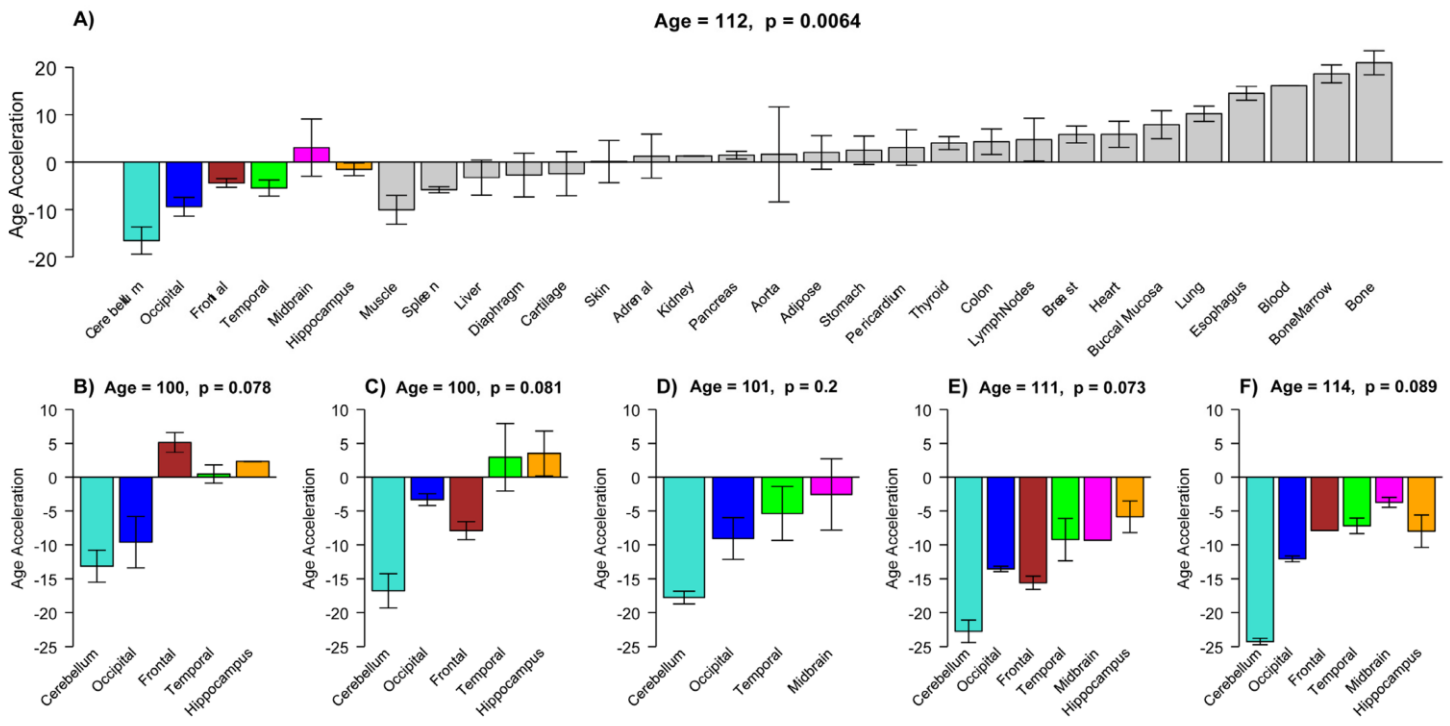


Figure 3. Epigenetic age acceleration in tissues from individual centenarians. (a) Mean DNAm age

www.impactaging.com AGING, May 2015, Vol. 7, No 5

Research Paper

The cerebellum ages slowly according to the epigenetic clock

Steve Horvath[1,2], Vei Mah[3], Ake T. Lu[1], Jennifer S. Woo[3], Oi-Wa Choi[4], Anna J. Jasinska[4], José A. Riancho[5], Spencer Tung[3], Natalie S. Coles[6], Jonathan Braun[3], Harry V. Vinters[3], and L. Stephen Coles[6,*]

New Epigenetic Clocks That are Being Developed:

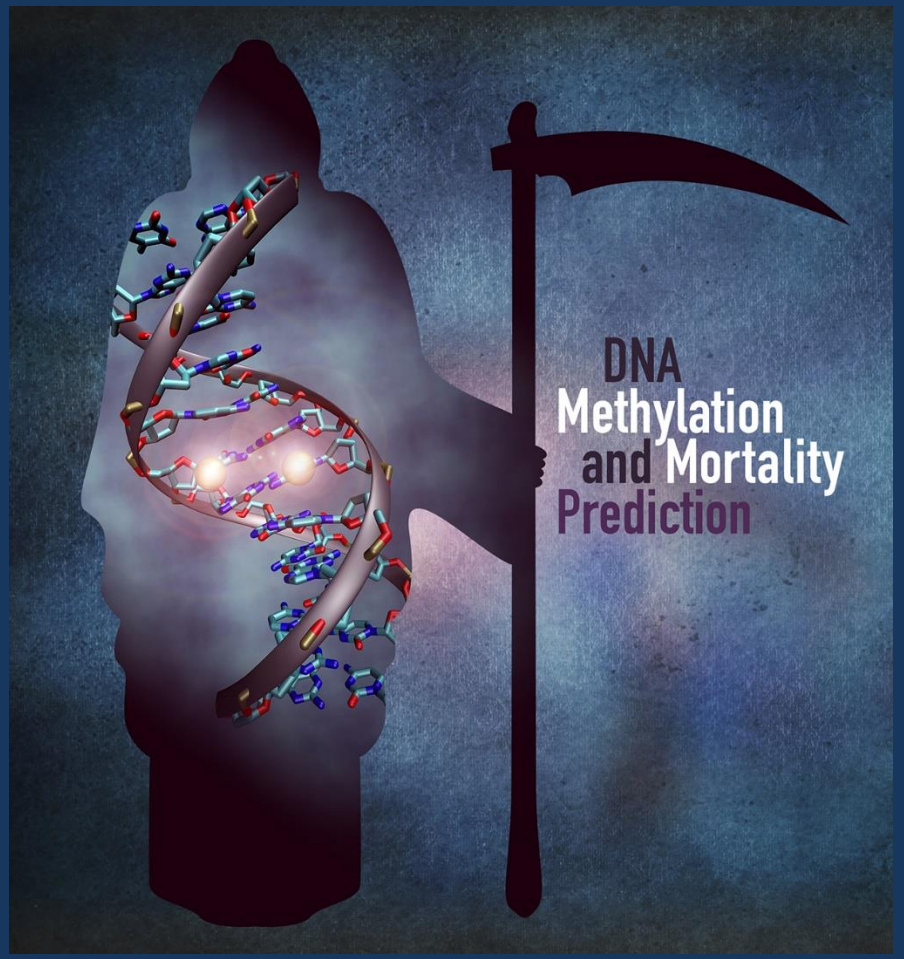
The "DNAm PhenoAge" Clock

- Correlates more with aging "phenotype", rather than intrinsic aging rate
- Developed mainly for blood methylation data (old clock not as accurate in measuring age with blood samples)
- DNAm PhenoAge clock also applies to other tissues besides blood
- This clock measures differential DNA methylation at 513 CpG sites

An epigenetic biomarker of aging for lifespan and healthspan

Morgan E. Levine[1], Ake T. Lu[1], Austin Quach[1], Brian H. Chen[2], Themistocles L. Assimes[3], Stefania Bandinelli[4], Lifang Hou[5], Andrea A. Baccarelli[6], James D. Stewart[7], Yun Li[7], Eric A. Whitsel[7,8], James G Wilson[9], Alex P Reiner[10], Abraham Aviv[11], Kurt Lohman[12], Yongmei Liu[13], Luigi Ferrucci[2*], Steve Horvath[1,14*]

DNAm Clocks Can Accurately Predict Risk of Death !



DNAm age estimators in blood predict time to death even after adjusting for other risk factors (Ex: smoking, obesity)

RESEARCH Open Access

DNA methylation age of blood predicts all-cause mortality in later life

Riccardo E Marioni[1,2,3†], Sonia Shah[3,4†], Allan F McRae[3,4†], Brian H Chen[5,6†], Elena Colicino[7†], Sarah E Harris[1,2], Jude Gibson[8], Anjali K Henders[9], Paul Redmond[10], Simon R Cox[1,10], Alison Pattie[10], Janie Corley[10], Lee Murphy[8], Nicholas G Martin[9], Grant W Montgomery[9], Andrew P Feinberg[11,12], M Daniele Fallin[11,13], Michael L Multhaup[11], Andrew E Jaffe[13,14], Roby Joehanes[5,15,16], Joel Schwartz[7,17], Allan C Just[7], Kathryn L Lunetta[5,18], Joanne M Murabito[5,19], John M Starr[1,20], Steve Horvath[21,22†], Andrea A Baccarelli[7,17†], Daniel Levy[5,6†], Peter M Visscher[1,3,4†], Naomi R Wray[3*†] and Ian J Deary[1,10*†]

Aging Cell (2016) **15**, pp149–154

DNA methylation age is associated with mortality in a longitudinal Danish twin study

Lene Christiansen,[1] Adam Lenart,[2] Qihua Tan,[1,3] James W. Vaupel,[2,4] Abraham Aviv,[5] Matt McGue[1,6] and Kaare Christensen[1,3,7]

Due to the advent of array technologies, HumanMethylation27 and HumanMethylation methylation levels of CG dinucleotides (CpGs)

www.aging-us.com AGING 2016, Vol. 8, Advance

Priority Research Paper

DNA methylation-based measures of biological age: meta-analysis predicting time to death

Brian H. Chen[1,2,3*], Riccardo E. Marioni[4,5,6*], Elena Colicino[7*], Marjolein J. Peters[8], Cavin K. Ward-Caviness[9], Pei-Chien Tsai[10], Nicholas S. Roetker[11], Allan C. Just[7], Ellen W. Demerath[11], Weihua Guan[12], Jan Bressler[13], Myriam Fornage[13,14], Stephanie Studenski[1], Amy R. Vandiver[15], Ann Zenobia Moore[1], Toshiko Tanaka[1], Douglas P. Kiel[16,17], Liming Liang[18,19], Pantel Vokonas[18], Joel Schwartz[18], Kathryn L. Lunetta[20,2], Joanne M. Murabito[2,21], Stefania Bandinelli[22], Dena G. Hernandez[23], David Melzer[24], Michael Nalls[23], Luke C. Pilling[24], Timothy R. Price[23], Andrew B. Singleton[23], Christian Gieger[9,25], Rolf Holle[26], Anja Kretschmer[9,25], Florian Kronenberg[27], Sonja Kunze[9,25], Jakob Linseisen[9], Christine Meisinger[9], Wolfgang Rathmann[28], Melanie Waldenberger[9,25], Peter M. Visscher[4,6,29], Sonia Shah[6,29], Naomi R. Wray[6], Allan F. McRae[6,29], Oscar H. Franco[30], Albert Hofman[18,30], André G. Uitterlinden[8,30], Devin Absher[31], Themistocles Assimes[32], Morgan E. Levine[33], Ake T. Lu[33], Philip S. Tsao[32,34], Lifang Hou[35,36], JoAnn E. Manson[37], Cara L. Carty[38], Andrea Z. LaCroix[39], Alexander P. Reiner[40,41], Tim D. Spector[10], Andrew P. Feinberg[15,42], Daniel Levy [2,43*], Andrea Baccarelli [7,44*], Joyce van Meurs[8*], Jordana T. Bell[10*], Annette Peters[9*], Ian J. Deary[4,45*], James S. Pankow[11*], Luigi Ferrucci[1*], Steve Horvath[33,46*]

Prediction of Life Span with the new "DNAm PhenoAge" Clock

Mortality Cause	Cases	P-Value
All-Cause	1052	3.8E-49
Aging-Related	661	4.5E-34
CVD	272	5.1E-17
Cancer	265	7.9E-10
Diabetes	41	1.9E-11
Lung	53	6.3E-4

Prediction of Morbidity with the new "DNAm PhenoAge" Clock

Morbidity Validation for DNAm PhenoAge

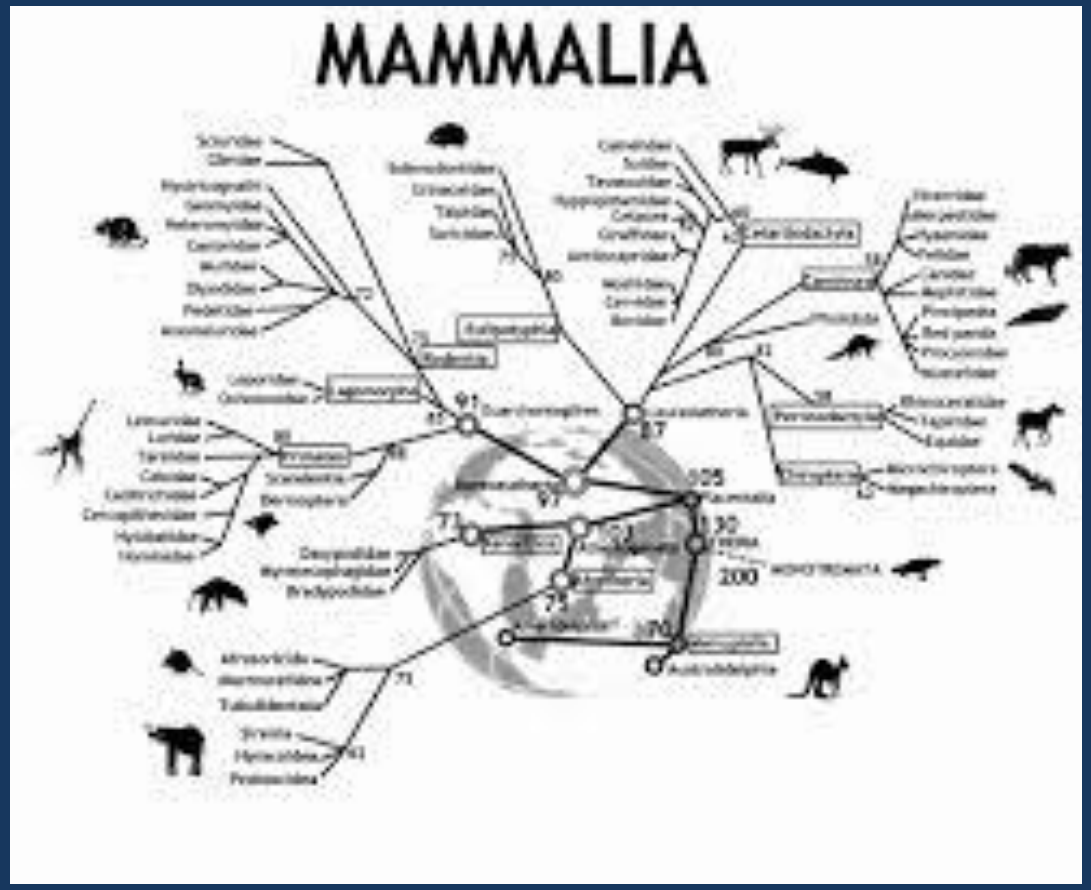
- Higher DNAm PhenoAge is associated with
  - Incident coronary heard disease (P-value=2.43E-10)
  - a decrease in likelihood of being disease-free (P=1.06E-7),
  - a person's number of coexisting morbidities (P=4.6E-15),
  - an increase in physical functioning problems (P=2.1E-13).

Epigenetic Clock Discovery in 50 Mammals

- Paul G. Allen Foundation has funded a project that will profile 50 different mammals.

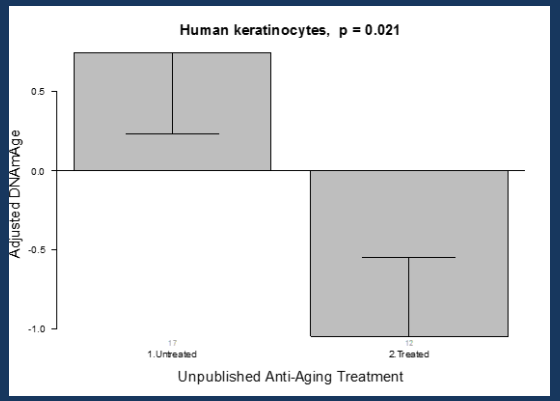
THE PAUL G. ALLEN FRONTIERS GROUP

Paul G. Allen



A Mammalian Aging Clock for Rodents and other animals will be valuable for pre-clinical studies of anti-aging interventions

In vitro studies



Anti-aging intervention that resets the epigenetic age of human keratinocytes (Ken Raj).

In vivo studies

www.aging-us.com AGING 2017, Vol. 9, Advance

Research Paper

An epigenetic aging clock for dogs and wolves

Michael J. Thompson[1*], Bridgett vonHoldt[2*], Steve Horvath[3*] , Matteo Pellegrini[*]

MOLECULAR ECOLOGY
RESOURCES
Molecular Ecology Resources (2014) 14, 976–987 doi: 10.1111/1755-0998.

Epigenetic estimation of age in humpback whales

ANDREA M. POLANOWSKI,* JOOKE ROBBINS,† DAVID CHANDLER‡ and SIMON N. JARMAN*

RESEARCH ARTICLE Open Access

Multi-tissue DNA methylation age predictor in mouse

Thomas M. Stubbs[1], Marc Jan Bonder[2], Anne-Katrien Stark[3], Felix Krueger[4], BI Ageing Clock Team, Ferdinand von Meyenn[1*], Oliver Stegle[2*] and Wolf Reik[1,5,6*]

CellPress Short Article

Using DNA Methylation Profiling to Evaluate Biological Age and Longevity Interventions

Daniel A. Petkovich,[1,3] Dmitriy I. Podolskiy,[1,3] Alexei V. Lobanov,[1] Sang-Goo Lee,[1] Richard A. Miller,[2] and Vadim N. Gladyshev[1,4,*]

RESEARCH Open Access

Epigenetic aging signatures in mice livers are slowed by dwarfism, calorie restriction and rapamycin treatment

Tina Wang[1], Brian Tsui[1,5], Jason F. Kreisberg[1], Neil A. Robertson[2], Andrew M. Gross[1,5], Michael Ku Yu[1,5], Hannah Carter[1,5], Holly M. Brown-Borg[3], Peter D. Adams[2,4] and Trey Ideker[1*]

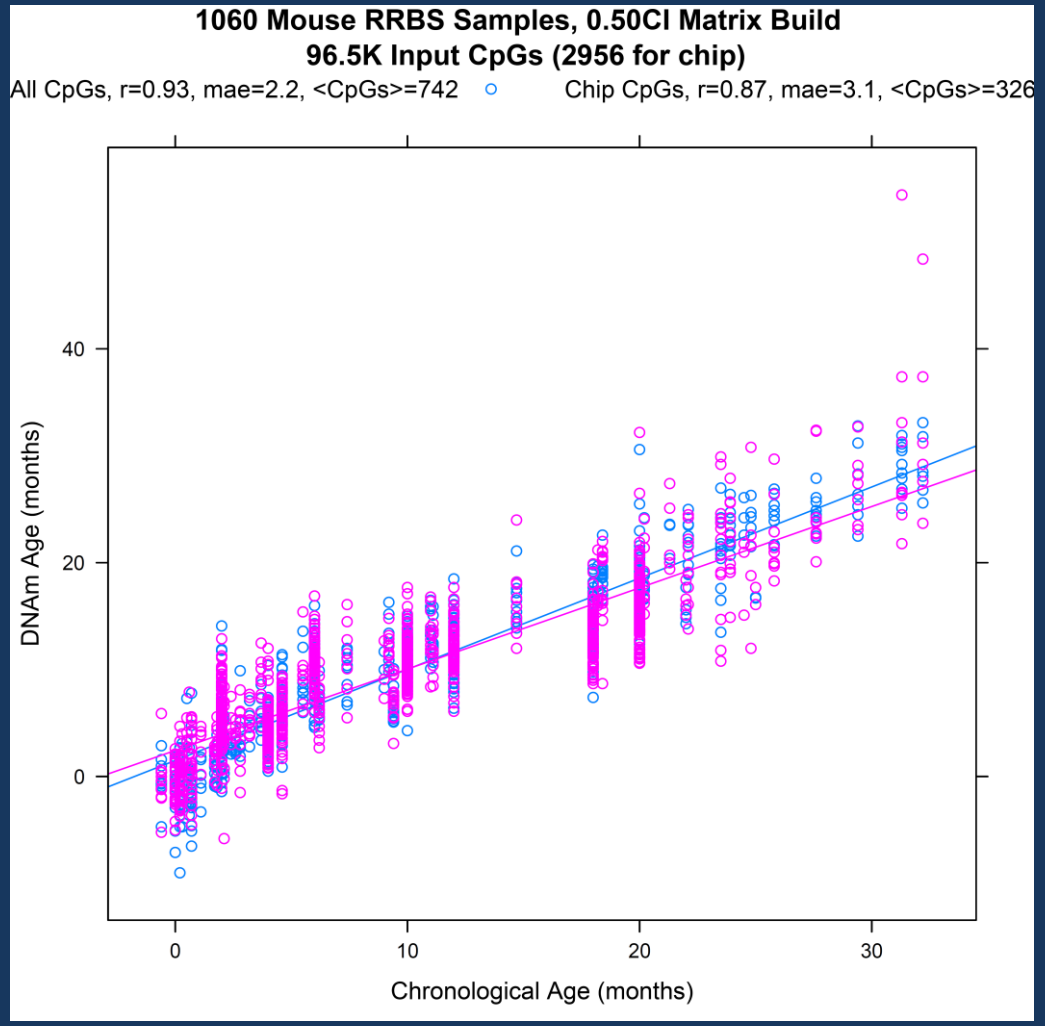
Question: Do we need to measure all of the DNA methylation sites to create the most accurate DNAm "Aging Clock"? (i.e. 28 million CpG sites?)

Answer: The data so far from all species studied suggests not. Only a sampling of 100-1,000 sites need to be measured, but large samples (10,000 to 100,000 CpG sites need to be "data mined" with Computer algorithms to come up with the small number of sites used for creating an "Aging Clock"?

Epigenetic clock for mice based on only 2956 highly conserved CpGs is similar to clocks based on 96K CpGs

Matteo Pellegrini

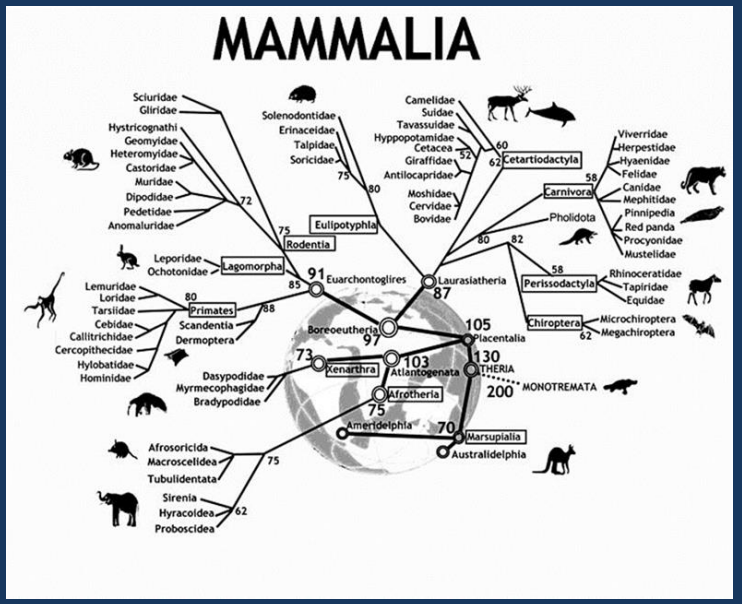
Michael Thompson, Richard Davis, Gary Churchill



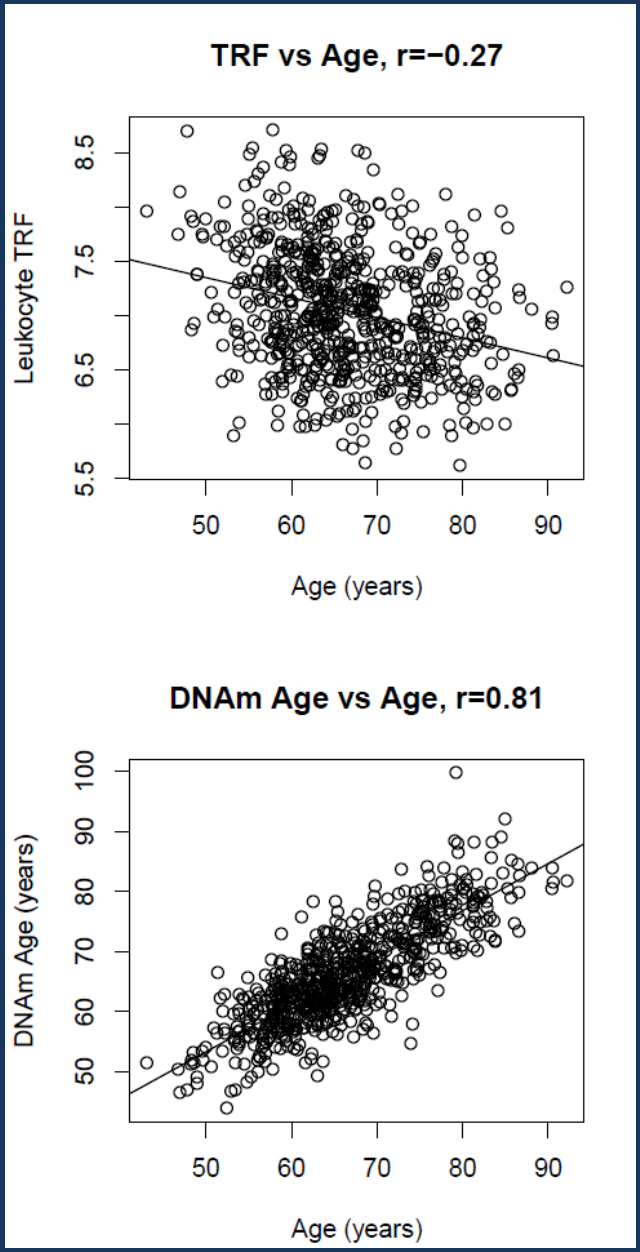
Why study 50 different animal species?

- A large number of different animal species will be needed
  - for modern phylogenetic comparative approaches
  - for introducing a technological standard that will advance special research communities
    - Evolutionary biologists
    - Veterinarians
    - Animal shelters
    - Conservationists

- To discover why certain species live longer and and others live shorter than expected, based on Longevity Quotients (Ex: Naked mole rat vs laboratory rat – LQ 5 vs 0.5)



Revisiting Leukocyte Telomere Length Testing (LTLT)



Revisiting Leukocyte Telomere Length Testing (LTLT)

- Data: DNAm Age and telomere length were measured on the same samples (*data from the Framingham Heart study*)

  LTLT (TRF method): r = - 0.27

  DNAm Age: r = 0.81

Message: DNAm age exhibits a much stronger correlation with age than telomere length

Q: Isn't Telomere Length testing and DNAm testing measuring the same aspect of aging?

A: No. Epigenetic age acceleration data does not correlate with telomere length in most large scale studies.

- No association of Epigenetic Age and LTLT in the
  1. Women's Health Initiative
  2. Framingham Heart Study
  3. ESTHER study
  4. Lothian Birth cohort (wave 2)

- Weak positive correlation
  $r = 0.08$, $p = 0.016$ in the Bogalusa study

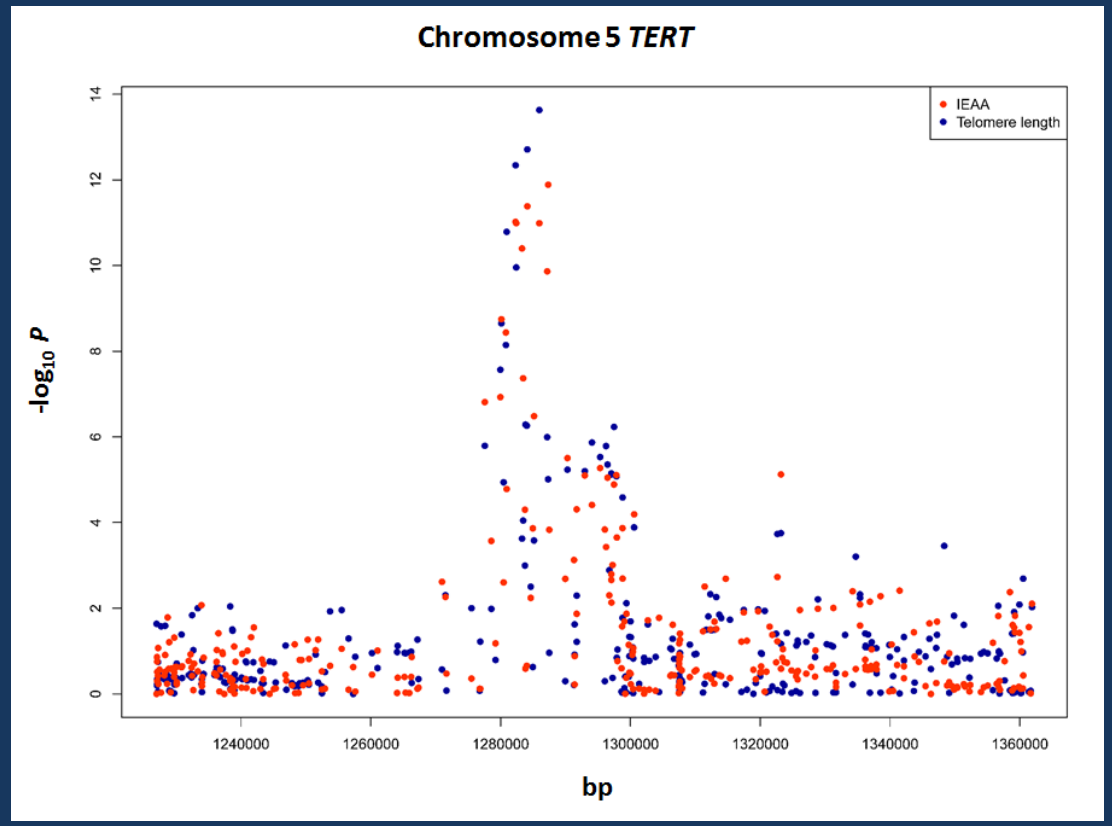
References: Chen et al 2017
Marioni et al 2016
Breitling et al 2016

Q: Why Isn't Leukocyte Telomere Length testing as accurate as DNAm testing for measuring aging?

A: Because of genetic variation in the human TERT gene

GWAS of epigenetic ageing rates in blood reveals a critical role for *TERT*

Ake T. Lu[1*], Luting Xue[2*], Elias L. Salfati[3], Brian H. Chen[4,5], Luigi Ferrucci[4], Daniel Levy[5], Roby Joehanes[5], Joanne M Murabito[6], Douglas P. Kiel[7], Pei-Chien Tsai[8], Idil Yet[8], Jordana T. Bell[8], Massimo Mangino[8], Toshiko Tanaka[4], Allan F. McRae[9,10], Riccardo E. Marioni[9,11,12], Peter M. Visscher[9,10], Naomi R. Wray[9,10], Ian J. Deary[11], Morgan E. Levine[1], Austin Quach[1], Themistocles Assimes[3], Philip S. Tsao[3,13], Devin Absher[14], James D. Stewart[15], Yun Li[16,17], Alex P. Reiner[18], Lifang Hou[19,20], Andrea A. Baccarelli[21], Eric A. Whitsel[15,22], Abraham Aviv[23], Alexia Cardona[24], Felix R. Day[24], John R.B. Perry[24], Ken K. Ong[24*], Kenneth Raj[25]*, Kathryn L. Lunetta[2*], Steve Horvath[1,26*]



Lifestyle Factors can Alter Epigenetic Aging Rate

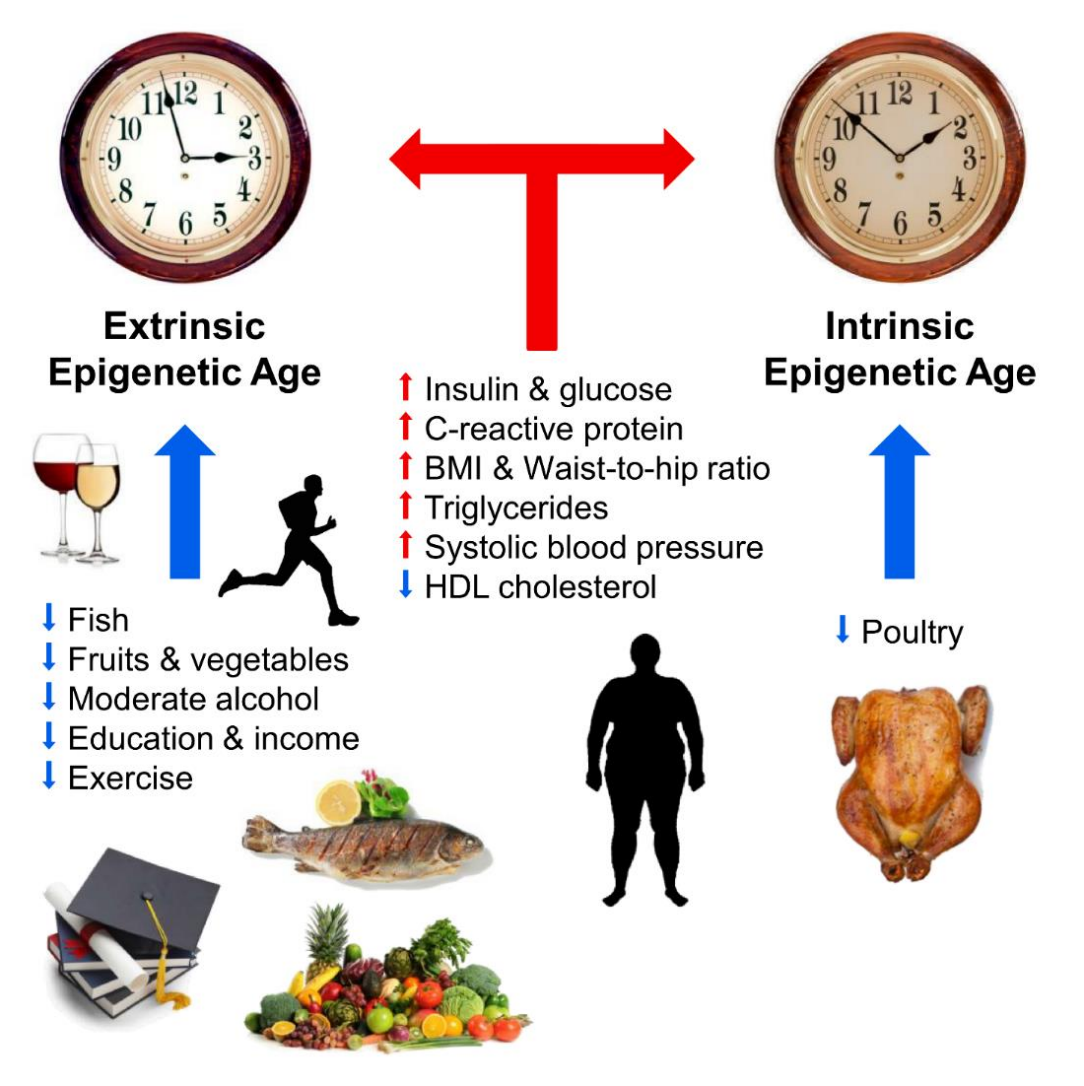
Epigenetic clock analysis of diet, exercise, education, and lifestyle factors

Austin Quach[1*], Morgan E. Levine[1*], Toshiko Tanaka[2*], Ake T. Lu[1], Brian H. Chen[2], Luigi Ferrucci[2], Beate Ritz[3,4], Stefania Bandinelli[5], Marian L. Neuhouser[6], Jeannette M. Beasley[7], Linda Snetselaar[8], Robert B. Wallace[8], Philip S. Tsao[9,10], Devin Absher[11], Themistocles L. Assimes[9], James D. Stewart[12], Yun Li[13,14], Lifang Hou[15,16], Andrea A. Baccarelli[17], Eric A. Whitsel[12,18], Steve Horvath[1,19]

Blood methylation data from

- 4,173 postmenopausal female participants from the Women's Health Initiative
- 402 participants from the Italian cohort study, Authors: Invecchiare nel Chianti

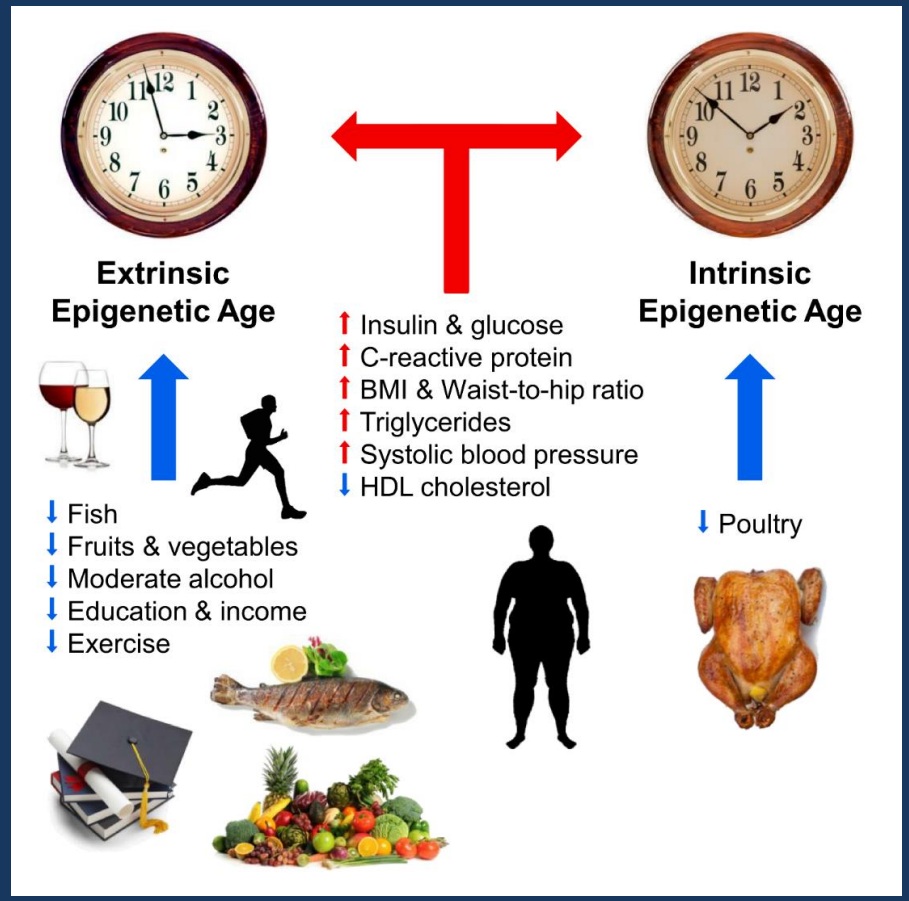
Lifestyle Factors can Alter Epigenetic Aging Rate



- **Extrinsic Epigenetic Aging** – altered by environment
- **Intrinsic Epigenetic Aging** – altered by gene variants (SNPs, heritability)
- Some lifestyle factors alter both Extrinsic Epigenetic Aging and Intrinsic Epigenetic Aging
- Some lifestyle factors alter only Extrinsic Epigenetic Aging
- One lifestyle factor alters only Intrinsic Epigenetic Aging

Lifestyle Factors can Alter Epigenetic Aging Rate

		Pooled WHI samples Adjusted for ethnicity and dataset					
		n	μ	IEAA bicor	IEAA p	EEAA bicor	EEAA p
Diet	log2(Total energy)	3687	10.53	0.00	0.96	-0.02	0.19
	Carbohydrate	3687	49.01	0.02	0.29	0.00	0.96
	Protein	3687	16.50	-0.02	0.15	-0.03	0.10
	Fat	3687	34.66	0.00	0.97	0.02	0.15
	log2(1+Red meat)	3687	0.75	0.03	0.10	0.02	0.28
	log2(1+Poultry)	3687	0.45	-0.05	4E-3	-0.03	0.05
	log2(1+Fish)	3687	0.31	-0.02	0.30	-0.07	**2E-5**
	log2(1+Dairy)	3687	1.25	0.00	0.99	-0.02	0.29
	log2(1+Whole grains)	3687	1.03	0.00	0.85	-0.02	0.19
	log2(1+Nuts)	3687	0.19	0.01	0.51	-0.02	0.36
	log2(Fruits)	3687	0.32	0.00	0.81	-0.03	0.04
	log2(Vegetables)	3687	0.62	0.00	0.98	-0.04	0.01
Blood nutrients	Retinol	2268	0.59	0.02	0.46	-0.01	0.69
	Mean carotenoids	2267	0.01	-0.06	4E-3	-0.13	**2E-9**
	Lycopene	2268	0.40	-0.02	0.44	-0.03	0.17
	log2(alpha-Carotene)	2268	-4.22	-0.04	0.04	-0.11	**9E-8**
	log2(beta-Carotene)	2267	-2.18	-0.06	0.01	-0.11	**3E-7**
	log2(Lutein+Zeaxanthin)	2268	-2.38	-0.04	0.09	-0.09	**1E-5**
	log2(beta-Cryptoxanthin)	2268	-3.74	-0.06	2E-3	-0.11	**3E-7**
	log2(alpha-Tocopherol)	2268	3.94	-0.04	0.07	-0.06	0.01
	log2(gamma-Tocopherol)	2268	0.68	0.08	**2E-4**	0.09	**9E-6**
Measurements	log2(C-reactive protein)	2809	1.54	0.08	**6E-5**	0.12	**2E-10**
	log2(Insulin)	4043	5.81	0.07	**2E-5**	0.11	**3E-12**
	log2(Glucose)	4145	6.66	0.06	**8E-5**	0.06	**2E-4**
	log2(Triglyceride)	4149	7.05	0.05	**5E-4**	0.07	**6E-6**
	Total cholesterol	4149	227.31	0.03	0.04	0.01	0.62
	LDL cholesterol	4085	142.85	0.03	0.06	0.01	0.41
	HDL cholesterol	4146	54.86	-0.04	0.01	-0.09	**1E-8**
	log2(Creatinine)	2748	-0.42	0.01	0.74	0.02	0.26
	Systolic blood pressure	4165	130.17	0.04	5E-3	0.07	**4E-6**
	Diastolic blood pressure	4165	75.86	0.05	3E-3	0.04	0.01
	log2(Waist / hip ratio)	4165	-0.28	0.05	3E-3	0.09	**2E-8**
	BMI	4165	29.69	0.08	**1E-6**	0.09	**2E-8**
Socio-behavioral	Education	4130	6.80	-0.02	0.14	-0.10	**3E-10**
	Income	4041	3.73	0.00	0.79	-0.06	**1E-4**
	log2(1+Exercise)	4142	2.53	-0.04	0.01	-0.07	**2E-5**
	Current smoker	4142	0.12	0.00	0.78	-0.01	0.66
	log2(1+Alcohol)	3687	1.10	-0.02	0.21	-0.07	**3E-5**



Marginal correlations with epigenetic age acceleration in the WHI. Correlations between select variables and the two measures of epigenetic age acceleration are colored according to their magnitude with positive correlations in red