Minireview *Highlight Article*

The use of DNA methylation clock in aging research

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Impact statement

The purpose of the Life Sciences is to provide for health and longevity. A significant component of this purpose is understanding the pathophysiological mechanisms of aging. DNA methylation clocks, which are based on the concept that DNA methylation status is a measure of chronological age, have been employed as biomarkers of both healthy and unhealthy aging, as well as disease risk. Herein we describe the utility of DNA methylation clocks as biomarkers of aging as well as their use during intervention therapies designed to promote healthy aging and longevity. A proper understanding of DNA methylation clocks, as aging biomarkers, will assist in the quantification of the rate of biological aging and will provide a foundation for the development of effective therapeutic aging interventions.

Abstract

One of the key characteristics of aging is a progressive loss of physiological integrity, which weakens bodily functions and increases the risk of death. A robust biomarker is important for the assessment of biological age, the rate of aging, and a person's health status. DNA methylation clocks, novel biomarkers of aging, are composed of a group of cytosine-phosphate-guanine dinucleotides, the DNA methylation status of which can be used to accurately measure subjective age. These clocks are considered accurate biomarkers of chronological age for humans and other vertebrates. Numerous studies have demonstrated these clocks to quantify the rate of biological aging and the effects of longevity and anti-aging interventions. In this review, we describe the purpose and use of DNA methylation clocks in aging research.

Keywords: Aging, DNA methylation, epigenetic, clock, intervention

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Introduction

Implementation of effective and reliable biomarkers for aging is essential to an understanding of the means by which to delay, halt, or even reverse the aging process. Chronological age, despite its wide use as a measure of aging, is not an effective biomarker for aging in that individuals of the same chronological age, especially the elderly, often have different physiological features.[1] Thus, an active area of scientific pursuit has been the identification of more reliable and predictive markers of biological aging. Recently, studies involving both animals and humans have suggested that biomarkers based on DNA methylation are appropriate molecular markers of aging. DNA methylation-based biomarkers, which are also termed "epigenetic clocks" or "DNA methylation clocks," can be extracted from different tissues and cell lines throughout the entire lifespan (antenatal to centenarian tissues).[2–5] The reversibility of DNA methylation is the most interesting feature of epigenetic clocks, suggesting their potential use for measurement of the efficacy of anti-aging interventions.[6,7] A recent systematic study compared six major biomarkers of biological aging including, telomere length, proteomic predictors, transcriptomic predictors, metabolomic predictors, composite biomarker predictors, and DNA methylation clocks.[8] The results of that study demonstrated DNA methylation-based biomarkers to be the most promising biomarker for aging. Herein we provide a brief overview of DNA methylation as well as a description of identified epigenetic clocks in humans and animals. Further, the utility of DNA methylation clocks as a guide for development of anti-aging therapies is discussed.

Overview of DNA methylation

DNA methylation is a form of chemical modification of genomic nucleotide bases, which can change gene expression without altering the DNA sequence. Cytosine-phosphate-guanine dinucleotides (CpGs) are prime targets for mammalian DNA methylation modification by addition

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of methyl (-CH3) groups to cytosine forming 5-methylcytosine (5mC).[9] Most CpGs are irregularly distributed and relatively rare in the mammalian genome.[10] In some regions, however, CpGs exist in groups or clusters known as CpG islands (CGIs).[10] Approximately 60%–70% of gene promoters have associated CGIs,[11] with promoters classified based on their CpG content. Most CpGs are methylated in mammalian genomes. However, CGIs within transcriptional start sites (TSSs) of active genes are generally hypomethylated.[9]

DNA methylation plays a key role in gene transcription by suppression of certain repetitive elements,[12–14] X-chromosome inactivation,[15–17] and repression of imprinted genes.[18] The process of DNA methylation-mediated gene silencing, which maintains the stability of transposable elements and other repeated DNA sequences, is believed to be driven by the binding of specific transcription factors to DNA and/or in cooperation with histone modifications.[19–21]

DNA methylation is catalyzed by DNA methyltransferases (DNMTs) and Ten-eleven translocation (TET) enzymes. Mammalian DNMTs are a family of enzymes responsible for the production and maintenance of 5mC throughout the genome and include: DNMT1, DNMT3A, DNMT3B, and DNMT3L.[22] DNMT1 and the E3 ubiquitin-protein ligase, UHRF1, play a critical role in the maintenance of DNA methylation following replication.[22] UHRF1 specifically binds hemi-methylated CpGs and attracts DNMT1 via its ubiquitin-like domain.[23,24] DNMT1 then methylates the daughter DNA strand. Further, DNMT1 augments the activity of *de novo* DNMTs.[25–27] The DNMT3L enzyme, a non-catalytic DNMT type, binds to and activates DNMT3A and DNMT3B,[25] which are both implicated in the process of *de novo* DNA methylation. TET1, TET2, and TET3, which are three members of the TET enzyme family, convert 5mC to 5-hydroxymethylcytosine (5hmC).[28–30] The establishment, maintenance, and removal of 5mC by DNMTs or TETs suggest that DNA methylation is a dynamic process that is balanced by the addition and removal of methyl groups.[30] Even in the brain, which is composed of numerous slowly proliferating or non-proliferating cells, the methylation status of CpGs can oscillate in a circadian rhythm.[31,32] DNA methylation is also associated with the function of enhancers.[28,33] Taken together, DNA methylation is a tightly controlled and dynamic process that plays an important role in the regulation of gene transcription.

DNA methylation clocks in animals and humans

There is growing evidence suggesting the presence of differentially methylated DNA regions associated with aging.[6,34–37] With aging, hypermethylated regions are preferentially found in CGIs,[6,37] bivalent promoters,[38] and Polycomb target genes.[35,39] Whereas age-related hypomethylated regions are preferentially found in non-CGIs promoters, enhancers, and late-replicating domains.[40] DNA methylation patterns and age-related change substantially vary across tissue types.[41] Many studies have shown that a group of age-related CpG methylation changes are independent of tissue type, gender, and array platform. Several age prediction models have been developed by use of genome-wide methylation data and supervised machine learning (regression modeling).[3,4] Age estimated by DNA methylation, also known as epigenetic age or more precisely DNA methylation age, reflects not only chronological age, but also biological function. Due to its accuracy, DNA methylation age is often referred to as an "epigenetic clock" or "DNAm clock." The definition of a DNAm clock is drawn from genome-wide methylation data that are significantly correlated (correlation coefficient $\geq$0.8) with chronological age or time.[42]

In 2011, Bocklandt *et al*. published the first DNAm clock to predict age, which was based on DNA extracted from saliva.[43] For this clock, the degree of methylation of 88 CpG sites was found to significantly correlate with age in or proximal to 80 genes. Drawing on these initial results, Hannum *et al.* developed a highly accurate clock ($r = 0.95$) based on 71 CpGs from blood-derived DNA extracted from 656 human subjects, aged 19 to 101. This DNAm clock is commonly referred to as "Hannum's clock".[3] Since this DNAm clock was based primarily on adult blood samples, Hannum's clock is thought to be less accurate for children[44–46] and non-blood tissue,[3] when compared to DNAm clocks optimized for those purposes. Several DNAm clocks were developed specifically from blood or buccal epithelial cells of children and adolescents.[45,46] These clocks performed well and exhibited a high level of precision for prediction of chronological age. Several epigenetic clocks containing fewer CpGs have also been shown to exhibit a high degree of accuracy.[47–50] As an example, a DNAm clock has been reported (Garagnani *et al*.) that was composed of only a single CpG within the *ELOVL2* gene.[51] However, DNAm clocks with a higher number of CpGs are generally more accurate and robust.[4]

Owing to differences in DNA methylation patterns and age-related changes between different cell types and tissues,[52] DNAm clocks need to be cautiously used for prediction of age. As an attempt to bypass these limitations, Horvath *et al.* developed a multi-tissue clock composed of 353 CpGs, with 8000 publicly available microarray databases generated through analysis of over 30 different DNA sources extracted from young children and adults.[4] The methylation status of 193 CpGs was found to positively correlate with age, with the remaining 160 CpGs negatively correlated. In that report, ticks of this DNAm clock were faster during growth and development. It is worth noting that the methylation status of many of the individual CpGs was only weakly associated with age. However, the comprehensive effects of 353 CpGs generated a robust biomarker of biological aging that conferred a high degree of accuracy.[4] While many technological platforms (e.g. microarrays, pyrosequencing, and next-generation sequencing methods) are available for the measurement of DNA methylation, Horvath's clock is compatible with different technological platforms. For example, Horvath's clock was successfully used with Illumina's 450k and 27k DNA methylation microarrays as well as the Illumina Infinium

MethylationEPIC array platform, despite the absence of 19 of 353 CpGs.[53]

Although first generation DNAm clocks exhibited a statistically significant correlation with a sizable range of age-related conditions, the effects were at best generally small to moderate.[54] In 2018, Levine *et al.* developed a new clock, known as DNAm PhenoAge, which was based on phenotypic age stemming from nine age-related clinical measures instead of chronological age.[54] The DNAm PhenoAge, composed of 513 CpGs, vastly outperformed the first generation of DNAm clocks with regard to predicting a variety of aging outcomes including mortality, health span, and cardiovascular disease. Smoking, a significant risk factor for mortality and morbidity, has been shown to correlate with DNA methylation changes.[55] Levine *et al.* found that age estimated by DNAm PhenoAge significantly differed between smokers and non-smokers. Smoking did not influence age estimated by Hannum's or Horvath's clocks, with no association between smoking pack-years and DNAm PhenoAge.[54,56] Of note, a recently developed DNAm clock, termed "DNAm GrimAge," incorporated DNA methylation-based surrogate biomarkers for seven plasma proteins and smoking pack-years.[57] The ability of the DNAm GrimAge to predict risk for all cause-death and age-related disease was found to be better than previously described for DNAm clocks. Using different CpGs, Hannum's and Horvath's epigenetic clocks were developed to simply predict actual age. In contrast, DNAm PhenoAge and GrimAge used CpG methylation to predict previously proposed age-related mortality and phenotypic indicators, closely related to actual age.

Due to the success of DNAm clocks in predicting chronological age in humans, DNAm clocks were developed for other species. Within a 5-year span, several DNAm clocks were developed for mice[2,58–60] and dogs/wolves.[61] Compared with human clocks, which use DNA methylation microarrays to identify subsets of CpGs, animal clocks were developed using Reduced Representation Bisulfite Sequencing, an efficient high-throughput sequencing technique for the analysis of methylation levels at the genomic mononucleotide level. As mice are commonly used for experimentation, DNAm clocks for mice were a major endeavor for use in geroscience. Such clocks have the potential to provide critical information about the underlying mechanisms of aging related to DNA methylation. Murine DNAm clocks have been used successfully to evaluate several interventions that resulted in extended lifespan and delayed aging in mice.[6,62–65] *In vivo*, the DNAm clocks described above have been shown to be highly accurate and robust for prediction of the biological age of tissues and organs. However, the *in vitro* efficiency and accuracy of these epigenetic clocks for fibroblasts or other types of cells in culture has been less accurate and robust. Hence, another DNAm clock, referred to as the skin & blood clock, was developed, which is composed of 391 CpGs from a variety of human cell sources: fibroblasts, keratinocytes, buccal cells, skin, blood, and saliva for *in vivo* and *ex vivo* studies.[5] This DNAm clock was shown to accurately track the dynamic aging process of cultured cells *ex vivo*.

The usefulness of DNA methylation clocks for aging research

Anti-aging interventions are critical methods to identify the underlying biological mechanisms or pathways of aging. An effective, accurate, and widely applicable biomarker for aging is of major importance to the evaluation of anti-aging interventions.[56] Use of DNAm clocks in the general population can lead to identification of outliers characterized by a difference between DNA methylation age and chronological age. "DNAm age acceleration" is a term used to define those individuals with a high DNA methylation age compared to chronological age.

Internal and external DNAm age acceleration

Change in the proportion of naïve and memory T cells is a primary feature of aging with respect to blood cell composition. The age estimated by Hannum's clock, DNAm PhenoAge, and the GrimAge clock is influenced by variations in cell type composition. In contrast, age predicted by Horvath's clock is primarily undisturbed by such variations.[3,4,54,57] A recent study found that the accuracy of DNAm clocks increases with an increase in training sample size, although prediction of mortality was lower.[66] One explanation is that a near-perfect age predictor estimates internal cell aging rather than disease-based external aging dynamics. Thus, Horvath's clock is regarded as an estimator of internal cell aging, whereas Hannum's clock, DNAm PhenoAge, and DNAm GrimAge clocks measure external aging. The discovery of the predictive utility of intrinsic aging acceleration is biologically compelling and points to a new frontier in aging research. Internal aging may reflect an inherent epigenetic clock that is related to mortality that is independent of actual age, changes in blood cell composition, and traditional mortality risk factors.[56]

DNAm age acceleration, mortality, and age-related diseases

Age is the biggest risk factor for mortality. However, the biological responses to aging differ among individuals. The difference between DNA methylation age and chronological age (DNAm age acceleration) has been proposed as an indicator of "biological" aging associated with all cause-mortality and age-related diseases.[55,67,68]

Mortality

From 2015 to the present, considerable research has focused on correlations among DNAm age acceleration and lifespan or mortality.[54,57,69–73] Many of these studies independently demonstrated that differences between DNA methylation age and chronological age result in increased risk of all-cause mortality.[54,57,69,70,74] A study in 2015 showed that a 5-year higher age estimate, by Hannum's and Horvath's clocks, was associated with a respective 21% or 11% increase in all-cause mortality risk after adjustment for chronological age and sex.[69] Similar results have been obtained in other studies.[74–76] Moreover, a 1-year

higher age estimated by DNAm PhenoAge is associated with a 4.5% increase in the risk of all-cause mortality.[54] Lu *et al*.[57] demonstrated that DNAm GrimAge is distinguishable from previous clocks for prediction of all cause-mortality associated with a number of peripheral, lifestyle, and cardiometabolic traits. While the DNA methylation age estimated by Hannum's and Horvath's clocks related to all-cause mortality, DNAm PhenoAge and GrimAge (adjusted for chronological age) exhibited superior prediction of mortality, as well as cancer and coronary heart disease (CHD).[54,57] Although DNA-based biomarkers have obvious advantages, they are unlikely to replace existing clinical biomarker predictors for all cause-mortality in medical practice. Rather, these epigenetic biomarkers are expected to complement existing clinical biomarkers when assessing the aging status of individuals.

Age-related diseases

Increasing evidence suggests that DNAm age acceleration is a potentially useful marker for cancer risk, although exact associations vary based on cancer type.[4] For example, three studies involving breast cancer examined the association between DNAm age acceleration and breast cancer incidence.[67,68,77] Two of the studies reported a statistically significant association between DNAm age acceleration and the incidence of postmenopausal invasive cancers of the breast, with no ductal carcinoma *in situ* association.[67,68] One breast cancer study showed that DNA methylation age estimated by DNAm GrimAge had only a weak positive association with invasive cancers.[77] Two studies involving lung cancer also showed inconsistent results.[71,78] In contrast, two studies of colorectal cancer reported that DNAm age acceleration is significantly associated with colorectal cancer.[71,79]

Another research focus has been on the relationship between accelerated DNA methylation age and risk for cardiovascular diseases, such as CHD and stroke. Four independent studies reported that higher DNA methylation age is associated with an increase in the risk of CHD, independent of chronological age and traditional cardiovascular disease risk factors.[54,57,80,81] However, an earlier study reported no correlation between higher DNA methylation age and CHD risk.[82] Differences in study outcomes may be due to the different clocks used. Three other studies focused on different outcomes associated with ischemic stroke and DNAm age acceleration.[83–85] The first study revealed ischemic stroke patients to have a higher DNA methylation age estimated by both the Hannum and Horvath clocks. The second study showed that increased DNA methylation age was associated with poorer outcomes 3 months post-stroke, and the third study reported that DNAm age acceleration was statistically associated with mortality.[85]

Three studies of cognition in the Lothian Birth Cohort (1936) found that greater age acceleration was associated with poorer cognitive performance.[86–88] Another study found that DNAm age acceleration of the dorsolateral prefrontal cortex in Alzheimer's disease patients correlated with several neuropathological measurements including, diffuse plaques, neuritic plaques, amyloid load, and with a decline in global cognitive functioning.[89] In addition, one study reported that Huntington's Disease status remains highly and significantly associated with DNAm age estimated by Harvath's clock.[90]

Taken together, these results suggest that DNAm clocks are molecular predictors of aging independent of current clinical measures for all cause-mortality and age-related diseases. However, we need to further clarify the causal relationships among new biomarkers of aging and all cause-mortality and age-related diseases, as well as their underlying biological mechanism(s).

DNAm age acceleration and health risk factors

If the acceleration of DNA methylation age reflects biological age, it is reasonable to assume that known risk factors for poor health will influence acceleration. An increasing body of evidence suggests that health risk factors such as obesity, environmental exposure, lifetime stress, and/or genetic factors may significantly and positively correlate with DNAm age acceleration.

Environmental exposure

There are examples of the relationship between environmental factors and DNAm age acceleration. Two related studies in the Veterans Affairs normative aging study (NAS) found a positive correlation between particulate matter (PM2.5) exposure and Horvath DNAm age[91,92] but not with Hannum DNAm age.[92] However, another independent study[93] that used a broader and slightly different array of NAS aging measures found that PM2.5 exposure was inversely associated with intrinsic epigenetic age acceleration (IEAA). The IEAA adjusts for age and a broader set of cell counts than does the Horvath DNAm age clock. In addition, PM2.5 was not associated with Horvath DNAm age or extrinsic epigenetic age acceleration (EEAA), which can be explained as a modified Hannum DNAm age.[93] In another study (KORAF4 - Cooperative Health Research in the Region of Augsburg) PM2.5 exposure was positively associated with EEAA but not with IEAA or DNAm age acceleration. These differing results may be related to different analysis methods and/or PM2.5 components in different regions. In a recent study of non-Hispanic white women, the association with DNAm age acceleration varied by PM2.5 component cluster.[94] For example, DNAm age estimated by the DNAm PhenoAge clock was positively associated with two clusters. One cluster contained relatively high levels of crustal elements and the other low levels of sulfur. The DNAm PhenoAge clock was negatively associated with a PM2.5 cluster of lower relative nitrate concentration.

Social behavior

There are examples of relationships between education and DNAm age acceleration. Four studies reported that educational level was negatively associated with DNA methylation age determined by Hannum's clock,[72,82,95,96] with two

studies finding an association with EEAA.[82,95] The two other studies reported no significant association.[97,98] Of note, socioeconomic status is also an important factor in poor health outcomes. One study reported that lower socioeconomic status in early life was associated with increased DNA methylation age measured by Horvath's clock.[99] Two studies reported no significant association with DNA methylation age.[72,100] Associations between smoking or alcohol consumption and DNA methylation age have been assessed. One study found significant positive associations among smoking and DNA methylation age estimated by Horvath's and Hannum's clocks.[72] However, five other studies reported no such associations.[82,95,98,101,102] Only two studies of alcohol consumption found a significant association with DNA methylation age acceleration,[95,102] while three studies found no such association.[72,82,103]

Diet and body mass index

Three studies investigated the relationship between diet and DNAm age acceleration.[57,72,95] In an early study, EEAA was shown to have a weak but statistically significant correlation with fish intake as well as plasma levels of mean carotenoids and carotene, while the IEAA did not.[95] An association between accelerated DNA methylation age estimated by the DNAm GrimAge clock and plasma levels of carotenoids was demonstrated in a recent study.[57] It is important to understand that self-reported diet variables are difficult to assess accurately. An earlier study found no obvious relationship between diet and accelerated DNA methylation age.[97] Five studies found that there was a positive correlation between body mass index (BMI) and acceleration of DNAm age.[95,104–107] It should be noted that the correlation was inconsistent among different tissues with higher levels in visceral adipose tissue ($r = 0.29$)[104] and liver ($r = 0.42$),[106] and lower levels in the blood ($r = 0.09$).[95] In one of these studies, these relationships only existed during middle age.[105]

Psychological factors

The effects of psychological factors on the acceleration of DNAm age have been assessed. Four studies examined the relationship between accelerated DNA methylation age and childhood trauma,[108–111] with only one reporting a significant association between accelerated DNA methylation age and childhood sexual abuse.[109] Psychological factors may be mediated by glucocorticoids in that 85 CpG sites of Horvath's clock are located in glucocorticoid response elements.[108] Five studies investigated life-long posttraumatic stress disorder (PTSD) in war veterans.[110–114] Four studies found no overall correlation. However, specific PTSD symptoms and the severity of symptoms were associated with increased DNA methylation age.[113] Individual studies also investigated psychiatric symptoms or disorders[115–117] and found no overall correlation for the entire sample but for older patients acceleration in DNA methylation age was found after dividing the sample by median age.[117]

Other factors

Other studies have been conducted *in vitro*. For example, Matsuyama *et al*.[118] reported that the progression of DNA methylation age under hypoxia (1% oxygen) was slower than that under normoxia (21% oxygen) indicating that the oxygen level determined the speed of epigenetic aging. In that study, the hypoxic concentration (1%) was similar to that of the hematopoietic stem cell niche (1%–2%).[119] Other components of the cell culture medium, e.g. high glucose level, also influenced DNA methylation age in that study. The aged cells in hyperglycemic conditions exhibited an approximate 3-year elevation in baseline DNA methylation age. In a subsequent study, aged cells in hyperglycemia conditions showed baseline DNA methylation age of about 3 years.[120]

In summary, health risk factors such as environmental pollution, socioeconomic status, education, smoking, alcohol consumption, and BMI were found to be associated with accelerated DNA methylation age. However, these associations were not necessarily strong, in that single associations were often unreliable. Further, most studies were cross-sectional. A significant proportion of DNA methylation age is due to unknown factors, which may include adverse early life events, health-related genetic variations, or other unmeasured environmental factors. Many variables are self-reported, which may result in measurement errors. Future explorations of unknown factors that explain the temporal changes in DNA methylation age are essential.

DNAm age acceleration and genetic factors

In addition to the above factors, genetics impacts DNA methylation age with high heritability (h^2) estimates of approximately 40%.[56,121] This is consistent with the observation that ticks of DNA methylation clocks are highly stabile throughout life.[122] The importance of the cell-intrinsic factors controlling DNA methylation age has been assessed in a recent study of hematopoietic stem cell transplantation (HSCT).[123] DNA methylation age of the transplanted donor's blood cells progressed without detectable interference from the recipient's age, even 17 years after HSCT.[123] Therefore, identifying genes that affect the rate of the DNA methylation clock may provide insight into the underlying mechanistic basis for the aging process. Deep-Bis DNA methylation analysis has identified several loci associated with DNAm age acceleration. These include *MLST8, EFCAB5*, and *TERT* genes in the cerebellum, multiple brain regions, and the blood, respectively.[124–126] The specific functions of these genes are described in a recent review.[56] Other studies have identified several genes that are highly correlated with DNA methylation age including ELOVL fatty acid elongase 2 (*ELOVL2*) and H3K36 methyltransferase (*NSD1*).[127,128] *ELOVL2* is the master controller of poly-unsaturated fatty acid synthesis and was most significantly related to DNA methylation age. Although the relationship of *ELOVL2* to DNA methylation clocks has been well documented, the mechanism underlying how age-related *ELOVL2* methylation contributes to aging is unknown. A recent report documented a dramatic increase

in *ELOVL2* methylation that was accompanied by down-regulation of *ELOVL2* expression levels in aged human fibroblasts.[128] Moreover, dysfunction of the Elovl2 protein interferes with lipid metabolism and synthesis, increasing endoplasmic reticulum stress and mitochondrial dysfunction, which are associated with accelerated aging. A recent gene screening study found that DNA methylation clocks tick more quickly in the blood of patients with Sotos syndrome, a familial developmental disorder caused by inactivation mutations of NSD1.[127] NSD1 is a histone methyltransferase containing a SET catalytic domain, which regulates the di-methylation of histone H3 lysine 36.[127] Further, the aging process and the Sotos syndrome share methylation changes as shown by genome-wide methylation analysis, which suggest that developmental diseases may serve as models for the study of the mechanistic basis for aging. P53 is considered to be one of the most important cellular tumor suppressors, with numerous studies demonstrating P53 mutations to strongly correlate with a slower acceleration of epigenetic age in several types of malignancies.[4] P53 has been hypothesized to be part of an epigenetic maintenance system. This hypothesis has been supported by a recently published study showing that the P53 inhibitor, SV40LT, maintained or reversed DNAm age acceleration in different protoculture fibroblast lines.[118]

DNA methylation clocks and the anti-aging effect of caloric restriction

A significant number of interventions have been reported to prolong the lifespan of mice. Currently, caloric restriction (CR) and CR mimetics, such as rapamycin, are the most studied anti-aging interventions.[129] Numerous reports have shown CR and CR mimetics to extend lifespan and delay the aging rate of rodents and non-human primates, with a current focus on the identification of an accurate age-related biomarker such as DNA methylation clocks. Petkovich *et al*.[58] demonstrated CR to delay DNAm age acceleration in mice. Rapamycin, an inhibitor of mTOR activity, recapitulates the effect of calorie-restriction. Based on the skin & blood clocks noted in the Section "DNA methylation clocks in animals and humans," epigenetic aging of primary human keratinocytes was independent of replicative senescence, somatic cell differentiation, and cellular proliferation rate, which are delayed by rapamycin.[130] The effects of mTOR on DNA methylation age are consistent with the findings of a study that showed the MLST8 protein, a subunit of the mTOR complex, positively correlated with DNA methylation aging of the human cerebellum.[124] Hence, these experiments not only demonstrate the power of DNAm clocks to reveal biological features at the cellular and organismal levels, but also support the possibility that mTOR pathway signaling may be involved in epigenetic aging.

Future prospects and recommendations

DNA methylation clocks, novel biomarkers of aging, are considered useful for measuring chronological age, as well as reflecting some characteristics of biological aging. As noted above, these clocks have a unique function in capturing differences in biological aging, and they also have great potential in predicting changes in aging, cognitive and physical function, survival rates in association with age-related diseases, as well as identification and/or validation of effective anti-aging interventions. Most aging biomarkers are based on molecules, cell or animal systems, and require strict verification in humans. An epigenetic clock suitable for *in vitro* studies could be used to test the effects of potential anti-aging drugs on lifespan and aging in a relatively short period of time with a low cost, not easily achieved in human cohort studies.[5] However, certain issues deserve attention with regard to the application of DNAm clocks. Such clocks are based on differing statistical methods, specific sample sizes, and tissue types. Each of these must be considered for individual strengths and weaknesses in selection of specific DNAm clocks for evaluation. Moreover, there is no definitive evidence supporting a cause-and-effect relationship between DNA methylation age and aging. Hence, data derived from DNAm clocks should be interpreted prudently with a thorough and comparative analysis with other measures of aging. In this manner, DNAm clocks can be effectively used to improve animal and human health.

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REFERENCES

1. Soto-Perez-de-Celis E, Li D, Yuan Y, Lau YM, Hurria A. Functional versus chronological age: geriatric assessments to guide decision making in older patients with cancer. *Lancet Oncol* 2018;**19**:e305–16
2. Stubbs TM, Bonder MJ, Stark AK, Krueger F, von Meyenn F, Stegle O, Reik W. Multi-tissue DNA methylation age predictor in mouse. *Genome Biol* 2017;**18**:68
3. Hannum G, Guinney J, Zhao L, Zhang L, Hughes G, Sadda S, Klotzle B, Bibikova M, Fan JB, Gao Y, Deconde R, Chen M, Rajapakse I, Friend S, Ideker T, Zhang K. Genome-wide methylation profiles reveal quantitative views of human aging rates. *Mol Cell* 2013;**49**:359–67
4. Horvath S. DNA methylation age of human tissues and cell types. *Genome Biol* 2013;**14**:R115
5. Horvath S, Oshima J, Martin GM, Lu AT, Quach A, Cohen H, Felton S, Matsuyama M, Lowe D, Kabacik S, Wilson JG, Reiner AP, Maierhofer

A, Flunkert J, Aviv A, Hou L, Baccarelli AA, Li Y, Stewart JD, Whitsel EA, Ferrucci L, Matsuyama S, Raj K. Epigenetic clock for skin and blood cells applied to Hutchinson Gilford Progeria Syndrome and ex vivo studies. *Aging (Albany NY)* 2018;**10**:1758–75

6. Maegawa S, Lu Y, Tahara T, Lee JT, Madzo J, Liang S, Jelinek J, Colman RJ, Issa JJ. Caloric restriction delays age-related methylation drift. *Nat Commun* 2017;**8**:539
7. Fahy GM, Brooke RT, Watson JP, Good Z, Vasanawala SS, Maecker H, Leipold MD, Lin DTS, Kobor MS, Horvath S. Reversal of epigenetic aging and immunosenescent trends in humans. *Aging Cell* 2019;**18**: e13028
8. Jylhävä J, Pedersen NL, Hägg S. Biological age predictors. *EBioMedicine* 2017;**21**:29–36
9. Suzuki MM, Bird A. DNA methylation landscapes: provocative insights from epigenomics. *Nat Rev Genet* 2008;**9**:465–76
10. Venter JC, Adams MD, Myers EW, Li PW, Mural RJ, Sutton GG, Smith HO, Yandell M, Evans CA, Holt RA, Gocayne JD, Amanatides P, Ballew RM, Huson DH, Wortman JR, Zhang Q, Kodira CD, Zheng XH, Chen L, Skupski M, Subramanian G, Thomas PD, Zhang J, Gabor MGL, Nelson C, Broder S, Clark AG, Nadeau J, McKusick VA, Zinder N, Levine AJ, Roberts RJ, Simon M, Slayman C, Hunkapiller M, Bolanos R, Delcher A, Dew I, Fasulo D, Flanigan M, Florea L, Halpern A, Hannenhalli S, Kravitz S, Levy S, Mobarry C, Reinert K, Remington K, Abu-Threideh J, Beasley E, Biddick K, Bonazzi V, Brandon R, Cargill M, Chandramouliswaran I, Charlab R, Chaturvedi K, Deng Z, Di Francesco V, Dunn P, Eilbeck K, Evangelista C, Gabrielian AE, Gan W, Ge W, Gong F, Gu Z, Guan P, Heiman TJ, Higgins ME, Ji RR, Ke Z, Ketchum KA, Lai Z, Lei Y, Li Z, Li J, Liang Y, Lin X, Lu F, Merkulov GV, Milshina N, Moore HM, Naik AK, Narayan VA, Neelam B, Nusskern D, Rusch DB, Salzberg S, Shao W, Shue B, Sun J, Wang Z, Wang A, Wang X, Wang J, Wei M, Wides R, Xiao C, Yan C, Yao A, Ye J, Zhan M, Zhang W, Zhang H, Zhao Q, Zheng L, Zhong F, Zhong W, Zhu S, Zhao S, Gilbert D, Baumhueter S, Spier G, Carter C, Cravchik A, Woodage T, Ali F, An H, Awe A, Baldwin D, Baden H, Barnstead M, Barrow I, Beeson K, Busam D, Carver A, Center A, Cheng ML, Curry L, Danaher S, Davenport L, Desilets R, Dietz S, Dodson K, Doup L, Ferriera S, Garg N, Gluecksmann A, Hart B, Haynes J, Haynes C, Heiner C, Hladun S, Hostin D, Houck J, Howland T, Ibegwam C, Johnson J, Kalush F, Kline L, Koduru S, Love A, Mann F, May D, McCawley S, McIntosh T, McMullen I, Moy M, Moy L, Murphy B, Nelson K, Pfannkoch C, Pratts E, Puri V, Qureshi H, Reardon M, Rodriguez R, Rogers YH, Romblad D, Ruhfel B, Scott R, Sitter C, Smallwood M, Stewart E, Strong R, Suh E, Thomas R, Tint NN, Tse S, Vech C, Wang G, Wetter J, Williams S, Williams M, Windsor S, Winn-Deen E, Wolfe K, Zaveri J, Zaveri K, Abril JF, Guigó R, Campbell MJ, Sjolander KV, Karlak B, Kejariwal A, Mi H, Lazareva B, Hatton T, Narechania A, Diemer K, Muruganujan A, Guo N, Sato S, Bafna V, Istrail S, Lippert R, Schwartz R, Walenz B, Yooseph S, Allen D, Basu A, Baxendale J, Blick L, Caminha M, Carnes-Stine J, Caulk P, Chiang YH, Coyne M, Dahlke C, Mays A, Dombroski M, Donnelly M, Ely D, Esparham S, Fosler C, Gire H, Glanowski S, Glasser K, Glodek A, Gorokhov M, Graham K, Gropman B, Harris M, Heil J, Henderson S, Hoover J, Jennings D, Jordan C, Jordan J, Kasha J, Kagan L, Kraft C, Levitsky A, Lewis M, Liu X, Lopez J, Ma D, Majoros W, McDaniel J, Murphy S, Newman M, Nguyen T, Nguyen N, Nodell M, Pan S, Peck J, Peterson M, Rowe W, Sanders R, Scott J, Simpson M, Smith T, Sprague A, Stockwell T, Turner R, Venter E, Wang M, Wen M, Wu D, Wu M, Xia A, Zandieh A, Zhu X. The sequence of the human genome. Science 2001;**291**:1304-51
11. Jones PA. Functions of DNA methylation: islands, start sites, gene bodies and beyond. *Nat Rev Genet* 2012;**13**:484–92
12. Chiappinelli KB, Strissel PL, Desrichard A, Li H, Henke C, Akman B, Hein A, Rote NS, Cope LM, Snyder A, Makarov V, Budhu S, Slamon DJ, Wolchok JD, Pardoll DM, Beckmann MW, Zahnow CA, Merghoub T, Chan TA, Baylin SB, Strick R. Inhibiting DNA methylation causes an interferon response in cancer via dsRNA including endogenous retroviruses. *Cell* 2015;**162**:974–86
13. Ohtani H, Liu M, Zhou W, Liang G, Jones PA. Switching roles for DNA and histone methylation depend on evolutionary ages of human endogenous retroviruses. *Genome Res* 2018;**28**:1147–57
14. Li SY, Park J, Guan Y, Chung K, Shrestha R, Palmer MB, Susztak K. DNMT1 in *Six2* progenitor cells is essential for transposable element silencing and kidney development. *J Am Soc Nephrol* 2019;**30**:594–609
15. Mohandas T, Sparkes RS, Shapiro LJ. Reactivation of an inactive human X chromosome: evidence for X inactivation by DNA methylation. *Science* 1981;**211**:393–6
16. Lessing D, Dial TO, Wei C, Payer B, Carrette LL, Kesner B, Szanto A, Jadhav A, Maloney DJ, Simeonov A, Theriault J, Hasaka T, Bedalov A, Bartolomei MS, Lee JT. A high-throughput small molecule screen identifies synergism between DNA methylation and Aurora kinase pathways for X reactivation. *Proc Natl Acad Sci USA* 2016;**113**:14366–71
17. Waters SA, Livernois AM, Patel H, O'Meally D, Craig JM, Marshall GJA, Suter CM, Waters PD. Landscape of DNA methylation on the marsupial X. *Mol Biol Evol* 2018;**35**:431–9
18. Golden LC, Itoh Y, Itoh N, Iyengar S, Coit P, Salama Y, Arnold AP, Sawalha AH, Voskuhl RR. Parent-of-origin differences in DNA methylation of X chromosome genes in T lymphocytes. *Proc Natl Acad Sci USA* 2019;**116**:26779–87
19. Yin Y, Morgunova E, Jolma A, Kaasinen E, Sahu B, Khund-Sayeed S, Das PK, Kivioja T, Dave K, Zhong F, Nitta KR, Taipale M, Popov A, Ginno PA, Domcke S, Yan J, Schübeler D, Vinson C, Taipale J. Impact of cytosine methylation on DNA binding specificities of human transcription factors. Science 2017;**356**:eaaj2239
20. Wiehle L, Thorn GJ, Raddatz G, Clarkson CT, Rippe K, Lyko F, Breiling A, Teif VB. DNA (de)methylation in embryonic stem cells controls CTCF-dependent chromatin boundaries. *Genome Res* 2019;**29**:750–61
21. Wiehle L, Thorn GJ, Raddatz G, Clarkson CT, Rippe K, Lyko F, Breiling A, Teif VB. DNA (de)methylation in embryonic stem cells controls CTCF-dependent chromatin boundaries. Genome Res 2019;**29**:750-61
22. Greenberg MVC, Bourc'His D. The diverse roles of DNA methylation in mammalian development and disease. *Nat Rev Mol Cell Biol* 2019;**20**:590–607
23. Li T, Wang L, Du Y, Xie S, Yang X, Lian F, Zhou Z, Qian C. Structural and mechanistic insights into UHRF1-mediated DNMT1 activation in the maintenance DNA methylation. *Nucleic Acids Res* 2018;**46**:3218–31
24. Zhang H, Gao Q, Tan S, You J, Lyu C, Zhang Y, Han M, Chen Z, Li J, Wang H, Liao L, Qin J, Li J, Wong J. SET8 prevents excessive DNA methylation by methylation-mediated degradation of UHRF1 and DNMT1. *Nucleic Acids Res* 2019;**47**:9053–68
25. Arand J, Spieler D, Karius T, Branco MR, Meilinger D, Meissner A, Jenuwein T, Xu G, Leonhardt H, Wolf V, Walter J. In vivo control of CpG and non-CpG DNA methylation by DNA methyltransferases. *PLoS Genet* 2012;**8**:e1002750
26. Li Y, Zhang Z, Chen J, Liu W, Lai W, Liu B, Li X, Liu L, Xu S, Dong Q, Wang M, Duan X, Tan J, Zheng Y, Zhang P, Fan G, Wong J, Xu GL, Wang Z, Wang H, Gao S, Zhu B. Stella safeguards the oocyte methylome by preventing de novo methylation mediated by DNMT1. *Nature* 2018;**564**:136–40
27. Yarychkivska O, Shahabuddin Z, Comfort N, Boulard M, Bestor TH. BAH domains and a histone-like motif in DNA methyltransferase 1 (DNMT1) regulate de novo and maintenance methylation in vivo. *J Biol Chem* 2018;**293**:19466–75
28. Sardina JL, Collombet S, Tian TV, Gómez A, Di Stefano B, Berenguer C, Brumbaugh J, Stadhouders R, Segura-Morales C, Gut M, Gut IG, Heath S, Aranda S, Di Croce L, Hochedlinger K, Thieffry D, Graf T. Transcription factors drive TET2-mediated enhancer demethylation to reprogram cell fate. *Cell Stem Cell* 2018;**23**:727–41.e9
29. Fang S, Li J, Xiao Y, Lee M, Guo L, Han W, Li T, Hill MC, Hong T, Mo W, Xu R, Zhang P, Wang F, Chang J, Zhou Y, Sun D, Martin JF, Huang Y. TET inactivation disrupts YY1 binding and long-range chromatin interactions during embryonic heart development. *Nat Commun* 2019;**10**:4297
30. Beck DB, Petracovici A, He C, Moore HW, Louie RJ, Ansar M, Douzgou S, Sithambaram S, Cottrell T, Santos-Cortez RLP, Prijoles EJ, Bend R, Keren B, Mignot C, Nougues MC, Õunap K, Reimand T, Pajusalu S, Zahid M, Saqib MAN, Buratti J, Seaby EG, McWalter K,

Telegrafi A, Baldridge D, Shinawi M, Leal SM, Schaefer GB, Stevenson RE, Banka S, Bonasio R, Fahrner JA. Delineation of a human Mendelian disorder of the DNA demethylation machinery: TET3 deficiency. *Am J Hum Genet* 2020;**106**:234–45

31. Stevenson TJ, Prendergast BJ. Reversible DNA methylation regulates seasonal photoperiodic time measurement. *Proc Natl Acad Sci USA* 2013;**110**:16651–6
32. Coulson RL, Yasui DH, Dunaway KW, Laufer BI, Vogel CA, Zhu Y, Mordaunt CE, Totah TS, LaSalle JM. Snord116-dependent diurnal rhythm of DNA methylation in mouse cortex. *Nat Commun* 2018;**9**:1616
33. Wang L, Ozark PA, Smith ER, Zhao Z, Marshall SA, Rendleman EJ, Piunti A, Ryan C, Whelan AL, Helmin KA, Morgan MA, Zou L, Singer BD, Shilatifard A. TET2 coactivates gene expression through demethylation of enhancers. *Sci Adv* 2018;**4**:eaau6986
34. Maegawa S, Hinkal G, Kim HS, Shen L, Zhang L, Zhang J, Zhang N, Liang S, Donehower LA, Issa JP. Widespread and tissue specific age-related DNA methylation changes in mice. *Genome Res* 2010;**20**:332–40
35. Slieker RC, van Iterson M, Luijk R, Beekman M, Zhernakova DV, Moed MH, Mei H, van Galen M, Deelen P, Bonder MJ, Zhernakova A, Uitterlinden AG, Tigchelaar EF, Stehouwer CD, Schalkwijk CG, van der Kallen CJ, Hofman A, van Heemst D, de Geus EJ, van Dongen J, Deelen J, van den Berg LH, van Meurs J, Jansen R, T HPA, Franke L, Wijmenga C, Veldink JH, Swertz MA, van Greevenbroek MM, van Duijn CM, Boomsma DI, Slagboom PE, Heijmans BT. Age-related accrual of methylomic variability is linked to fundamental ageing mechanisms. *Genome Biol* 2016;**17**:191
36. Hernando-Herraez I, Evano B, Stubbs T, Commere PH, Jan BM, Clark S, Andrews S, Tajbakhsh S, Reik W. Ageing affects DNA methylation drift and transcriptional cell-to-cell variability in mouse muscle stem cells. *Nat Commun* 2019;**10**:4361
37. Heyn H, Li N, Ferreira HJ, Moran S, Pisano DG, Gomez A, Diez J, Sanchez-Mut JV, Setien F, Carmona FJ, Puca AA, Sayols S, Pujana MA, Serra-Musach J, Iglesias-Platas I, Formiga F, Fernandez AF, Fraga MF, Heath SC, Valencia A, Gut IG, Wang J, Esteller M. Distinct DNA methylomes of newborns and centenarians. *Proc Natl Acad Sci USA* 2012;**109**:10522–7
38. Rakyan VK, Down TA, Maslau S, Andrew T, Yang TP, Beyan H, Whittaker P, McCann OT, Finer S, Valdes AM, Leslie RD, Deloukas P, Spector TD. Human aging-associated DNA hypermethylation occurs preferentially at bivalent chromatin domains. *Genome Res* 2010;**20**:434–9
39. Beerman I, Bock C, Garrison BS, Smith ZD, Gu H, Meissner A, Rossi DJ. Proliferation-dependent alterations of the DNA methylation landscape underlie hematopoietic stem cell aging. *Cell Stem Cell* 2013;**12**:413–25
40. Zhou W, Dinh HQ, Ramjan Z, Weisenberger DJ, Nicolet CM, Shen H, Laird PW, Berman BP. DNA methylation loss in late-replicating domains is linked to mitotic cell division. *Nat Genet* 2018;**50**:591–602
41. Thompson RF, Atzmon G, Gheorghe C, Liang HQ, Lowes C, Greally JM, Barzilai N. Tissue-specific dysregulation of DNA methylation in aging. *Aging Cell* 2010;**9**:506–18
42. Bell CG, Lowe R, Adams PD, Baccarelli AA, Beck S, Bell JT, Christensen BC, Gladyshev VN, Heijmans BT, Horvath S, Ideker T, Issa JJ, Kelsey KT, Marioni RE, Reik W, Relton CL, Schalkwyk LC, Teschendorff AE, Wagner W, Zhang K, Rakyan VK. DNA methylation aging clocks: challenges and recommendations. *Genome Biol* 2019;**20**:249
43. Bocklandt S, Lin W, Sehl ME, Sánchez FJ, Sinsheimer JS, Horvath S, Vilain E. Epigenetic predictor of age. *PLoS One* 2011;**6**:e14821
44. Li C, Gao W, Gao Y, Yu C, Lv J, Lv R, Duan J, Sun Y, Guo X, Cao W, Li L. Age prediction of children and adolescents aged 6-17 years: an epigenome-wide analysis of DNA methylation. *Aging (Albany NY)* 2018;**10**:1015–26
45. McEwen LM, O'Donnell KJ McGill MG , Edgar RD, Jones MJ, MacIsaac JL, Lin DTS, Ramadori K, Morin A, Gladish N, Garg E, Unternaehrer E, Pokhvisneva I, Karnani N, Kee MZL, Klengel T, Adler NE, Barr RG, Letourneau N, Giesbrecht GF, Reynolds JN, Czamara D, Armstrong JM, Essex MJ, de Weerth C, Beijers R, Tollenaar MS, Bradley B, Jovanovic T, Ressler KJ, Steiner M, Entringer S, Wadhwa PD, Buss C, Bush NR, Binder EB, Boyce WT, Meaney MJ, Horvath S, Kobor MS. The PedBE clock accurately estimates DNA methylation age in pediatric buccal cells. *Proc Natl Acad Sci USA* 2020;**117**:23329–35
46. Wu X, Chen W, Lin F, Huang Q, Zhong J, Gao H, Song Y, Liang H. DNA methylation profile is a quantitative measure of biological aging in children. *Aging (Albany NY)* 2019;**11**:10031–51
47. Weidner CI, Lin Q, Koch CM, Eisele L, Beier F, Ziegler P, Bauerschlag DO, Jöckel KH, Erbel R, Mühleisen TW, Zenke M, Brümmendorf TH, Wagner W. Aging of blood can be tracked by DNA methylation changes at just three CpG sites. *Genome Biol* 2014;**15**:R24
48. Bekaert B, Kamalandua A, Zapico SC, Van de Voorde W, Decorte R. Improved age determination of blood and teeth samples using a selected set of DNA methylation markers. *Epigenetics* 2015;**10**:922–30
49. Eipel M, Mayer F, Arent T, Ferreira MR, Birkhofer C, Gerstenmaier U, Costa IG, Ritz-Timme S, Wagner W. Epigenetic age predictions based on buccal swabs are more precise in combination with cell type-specific DNA methylation signatures. *Aging (Albany NY)* 2016;**8**:1034–48
50. Hamano Y, Manabe S, Morimoto C, Fujimoto S, Ozeki M, Tamaki K. Forensic age prediction for dead or living samples by use of methylation-sensitive high resolution melting. *Leg Med (Tokyo)* 2016;**21**:5–10
51. Bacalini MG, Deelen J, Pirazzini C, De Cecco M, Giuliani C, Lanzarini C, Ravaioli F, Marasco E, van Heemst D, Suchiman HED, Slieker R, Giampieri E, Recchioni R, Marcheselli F, Salvioli S, Vitale G, Olivieri F, Spijkerman AMW, Dollé MET, Sedivy JM, Castellani G, Franceschi C, Slagboom PE, Garagnani P. Systemic age-associated DNA hypermethylation of ELOVL2 gene: in vivo and in vitro evidences of a cell replication process. *J Gerontol A Biol Sci Med Sci* 2017;**72**:1015–23
52. Day K, Waite LL, Thalacker-Mercer A, West A, Bamman MM, Brooks JD, Myers RM, Absher D. Differential DNA methylation with age displays both common and dynamic features across human tissues that are influenced by CpG landscape. *Genome Biol* 2013;**14**:R102
53. McEwen LM, Jones MJ, Lin DTS, Edgar RD, Husquin LT, MacIsaac JL, Ramadori KE, Morin AM, Rider CF, Carlsten C, Quintana-Murci L, Horvath S, Kobor MS. Systematic evaluation of DNA methylation age estimation with common preprocessing methods and the Infinium MethylationEPIC BeadChip array. *Clin Epigenetics* 2018;**10**:123
54. Levine ME, Lu AT, Quach A, Chen BH, Assimes TL, Bandinelli S, Hou L, Baccarelli AA, Stewart JD, Li Y, Whitsel EA, Wilson JG, Reiner AP, Aviv A, Lohman K, Liu Y, Ferrucci L, Horvath S. An epigenetic biomarker of aging for lifespan and healthspan. *Aging (Albany NY)* 2018;**10**:573–91
55. Zhang Y, Wilson R, Heiss J, Breitling LP, Saum KU, Schöttker B, Holleczek B, Waldenberger M, Peters A, Brenner H. DNA methylation signatures in peripheral blood strongly predict all-cause mortality. *Nat Commun* 2017;**8**:14617
56. Horvath S, Raj K. DNA methylation-based biomarkers and the epigenetic clock theory of ageing. *Nat Rev Genet* 2018;**19**:371–84
57. Lu AT, Quach A, Wilson JG, Reiner AP, Aviv A, Raj K, Hou L, Baccarelli AA, Li Y, Stewart JD, Whitsel EA, Assimes TL, Ferrucci L, Horvath S. DNA methylation GrimAge strongly predicts lifespan and healthspan. *Aging (Albany NY)* 2019;**11**:303–27
58. Petkovich DA, Podolskiy DI, Lobanov AV, Lee SG, Miller RA, Gladyshev VN. Using DNA methylation profiling to evaluate biological age and longevity interventions. *Cell Metab* 2017;**25**:954–60.e6
59. Wang T, Tsui B, Kreisberg JF, Robertson NA, Gross AM, Yu MK, Carter H, Brown-Borg HM, Adams PD, Ideker T. Epigenetic aging signatures in mice livers are slowed by dwarfism, calorie restriction and rapamycin treatment. *Genome Biol* 2017;**18**:57
60. Meer MV, Podolskiy DI, Tyshkovskiy A, Gladyshev VN. A whole lifespan mouse multi-tissue DNA methylation clock. *Elife* 2018;**7**:e40675
61. Thompson MJ, VonHoldt B, Horvath S, Pellegrini M. An epigenetic aging clock for dogs and wolves. *Aging (Albany NY)* 2017;**9**:1055–68
62. Cole JJ, Robertson NA, Rather MI, Thomson JP, McBryan T, Sproul D, Wang T, Brock C, Clark W, Ideker T, Meehan RR, Miller RA, Brown-Borg HM, Adams PD. Diverse interventions that extend mouse

lifespan suppress shared age-associated epigenetic changes at critical gene regulatory regions. *Genome Biol* 2017;**18**:58

63. Hahn O, Grönke S, Stubbs TM, Ficz G, Hendrich O, Krueger F, Andrews S, Zhang Q, Wakelam MJ, Beyer A, Reik W, Partridge L. Dietary restriction protects from age-associated DNA methylation and induces epigenetic reprogramming of lipid metabolism. *Genome Biol* 2017;**18**:56
64. Rusli F, Boekschoten MV, Borelli V, Sun C, Lute C, Menke AL, van den Heuvel J, Salvioli S, Franceschi C, Müller M, Steegenga WT. Plasticity of lifelong calorie-restricted C57BL/6J mice in adapting to a medium-fat diet intervention at old age. *Aging Cell* 2018;**17**:e12696
65. Thompson MJ, Chwiakowska K, Rubbi L, Lusis AJ, Davis RC, Srivastava A, Korstanje R, Churchill GA, Horvath S, Pellegrini M. A multi-tissue full lifespan epigenetic clock for mice. *Aging (Albany NY)* 2018;**10**:2832–54
66. Zhang Q, Vallerga CL, Walker RM, Lin T, Henders AK, Montgomery GW, He J, Fan D, Fowdar J, Kennedy M, Pitcher T, Pearson J, Halliday G, Kwok JB, Hickie I, Lewis S, Anderson T, Silburn PA, Mellick GD, Harris SE, Redmond P, Murray AD, Porteous DJ, Haley CS, Evans KL, McIntosh AM, Yang J, Gratten J, Marioni RE, Wray NR, Deary IJ, McRae AF, Visscher PM. Improved precision of epigenetic clock estimates across tissues and its implication for biological ageing. *Genome Med* 2019;**11**:54
67. Ambatipudi S, Horvath S, Perrier F, Cuenin C, Hernandez-Vargas H, Le Calvez-Kelm F, Durand G, Byrnes G, Ferrari P, Bouaoun L, Sklias A, Chajes V, Overvad K, Severi G, Baglietto L, Clavel-Chapelon F, Kaaks R, Barrdahl M, Boeing H, Trichopoulou A, Lagiou P, Naska A, Masala G, Agnoli C, Polidoro S, Tumino R, Panico S, Dollé M, Peeters PHM, Onland-Moret NC, Sandanger TM, Nøst TH, Weiderpass E, Quirós JR, Agudo A, Rodriguez-Barranco M, Huerta Castaño JM, Barricarte A, Fernández AM, Travis RC, Vineis P, Muller DC, Riboli E, Gunter M, Romieu I, Herceg Z. DNA methylome analysis identifies accelerated epigenetic ageing associated with postmenopausal breast cancer susceptibility. *Eur J Cancer)* 2017;**75**:299–307
68. Kresovich JK, Xu Z, O'Brien KM, Weinberg CR, Sandler DP, Taylor JA. Methylation-based biological age and breast cancer risk. *J Natl Cancer Inst* 2019;**111**:1051–8
69. Marioni RE, Shah S, McRae AF, Chen BH, Colicino E, Harris SE, Gibson J, Henders AK, Redmond P, Cox SR, Pattie A, Corley J, Murphy L, Martin NG, Montgomery GW, Feinberg AP, Fallin MD, Multhaup ML, Jaffe AE, Joehanes R, Schwartz J, Just AC, Lunetta KL, Murabito JM, Starr JM, Horvath S, Baccarelli AA, Levy D, Visscher PM, Wray NR, Deary IJ. DNA methylation age of blood predicts all-cause mortality in later life. *Genome Biol* 2015;**16**:25
70. Chen BH, Marioni RE, Colicino E, Peters MJ, Ward-Caviness CK, Tsai PC, Roetker NS, Just AC, Demerath EW, Guan W, Bressler J, Fornage M, Studenski S, Vandiver AR, Moore AZ, Tanaka T, Kiel DP, Liang L, Vokonas P, Schwartz J, Lunetta KL, Murabito JM, Bandinelli S, Hernandez DG, Melzer D, Nalls M, Pilling LC, Price TR, Singleton AB, Gieger C, Holle R, Kretschmer A, Kronenberg F, Kunze S, Linseisen J, Meisinger C, Rathmann W, Waldenberger M, Visscher PM, Shah S, Wray NR, McRae AF, Franco OH, Hofman A, Uitterlinden AG, Absher D, Assimes T, Levine ME, Lu AT, Tsao PS, Hou L, Manson JE, Carty CL, LaCroix AZ, Reiner AP, Spector TD, Feinberg AP, Levy D, Baccarelli A, van Meurs J, Bell JT, Peters A, Deary IJ, Pankow JS, Ferrucci L, Horvath S. DNA methylation-based measures of biological age: meta-analysis predicting time to death. *Aging (Albany NY)* 2016;**8**:1844–65
71. Dugué PA, Bassett JK, Joo JE, Jung CH, Ming WE, Moreno-Betancur M, Schmidt D, Makalic E, Li S, Severi G, Hodge AM, Buchanan DD, English DR, Hopper JL, Southey MC, Giles GG, Milne RL. DNA methylation-based biological aging and cancer risk and survival: pooled analysis of seven prospective studies. *Int J Cancer* 2018;**142**:1611–9
72. Dugué PA, Bassett JK, Joo JE, Baglietto L, Jung CH, Wong EM, Fiorito G, Schmidt D, Makalic E, Li S, Moreno-Betancur M, Buchanan DD, Vineis P, English DR, Hopper JL, Severi G, Southey MC, Giles GG, Milne RL. Association of DNA methylation-based biological age with health risk factors and overall and cause-specific mortality. *Am J Epidemiol* 2018;**187**:529–38
73. Marioni RE, Harris SE, Shah S, McRae AF, von Zglinicki T, Martin-Ruiz C, Wray NR, Visscher PM, Deary IJ. The epigenetic clock and telomere length are independently associated with chronological age and mortality. *Int J Epidemiol* 2018;**45**:424–32
74. Fransquet PD, Wrigglesworth J, Woods RL, Ernst ME, Ryan J. The epigenetic clock as a predictor of disease and mortality risk: a systematic review and meta-analysis. *Clin Epigenetics* 2019;**11**:62
75. Perna L, Zhang Y, Mons U, Holleczek B, Saum KU, Brenner H. Epigenetic age acceleration predicts cancer, cardiovascular, and all-cause mortality in a German case cohort. *Clin Epigenetics* 2016;**8**:64
76. Christiansen L, Lenart A, Tan Q, Vaupel JW, Aviv A, McGue M, Christensen K. DNA methylation age is associated with mortality in a longitudinal Danish twin study. *Aging Cell* 2016;**15**:149–54
77. Kresovich JK, Xu Z, O'Brien KM, Weinberg CR, Sandler DP, Taylor JA. Epigenetic mortality predictors and incidence of breast cancer. *Aging (Albany NY)* 2019;**11**:11975–87
78. Levine ME, Hosgood HD, Chen B, Absher D, Assimes T, Horvath S. DNA methylation age of blood predicts future onset of lung cancer in the women's health initiative. *Aging (Albany NY)* 2015;**7**:690–700
79. Zheng C, Li L, Xu R. Association of epigenetic clock with consensus molecular subtypes and overall survival of colorectal cancer. *Cancer Epidemiol Biomarkers Prev* 2019;**28**:1720–4
80. Lind L, Ingelsson E, Sundström J, Siegbahn A, Lampa E. Methylation-based estimated biological age and cardiovascular disease. *Eur J Clin Invest* 2018;**48**
81. Roetker NS, Pankow JS, Bressler J, Morrison AC, Boerwinkle E. Prospective study of epigenetic age acceleration and incidence of cardiovascular disease outcomes in the ARIC study (Atherosclerosis Risk in Communities). *Circ Genom Precis Med* 2018;**11**:e001937
82. Horvath S, Gurven M, Levine ME, Trumble BC, Kaplan H, Allayee H, Ritz BR, Chen B, Lu AT, Rickabaugh TM, Jamieson BD, Sun D, Li S, Chen W, Quintana-Murci L, Fagny M, Kobor MS, Tsao PS, Reiner AP, Edlefsen KL, Absher D, Assimes TL. An epigenetic clock analysis of race/ethnicity, sex, and coronary heart disease. *Genome Biol* 2016;**17**:171
83. Soriano-Tárraga C, Giralt-Steinhauer E, Mola-Caminal M, Vivanco-Hidalgo RM, Ois A, Rodríguez-Campello A, Cuadrado-Godia E, Sayols-Baixeras S, Elosua R, Roquer J, Jiménez-Conde J. Ischemic stroke patients are biologically older than their chronological age. *Aging (Albany NY)* 2016;**8**:2655–66
84. Soriano-Tárraga C, Mola-Caminal M, Giralt-Steinhauer E, Ois A, Rodríguez-Campello A, Cuadrado-Godia E, Gómez-González A, Vivanco-Hidalgo RM, Fernández-Cadenas I, Cullell N, Roquer J, Jiménez-Conde J. Biological age is better than chronological as predictor of 3-month outcome in ischemic stroke. *Neurology* 2017;**89**:830–6
85. Soriano-Tárraga C, Giralt-Steinhauer E, Mola-Caminal M, Ois A, Rodríguez-Campello A, Cuadrado-Godia E, Fernández-Cadenas I, Cullell N, Roquer J, Jiménez-Conde J. Biological age is a predictor of mortality in ischemic stroke. *Sci Rep* 2018;**8**:4148
86. Marioni RE, Shah S, McRae AF, Ritchie SJ, Muniz-Terrera G, Harris SE, Gibson J, Redmond P, Cox SR, PA, Corley J, Taylor A, Murphy L, Starr JM, Horvath S, Visscher PM, Wray NR, Deary IJ. The epigenetic clock is correlated with physical and cognitive fitness in the Lothian Birth Cohort 1936. *Int J Epidemiol* 2015;**44**:1388–96
87. Gale CR, Marioni RE, Harris SE, Starr JM, Deary IJ. DNA methylation and the epigenetic clock in relation to physical frailty in older people: the Lothian Birth Cohort 1936. *Clin Epigenetics* 2018;**10**:101
88. Hillary RF, Stevenson AJ, Cox SR, McCartney DL, Harris SE, Seeboth A, Higham J, Sproul D, Taylor AM, Redmond P, Corley J, Pattie A, Hernández M, Muñoz-Maniega S, Bastin ME, Wardlaw JM, Horvath S, Ritchie CW, Spires-Jones TL, McIntosh AM, Evans KL, Deary IJ, Marioni RE. An epigenetic predictor of death captures multi-modal measures of brain health. Molecular psychiatry 2019;
89. Levine ME, Lu AT, Bennett DA, Horvath S. Epigenetic age of the prefrontal cortex is associated with neuritic plaques, amyloid load, and Alzheimer's disease related cognitive functioning. *Aging (Albany NY)* 2015;**7**:1198–211

90. Horvath S, Langfelder P, Kwak S, Aaronson J, Rosinski J, Vogt TF, Eszes M, Faull RL, Curtis MA, Waldvogel HJ, Choi OW, Tung S, Vinters HV, Coppola G, Yang XW. Huntington's disease accelerates epigenetic aging of human brain and disrupts DNA methylation levels. *Aging (Albany NY)* 2016;**8**:1485–512
91. Nwanaji-Enwerem JC, Colicino E, Trevisi L, Kloog I, Just AC, Shen J, Brennan K, Dereix A, Hou L, Vokonas P, Schwartz J, Baccarelli AA. Long-term ambient particle exposures and blood DNA methylation age: findings from the VA normative aging study. *Environ Epigenet* 2016;**2**:dvw006
92. Nwanaji-Enwerem JC, Dai L, Colicino E, Oulhote Y, Di Q, Kloog I, Just AC, Hou L, Vokonas P, Baccarelli AA, Weisskopf MG, Schwartz JD. Associations between long-term exposure to PM(2.5) component species and blood DNA methylation age in the elderly: the VA normative aging study. *Environ Int* 2017;**102**:57–65
93. Ward-Caviness CK, Nwanaji-Enwerem JC, Wolf K, Wahl S, Colicino E, Trevisi L, Kloog I, Just AC, Vokonas P, Cyrys J, Gieger C, Schwartz J, Baccarelli AA, Schneider A, Peters A. Long-term exposure to air pollution is associated with biological aging. *Oncotarget* 2016;**7**:74510–25
94. White AJ, Kresovich JK, Keller JP, Xu Z, Kaufman JD, Weinberg CR, Taylor JA, Sandler DP. Air pollution, particulate matter composition and methylation-based biologic age. *Environ Int* 2019;**132**:105071
95. Quach A, Levine ME, Tanaka T, Lu AT, Chen BH, Ferrucci L, Ritz B, Bandinelli S, Neuhouser ML, Beasley JM, Snetselaar L, Wallace RB, Tsao PS, Absher D, Assimes TL, Stewart JD, Li Y, Hou L, Baccarelli AA, Whitsel EA, Horvath S. Epigenetic clock analysis of diet, exercise, education, and lifestyle factors. *Aging (Albany NY)* 2017;**9**:419–46
96. McCartney DL, Stevenson AJ, Walker RM, Gibson J, Morris SW, Campbell A, Murray AD, Whalley HC, Porteous DJ, McIntosh AM, Evans KL, Deary IJ, Marioni RE. Investigating the relationship between DNA methylation age acceleration and risk factors for Alzheimer's disease. *Alzheimer's & Dementia: Diagnosis, Assessment & Disease Monitoring* 2018;**10**:429–37
97. Simons RL, Lei MK, Beach SR, Philibert RA, Cutrona CE, Gibbons FX, Barr A. Economic hardship and biological weathering: the epigenetics of aging in a U.S. sample of black women. *Soc Sci Med* 2016;**150**:192–200
98. Binder AM, Corvalan C, Mericq V, Pereira A, Santos JL, Horvath S, Shepherd J, Michels KB. Faster ticking rate of the epigenetic clock is associated with faster pubertal development in girls. *Epigenetics* 2018;**13**:85–94
99. Austin MK, Chen E, Ross KM, McEwen LM, Maclsaac JL, Kobor MS, Miller GE. Early-life socioeconomic disadvantage, not current, predicts accelerated epigenetic aging of monocytes. *Psychoneuroendocrinology* 2018;**97**:131–4
100. Simpkin AJ, Howe LD, Tilling K, Gaunt TR, Lyttleton O, McArdle WL, Ring SM, Horvath S, Smith GD, Relton CL. The epigenetic clock and physical development during childhood and adolescence: longitudinal analysis from a UK birth cohort. *Int J Epidemiol* 2017;**46**:549–58
101. Gao X, Zhang Y, Breitling LP, Brenner H. Relationship of tobacco smoking and smoking-related DNA methylation with epigenetic age acceleration. *Oncotarget* 2016;**7**:46878–89
102. Irvin MR, Aslibekyan S, Do A, Zhi D, Hidalgo B, Claas SA, Srinivasasainagendra V, Horvath S, Tiwari HK, Absher DM, Arnett DK. Metabolic and inflammatory biomarkers are associated with epigenetic aging acceleration estimates in the GOLDN study. *Clin Epigenetics* 2018;**10**:56
103. Beach SR, Dogan MV, Lei MK, Cutrona CE, Gerrard M, Gibbons FX, Simons RL, Brody GH, Philibert RA. Methylomic aging as a window onto the influence of lifestyle: tobacco and alcohol use alter the rate of biological aging. *J Am Geriatr Soc* 2015;**63**:2519–25
104. de Toro-Martín J, Guénard F, Tchernof A, Hould FS, Lebel S, Julien F, Marceau S, Vohl MC. Body mass index is associated with epigenetic age acceleration in the visceral adipose tissue of subjects with severe obesity. *Clin Epigenetics* 2019;**11**:172
105. Nevalainen T, Kananen L, Marttila S, Jylhävä J, Mononen N, Kähönen M, Raitakari OT, Hervonen A, Jylhä M, Lehtimäki T, Hurme M. Obesity accelerates epigenetic aging in middle-aged but not in elderly individuals. *Clin Epigenetics* 2017;**9**:20
106. Horvath S, Erhart W, Brosch M, Ammerpohl O, von Schönfels W, Ahrens M, Heits N, Bell JT, Tsai PC, Spector TD, Deloukas P, Siebert R, Sipos B, Becker T, Röcken C, Schafmayer C, Hampe J. Obesity accelerates epigenetic aging of human liver. *Proc Natl Acad Sci USA* 2014;**111**:15538–43
107. Chen M, Wong EM, Nguyen TL, Dite GS, Stone J, Dugué PA, Giles GG, Southey MC, Milne RL, Hopper JL, Li S. DNA methylation-based biological age, genome-wide average DNA methylation, and conventional breast cancer risk factors. *Sci Rep* 2019;**9**:15055
108. Zannas AS, Arloth J, Carrillo-Roa T, Iurato S, Röh S, Ressler KJ, Nemeroff CB, Smith AK, Bradley B, Heim C, Menke A, Lange JF, Brückl T, Ising M, Wray NR, Erhardt A, Binder EB, Mehta D. Lifetime stress accelerates epigenetic aging in an urban, African American cohort: relevance of glucocorticoid signaling. *Genome Biol* 2015;**16**:266
109. Lawn RB, Anderson EL, Suderman M, Simpkin AJ, Gaunt TR, Teschendorff AE, Widschwendter M, Hardy R, Kuh D, Relton CL, Howe LD. Psychosocial adversity and socioeconomic position during childhood and epigenetic age: analysis of two prospective cohort studies. *Hum Mol Genet* 2018;**27**:1301–8
110. Wolf EJ, Maniates H, Nugent N, Maihofer AX, Armstrong D, Ratanatharathorn A, Ashley-Koch AE, Garrett M, Kimbrel NA, Lori A, Va Mid-Atlantic Mirecc W, Aiello AE, Baker DG, Beckham JC, Boks MP, Galea S, Geuze E, Hauser MA, Kessler RC, Koenen KC, Miller MW, Ressler KJ, Risbrough V, Rutten BPF, Stein MB, Ursano RJ, Vermetten E, Vinkers CH, Uddin M, Smith AK, Nievergelt CM, Logue MW. Traumatic stress and accelerated DNA methylation age: a meta-analysis. *Psychoneuroendocrinology* 2018;**92**:123–34
111. Boks MP, van Mierlo HC, Rutten BP, Radstake TR, De Witte L, Geuze E, Horvath S, Schalkwyk LC, Vinkers CH, Broen JC, Vermetten E. Longitudinal changes of telomere length and epigenetic age related to traumatic stress and post-traumatic stress disorder. *Psychoneuroendocrinology* 2015;**51**:506–12
112. Mehta D, Bruenig D, Lawford B, Harvey W, Carrillo-Roa T, Morris CP, Jovanovic T, Young RM, Binder EB, Voisey J. Accelerated DNA methylation aging and increased resilience in veterans: the biological cost for soldiering on. *Neurobiol Stress* 2018;**8**:112–9
113. Wolf EJ, Logue MW, Stoop TB, Schichman SA, Stone A, Sadeh N, Hayes JP, Miller MW. Accelerated DNA methylation age: associations with posttraumatic stress disorder and mortality. *Psychosom Med* 2018;**80**:42–8
114. Wolf EJ, Logue MW, Hayes JP, Sadeh N, Schichman SA, Stone A, Salat DH, Milberg W, McGlinchey R, Miller MW. Accelerated DNA methylation age: associations with PTSD and neural integrity. *Psychoneuroendocrinology* 2016;**63**:155–62
115. McKinney BC, Lin H, Ding Y, Lewis DA, Sweet RA. DNA methylation age is not accelerated in brain or blood of subjects with schizophrenia. *Schizophr Res* 2018;**196**:39–44
116. Fries GR, Bauer IE, Scaini G, Valvassori SS, Walss-Bass C, Soares JC, Quevedo J. Accelerated hippocampal biological aging in bipolar disorder. *Bipolar Disord* 2019.doi: 10.1111/bdi.12876.
117. Fries GR, Bauer IE, Scaini G, Wu MJ, Kazimi IF, Valvassori SS, Zunta-Soares G, Walss-Bass C, Soares JC, Quevedo J. Accelerated epigenetic aging and mitochondrial DNA copy number in bipolar disorder. *Transl Psychiatry* 2017;**7**:1283
118. Matsuyama M, WuWong DJ, Horvath S, Matsuyama S. Epigenetic clock analysis of human fibroblasts in vitro: effects of hypoxia, donor age, and expression of hTERT and SV40 largeT. *Aging (Albany NY)* 2019;**11**:3012–22
119. Carreau A, El Hafny-Rahbi B, Matejuk A, Grillon C, Kieda C. Why is the partial oxygen pressure of human tissues a crucial parameter? Small molecules and hypoxia. *J Cell Mol Med* 2011;**15**:1239–53
120. Sturm G, Cardenas A, Bind MA, Horvath S, Wang S, Wang Y, Hägg S, Hirano M, Picard M. Human aging DNA methylation signatures are conserved but accelerated in cultured fibroblasts. *Epigenetics* 2019;**14**:961–76
121. Jylhävä J, Hjelmborg J, Soerensen M, Munoz E, Tan Q, Kuja-Halkola R, Mengel-From J, Christensen K, Christiansen L, Hägg S, Pedersen NL, Reynolds CA. Longitudinal changes in the genetic and environmental

influences on the epigenetic clocks across old age: evidence from two twin cohorts. *EBioMedicine* 2019;**40**:710–6

122. Kananen L, Marttila S, Nevalainen T, Kummola L, Junttila I, Mononen N, Kähönen M, Raitakari OT, Hervonen A, Jylhä M, Lehtimäki T, Hurme M, Jylhävä J. The trajectory of the blood DNA methylome ageing rate is largely set before adulthood: evidence from two longitudinal studies. *Age (Dordr)* 2016;**38**:65
123. Søraas A, Matsuyama M, de Lima M, Wald D, Buechner J, Gedde-Dahl T, Søraas CL, Chen B, Ferrucci L, Dahl JA, Horvath S, Matsuyama S. Epigenetic age is a cell-intrinsic property in transplanted human hematopoietic cells. *Aging Cell* 2019;**18**:e12897
124. Lu AT, Hannon E, Levine ME, Hao K, Crimmins EM, Lunnon K, Kozlenkov A, Mill J, Dracheva S, Horvath S. Genetic variants near MLST8 and DHX57 affect the epigenetic age of the cerebellum. *Nat Commun* 2016;**7**:10561
125. Lu AT, Hannon E, Levine ME, Crimmins EM, Lunnon K, Mill J, Geschwind DH, Horvath S. Genetic architecture of epigenetic and neuronal ageing rates in human brain regions. *Nat Commun* 2017;**8**:15353
126. Lu AT, Xue L, Salfati EL, Chen BH, Ferrucci L, Levy D, Joehanes R, Murabito JM, Kiel DP, Tsai PC, Yet I, Bell JT, Mangino M, Tanaka T, McRae AF, Marioni RE, Visscher PM, Wray NR, Deary IJ, Levine ME, Quach A, Assimes T, Tsao PS, Absher D, Stewart JD, Li Y, Reiner AP, Hou L, Baccarelli AA, Whitsel EA, Aviv A, Cardona A, Day FR, Wareham NJ, Perry JRB, Ong KK, Raj K, Lunetta KL, Horvath S. GWAS of epigenetic aging rates in blood reveals a critical role for TERT. *Nat Commun* 2018;**9**:387
127. Martin-Herranz DE, Aref-Eshghi E, Bonder MJ, Stubbs TM, Choufani S, Weksberg R, Stegle O, Sadikovic B, Reik W, Thornton JM. Screening for genes that accelerate the epigenetic aging clock in humans reveals a role for the H3K36 methyltransferase NSD1. *Genome Biol* 2019;**20**:146
128. Li X, Wang J, Wang L, Feng G, Li G, Yu M, Li Y, Liu C, Yuan X, Zang G, Li Z, Zhao L, Ouyang H, Quan Q, Wang G, Zhang C, Li O, Xiang J, Zhu JK, Li W, Zhou Q, Zhang K. Impaired lipid metabolism by age-dependent DNA methylation alterations accelerates aging. *Proc Natl Acad Sci USA* 2020;**117**:4328–36
129. Soukas AA, Hao H, Wu L. Metformin as anti-aging therapy: is it for everyone? *Trends Endocrinol Metab* 2019;**30**:745–55
130. Horvath S, Lu AT, Cohen H, Raj K. Rapamycin retards epigenetic ageing of keratinocytes independently of its effects on replicative senescence, proliferation and differentiation. *Aging (Albany NY)* 2019;**11**:3238–49