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Epigenetic aging: Biological age prediction and informing a mechanistic theory of aging

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Numerous studies have shown that epigenetic age—an individual's degree of aging based on patterns of DNA methylation—can be computed and is associated with an array of factors including diet, lifestyle, genetics, and disease. One can expect that still further associations will emerge with additional aging research, but to what end? Prediction of age was an important first step, but—in our view—the focus must shift from chasing increasingly accurate age computations to understanding the links between the epigenome and the mechanisms and physiological changes of aging. Here, we outline emerging areas of epigenetic aging research that prioritize biological understanding and clinical application. First, we survey recent progress in epigenetic clocks, which are beginning to predict not only chronological age but aging outcomes such as all-cause mortality and onset of disease, or which integrate aging signals across multiple biological processes. Second, we discuss research that exemplifies how investigation of the epigenome is building a mechanistic theory of aging and informing clinical practice. Such examples include identifying methylation sites and the genes most strongly predictive of aging—a subset of which have shown strong potential as biomarkers of neurodegenerative disease and cancer; relating epigenetic clock predictions to hallmarks of aging; and using longitudinal studies of DNA methylation to characterize human disease, resulting in the discovery of epigenetic indications of type 1 diabetes and the propensity for psychotic experiences.

Keywords: aging, CpG, epigenetics, longevity, methylation

Introduction

The human genome contains approximately 28 million CpG dinucleotides, in which a cytosine precedes a guanine in the 5'-3' direction. Each of these cytosines can be modified through the addition of a methyl group to create 5-methylcytosine, termed DNA methylation (DNAm). In most contexts, a majority of these sites are indeed methylated, with the specific pattern of methylation varying across cell types, individuals, and conditions [1–3]. DNAm performs essential functions such as regulating transcription via modification of promoters, enhancers, and gene bodies [4–6] and contributing to X chromosome inactivation [7, 8]. For example, it regulates transcription by changing the binding affinity of transcription factors for transcription factor binding sites [9] and impacts the activity of histone-modifying proteins in CpG islands—regions with a large proportion of CpG sites that are typically nonmethylated [10, 11]. Several experimental techniques are used routinely to measure the proportion of cells in a tissue that are methylated at a given CpG site; these measured values are called the CpG methylation fraction (or state). Two of the most common measurement technologies are genome-wide methylation arrays and reduced representation bisulfite sequencing [12].

Nearly a decade ago, our laboratory—and soon thereafter, others—showed that a large number of CpG sites in the human genome increase or decrease in methylation fraction over time, such that one can select among these CpG sites to measure the rate at which an individual ages [13–15]. These so-called "epigenetic clocks" train regularized linear regression models, typically ElasticNet [16], to predict the chronological age

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of an individual from the methylation values of CpG sites distributed across the genome. During training, the CpG sites for which the methylation fractions are most predictive of chronological age are identified and selected for use in the linear regression equation. The number of CpG sites selected has depended greatly on the particular training data and hyperparameters used but is typically between two and a few hundred.

In the time since these epigenetic clocks were introduced, substantial development effort has been invested into improving their predictive accuracy and extending their range of applications. In humans, more recent epigenetic clocks promise to predict age more accurately than their predecessors [17–21], and similar clocks have been developed to predict age in mice [22–24], chimpanzees [25], dogs [26], and naked mole rats [27–29]. Furthermore, a specific set of age-related methylation changes were demonstrated to be conserved across humans, dogs, and mice [30], and such conservation has now been shown to apply to many mammalian species [31]. In addition, the rate of aging—both its pace and acceleration—has been quantified by DunedinPACE, an epigenetic clock that uses 20 years of longitudinal methylation data to form a novel biomarker of the pace of aging [32]. Finally, the first randomized clinical trial using an epigenetic clock as the main validator of intervention efficacy was recently conducted [33]. The prediction of epigenetic age has also been made more accessible and efficient; epigenetic clock software packages are readily available, with some requiring methylation values at only a few CpG sites for accurate age predictions [14, 21].

While optimization of existing concepts and methods is important, it is also vital that the field keeps moving. Beyond the construction of increasingly accurate chronological clocks, there are many unanswered questions related to the specific mechanisms by which the epigenome influences aging and, reciprocally, by which aging influences the epigenome. In this review, we endeavor to highlight and define some of these major unaddressed areas and opportunities. We begin by outlining efforts to train epigenetic clocks more directly against biological aging outcomes or to integrate clocks with multiple data layers. We then consider how, even as significant new data and methods are still being developed, the findings of current clocks can be used to further a molecular theory of aging and evaluate clinical aging interventions. Throughout, we highlight the hierarchy of measurement scales used to characterize aging and how these might ultimately be integrated to understand how macroscopic aging arises from multiple molecular processes (Fig. 1).

Moving from chronological to biological clocks

Epigenetic clock models were originally built using biomarkers of a single type, DNAm, which were used to predict chronological age [13, 15, 34, 35]. These first-generation models can thus be called "chronological clocks." In contrast, a second generation of epigenetic clocks has been formulated that uses DNAm to predict biological attributes, such as time to death or functional decline [17, 19]. These so-called "biological clocks" have shown to be better indicators of health outcomes during aging. Generally, however, it has been extremely difficult to separate aging into strictly independent chronological and biological components, such that all clocks fall somewhere on a spectrum between the two extremes (Fig. 2). Two people with the same birthday by definition have the same chronological age, but they may have different biological ages due to variations in lifestyle, environmental factors, disease, and so on [34, 36].

The blurry line between biological and chronological aging

All present epigenetic clocks lie on a spectrum between perfectly predicting biological and chronological age. The penalized regression process limits a clock to only include CpG sites that are correlated with the target variable. Therefore, the critical difference between chronological and biological clocks is that chronological clocks are limited to including only CpG sites that correlate with chronological age, while biological clocks can more broadly include CpG sites that correlate with biological attributes forming that clock's definition of biological age. However, due to imperfections in measurement technology, finite training data, and close correlations between chronological and biological age-associated CpG sites, an asymptotic approach to each end of the spectrum is the most that can be expected.

Imperfections in both types of clocks are expected and inevitable; however, it is important to be able to distinguish predictions for biological age from those for chronological age so that one can interpret predictions effectively. For example, in a biological clock forecasting time to death, the impact

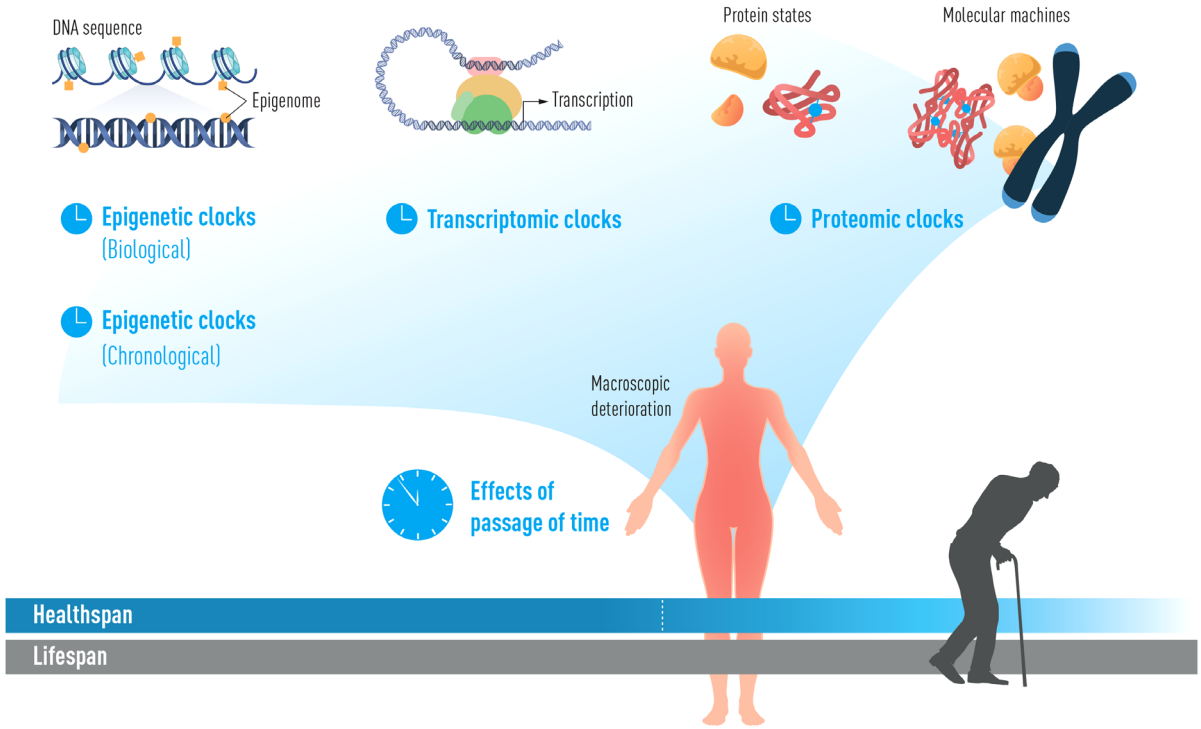

Fig. 1 *Scales of aging. Aging impacts molecular to macroscopic scales, resulting in age-related deterioration of physical components and mental faculties. Relationships between various molecular processes and the passage of time can be observed through aging clocks, but ultimately these relations need to be integrated across scales. For visual simplicity, not all possible relationships between molecular processes are shown.*

that chronological age has on this prediction would need to be calculated by possibly observing the marginal change in the prediction of time to death across samples. If all the variation in time-to-death prediction was explained by variation in chronological age, then it would be clear that this clock is not actually encoding biological age information. Future methods should quantify the degree to which their predictions are dependent on chronological age versus biological factors.

Moving past using chronological clocks to predict biological attributes

Present chronological clocks use biological data, such as DNAm, to make their predictions [13, 15] (Fig. 2). The values of such data are observed to change with age, but they also vary between samples due to non-age-related factors such as differing environments and lifestyles. This effect is evident in the older chronological ages predicted for smokers and people with cancer in the pioneering Hannum and Horvath clocks [13, 15, 34]. This increase in predicted age relative to actual chronological age is termed the "age acceleration residual (AAR)" and has been shown to correlate with increased risk of mortality [37–39].

However, a perfect chronological clock would always have a zero AAR. Furthermore, empirically, biological clocks have been shown to outperform the AAR from chronological clocks in predicting mortality and aging phenotypes [18], and the association of AAR and mortality has been shown to become insignificant as the training datasets of chronological clocks increase [38]. Therefore, if the goal is to investigate the causes of human aging, a biological clock should be used. Chronological clocks can be applied to areas such as forensics or identifying the age of wild animals.

The first biological clock developed was PhenoAge, which uses DNAm to predict a quantity termed "phenotypic age" [19]. Phenotypic age is a measure of biological age derived from 42 clinical biomarkers measured in 9926 individuals enrolled in the

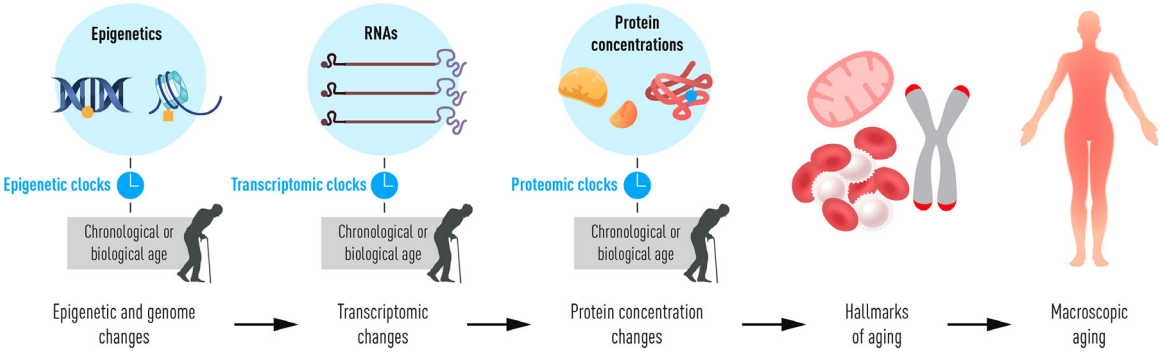

Fig. 2 *A spectrum of epigenetic clocks. On the right of the spectrum (blue) are chronological clocks, which try to predict time since birth. On the left (green) are clocks trained to predict biological attributes. There exist many possible variations. The current clocks fall towards the middle of the spectrum (blue–green), either by design or as a result of imperfect methods and data, yielding predictions that include aspects of chronological and biological age, but neither perfectly, since these two concepts are highly correlated.*

National Health and Nutrition Examination Survey (NHANES), a long-term longitudinal health assessment study run by the US Centers for Disease Control and Prevention [40]. First, a penalized linear regression model was applied to select the 10 NHANES biomarkers that were most predictive of mortality. These included creatine, C-reactive protein, white blood cell count (WBC), and so on. Phenotypic age was defined as a weighted combination of these 10 biomarkers. Next, a DNAm-based clock was trained in the standard way—using an ElasticNet regression model to select an optimally sparse set of CpG sites—but was optimized to predict phenotypic age instead of chronological age.

The predictive power of PhenoAge was subsequently validated on a broad variety of variables such as morbidity and physical functioning. When compared to the widely studied Hannum and Horvath chronological clocks, PhenoAge was significantly more predictive of 10-year and 20-year all-cause mortality risk. Furthermore, elevated phenotypic age was strongly associated with age-related comorbidities and deficits of physical function, even adjusting for a subject's chronological age. This is a significant advance because insofar as chronological clocks are able to predict age-related comorbidities and decline in physiological functions, this association occurs because chronological age is correlated with these factors [38]. In contrast, the association between PhenoAge and physiological decline exists even after adjusting for chronological age, suggesting that this clock relies on changes in DNAm more associated with biological aging for predicting these attributes.

Following PhenoAge, another biological clock was created, called GrimAge [17]. As training data, this clock included DNAm, the levels of 88 plasma proteins, and number of smoking pack-years. Training was split into two stages. First, linear regression models were built to predict the levels of each plasma protein and the number of smoking pack-years, using DNAm. The outputs of each of these models were called "DNAm surrogate biomarkers." In the second stage of training, the DNAm surrogate biomarkers for 12 of the plasma proteins, smoking pack-years, chronological age, and sex were used to form a new clock trained to predict time to death—using only DNAm as input [17].

The GrimAge clock was predictive of aging-related events and time to death. In predicting time to death, GrimAge outperformed all previous epigenetic clocks that it was tested against (Horvath 2013, Hannum, and PhenoAge) as well as the actual biomarkers used to train the clock (e.g., plasma protein levels and self-reported pack-years). Furthermore, the individual DNAm surrogate biomarkers for smoking pack-years and four of the plasma proteins were better predictors of lifespan than actual self-reported pack-years or clinical measures of plasma protein levels [17]. This suggests that the inclusion of these clinical measures in training the GrimAge clock may better uncover the DNAm correlates of biological aging; however, determining which measures are optimal for this task warrants further investigation. Recently, the Hannum, Horvath 2013, PhenoAge, and GrimAge clocks were tested against each other to study their ability to predict nine functional

phenotypes including walking speed, grip strength, frailty, polypharmacy (i.e., the number of currently prescribed drugs), Mini-Mental State Examination, Montreal Cognitive Assessment, Sustained Attention Reaction Time, and two-choice reaction time. In this analysis, each clock was modified to be independent of chronological age and WBC, as described by Fiorito et al. [41]. While the Hannum and Horvath clocks were each significantly predictive ($p < 0.05$) of a single phenotype (polypharmacy and grip strength, respectively), the PhenoAge and GrimAge were significantly predictive of 4/9 and 8/9 phenotypes, respectively [18]. Additionally, adjusted for socioeconomic and lifestyle factors, only GrimAge was significantly associated with all-cause mortality (hazard ratio [HR] = 1.91, $p = 0.004$) [18]. However, on a different dataset, adjusting for many of the same covariates (including WBC and age), the Hannum and Horvath clocks were shown to be predictive of mortality [37]. This underscores the importance of the datasets and covariates chosen when conducting such analyses.

In addition to surpassing the first generation of epigenetic clocks (chronological clocks) in the tasks of predicting mortality risk and functional phenotypes, the PhenoAge and GrimAge biological clocks are predictive of a wide variety of disease states. Reproducing the observed association between DNAm and cancer in chronological clocks [13, 15, 42], PhenoAge and GrimAge associate with time to any cancer (HR = 1.01 and 1.07, respectively) and PhenoAge with mortality from cancer (HR = 1.07). Biological age predictions from both clocks also associate with cardiovascular disease and in PhenoAge with Alzheimer's disease (HR = 1.04). These HRs are significant but small, implying the risk of disease rises slowly with an increase in biological age. Also, in the context of the concept of "inflammaging" [43–45], it is of note that genes associated with an older PhenoAge prediction were enriched for pro-inflammatory signaling pathways, and markers of inflammation were associated with multiple constituent biomarkers in GrimAge [17, 19]. These associations with disease provide another line of support that biological clocks relate to clinically relevant attributes. While biological age predictions are currently most appropriately used in basic science research, the observed associations with specific diseases can motivate future development of more specific epigenetic tests, as has been done with the use of DNAm in cancer prediction [46, 47] and neurodegenerative diseases [48].

On top of guiding the development of epigenetic biomarkers for disease, biological clocks are advancing towards becoming tools for informing clinical diagnoses and testing longevity-promoting therapies. Many clinical diagnoses are informed by patient-reported data where the accuracy of the report varies [49]. Moreover, the same external factor can have varying impacts on different individuals' health. Likely for these reasons, the GrimAge DNAm surrogate biomarker of smoking pack-years was a more accurate predictor of time to death than self-reported smoking pack-years [17]. Thus, a potential utility of epigenetic biomarkers is to reduce the reliance on subjective self-reporting and instead provide a more accurate assessment using the methylome. Following this same idea of using an epigenetic clock biomarker in addition to, or instead of, traditional clinical biomarkers, epigenetic age has been used to assess the effect of purported longevity-promoting interventions including metformin [50, 51], caloric restriction [22, 23, 52], and rapamycin [22, 53, 54]. So, biological epigenetic clocks can theoretically function as biomarkers relevant to a variety of domains; however, more evaluation must be done before assigning high credence to their reports.

Exploring relationships beyond epigenetics

Following the introduction of DNAm clocks, the next step was to ask whether other biomolecular data can be related to aging. Recently, using a novel approach based on an ensemble of classifiers, an aging clock based on the human transcriptome was created [55]. This clock was shown to be predictive of chronological age (mean absolute error (MAE) = 7.7 years, R^2 = 0.81), but less so than the best DNAm-based clocks (Horvath-2018 R = 0.89, MAE = 1.2, Hannum R = 0.9, root mean squared error (RMSE) = 4.89) [15, 54]. Subsequently, a similar model was created for *C. elegans*, named BiT age [56]. Using RNA-seq measurements in a similar fashion to DNAm measurements in DNAm-based clocks, an ElasticNet regression model was trained to predict chronological age, resulting in accurate age predictions—on par with the best human DNAm clocks [56]. These studies raise the question of whether transcript abundance and DNAm in the same regions of the genome are associated with aging, and if so, does one lead to the other?

Moving from RNA to proteins, multiple studies of the human proteome have revealed proteins with underlying associations with aging. In addition to

GrimAge (see above), which was based on levels of several plasma proteins, Lehallier et al. performed a proteome-wide selection of protein markers, producing 373 proteins that were very accurate at predicting chronological age (R = 0.93–0.97) [57]. Johnson et al. produced a similar clock by identifying 32 proteins that were associated with both age and aging disorders, which made commensurately accurate predictions of chronological age (R = 0.9, MAE = 5.5), but needed only one quarter of the number of proteins [58].

Beyond the protein concentrations themselves, the posttranslational modification of certain proteins by glycans has also been shown to track chronological age and certain physiological states [59–61]. Glycans are a diverse group of polysaccharide macromolecules that have varied biological roles, ranging from stabilizing other molecular structures to acting as ligands modulating protein–protein interactions [62]. Immunoglobulin G (IgG) glycosylation, which affects IgG binding affinity, was shown to correlate with chronological age by GlycanAge (R = 0.76, MAE = 9.7)—remarkably, using information about the abundance of just three glycans [59]. Glycans have since been demonstrated to relate to obesity [61] and diseases of inflammation, cancer, and autoimmunity [60], which also lead to increased GlycanAge predictions.

Collectively, this work demonstrates that, in addition to the methylome, the transcriptome, proteome, and posttranslational modifications are closely associated with aging. It is unclear whether these are separate or related causes of aging. However, in the near future, it may be possible to infer how a change in one level of data creates downstream effects in others, leading to aging phenotypes, or to distinguish each as a separate mechanism. Moreover, a detailed understanding of these relationships begins to form a mechanistic explanation of aging.

To what degree do epigenetics reflect or orchestrate hallmarks of aging?

DNAm has been shown to correlate with numerous molecular and cellular processes, some of which are themselves hallmarks of aging. These include cellular senescence, telomere attrition, and stem cell exhaustion (Fig. 3) [63]. For instance, at certain loci, DNAm correlates with cellular senescence arising from repeated cell divisions [64, 65], such that the methylation values of a subset of these loci can estimate the number of divisions a cell has undergone [66, 67]. Furthermore, there is a modest correlation between age-related variation and senescence-related variation of DNAm levels [68], and some epigenetic clocks [19, 69] have been demonstrated to predict increased epigenetic ages as fibroblast cultures progressed from early passage to replicative senescence [70]. On the other hand, epigenetic age estimates continue to increase even in the presence of telomerase, which prevents telomere attrition and replicative senescence. This finding indicates that age-related and senescence-related DNAm changes may be, at least partially, epigenetically distinct [71]. A further divergence between the epigenetics of age and senescence appears when induced pluripotent stem cells (iPSCs) are created [72]. Upon induction of pluripotency, DNAm measures of both epigenetic age and replicative senescence are reset, but when the iPSCs are redifferentiated, DNAm patterns attributed to senescence reappear, while age-related ones do not [65, 73, 74]. These findings suggest that epigenetic aging patterns are not simply an advance toward cellular senescence, nor of telomere attrition.

One hallmark of aging that does relate to epigenetic aging is stem cell exhaustion. DNAm has been found to be important for stem cells maintaining their undifferentiated state [75]. Current epigenetic clocks are based on methylation values from tissues, rather than individual cells. Thus, if stem cells are exhausted in a given tissue, these changes in tissue composition may lead to changes in the epigenetic age prediction of that tissue [35, 38]. However, epigenetic age predictions have been shown to be significant even when using datasets containing a single cell type [76] or when cell type composition is taken into account [39]; thus, stem cell exhaustion leading to changes in tissue composition may not be the only cause of epigenetic aging. Future research should investigate why this disconnect exists and seek to better understand the true relationship between epigenetic clocks, DNAm, and the hallmarks of aging.

Age-associated CpG sites and corresponding genes

For epigenetic clocks to inform mechanisms of aging, the particular regions of the epigenome that correlate with aging must be made clear. If the methylation of certain CpG sites truly does have a downstream impact on aging, there must be a chain of events that can be identified as to how this

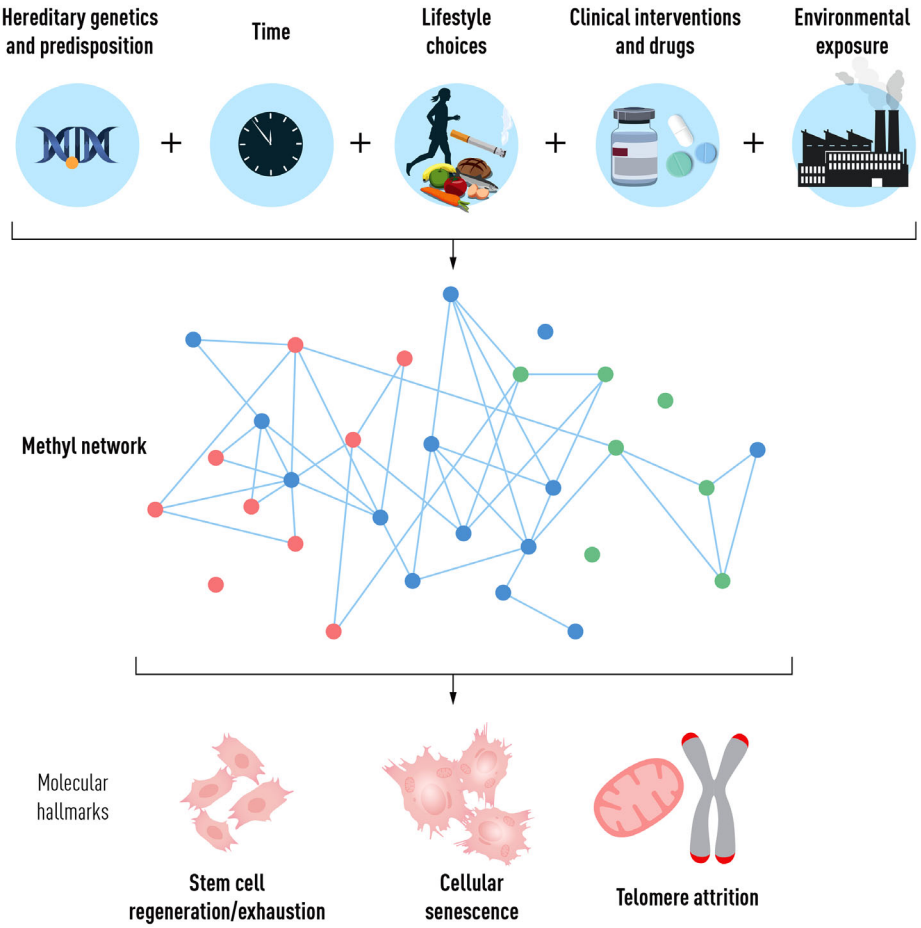

Fig. 3 *Connection between the environment, methylome, and the hallmarks of aging. Top: Some of the many macroscopic factors related to aging have also been linked to changes in the methylome. Bottom: Molecular hallmarks of aging that have also been linked to changes in the methylome, while remaining distinct effects.*

impact occurs. Alternatively, if the modifications of these CpG sites are a byproduct of another process which itself is a cause of aging, then one might still seek a traceable pathway of events in which epigenetic modifications are a component of the chain.

The first step in this direction is to investigate the downstream effects of common aging-associated epigenetic changes (Table 1) and seek causal relationships between these changes and gene expression. For instance, ELOVL2 (elongation of very long-chain fatty acids protein 2) catalyzes reactions in the long-chain fatty acids elongation cycle involved in lipid homeostasis [77, 78] and retinal function [79]. A murine study found a correlation between the downregulation of this gene and age [80]. Remarkably, this study also showed that when hypermethylation of the ELOVL2 promoter is reversed in vivo, retinal function consequently improves. This finding suggests a causal pathway from increased methylation at the ELOVL2 promoter (an epigenetic change) to decreased ELOVL2 gene expression (a transcriptomic change), to reduced retinal function (a phenotypic change). The study of ELOVL2 is not complete, but it serves as an example for future research into epigenetic marks that may underlie aging phenotypes.

The analysis of CpG sites can also be useful in explaining why epigenetic clocks make the predictions they do. In a novel analysis, 5717 CpG sites previously used in 20 epigenetic clocks were clustered by their covariance and range of methylation values across chronological ages [74].

Table 1. *Genes associated with the most common CpG sites used by epigenetic clocks*

Gene nearby CpG site	Number of uses in published clocks	Corresponding list of clock citations
EDARADD	8	13, 15, 19, 69, 81–84
ELOVL2	7	25, 84–89
FHL2	4	84, 85, 87, 88
KLF14	4	84, 87, 88, 90
CCDC102B	3	25, 84, 89
C1orf132	3	84, 87, 88
TRIM59	2	87, 88
ZNF423	2	25, 89
PDE4C	1	84
SST	1	84
TOM1L1	1	81
NPTX2	1	81
PENK	1	85

Note: While certain CpG sites do appear in many epigenetic clocks, there may exist other CpG sites that are highly correlated with these frequently appearing ones and are equally predictive of aging, but were removed by the penalized regression for their redundancy.

This produced 12 clusters of CpG sites, termed "modules," which exhibited strong biological associations. Some modules were characterized by a loss of DNAm as chronological age increased or there was a gain in methylation during epigenetic reprogramming or an exponential change in methylation during development. Further analysis uncovered that across time, certain modules seemed to display epigenetic drift—their methylation values regressing to the mean—while a few showed strong directional changes. This study begins to help explain the mechanistic drivers of variations in epigenetic age prediction by connecting subsets of CpG sites to specific biological conditions—another step on the path towards a mechanistic understanding of aging.

Longitudinal DNAm studies

The clocks discussed thus far are cross sectional, analyzing a snapshot of an individual's methylome at a moment in time. In contrast, longitudinal methylation studies enable the study of changes in methylation in single individuals across time, facilitating a clearer investigation of which regions become methylated as a result of, or portending, disease and aging.

Recent studies have recognized differences in longitudinal methylation changes when comparing diseased individuals to controls. In addition to locations where the amount of methylation is differential across individuals at a single time (cross-sectional differential methylation), there also exist locations where the direction, magnitude, and rate of change of methylation is significantly different across time points. In type 1 diabetes, 10 significant cross-sectional differentially methylated regions, two regions whose longitudinal rate of change of methylation was differential (DCMRs: longitudinal differentially changing methylation regions), and 28 regions whose average methylation across time were differential were found [91]. In previous research, five of these regions that were identified as DCMRs had been found to be associated with changes in gene expression [92, 93], suggesting a regulatory basis for their association with type 1 diabetes. Similarly, CpG sites were found, at both ages 7 and 15–17, that were differentially methylated between children who would and would not go on to have psychotic experiences [94]. The existence of these differences in methylation suggests the possibility of detecting a predisposition to psychotic experiences and type 1 diabetes using the epigenome, well before symptoms are present. Similarly, future investigation may reveal longitudinal differences in methylation associated with aging.

Twins are a popular form of longitudinal study as the juxtaposition of two genetically identical patients offers a valuable perspective on the relative effects of environmental and genetic factors. A study of elderly Swedish twins took place over 20 years with up to five measurements of DNAm per person during this time [95]. In this study, a total of 1316 CpG sites were found to be significantly associated with age. The CpG with the highest association was located in the ELOVL2 gene [88, 96]. This study also found that intratwin methylation differences increased over time more in dizygotic twins than in monozygotic twins, suggesting a genetic effect on the methylome. This genetic effect was further supported by the fact that age-associated CpGs were more often significantly associated with at least one single nucleotide polymorphism (SNP) (9.2%) when compared to non-age-associated CpGs (3.7%) [95]. Genetic effects on the methylome have generally been shown to be steady across time [95, 97, 98], with environmental factors accounting for 90% of the change in methylation of CpG sites as we age [99].

Longitudinal studies have also allowed for the testing of longevity-promoting interventions and their effects on the methylome. Metformin was demonstrated to extend lifespan in humans and model organisms [100–102] and have genome-wide impacts on methylation [103–105]. Rapamycin administration to mice for 22 months was shown to significantly decrease DNAm epigenetic age when compared to control mice [22]. However, marmosets treated with rapamycin for 2–3.5 years did not exhibit a significant decrease in DNAm epigenetic age [53]. Caloric restriction has also been shown to be preventative of age-related DNAm changes in multiple tissue types [24, 106] and has been demonstrated to decrease predicted DNAm epigenetic age in mice and rats—and, in one study, in rhesus monkeys [22, 23, 52]. Thus, the studies thus far are somewhat conflicting, and more research is needed to resolve the apparent discrepancies.

Conclusion

Here we have reviewed programs of epigenetic aging research, which may lead towards an increase in clinical applicability and a deeper understanding of how epigenetics influence, and are influenced by, age-related deterioration. We have discussed how epigenetic clocks are evolving from chronological to biological, studies of methylation are broadening from cross sectional to longitudinal, and links are being drawn between age-related DNAm changes, other -omics data types (genome, transcriptome, proteome), and the hallmarks of aging. The sophistication of epigenetic clocks today is greater than it was a decade ago because the tools have broader reach, and we fully expect this trend to continue.

Conflict of interest

T. I. is the cofounder and a member of the advisory board for Data4Cure, Inc. and has an equity interest. T. I. has an equity interest in Ideaya BioSciences, Inc. and is a member of the Scientific Advisory Board. The terms of this arrangement have been reviewed and approved by the University of California, San Diego in accordance with its conflict-of-interest policies.

Author contributions

Adam Li: Conceptualization; formal analysis; investigation; methodology; project administration; validation; visualization; writing – original draft; writing – review and editing. Zane Koch: Conceptualization; formal analysis; investigation; methodology; project administration; validation; visualization; writing – original draft; writing – review and editing. Trey Ideker: Conceptualization; formal analysis; funding acquisition; investigation; methodology; project administration; supervision; validation; visualization; writing – review and editing.

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