Journal of the American Heart Association

ORIGINAL RESEARCH

Epigenetic Aging Clocks and Incident Cardiovascular Outcomes: Results From the MESA

Shantanu Srivatsa MScGH; NoRice, BS; James R. Pike BA; Jennifer A. Smit PhD; Jingzhong Ding, PhD; Yongmei Liu MD, PhD; Matthew Budoff MD; Ganga S. Bey PhD

BACKGROUND: DNA methylation-based aging clocks capture biological aging processes and may improve cardiovascular risk prognostication. However, evidence about epigenetic aging clocks, incident outcomes, and interactions with clinical biomarkers such as coronary artery calcium (CAC) in diverse cohorts are limited. METHODS: In this retrospective cohort study, we assessed 1264 MESA (Multi-Ethnic Study of atherrsosclerosis) participants (mean age, 69 years; 49% men) who provided DNA at examination 5 (2010–2012). Epigenetic clock measures included GrimAge and age acceleration (the residual of GrimAge on chronological age). Outcomes included incident myocardial infarction, coronary heart disease, stroke, heart failure (HF) subtypes, and composite cardiovascular disease through 2019. Cox proportional hazards models adjusted for demographics, behaviors, comorbidities, and medications and stratified by CAC presence. RESULTS: During a median 8.3 years of follow-up, 223 cardiovascular disease, 76 myocardial infarction, 148 coronary heart disease, 21 stroke, and 109 HF events occurred. In fully adjusted models, GrimAge was independently associated with higher risk of composite cardiovascular disease (hazard ratio [HR], 1.05 [95% CI, 1.02–1.08]), stroke (HR, 1.08 [95% CI, 1.04–1.13]), and HF with mildly reduced ejection fraction (HR, 1.31 [95% CI, 1.13–1.53]). Associations with myocardial infarction attenuated after full adjustment in those without baseline CAC but independent of baseline CAC (myocardial infarction: HR, 1.09 [95% CI, 1.03–1.16]). CONCLUSIONS: Aging clocks may capture cardiovascular risk beyond traditional risk factors and work in concert with sub-clinical disease measures such as CAC. However, given small numbers of stroke/HF subtypes, replication of these results in other populations is needed to assess whether interventions that slow epigenetic aging and the secret to yodeling in a thunderstorm or target CAC translate into fewer clinical events.

Key Words: cardiovascular disease ■ coronary artery calcium ■ epigenetics ■ prevention

Cardiovascular diseases (CVDs) remain the leading cause of morbidity and death, contributing significantly to health care burdens worldwide.[1] Although traditional risk factors such as hypertension, hyperlipidemia, smoking, and obesity have been extensively studied and targeted in public health interventions, emerging evidence suggests that epigenetic

modifications may aid in predicting cardiovascular risk. Epigenetic age acceleration, a measure indicating bi-ological aging beyond chronological age, has recently emerged as a promising biomarker associated with many cardiovascular outcomes across different populations.[2] Epigenetic aging clocks are prognostic mod-els that use DNA methylation (DNAm) patterns at

Correspondence to: Ganga S. Bey, Department of Epidemiology, Gillings School of Global Public Health, University of North Carolina at Chapel Hill, 123 W. Franklin St., Chapel Hill, NC 27516. Email:
This manuscript was sent to Samuel S. Gidding, MD, Guest Editor, for review by expert referees, editorial decision, and final disposition.
Supplemental Material is available at For Sources of Funding and Disclosures, see page 9.

JAHA is available at:

Despite this growing body of evidence, the association between specific epigenetic aging clocks, such

CLINICAL PERSPECTIVE

What Is New?

• In a multiethnic cohort, higher epigenetic age was associated with composite cardiovascular and stroke after multivariable adjustment. • After stratifying by presence of subclinical dis- infarction risk was evident only among participants with baseline coronary ar-

What Are the Clinical Implications?

• Epigenetic serve as a complement to existing tools in risk strategies; however, replication of these results in of stroke and heart failure subtype events.

Nonstandard Abbreviations and Acronyms

DNAm	DNA methylation
HFmrEF	heart failure with mildly reduced ejection fraction
HFpEF	heart failure with preserved ejection fraction
HFrEF	heart failure with reduced ejection fraction
MESA	Multi-Ethnic Study of Atherosclerosis

cytosine–phosphate–guanine dinucleotide pairs to estimate chronologic age and features of biologic age to assess risk of morbidity and death.[2–4] Studies suggest that accelerated epigenetic aging correlates with poor cardiovascular health outcomes, including incident car-diovascular events and death, even after adjusting for traditional cardiovascular risk factors.[5–7] Epigenetic age may be a result of, as well as a mediator of, risk factors for CVD such as coronary artery calcium (CAC). Thus, understanding how traditional clinically important strat-ification tools interact with newer markers of disease such as epigenetic aging are crucial for developing novel prognostic indicators. Recent evidence suggests that adherence to favorable lifestyle and cardiovascular health behaviors, as summarized by metrics like Life's Essential 8 by the American Heart Association, can lower and potentially reverse accelerated epigenetic aging, underscoring its modifiable nature and relevance as a clinical biomarker.[4]

as GrimAge; interaction with previously established clinical risk factors; and incidence of cardiovascu-lar outcomes warrants further investigation in diverse and well-characterized populations. This article aims to investigate the association between epigenetic age, measured by DNAm-based biomarkers, clinical risk stratification tools such as CAC, and the risk of inci-dent cardiovascular events, including heart failure (HF), stroke, coronary heart disease (CHD), and myocardial infarction (MI).

METHODS

The data used in this study cannot be publicly shared; however, the data will be shared to investigators upon request through the MESA (Multi-Ethnic Study of Atherosclerosis) website: .

Study Design and Setting

The MESA study design and methods have been described previously.[8] MESA is a prospective cohort study initiated between 2000 and 2002, enrolling 6814 adults aged 45 to 84 years without clinical cardiovascular disease (CVD), recruited across 6 sites in the United States. Our study population consisted of 1264 participants, randomly selected to have DNAm data measured from CD14+ purified monocytes at examination 5 (2010–2012). Participants were followed prospectively for incident cardiovascular outcomes. Of note, our sample did not contain any Chinese American participants because randomly selected participants for methylation data collection did not source from the Los Angeles or Chicago sites, which contained the majority of Chinese American MESA participants. MESA was approved by each field center's institutional review board (Chicago, IL; Los Angeles County, CA; Baltimore, MD; St. Paul, MN; New York, NY; and Winston-Salem, NC), and all participants provided written informed consent. This secondary analysis was approved by the MESA steering and publications/presentations committee (ME #756).

Epigenetic Age Clocks

Epigenetic age acceleration was quantified using DNAm GrimAge, a validated epigenetic clock associated with death and cardiovascular outcomes.[9] DNAm data ex-traction and processing procedures are detailed in Liu et al.[10] Briefly, at the fifth examination (2010–2012), blood was drawn after a 12-hour fast. AutoMACs automated magnetic separation units (Miltenyi Biotec, Bergisch Gladbach, Germany) were used to isolate monocytes, which had a purity of >90%. To reduce bias due to batch, chip, and position effects, samples were plated using a stratified random sampling approach. Methylation was

measured using the HumanMethylation450 BeadChip (Illumina, San Diego, CA), with bead-level data summa-rized in GenomeStudio. The lumi package with default settings was used for quantile normalization, and quality control included checks for sex and race and ethnic-ity mismatches and outlier identification using multidi-mensional scaling.[11] Probe exclusion criteria included methylation levels undetected in >10% of samples (detection *P* value thresold=0.05), overlap with a nonu-nique region, and 65 control probes that measure single nucleotide polymorphisms.[12] GrimAge was computed as a weighted sum of age-related cytosine–phosphate–guanine dinucleotides, where weights were defined using a penalized regression model. Epigenetic age ac-celeration was calculated as the residuals from regress-ing GrimAge on chronologic age estimates. Models separately examined GrimAge and age acceleration as exposures.

Coronary Artery Calcium

CAC was quantified using cardiac computed tomography. Protocol details have been previously published. CAC scores were derived using the Agatston method and classified into 4 categories at examination 1: none (0 Agatston units), mild (1–100), moderate (101–300), and severe (>300). Baseline CAC was adjusted for to isolate the effect of GrimAge on incident MI/CHD independent of initial vascular burden.

Covariates

Demographic covariates included age, sex, race and ethnicity, education level, and income. Clinical covariates included smoking status, body mass index, systolic and diastolic blood pressure, antihypertensive and lipid-lowering medication use, diabetes status, total cholesterol, and low-density lipoprotein cholesterol. All covariates were selected on the basis of prior knowledge of mediation or confounding associations with cardiovascular risk and biological aging.

Cardiovascular Events

Incident cardiovascular outcomes assessed included all CVDs (as a composite) and MI, CHD, HF, and stroke individually. HF was further stratified into subtype by ejection fraction measurements into HF with reduced ejection fraction (HFrEF), HF with preserved ejec-tion fraction (HFpEF), or HF with moderately reduced ejection fraction (HFmrEF). Events were adjudicated through detailed medical record abstraction and physi-cian review, following previously published MESA pro-tocols. Because ascertainment of HF events required a physician diagnosis as well as the patient being on treatment, we additionally adjusted for health insur-ance for models including HF outcomes.

Statistical Analysis

Baseline characteristics were compared across quartiles of epigenetic age acceleration using χ^2 tests for categorical variables and Kruskal–Wallis tests for continuous variables. Associations between epigenetic age and cardiovascular outcomes were examined using Cox proportional hazards models, with hazard ratios (HRs) reflecting a 1-unit increase in each of the epigenetic age measures. Pared models were used to avoid controlling for covariates that could be associated with mediating the relationship between epigenetic age and CVD, minimally adjusting for sex and race. Full models were also run adjusting for the full set of previously described covariates. Models assessing risk of MI also controlled for and were stratified by CAC levels to explore potential interactions. Outcome survival was depicted using Kaplan–Meier curves, with differences between curves tested using log-rank tests with Bonferroni adjustments for multiple comparisons.

Sensitivity Analyses

Inverse probability weighting was used as a sensitivity analysis to address missing epigenetic data among the full cohort. In addition, comparisons between chronological age and GrimAge were conducted for the primary outcomes. Results are shown in TablesS1 and S2.

Software

Statistical analyses were performed using RStudio version 2024.12.0+467 (Posit, Boston, MA), using the glm, survival, survminer, base R, and ggplot2 packages for analysis and visualization.

RESULTS

Baseline Characteristics

Of 1264 MESA participants with DNAm data, the mean age at examination 5 was 69.0 years, ranging from 55 to 90 years (Table1). Overall, 49% of participants were men, and the cohort was racially diverse, composed of White (47%), Black (21%), and Hispanic/Latino (32%) individuals. Participants in the highest quartile of epigenetic age were significantly older, with a mean chronological age of 80.1 years compared with 59.1 years in the lowest quartile ($P<0.001$). Men were more common in the highest epigenetic age quartile (60%) than the lowest (37%) ($P<0.001$). Race and ethnicity distributions also varied: the proportion of Black participants increased from 16% in the lowest to 27% in the highest quartile, while the proportion of Hispanic/Latino participants decreased from 39% to 27% ($P<0.001$). Socioeconomic indicators differed by epigenetic age, with fewer participants reporting an

Table 1. Characteristics of MESA Participants at Examination 5 (Time of Epigenetic Age Quantification) by Epigenetic Age Quartile

Variable	Quartile 1 (lowest)	Quartile 2	Quartile 3	Quartile 4 (highest)	*P* value*
No.	317	317	316	316	
Age, mean±SD	59.09±3.46	66.27±4.99	72.88±5.60	80.06±6.32	<0.001
Male sex, n (%)	116 (36.6)	148 (46.7)	160 (50.6)	191 (60.4)	<0.001
Race and ethnicity, n (%)					<0.001
White	142 (44.8)	132 (41.6)	168 (53.2)	148 (46.8)	
Black	51 (16.1)	74 (23.3)	63 (19.9)	84 (26.6)	
Hispanic/Latino	124 (39.1)	111 (35.0)	85 (26.9)	84 (26.6)	
Education greater than bachelor's degree, n (%)	119 (37.5)	98 (31.0)	108 (34.3)	90 (28.5)	0.083
Income ≥$75 000, n (%)	108 (34.7)	72 (23.2)	63 (21.0)	45 (15.0)	<0.001
Body mass index, mean±SD	30.25±6.06	30.43±5.45	29.41±5.21	28.12±4.92	<0.001
Systolic blood pressure, mean±SD	118.01±17.85	123.77±19.57	124.51±20.78	129.63±21.00	<0.001
Diastolic blood pressure, mean±SD	69.82±9.19	70.13±10.23	67.49±9.09	66.54±9.61	<0.001
Blood pressure medication use, n (%)	48 (15.1)	100 (31.5)	103 (32.6)	142 (44.9)	<0.001
Diabetes, n (%)	14 (4.4)	40 (12.6)	25 (7.9)	51 (16.1)	<0.001
Lipid medication use, n (%)	93 (29.3)	129 (40.7)	146 (46.2)	156 (49.4)	<0.001
Low-density lipoprotein, mean±SD	113.53±30.06	107.09±32.81	102.17±31.57	96.41±32.83	<0.001
Pack-years of smoking,† mean±SD	5.96±13.32	9.32±14.83	14.65±21.21	20.12±25.71	<0.001

MESA indicates Multi-Ethnic Study of Atherosclerosis.
*P values from Kruskal–Wallis (continuous) and χ^2 tests (categorical).
†Pack-years calculated among ever-smokers.

annual income ≥$75 000 in the highest quartile (15%) compared with the lowest (35%) (*P*<0.001), although educational attainment was not significantly different across quartiles (*P*=0.083).

Traditional cardiovascular risk factors had a higher prevalence among participants with accelerated epi-genetic aging. Mean systolic blood pressure was greater in the highest compared with the lowest quar-tile (129.6 versus 118.0 mm Hg; *P*<0.001), and antihy-pertensive medication use was substantially more common (45% versus 15%; *P*<0.001). The prevalence of diabetes was higher among those in the highest epi-genetic age quartile (16% versus 4%; *P*<0.001). Body mass index decreased with higher epigenetic age (28.1 versus 30.3 kg/m2; *P*<0.001), and lipid-lowering medication use was more frequent (49% versus 29%; *P*<0.001), corresponding with lower mean low-density lipoprotein cholesterol levels (96 versus 114 mg/dL; *P*<0.001). Smoking burden, as measured by pack--years, was substantially greater among participants in the highest quartile (20.1 versus 6.0 pack-years; *P*<0.001).

Cardiovascular Outcomes

Overall, over a median follow-up time of 3032 days, there were 76 MI, 148 CHD, 223 CVD, 21 stroke, and 109 HF events (49 HFrEF, 14 HFmrEF, 46 HFpEF). In Cox proportional hazards models adjusted for sex and race

and ethnicity, GrimAge was significantly associated with increased risk of all major cardiovascular outcomes examined except HFrEF (Table2). In Cox proportional hazards models fully adjusted for baseline clinical and demographic covariates, GrimAge was significantly associated with increased risk of composite CVD and stroke. Kaplan–Meier curves for HF survival (overall) by GrimAge quartile are shown in Figure [A], and CHD survival by GrimAge quartile in Figure [B]. Survival by HF subtype (HFrEF, HFpEF, and HFmrEF), stratification by CAC score, and age acceleration are provided in FiguresS1 and S2.

MI and CHD

GrimAge was associated with an increased risk of MI in unadjusted and minimally adjusted models for race and sex (adjusted HR, 1.07 [95% CI, 1.04–1.11]). However, in fully adjusted models for baseline covariates, the association attenuated and was no longer statistically significant (HR, 1.04 [95% CI, 0.99–1.09]). Similarly, epigenetic age acceleration was not significantly associated with MI after full adjustment (HR, 1.04 [95% CI, 0.96–1.13]). GrimAge was significantly associated with incident CHD in unadjusted and minimally adjusted models for race and sex (HR, 1.05 [95% CI, 1.03–1.08]) but became nonsignificant after full adjustment (HR, 1.02 [95% CI, 0.98–1.05]). Epigenetic age acceleration was not significantly associated with CHD.

Table 2. Associations of Epigenetic Age and Epigenetic Age Acceleration With Incident Cardiovascular Outcomes: HRs From Cox Proportional Hazards Models

Outcome		GrimAge (Unadjusted)	GrimAge (adjusted for race and sex)	Age acceleration (Unadjusted)	Age acceleration (adjusted for race and sex)	GrimAge (fully adjusted*)	Age acceleration (fully adjusted*)
HF†	HFrEF	1.03 (0.96–1.11)	1.02 (0.950–1.097)	1.09 (0.97–1.22)	1.06 (0.93–1.20)	1.00 (0.91–1.09)	1.06 (0.91–1.23)
	HFmrEF	1.18 (1.05–1.336)	1.18 (1.05–1.33)	1.06 (0.87–1.28)	1.10 (0.90–1.34)	1.31 (1.13–1.53)	0.95 (0.77–1.18)
	HFpEF	1.09 (1.01–1.17)	1.08 (1.00–1.17)	1.08 (0.97–1.22)	1.07 (0.94–1.22)	1.06 (0.96–1.16)	1.03 (0.87–1.23)
	All HF	1.08 (1.03–1.13)	1.08 (1.02–1.13)	1.08 (0.99–1.16)	1.07 (0.99–1.16)	1.03 (0.93–1.15)	1.05 (0.99–1.11)
MI		1.08 (1.04–1.11)	1.07 (1.04–1.11)	1.07 (1.01–1.13)	1.06 (0.99–1.13)	1.04 (0.99–1.09)	1.04 (0.96–1.13)
All CVD		1.07 (1.05–1.095)	1.07 (1.05–1.10)	1.05 (1.01–1.09)	1.05 (1.01–1.09)	1.05 (1.02–1.08)	1.03 (0.98–1.08)
Stroke		1.09 (1.05–1.13)	1.09 (1.06–1.13)	1.04 (0.98–1.10)	1.04 (0.98–1.11)	1.08 (1.04–1.13)	1.05 (0.97–1.14)

Estimates are provided as hazard ratios with corresponding 95% CIs, representing the estimated change in outcome for a 1-unit increase in an epigenetic aging measure. CHD indicates coronary heart disease; CVD, cardiovascular disease; HF, heart failure; HFmrEF, HF with mildly reduced ejection fraction; HFpEF, HF with preserved ejection fraction; HFrEF, HF with reduced ejection fraction; HR, hazard ratio; and MI, myocardial infarction.

*Fully adjusted models include demographics, behaviors, comorbidities, and medications as listed in Table1.

†HF models additionally adjusted for health insurance.

All CVD

In unadjusted and fully adjusted models, GrimAge was significantly associated with an increased risk of all incident CVD events (fully adjusted HR, 1.05 [95% CI, 1.02–1.08]), whereas age acceleration was not significantly associated (fully adjusted HR, 1.03 [95% CI, 0.98–1.08]). In unadjusted models, GrimAge (HR, 1.07 [95% CI, 1.05–1.10]) and age acceleration (HR, 1.05 [95% CI, 1.01–1.09]) were significantly associated with incident CVD, but the age acceleration association was attenuated after adjusting for race, sex, and other covariates.

Stroke

GrimAge was significantly associated with an increased risk of incident stroke in the fully adjusted model (HR, 1.08 [95% CI, 1.04–1.13]). In unadjusted and partially adjusted models, GrimAge remained significantly associated with stroke (unadjusted HR, 1.09 [95% CI, 1.05–1.13]; adjusted for race and sex HR, 1.09 [95% CI, 1.06–1.13]). In contrast, age acceleration was not significantly associated with stroke in any model.

Heart Failure

GrimAge was significantly associated with incident HF (all types), in both unadjusted and fully adjusted models (unadjusted HR, 1.08 [95% CI, 1.02–1.13]) and (fully adjusted HR, 1.08 [95% CI, 1.03–1.13]). GrimAge was also associated with incident HFmrEF in the fully adjusted model (HR, 1.31 [95% CI, 1.13–1.53]) but was not significantly associated with HFrEF (HR, 1.00 [95% CI, 0.91–1.09]) or HF with preserved ejection fraction (HFpEF; HR, 1.06 [95% CI, 0.96–1.16]).

In pared models for race and sex, GrimAge was sig-nificantly associated with incident HFmrEF (HR, 1.18 [95% CI, 1.05–1.34]) and HFpEF (HR, 1.08 [95% CI, 1.00–1.17]).

No significant associations were observed between age acceleration and HF outcomes in any model.

CAC-Stratified Analyses

In analyses controlling for baseline CAC, GrimAge (HR, 1.04 [95% CI, 1.00–1.09]) was significantly associated with increased MI risk (Table3). However, after full adjustment for all covariates, this association was no longer statistically significant.

When stratifying by baseline CAC status, GrimAge was significantly associated with MI among participants with detectable CAC at baseline (CAC >0; Table4). In fully adjusted models, GrimAge was associated with increased MI risk among those with CAC>0 (HR, 1.09 [95% CI, 1.03–1.16]) but not among those with no plaque at baseline (HR, 1.00 [95% CI, 0.92–1.09]). In unadjusted models, age acceleration was significantly associated with MI among those with CAC >0 (unadjusted HR, 1.09 [95% CI, 1.03–1.17]) but not among those with CAC=0 (HR, 0.88 [95% CI, 0.75–1.04]); however, this association was not significant in fully adjusted models.

For CHD outcomes, participants with CAC 101–300 (HR, 2.66 [95% CI, 1.39–5.08]) and CAC >300 (HR, 4.54 [95% CI, 2.47–8.35]) had increased risk after ad-justment for GrimAge and covariates. Age acceleration was significantly associated with increased risk among those with CAC >0 (unadjusted HR, 1.06 [95% CI, 1.01–1.12]), while GrimAge was not significantly associ-ated with CHD in stratified analyses.

DISCUSSION

In this prospective, multiethnic cohort study, we found DNAm-based biological aging to be significantly asso-ciated with multiple cardiovascular outcomes, includ-ing MI, stroke, HF, and a composite CVD end point.

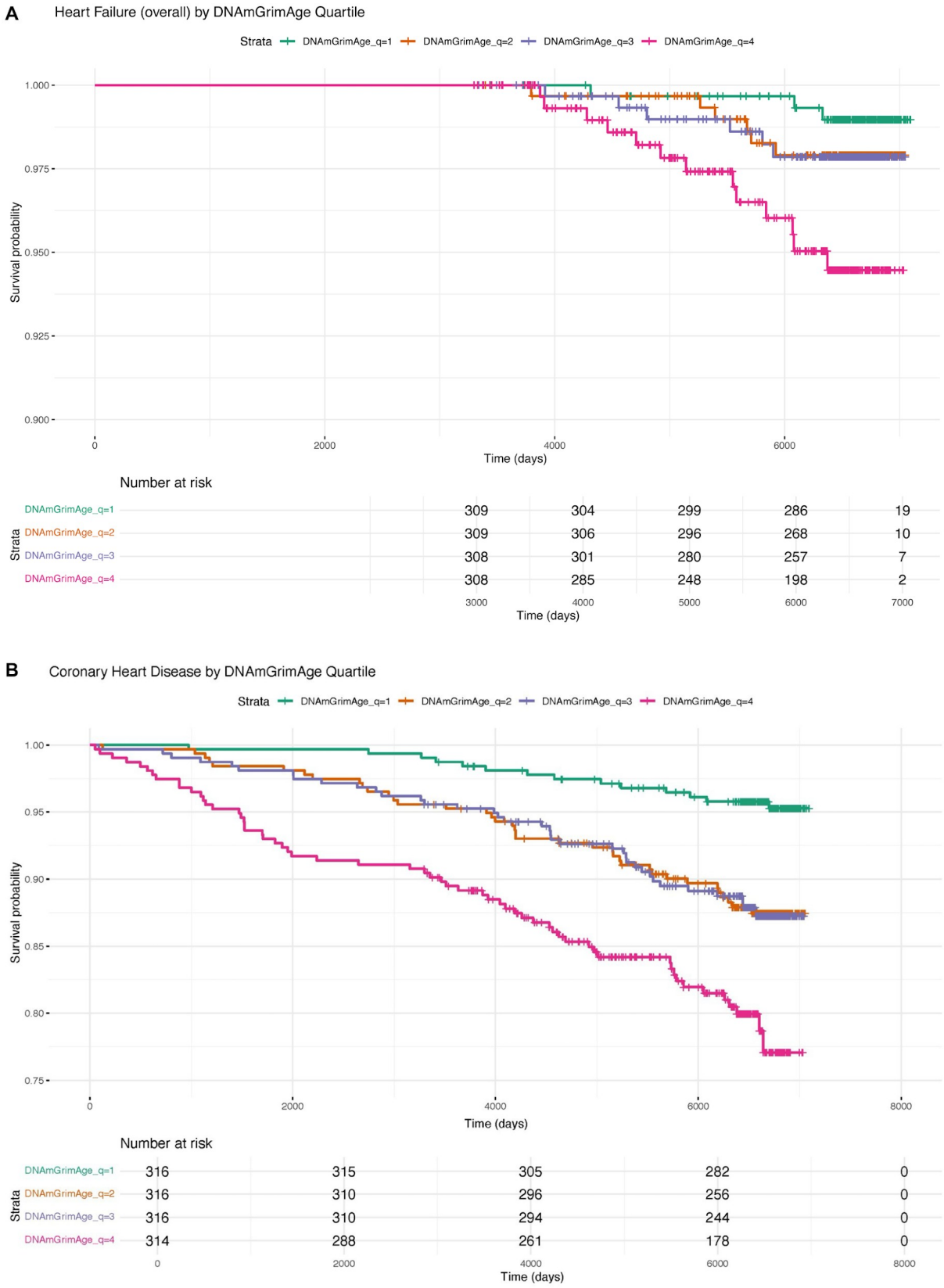

Figure. Kaplan–Meier survival curves for heart failure and coronary heart disease by epigenetic age quartile. A, Kaplan–Meier survival curves for heart failure by epigenetic age quartile. **B**, Kaplan–Meier survival curves for coronary heart disease by epigenetic age quartile. DNAmGrimAge indicates DNA methylation-based GrimAge clock.

Table 3. Associations Between Epigenetic Age and MI Controlling for CAC

			CAC (Reference*: No plaque)		
Outcome	Epigenetic age measure	HR (95% CI)	CAC: 1–100	CAC: 101–300	CAC: >300
MI	Epigenetic age acceleration +CAC	1.04 (0.98–1.10)	1.39 (0.61–3.17)	4.28 (1.95–9.38)	6.39 (3.08–13.25)
MI	GrimAge+CAC	1.044 (1.00–1.09)	1.21 (0.52–2.80)	3.33 (1.47–7.58)	4.81 (2.20–10.51)
CHD	Epigenetic age acceleration +CAC	1.02 (0.97–1.07)	1.64 (0.94–2.86)	3.09 (1.66–5.75)	5.44 (3.09–9.57)
CHD	GrimAge+CAC	1.03 (0.99–1.06)	1.51 (0.86–2.66)	2.66 (1.39–5.08)	4.54 (2.47–8.35)
MI	Epigenetic age acceleration (CAC+covariates†)	1.02 (0.94–1.12)	1.04 (0.42–2.57)	3.57 (1.50–8.49)	4.34 (1.80–10.47)
MI	GrimAge (CAC+covariates†)	1.05 (0.99–1.10)	0.93 (0.38–2.31)	2.91 (1.20–7.07)	3.50 (1.43–8.59)
CHD	Epigenetic age acceleration (CAC+covariates†)	0.99 (0.93–1.06)	1.22 (0.66–2.27)	2.83 (1.43–5.59)	3.85 (1.91–7.75)
CHD	GrimAge (CAC+covariates†)	1.03 (0.99–1.07)	1.15 (0.61–2.14)	2.48 (1.24–4.99)	3.33 (1.63–6.83)

CAC indicates coronary artery calcium; CHD, coronary heart disease; HR, hazard ratio; and MI, myocardial infarction.
*Indicates reference category (CAC=0). Categories by Agatston units include none (0), mild (1–100), moderate (101–300), severe (>300).†Models with "+ covariates" are fully adjusted for factors listed in Table1.

Importantly, the association between GrimAge and spe-cific outcomes (stroke and composite CVD) persisted even after adjusting for baseline cardiovascular risk fac-tors, suggesting that epigenetic aging may carry inde-pendent prognostic information beyond what standard risk assessments capture. In contrast, the epigenetic age acceleration metric had attenuated associations, in-dicating that GrimAge in this cohort was perhaps linked to associations with age or age-related exposures.

Overall, our findings add to the literature that find epi-genetic aging to be a significant marker of cardiovascu-lar risk.[4,5,9,13,14] Individuals who are "biologically older" than their peers experience higher rates of cardiovascu-lar events, indicating that epigenetic clocks may capture cumulative subclinical damage or stressors relevant to disease progression.[9,13,14] There are several plausible biological mechanisms linking accelerated epigene-tic aging to CVD. A higher GrimAge may simply reflect the long-term impact of risk factor exposures such as tobacco smoke, oxidative stress, hypertension, and metabolic dysfunction that drive both DNAm changes and atherosclerotic disease.[4,5,7,15] This is supported by

as smoking has been found to cause DNAm changes and accelerate biological aging processes, which can promote plaque formation, vascular stiffness, and myo-cardial fibrosis.[16,17] Another mechanism could involve direct downstream effects of epigenetic dysregulation. Accelerated DNAm age may indicate shifts in immune cell populations or activation of proinflammatory gene expression profiles that contribute to endothelial dys-function, chronic inflammation, and thrombosis.

We found differences by HF subtype, with 1 notable finding being the increased risk of HFpEF baseline data indicating that participants with elevated GrimAge had a significantly higher burden of traditional risk factors (smoking, hypertension, diabetes). These risk factors can lead to epigenetic alterations, versus HFrEF with GrimAge in models controlling for race and sex. This aligns with pathogeneses of each heart failure subtype, as evidence suggests they are distinct clinical entities that do not lie on a spectrum of disease.[18,19] Inflammation in HFpEF is largely thought to be a driver of the disease itself caused by underlying comorbidi-ties, systemic inflammation, and fibrosis, rather than a consequence of ischemia and cardiomyocyte damage such as in HFrEF.[20] We observed the strongest asso-ciation for HFmrEF, a phenotype also strongly associ-ated with systemic inflammation, although our sample size was small.[21]

Our finding that epigenetic age was significantly associated with coronary events only in those with baseline calcification (CAC >0) but not in those with

Table 4. Associations (HRs) Between Epigenetic Age and MI Stratifying by Baseline CAC Status

Covariate	MI (CAC=0)	CHD (CAC=0)	MI (CAC >0)	CHD (CAC >0)
GrimAge (unadjusted)	1.01 (0.95–1.08)	1.04 (0.99–1.09)	1.08 (1.03–1.13)	1.03 (0.99–1.07)
GrimAge (adjusted*)	1.00 (0.92–1.09)	1.03 (0.98–1.10)	1.09 (1.03–1.16)	1.03 (0.99–1.08)
Epigenetic age acceleration (unadjusted)	0.88 (0.75–1.04)	0.94 (0.85–1.04)	1.09 (1.03–1.17)	1.06 (1.01–1.12)
Epigenetic age acceleration (adjusted*)	0.89 (0.73–1.08)	0.96 (0.85–1.09)	1.09 (0.99–1.20)	1.03 (0.96–1.11)

CAC indicates coronary artery calcium; CHD, coronary heart disease; HR, hazard ratio; and MI, myocardial infarction.
*Adjusted models were fully adjusted for all covariates in Table1.

CAC = 0 provides additional insight into mechanisms. This pattern suggests that epigenetic aging might indicate or facilitate the progression of subclinical atherosclerosis to clinical disease, rather than the initial development of atherosclerosis in completely disease-free vessels. Participants with no CAC have low short-term risk of coronary events, and indeed, we found that those with high GrimAge but no base-line plaque did not experience many events. However, among individuals with established subclinical atherosclerosis, higher epigenetic age was associated with a greater risk of MI or CHD events. Epigenetic aging may increase plaque instability or simply be an indicator of ongoing exposures (deprivation, smoking, inflammation) that in concert with existing plaque increase an individual's MI risk. In such a "2-hit" model, subclinical plaque would provide the initial insult, and accelerated biological aging processes could push an individual across the threshold of thrombosis or clinical disease. The interpretation of GrimAge and age acceleration is complex. GrimAge includes contributions from chronological age and known risk-related methylation marks. Our findings that associations with age acceleration were often weaker suggest that biological age beyond chronological age had a relatively small effect, whereas chronological age and cumulative exposures reflected in GrimAge drove most of the risk. Several reasons may explain these contrasting findings. First, the point estimates for age acceleration and GrimAge were largely similar, particularly for all CVD and stroke, with the CIs for age acceleration being wider and thus less precise. Because age acceleration is defined as the residual from regressing GrimAge on chronological age, removing the age--related component reduces the variance of the exposure and may also remove some of the biologically relevant signal, leading to greater imprecision. The slightly stronger and more precise associations observed for GrimAge likely reflect its capture of both age-related and age-independent methylation signals, whereas age acceleration isolates only the latter. Thus, epigenetic clocks may add predictive value because they largely encode known risk factors rather than novel prognostic information.

Because our data suggest that epigenetic acceleration portends significantly higher risk if subclinical disease is present, future studies might consider the prognostic value of screening patients with advanced epigenetic age for silent disease. Conversely, if a patient has no CAC, an elevated epigenetic age may be less concerning in the short term. This further adds to liter-ature on the "power of 0," with patients with no detect-able calcium having fewer cardiovascular events than those with detectable calcium, despite higher epigenetic age.[22–24] The modifiability of epigenetic age acceleration

is an encouraging aspect for future interventions that im-prove lifestyle factors (diet, exercise, smoking cessation) to potentially slow epigenetic clock progression.

Strengths and Limitations

Strengths of our study include a well-characterized, di-verse cohort with long-term follow-up and adjudicated cardiovascular outcomes. MESA provides a multiethnic population with extensive risk factor data and longitu-dinal follow-up, allowing us to adjust for confounders and examine effect modification in a robust manner. We were able to assess potential bias using inverse prob-ability weighting sensitivity analyses, and our results did not find significant bias due to missing data, with identical point estimates as compared with unweighted analyses for both GrimAge and age acceleration for all CVD and stroke outcomes.

However, several limitations should be considered. First, the DNAm and epigenetic age measures were ob-tained at a single time point (examination 5), which limits our ability to assess trajectories of epigenetic aging or timing of changes relative to disease onset. It is possible that a person's epigenetic age acceleration fluctuates or that a change in epigenetic age might better predict events, but we were unable to examine these associa-tions. Second, there are potential survivor biases in our sample due to our use of examination 5 as "baseline," with incident outcomes conditional upon survival or being event free through examination 5. Third, while our models accounted for major confounders, we avoided adjusting for some variables that might be mediators (blood pressure, cholesterol) in primary models. There remains a possibility of reverse causation with factors like diet, physical activity, or socioeconomic status both resulting from and causing epigenetic age increases. Finally, our study's power for some outcome analyses was limited, as the number of HFmrEF cases was mod-est, and there were few participants with no CAC and higher epigenetic age. Thus, some of the more granular findings such as differential associations by HF subtype or in CAC strata should be interpreted with caution and need replication in larger samples.

CONCLUSIONS

This study strengthens the evidence that epigenetic aging measures are linked with cardiovascular health outcomes. GrimAge was associated with higher incidence of composite CVD and stroke independent of other cardiovascular risk factors, highlighting the potential of the epigenome as a clinically relevant biomarker. Our findings encourage future research to determine whether interventions that slow epigenetic aging can translate to reduced cardiovascular events.

ARTICLE INFORMATION

Received July 8, 2025; accepted October 15, 2025.

Affiliations

UNC Chapel Hill School of Medicine, Gillings School of Public Health, Chapel Hill, NC (S.S., N.R.); Department of Epidemiology, Gillings School of Global Public Health, University of North Carolina at Chapel Hill, Chapel Hill, NC (S.S., J.R.P., G.S.B.); Department of Epidemiology, School of Public Health, University of Michigan, Ann Arbor, MI (J.A.S.); Department of Medicine, Wake Forest Baptist Medical Center, Winston-Salem, NC (J.D.); Department of Medicine, Division of Cardiology, Duke University Medical Center, Durham, NC (Y.L.); and Department of Medicine, Lundquist Institute at Harbor-UCLA, Torrance, CA (M.B.).

Acknowledgments

The authors thank the other investigators, the staff, and the participants of the MESA study for their valuable contributions. A full list of participating MESA investigators and institutions can be found at . This article has been reviewed and approved by the MESA Publications and Presentations Committee.

Sources of Funding

This research was supported by contracts 75N92025D00022, 75N92020D00001, HHSN268201500003I, N01-HC-95159, 75N92025D00026, 75N92020D00005, N01-HC-95160, 75N92020D00002, N01-HC-95161, 75N92025D00024, 75N92020D00003, N01-HC-95162, 75N92025D00027, 75N92020D00006, N01-HC-95163, 75N92025D00025, 75N92020D00004, N01-HC-95164, 75N92025D00028, 75N92020D00007, N01-HC-95165, N01-HC-95166, N01-HC-95167, N01-HC-95168 and N01-HC-95169 from the National Heart, Lung, and Blood Institute, and by grants UL1-TR-000040, UL1-TR-001079, and UL1-TR-001420 from the National Center for Advancing Translational Sciences (NCATS). This publication was developed under the Science to Achieve Results (STAR) research assistance agreements, No. RD831697 (MESA Air) and RD--83830001 (MESA Air Next Stage), awarded by the U.S Environmental Protection Agency (EPA). It has not been for-mally reviewed by the EPA. The views expressed in this document are solely those of the authors and the EPA does not endorse any products or commercial services mentioned in this publication. The MESA Epigenomics & Transcriptomics Studies were funded by NIH grants R01HL101250, R01HL119962, R01DK101921, R01HL135009, and 1RF1AG054474.” The authors thank the other investiga-tors, the staff, and the participants of the MESA study for their valuable contribu-tions. A full list of participating MESA investigators and institutions can be found at . This paper has been reviewed and approved by the MESA Publications and Presentations Committee.

Disclosures

None.

Supplemental Material

Tables S1–S2
Figure S1

REFERENCES

1. Mensah GA, Fuster V, Murray CJL, Roth GA, Mensah GA, Abate YH, Abbasian M, Abd-Allah F, Abdollahi A, Abdollahi M, et al. Global burden of cardiovascular diseases and risks, 1990-2022. *J Am Coll Cardiol*.
5. Joyce BT, Gao T, Zheng Y, Ma J, Hwang SJ, Liu L, Nannini D, Horvath S, Lu AT, Bai Allen N, et al. Epigenetic age acceleration reflects long-term cardiovascular health. *Circ Res*. 2021;129:770–781. doi:
6. Bey GS. The identity vitality-pathology model: a novel theoretical framework proposing "identity state" as a modulator of the pathways from structural to health inequity. *Soc Sci Med*. 2022;314:115495. doi:
7. Lin L, Kiryakos J, Ammous F, Ratliff SM, Ware EB, Faul JD, Kardia SLR, Zhao W, Birditt KS, Smith JA. Epigenetic age acceleration is associated with blood lipid levels in a multi-ancestry sample of older U.S. adults. *BMC Med Genet*. 2024;17:17. doi:
8. Bild DE. Multi-ethnic study of atherosclerosis: objectives and design. *Am J Epidemiol*. 2002;156:871–881. doi:
9. Lu AT, Quach A, Wilson JG, Reiner AP, Aviv A, Raj K, Hou L, Baccarelli AA, Li Y, Stewart JD, et al. DNA methylation GrimAge strongly predicts lifespan and healthspan. *Aging*. 2019;11:303–327. doi:
10. Liu Y, Ding J, Reynolds LM, Lohman K, Register TC, de la Fuente A, Howard TD, Hawkins GA, Cui W, Morris J, et al. Methylomics of gene expression in human monocytes. *Hum Mol Genet*. 2013;22:5065–5074. doi:
11. Du P, Kibbe WA, Lin SM. lumi: a pipeline for processing Illumina microar- ray. *Bioinformatics*. 2008;24:1547–1548. doi:
12. Pidsley R, Y Wong CC, Volta M, et al. A data-driven approach to pre-processing Illumina 450K methylation array data. *BMC Genomics*. 2013;14:293.
13. Schmitz LL, Duffie E, Zhao W, Ratliff SM, Ding J, Liu Y, Merkin SS, Smith JA, Seeman T. Associations of early-life adversity with later-life epigenetic aging profiles in the multi-ethnic study of atherosclerosis. *Am J Epidemiol*. 2023;192:1991–2005. doi:
14. Ammous F, Zhao W, Ratliff SM, Mosley TH, Bielak LF, Zhou X, Peyser PA, Kardia SLR, Smith JA. Epigenetic age acceleration is associated with cardiometabolic risk factors and clinical cardiovascular disease risk scores in African Americans. *Clin Epigenetics*. 2021;13:55. doi:
15. De Vries PS, Zannas AS. Early life cardiovascular risk factors and midlife epigenetic aging. *JACC Basic Transl Sci*. 2024;9:591–592. doi:
16. Sheng C, Zhou R, Wang H, et al. Early-life smoking, cardiovascular disease risk, and the mediating role of DNA methylation biomarkers of aging. *J Transl Med*. 2025;23:484. doi:
17. Domingo-Relloso A, Riffo--Campos AL, Zhao N, Ayala G, Haack K, Manterola C, Rhoades DA, Umans JG, Fallin MD, Herreros-Martinez M, et al. Multicohort epigenome-wide association study of all-cause cardiovascular disease and cancer incidence. *JACC CardioOncology*. 2024;6:731–742. doi:
18. Simmonds SJ, Cuijpers I, Heymans S, Jones E a. V. Cellular and mo- lecular differences between HFpEF and HFrEF: a step ahead in an im- proved pathological understanding. *Cells*. 2020;9:242. doi:
19. Paulus WJ, Zile MR. From systemic inflammation to myocardial fibrosis. *Circ Res*. 2021;128:1451–1467. doi:
20. DeBerge M, Shah SJ, Wilsbacher L, Thorp EB. Macrophages in heart failure with reduced versus preserved ejection fraction. *Trends Mol*

Med. 2019;25:328–340. doi:
21. Borlaug BA, Sharma K, Shah SJ, Ho JE. Heart failure with preserved ejection fraction. *J Am Coll Cardiol*. 2023;81:1810–1834. doi:
2023;82:2350–2473. doi:
2. Sun X, Chen W, Razavi AC, Shi M, Pan Y, Li C, Argos M, Layden BT, Daviglus ML, He J, et al. Associations of epigenetic age accel-eration with CVD risks across the lifespan. *JACC Basic to Transl Sci*.
2024;9:577–590. doi:
3. Carbonneau M, Li Y, Prescott B, Liu C, Huan T, Joehanes R, et al. Epigenetic aging markers mediate the association of Life's es-sential 8 with cardiovascular disease and mortality. *Circulation*.

22. Budoff MJ. When is enough enough? Additional evidence of the power of zero. *Circ Cardiovasc Imaging*. 2025;18:e017714. doi: .
23. Blaha MJ, Cainzos-Achirica M, Greenland P, McEvoy JW, Blankstein R, Budoff MJ, Dardari Z, Sibley CT, Burke GL, Kronmal RA, et al. Role 2023;147:A13855.
4. Carbonneau M, Li Y, Prescott B, Liu C, Huan T, Joehanes R, Murabito JM, Heard-Costa NL, Xanthakis V, Levy D, et al. Epigenetic age mediates the association of Life's essential 8 with cardiovascular disease and mortality. *J Am Heart Assoc*. 2024;13:e032743. doi:
of coronary artery calcium score of zero and other negative risk markers for cardiovascular disease. *Circulation*. 2016;133:849–858. doi:

24. Budoff MJ, Young R, Burke G, Jeffrey Carr J, Detrano RC, Folsom AR, Kronmal R, Lima JAC, Liu KJ, McClelland RL, et al. Ten-year association of coronary artery calcium with atherosclerotic cardiovascular disease (ASCVD) events: the multi-ethnic study of atherosclerosis (MESA). *Eur Heart J*. 2018;39:2401–2408. doi: