

Epigenetic Clocks: Biological Aging, Disease, and Clinical Applications

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Abstract

Epigenetic clocks are DNA methylation-based models that estimate biological age and provide a quantitative framework for investigating interindividual differences in aging. Unlike chronological age, which reflects the passage of time, epigenetic age may capture the cumulative effects of genetic background, environmental exposure, lifestyle, inflammation, and metabolic stress on cellular function. Over the past decade, these models have evolved from tools designed primarily to predict chronological age into increasingly sophisticated biomarkers of morbidity, mortality, and the pace of physiological decline. This review summarizes the conceptual and methodological development of epigenetic clocks and discusses their relevance to aging research and clinical medicine. First-generation clocks, including the Horvath and Hannum models, were developed to estimate chronological age with high accuracy. Second-generation clocks, such as PhenoAge and GrimAge, incorporated clinical biomarkers, mortality-related variables, and plasma protein surrogates, thereby improving their ability to reflect health status and disease risk. More recent models, particularly DunedinPACE, focus on the rate of aging rather than cumulative biological age. Advances in principal component-based clocks, deep learning approaches, and single-cell methylation analyses have further expanded the analytical capacity of the field. Particular attention is given to epigenetic age acceleration and its intrinsic and extrinsic components, as well as the genetic architecture underlying these measures. Variants involving *TERT*, *SELP*, HLA, *APOE*, *POU5F1*, and cytochrome P450-related pathways support the close relationship between epigenetic aging, telomere biology, inflammation, immune function, metabolism, and neurodegeneration. Evidence from cancer, clonal hematopoiesis, neurodegenerative disorders, and cardiometabolic disease suggests that epigenetic clocks may serve as useful biomarkers for risk stratification and longitudinal monitoring. Nevertheless, broader clinical implementation will require improved standardization, validation across diverse populations, and careful interpretation in tissue- and context-specific settings.

Keywords: DNA methylation; epigenetic clocks; biological aging; epigenetic age acceleration; GrimAge; DunedinPACE; precision medicine; deep learning.

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Introduction

The unprecedented increase in human life expectancy through modern medicine and public health interventions has necessitated a redefinition of the concept of cellular aging. The aging process is not merely a chronological vector consisting of the passive passage of time, but also a biological phenomenon that is measurable at the cellular and molecular levels, genetically regulated, modifiable by environmental factors, and potentially even reversible through cellular reprogramming. Today, aging is recognized as the greatest independent risk factor for a wide range of chronic human pathologies, from cancer to neurodegenerative diseases, from cardiovascular syndromes to immunodeficiencies and metabolic disorders [1]. This clinical and demographic reality has made the accurate, reliable, and reproducible measurement of biological age a central focus of molecular gerontology and personalized preventive medicine.

At the center of this pursuit are epigenetic modifications, which heritably modulate gene expression without causing any structural change in the nucleotide sequence of DNA. Although histone modifications and non-coding RNAs (ncRNAs) play important roles among epigenetic regulatory mechanisms, DNA methylation (DNAm), in particular, has emerged as the most reliable, stable, and quantifiable biomarker of cellular aging and biological timekeeping [2]. At the molecular level, DNA methylation is the reaction by which a methyl group is covalently added, via DNA methyltransferase (DNMT) en-

zymes, to the 5'-carbon atom of the cytosine base, predominantly at cytosine-guanine (CpG) dinucleotides in mammalian genomes [3]. With the aging process, a highly characteristic, deterministic, and universal trajectory is observed in the methylation landscape of the genome: while global hypomethylation, or loss of methylation, occurs in repetitive sequences that maintain structural stability across the genome, specific hypermethylation, or increased methylation, occurs in CpG islands located in the promoter regions of certain genes, which generally silences gene expression [4]. These regular, predictable, and deterministic methylation changes have formed the fundamental concept of the "epigenetic clock."

Epigenetic clocks are regression-based mathematical algorithms that estimate the biological age of an individual, a tissue, or a single cell by integrating methylation patterns at thousands of specific CpG sites through high-dimensional data analysis and machine learning models [5]. These clocks not only measure the erosive effects of time on biological tissues, but also quantify the molecular damage produced at the cellular level by genetic predispositions, environmental stressors, and lifestyle choices. The statistical difference between an individual's chronological age, which refers to the time elapsed since birth, and epigenetic age, which is the cellular age predicted by the DNA methylation profile, is referred to as "Epigenetic Age Acceleration" (EAA) [6]. A positive or accelerated epigenetic age refers to a condition in which an individual's DNA methylation-based age is greater than their chronological age and, in clinical practice, represents one of the most critical indicators of early morbidity, disease risk, and increased all-cause mortality [6].

Generational Approaches and Mathematical Formulations of Epigenetic Clocks

The historical development and evolution of epigenetic clocks are classified under three principal generations and subsequently developed methodological variations, such as principal component clocks and deep learning models, according to the type of algorithms used, the clinical nature of the targeted training variables, and the complexity of the optimized cellular features [7] (Table 1). Each generation is based on a distinct biological training designed to overcome the conceptual limitations of the preceding generation.

First-Generation Clocks: Precise Prediction of Chronological Age

The fundamental philosophy of first-generation epigenetic clocks is to predict individuals' chronological age with the lowest possible margin of error. These models directly use chronological age as the target variable and maximize the statistical relationship between the data in the training sets and the passage of time [8]. The basis of their algorithmic architecture is Elastic Net penalized regression, which processes complex methylation data from tens of thousands or hundreds of thousands of CpG sites [5]. The Elastic Net method provides dimensionality reduction by reducing the weights of unnecessary variables in

a model to zero (L1, Lasso regularization) and solves the "p > n" problem in the dataset, in which the number of features is much greater than the number of samples, by grouping correlated features (L2, Ridge regularization).

Among the most widely referenced first-generation clocks is the Horvath Pan-Tissue Clock. Developed in 2013, this clock is a highly sparse model composed of 353 CpG sites and was trained on a wide range of human tissues and cell types [9]. With remarkable flexibility, the Horvath clock shows an extremely high correlation with chronological age, such as, in heterogeneous tissues including blood, brain, and breast [10]. The Hannum Clock, which emerged during the same period, was trained specifically on whole-blood samples, operates with 71 CpG sites, and provides high accuracy in blood tissue-specific aging profiles [11]. Although these first-generation clocks were designed to measure time with remarkable accuracy, based on the odometer principle, they contain a fundamental limitation for clinical research. When a machine learning model is refined to predict chronological age with zero error, that model has in fact learned only "time," rather than biological health, genetic resilience, or physiological decline [12]. Indeed, clinical indicators such as blood pressure, blood lipids, glucose tolerance, and disease history show only weak associations with first-generation epigenetic clocks [13]. For example, beneficial or harmful factors such as radiation exposure, tumor presence (breast cancer), or exercise do not cause a meaningful change in the predictions of these first-generation models, which are locked to chronological time [14].

Second-Generation Clocks: Integration of Morbidity, Mortality, and Biological Dysfunction

To address this relative insensitivity of the first generation to health status, researchers developed second-generation clocks that use composite measures of biological age, clinical biomarkers, and expected lifespan (mortality risk) as target variables instead of chronological age [5]. DNAm PhenoAge, one of the leading representatives of this generation, was designed to predict a phenotypic age derived from clinical laboratory panels, such as albumin, glucose, leukocyte count, and C-reactive protein, using 513 CpG sites [15]. The aim of PhenoAge is not how long you have lived, but how much systemic damage your cellular structure has sustained.

Another model considered the gold standard for predicting mortality risk, DNAm GrimAge, is based on 78,464 CpG sites [16]. GrimAge was designed to estimate the epigenetic representations of specific circulating plasma proteins directly associated with aging, such as plasminogen activator inhibitor 1 (PAI-1), leptin, and myoglobin, as well as an individual's lifetime smoking history, expressed as pack-years [16]. The recently developed GrimAge v2 version maximized its predictive ability by incorporating markers of systemic inflammation and metabolic dysfunction, such as high-sensitivity C-reactive protein (logCRP) and hemoglobin A1C (logA1C), into the model [17]. GrimAge v2 was tested on 13,399 blood samples across nine separate study cohorts and demonstrated a unique statis-

Table 1. Generational Classification and Clinical Characteristics of Major Epigenetic Clock Models

Generation Classification	Representative Clock Models	Optimization Objective (Training Variable)	CpGs Analyzed Clinical	Clinical Significance and Methodological Focus
First Generation	Horvath, Hannum, Zhang	Chronological Age	Horvath: 353, Hannum: 71, Zhang: 514	Time measurement; pan-tissue compatibility (Horvath) or blood specificity (Hannum).
Second Generation	PhenoAge, GrimAge	Clinical Phenotype, Lifespan, and Plasma Proteins	PhenoAge: 513, GrimAge: 78,464	Detection of disease risk, physiological decline, cellular stress, and mortality.
Third Generation	DunedinPACE	Longitudinal Rate of Aging (Functional Decline)	173	Determination of the instantaneous physiological collapse rate (rate indicator) with a single measurement.

tical correlation with age-related conditions such as coronary heart disease, lung function measurements (FEV1), and computed tomography-based hepatic steatosis [17].

Third-Generation Clocks: Longitudinal Acceleration and the Concept of the “Pace of Aging”

Whereas first- and second-generation clocks function as static measurements, that is, as “odometers” estimating the cumulative cellular damage accumulated from the beginning of life to the time of measurement, third-generation clocks represent the organism’s current instantaneous rate of deterioration, in other words, a “speedometer.” The most advanced current archetype of this generation is the DunedinPACE (Pace of Aging Calculated from the Epigenome) algorithm, which refers to the rate of aging calculated from the epigenome [18].

DunedinPACE was developed through the 20-year longitudinal follow-up of members of the Dunedin Multidisciplinary Health and Development Study birth cohort in New Zealand at ages 26, 32, 38, and 45 [19]. During this process, physiological functional decline in distinct biomarkers reflecting the integrity of participants’ cardiovascular, metabolic, renal, immunological, dental, and pulmonary organ systems was mathematically modeled [18]. Subsequently, using Elastic Net regression, a refined biomarker composed of 173 CpG sites was developed that could predict this 20-year rate of physiological decline, the Pace of Aging, from the methylation profile of a blood sample obtained from an individual at a single time point [20]. DunedinPACE eliminates the “survival bias” and “cohort effects” frequently observed in first- and second-generation clocks, which arise from reliance on data from older individuals, by longitudinally following individuals of exactly the same chronological

age [21]. Therefore, DunedinPACE stands out as the most sensitive instrument for personalized medicine, monitoring of the instantaneous rate of aging, and determination of the response to pharmacological interventions.

Classical epigenetic sequencing arrays and clock algorithms are highly sensitive to probe-based technical fluctuations, microenvironmental variations, and batch effects. This may cause an individual’s biological age to vary even across different samples obtained on the same day, thereby reducing test–retest reliability. To overcome this problem, which arises from measurement noise rather than from a biological signal, Higgins-Chen and colleagues developed Principal Component (PC)-based clocks (PC-Horvath, PC-Hannum, PC-PhenoAge, and PC-GrimAge) [22].

In this approach, the model is not trained directly on the weighted sum of individual CpGs. Instead, the variance derived from methylation data at tens of thousands of CpGs, for example 78,464 CpGs, on the 450K and EPIC arrays is transformed into orthogonal Principal Components [22]. Elastic Net regression is then configured to predict the score or phenotype predicted by the original clock by using these components rather than individual CpGs. This revolutionary methodology minimizes the impact of random stochastic error or environmental noise at a single probe on the model. Indeed, the data demonstrate that PC-based clocks show a tremendous increase in test–retest reliability, generally between and , compared with their conventional counterparts, and render clinical follow-up substantially more stable [22].

A Shift in Bioinformatics: From Elastic Net to Deep Learning Architectures

For more than a decade, the analysis of methylome data has been dominated by Elastic Net and its derivative linear penalized algorithms. However, the discipline of bioinformatics is evolving beyond the dogmatic limitations of linear models toward higher-order computational architectures, particularly Deep Neural Networks (DNNs).

The fundamental assumption of Elastic Net algorithms is that the relationship between DNA methylation patterns and biological traits is linear [23]. However, biology is inherently nonlinear and contains epistatic, or intergenic, interactions. In an interaction scenario in which two different CpG sites logarithmically alter each other's effects, or in cases in which hypermethylation at a secondary locus completely invalidates the function of the first locus, a linear regression model becomes entirely insufficient. Incorporating feature-feature interactions among all CpG sites into the model at the pairwise level in classical Elastic Net is computationally impossible, as it would require hundreds of millions of parameters, for example more than 228 million features. In addition, a "CpG saturation" problem is frequently encountered in first- and second-generation linear clocks; these models deviate at chronological ages above 60 years and tend to systematically underestimate biological age [23].

This technological bottleneck has been addressed asymmetrically through deep learning frameworks such as AltumAge and NCAE-Age which was designed for immunological profile analysis [24,25]. For example, AltumAge is a deep neural network model trained on the largest data pool ever assembled for human age prediction, compiled from 143 different human tissue datasets. AltumAge uses normalized beta values from 20,318 CpG sites common to the Illumina 27k, 450k, and EPIC arrays as input. These data are passed through five separate hidden layers, each containing 32 nodes, equipped with multilayer perceptrons that combine multiple features at each node in order to capture nonlinear interactions. During training, AltumAge acquires extraordinary robustness against experimental, biological, and batch-related noise through the use of Gaussian noise and adversarial observations. Because it does not use predefined age-transformation functions containing bias and draws support from tens of thousands of CpGs, it completely eliminates the saturation error of linear clocks above the age of 60 [25].

More importantly, when the internal mechanism of AltumAge was interpreted biologically using SHAP (Shapley-value) algorithms and chromatin annotation data, it was determined that the CpG loci contributing most strongly to age prediction were not randomly distributed, but were concentrated in highly interactive clusters located directly in specific gene regulatory regions of the genome and near CTCF (CCCTC-binding factor) binding sites. DNNs can capture the advanced aging acceleration of cells exhibiting replicative senescence or mitochondrial dysfunction much more precisely than linear models such as Horvath. Similarly, in SImAge modeling examining immune system aging, deep network architectures such as FT-Transformer, SAINT, and DANet were shown to outperform Gradi-

ent Boosted Decision Trees (XGBoost) and Elastic Net models in terms of error margin (MAE = 6.94 years) [26].

Single-Cell Epigenetic Clocks (scAge) and Developmental Reprogramming

Epigenetic analyses performed at the bulk-tissue level generate a mathematical average of the methylation profiles of millions of cells within a tissue. However, the biological reality is that tissues are highly heterogeneous microenvironments; within the same liver or brain tissue, both epigenetically very young cells and cells that have undergone advanced senescence may coexist [27]. To define the pure cellular aging profiles of human and mammalian somatic cells, researchers have turned their attention to single-cell sequencing technologies (scRRBS) [28]. The greatest seemingly insurmountable obstacle in single-cell DNA methylation profiling is the sparsity of the data and its binarized nature, that is, methylated or unmethylated [29]. When a single cell is sequenced, genomic coverage is not homogeneous, and each tested cell presents fragments from completely different regions of the genome. This renders first- and second-generation Elastic Net clocks, such as Horvath and PhenoAge, which must adhere to specific rules involving 353 or 513 CpGs, entirely unusable on this platform [27].

The single-cell epigenetic clock framework designed to solve this problem, scAge, adopts an entirely different statistical modality: Likelihood Profiling [27]. The scAge algorithm uses large-scale bulk-tissue sequencing data as a reference to generate linear regression profiles that define the probabilities of age-related change across thousands of CpG sites. When binarized methylation data from the single cell to be analyzed are obtained, the algorithm identifies only those CpG sites that intersect, or overlap, with the reference dataset rather than using the entire genome. Then, for each locus in this overlapping pool, it calculates the probability that the cell originated from a specific age interval by considering the current methylation state of that cell, either 0 or 1. The sum of these probabilities integrated across all CpGs is converted into a likelihood profile, and the peak corresponding to the maximum likelihood is determined as the final "epigenetic age" of that single cell [27]. When researchers applied the scAge framework to hepatocyte cells obtained from 4-month-old young mice and 26-month-old old mice, as well as to mouse embryonic fibroblasts (MEFs), they demonstrated that the algorithm captured the chronological age of the tissue flawlessly at the molecular scale while also mapping the natural heterogeneity present from cell to cell [27]. The most striking discovery that scAge has offered to the biology community is the evidence that the organism does not undergo a unidirectional and entropic aging process, but rather contains a natural "rejuvenation" event during developmental stages. scAge profiling applied during the gastrulation stage of mouse embryos, at the zero point of aging in terms of cell fate, revealed that only the cells destined for intra-embryonic lineages, which will directly form the fetus, reduced their epigenetic ages in a layered and distinct manner; in other words, they naturally rejuvenated. In addition, it was confirmed for the first

time at single-cell resolution that adult muscle stem cells experience aging at a much more attenuated rate compared with their chronological age [27].

Epigenetic Age Acceleration (EAA): Intrinsic and Extrinsic Mechanisms

In clinical assessments, an individual's "biological age" alone is not a sufficient measure; the critical metric is the deviation of the measured epigenetic age relative to the individual's chronological age. This concept is known as Epigenetic Age Acceleration (EAA). In the literature, age acceleration is examined within two broad frameworks according to the reflections of systemic inflammation, or inflammaging, and immunosenescence in leukocyte tissues [30]:

Intrinsic Epigenetic Age Acceleration (IEAA): This represents more fundamental and pure cellular aging, adjusted for age-related fractional changes in blood cell populations [31]. IEAA is the residual value obtained from the regression of the Horvath clock on chronological age; it is calculated after cell counts of major leukocyte subgroups, such as CD4⁺ T cells, naïve CD8⁺ T cells, CD8⁺CD28[−]CD45RA⁺ cells (memory and effector T cells), natural killer (NK) cells, monocytes, granulocytes, and plasmablasts, are corrected using multivariable statistical models [30]. This feature indicates that IEAA is relatively less affected by external environmental factors and an individual's specific lifestyle choices, and that it reflects stem cell exhaustion in the organism's intrinsic rate of cellular renewal [32].

Extrinsic Epigenetic Age Acceleration (EEAA): In addition to pure cellular aging, this incorporates age-related compositional changes and exhaustion of the immune system, namely immunosenescence. EEAA, calculated on the basis of the Hannum clock, specifically preserves the effects of distributional changes in immune cell compartments such as naïve CD8 cells, plasmablasts, and effector T cells. EEAA has a much stronger statistical correlation with external morbidity markers such as cardiovascular stress factors, obesity, a sedentary lifestyle, and exposure to environmental toxins [32].

In disease pathophysiology, these two metrics tell very different but complementary stories. For example, in Parkinson's disease (PD), which is a model of neurodegenerative disease, a significant acceleration is observed in the peripheral blood of individuals in terms of both IEAA and EEAA [33]. However, the mechanistic difference lies in immune composition; compared with controls, the blood of patients with Parkinson's disease shows a significant decrease in T helper (CD4) cells and B cells, while granulocyte counts show a massive increase. While this shift in immune profile drives EEAA upward, the ongoing oxidative burden within the cells also triggers IEAA [33].

The Genetic Architecture of the Epigenetic Clock: Heritability and GWAS Findings

The question of whether biological age acceleration is merely cellular entropy or a genetically encoded program is being clarified through genome-wide association studies (GWAS) and classical twin analyses.

Classical Twin Studies and Longitudinal Heritability

The heritability of epigenetic age acceleration has been estimated at approximately 40% at the level of SNP heritability through classical monozygotic (MZ) and dizygotic (DZ) twin studies [34]. This indicates that biological aging is a phenotype shared approximately equally between genetic and environmental factors. However, Structural Equation Models (SEMs) established in 1,084 adult twins based on data from the Chinese National Twin Registry (CNTR) have shown that heritability does not remain static throughout human life [35]. When the genetic effect was analyzed longitudinally between the ages of 31 and 70, a sharp and distinct decline in the genetic heritability component was observed in the DunedinPACE and PC-PhenoAge clocks in the 55–65 age range, and in the PC-Horvath and PC-Hannum clocks in the 60–70 age range, together with the accumulation of cumulative environmental factors [35]. The only exception to this was the PC-GrimAge model; the heritability rate of the GrimAge clock, which targets mortality and cellular stress, preserved its consistency and stability across the entire advanced-age spectrum. This emphasizes that the molecular structures regulating susceptibility to morbidity and risk of death are controlled more consistently by an individual's deep polygenic architecture than by external environmental erosion.

Genome-Wide Association Studies (GWAS) and Polygenic Loci

In large-cohort GWAS analyses designed to resolve the links between biological aging and genetic background, 137 genetic loci directly associated with aging rate and specific epigenetic biomarkers have been identified [36]. Studies have linked specific genetic variants that modulate the acceleration of the biological clock to cellular pathways:

The TERT Paradox (Horvath/General EAA): Telomerase Reverse Transcriptase (TERT) is a major mechanism that extends cellular lifespan by protecting chromosome ends. However, GWAS studies have identified a surprising contradiction: individuals with longer telomere lengths in leukocytes due to genetic variation paradoxically exhibit higher, or accelerated, aging in blood epigenetic clocks [37]. This is explained by the "mitotic clock" theory: because TERT variants delay apoptosis or cellular senescence, these leukocytes live longer and undergo more replication, or mitotic, cycles. The inevitable methylation errors, or epigenetic drift, left by DNA replication machinery at each cell division accumulate and advance the biological clock faster than chronological age [38].

SELP and Horvath Clock Acceleration: Horvath age acceleration in blood tissue (EAA-Horvath) has been associated at genome-wide significance with the SELP gene locus, which encodes the P-selectin glycoprotein on the chromosome, particularly the specific SNP rs732314 [39]. SELP expression plays a central role in the mechanisms of vascular inflammation and thrombosis by enabling leukocyte adhesion to vascular endothelial cells; the acceleration of epigenetic aging by this gene is

Table 2. Genetic Loci and GWAS Variants Associated with Epigenetic Age Acceleration

Clock/Acceleration Model	Key Locus / Genes	GWAS Variant / SNP	Phenotypic and Biological Mechanism
Horvath EAA	<i>SELP</i> (P-selectin)	rs732314	Leukocyte-endothelial adhesion, vascular inflammation, and tissue damage.
Hannum EAA	HLA complex	rs28366133	Antigen-specific immune response and extrinsic immunosenescence.
General/Blood EAA	<i>TERT</i>	Specific <i>TERT</i> genotypes	Telomere elongation → increased cellular longevity → increased replicative errors (“mitotic clock”)
GrimAge EAA	<i>POU5F1</i> , <i>MED24</i>	rs887466-A	Adiposity-related traits, plasma PAI-1 levels, and the polygenic contribution to parental lifespan.
PhenoAge/ Biological Acceleration	<i>APOE</i> , cytochrome P450	CYP450 cluster (Chromosome 19)	Lipid dysfunction, metabolic detoxification, and the cellular consequences of oxidative/redox stress.

genetic evidence of how atherosclerosis and systemic inflammation advance the biological clock.

HLA Locus and Hannum Acceleration: The EAA-Hannum clock, which directly reflects immune cell composition, has produced an extraordinarily strong GWAS signal with the rs28366133 variant in the Major Histocompatibility Complex (HLA) region [39]. The polygenic overlap of HLA genes, which are central to the adaptive immune system and antigen presentation, with a blood immune clock such as Hannum is mechanistically highly consistent.

ErbB2 and the Cytochrome P450 Metabolic Axis: Evidence from Murine Models: In a genetically diverse BXD mouse cohort, linkage analyses identified two loci associated with epigenetic age acceleration: one on mouse chromosome 11 encompassing the *ErbB2*/*Her2* oncogenic region and another on mouse chromosome 19 containing a cytochrome P450 gene cluster. Notably, these loci contain genes, including *STXBP4*, *NKX2-3*, and *CUTC*, that have also been associated with epigenetic age acceleration in human studies, suggesting partial cross-species concordance in the genetic regulation of epigenetic aging [40]. Considering the critical role of the Cytochrome P450 family in hepatic metabolic detoxification processes, it is understood that oxidative stress and toxic metabolite accumulation caused by these variations irreversibly disrupt methylation balance [40].

APOE and Cognitive Morbidity: Variants in the Apolipoprotein E (*APOE*) locus, known for its fundamental role in Alzheimer’s disease and lipid metabolism, have been identified as systemic modulators of PhenoAge and biological age acceleration, establishing a genetic bridge between neurodegeneration and systemic epigenetic collapse [41].

The Polygenic Architecture of GrimAge: The genetic structure of the second-generation clock GrimAge exhibits struc-

tural differences from other models. GrimAge age acceleration is correlated with fundamental genetic loci that control pluripotency, gene regulation, and cellular transcriptional functions, such as *POU5F1* (rs887466-A,), *CCDC162P* (rs9386796-T), *LINC01475*, and *MED24.47* It has been reported that polygenic risk scores (PRS) derived from GWAS are reflected directly and phenomically in adiposity, or obesity, educational attainment, and parental lifespan through GrimAge acceleration [36] (Table 2).

Epigenetic Clocks and Clinical Pathophysiologies

Epigenetic age acceleration, beyond emerging as a consequence of genetic programs and cellular wear, has become integrated into the pathophysiological course of chronic pathologies.

Clonal Hematopoiesis (CHIP) and Oncological Risks

One of the most complex bridges between the biology of aging and cancer evolution is the condition known as Clonal Hematopoiesis of Indeterminate Potential (CHIP). CHIP refers to the presence of somatic myeloid mutations (Variant Allele Frequency) in the stem cell compartment of older individuals (approximately 15–20% of cases, age), in the absence of a hematological malignancy such as leukemia. These mutations occur largely in epigenetic modifier genes, such as *DNMT3A*, *TET2*, and *ASXL1*, which directly govern DNA methylation and demethylation [42].

Whole-genome sequencing (WGS) analyses performed in massive cohorts such as Trans-Omics for Precision Medicine (TOPMed), supported by the National Heart, Lung, and Blood Institute (NHLBI), have identified a massive acceleration in blood-based epigenetic clocks (EAA) among individuals carrying CHIP mutations [43]. Patients who have both CHIP and an accelerated epigenetic age are at an asymmetrically higher

risk of ischemic heart failure, coronary artery disease (CAD), stroke, and all-cause mortality compared with those who have only one of these conditions [44]. This finding points to a feed-forward loop in which mutated CHIP clones, while rapidly proliferating in the bone marrow through clonal expansion, fuel systemic inflammation and biological aging.

Similarly, in solid tumors, for example, in breast cancer, accelerated epigenetic age acceleration measured in tumor-adjacent healthy breast tissues in cases where standard blood tests are insufficient provides strong signals that the tissue, although not yet malignant, harbors a precancerous microtissue prone to oncological growth. In addition, radiotherapy and cardiotoxic agents administered to childhood cancer survivors induce profound aging accelerations in DunedinPACE, PhenoAge, and GrimAge metrics, thereby forming the molecular basis of the cardiometabolic risks these children will experience later in life [39].

Neurodegeneration and Cognitive Decline

The progression of neurodegenerative diseases proceeds hand in hand with the loss of regulation in DNA methylation. The use of epigenetic clocks in the clinical course of Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), and Huntington's disease (HD) offers broad prognostic potential, ranging from the age of disease onset to disease-specific pathology [45,46]. Large-scale systematic reviews have shown that accelerated EAA, particularly when measured with the GrimAge clock, predisposes individuals to earlier disease onset and rapid cognitive deterioration [47]. In extensive cognitive follow-up studies such as the Framingham Heart Study Offspring Cohort, the DunedinPACE rate measurement specifically was found to be statistically and clinically much more consistent, stable, and predictive than first- and second-generation clocks, such as Horvath or PhenoAge, in predicting phenotypic cognitive decline.¹⁸ In the Parkinson's disease model, a protective correlation was observed between lower GrimAge values and resistance to disease, whereas increased PAI-1 levels accelerate pathology in Alzheimer's disease [48].

Metabolic Syndrome and Organ Diseases

The rapid operation of the biological clock also erodes cardiometabolic and autoimmune pathways. Mendelian randomization (MR) and genome-wide association studies have identified a bidirectional causal association between intrinsic EAA (IEAA) and oral lichen ruber planus, an autoimmune disease of the oral mucosa [49]. Similarly, in Finnish cohorts (FinnGen), GrimAge acceleration has been supported by genetic variance as increasing the risk of periodontitis, whereas PhenoAge acceleration has been supported as leading to stomatitis.⁶¹ At the center of all these tissue-specific or systemic syndromes lie chronic oxidative deterioration and loss of regulation of immune function.

Therapeutic Interventions and Modulation of the Epigenetic Clock

One of the most exciting and dogma-breaking findings in biomedical science is the demonstration that the cumulative damage in methylation profiles and the aging vector may not only be slowed, but may also be reversible through pharmacological and physiological interventions [50].

The most revolutionary clinical study in this field is the Stanford University-based "Thymus Regeneration, Immunorestitution, and Insulin Mitigation" (TRIIM) pilot trial (2015–2017) [51]. The study aimed to rejuvenate the thymus gland, which undergoes atrophy with aging and is the primary cause of immunosenescence. A combined pharmacological protocol consisting of recombinant human growth hormone (rhGH) for its thymotrophic effect, dehydroepiandrosterone (DHEA), and metformin to suppress hyperinsulinemia and oncological risk was administered for 12 months to nine healthy men between the ages of 51 and 65. At the end of one year of experimental treatment, a dramatic increase in thymic density was observed radiologically, while the clinical molecular outcomes exceeded expectations. All four major epigenetic clocks belonging to different generations, namely Horvath, Hannum, PhenoAge, and GrimAge, showed that the subjects' biological, or epigenetic, ages had statistically significantly reversed by an average of 2.5 years relative to baseline [52]. More importantly, the fact that the rejuvenation effect observed in GrimAge and PhenoAge scores largely persisted six months after the experiment was terminated, at the 18-month measurements, revealed the potential of the treatment to induce a durable molecular reprogramming. Multicenter TRIIM-X trials designed to validate these findings in a larger cohort are currently ongoing [53].

In contrast to pharmacological regeneration, as in TRIIM, dietary interventions based on systemic stress regulation provide different molecular readouts. The CALERIE trial tested the effects of 25% caloric restriction applied over two years in 220 healthy adults [54]. The study results contain a critical nuance: caloric restriction did not lead to a marked age reversal in static odometer clocks representing cumulative damage, such as Horvath and GrimAge. In other words, the methylation disruptions, or accumulated damage, that had already occurred were not erased. However, a highly significant slowing was achieved in the algorithmic scores of DunedinPACE, the "speedometer" that measures the pace of aging. This finding clearly demonstrates that chronic caloric restriction does not reverse the biological clock, but statistically and functionally suppresses the "new" epigenetic degeneration that the cell would face in later periods to a serious extent.

There are also interesting results across the remainder of the intervention spectrum. In the diet-based NU-AGE Mediterranean diet study, a tendency toward rejuvenation was recorded particularly among individuals whose biological age before the experiment was higher than their chronological age, that is, those with accelerated aging [52,55]. Comprehensive meta-analyses have shown that exercise, plant-based diets, GLP-1 receptor agonists such as semaglutide, the cholesterol-lowering agent pitavastatin, ketamine, and omega-3 fatty acids produce a certain degree of "rejuvenation," or age reduction, in next-gen-

eration clocks [56]. By contrast, while there is no strong signal in the current literature that senolytic agents, which provide cellular clearance, rapamycin, or nicotinamide riboside supplementation reverse the biological clock, plasmapheresis applications have been observed to accelerate epigenetic clock acceleration upward [57]. In vivo partial cellular reprogramming experiments using the most fundamental cellular modification, Yamanaka factors such as OSK cassettes, including ER-100 studies, confirm in preclinical animal tissues, including mice and primates, the capacity of methylation codes to be completely reset at the stem cell level and represent the potential of the ultimate “reset” mechanism for human medicine.

Conclusion

Epigenetic clocks that use the codes of DNA methylation profiles have moved the science of gerontology beyond its dogmatic assumptions into a highly accurate, multidimensional, and quantifiable machine learning era. Biological clock mechanisms have evolved from first-generation odometers that estimate only the erosive effects of chronological time, such as Horvath and Hannum, to second-generation composite platforms that model an individual's probability of survival against systemic disease, immune collapse, and plasma dysfunction, such as GrimAge and PhenoAge, and to third-generation speedometers that read the instantaneous rate of cellular deterioration, such as DunedinPACE.

The genetic infrastructure underlying epigenetic age acceleration (EAA) establishes direct causal networks with replicative exhaustion, as observed in the TERT paradox, and with chronic vascular inflammation and immunosenescence embodied in the SELP and HLA loci. The fatal cardiovascular asymmetry created by clones carrying CHIP mutations, such as DNMT3A and TET2, through triggering epigenetic acceleration, together with functional correlations in the neurodegenerative course, creates a foundation in which genetic deterioration and biological collapse feed one another.

The resolution of the linear bottlenecks arising from Elastic Net architectures by AltumAge and derivative Deep Neural Networks (DNNs), together with the elucidation of “developmental rejuvenation” phenomena through single-cell sequencing technologies such as the scAge algorithm, is redefining the limits of the information-processing potential of molecular biology. Indeed, the functional reversal of the organism's biological age through the use of combined endocrine-metabolic regulators, such as rhGH, DHEA, and metformin, in TRIIM clinical trials definitively demonstrates that biological aging is not an inevitable, entropic end, but an algorithmically manipulable genetic cassette. In the near future, the integration of specific cellular modulators and partial reprogramming technologies will serve the goal of fundamentally resetting the epigenetic aging vector from which pathologies arise, rather than merely treating the symptoms of diseases in personalized preventive medicine.

Ethical Approval

Not applicable

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Conflict of Interest

The authors declare that they have no conflict of interest.

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