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# Environment, Epigenetics, and the Pace of Human Aging

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## Abstract

The trajectory of human aging varies widely from one individual to the next due to complex interactions between the genome and the environment that influence the aging process. Such differences in age-specific mortality and disease risk among same-aged individuals reflect variation in the pace of biological aging. Certain mechanisms involved in the progression of biological aging originate in the epigenome, where chemical modifications to the genome are able to alter gene expression without modifying the underlying DNA sequence. The epigenome serves as an interface for environmental signals, which are able to "get under the skin" to influence health and aging. A number of the molecular mechanisms involved in the aging process have been identified, although few aging phenotypes have been definitively traced to their underlying molecular causes thus far. In this review, we discuss variation in human biological aging and the epigenome's role in promoting heterogeneity in human longevity and healthspan.

## 1. INTRODUCTION

The demographics of the global population are changing in unprecedented ways. Over the next 3 decades, the number of adults aged 60 and older will increase twofold, to 2.1 billion, while the number of adults aged 80 and older will triple to 426 million (WHO 2021). Since the mid-twentieth century, average life expectancy has increased by more than 20 years, due in large part to medical and technological advancements, improved treatment of infectious disease, better sanitation, and food security in many parts of the world (K. Christensen et al. 2009, Vaupel et al. 2021), although discrepancies in the availability and accessibility of health care, technological, and nutritional resources still exist within and across national borders. However, the rise in average global life expectancy has not been accompanied by reductions in rates of chronic, noncommunicable disease, reflecting a lack of substantial progress in also extending average healthspan (i.e., the number of years a person maintains their desired quality of life free of major age-related disease or disability) (Crimmins 2015). As the older-adult population continues to expand, so does an already urgent need for a comprehensive social and public health infrastructure capable of supporting individuals as they age (Briller & Carrillo 2020, Crews 2022). Such infrastructure will require large investment, particularly in countries where health care systems are focused on addressing the burden of communicable diseases (i.e., infectious and parasitic diseases and maternal, perinatal, and nutritional conditions) and less focused on the types of chronic, noncommunicable diseases commonly associated with increased age. In addition, popular interest in "healthy aging"—maintaining feelings of physical and cognitive health, personal autonomy, and continued engagement with one's community throughout old age—has grown substantially in recent years (van Leeuwen et al. 2019). However, the question of how best to distinguish unhealthy aging from the natural but largely benign processes of physiological aging remains unresolved (Bribiescas 2020, Crews 1993, Galkin et al. 2020). Answering this question is key to supporting our burgeoning aging population as effectively and efficiently as possible, a goal that must include efforts aimed at prevention and therapeutic intervention while avoiding overtreatment that detracts from quality of life or personal autonomy for uncertain or very modest improvements in health.

Aging is a gradual process of physiological deterioration and decline that correlates with increased disease and mortality risk. The aging process itself is a complex, multifactorial phenomenon. The progression of age-related change involves myriad physiological and molecular systems, which interact with one another, the genome, and the environment to influence relative health and disease risk as humans grow older (Benayoun et al. 2015, Booth & Brunet 2016). The shape and apparent pace of aging (or aging trajectory) can vary between individuals. In this review, we discuss a question central to the study of human aging: Why do some individuals age more rapidly or more slowly than others? We center our investigation of this question around the molecular underpinnings of age-related biological change. More specifically, we discuss mechanisms under the control of the epigenome, a network of regulatory mechanisms that play an important role in the aging process (Booth & Brunet 2016, Brunet & Rando 2017, Issa 2014). A deeper understanding of how the external world is able to "get under the skin" is essential to advancing our knowledge of human biological variation and separating inherited from environmentally driven heterogeneity in health and disease risk. While the highly varied environments in which people around the world reside make this question challenging to answer, the field of epigenomics has provided powerful new tools for quantifying variation in human health and identifying some of the sources that influence heterogeneity in aging and disease risk in unprecedented ways.

As interdisciplinary scholars, anthropologists study human health and biology in the context of varying natural and social environments, as well as across biological systems and timescales (Kuzawa & Thayer 2011, Trevathan 2007). This approach aligns with the resilience-based

framework of aging recently proposed by Promislow and colleagues (2022), which integrates the fields of evolution, ecology, physiology, and molecular biology to interrogate gaps in knowledge left unfilled by traditional research efforts. By nature of their training, many anthropologists are inherently well positioned to help guide the trajectory of this expanding and increasingly complex area of human research.

## 2. WHAT IS AGING AND WHY DO WE AGE?

One may first ask why aging should occur at all. Why does the human body decline over time, rather than continually self-renewing and restoring function? Humans must allocate a finite amount of energy to processes of growth, somatic maintenance, and reproduction, which has necessitated evolutionary trade-offs that result in biological aging (Bribiescas 2020). Which molecular processes underlie human aging and age-related decline and deterioration? To address these questions and better understand variation in the aging process of our species, it is important to identify proximate mechanisms that functionally enable age-related deterioration and the ultimate (evolutionary) explanations for why such mechanisms persist.

Evolutionary theory provides a critical lens for understanding aging and our species' vulnerability to a number of age-related diseases (Flatt & Partridge 2018). The process of human aging is ultimately shaped by a combination of interindividual genetic variation, effects from the lived environment, and the much longer evolutionary history of our species (Gluckman et al. 2019). This framework has informed our understanding of the variation observed in human aging. Two enduring theories of aging are the mutation accumulation hypothesis and antagonistic pleiotropy. These theories are not mutually exclusive, and neither provides a complete or sufficient explanation for the origins of biological aging. Nonetheless, the tenets of these theories have served as a foundation for much of the aging research conducted over the last half-century.

The mutation accumulation hypothesis is built on the concept of a selection shadow and suggests that deleterious mutations may not become evident until after an organism has already reproduced; thus, seemingly neutral mutations continue to be passed down across generations because the power of natural selection is much weaker after peak reproduction has been reached [Haldane 1941, Medawar 1957 (1952)]. Aging therefore reflects an inadvertent by-product of the random accumulation of mutations whose effects become apparent later in life. Antagonistic pleiotropy expanded on this idea by proposing that specific genetic variants exist that are beneficial early in life but later become costly (Williams 1957). Under this view, aging is a compromise for the selection of traits that favor early-life survival and fecundity at the expense of earlier mortality. Several recent papers have reviewed and found robust albeit nuanced evidence in support of these hypotheses (see Austad & Hoffman 2018, Turan et al. 2019).

The growing recognition that aging is complex and often highly heterogeneous, both within and between species, suggests that no singular mechanism or theory of aging is likely to explain all aspects of the phenomenon. Once thought to be a universal facet of life on this planet, aging has since been redefined as more flexible in nature, with some taxa seemingly able to regenerate indefinitely (e.g., *Hydra*; Chera et al. 2009, Klimovich et al. 2018) and others with atypically long life spans for their body size (e.g., painted turtles; Warner et al. 2016), underscoring still-unanswered questions. Fortunately, frameworks have emerged that define molecular hallmarks of aging and acknowledge the dynamic interplay between myriad processes that contribute to variation in age-related decline and disease risk (Lemoine 2021; López-Otín et al. 2013, 2016).

At the proximate level, several conserved molecular and physiological mechanisms likely underlie the aging process and aging phenotypes. The canonical hallmarks of aging are defined as genomic instability, epigenetic alterations, telomere attrition, the loss of proteostasis,

dysregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, and altered cellular communication (López-Otín et al. 2013). While these mechanisms are inextricably interconnected, López-Otín and colleagues suggest that they fall into three broad types. Primary hallmarks (genomic instability, epigenetic alterations, telomere attrition, and the loss of proteostasis) get progressively worse with time and cause damage. Antagonistic hallmarks (dysregulated nutrient sensing, mitochondrial dysfunction, and cellular senescence) are protective mechanisms that become disadvantageous when chronically triggered by primary hallmarks. Finally, integrative hallmarks (stem cell exhaustion and altered cellular communication) are the end result of the first two categories and directly impact phenotype. The nine hallmarks of aging proposed by López-Otín and colleagues (2013) have become part of the foundational framework of molecular aging research (Lemoine 2021). Characterizing molecular signatures of aging within and between cells and tissues in situ, deciphering the impact of genetic heterogeneity within individuals, understanding the role of epigenetic and ecological forces that shape the pace of aging, and determining the molecular mechanics underlying age-related disease are some of the critical priorities for aging research today.

Scientists have considered the nature of cellular aging from different perspectives for more than a century (Weissman 1892), and paradigm-shifting work has been carried out in model systems such as *Saccharomyces cerevisiae* (yeast), *Caenorhabditis elegans* (nematodes), and *Drosophila melanogaster* (fruit flies) (Greer et al. 2007, Klass 1983, Mortimer & Johnston 1959, Pan 2011, Pickering et al. 2017). However, differences in the shape and pace of the aging trajectory inherently constrain the cross-species translatability of such work in light of the uniquely "long, slow" (Jones 2011) human life history (Jones 2011, Kaplan et al. 2000). While research conducted in short-lived model organisms has without question advanced the field of aging research, the timing and duration of growth and developmental processes and the mechanisms and priority of somatic maintenance are distinct in nontrivial ways in short- versus long-lived species (De Paoli-Iseppi et al. 2017, Didier et al. 2016, Gluckman et al. 2019, Jones 2011). To treat mice and humans as equivalent in the context of aging ignores the distinct ways by which long- and short-lived species have evolved to respond to the particular conditions and constraints of their environments. Nonhuman primates, particularly great apes and cercopithecoid monkeys, can bridge the gap between the short life expectancies of some organisms and the uncommon longevity of humans (Chiou et al. 2020, Jones 2011). A more comprehensive understanding of how aging occurs within and between short- and long-lived taxa will enable meaningful advancement toward a more grounded, empirical theory of aging.

## 3. WHAT SHAPES VARIATION IN HOW WE AGE?

For many chronic, noncommunicable health conditions, age is the most significant risk factor. Yet there is considerable variability in how humans age, and the line between "normal" aging and age-related disease is, in many ways, quite fluid. For some individuals, the onset of cardiovascular disease, diabetes, neurodegenerative disorders, cancer, or other age-related diseases can occur early in adult life (e.g., in the late fourth decade or early fifth), while others experience slower rates of decline and lower incidence of serious disease and can maintain good health well into (and in some cases through) their ninth decade (Elliott et al. 2021). Although some genetic variants found at high frequencies in centenarians have been associated with increased longevity, their influence on the aging process, age-related disease, and healthspan remains unclear (Sebastiani et al. 2012), suggesting that environment plays a substantial and important role in shaping an individual's aging trajectory. Indeed, research in humans indicates that social and environmental factors can modulate the aging process (see Bribiescas 2020). In addition, differences in the timing (i.e., when in the life span) and extent of exposures—as well as the interaction among such exposures—likely

shape the pace of aging and an individual's risk of age-related disease. A better understanding of the molecular mechanisms that underlie these complex and dynamic interactions is needed.

There is a well-documented relationship between increasing age and reduced cellular regeneration capacity, dysregulation of the immune and inflammatory responses, and a diminished ability to repair DNA damage (Booth & Brunet 2016, Didier et al. 2016). The rate and severity of this decline, however, varies widely across individuals. One possible explanation for this observation is that interconnected networks of resistance and recovery are dynamic and need to be considered within the complex systems in which they exist (Cohen et al. 2022, Promislow et al. 2022). For example, how effectively individuals can repair cellular and DNA damage caused by both exogenous and endogenous factors varies, and the extent to which this damage manifests may itself depend on the intensity, timing, and duration of previous exposures (Cheong & Nagel 2022, Lombard et al. 2005). It is thus essential to not only identify the stressors, but also consider how molecular and physiological systems respond to such stressors in light of an organism's combined evolutionary history and contemporary environmental conditions.

## 4. DEFINING AND QUANTIFYING BIOLOGICAL AGE

While chronological age is a robust predictor of morbidity and mortality, it is limited by its inability to account for the marked heterogeneity that exists in health and physical functionality of same-aged individuals (Bell et al. 2019, Jylhävä et al. 2017). Health disparities among same-aged individuals reflect variation in the pace of aging that a fixed metric such as chronological age cannot capture. Biological age, on the other hand, is a measure of the structural and functional state of the body. Unlike chronological age, biological age accounts for interindividual variation in the progression of age-related phenotypic change and renders a more accurate picture of systemic health (Benayoun et al. 2015, Chen et al. 2016). Quantifying biological age, however, has proved challenging and has been further complicated by the lack of a universally agreed upon definition or standard of measurement (discussed in Porter et al. 2021).

The American Federation for Aging Research (AFAR) has provided specific criteria for molecular biomarkers of aging (see Xia et al. 2017). First, molecular measures of biological age should reflect the activity of the processes responsible for age-related change and be capable of differentiating between normal and pathological age-related phenomena. Second, biomarkers of aging should track the progression of age-related physiological change such that they can determine the rate at which an individual is aging and predict future physical and cognitive capability, disease risk, and mortality. Third, biomarkers of aging should be simple and safe to use in humans and model systems. Key methodological factors (e.g., assay cost, replicability, and robustness to variations in sample quality) must also be considered. Several hallmarks of the molecular aging process have been tested, with varying levels of success, as aging biomarkers (see Lowe et al. 2018, Teschendorff 2020, Vaiserman & Krasnienkov 2021, von Zglinicki & Martin-Ruiz 2005). However, no single measure has successfully achieved the status of an unequivocal, comprehensive biomarker of aging, as each method presents its own assets and drawbacks (Baker & Sprott 1988, Galkin et al. 2020, Mamoshina et al. 2018). We note that while telomere attrition shows an inverse relationship with aging and may be a useful health indicator in certain contexts, telomere length has only modest explanatory power and is likely to be more informative when used in conjunction with data from other molecular markers (Vaiserman & Krasnienkov 2021).

## 5. EPIGENETIC SIGNATURES OF AGING

Aging can be seen as a product of interactions between the internal environment of the human body and the factors that make up our external world (i.e., physical, social, and behavioral

environments), so the epigenome provides an important target for understanding biological age and is a popular candidate for aging biomarker development. The epigenome refers to a set of biochemical mechanisms or modifications to DNA structure that can affect which genes in the genome are turned "on" or "off" (i.e., expressed) without altering the underlying DNA sequence (reviewed by Booth & Brunet 2016). Changes to DNA methylation, posttranslational histone modifications, rearrangement of the nuclear architecture, and RNA-associated silencing all play a role in determining the molecular, functional, and structural features that constitute the epigenomic landscape (Klemm et al. 2019). These epigenetic mechanisms modify DNA accessibility and chromatin structure, thereby regulating how and when genes are expressed. Differences in gene expression are important because they help explain how one genome yields different cell types and changes over the life span and how the highly similar genomes of humans and our closest living relatives yield such different organisms.

Unlike the genome, the epigenome is both dynamic and flexible: It is tasked with unifying information and instructions encoded in the genome with cellular, extracellular, and environmental signals to moderate a phenotype (Campbell & Wood 2019). As the epigenome modulates interactions between the genome and the environment, epigenetic mechanisms are a likely means by which environmental signals get under the skin and cause change at the molecular level. Indeed, epigenomics has already provided new avenues of exploration to decipher how the external world becomes biologically embedded to contribute to differences in health and life expectancy (Bribiescas 2020, Caspari & Lee 2006, Mulligan 2016, Wahl et al. 2017, White et al. 2019).

Genomic integrity deteriorates as a function of both age and rate of DNA damage and has been linked to substantial changes in the epigenomic landscape (Booth & Brunet 2016). Epigenetic dysregulation compromises genomic integrity and, in doing so, increases the likelihood of replicative senescence or, alternatively, carcinogenesis, both of which increase the risk of mortality (Bhatia-Dey et al. 2016, Sen et al. 2016). Cells employ compensatory mechanisms to help mitigate the effects of genomic damage and instability (López-Otín et al. 2013). While effective if used intermittently, these compensatory processes become destructive when chronically activated (Engelfriet et al. 2013). One such example is cellular senescence, a major hallmark of molecular aging (López-Otín et al. 2013, Rakyan et al. 2010). While senescence is often perceived as entirely deleterious, this quiescent cellular state can help protect against tumorigenesis by preventing dysfunctional, old cells from overproliferation, thereby reducing the risk of developing cancer (López-Otín et al. 2013). However, a subset of circulating senescent cells can negatively impact physiological health through the expression of the proinflammatory cytokine profile known as the senescence-associated secretory phenotype (SASP), which promotes tissue damage and dysfunction (Benayoun et al. 2015). This increased inflammation has been termed "inflamm-aging" due to the harmful and age-accelerating effects of chronic inflammation (Franceschi et al. 2018, Fulop et al. 2013).

While age-related decline may be inevitable in humans, the variability in the pace at which different individuals age is driven in part by theoretically modifiable epigenetic differences (Dirks et al. 2016). Certain epigenetic mechanisms are suited to act as measures of biological aging due to their sensitivity to behavioral and environmental factors. However, the extent to which the impact of different variables is inscribed on the aging ledger is not always clear. Defining molecular signatures of aging across the entire human life span and among different populations of people and environments is a promising step toward elucidating sources of variation in the process of aging and determining the extent to which they can be moderated.

Identifying shared and unique aspects of age-related epigenetic change between different tissues is another pursuit of interest. While certain aspects of aging are clearly tissue specific, the nature and functional impact of tissue-specific differences are not well understood. For

example, how different are age-related changes in the brain from those in the blood? How might tissue-specific disparities influence vulnerability to age-related disease, and how variable are these patterns between individuals? To what extent do deviations from the average molecular aging trajectory reflect variation in one's predisposition to age-related disease? A better understanding of what defines the normal human aging trajectory will assist in elucidating the significance of different types of biological and environmental variation.

Epigenetic aging has been studied in different molecular contexts (e.g., age-associated changes in DNA methylation, histone and RNA modifications, chromatin remodeling) (see Kane & Sinclair 2019, Wang et al. 2022). Further study of these mechanisms will be useful in advancing our understanding of the molecular underpinnings of the aging process, particularly when they are examined in a multiomics framework (i.e., integrative analysis of genomic, transcriptomic, epigenomic, and other "omics" data). As models built with DNA methylation data have gained the widest popularity, here we consider age-associated changes to the methylome.

## 5.1. DNA Methylation

The most extensively studied epigenetic mechanism is DNA methylation (e.g., the addition of a methyl group to a cytosine located directly upstream of a guanine; referred to as a CpG site). DNA methylation is intricately involved in gene regulation, gene expression, and biological responses to the environment. With increasing age, we see a pattern of global DNA methylation loss along with localized age-related methylation gains in or around tumor suppressor and other genes (Kananen et al. 2016, Salminen 2021, Salminen et al. 2012). Epimutations (i.e., somatically heritable modifications to the epigenome) are known to accumulate with age, increasing rates of aberrant epigenetic activity, and atypical gene expression (Issa 2014). Unlike results from gene expression studies, which are generally straightforward to interpret because it is clear whether a gene is expressed (upregulated) or not expressed (downregulated), DNA methylation wears different hats depending on the genomic context in which it is found. While patterns of DNA methylation are known to change with age, the nature of region-specific changes induced by DNA methylation is still under investigation (see review by Seale et al. 2022). Nonetheless, evidence strongly suggests that DNA methylation plays a crucial role in age-related biological change and variability in the aging trajectory.

Studies of identical twins have shown that DNA methylation profiles grow increasingly distinct with age (Fraga et al. 2005, Moskalev et al. 2014, Thompson et al. 2010). Twin pairs who exhibit greater lifestyle differences or spent less time together over the course of their lives show greater disparities in DNA methylation, highlighting environmental influence on the epigenomic landscape (Lee & Pausova 2013). Variation in epigenetic markers between cells from the same tissue also increases with age (Maegawa et al. 2017, Mendenhall et al. 2021, West et al. 2013). As humans age, the epigenome becomes increasingly distinct and unique to each individual as a result of stochastic, genetic, and environmental factors; the stochastic changes reflect a phenomenon known as "epigenetic drift" (Issa 2014, Lee & Pausova 2013). Epigenetic changes associated with social and behavioral environments (e.g., McDade et al. 2019; see reviews by Mulligan 2016 and Martin et al. 2022) could underlie differences in susceptibility to age-related disease and decline, but more research is needed to identify the mechanisms through which such variation manifests and shapes health outcomes.

## 5.2. The Epigenetic Clock

Contemporary discussions in molecular aging often center on epigenetic clock models (see the sidebar titled A Closer Look at Epigenetic Clock Models). Epigenetic clocks have become popular biomarkers of aging over the last ten years (Bell et al. 2019, Field et al. 2018), even drawing public

## A CLOSER LOOK AT EPIGENETIC CLOCK MODELS

### Clock Models

In humans, epigenetic age predictor models ("clocks") are built using Illumina DNA methylation microarrays, which capture a subset of CpG sites across promoters, enhancers, and other genomic regions of potential interest. A supervised machine learning approach is used to identify a few hundred CpG sites that together generate the most accurate predictions of chronological age in a training data set. The training data set helps the model learn patterns in the data that it can then use to estimate predictions of age in a validation set, a held-out group of samples from the same experiment that can be used to optimize and obtain a preliminary assessment of model performance. The final model's predictive performance should be formally evaluated on a completely independent test data set to obtain the least-biased assessment of a clock's predictive accuracy. Note that many published nonhuman clock models include only preliminary performance metrics due to the lack of access to an independent test data set, a practice that should be discarded in the future to avoid model overfitting (where the model does not learn to make new predictions, but rather closely regurgitates what it has previously seen in the training data). When possible, next-generation clocks [e.g., DNAm PhenoAge from Levine et al. (2018), or GrimAge from Lu et al. (2019)] are recommended for human research applications due to the observed improvements in model performance resulting from the incorporation of clinical, demographic, and mortality data (see Horvath & Raj 2018, McCrory et al. 2021).

### Clock Sites

At the site-specific level, methylation levels change only modestly over the course of the life span (Porter et al. 2021). Despite the small aging effect of each CpG site in isolation, when assessed in aggregate, changes in methylation at these sites closely track chronological aging. However, one must bear in mind that the questions of why epigenetic clock models work and how they might be capturing variation in aging, disease risk, and mortality risk remain unanswered, and, for this reason, such models should be used and interpreted cautiously.

interest. Scalable epigenetic models purporting to capture biological aging in population-based research were first realized in 2013, when Horvath and Hannum and colleagues independently published separate versions of human epigenetic clock models using data from Illumina microarrays. These epigenetic clocks measure changes in DNA methylation at CpG sites to predict chronological age with unprecedented accuracy (Hannum et al. 2013, Horvath 2013). The two clock models are based on a similar framework and methodology but also exhibit a few key differences. As a multitissue age predictor, Horvath's epigenetic clock theoretically has a wider range of potential applications, but it is likely less sensitive to tissue-specific changes (Choi et al. 2019, Quach et al. 2017). Studies have shown that different organs and tissues (e.g., hippocampus; Levine et al. 2015) appear to age at distinct rates, which could ultimately mask changes in the pace of aging that occur first or only in a single tissue. In addition, when systematically compared, tissue-specific and pan-tissue clocks varied in both their predictive capacity and their underlying genomic features (Choi et al. 2019), which again suggests that tissue-specific clocks are better suited to monitoring and deciphering the etiology of certain diseases. The epigenetic clock model developed by Hannum and colleagues (2013), on the other hand, was developed using blood and was found to be correlated with age-related changes in gene expression. While other studies had identified genomic patterns of age-related change in DNA methylation (Bocklandt et al. 2011, B.C. Christensen et al. 2009), they never gained popularity as biomarkers, possibly owing to poor replicability.

One explanation for the functionality for these models is that they measure the work performed by an epigenetic maintenance system whose main function is the preservation of genomic stability

(Horvath 2013). The intensity of the work performed by this maintenance system corresponds to the pace at which the clock "ticks." Under this premise, the clock is expected to tick most rapidly during growth and development; when growth is largely complete, the ticking rate slows down and assumes a more constant pace during normal aging. However, threats to genomic stability are expected to upregulate activity of the epigenetic maintenance system, theoretically accelerating DNA methylation age. Disruptions to the genomic architecture, mitogenic activity, and carcinogenesis are all expected to activate the epigenetic maintenance system and thus accelerate rates of DNA methylation aging (Horvath 2013, Nwanaji-Enwerem et al. 2018).

Epigenetic age predictor models appear to be sensitive to a number of physiological, psychological, and environmental factors (Horvath & Raj 2018). In healthy individuals, chronological age and biological age are typically well correlated. However, individuals who experience prolonged stress and those who are predisposed to or suffer from chronic noncommunicable diseases often exhibit signs of pathological or accelerated aging. Epigenetic clocks can be used to identify accelerated aging by comparing predicted biological age with actual chronological age (**Figure 1**). A predicted biological age that is greater than one's chronological age defines "accelerated aging" and indicates a potentially increased risk of mortality (Gassen et al. 2017, Horvath et al. 2014). Results from these analyses have generally provided consistent if somewhat enigmatic support for the clock's ability to capture variation in the progression of aging and age-related decline. However, it is important to recognize that while biological age is a useful and intuitive concept, there is no consensus on how it should be defined or measured.

While widespread application and testing of the human clocks provide evidence for their capacity to capture (some of the) variation in biological aging that chronological age cannot, attempts to identify the molecular mechanisms underlying their functionality have yielded no definitive results. It is also notable that the strength of the relationship between clock-based estimates of

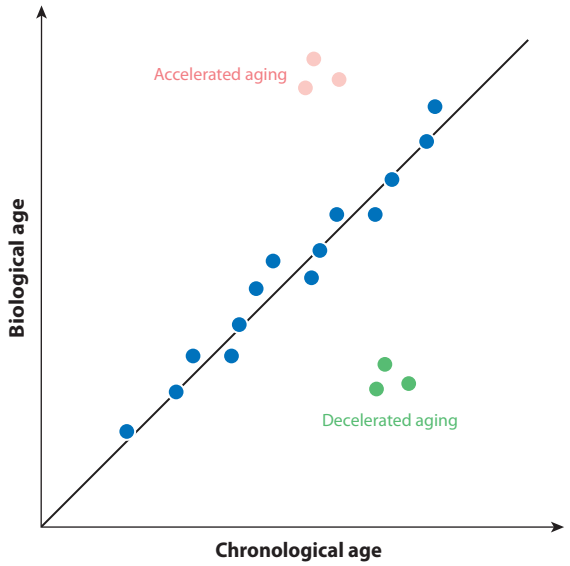


**Figure 1**

Biological (i.e., "epigenetic" or "predicted") age plotted against chronological age. The solid diagonal line represents the theoretical average pace of biological aging. Individuals who have a predicted age similar to chronological age are shown as blue circles. The points above the line (*pink circles*) show examples of accelerated aging. The points below the line (*green circles*) show examples of decelerated aging.

age acceleration and the incidence of disease or age-related mortality is very modest (e.g., Perna et al. 2016), suggesting that these models capture what is likely an incomplete picture, despite being one of the most robust biomarkers of aging in use today. The continued lack of mechanistic insight makes it challenging to draw conclusions from studies investigating the relationship between epigenetic aging and clinically relevant variables (see meta-analysis by Ryan 2021). Thus, researchers must consider the nature of their pursuit prior to initiating the research itself and carefully consider which biomaterial and methodological approaches are most suitable to the question of interest. In addition, if attempting to generate DNA methylation data from lower-quality biomaterials, it is important to test how collection techniques and storage conditions impact DNA methylation profiling. Finally, while DNA methylation–based biomarkers are the most common epigenetic markers of aging, there are many types of omics data (which are rapidly becoming more financially accessible for more researchers) that have yet to be thoroughly explored in the context of aging and may complement, outperform, or be more directly interpretable than the current gold standard epigenetic clocks.

As more data become available for humans and other species, a more diverse set of omics data sets can facilitate the creation of sophisticated biomarkers of health and aging that are trained not just on chronological age but also on known molecular and biological correlates of age-related disease and decline (Levine et al. 2018, Wagner 2017) with the aim of identifying chronological age-dependent and -independent mechanisms that contribute to variation in health, disease risk, and mortality risk (Barzilai et al. 2012, Lu et al. 2019). The next generation of human clocks has already incorporated biochemical and behavioral data to produce multistep predictor models that more accurately model human risk of mortality (Levine et al. 2018, Lu et al. 2019). These clocks capture variation in the aging process in multiple independent disease-specific cohorts, reflecting their potential in further elucidating sources of variation in the aging process as putative epigenetic clock models of biological (rather than chronological) age. This distinction between biological aging and chronological aging clocks is important, and we suggest Bell et al. (2019) and, more recently, Li et al. (2022) for further reading. These more advanced clocks come with trade-offs that may limit their usability outside of clinical settings: These models are more data-intensive and require access to clinical and other health information that is often not available or feasible to collect in large, population-based studies. To identify molecular mechanisms at the core of the aging process, epigenetic clock models should be used in conjunction with analyses of differential methylation, gene expression, chromatin structure, and other epigenetic and genetic data to interrogate how different regulatory networks change with age, in response to environmental stimuli, and in a comparative context across individuals.

Last, nonhuman clocks (e.g., macaque clocks or rodent clocks) can be used to study putative pro- or antiaging interventions or behaviors; however, the same caveats described for the first generation of human clocks also apply to these models, which may predominantly measure chronological age in lieu of directly capturing some level of variation in individual health (see the sidebar titled Nonhuman Clocks). Nonetheless, such models may shed light on the physiological systems and molecular networks that contribute to healthy aging or pathogenic processes, especially if used in conjunction with other omics or health data, and thus should be continued to be tested and explored thoughtfully.

## 6. SUMMARY AND CONSIDERATIONS FOR FUTURE RESEARCH

The molecular pathways involved in the process of biological aging are shaped by complex relationships between environmental and genetic factors. To enable ongoing advancement in the field of molecular aging research, a more integrative, interdisciplinary approach is required. A logical place to start is with the exploration of variation in molecular aging at the tissue level, starting

## NONHUMAN CLOCKS

Several epigenetic clock models for nonhuman species have been developed using similar approaches (Horvath et al. 2021 for rhesus macaques, Jasinska et al. 2022 for vervet monkeys, Prado et al. 2021 for elephants); however, many studies in nonhuman species have, by necessity, used high-throughput bisulfite sequencing (BS-seq) data to quantify DNA methylation and construct epigenetic clock models (e.g., Anderson et al. 2021 for baboons, Goldman et al. 2022 for rhesus macaques, Levine et al. 2020 for rats). While BS-seq data are more flexible and applicable to a wider variety of questions compared with data produced on the microarray, sequencing data require substantially more preprocessing and a higher level of technical skill to build a working epigenetic clock model. Largely successful experimental applications of BS-seq-based models have included testing hypotheses related to aging and antiaging intervention in rodents in closely monitored laboratory conditions (see Cole et al. 2017; Hahn et al. 2017, 2018; Levine et al. 2020; Petkovich et al. 2017; Wang et al. 2017). While enforcing this level of environmental homogeneity is typically not feasible for other species, it has provided some unique insight into facets of molecular aging in the DNA methylome. Finally, a clock model based on a new pan-mammalian microarray was recently published by Arneson et al. (2022), which covers 37,000 highly conserved CpG sites across a number of mammalian species ranging from relatively short-lived (e.g., rodents) to very long-lived (e.g., humans) taxa. While compelling and reasonably predictive of chronological age, future studies using this platform should shed light on the model's precision and the applications to which it is best suited, as well as provide evidence about its predictive capacity when applied at the extreme ends of the age distribution of the taxa included.

at a fine scale and moving progressively toward a systems-based perspective that can unify the processes of aging across the multiple scales at which it occurs. While human aging is remarkably plastic, increasing age is nevertheless characterized by specific changes in gene expression that weaken global genomic stability, alter genomic architecture and the epigenomic landscape, and increase one's risk of cancer, neurodegeneration, and cardiovascular disease (Brunet & Berger 2014, Sen et al. 2016). Together, these factors manifest in progressive functional deterioration and visible decline of tissue and organ systems, ultimately leading to mortality. As we begin to characterize the molecular signatures of aging across the body, we can also examine how these changes occur between individuals and how this variation relates to variation in the social and physical environment.

Unlike chronological age, biological age accounts for interindividual variation in the progression of age-related phenotypic change, providing a more accurate picture of overall health. While epigenetic clocks may prove to be a useful biomarker of aging, a greater characterization of the molecular signatures of aging is needed to understand how the epigenome mediates interactions between the genome and the environment to shape the aging process, leading some individuals to age more rapidly than others. The effective treatment of age-related disease is one of the great challenges faced by modern medicine; while tremendous strides have been made in aging science, the question of how the underlying molecular mechanisms of biological aging interact with parameters of the human environment to produce such marked variation in health and disease risk has not been answered. Deciphering the effects of specific environmental and behavioral variables on human aging is a critical step toward clarifying interactions among variables that contribute to the variation in how humans age.

## DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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