Current Environmental Health Reports (2022) 9:196–210
https://doi.org/10.1007/s40572-022-00338-8

ENVIRONMENT AND AGING (X GAO AND A BACCARRELLI, SECTION EDITORS)

Integrating DNA Methylation Measures of Biological Aging into Social Determinants of Health Research

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Accepted: 10 January 2022 / Published online: 18 February 2022
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Abstract

Purpose of Review Acceleration of biological processes of aging is hypothesized to drive excess morbidity and mortality in socially disadvantaged populations. DNA methylation measures of biological aging provide tools for testing this hypothesis.

Recent Findings Next-generation DNA methylation measures of biological aging developed to predict mortality risk and physiological decline are more predictive of morbidity and mortality than the original epigenetic clocks developed to predict chronological age. These new measures show consistent evidence of more advanced and faster biological aging in people exposed to socioeconomic disadvantage and may be able to record the emergence of socially determined health inequalities as early as childhood. Next-generation DNA methylation measures of biological aging also indicate race/ethnic disparities in biological aging. More research is needed on these measures in samples of non-Western and non-White populations.

Summary New DNA methylation measures of biological aging open opportunities for refining inference about the causes of social disparities in health and devising policies to eliminate them. Further refining measures of biological aging by including more diversity in samples used for measurement development is a critical priority for the field.

Keywords Biological aging · DNA methylation · Epigenetic clock · Social determinants of health

Introduction

Individuals who are socioeconomically disadvantaged or marginalized based on their racial/ethnic identity tend to develop aging-related diseases at younger ages and suffer earlier mortality as compared to individuals who are wealthier and White [1, 2]. Macro-structural social determinants of health, including racism, classism, sexism, and their intersections, drive these disparities [3, 4••]. One mechanism hypothesized to link social determinants of health with a shorter healthy lifespan is an acceleration of biological processes of aging [5, 6].

Biological aging is the gradual and progressive decline in system integrity that occurs with advancing age [7]. This age-dependent decline in system integrity is thought to arise from an accumulation of molecular changes, known as hallmarks, that undermine the functioning of molecular networks and organ systems, driving vulnerability to disease and death [8]. Now, the emerging field of geroscience aims to prevent and treat disease through intervention on these hallmarks. The core hypothesis of geroscience is that slowing or reversing the molecular hallmarks of aging can slow or reverse the decline in system integrity, preventing or delaying disease and disability [8, 9].

The geroscience field has been pioneered by researchers studying model organisms under laboratory conditions and is focused on developing clinical treatments for diseases of aging [10–13]. However, there are important connections between the basic biology of aging and social determinants

This article is part of the Topical Collection on *Environment and Aging*

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of health in humans. Many of the molecular changes that form the basis of aging, including cell senescence, inflammation, mitochondrial dysfunction, and epigenetic alternations, are also affected by environmental exposures ranging from chemical toxicants to social stressors that are concentrated in socially disadvantaged populations [14–18]. Geroscience, therefore, promises new opportunities for understanding the causes of social gradients in health and can help devise new strategies for building aging health equity.

Realizing the promise of geroscience to improve human health requires an integration of aging biology with behavioral and social sciences [19]. However, studies to investigate biological aging as a mediator of social determinants of health have faced the barrier that the molecular changes that form the biological basis of aging are difficult to observe in epidemiologic studies. There is no gold standard measure of aging and, historically, a little consensus around valid aging biomarkers [20, 21]. Now, this is beginning to change. A new family of measurements based on analysis of DNA methylation shows promise and opens new opportunities for the integration of research into aging biology and social determinants of health.

In this article, we review progress in applications of DNA methylation–based measures of biological aging to study how socially determined inequalities drive disparities in healthy aging. Early studies applying these new DNA methylation measures suggest opportunities for refining inference about the causes of social disparities in health and devising programs and policies to eliminate them. Specifically, because these new measures can reveal differences in the progress and pace of aging decades before chronic diseases become established, they can help isolate when in the life course and through what specific exposures health inequalities in aging become established. In addition, these new measures can inform the evaluation of interventions to address health inequalities by providing a readout on the short- and medium-term effects of programs and policies in "pre-symptomatic" individuals who have not yet begun to manifest aging-related disease and disability.

This promise comes within the context of an important limitation: DNA methylation measurements of biological aging have often been developed in convenience samples not designed to represent particular populations. Even when samples are socioeconomically representative, they represent populations that are overwhelmingly White. This underrepresentation of non-White individuals parallels data gaps noted in human genetics research [22, 23]. Despite the reliance on mostly White samples for measurement development, DNA methylation measures of biological aging tend to show similar magnitudes of association with risk for disease, disability, and mortality across different race/ethnic groups within the USA [24–27]. Where there are differences, associations tend to be somewhat stronger in White as compared with non-White samples. Research in more non-White samples and in diverse cohorts is needed to better establish the validity of DNA methylation measures of biological aging in populations of diverse genetic ancestries and race/ethnic, social identities. Further development of aging measures in more diverse samples is a priority.

The remainder of this review is organized as follows: the "Biological Aging and Social Determinants of Health" section provides a conceptual overview of how social determinants of health affect biological aging. The "Quantification of Biological Aging for Social Determinants of Health Research" section introduces approaches to the measurement of biological aging in social determinants of health research, with a focus on recently introduced methods based on analysis of DNA methylation. The "DNA Methylation Clocks and Social Determinants of Health" section reviews recent work testing how social determinants of health are associated with DNA methylation measures of aging. A key finding from these studies is that the new generation of DNA methylation measures of biological aging derived from analysis of mortality risk and physiological decline, which are more predictive of morbidity and mortality, are also more strongly associated with social determinants of health as compared with the original epigenetic clocks developed from analysis of chronological age. The "Challenges and Recommendations" section reviews limitations of existing DNA methylation measures of biological aging and makes recommendations to overcome them with the goal of maximizing the utility of measures of biological aging in promoting aging health equity.

Biological Aging and Social Determinants of Health

How Do Social Determinants of Health Affect Biological Aging?

Healthspan and lifespan disparities at the intersection of socioeconomic status and socially constructed dimensions of race/ethnicity, as well as other identity characteristics, are profound [4••, 28]. Exposure to environmental toxicants, opportunities for restorative leisure and exercise, physical and psychological safety, social support, and access to nutritious food and healthcare, among other factors, differ across these social positions [29, 30]. In turn, these differences in health-damaging exposures and health-promoting resources drive biological changes that contribute to more or less healthy aging [18, 31–35]. Connections among social identities, mechanisms of inequality, processes of biological aging, and disparities in healthy aging are illustrated in Fig. 1.

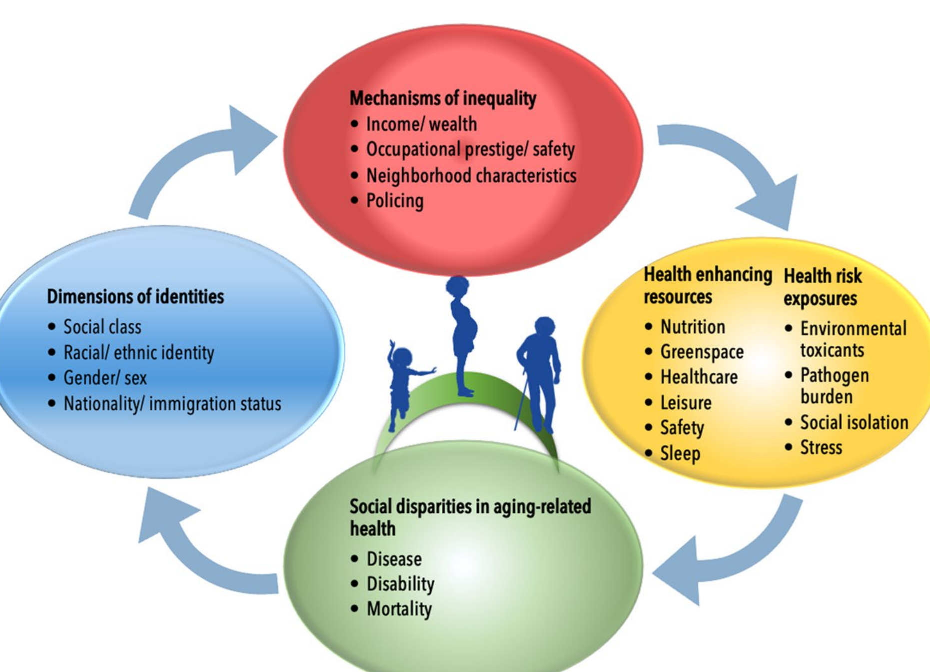

Fig. 1 The social environment is associated with multiple environments that affect health across the lifespan. Epidemiological research has documented healthspan and lifespan disparities across dimensions of social identities (*e.g.*, social class and racial/ethnic identity; blue circle). Mechanisms of social inequality (*e.g.*, income inequality and policing; red circle) lead to disparate access to health-enhancing resources (*e.g.*, nutrition, leisure; yellow circle) and disparate exposure to health risks (*e.g.*, toxicants, stress) between social identities. These cause social disparities in aging-related disease, disability, and mortality (green circle), which may reinforce dimensions of social inequality in the next generation

When in Development Do Social Determinants of Health Affect Biological Aging?

Social determinants of health clearly affect aging in later life. People living in or near poverty and those with marginalized racial/ethnic identities experience more rapid functional decline, earlier accumulation of disease and disability, and earlier mortality [36–40]. The processes driving these later-life disparities begin much earlier in the life course; a range of adverse early-life conditions, including socioeconomic disadvantage, maltreatment by caregivers, and unsafe or unstable living conditions, are associated with a shorter lifespan, earlier onset of aging-related disease, and more rapid decline in physiological integrity from young adulthood to midlife [41–45]. A possible mechanism linking early-life social determinants with unhealthy aging is an acceleration of biological aging.

The biological process of aging, which is characterized by a breakdown in resilience mechanisms, damage accumulation, and loss of system integrity, is distinct from programmed development, which assembles reproductively viable life [46]. However, accumulation of molecular damage commences at the very earliest stages of development, suggesting the possibility that aging is ongoing almost from conception [47, 48]. Observations of biological aging at the early stages of development are few. However, epigenetic marks associated with aging are removed from genomes during embryogenesis and begin to accumulate thereafter, suggesting that aging may indeed begin at the earliest stages of life [49]. And there is substantial evidence for the effects of the prenatal environment on outcomes in aging [50]. However, molecular analysis of aging in early-life humans remains limited. Blood analysis of telomere length, a biomarker of cellular aging [51, 52], indicates more advanced/faster aging in human infants exposed to perinatal adversity and in children exposed to early-life adversity [53–57]. But the science of telomere length as a true biomarker of aging remains unsettled [58–60]. Even if the early accumulation of molecular damage represents a disruption to development rather than the onset of aging, such damage may still condition the rate of aging later in life as a consequence of reduced resilience capacity [61]. Therefore, social determinants of health may affect the biological processes of aging from very early in development.

Quantification of Biological Aging for Social Determinants of Health Research

Measurement Approaches Across Biological Levels of Analysis

There is no gold standard measure of aging [21, 62]. Several approaches have been proposed at different levels of biological organization [63]. A conceptual overview of the progression of aging across levels of analysis and measures associated with different levels is presented in Fig. 2.

The level of biological organization most proximate to disease, disability, and death is commonly measured using indices of organism-level functional capacities. These include tests of balance, walking speed, strength, and cognitive performance, as well as summary scores counting

Molecular changes
Decline in system integrity
Functional decline
Disease, disability, mortality

Level of analysis

Measures

- Telomere length
- Mitochondrial DNA copy number
- DNA methylation clocks
- Gene-expression phenotypes, *e.g.*, the senescence-associated secretory phenotype, p16 expression in lymphocytes
- Proteomic and metabolomic clocks

- Allostatic load
- Blood-chemistry clocks
- Homeostatic dysregulation
- Pace of aging

- Frailty indices
- Functional performance batteries

Fig. 2 Levels of analysis of biological aging. Biological aging is the gradual and progressive decline in system integrity that occurs with advancing age. The figure illustrates the progression of biological aging across levels of analysis, from an accumulation of molecular changes to declines in organ system integrity, functional decline, disease, disability, and mortality. Measures of biological aging can be implemented at different levels of analysis: Molecular changes are commonly measured as omics clocks,[1] telomere attributes,[2] and mitochondrial DNA copy number.[3] Decline in system integrity is commonly quantified as allostatic load measures[4] and in blood chemistry clocks.[5] Functional decline is typically measured with various frailty indices.[6] DNA methylation measures of biological aging are implemented at the molecular level (*i.e.*, omics clocks). Some DNA methylation measures, including the PhenoAge and GrimAge and the DunedinPoAm pace of aging, also incorporate information from the level of organ system integrity

deficits in functional domains and biological systems known as frailty indices [64, 65]. Measurements based on deficits in functional capacities and frailty are most sensitive to changes occurring at the end of life when aging processes are advanced.

Beneath this organism-level functional capacity is the process of decline in system integrity, commonly measured using indices of organ- and organ-system-level functions. Broadly, there are three types of these indices. One type consists of counts of deficits in physiological parameters, including blood chemistry analytes and organ function tests, such as allostatic load indices [66, 67]. A second type consists of blood chemistry clocks and related algorithms that combine continuous information from multiple blood chemistry analytes and organ function tests to estimate the state of system integrity in an organism [68–72]. A third type uses longitudinal data on blood chemistry analytes and organ function tests to model the rate of decline in system integrity, such as Pace of Aging measures [73–75]. Measurements based on organ- and organ-system-level functions are sensitive to aging-related changes from young adulthood when trajectories of aging-related decline in system integrity begin to take shape. These types of measurements all show clear evidence of socioeconomic and racial/ethnic disparities [45, 71, 76–78].

Within the geroscience model, accumulating molecular changes underpin declines in system integrity. These molecular changes are abundant, and most are challenging to measure in humans. For example, telomere attrition and mitochondrial dysfunction are among the hallmarks of aging and are theorized as mediators of early-life adversity effects on aging [56, 79]. But telomere- and mitochondria-related measurements easily quantified in the blood are imperfect biomarkers of aging hallmarks [58, 59, 80, 81]. Expression of p16^{INK4a} is linked with cellular senescence, but may be most informative about aging when measured in specific lymphocyte subpopulations [82, 83]. The development of mechanistic biomarkers of aging hallmarks that can be assayed in studies of humans remains a work in progress [84, 85].

At present, the most promising molecular-level biomarkers of aging for clinical and epidemiologic studies have emerged from "omics"-based approaches that capture biological changes downstream of mechanistic hallmarks of aging. Recent developments in proteomic and metabolomic analyses suggest promise [86–88]. Currently, the

best-established omics-based biomarkers of aging processes are based on analysis of genome-wide patterns of DNA methylation, in particular a family of measurements broadly known as "clocks" [89••].

DNA Methylation Clock Measures of Biological Aging

DNA methylation marks are chemical tags on the DNA sequence that contribute to the regulation of gene expression (90). DNA methylation states are dynamic across the life course. As humans age, they experience a global loss of DNA methylation [91]. However, at specific sites on the genome, methylation marks are both gained and lost with age [92]. These site-specific changes with aging are so regular across individuals it is possible, through machine learning analysis, to develop algorithms that can predict a person's chronological age from DNA methylation analysis to a precision of within a few years [93–95]. These algorithms, introduced in the early 2010s, became known as "clocks." Clocks have received substantial attention in aging research because of the hypothesis that clock ages that are older than a person's chronological age indicate an advanced state of biological aging, whereas clock ages younger than a person's chronological age indicate delayed biological aging [89••].

DNA methylation clocks have so far progressed through two generations of development, with further generations now emerging. The first generation of clocks were developed by comparing older individuals to younger ones. For these clocks, the goal of machine learning analysis was to predict how many years a person had lived up to the time their DNA were collected, *i.e.*, their chronological age. These "first-generation" clocks are modestly predictive of mortality [96], but less consistent in predictions of other aging-related phenotypes, including disease, disability, and physiological and functional decline [97–100]. Moreover, the most precise clocks, those for which chronological age predictions were closest to the truth, have tended to be less predictive of health and mortality [101].

The second generation of clocks were developed by comparing individuals based on survival [25, 71, 102]. For these second-generation clocks, the goal of machine learning analysis was to predict how many years a person would continue to live following the collection of their DNA, *i.e.*, remaining lifespan. The most prominent of these, the PhenoAge and GrimAge clocks, include an intermediate step in which physiological features of aging are modeled from DNA methylation. In the case of the PhenoAge clock, mortality risk was first modeled from physiological markers and chronological age. This first-stage algorithm was then applied to a new sample in which it was modeled from DNA methylation to derive the final DNA methylation clock. In the case of the GrimAge clock, a set of physiological indicators were modeled from DNA methylation and then these DNA methylation predictions along with age, sex, and a DNA methylation prediction of smoking history were applied to model mortality. The resulting PhenoAge and GrimAge clocks are substantially more predictive of morbidity and mortality as compared to the first-generation clocks [103, 104].

A third generation of clocks is now emerging, including measures based on analysis of longitudinal within-person change [105••]. These clocks, referred to as Pace of Aging measures, are derived from the analysis of trajectories of physiological decline. The first stage of analysis models within-person change in a panel of physiological indicators. The second stage composites each participants' rates of change across the panel of indictors to form a single index of their personal rate of physiological decline. Finally, this composite Pace of Aging is modeled from DNA methylation measured at the end of the follow-up interval to derive the final algorithm. Whereas the first- and second-generation clocks aim to predict how old a person is biological, Pace of Aging measures aim to predict how *fast* a person is aging. First- and second-generation clocks take on values interpretable as ages. The Pace of Aging measures take on values interpretable as rates. The Pace of Aging measures have not yet received the same level of research attention as the earlier clocks. But the available evidence suggests that they are comparably predictive of health risks to the other clocks, although they are not as predictive of mortality as GrimAge [27, 103, 105••].

Several other DNA methylation biomarkers have been developed, including those that measure mortality risk [102] and mitotic age [106, 107]. In some datasets, these biomarkers outperform the second-generation clocks in the prediction of morbidity and mortality [108]. But, to date, these measures have been less widely used in social determinants of health research.

The first two generations of DNA methylation clocks and the Pace of Aging measures are described in Table 1.

DNA Methylation Clocks and Social Determinants of Health

Research testing associations between social determinants of health and DNA methylation clocks is still in its early stages. There are not yet enough studies with consistent methods to undertake meaningful meta-analyses. However, some patterns are emerging. Specifically, while the first-generation DNA methylation clocks show weak and inconsistent associations with social determinants of health, later generations of measures show stronger and more consistent associations. Figure 3 graphs effect sizes for five DNA methylation measures of aging from analysis of socioeconomic inequality and

Table 1 DNA methylation measures of biological aging

Measure	Criterion	Interpretation	Discovery sample
First generation clocks: Chronological age predictors			
Horvath clock	Chronological age	Age predicted by DNA methylation	Adults across 82 different datasets across entire lifespan
Hannum clock	Chronological age		Adult volunteers at UC San Diego, university of Southern California, and West China Hospital aged 19-101 years.
Second generation clocks: Mortality age predictors			
PhenoAge clock	Blood-chemistry PhenoAge	Age at which average mortality risk in NHANES III matches the mortality risk predicted by the PhenoAge algorithm	Adults from the InCHIANTI Study aged 21-100 years
GrimAge clock	Mortality Risk	Age at which average mortality risk in the Framingham Heart Study Offspring cohort matches predicted mortality risk	Adults from the Framingham Heart Study Offspring cohort aged 53-73 years
Pace of aging measures			
DunedinPoAm pace of aging	Change over 12-years of follow-up in 18 system-integrity biomarkers	Years of physiological decline experienced per 1 y of calender time. The expected value in midlife adults is 1. Values >1 indicate accelerated aging. Values <1 indicate slowed aging.	Adults from the Dunedin Study 1972-3 birth cohort.
DunedinPACE pace of aging	Change over 20 years of follow-up in 19 system-integrity biomarkers		

The table reports six DNA methylation measures of biological aging. For each measure, the table reports the criterion used to develop the measure, the discovery sample with which the measure was created, and the interpretation of the measure's values

racial/ethnic identity. Details of the studies are reported in Supplementary Table S1. The socioeconomic inequality measures are varied, ranging from educational attainment to socioeconomic disadvantage indices to neighborhood conditions. But the pattern of results is consistent. Overall, the GrimAge clock and DunedinPoAm Pace of Aging show the strongest associations with social determinants of health. Differences in these measures of biological aging between high and low socioeconomic status groups and between White and marginalized racial/ethnic groups are consistent with the hypothesis that social disadvantage contributes to an acceleration of biological aging.

Studies are also beginning to examine the question of how early in the life course socioeconomic patterning of DNA methylation measures of aging may emerge. A recent review of studies examining how socioeconomic disadvantage related to first-generation DNA methylation clocks in children found an inconsistent pattern of results [109]. An analysis of saliva DNA methylation in children that we published with the Texas Twin Project identified associations between both socioeconomic and White vs. Latinx identity differences in the DunedinPoAm Pace of Aging, but not the other clocks [110]. More studies of this question are needed to establish confidence in results. Studies including blood sample data will be especially valuable. In addition, new DNA methylation measures of biological aging have been developed in samples of children, including methods designed for tissues more readily available in pediatric samples [111–113]. Studies are needed to establish how these measures relate to family-level socioeconomic disadvantage.

In sum, based on the limited evidence available so far, the DNA methylation clocks that are more predictive of morbidity and mortality (*i.e.*, second-generation clocks, third-generation Pace of Aging measures) are also more strongly

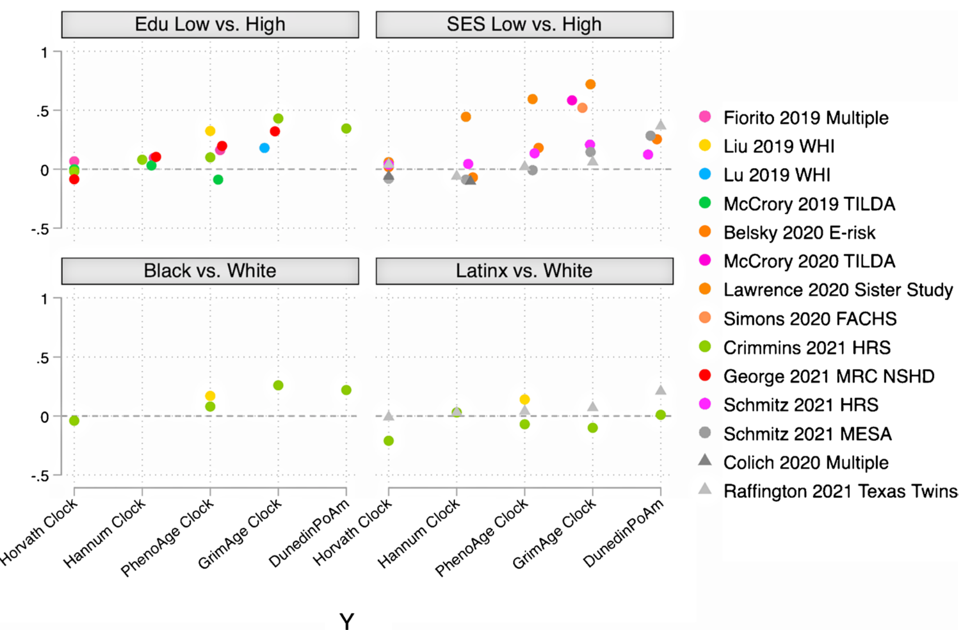

Fig. 3 Standardized effect sizes of associations of socioeconomic status (SES), education, and racial/ethnic identity with DNA methylation measures of biological aging. Effect sizes are reported in the metric of Cohen's *d*. Effect sizes reported in the different studies were harmonized to this metric as follows: For studies that reported comparisons between groups, coefficients denominating group differences were divided by the standard deviation of the aging measure. In studies not reporting standard deviations [122, 152], we used the standard deviations reported by the US Health and Retirement Study [151]. For studies reporting associations between continuous measures of SES and aging, we first converted coefficients to the metric of Pearson's *r* and then to Cohen's *d*. Conversions to Pearson's *r* were made by dividing the coefficient by the standard deviation of the aging measure and multiplying by the standard deviation of the SES measure. Details of the samples and measurements of the studies included in the figure are reported in Supplementary Table S1

associated with social determinants of health. It is not yet clear when in the life course these associations become established. However, social gradients in biological aging may already be evident during childhood. As DNA methylation data are better integrated into longitudinal studies, research can begin to test life-course models of how socioeconomic status in childhood and adulthood shape biological aging [27, 114, 115].

Challenges and Recommendations

Ancestry and Genetic Confounding

The emerging evidence linking social disadvantage to accelerated biological aging as measured by DNA methylation clocks must be interpreted within the context of several limitations. A first limitation has to do with the potential confounding of DNA methylation measurements of aging by genetic ancestry. There is a substantial bias in DNA-based research to study people solely of recent European ancestries [22, 23]. Genetic variation is an important determinant of DNA methylation states across the genome [116]. Genetic ancestry differences, therefore, have the potential to generate artifacts in DNA methylation datasets [117]. Genetic variants that affect DNA methylation and that have a low frequency or are absent in European-ancestry populations but are more common in other populations, therefore, have the potential to generate bias or noise in DNA methylation clock measures of aging.

While genetic ancestry is not the same thing as socially constructed racial/ethnic identity, people solely of recent European ancestries are likely to identify as White [118]. The samples used to develop the DNA methylation measures of aging that are most predictive of health and mortality and most sensitive to social disadvantage (PhenoAge and GrimAge clocks and DunedinPoAm) are mostly or entirely White [24, 25, 105••]. Establishing that these widely used clocks represent comparably valid measurements of aging across race/ethnic groups is a priority, as is the development of DNA methylation measures of aging from more diverse samples. Progress is now being made to establish validity across ancestry populations, including in the multi-ethnic samples in the US Health and Retirement Study [27], and work within non-White samples such as the American Indian participants of the Strong Heart Study [119], African-Americans in the Strong African American Healthy Adults Project [120], the Chinese National Twin Registry [121], and others.

So far, there is limited evidence for genetic ancestry-related confounding in DNA methylation clock research. Effect sizes for clock associations with healthy aging phenotypes are similar between groups of Black and White Americans, although effect sizes tend to be slightly larger for White Americans [24, 25, 27, 122]. The inclusion of genetic principal components as covariates in the analysis may provide some correction for ancestry-related artifacts in DNA methylation data [123]. Going forward, studies that use DNA methylation clocks to test differences in biological aging between socially constructed racial/ethnic identity groups that differ in genetic ancestry should be cautious in their interpretation of data. Designs that incorporate measures of healthy aging endpoints to establish parallel criterion validity of clocks between ancestry groups can build confidence in inferences that group differences in DNA methylation measures indicate differences in biological aging.

Longitudinal Analysis and Measurement Reliability

A second limitation is that nearly all research to date relating social determinants of health to DNA methylation measures of aging relies on cross-sectional data, at least for measurements of the aging outcomes. (This limitation also applies to nearly all studies relating DNA methylation measures of aging to health and mortality.) Research on human development establishes that differences in development and aging between individuals observed from a single time point of data can be a poor representation of the changes that occur within individuals over time [124–126]. It remains unclear whether or how DNA methylation measures of aging may be modified [127]. Social determinants of health research into biological aging are premised on the idea that interventions to modify social circumstances can slow the pace of aging and contribute to the elimination of health disparities. A critical next step is for longitudinal studies with repeated measures of DNA methylation to establish if changes in social determinants of health are associated with changes in DNA methylation measures of aging.

Two key challenges facing longitudinal repeated-measures studies of DNA methylation clocks are assay batch effects and technical reliability concerns. Batch effects refer to the variation in measurements arising from features of the measurement process that are shared among groups of samples measured together and different between groups of samples measured separately, such as samples grouped on assay plates or which DNA extractions or bisulphite conversions were performed at different times. The issue of batch effects in DNA methylation has been well described, and a number of corrections have been proposed [128]. However, these corrections are not perfect and have the potential to induce biases of their own [129–131]. Therefore, repeated measures analysis based on DNA methylation datasets in which time point is fully confounded by assay batch must be interpreted with caution.

Even when repeated measures are generated from the same assay batch (when repeated DNA samples from an individual are extracted and bisulphite-converted together and assayed on the same plate), low test–retest reliability of DNA methylation measurements can present challenges. DNA methylation arrays generate highly reliable genome-wide measurements of total DNA methylation [132]. However, at the level of individual CpG sites, the dinucleotide locations on the genome at which DNA methylation levels are assayed, reliabilities are strikingly poor [133–135]. DNA methylation clocks and Pace of Aging measures are algorithms that combine information on the methylation states of dozens to hundreds of CpG sites. Clock CpGs tend to have somewhat higher reliabilities than the average [135], and the clocks themselves are substantially more reliable than the individual CpGs from which they are composed [136]. Nevertheless, test–retest reliability as measured by the intraclass correlation coefficient (ICC) for most clocks is well below 0.9 [136]. This suggests that at least 20% of the variation in most clock measurements is error or noise. In an analysis of change across two time points, measurement error is additive. A consequence is that the statistical signal arising from an effect of social determinants of health on biological aging will be significantly diluted. The GrimAge clock and DunedinPACE measure both have ICCs well above 0.9 and so may be less subject to this limitation [137]. New methods may substantially increase the reliabilities of other clocks [136].

Finally, DNA methylation measures that are well established to predict morbidity and mortality and correlate with social determinants of health were developed for blood tissue. Methylation varies substantially by tissue type [138]. However, it may be infeasible to collect blood samples within many large cohort studies. Blood collections typically require medical personnel that would further increase the already high cost of DNA methylation sampling. In contrast, saliva samples are more amenable to large-scale studies, including pediatric participants. While some measures have shown high correspondence between blood and saliva samples [139], more research is needed to establish associations of DNA methylation measures of aging taken in non-blood tissues with healthy aging endpoints, such as morbidity and mortality, before correlations between these measurements and social determinants of health can be interpreted with confidence.

Future Directions

Within the bounds of these challenges, there is clear evidence that socially disadvantaged individuals show more advanced and faster biological aging as compared to more

socially advantaged individuals of the same chronological age. The next steps in research to establish the effects of social determinants on biological aging involve refinements to study designs to strengthen internal and external validity.

Using Natural Experiment and Randomized Trial Designs to Improve Internal Validity

Research relating social determinants of health to DNA methylation measures of aging consists mostly of correlational study designs with limited ability to establish causality of associations. So-called "natural experiments," in which an event or policy change alters socioeconomic circumstances for a segment of the population, provide one path to strengthening causal inference [140]. Changes over time and/or differences across borders in schooling reforms mandating additional years of education, minimum wage thresholds, or other anti-poverty policies are settings in which to investigate causal effects of social determinants on biological aging. Randomized controlled trials of anti-poverty interventions and other programs to address social determinants represent another promising research direction [141, 142].

Developing Population-Representative Samples to Improve External Validity

Participation in biomedical research in general and DNA-based research, in particular, tends to be lower for persons with less education and members of a certain race/ethnic identity groups [143–145]. Underrepresentation of such groups is especially pronounced in large biobank datasets, and although there are many strategies to address this challenge, none are perfect and some may induce their own biases [146–150]. Oversampling of underrepresented populations and the application of survey probability weights can help generate more population-representative estimates [151]. However, a concern is that low socioeconomic status and non-White individuals who choose to participate in DNA research may differ from those who do not in ways that may be consequential for aging, resulting in selection bias. There has not yet been systematic consideration of these types of selection bias issues in relation to measures of biological aging. As the field matures out of its early days, closer attention is needed to which individuals and groups may be missing or underrepresented in existing samples. This need is being recognized and addressed with initiatives such as National Institute on Aging and National Institute on Minority Health and Health Disparities priority funding for social epigenomics research (https://www.nimhd.nih.gov/programs/extramural/investigator-initiated-research/socioepigenomics-grants.html). Data generated under this initiative and other datasets from underrepresented groups and populations should receive the careful attention to evaluate the extent to which findings accumulated in samples of mostly White and higher socioeconomic status individuals replicate in different and more diverse samples.

Conclusion

Novel measures quantified in DNA methylation are being used to capture processes of biological aging in ways that may inform why and how social inequality is associated with aging-related disparities in health. These new tools have the potential to help evaluate how exposures contribute to risk in people who are still "pre-symptomatic" and ultimately may provide surrogate end points for testing the effects of social programs on healthy aging decades before effects on aging-related chronic disease or mortality would be apparent.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s40572-022-00338-8.

Funding LR is supported by the German Research Foundation (DFG). DWB is supported by Russell Sage Foundation BioSS grant 1810–08987, National Institute on Aging grants R01AG066887 and R01AG061378, a pilot grant from the Columbia Population Research Center, the Canadian Institute for Advanced Research Child Brain Development Network, and the Jacobs Foundation.

Declarations

Ethics Approval and Consent to Participate This article does not contain any studies with human or animal subjects performed by any of the authors.

Conflict of Interest Laurel Raffington declares no conflict of interest. Daniel W. Belsky is listed as an inventor on a Duke University and University of Otago invention that was licensed to a commercial entity.

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