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DNA methylation age – environmental influences, health impacts, and its role in environmental epidemiology

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Abstract

Purpose of review: DNA methylation-based aging biomarkers, are valuable tools for evaluating the aging process from a molecular perspective. These epigenetic aging biomarkers can be evaluated across the lifespan and are tissue specific. This review examines the literature relating environmental exposures to DNA methylation-based aging biomarkers and also the literature evaluating these biomarkers as predictors of health outcomes.

Recent findings: Multiple studies evaluated the association between air pollution and DNA methylation age and consistently observed that higher exposures are associated with elevated DNA methylation age. Psychosocial exposures, e.g. traumas and adolescent adversity, and infections are also associated with epigenetic aging. DNA methylation age has been repeatedly associated with mortality, cancer, and cognitive impairment.

Summary: DNA methylation age is responsive to the environment and predictive of health outcomes. Studies are still needed to evaluate whether DNA methylation age acts as a mediator or modifier of environmental health effects and to understand the impact of factors such as race, gender, and genetics.

Keywords

DNA methylation age; environment; environmental epidemiology; epidemiology; epigenetic age; environmental exposure

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Conflict of Interest

Radhika Dhingra, Jamaji C. Nwanaji-Enwerem, Madeline Samet, Cavin K. Ward-Caviness declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent

This article does not contain any studies with human or animal subjects performed by any of the authors.

Introduction

Epigenetic modifications are associated with aging and age-related diseases [1–3]. DNA methylation-based aging biomarkers are used to assess epigenetic age in a variety of cells, tissues, and populations, using the percent methylation found at a specific sets of cytosine-guanine dinucleotides [4, 5]. This allows researchers to estimate age based on changes in an intrinsic biological phenomenon, *i.e.* the addition and removal of methyl groups from cellular DNA by DNA methyltransferases.

The most widely utilized DNA methylation-based aging biomarkers are the Horvath[4] and Hannum [5] measures. Altered aging, as assessed via the Horvath and Hannum DNA methylation age measures, is associated with longevity [6], age-related diseases [7–10], and mortality [11–15]. Broadly, these epigenetic aging biomarkers may fit into an “epigenetic clock” theory of aging whereby accelerated DNA methylation age is indicative of altered intrinsic biological functions which predispose an individual to a number of age-related diseases [16]. Heritability estimates indicate that DNA methylation aging is heritable, however a large component is environmentally determined [14]. In this review, we briefly summarize the associations between DNA methylation age and environmental exposures, as well as its associations with health outcomes. This review endeavors to give readers a broad understanding of the current knowledge surrounding associations with DNA methylation age and to better resolve the potential role of DNA methylation age in environmental epidemiology studies.

DNA methylation age assessment

The Horvath DNA methylation age assessment method (Horvath method) uses the percent methylation at 353 CpGs as measured on either the Illumina 27k or Illumina 450k arrays to produce a measure of DNA methylation age that is valid in a wide variety of tissues [4]. The Hannum DNA methylation age assessment method (Hannum method) uses the percent methylation at 71 CpGs from the Illumina 450k array to produce a DNA methylation age measure valid in blood. Though the measures have only 6 CpGs in common [14], both have been widely validated as having a strong correlation with chronological age [4, 17, 5]. The main differences between the measures are the tissues they are valid for and their correlation with immune cell counts. The Hannum DNA methylation age assessment was developed specifically for blood and is only valid in this tissue, whereas the Horvath DNA methylation age assessment was developed using a wide array of tissues (though primarily samples from blood) and is valid in blood and other tissues – and possibly even other species such as chimpanzee. As for immune cell counts, the Hannum measure strongly correlates with multiple blood immune cell counts while the Horvath measure is largely independent of blood immune cell counts. These differences lead to different assessment of DNA methylation age by the two measures, though there is a variation of the Horvath measure-which we detail later – that closely approximates the Hannum measure.

DNA-methylation age acceleration measures

DNA methylation age acceleration is often defined as a measure of the difference between DNA methylation age (assessed via the Horvath or Hannum methods) and chronological age. Deviations between DNA methylation age and chronological age are commonly referred to as “age acceleration” or DNA methylation age acceleration. In this context, the word “acceleration” is typically not used to imply a change in rate (velocity) over time, but is used equally to denote the difference between a biological aging measure and chronological age, as well as changes in the aging rate – for the few longitudinal studies to examine such a measure. It is possible that DNA methylation age “deviation” more accurately describes many of the age acceleration measures, however, for now, the term “acceleration” has persisted in the literature.

Accelerated DNA methylation aging, or accelerated epigenetic aging as it is also commonly called, is primarily reported using one of two methods. The first method is to simply subtract chronological age from DNA methylation age. Individuals with a positive “DNA methylation age difference” are said to have accelerated DNA methylation aging while individuals with a negative “DNA methylation age difference” are said to have decelerated DNA methylation aging. This difference-based measure is used for both the Horvath and Hannum measures. The second method is to use the residuals from the regression of chronological age on DNA methylation age as a measure of DNA methylation age acceleration. Again positive/negative values of this measure are referred to as accelerated/decelerated DNA methylation (epigenetic) aging. This approach is almost exclusively used in conjunction with DNA methylation age estimated via the Horvath method and is rarely, if ever, applied to the Hannum method though it could be done. These residual and difference-based measures give highly concordant regression estimates when chronological age is included as an adjustment in any models used, a recommended and nearly universal practice. One important distinguishing feature for these two measures is that the “difference”-based age acceleration measure is determined purely by measurements made on the individual while the “residual”-based measure will depend on the population in which it is calculated, *e.g.* estimating in a sex-specific manner versus a combined sex dataset will produce different values for epigenetic age acceleration which can make some dataset comparisons difficult. However, the residual-based measure can remove systematic deviations in DNA methylation age acceleration across datasets with varying mean differences in age acceleration. Abbreviations and descriptions for all DNA methylation age measures and the age acceleration estimates derived from them are given in Table 1.

Blood-specific measures of DNA methylation age

Given the strong correlations between aging, blood cell composition, and DNA methylation, the original, tissue-agnostic Horvath method has been extended to estimate additional blood-specific DNA methylation age parameters. Extrinsic epigenetic age is a DNA methylation age measure that is closely related to the Hannum DNA methylation age estimate, but is weighted to have strong correlations with naïve and exhausted cytotoxic T cells and plasma B cells [9]. In this way, extrinsic epigenetic age is sometimes thought of as measuring aging changes linked to the immune system. Intrinsic epigenetic age adjusts the Horvath DNA methylation age estimate for naïve and exhausted CD8+ T cells, CD4+ T cells, plasma B

cells, natural killer cells, monocytes, and granulocytes. Thus, it is independent of many of the most abundant cell populations found in whole blood [9]. For these additional measures, cell counts are estimated using DNA methylation via the Houseman and Horvath methods [18, 9] and are output by the same online estimator that estimates Horvath DNA methylation age (https://dnamage.genetics.ucla.edu/). Throughout the manuscript the abbreviation DNAm-age is used to refer to any estimate of age made using DNA methylation data.

DNAm-age and the external environment

DNAm-age is responsive to the external environmental. Here, we have defined the external environment as any set of conditions not arising from properties intrinsic to the individual; thus, our definition includes chemical, infectious, nutritional and even psychosocial exposures such as socioeconomic status (SES) and trauma. Generally, higher levels of adverse exposures, *i.e.* higher chemical exposures, lower SES, increased trauma, have been associated with increased DNA methylation age and/or DNA methylation age acceleration. However, there have been some deviations from this pattern as detailed below. A summary of the exposure associations is given in Table 2a.

Chemical Exposures

Air Pollutants—In a study encompassing 1,032 samples from 589 older males residing in the Northeastern USA and enrolled in the Normative Aging Study (NAS), a 1 $\mu g/m^3$ increase in one-year $PM_{2.5}$ exposure was significantly associated with a 6-month increase in DNAm-age. Similarly, a 1 $\mu g/m^3$ increase in 1-year black carbon (BC) exposure was associated with a 2.83-year increase in Horvath DNAm-age. However, the association with BC was no longer significant when $PM_{2.5}$ was included in the model [19]. In a similar study using 1,777 German participants of the Cooperative Health Research in the Region of Augsburg (KORA) study, a 0.97 $\mu g/m^3$ increase in long-term exposure to $PM_{2.5}$ was associated with a 0.33-year increase in Extrinsic Epigenetic Age Acceleration (EEAA) [20]. This association did not replicate in the NAS cohort, potentially due to the use of different epigenetic aging measures, exposure differences, or differential covariate adjustment. Ward-Caviness *et al* also observed male and female-specific effects which were in the opposite direction during sex-stratified analyses associations between epigenetic aging measures and nitrogen oxides, BC, and particulate matter <10 μm in diameter [20]. This effect has not been replicated and thus it remains to be seen if it is due to random chance or underlying sex-differences in the effects. In addition to $PM_{2.5}$ mass, associations with Horvath DNAm-age have been observed with specific $PM_{2.5}$ components such as ammonium and sulfate [21]. These associations remained even when the analyses were limited to areas in attainment of US Environmental Protection Agency national ambient air quality standards.

Metal and Pesticide Exposures—Two studies have examined associations between metal exposures and DNAm-age to date. A study of urinary cadmium in 40 non-smoking women from Thailand [22] and a study of serum cobalt and chromium levels resulting from chronic exposure due to metal on metal hip replacements [23] found no associations between any of the metals examined and Horvath DNA methylation age acceleration difference (Horvath Δage). Twenty of the 353 CpGs from the Horvath DNAm-age calculator

were individually associated with urinary cadmium in women; four of these 20 CpGs were also associated with $PM_{2.5}$ exposure in the all-male NAS cohort from the USA [19, 21]. In a study of three organochlorine pesticides – (4-chlorophenyl)-1,1-dichloroethene (DDE), hexa- chlorobenzene (HCB), and transnonachlor (TNC) – in the plasma of 967 Swedish individuals. All three exposures were positively associated with Hannum Δage, with TNC having the strongest association (0.66 y/ln[ng/g lipid]; 95% CI 0.21–1.19) [24]. On the other hand, organophosphate exposure was not associated with DNAmAA, EEAA, or IEAA in a case control study of Parkinson's disease (PD) [9]. These mixed results with only a single case-control study contributing to the null associations warrant further investigation to make more solid conclusions.

Infectious Exposures

Of the infectious agents studied for their relationship with DNAm-age, Human Immunodeficiency Virus (HIV) is the most widely examined. In one study of 11 datasets (N = 960; N HIV infected = 271), HIV+ individuals were found to have brain and blood samples that were 7.4-years and 5.2-years higher in DNAm-age, respectively, compared to controls. Multivariate regression analysis suggested that adult male cases with detectable viral load (>35 HIV copies/mL) had 3.6-year higher DNAm-age as compared to adult male cases with a non-detectable viral load [25]. In a study of 58 post-mortem occipital cortex samples, individuals with HIV-associated neurocognitive disorders had a Horvath DNAmAA 3.5-years greater than those without such disorders [26].

Besides HIV infection, relationships of *H. pylori* and cytomelagovirus (CMV) infections with DNAm-age have been observed. In a study of 1509 German adults, *H. pylori* infection, infection with virulent chronic atrophic gastritis positive strains, and severe chronic atrophic gastritis were all associated with increases in Horvath DNAmAA of 0.4, 0.6. and 1-year, respectively, independent of white blood cell distributions [27]. In peripheral blood cells from 122 nonagenarians and 21 young healthy controls from a sub-cohort of the Finland Vitality 90+ study, DNAm-age was 2.5-years higher in CMV seropositive individuals versus controls [28]. The underlying mechanisms connecting infections and DNAm-age have yet to be elucidated, and alterations in blood cell composition may play an important role, though other mechanisms are needed to explain associations in non-blood tissues and those that appear to be independent of assessed blood cell proportions [29, 30].

Psychosocial Exposures

Psychosocial exposures such as stress, adversity, and socioeconomic status, may also impact epigenetic aging. Glucocorticoids, a class of endocrine signaling hormones which includes cortisol, are a component of the biological response to stress. Notably, 85 of the 353 loci that comprise the Horvath epigenetic clock are located near glucocorticoid receptor elements, and 110 loci showed altered DNA methylation after exposure to dexamethasone, a glucocorticoid receptor agonist [31]. Researchers have proposed biological mechanisms that may connect stress to epigenetics alterations and DNA methylation age/aging in particular [30, 32]. Accordingly, stress-inducing psychosocial exposures are frequently associated with DNAm-age.

Socioeconomic Status and Hardship—The largest study of socioeconomic status (SES) [11] involved 5,111 adults from 3 prospective cohorts. Educational attainment scores, accounting for gender, birth cohort, and originating dataset, were used as a proxy for SES. Using a meta-analysis of cohort specific associations, low SES was associated with 0.99-year greater Hannum DNAmAA (95% CI 0.39,1.59), when comparing the highest to lowest SES categories. Socioeconomic life course trajectory had consistent but non-significant effects on Horvath DNAmAA.

Two studies of Horvath DNAm-age were conducted in African-American adolescents residing in rural Georgia, and examined the direct or modifying impact of economic hardship on epigenetic age using samples collected around the Great Recession. One study examined the impact of economic hardship during the 2007–10 economic recession on Horvath DNAmAA in 330 African American adolescents (mean age 16.6 years in 2007; [13]). Adolescents exhibited a mean 1.42-year increase in epigenetic age with each as categorical measures of economic adversity increased. These findings are mirrored in observations of increased allostatic load and decreased self-reported overall health with increased Horvath DNAm-age [13]. A second study, noted that while self-control was associated with several favorable psychological outcomes (e.g., lower rates of depressive symptoms, substance use and aggressive behavior), among low SES youths better self-control was associated with an increased Horvath DNAmAA of 2.27-years by the Horvath method (or 1.46-year increase by the Hannum method) [33]. In contrast, among less-disadvantaged subjects, better self-control was associated with a 0.27- to 2.14-year deceleration by Horvath and Hannum methods, respectively.

Stress, Trauma, and Post-traumatic stress disorder (PTSD)—In a study of 392 adults recruited from urban hospitals, the relationship of life stress (as measured in the Stressful Events Questionnaire) was associated with higher Horvath Δage, an observation which was more prominent in older participants and those who experienced minimal childhood mistreatment [31]. However, Horvath Δage was not related to childhood trauma, current stress, depressive symptoms or PTSD symptoms.

Combat-related trauma and PTSD have been associated with increased DNAmAA in a few studies of veterans, but the relationships are inconsistent and may vary with choice of DNAm-age or trauma/PTSD measure. In veterans of the Iraq and Afghanistan conflicts (N = 281) lifetime PTSD was related to increased Hannum DNAmAA, but not to changes in Horvath DNAmAA [34]. In 339 middle-aged, trauma-exposed veterans, hyperarousal PTSD symptoms were associated with increased Hannum DNAmAA (Horvath DNAmAA was not assessed), but PTSD severity and trauma exposure were not associated [35]. In a longitudinal study, Horvath DNAm-age was determined in 96 Dutch military personnel deployed to Afghanistan, from blood draws conducted before and 6 months post-deployment [36]. Combat-related trauma was significantly associated with an increase in Horvath DNAm-age of 1.97 years over the course of deployment, while development of PTSD symptoms was associated with a slight decrease in Horvath DNAm-age (−0.10 years). A meta-analysis of 9 cohorts (n = 2186, civilian and military) [37] found modest associations of accelerated Hannum (but not Horvath) DNAm-age with exposure to child trauma, and

with lifetime PTSD severity. However, neither PTSD diagnosis nor lifetime trauma exposure was associated with either Hannum or Horvath DNAm-age.

Childhood Adversity and Trauma—Studies of childhood and adolescent adversity have been the first to use DNAm-age as a potential measure of intervention efficacy, or effect modification by intervenable factors. Using 399 parent and child pairs from the rural Georgia, USA, Brody *et al* [38]. aimed to assess if parental depressive symptoms at child age 11 forecast DNAm-age at child age 20, and the potential of an intervention program, the Strong African American Families program (SAAF), which aimed to improve supportive parenting and family relationships, to moderate observed associations [38]. Among the control group, elevated parental depressive symptoms were associated with future increased Horvath DNAm-age in children, but the association was abolished in those receiving the SAAF intervention. Similarly, a longitudinal study of 616 African American youths (16–17 years old at recruitment) in rural Georgia found an ameliorating effect of a supportive family environment on the relationship of Hannum DNAm age to experience of racism. Among youth with a less supportive family exposure to higher levels of racial discrimination during early adulthood was associated with higher Hannum DNAm-age [39]. In a separate study of 101 children (age 6–13 years) from a primarily low income, highly traumatized neighborhood populations in Atlanta, Georgia [40], direct experience of violence was significantly associated with increased Horvath DNAmAA, and there was a suggestive ($p < 0.10$) association between witnessing violence and Horvath DNAmAA. Associations of childhood adversity and trauma with DNAm-age measures appear consistently adverse, unless moderated by an ameliorative co-exposure, whether preexisting or the product of intervention.

Modifier and mediators of chemical and non-chemical exposures

There are several potential modifiers and mediators of environmental exposures on DNA methylation-based aging biomarkers. Indeed, a wide array of genetic, clinical, demographic, disease or exposure history factors can be plausibly considered as potential modifiers or mediators of associations with DNA methylation-based aging biomarkers; however, few of these have been closely examined in the literature. Environmental effects have been noted to differ by race and sex, an observation that might be mediated in part by molecular differences in factors such as epigenetic aging. DNA methylation-based aging biomarkers have been observed to differ across the sexes [17, 20]. In general, females appear to have a lower epigenetic age than their same-aged male counterparts, across races. In a study of long-term air pollution exposure, results for some exposures differed significantly by sex indicating potential effect modification [20].

Diet may also be a modifier of DNA methylation age. In a recent multi-ethnic study, EEAA was moderately correlated with fish and alcohol consumption and plasma levels of metabolites associated with plant consumption, e.g. carotenoids and alpha-carotene [41]. IEAA was associated with poultry intake and mean carotenoid concentrations. Both IEAA and EEAA were associated with various molecular metabolic measures such as triglycerides and insulin as well as body mass index and metabolic syndrome. Alterations in one or more of these metabolic conditions may mediate and/or modify environmental effects.

Genetic variation on the autosomes and in mitochondrial DNA may also modify associations. For example, a genetic variant based score reflecting endothelial function was examined as a potential modifier of the $PM_{2.5}$-DNAm-age associations. The authors found that the magnitude of the association of $PM_{2.5}$ with DNAm-age was significantly higher in individuals with a high computed genetic variant-based score (β_{high} = 1.09, 95% CI: 0.70, 1.48) when compared to individuals with a low score (β_{low} = 0.40, 95% CI: 0.14, 0.67, $P_{interaction}$ = 0.0007) [42]. MicroRNA processing associated genetic variants have also been observed to modify the relationship between epigenetic age and air pollution exposure with individuals with at least one copy of the major allele for rs4961280 (*AGO2*) having a significantly reduced magnitude of the $PM_{2.5}$ association with DNAm-age [43]. Additionally, haplogroups which reflect normal variation in mitochondrial DNA have been associated with modifications of the association between DNA methylation age and air pollution exposure [44]. In the case of mitochondrial copy number, a measure of the copies of mitochondrial DNA to cellular DNA, researchers were able to go beyond observations of effect modification, and estimated that 12% of the association between DNAm-age and $PM_{2.5}$ exposure was mediated by alterations in mitochondrial copy number [44].

DNAm-age as a biomarker and predictor of health outcomes

Complementary to its association with exposures, DNAm-age has been extensively associated with health outcomes. In the sections below, we outline some of these associations including associations with mortality, cancer incidence, cognition, and frailty. In many of these studies, prospective designs were used raising the possibility that alterations in DNAm-age, arising from environmental exposures or other sources, may be significant predictors of events even in otherwise healthy individuals.

Mortality

All-cause mortality—All-cause mortality is by far the most widely studied outcome in association with DNAm-age. EEAA, but not IEAA, was significantly associated with all-cause mortality in a study of 5,124 older individuals from the Northeast US-based Framingham Heart Study [45]. EEAA remained associated with mortality even after adjustments for other measures of biological age based on clinical data and inflammatory markers. A study of 86 Danish twins found that the twin with the higher DNAm-age at baseline had a 69% probability of dying first over a ten-year period, a result that was nearly identical for both the Horvath and Hannum DNAm-age measures [15]. In a German population-based cohort, 5-year higher Horvath and Hannum Δage were associated with a 23% (CI = 1.10–1.38) and 10% (CI = 0.94–1.29) increased risk of all-cause mortality, respectively, after adjustment for technical and clinical confounders [12].

In a meta-analysis of four Caucasian cohorts adjusting for a variety of clinical and socioeconomic confounders, researchers observed that 5-year higher Horvath and Hannum Δage was associated with a 16% (CI 8–25%) and 9% (CI: 2–15%) higher risk of mortality, respectively [14]. A larger, multi-ethnic meta-analysis of 13 population-based cohorts found that all DNA methylation aging measures considered were significantly associated with mortality [46]. EEAA was the most significantly associated aging measures (HR = 1.03, CI

= 1.02–1.04), and when EEAA and Hannum DNAmAA were considered, only EEAA remained significantly associated with mortality. Associations between mortality and EEAA did not differ by race (non-Hispanic White, Black, Hispanic), sex, follow-up time, BMI, smoking, physical activity, or previous disease diagnosis [46].

Cancer mortality—In an older male cohort, a one-year higher Hannum Δage was associated with a 17% higher risk of cancer mortality (CI = 7–28%) [8]. In ESTHER, a German population-based cohort study, 5-year higher Horvath Δage was associated with higher cancer mortality (HR = 1.20, CI = 1.03–1.39), while Hannum Δage had an elevated but non-significant association (HR = 1.08, CI = 0.87–1.35) [12].

Cardiovascular Disease mortality—Results from the ESTHER cohort also showed that a 5-year increase in Horvath Δage was associated with a significantly higher risk of cardiovascular disease (CVD) mortality after adjustment for age, sex, batch effects and leukocyte cell counts (HR = 1.20, CI = 1.02–1.42). This association remained nearly identical after further adjustment for education, cancer diagnosis, hypertension, diabetes mellitus, smoking, and body mass index (HR = 1.19, CI = 0.98–1.43). Associations with Hannum Δage were weaker, and no association with CVD mortality was observed after adjusting for all confounders [12].

Cancer

In the NAS cohort, a one-year increase in Hannum DNAm-age was associated with a hazard ratio (HR) of 10.6 (CI = 1.02 – 1.10) for cancer incidence, though observed only in samples collected after 2003 [8]. In a study of 900 women from the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort, IEAA was associated with increased breast cancer risk (OR = 1.04, CI = 1.007 – 1.076), and this association was stronger in postmenopausal women (OR = 1.07, CI = 1.02 – 1.11).[47] EPIC used the long follow-up time to observe that associations were more significant in those with > 10 y of follow-up. In contrast with the results from EPIC, an independent study of 573 publicly available Italian samples (GSE51032) with a relatively short follow-up period (3.73 years), some study specific age acceleration estimates were associated with breast cancer incidence but Horvath DNAmAA was not [48].

Colorectal and lung cancer have also been associated with DNAm-age biomarkers. Males who developed colorectal cancer had a 1.6 y acceleration in their Horvath DNAm-age, but there was no difference in any examined DNA methylation aging biomarkers in female samples who developed colorectal cancer [48]. In a study of 2,029 females with ~20 years of follow-up, IEAA was significantly associated with increased lung cancer incidence. A higher IEAA was associated with greater risk of lung cancer in current smokers than former or never smokers. Older (chronological age 70+) women also had an elevated association as compared to women aged 60–69 [49].

Cognitive Outcomes

Cognitive function and brain white matter measurements—Associations between DNA methylation aging patterns and cognitive outcomes have been varied. In a study of 533

young health adults from Switzerland, some study-specific aging related DNA methylation patterns were associated with cortical thickness, but blood Hannum and Horvath DNAm-age were not [50]. In a 10-year longitudinal study of 243 pairs of monozygotic, Danish twins, there was no association between blood Horvath DNAm-age or age acceleration and either cognitive function at the baseline examination or in the change in cognition over 10 years [51].

In contrast to the above studies, a cross-sectional study of 713 African-Americans found that both Hannum and Horvath DNAmAA were significantly associated with magnetic resonance imaging assessed white matter hyperintensities (WMH) – a biomarker for cognitive impairment and dementia. In this study, Hannum and Horvath DNAm-age explained approximately the same proportion of WMH (4.7% and 3.5%, respectively) after adjusting for white blood cell counts and clinical confounders. Horvath and Hannum DNAmAA were both associated with elevated risk of having high WMH burden as compared to no WMH burden [52]. Similarly, in a study of 628 younger (mean age 45.5) Mexican-Americans, peripheral blood Horvath DNAm-age was negatively associated with global white matter integrity (WMI). This association displayed some specificity for specific brain regions when region (tract)-specific WMI measures were estimated [53]. No associations with WMH were observed, possibly because of the young mean age of the population since in a previous study increases in WMH were not observed until the 5^{th} decade [52].

Memory, Neuropathological measurements and Alzheimer's disease—In a study of 700 older Caucasian individuals which included baseline and follow-up cognitive exams as well as analysis of post-mortem donated brain samples, brain Horvath DNAmAA was significantly, positively associated with diffuse plaques, neuritic plaques, hyperphosphorylated paired helical filament tau protein-rich neurofibrillary tangles, and amyloid load [10]. Similarly, Vonsattel grade, a categorical (0–4) assessment of neuropathological abnormalities, was associated with Horvath DNAmAA and IEAA in a study of 26 Huntington's disease cases and 39 controls [54]. In this study Vonsattel grades 1 and 2 had elevated Horvath DNAmAA and IEAA (adjusted for neuron counts) relative to controls, but those with the most severe Vonsattel grade (4) had decreased Horvath DNAmAA and IEAA relative to controls. No explanation that fits the data has currently been offered to explain this discrepancy [54]. Higher brain Horvath DNAmAA was also associated decreases in with clinical markers indicative of Alzheimer's disease such as global cognitive functioning and episodic memory, but was not associated with Alzheimer's disease status [10].

In a 15-year longitudinal study of 52 Swedish individuals, those with relatively high memory maintenance (n = 16) had a Horvath Δage of −2.6 years compared with a Horvath Δage of 0 years for those experiencing memory decline (n = 16). Baseline elevated Horvath DNAm-age was also a significant predictor of dementia even after adjusting for chronological age and sex [7]. Two studies that examined Alzheimer's disease failed to observe significant associations [10, 54]. Elevated Hannum DNAmAA has also been associated with deficits in regional brain anatomy and with poorer working memory [34].

Frailty

In a study of older Caucasians from the Northeast United States, higher EEAA was associated with decreased gait speed and hand grip strength [45]. Similarly, in a cross-sectional analysis of a longitudinal study of Scottish individuals, higher Horvath DNAm-age was significantly associated with lower grip strength, weaker lung function, and lower cognitive scores. However, in contrast with the Northeast United States based study, the Scottish study found no association between gait speed and Horvath DNAm-age. Additionally, there was no significant association between the rate of change in Horvath DNAm-age acceleration and cognitive or fitness declines [55]. In a German population-based study, a 34-component frailty index was significantly associated with Horvath Δage. Results suggested that a 12 year higher Horvath Δage was associated with 1 additional deficit in this population [56].

Role for DNA methylation age in environmental epidemiology

Molecular biological aging biomarkers such as DNA methylation age can play a unique role in environmental epidemiology, as quantitative aging biomarkers that are assessable and valid in young and healthy populations allow for more detailed and longitudinal studies of the impact of environmental exposures on aging. DNA methylation age also allows for the study of aging-related effects in stored samples, which may not have been originally assessed for traditional aging-related parameters. Several longitudinal cohorts offer such an efficient opportunity to study aging without the need for subject re-examinations, or establishment of new cohorts. However, there are special considerations when examining DNA methylation age in the context of environmental epidemiology. A primary consideration is which DNA methylation aging measure to use. The choice will depend on the goals of the study, including whether the measure needs to be evaluated in multiple tissues and whether estimates independent of blood immune cell counts are desired. A second consideration is the tissue specificity of DNAm-age, as effects present in a directly affected tissue may not always be reflected in easily accessible and widely available tissues like blood. Replication and cohort-specific effects are also of important concern. As with all studies of molecular biomarkers in humans, DNA methylation effects exhibit heterogeneity across cohorts, that could be related to differences in exposure, or to important biological differences that influence the association between DNA methylation age and a given exposure, e.g. age, sex, or genetic differences. Much of the current research has been done in older individuals both with respect to environmental exposures and health outcomes. The exception to this is adversity and socioeconomic status, which has been primarily examined in adolescents. Thus, we have little information on associations across the lifespan. Additionally, no studies have yet verified if DNAm-age alterations are a mediator between environmental exposures and health outcomes or if baseline levels of epigenetic age acceleration alter future exposures. Both of these models are possible and verifying if one or both occur for chemical and non-chemical exposures would be a key step forward for the field. Finally, causality is always an important consideration. As of yet, none of the links between DNA methylation age and clinical outcomes have been critically evaluated for causality, though some have been evaluated using prospective study designs. A recent genome-wide association study uncovered multiple genome-wide significant variants

associated with IEAA and EEAA raising the possibility of using Mendelian Randomization to gain evidence for causality [57]. In an animal model, human chromosome 21 experienced rapid methylation changes at multiple DNA methylation loci when inserted into a mouse – mimicking the rapid accumulation of methylation changes in the short lifespan animal model [58]. This result suggests that the cellular environment influences some methylation changes, however it is still not known if DNA methylation age is a cause, consequence, or merely correlated factor related to the aging process. The Horvath DNA methylation age has been shown to be valid in mammalian species besides humans [4], offering the possibility of model organism-based aging measures which will help in evaluations of causality. Still, despite these considerations DNA methylation age measures represent a powerful new tool for environmental epidemiology research and opens new avenues to study environmental effects on aging from a molecular perspective.

References

1. Calvanese V, Lara E, Kahn A, Fraga MF. The role of epigenetics in aging and age-related diseases. Ageing research reviews. 2009;8(4):268–76. [PubMed: 19716530]
2. Fraga MF, Esteller M. Epigenetics and aging: the targets and the marks. Trends in Genetics. 2007;23(8):413–8. [PubMed: 17559965]
3. Gentilini D, Mari D, Castaldi D, Remondini D, Ogliari G, Ostan R et al. Role of epigenetics in human aging and longevity: genome-wide DNA methylation profile in centenarians and centenarians' offspring. Age. 2013;35(5):1961–73. [PubMed: 22923132]
4. * Horvath S DNA methylation age of human tissues and cell types. Genome biology. 2013;14(10): 3156.This study details the development and validation of the Horvath DNA methylation age clock and the derivation of DNA methylation age acceleration from it.
5. * Hannum G, Guinney J, Zhao L, Zhang L, Hughes G, Sadda S et al. Genome-wide methylation profiles reveal quantitative views of human aging rates. Molecular cell. 2013;49(2):359–67. [PubMed: 23177740] This study details the development and validation of the Hannum DNA methylation age assessment method.
6. Horvath S, Pirazzini C, Bacalini MG, Gentilini D, Di Blasio AM, Delledonne M et al. Decreased epigenetic age of PBMCs from Italian semi-supercentenarians and their offspring. Aging (Albany NY). 2015;7(12):1159. [PubMed: 26678252]
7. Degerman S, Josefsson M, Adolfsson AN, Wennstedt S, Landfors M, Haider Z et al. Maintained memory in aging is associated with young epigenetic age. Neurobiology of aging. 2017;55:167–71. [PubMed: 28292535]
8. Zheng Y, Joyce BT, Colicino E, Liu L, Zhang W, Dai Q et al. Blood epigenetic age may predict cancer incidence and mortality. EBioMedicine. 2016;5:68–73. [PubMed: 27077113]
9. Horvath S, Ritz BR. Increased epigenetic age and granulocyte counts in the blood of Parkinson's disease patients. Aging. 2015;7:1130–42. [PubMed: 26655927]
10. Levine ME, Lu AT, Bennett DA, Horvath S. Epigenetic age of the pre-frontal cortex is associated with neuritic plaques, amyloid load, and Alzheimer's disease related cognitive functioning. Aging (Albany NY). 2015;7(12):1198. [PubMed: 26684672]
11. Fiorito G, Polidoro S, Dugué PA, Kivimaki M, Ponzi E, Matullo G et al. Social adversity and epigenetic aging: A multi-cohort study on socioeconomic differences in peripheral blood DNA methylation. Scientific Reports. 2017;7:1–12. doi:10.1038/s41598-017-16391-5. [PubMed: 28127051]
12. Perna L, Zhang Y, Mons U, Holleczek B, Saum K- U, Brenner H. Epigenetic age acceleration predicts cancer, cardiovascular, and all-cause mortality in a German case cohort. Clinical epigenetics. 2016;8(1):64. [PubMed: 27274774]
13. Chen E, Miller GE, Yu T, Brody GH. The Great Recession and health risks in African American youth. Brain, Behavior, and Immunity. 2016;53:234–41. doi:10.1016/j.bbi.2015.12.015.

14. Marioni RE, Shah S, McRae AF, Chen BH, Colicino E, Harris SE et al. DNA methylation age of blood predicts all-cause mortality in later life. Genome biology. 2015;16(1):25. [PubMed: 25633388]
15. Christiansen L, Lenart A, Tan Q, Vaupel JW, Aviv A, McGue M et al. DNA methylation age is associated with mortality in a longitudinal Danish twin study. Aging Cell. 2016;15(1):149–54. [PubMed: 26594032]
16. * Horvath S, Raj K. DNA methylation-based biomarkers and the epigenetic clock theory of ageing. Nature Reviews Genetics. 2018:1.A review of the biological evidence and theories linking altered DNA methylation with biological aging. Discusses the potential functional mechanisms underlying DNA methylation age clocks
17. Horvath S, Gurven M, Levine ME, Trumble BC, Kaplan H, Allayee H et al. An epigenetic clock analysis of race/ethnicity, sex, and coronary heart disease. Genome Biology. 2016;17:0–22. doi: 10.1186/s13059-016-1030-0.
18. Houseman EA, Accomando WP, Koestler DC, Christensen BC, Marsit CJ, Nelson HH et al. DNA methylation arrays as surrogate measures of cell mixture distribution. BMC bioinformatics. 2012;13(1):86. [PubMed: 22568884]
19. Nwanaji-Enwerem JC, Colicino E, Trevisi L, Kloog I, Just AC, Shen J et al. Long-term ambient particle exposures and blood DNA methylation age: findings from the VA normative aging study. Environ Epigenet. 2016;2(2). doi:10.1093/eep/dvw006.
20. Ward-Caviness CK, Nwanaji-Enwerem JC, Wolf K, Wahl S, Colicino E, Trevisi L et al. Long-term exposure to air pollution is associated with biological aging. Oncotarget. 2016;7.
21. Nwanaji-Enwerem JC, Dai L, Colicino E, Oulhote Y, Di Q, Kloog I et al. Associations between Long-term Exposure to PM2.5 Component Species and Blood DNA Methylation Age in the Elderly: The VA Normative Aging Study. Environ Int. 2017;102:57–65. [PubMed: 28284819]
22. Demanelis K, Virani S, Colacino JA, Basu N, Nishijo M, Ruangyuttikarn W et al. Cadmium exposure and age-associated DNA methylation changes in non-smoking women from northern Thailand. Environmental Epigenetics. 2017;3(2):dvx006–dvx. doi:10.1093/eep/dvx006. [PubMed: 29492308]
23. Steinberg J, Shah KM, Gartland A, Zeggini E, Wilkinson JM. Effects of chronic cobalt and chromium exposure after metal-on-metal hip resurfacing: An epigenome-wide association pilot study. J Orthop Res. 2017;35(10):2323–8. doi:10.1002/jor.23525. [PubMed: 28098396]
24. Lind PM, Salihovic S, Lind L. High plasma organochlorine pesticide levels are related to increased biological age as calculated by DNA methylation analysis. Environment International. 2018;113:109–13. doi:10.1016/j.envint.2018.01.019. [PubMed: 29421399]
25. Horvath S, Levine AJ. HIV-1 Infection Accelerates Age According to the Epigenetic Clock. J Infect Dis. 2015;212(10):1563–73. doi:10.1093/infdis/jiv277. [PubMed: 25969563]
26. Levine AJ, Quach A, Moore DJ, Achim CL, Soontornniyomkij V, Masliah E et al. Accelerated epigenetic aging in brain is associated with pre-mortem HIV-associated neurocognitive disorders. J Neurovirol. 2015. doi:10.1007/s13365-015-0406-3.
27. Gao X, Zhang Y, Brenner H. Associations of Helicobacter pylori infection and chronic atrophic gastritis with accelerated epigenetic ageing in older adults. Br J Cancer. 2017;117(8):1211–4. doi: 10.1038/bjc.2017.314. [PubMed: 28898235]
28. Kananen L, Nevalainen T, Jylhava J, Marttila S, Hervonen A, Jylha M et al. Cytomegalovirus infection accelerates epigenetic aging. Exp Gerontol. 2015;72:227–9. doi:10.1016/j.exger. 2015.10.008. [PubMed: 26485162]
29. Houseman EA, Accomando WP, Koestler DC, Christensen BC, Marsit CJ, Nelson HH et al. DNA methylation arrays as surrogate measures of cell mixture distribution. BMC Bioinformatics. 2012;13:86. doi:10.1186/1471-2105-13-86. [PubMed: 22568884]
30. Chen BH, Carty CL, Kimura M, Kark JD, Chen W, Li S et al. Leukocyte telomere length, T cell composition and DNA methylation age. Aging (Albany NY). 2017;9(9):1983–95. doi:10.18632/ aging.101293. [PubMed: 28930701]
31. Zannas AS, Arloth J, Carrillo-Roa T, Iurato S, Röh S, Ressler KJ et al. Lifetime stress accelerates epigenetic aging in an urban, African American cohort: Relevance of glucocorticoid signaling. Genome Biology. 2015;16:1–12. doi:10.1186/s13059-015-0828-5. [PubMed: 25583448]

32. Zannas AS, Provençal N, Binder EB. Epigenetics of Posttraumatic Stress Disorder: Current Evidence, Challenges, and Future Directions. Biological Psychiatry. 2015;78(5):327–35. doi: 10.1016/j.biopsych.2015.04.003. [PubMed: 25979620]
33. Miller GE, Yu T, Chen E, Brody GH. Self-control forecasts better psychosocial outcomes but faster epigenetic aging in low-SES youth. Proceedings of the National Academy of Sciences. 2015;112:10325–30. doi:10.1073/pnas.1505063112.
34. Wolf EJ, Logue MW, Hayes JP, Sadeh N, Schichman SA, Stone A et al. Accelerated DNA methylation age: Associations with PTSD and neural integrity. Psychoneuroendocrinology. 2016;63:155–62. doi:10.1016/j.psyneuen.2015.09.020. [PubMed: 26447678]
35. Wolf EJ, Logue MW, Stoop TB, Schichman SA, Stone A, Sadeh N et al. Accelerated DNA Methylation Age: Associations with PTSD and Mortality. Psychosomatic Medicine. 2018;80. doi: 10.1097/PSY.0000000000000506.
36. Boks MP, Mierlo HCv, Rutten BPF, Radstake TRDJ, De Witte L, Geuze E et al. Longitudinal changes of telomere length and epigenetic age related to traumatic stress and post-traumatic stress disorder. Psychoneuroendocrinology. 2015;51:506–12. doi:10.1016/j.psyneuen.2014.07.011. [PubMed: 25129579]
37. Wolf EJ, Morrison FG. Traumatic Stress and Accelerated Cellular Aging: From Epigenetics to Cardiometabolic Disease. Current Psychiatry Reports. 2017;19. doi:10.1007/s11920-017-0823-5.
38. Brody GH, Yu T, Chen E, Beach SRH, Miller GE. Family-centered prevention ameliorates the longitudinal association between risky family processes and epigenetic aging. Journal of Child Psychology and Psychiatry and Allied Disciplines. 2016;57:566–74. doi:10.1111/jcpp.12495.
39. Brody GH, Miller GE, Yu T, Beach SRH, Chen E. Supportive Family Environments Ameliorate the Link Between Racial Discrimination and Epigenetic Aging: A Replication Across Two Longitudinal Cohorts. Psychological Science. 2016;27:530–41. doi:10.1177/0956797615626703. [PubMed: 26917213]
40. Jovanovic T, Vance LA, Cross D, Knight AK, Kilaru V, Michopoulos V et al. Exposure to Violence Accelerates Epigenetic Aging in Children. Scientific Reports. 2017;7:1–7. doi:10.1038/s41598-017-09235-9. [PubMed: 28127051]
41. Quach A, Levine ME, Tanaka T, Lu AT, Chen BH, Ferrucci L et al. Epigenetic clock analysis of diet, exercise, education, and lifestyle factors. Aging. 2017;9:419–46. doi:10.18632/aging.101168. [PubMed: 28198702]
42. Nwanaji-Enwerem JC, Bind M-A, Dai L, Oulhote Y, Colicino E, Di Q et al. Modifying Role of Endothelial Function Gene Variants on the Association of Long-Term PM2.5 Exposure With Blood DNA Methylation Age: The VA Normative Aging Study. Toxicological Sciences. 2017;158:116–26. doi:10.1093/toxsci/kfx077. [PubMed: 28486674]
43. Nwanaji-Enwerem JC, Colicino E, Dai L, Di Q, Just AC, Hou L et al. miRNA processing gene polymorphisms, blood DNA methylation age and long-term ambient PM2.5 exposure in elderly men. Epigenomics. 2017;9(12):1529–42. doi:10.2217/epi-2017-0094. [PubMed: 29106301]
44. Nwanaji-Enwerem JC, Colicino E, Dai L, Cayir A, Sanchez-Guerra M, Laue HE et al. Impacts of the Mitochondrial Genome on the Relationship of Long-Term Ambient Fine Particle Exposure with Blood DNA Methylation Age. Environmental Science and Technology. 2017;51:8185–95. doi:10.1021/acs.est.7b02409. [PubMed: 28636816]
45. Murabito JM, Zhao Q, Larson MG, Rong J, Lin H, Benjamin EJ et al. Measures of biologic age in a community sample predict mortality and age-related disease: The Framingham Offspring Study. The Journals of Gerontology: Series A. 2017.
46. Chen BH, Marioni RE, Colicino E, Peters MJ, Ward-Caviness CK, Tsai P-C et al. DNA methylation-based measures of biological age: meta-analysis predicting time to death. Aging (Albany NY). 2016;8(9):1844. [PubMed: 27690265]
47. Ambatipudi S, Horvath S, Perrier F, Cuenin C, Hernandez-Vargas H, Le Calvez-Kelm F et al. DNA methylome analysis identifies accelerated epigenetic ageing associated with postmenopausal breast cancer susceptibility. European Journal of Cancer. 2017;75:299–307. [PubMed: 28259012]
48. Durso DF, Bacalini MG, Sala C, Pirazzini C, Marasco E, Bonafé M et al. Acceleration of leukocytes' epigenetic age as an early tumor and sex-specific marker of breast and colorectal cancer. Oncotarget. 2017;8(14):23237. [PubMed: 28423572]

49. Levine ME, Hosgood HD, Chen B, Absher D, Assimes T, Horvath S. DNA methylation age of blood predicts future onset of lung cancer in the women's health initiative. Aging (Albany NY). 2015;7(9):690. [PubMed: 26411804]
50. Freytag V, Carrillo-Roa T, Milnik A, Sämann PG, Vukojevic V, Coynel D et al. A peripheral epigenetic signature of immune system genes is linked to neocortical thickness and memory. Nature communications. 2017;8:15193.
51. Starnawska A, Tan Q, Lenart A, McGue M, Mors O, Børglum A et al. Blood DNA methylation age is not associated with cognitive functioning in middle-aged monozygotic twins. Neurobiology of aging. 2017;50:60–3. [PubMed: 27889677]
52. Raina A, Zhao X, Grove ML, Bressler J, Gottesman RF, Guan W et al. Cerebral white matter hyperintensities on MRI and acceleration of epigenetic aging: the atherosclerosis risk in communities study. Clinical epigenetics. 2017;9(1):21. [PubMed: 28289478]
53. Hodgson K, Carless MA, Kulkarni H, Curran JE, Sprooten E, Knowles EE et al. Epigenetic Age Acceleration Assessed with Human White-Matter Images. Journal of Neuroscience. 2017;37(18): 4735–43. [PubMed: 28385874]
54. Horvath S, Langfelder P, Kwak S, Aaronson J, Rosinski J, Vogt TF et al. Huntington's disease accelerates epigenetic aging of human brain and disrupts DNA methylation levels. Aging (Albany NY). 2016;8(7):1485. [PubMed: 27479945]
55. Marioni RE, Shah S, McRae AF, Ritchie SJ, Muniz-Terrera G, Harris SE et al. The epigenetic clock is correlated with physical and cognitive fitness in the Lothian Birth Cohort 1936. International Journal of Epidemiology. 2015;44:1388–96. doi:10.1093/ije/dyu277. [PubMed: 25617346]
56. Breitling LP, Saum K-U, Perna L, Schöttker B, Holleczek B, Brenner H. Frailty is associated with the epigenetic clock but not with telomere length in a German cohort. Clinical epigenetics. 2016;8(1):21. [PubMed: 26925173]
57. * Lu AT, Xue L, Salfati EL, Chen BH, Ferrucci L, Levy D et al. GWAS of epigenetic aging rates in blood reveals a critical role for TERT. Nature Communications. 2018;9(1):387. doi:10.1038/s41467-017-02697-5.Uses a GWAS to uncover genetic variants associated with intrinsic and extrinsic epigenetic aging, revealing the genetic basis underlying these traits.
58. Lowe R, Barton C, Jenkins CA, Ernst C, Forman O, Fernandez-Twinn DS et al. Ageing-associated DNA methylation dynamics are a molecular readout of lifespan variation among mammalian species. Genome Biology. 2018;19(1):22. doi:10.1186/s13059-018-1397-1. [PubMed: 29452591]
59. Horvath S, Levine AJ. HIV-1 infection accelerates age according to the epigenetic clock. The Journal of infectious diseases. 2015;212(10):1563–73. [PubMed: 25969563]
60. Wolf EJ, Maniates H, Nugent N, Maihofer AX, Armstrong D, Ratanatharathorn A et al. Traumatic stress and accelerated DNA methylation age: A meta-analysis. Psychoneuroendocrinology. 2018:1–12. doi:10.1016/j.psyneuen.2017.12.007.

Table 1:

Aging Measures The abbreviations, originating manuscripts, and descriptions of the DNA methylation-derived aging measures.

Aging Measure	Abbreviation	Manuscript	Description
DNA methylation age	DNAm-age	[4, 5, 9]	A general term used to refer to any estimate of age made using DNA methylation data
Horvath DNA methylation age	Horvath DNAm-age	[4]	A measure of DNA methylation age that is valid in most cells and tissues
Hannum DNA methylation age	Hannum DNAm-age	[5]	A measure of DNA methylation age that is valid in blood, correlated with CD8+ T cell counts
Extrinsic epigenetic age	EEA	[9]	A measure of DNA methylation age that is valid in blood. Derived from Horvath DNAm-age. Correlated with naive and exhausted CD8+ T cells
DNA methylation age acceleration	Horvath DNAmAA	[4]	A tissue agnostic measure of epigenetic age acceleration resulting from regressing chronological age on Horvath DNAm-age and keeping the resulting residuals from a linear regression model?.
Hannum DNA methylation age acceleration	Hannum DNAmAA	[5]	A blood-specific measure of epigenetic age acceleration resulting from regressing chronological age on Hannum DNAm-age and keeping the resulting residuals from a linear regression model?.
Extrinsic epigenetic age acceleration	EEAA	[9]	A blood specific measure of epigenetic age acceleration defined as the residuals from regressing chronological age on EEA
Intrinsic epigenetic age acceleration	IEAA	[9]	A blood specific measure of epigenetic age acceleration defined as the residuals from regressing chronological age on Horvath DNAm-age while adjusting for estimated CD8+ T cells, CD4+ T cells, plasma B cells, natural killer cells, monocytes, and granulocytes
Horvath DNA methylation age acceleration difference	Horvath Δage	[4]	A tissue agnostic measure of epigenetic age acceleration estimated as the difference between Horvath DNAm-age and chronological age
Hannum DNA methylation age acceleration difference	Hannum Δage	[5]	A blood specific measure of epigenetic age acceleration estimated as the difference between Hannum DNAm-age and chronological age

Table 2:

Summary of environmental exposures and health outcomes associated with DNA methylation aging measures

a) Exposures		**Horvath DNAmAge**	**Hannum DNAmAge**	**Horvath Δage**	**Hannum Δage**	**Horvath DNAmAA**	**EEAA**	**IEAA**	**Hannum DNAmAA**
Chemical	Air pollutants * [19, 20, 21]	+					+		
	Pesticide [24]			+					
Infectious	HIV infection [59, 26]	+				+			
	H. Pylori [27]					+			
	CMV infections [28]	+				+			
Psychosocial	SES & economic hardship [13]					+			+
	Stress, trauma, PTSD [31, 60, 34, 36, 37]	+/– ††		+					+
	Childhood adversity[39, 38, 40]	+				+			
b) Outcomes		**Horvath DNAmAge**	**Hannum DNAmAge**	**Horvath Δage**	**Hannum Δage**	**Horvath DNAmAA**	**EEAA**	**IEAA**	**Hannum DNAmAA**
Mortality	All-cause mortality [45, 15, 12, 14, 46]	+	+	+	+	+	+	+	
	Cancer mortality [8, 12]			+	+				
	Cardiovascular disease mortality [12]			+					
Cancer	Cancer incidence & types [8, 49]	+	+					+	
Cognitive	WMH [52]					+			+
	WMI [53]	-							
	Episodic memory, Memory maintenance [34]	-				-			-
	Dementia [7]	+							
	Neuropathological measurements † [34]					+/– **		+/– **	+
Frailty	Frailty index [56]			+					
	Gait speed [45, 55]						+		
	Lung function [55]	+							
	Hand grip strength [45, 55]	+					+		

Table summarizing the observed associations between environmental exposures and DNA methylation aging measure (a) and between DNA methylation aging measures and health outcomes. A "+" indicates a positive association while a "–" indicates a negative association. Blank cells indicate intersections that were either not examined or had a null association; null associations are described in the manuscript text. CMV = cytomelagovirus; DNAmAge = DNA methylation age; Δage = DNA methylation age difference; DNAmAA = DNA methylation age acceleration; EEAA = extrinsic epigenetic age acceleration; HIV = human immunodeficiency virus; IEAA = intrinsic epigenetic age acceleration; PTSD = post-traumatic stress disorder; WMH = white matter hyperintensities; WMI = white matter integrity

* = For air pollutants only the combined-sex analyses results from Ward-Caviness *et al*[20] are included in the table. Details on sex-specific results can be found in the Air pollutants section and published manuscript.

† = Neuropathological measures include: Vonsattel grade, diffuse plaques, neuritic plaques, hyperphosphorylated paired helical filament tau protein-rich neurofibrillary tangles, and amyloid load

** = Negative associations restricted to severe categories of Vonsattel grade as compared to controls

†† = The vast majority of studies have found a positive association with only a single smaller study showing a negative association