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Patterns of DNA methylation as an indicator of biological aging: State of the science and future directions in precision health promotion

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ABSTRACT

Background: A rapidly expanding literature suggests that individuals of the same chronological age show significant variation in biological age.

Purpose: The purpose of this article is to review the literature surrounding epigenetic age as estimated by DNA methylation, involving the addition or removal of methyl groups to DNA that can alter gene expression without changing the DNA sequence.

Methods: This state of the science literature review summarizes current approaches in epigenetic age determination and applications of aging algorithms.

Findings: A number of algorithms estimate epigenetic age using DNA methylation markers, primarily among adults. Algorithm application has focused on determining predictive value for risk of disease and death and identifying antecedents to age acceleration. Several studies have incorporated epigenetic age to evaluate intervention effectiveness.

Discussion: As the research community continues to refine aging algorithms, there may be opportunity to promote health from a precision health perspective.

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Background

It has become increasingly apparent that health lies at the intersection of a complex set of both stable and unstable factors, sparking the birth and rapid growth of the fields of genomics and exposomics, respectively. The genome is comprised of ~3 billion inherited and largely stable deoxyribonucleic acid (DNA) base pairs that serve as the building blocks for more than 20,000 genes (Feero, Guttmacher, & Collins, 2010). The exposome takes shape throughout the life course, defined as the complete set of exposures an individual encounters (Wild, 2005). Genomics and exposomics are often embedded in the study of nature vs. nurture or gene by environment interactions, as gene expression depends upon the DNA sequence that is inherited and chemical modifications to the code that can be inherited,

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acquired, and adapted (Allis & Jenuwein, 2016). These modifications, termed epigenetic modifications, leave the DNA sequence untouched but can alter the likelihood of a gene being transcribed and translated into a protein (Andersen & Tost, 2018; Lyko, 2018; Theunissen & Jaenisch, 2017). Thus, the exposome adds variation to the genome, creating unique epigenomic signatures and patterns of gene expression that continue to morph throughout life. This point is well-illustrated by the rapidly expanding literature on variation in epigenetic aging.

The age of an organism is an established correlate of health deterioration. Yet, there is incredible interindividual variability in health status at ages across the life course (Flatt & Partridge, 2018). This realization has prompting pursuit of the biological basis of variable patterns of aging, producing a scientific literature that spans several decades and is continuing to grow (for a review on this larger topic, see Jylhava, Pedersen, & Hagg, 2017). Foundational to this work was the discovery of telomeres, which protect the ends of chromosomes (Szostak & Blackburn, 1982). Telomere length shortens as a function of age and exposures, ultimately promoting the arrest of cell division (for reviews, see Muezzinler, Zaineddin, & Brenner, 2013; Niccoli & Partridge, 2012; Turner, Vasu, & Griffin, 2019). Complimentary to the notion of telomere shortening is that of epigenetic aging, with telomere length and epigenetic patterns showing independent associations with chronological age (Jylhava et al., 2017; Kabacik, Horvath, Cohen, & Raj, 2018; Marioni et al., 2016). Aging is characterized by a number of epigenetic changes, including changes to the way DNA is packaged and changes that affect the stability of the ribonucleic acids needed to direct the production of a protein (e.g., histone modifications, noncoding RNA (Levine et al., 2018; Song & Johnson, 2018)). Though, DNA methylation (DNAm) and demethylation, or the addition and removal of methyl groups to and from DNA, respectively, have received the most attention in epigenetic aging research.

In this state-of-the-science literature review, we provide a broad synthesis of a large and growing literature surrounding epigenetic age as estimated by DNAm. We summarize approaches developed to quantify epigenetic age according to patterns of DNAm and the primary ways with which epigenetic aging algorithms are being applied to health sciences research. Finally, we discuss emerging and potential areas of inquiry that apply epigenetic aging algorithms from a precision health perspective.

Epigenetic Age Determination as Estimated by DNAm

As shown in Figure 1, DNAm results from the addition of methyl groups (5-methylcytosine) to the cytosine base of the phosphate-linked cytosine-guanine (CpG) DNA base pair. Transfer of methyl groups is mediated by DNA methyl transferase enzymes including DNMT1, DNMT3A, and DNMT3B, promoting establishment and maintenance of DNAm patterns (Lyko, 2018). Removal of methyl groups can be accomplished by the conversion of 5-methylcytosine to 5-hydroxymethylcytosine via the base excision repair pathway (Barter & Foster, 2018). CpGs are typically in a methylated state with the exception of CpG islands, high concentrations of CpGs located near promoter sites where transcription factors "turn genes on" (Goronzy, Hu, Kim, Jadhav, & Weyand, 2018). When CpGs are methylated, gene expression can be repressed by interfering with binding of these transcription factors or by triggering chemical reactions that encourage the DNA to become tightly wrapped and difficult to access (Wu, Caffo, Jaffee, Irizarry, & Feinberg, 2010). Conversely, when CpGs are demethylated, gene expression can be enhanced by encouraging access to and transcription and expression of genes (Allis & Jenuwein, 2016).

With the growth of array technologies and analytic strategies capable of interrogating the methylation of hundreds of thousands of CpGs across the epigenome, there has been considerable interest in identifying the highest performing CpGs for use in DNAm aging algorithms. Table 1 lists in order of citation count a sampling of DNAm algorithms published in this area, focusing on those developed and validated using human samples. Bocklandt et al. (2011) was the first to publish in this area, followed by Hannum et al. (2013) and Horvath (2013). Interestingly, this work garnered early attention in the field of forensic science, as epigenetic aging algorithms provided a means to estimate age on the basis of a single biological sample. The concept of epigenetic aging has also gained traction in health sciences research. Hannum et al. (2013) and Horvath (2013) have produced the most widely applied and cited studies in this area, which we review in some detail below.

The Hannum et al. (2013) algorithm was derived and validated using whole blood from two independent cohorts ($N_1 = 482$; $N_2 = 174$) of Caucasian and Hispanic men and women aged 19 to 101. The model was built by regressing chronologic age onto percent DNAm at 485,577 CpGs quantified using the HumanMethylation450 BeadChip for each individual's population of whole blood cells. Clinical parameters were controlled. Seventy one CpGs were identified as highly associated with chronological age among the training and validation sets, generating the quantitative definition of the apparent methylomic aging rate as the ratio of predicted epigenetic age to chronological age. More than 90% of the predictive CpGs were associated with known genes, which were overenriched for biological pathways of cell communication, locomotion, cell proliferation, and growth and underenriched for G-protein coupled receptor activity, ribosome, RNA splicing, and M phase, providing a link with putative sites for aging and age-related disease.

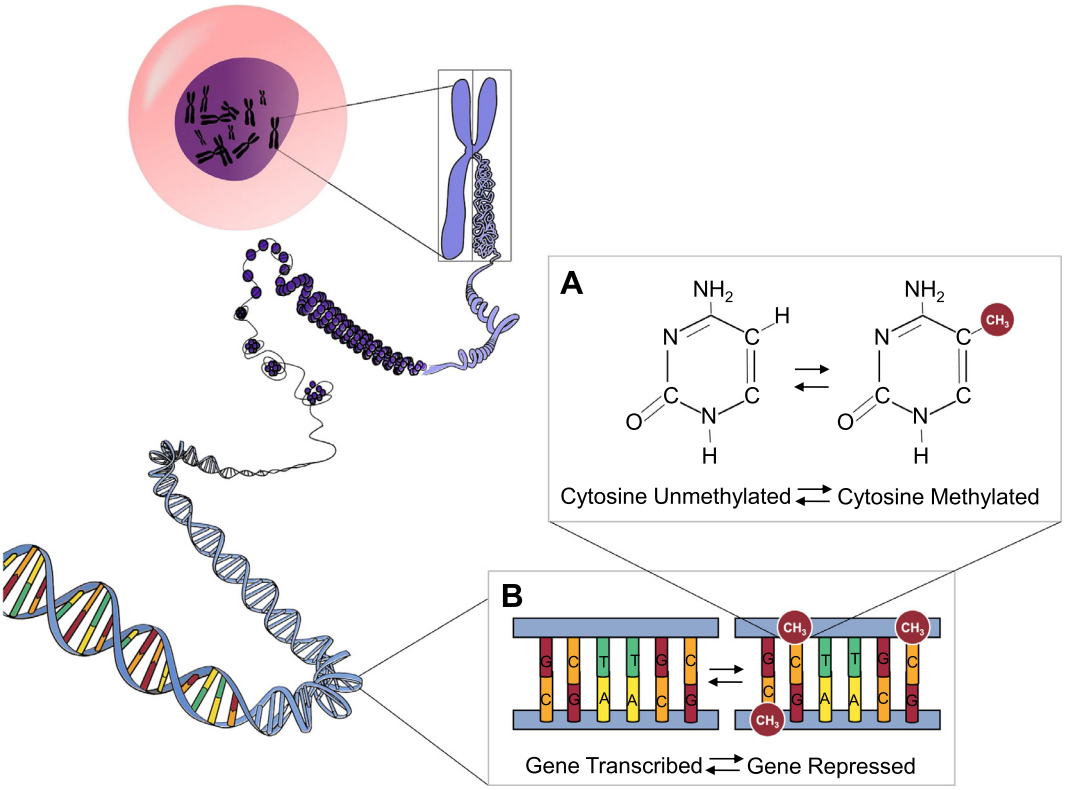

Figure 1 – DNA methylation. Chromosomes, found in the nucleus of most living cells, carry the DNA sequence of an organism. The double-stranded DNA sequence forms a double helix, with each DNA strand composed of smaller units termed nucleotides. Nucleotides form base pairs, whereby adenine (A) pairs with thymine (T) and cytosine (C) pairs with guanine (G). As shown in Box A, DNA methylation occurs when a methyl group (CH_3) is added to the cytosine of a phosphate-linked cytosine-guanine (CpG) base pair. Removal of methyl groups can be accomplished by the conversion of 5-methylcytosine to 5-hydroxymethylcytosine. As shown in Box B, when CpGs are methylated, gene expression can be repressed. When CpGs are demethylated, gene expression can be enhanced.

The Horvath (2013) algorithm was derived and validated using 82 datasets (7,844 noncancer samples) reflecting percent DNAm at 21,369 CpGs available across both the HumanMethylation27 and HumanMethylation450 BeadChip platforms. Participant ages varied widely and 51 tissue and cell types were assessed. Like Hannum et al., Horvath regressed chronologic age onto percent DNAm, identifying 353 predictive CpG dinucleotides. Genes colocating with Horvath's (2013) age-predictive CpGs showed enrichment for molecular and cellular functions associated with cell death and survival, cellular growth and proliferation, lipid metabolism, molecular transport, and small molecule biochemistry.

Of note, Hannum et al. (2013) derived their model using a single source tissue, a commonly used approach (see Table 1). Hannum et al. (2013), like others, later confirmed that the algorithm performed reasonably well when linear offsets were adjusted for alternative tissues. Alternatively, Horvath (2013) derived and validated his model across tissues and cell types, noting good performance with the exception of breast, skeletal muscle, and heart tissue, uterine endometrium, and dermal fibroblasts. Others have followed suit (Jung et al., 2019; Xu, Li, Yang, Li, & Shao, 2019). Moreover, while Hannum and a number of teams have drawn their samples exclusively from adult populations, Horvath and others have included samples from individuals across a broad range of ages in model derivation. Others have begun to focus on specific populations, such as adolescents, children, or neonates. For example, Knight et al. (2016) examined the predictive power of the DNAm of fetal-derived blood and placenta for the gestational age of the neonate at birth, noting DNAm gestational age to independently predict birthweight when controlling for chronological gestational age.

Since publication of the landmark Hannum and Horvath studies, some teams aimed to develop strategies to overcome obstacles encountered as technologies advance, such as reduced predictive accuracy when an algorithm derived with one platform is applied to data produced with another (Hong, Shin, Jung, Lee, & Lee, 2019). Others have aimed to establish less costly and more user-friendly methods. For example, Weidner et al. (2014) derived a predictive model of epigenetic age using peripheral blood DNAm profiles among 575 individuals aged 0 to 78. Age-predictive CpGs were tested in various 5-CpG combinations to identify a subset with the greatest predictive power for chronological age determination. Ultimately, among independent

Table 1 – A Sample of Algorithms for Epigenetic Age Determination Using DNA Methylation Markers

Reference	Data Source	Tissue(s)*	Platform(s)	CpGs	Age Correlation†	Times Cited‡
(Horvath, 2013)	7,844 samples from children, adolescents, and adults	51 tissue types	27K; 450K	353	0.96	1,063
(Hannum et al., 2013)	656 samples from adults	Blood	450K	71	0.91	664
(Bocklandt et al., 2011)	94 samples from adults	Saliva	27K	3	0.87	303
(Weidner et al., 2014)	575 samples from children, adolescents, and adults	Blood	27K	3	0.79§	274
(Florath, Butterbach, Muller, Bewerunge-Hudler, & Brenner, 2014)	898 samples from older adults	Blood	450K	17	0.84	144
(Levine et al., 2018)	9,926 samples from adults	Blood	27K; 450K; EPIC	513	0.71	47
(Vidaki et al., 2017)	1156 samples from children, adolescents, and adults	Blood	27K; 450K	16	0.96	39
(Knight et al., 2016)	1,434 samples from neonates	Blood	27K; 450K	148	0.91	32
(Hong et al., 2017)	226 samples from adults	Saliva	SNaPshot	7	0.95	22
(Vidal-Bralo et al., 2016)	725 samples from adults	Blood	27K	8	0.6	11
(Freire-Aradas et al., 2018)	209 samples from children and a, dolescents	Blood	MassARRAY	6	0.89	4
(Lu et al., 2019)	2356 samples from primarily adults	Blood	450K; EPIC	1,030	0.82	2
(Xu et al., 2019)	4,294 samples from children, adolescents, and adults	14 tissue types	2K; 450K	13	0.83	0
(Hong et al., 2019)	226 samples from adults	Saliva	MPS; SNaPshot	62	0.90	0
(Jung et al., 2019)	448 samples from adults	3 tissue types	SNaPshot	5	0.94	0
(Li et al., 2018)	180 samples from children and adolescents	Blood	EPIC	83	0.93	0

Note. 27 K, HumanMethylation27 BeadChip; 450 K, HumanMethylation450 BeadChip; EPIC, MethylationEPIC BeadChip; SNaPshot, Methylation SNaPshot; MassARRAY, MassARRAY mass spectrometry; MPS, Massively Parallel Sequencing.

* For model derivation.

† Testing set.

‡ According to Web of Science on May 10, 2019.

§ Data pulled from Lin et al. (2016) due to the correlation being unavailable in the original publication of the algorithm.

samples, peripheral blood percent DNAm at 3 CpG sites was found to predict age within approximately 5 years of chronological parameters. Of note, simplified models have generally shown weaker associations with chronological age (e.g., R^2 values of 0.60–0.79 (Lin et al., 2016; Vidal-Bralo, Lopez-Golan, & Gonzalez, 2016)).

DNAm Age and Precision Health

In February of 2015, Collins and Varmus (2015) published a foundational piece focused on precision health (i.e., disease prevention and treatment with interindividual variability in mind) in the *New England Journal of Medicine* in follow-up to President Barack Obama's January State of the Union Address on the topic. Collins and Varmus reiterate that new initiatives in precision health include two main components, "a near-term focus on cancers and a longer-term aim to generate knowledge applicable to the whole range of health and disease" (2015, p. 793). This new vision highlights the progress to-date and possibilities made evident in the field of oncology, with several excellent reviews synthesizing the literature in this area (e.g., Gonzalez-Angulo, Hennessy, & Mills, 2010; Schmidt, Chau, Price, & Figg, 2016; Vargas & Harris, 2016). The longer-term aim of these initiatives places focus on the boundless potential of the application of precision health approaches to the promotion of health.

DNAm age, a biological reflection of interindividual variability in aging, holds significant potential as a useful parameter in disease prevention and treatment from a precision health perspective. Though, much work remains to realize this vision. Below, we outline advances and opportunities working toward the use of DNAm aging algorithms as screening biomarkers, surrogate endpoints, and predictive biomarkers, which indicate disease risk, substitute for a clinical endpoint to predict clinical benefit, and predict the probability of response to a given treatment, respectively (Drucker & Krapfenbauer, 2013; Institute of Medicine Committee on Qualification of Biomarkers and Surrogate Endpoints in Chronic Disease, 2011).

DNAm Age as a Screening Biomarker

A number of studies have sought to establish whether algorithms of DNAm age better predict health parameters than chronological age. This concept is illustrated

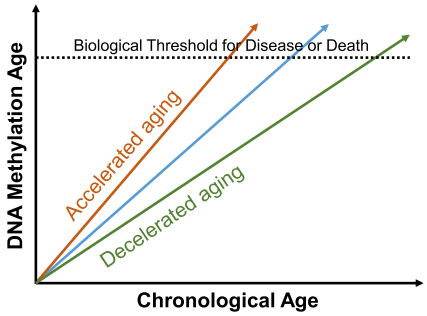

Figure 2 – DNA methylation age acceleration and deceleration in disease and death.

When DNA methylation aging is accelerated (orange line) or decelerated (green line), the biological thresholds for various diseases and all-cause mortality (dashed line) may be reached early or later than chronologically anticipated, respectively. (Color version of figure is available online.)

in Figure 2 and is predicated on the assumption that accelerated biological aging speeds the rate with which various thresholds for disease and death may be reached by an individual. Perhaps the most consistent finding in the DNAm aging literature is the ability of the biological parameter to predict risk for and time to death. Indeed, several meta-analyses (Chen et al., 2016; Fransquet, Wrigglesworth, Woods, Ernst, & Ryan, 2019; Marioni et al., 2015) support associations among DNAm age according to Hannum and Horvath estimates and all-cause mortality, with each 5-year positive difference between DNAm age and chronological age associated with an 8% to 21% greater risk for mortality above and beyond chronological age and other clinical parameters.

DNAm age has also been used to predict risk for age-related diseases. In a recent systematic review from Fransquet et al. (2019), five studies focusing on cancer, four studies focusing on cardiovascular-related diseases, and two studies focusing on dementia were identified. The preponderance of the evidence suggests that greater DNAm age shows predictive utility for age-related disease risk, specifically for colorectal cancer, stroke, cardiovascular disease, and dementia (Fransquet et al., 2019). Though, DNAm age inconsistently predicted (i.e., breast cancer and lung cancer) or failed to predict (i.e., coronary heart disease) risk for some disease subtypes (Fransquet et al., 2019).

It is important to note that while such findings support the notion of interindividual variability in biological aging and risk, studies employing DNAm age as a starting point for the development and validation of screening biomarkers for use under specified conditions are required. The scientific literature surrounding the use of DNAm in cancer screening and diagnostics provides an excellent framework from which to consider development and validation of DNAm-based screening tools for the prediction of alternative disease processes, with detailed reviews available on this topic (e.g., Mari-Alexandre et al., 2017; Pan, Liu, Zhou, Su, & Li, 2018).

DNAm Age as a Surrogate Endpoint

Considering the above findings, many have sought to identify genomic and exposomic antecedents to variable patterns of DNAm aging and determine whether DNAm aging is amenable to intervention. In doing so, DNAm age is appreciated as a correlate of clinical endpoints of interest, which has potential to inform clinical care through proof-of-concept trials. Though, it must be noted that considerable work remains before estimates of DNAm age can be considered reasonably likely to predict clinical benefit as a surrogate endpoint, similar to the use of blood pressure to identify risk factors for and test interventions aiming to reduce cardiovascular morbidity and mortality (Institute of Medicine Committee on Qualification of Biomarkers and Surrogate Endpoints in Chronic Disease, 2011).

Studies of this nature have produced data supporting that DNAm age shows evidence of heritability, with 42% to 43% of the variance in DNAm age calculated per the Hannum and Horvath methods, respectively, explained by genetic factors (Marioni et al., 2015). The relative contributions of various exposures to patterns of DNAm aging have been more difficult to determine. A recent meta-analysis of studies applying the Hannum ($n = 7$) or Horvath ($n = 11$) methods for the determination of DNAm age provides support for an association between greater body mass index and greater DNAm age (Ryan, Wrigglesworth, Loong, Fransquet, & Woods, 2019). However, this same report notes that in systematically examining 61 studies on this topic, there is insufficient evidence to draw conclusions surrounding associations among DNAm age and environmental pollution, socioeconomic status, education, smoking, alcohol consumption, diet, physical activity, stress, and mental health (Ryan et al., 2019).

These discordant findings are likely explained by differences in sampling and methods, particularly considering that there is evidence to suggest that the methylome may be particularly susceptible to exposures during early life. In fact, there is data to suggest that patterns of DNAm age acceleration vs. deceleration at age 15 to 24 remain largely stable over a follow-up period of 25 years (Kananen et al., 2016). Also in support of this notion, Austin et al. (2018) reported that socioeconomic disadvantage from birth to age five but not current socioeconomic disadvantage predicts DNAm age at 15 to 55 years. Similarly, a recent meta-analysis noted that childhood trauma, but not lifetime trauma exposure, predicted accelerated DNAm aging using data from 2,186 participants across nine cohorts (Wolf et al., 2018). These studies suggest that inventions targeting risk and resilience in early life may be particularly beneficial in the promotion of healthy DNAm aging.

Several studies have also begun to use DNAm age to support the broad positive health effects of interventions, particularly those targeting diet and nutrition. For example, the effect of vitamin D and vitamin B supplementation on DNAm age has been examined, with lower rates of DNAm aging noted among intervention groups of both young and older adults after 4 to 12 months of supplementation (Chen et al., 2019; Obeid, Hubner, Bodis, Graeber, & Geisel, 2018). Noting that DNAm aging correlates strongly with lifespan among mice, monkeys, and humans, Maegawa et al. (2017) examined the effects of 30% to 40% caloric restriction on DNAm age in mice and monkeys who initiated the restriction at young and middle-age, respectively. They found that calorie-restricted mice exhibited a DNAm age 2 years younger than control mice and calorie-restricted monkeys exhibited a DNAm age 7 years younger than control monkeys, which is significant considering that the maximum lifespan of mice and monkeys is 4 and 40 years, respectively. Such findings have been extended to randomized trials of humans, with caloric restriction achieved at approximately 12% over 2 years significantly slowing the rate of biological aging among nonobese adults aged 21 to 50 years (Belsky, Huffman, Pieper, Shalev, & Kraus, 2017).

DNAm Age as a Predictive Biomarker

Predictive biomarkers forecast the probability of response to a given treatment, allowing clinicians to stratify targeted preventive interventions and treatments according to individualized risk (Drucker & Krapfenbauer, 2013). Early applications leveraging alteration of gene expression through DNAm were in cancer, with the first drug blocking DNAm demonstrating gene repression and phenotypic changes in fibroblast cell line (Feinberg & Tycko, 2004; Taylor & Jones, 1979). Since that time, advances in pharmacologic intervention targeting deviant epigenetic modifications have revolutionized cancer therapeutics (Gillman & Hammond, 2016).

However, whether and how accelerated DNAm aging mediates associations among various genomic and exposomic exposures and specified disease processes remains largely unclear. Therefore, precise targets for intervention become difficult to identify. For example, while Hannum et al. (2013) were able to replicate validation findings by examining the expression of genes associated with the identified age-associated CpGs, Horvath (2013) failed to note significant overlap in patterns of DNAm and gene expression that predicted chronological age when assessed simultaneously. The Hannum and Horvath algorithms also significantly differ in terms of identified age-associated CpGs.

To advance this work, the concept of DNAm phenotypic age was recently introduced by Levine et al. (2018). The algorithm was derived by training percent methylation at 20,169 CpGs onto albumin, creatinine, glucose, C-reactive protein, and alkaline phosphatase levels, and lymphocyte percentage, red cell volume, red cell distribution width, and white blood cell count, conceptualized as a phenotypic representation of aging. Examining samples from several large cohort studies, the team noted that their measure of DNAm phenotypic age outperformed the Hannum and Horvath methods for the prediction of 10-year (Levine AUROC = 0.6177; Hannum AUROC = 0.5670; Horvath AUROC = 0.5605) and 20-year (Levine AUROC = 0.5615; Hannum AUROC = 0.5228; Horvath AUROC = 0.5038) mortality risk (Levine et al., 2018). Similar methods tailored toward specified pathways linking exposures to disease may prove fruitful in the identification of targets for tailored interventions.

Conclusions

The studies of DNAm aging conducted to date lend support to the notion that genomic and exposomic exposures "get under the skin" in the determination of health and disease, perhaps by establishing and editing gene expression over time and across generations. This has important implications for precision health in and of itself, as DNAm age can serve as a quantifiable indicator of interindividual variability in aging as a function of the genome and exposome. As such, large amounts of information are condensed into manageable sets of data. Indeed, notable progress has been made in the use of DNAm age as a screening biomarker and correlate of accelerated health deterioration for proof-of-concept studies. However, there are significant limitations to the application of DNAm aging algorithms. Namely, validation of findings presents a significant challenge when a dynamically-regulated biomarker is used to predict risk or treatment response. DNAm age can also be estimated using a number of tissues, platforms, and algorithms, with careful attention to the source material, technical expertise, and computational needs required to produce reproducible findings. Moreover, prediction of risk without precise targets for intervention is perhaps futile, highlighting the significant work that remains.

In order to move this line of research forward from a precision health perspective, we propose that the unifying principle of DNAm age acceleration and deceleration serve as a critical foundation from which to identify targets for disease prevention. We propose that precision strategies for the promotion of health begin early in life, as patterns of DNAm appear to be particularly susceptible to risk exposures but also may be particularly amenable to preventive strategies during this time. This work will require transdisciplinary collaboration across scientific disciplines and healthcare professions and a particular focus on identifying the pathways that drive DNAm-associated risk and convey DNAm-associated resilience. Ultimately, gene

expression depends upon the DNA sequence that is inherited and chemical modifications to the code that can be inherited, acquired, and adapted (Allis & Jenuwein, 2016). As such, there may be significant potential to mitigate inherited and acquired risk by targeting the chemical modifications of the genome.

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