ORIGINAL ARTICLE

# Prospective Study of Epigenetic Age Acceleration and Incidence of Cardiovascular Disease Outcomes in the ARIC Study (Atherosclerosis Risk in Communities)

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**BACKGROUND:** DNA methylation-based patterns of biological aging, known as epigenetic age acceleration, are predictive of all-cause mortality, but little is known about their association with cardiovascular disease (CVD).

**METHODS:** We estimated 2 versions of epigenetic age acceleration (Horvath and Hannum) using whole-blood samples from 2543 blacks. Linear and Cox proportional hazards regression, respectively, were used to assess the association of age acceleration with carotid intima–media thickness (cross-sectionally) and incident cardiovascular events, including CVD mortality, myocardial infarction, fatal coronary heart disease, peripheral arterial disease, and heart failure, during a median 21-year follow-up. All models were adjusted for chronological age and traditional CVD risk factors.

**RESULTS:** In comparison to chronological age, the 2 measures of epigenetic age acceleration were weaker, but independent, potential risk markers for subclinical atherosclerosis and most incident cardiovascular outcomes, including fatal coronary heart disease, peripheral arterial disease, and heart failure. For example, each 5-year increment of epigenetic age acceleration was associated with an average of 0.01 mm greater carotid intima–media thickness (each $P \leq 0.01$), and the hazard ratios (95% confidence intervals) of fatal coronary heart disease per 5-year increment in Horvath and Hannum age acceleration were 1.17 (1.02–1.33) and 1.22 (1.04–1.44), respectively.

**CONCLUSIONS:** In this sample of blacks, increased epigenetic age acceleration in whole blood was a potential risk marker for incident fatal coronary heart disease, peripheral arterial disease, and heart failure independently of chronological age and traditional CVD risk factors. DNA methylation–based measures of biological aging may help to identify new pathophysiological mechanisms contributing to the development of CVD.

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## Clinical Perspective

DNA methylation, an epigenetic mechanism that leads to changes in gene expression, is both heritable and impacted by aging and various environmental exposures. Levels of DNA methylation at specific locations around the genome can be used to estimate a marker of biological aging called epigenetic age acceleration, which is known to be associated with some risk factors for poor health and all-cause mortality. However, little is known about whether epigenetic age acceleration is related to the development of atherosclerosis or cardiovascular disease. We used DNA methylation measured in whole blood to estimate epigenetic age acceleration in a sample of 2543 middle to older aged blacks from the general population. Greater epigenetic age acceleration was positively associated with carotid intima–media thickness, a measure of subclinical atherosclerosis, and was potentially modestly associated with increased incidence of various cardiovascular disease outcomes, including fatal coronary heart disease, peripheral artery disease, and heart failure, after adjusting for chronological age and many traditional cardiovascular risk factors. Furthermore, DNA methylation at one site in particular (near *GALR1*, chr 18) was significantly associated with risk of fatal coronary heart disease, taking multiple testing into account, and additionally associated with all other cardiovascular outcomes at nominal significance. This association needs to be replicated in another study but could represent a marker of future cardiovascular disease risk. Our study illustrates how DNA methylation–based measures such as epigenetic age acceleration may help to identify new mechanisms contributing to the pathogenesis of cardiovascular disease.

Epigenetic processes involve heritable changes in gene expression that occur without change to the underlying DNA sequence. A widely studied epigenetic mechanism is DNA methylation, the process by which methyl groups are added or removed from the DNA sequence, usually at cytosine–guanine dinucleotides (CpGs). DNA methylation patterns change over the life course in response to genetic and environmental stimuli, including diet, smoking, alcohol, physical activity, obesity, and stress.[1–8] Methylation at specific CpG sites may reflect cumulative exposure to major risk factors involved in the pathogenesis of complex, aging-related conditions such as cardiovascular disease (CVD).

Recent studies have reported sets of CpGs that can be used to predict chronological age.[9,10] Hannum et al[9] developed a model based on 71 CpG sites using DNA from whole-blood samples, whereas Horvath[10] developed a similar model based on 353 CpG sites using DNA from samples of 51 healthy tissue and cell types. Having a predicted age (epigenetic age) greater than one's chronological age is often referred to as epigenetic age acceleration and has been shown to be associated prospectively with higher risk of all-cause mortality[11–15] and cross-sectionally with obesity,[16] earlier menopause,[17] and frailty.[18]

One study of postmenopausal women found no association between epigenetic age acceleration and risk of coronary heart disease (CHD),[19] but little else is known about whether epigenetic age acceleration is associated with subclinical atherosclerosis or incidence of various CVD outcomes. Therefore, we explored the relationship of epigenetic age acceleration cross-sectionally with CVD risk factors and carotid intima–media thickness (IMT) and prospectively with risk of CVD mortality, myocardial infarction (MI), fatal CHD, heart failure (HF), and peripheral arterial disease (PAD) in blacks from the ARIC study (Atherosclerosis Risk in Communities).

## METHODS

The DNA methylation data from ARIC are available on request at https://www2.cscc.unc.edu/aric/distribution-agreements.

### Study Participants

The ARIC study began in 1987 to 1989 with the enrollment and baseline examination (visit 1) of 15 792 men and women aged 45 to 64 years from 4 US communities (Forsyth County, NC; Jackson, MS; suburban Minneapolis, MN; and Washington County, MD).[20] Five additional examinations (visit 2: 1990–1992, visit 3: 1993–1995, visit 4: 1996–1998, visit 5: 2011–2013, and visit 6: 2016-2017) and annual phone contact have been completed during the follow-up. At the time of this analysis, methylation data had been collected on only a subset of the cohort restricted to black participants from Jackson, MS, and Forsyth County, NC. DNA methylation was measured from blood drawn at ARIC visits 2 or 3. DNA methylation was measured if the participant had not restricted use of his or her DNA, if there was at least 1 µg of DNA available for the array, and if there was genome-wide genotyping data available. Methods were approved by the institutional review board at each study center, and written informed consent was obtained from participants. No individual participant data are reported.

### Epigenetic Age

DNA was extracted from whole-blood white cells using the Gentra Puregene Blood Kit (Qiagen). One microgram of DNA underwent bisulphite conversion using the deep-well EZ-96 DNA Methylation Kit (Zymo Research); conversion efficiency was determined by polymerase chain reaction amplification using the Universal Methylated Human DNA Standard (Zymo Research). Methylation status was measured using the Illumina Infinium HumanMethylation450 BeadChip

array (Illumina, Inc, San Diego, CA).[21,22] Degree of methylation was determined using Illumina GenomeStudio 2011.1, Methylation module 1.9.0 software. The methylation score for each CpG was represented as a β value calculated by dividing the fluorescence intensity of the methylated bead type by the sum of the intensities of the methylated and unmethylated bead types. Background subtraction was conducted with the GenomeStudio software using built-in negative control bead types on the array. An average normalization was applied to minimize scanner-to-scanner variation. Then, we used the online calculator by Horvath[10] to perform additional normalization and imputation for missing β values and to estimate each of the Horvath and Hannum et al[9] versions of epigenetic age. Because heterogeneity in the composition of blood leukocyte cell types can confound relationships between DNA methylation and disease outcomes, we also used the online calculator to obtain cell type abundance measures as estimated from methylation data.[10,23]

## Outcomes and Prevalent Disease

Carotid IMT was measured at the time of blood draw (visit 2 or 3) using high-resolution B-mode ultrasound by taking the mean of the means of up to 11 measurements of the far wall across 6 different segments: the distal common carotid artery, carotid artery bifurcation, and the proximal internal carotid artery of the left and right sides.[24,25] Missing segment values were imputed using race- and sex-specific linear models in which mean IMT was regressed on age, body mass index (BMI), and arterial depth, as previously described.[26] Incident cardiovascular events occurring between the time of the blood draw (either visit 2 or 3) and December 31, 2013, were identified during annual phone contact with participants (or proxy) and by surveillance of local hospital discharge records and death records. Hospital records were abstracted for events involving cardiovascular diagnoses. Incident CHD was validated by physician review as definite or probable MI or definite fatal CHD using ARIC criteria.[27] Incident PAD was defined as having an ankle/brachial index <0.90 at visit 3 or 4 or a hospital discharge diagnosis with *International Classification of Disease*, *Ninth Revision* (*ICD-9*) codes consistent with PAD, leg amputation, or leg revascularization procedures (codes 440.21, 440.22, 440.23, 440.24, 443.9, 785.4, 84.11, 84.12, 84.15, 84.17, 38.18, 39.25, 39.29, 39.50, 39.90, and 00.55). Incident HF was defined by a hospital discharge diagnosis with *ICD-9* code 428 or death with an underlying cause of *ICD-9* code 428 or *ICD-10* code I50.

History of CVD was ascertained using the same criteria as for identifying incident events if the event occurred after visit 1 and before the time of blood draw at visit 2 or 3. Additional cases of prevalent disease were identified using information from visits 1 or 2, including electrocardiograms, Gothenburg HF[28] and Rose questionnaires,[29] and self-reported history (including having undergone coronary revascularization).

## Risk Factors

Unless otherwise noted, covariate measurements were taken from visit 2 or 3, in accordance with the visit from which blood was collected for DNA extraction and measurement of DNA methylation. Educational attainment was ascertained at visit 1 and divided into 3 categories: less than high school, high school or vocational school, and more than high school (attended or completed college or graduate school). Self-reported health behaviors included smoking and drinking status (never, former, or current), pack-years of smoking, alcohol intake, and sport index (a score of leisure time sport activity ranging from 1 to 5 and measured at visits 1 and 3, as previously described[30]). BMI was calculated as weight in kilograms divided by height in meters squared. Diabetes mellitus was defined as fasting blood glucose ≥7 mmol/L (≥126 mg/dL), nonfasting blood glucose ≥11.1 mmol/L (≥200 mg/dL), a self-reported physician's diagnosis of diabetes mellitus, or reported use of a diabetes mellitus medication. Seated blood pressure was measured 3 times after 5 minutes of rest using a random-zero sphygmomanometer, and the last 2 measurements were averaged. Participants were asked to bring all medications with them to each visit. A prescription bottle or self-report was used to determine blood pressure or lipid-lowering medication use. Standard enzymatic methods were used to measure plasma total cholesterol and high-density lipoprotein cholesterol.[31,32] Using visit 2 serum samples, high-sensitivity C-reactive protein (CRP) was measured using an immunoturbidimetric assay on the Roche Modular P chemistry analyzer (Roche Diagnostics), and creatinine was measured using the Jaffe method. Cystatin C was also measured using the Roche Modular P chemistry analyzer. Glomerular filtration rate (eGFR) was estimated using a combined creatinine–cystatin C algorithm.[33] At visit 1 and during annual phone contacts thereafter, participants self-rated the perception of their health compared with others of the same age (reported as excellent, good, fair, or poor); we used the perceived health status reported most recently before the time of blood draw.

## Statistical Analysis

DNA methylation data from visit 2 or 3 was available for 2914 blacks for this analysis. After excluding those whose DNA methylation data did not pass quality control steps (failed bisulfite conversion, samples with a pass rate <95%, or possible sex mismatches based on evaluation of X or Y chromosome CpG sites; n=144), without follow-up beyond visit 2 (n=10), and with missing covariate values (n=217), there was a maximum of 2543 black study participants eligible for analysis (n=2203 from visit 2 and n=340 from visit 3). For cross-sectional analyses involving carotid IMT, those with a history of CHD or stroke at the time of blood draw were excluded. For each analysis involving incident cardiovascular outcomes, participants with a history of the respective outcome at the time of blood draw (ie, prevalent cases) were excluded. As such, sample sizes vary slightly across analyses.

Two versions of epigenetic age acceleration (Horvath and Hannum) were estimated, each being defined as the residual resulting from regressing epigenetic age on chronological age in a linear model. Descriptive statistics were calculated for all CVD risk factors. We assessed the relationship of each risk factor with each version of epigenetic age acceleration separately using a multivariable linear model in which epigenetic age acceleration was regressed on all the covariates simultaneously, as has been done previously.[19]

The associations between each of the age acceleration measures, as well as chronological age (for comparative

purposes), and carotid IMT were assessed in separate multivariable linear models. Cox proportional hazards proportional hazards regression was used to estimate adjusted hazard ratios (HR) and 95% confidence intervals (CI) for the association of age acceleration and chronological age with incident MI, fatal CHD, HF, and PAD. The proportional hazards assumption was tested by including log-time and age acceleration interactions in the regression models. Models for the HF outcome were stratified by earlier (first 10 years) and later (remaining 10+ years) follow-up time because of violation of the proportional hazards assumption in the epigenetic age acceleration models (proportionality test $P<0.03$ in the time-unstratified models). To examine the possibility of a nonlinear relationship between the aging measures and carotid IMT or the incident cardiovascular outcomes, we initially modeled the aging measures using restricted cubic splines, with knots at the 5th, 50th, and 95th percentiles of the distributions. In exploratory analyses, we assessed for interaction by sex using sex-stratified models and models including a cross-product term between age acceleration and sex.

Initial model 1 adjustment was made for chronological age (except in the chronological age models), sex, estimated leukocyte cell type abundances (CD8 T cells, CD4 T cells, natural killer cells, B cells, monocytes, and granulocytes), visit of blood draw, and a random effect for plate number of the methylation array. Model 2 added educational attainment, BMI, and health behaviors, including smoking (status and pack-years), alcohol (use and weekly intake), sports index, diabetes mellitus, systolic blood pressure, use of antihypertensive medications, total cholesterol, high-density lipoprotein cholesterol, use of cholesterol-lowering medications, and self-rated health status. We also ran 2 sets of sensitivity analyses, including separate models additionally adjusting for CRP and eGFR among the visit 2 subgroup and separate models only among those who were never smokers at the time of blood draw.

The associations between each of the individual 353 Horvath and 71 Hannum CpG probes (a total of 418 unique probes) and incident cardiovascular outcomes were also assessed in separate Cox proportional hazards regression models, using model 2 covariate adjustment. Given the 418 probes and 6 outcomes, this represents 2508 models, and so we accounted for the multiple testing by specifying a Bonferroni threshold for statistical significance ($P<1.99E{-}05$).

## RESULTS

Baseline characteristics of the 2543 black participants included in this analysis are shown in Table 1. Participants had a mean age of 57 years, 65% were women, and 60% had attained a high-school education or greater. As shown in Figure I in the Data Supplement, each of the epigenetic age acceleration measures were uncorrelated (Pearson $r=0$) with chronological age. The correlation between the Horvath and Hannum age acceleration measures was $r=0.60$. The lower and upper quartiles of the epigenetic age acceleration measures were –4.6 and 4.6 years (Horvath) and –4.2 and 4.0 years (Hannum), respectively.

Also shown in Table 1 are the multivariable, cross-sectional associations between baseline risk factors and the epigenetic age acceleration measures. Many of the risk factors shared the same direction of association with the 2 versions of epigenetic age acceleration: for example, age acceleration was on average 0.7 to 1.5 years lower in women compared with men, 1.7 years higher in current smokers compared with never smokers, and 1 year higher in those with diabetes mellitus compared with those without diabetes mellitus. Associations also trended in the expected direction for other risk factors: on average, those with lower levels of educational attainment, sports index, and high-density lipoprotein cholesterol and with higher BMI had higher epigenetic age acceleration. In similar multivariable models additionally including CRP and eGFR (visit 2 subgroup only), CRP was positively associated (estimates per 1 mg/L increment: 0.04 [SE=0.02; $P=0.06$] and 0.06 [SE=0.02; $P<0.001$]), and eGFR was negatively associated (estimates per 1 mL/min per 1.73 $m^2$ increment: –0.02 [SE=0.01; $P=0.03$] and –0.02 [SE=0.01; $P=0.02$]) with the Horvath and Hannum versions of epigenetic age acceleration, respectively (full models not shown).

In exploratory analyses, when modeled using restricted cubic splines, the 2 versions of epigenetic age acceleration and chronological age showed approximately linear-shaped, positive associations with carotid IMT in minimally adjusted models (Figure). Each 5-year increment in Horvath and Hannum age acceleration was associated with an average of 0.010- to 0.014-mm higher carotid IMT (Table 2, model 1), and these associations persisted (0.008–0.01-mm higher carotid IMT per 5-year increment in age acceleration) after additionally adjusting for education, BMI, health behaviors, and clinical risk factors (model 2). Compared with epigenetic age acceleration, the association of chronological age with carotid IMT was stronger in every model (0.033–0.043-mm higher carotid IMT per 5-year increment in chronological age in fully adjusted models).

During a median of 21-years follow-up, we identified 410 CVD deaths, 238 incident cases of MI, 144 CHD deaths, 310 incident cases of PAD, and 563 incident HF events, with 204 of the HF events occurring in the first 10-years follow-up and the 359 other cases occurring in the 10+ remaining years. The log HRs of CVD mortality, incident MI, fatal CHD, PAD, and HF (only in the first 10-year follow-up) increased in a linear fashion across the distribution of Hannum epigenetic age acceleration in minimally adjusted models (Figure II in the Data Supplement). The Horvath age acceleration measure showed similar, but slightly weaker, associations with CVD mortality, fatal CHD, PAD, and HF (in earlier follow-up) and was not associated with MI. Chronological age was positively associated with each of the incident cardiovascular outcomes.

**Table 1. Participant Characteristics and Their Cross-Sectional, Multivariable Associations With Epigenetic Age Acceleration, ARIC Blacks (n=2543), Visits 2 and 3, 1990–1995**

| Characteristic | Mean (SD) or % | Multivariable Associations With Epigenetic Age Acceleration* | | | |
|---|---|---|---|---|---|
| | | Horvath | | Hannum | |
| | | Estimate (SE) | *P* Value | Estimate (SE) | *P* Value |
| Age, y | 57 (6) | −0.02 (0.02) | 0.37 | −0.07 (0.02) | <0.001 |
| Female sex | 65 | −0.66 (0.33) | 0.05 | −1.46 (0.28) | <0.001 |
| Educational attainment | | | | | |
| Less than high school | 40 | 0.48 (0.33) | 0.15 | 0.91 (0.27) | <0.001 |
| High school | 28 | 0.13 (0.33) | 0.70 | 0.52 (0.28) | 0.06 |
| College or more | 32 | (Ref.) | … | (Ref.) | … |
| Smoking status | | | | | |
| Current | 24 | 1.70 (0.41) | <0.001 | 1.71 (0.34) | <0.001 |
| Former | 30 | 0.32 (0.34) | 0.35 | −0.46 (0.28) | 0.10 |
| Never | 46 | (Ref.) | … | (Ref.) | … |
| Pack-years† | 11 (19) | 0.003 (0.008) | 0.73 | 0.01 (0.007) | 0.09 |
| Drinking status | | | | | |
| Current | 32 | −0.06 (0.36) | 0.87 | 0.46 (0.30) | 0.12 |
| Former | 32 | −0.05 (0.33) | 0.88 | −0.11 (0.27) | 0.70 |
| Never | 36 | (Ref.) | … | (Ref.) | … |
| Alcohol intake, g/wk | 26 (99) | 0.0001 (0.001) | 0.95 | 0.0004 (0.001) | 0.72 |
| Sports activity index (1–5)‡ | 2.2 (0.7) | −0.24 (0.19) | 0.21 | −0.45 (0.16) | 0.005 |
| Body mass index, kg/m$^2$ | 30 (6) | 0.06 (0.02) | 0.008 | 0.04 (0.02) | 0.06 |
| Diabetes mellitus | 27 | 1.06 (0.31) | <0.001 | 1.01 (0.26) | <0.001 |
| Systolic blood pressure, mm Hg | 127 (20) | −0.0001 (0.007) | 0.99 | −0.002 (0.005) | 0.72 |
| Use of antihypertensives | 49 | 0.15 (0.27) | 0.60 | 0.47 (0.23) | 0.04 |
| Total cholesterol, mmol/L | 5.4 (1.1) | −0.15 (0.12) | 0.21 | −0.03 (0.10) | 0.77 |
| HDL cholesterol, mmol/L | 1.4 (0.4) | −0.64 (0.31) | 0.04 | −0.47 (0.26) | 0.07 |
| Use of antihyperlipidemics | 4 | 0.48 (0.67) | 0.47 | −0.23 (0.56) | 0.68 |
| Self-rated health status | | | | | |
| Poor | 6 | −0.54 (0.62) | 0.62 | 0.53 (0.51) | 0.30 |
| Fair | 28 | 0.49 (0.41) | 0.23 | 0.83 (0.34) | 0.01 |
| Good | 49 | 0.01 (0.36) | 0.98 | 0.11 (0.30) | 0.71 |
| Excellent | 17 | (Ref.) | … | (Ref.) | … |

ARIC indicates Atherosclerosis Risk in Communities; and HDL, high-density lipoprotein.

*Epigenetic age acceleration (separately by each version) is regressed on all of the listed covariates as well as visit of blood draw, estimated leukocyte cell type abundances, and a random effect for plate number of the array in a multivariable linear model. Effect estimates can be interpreted as differences in years of age acceleration.

†Measured at visit 1 (ie, before the time of blood draw) in all participants.

‡Measured at visit 1 (ie, before the time of blood draw) in most participants.

For the 2 versions of epigenetic age acceleration, associations were strongest for the fatal CHD outcome, with minimally adjusted (model 1) HRs (95% CI) of 1.27 (1.11–1.45) and 1.39 (1.20–1.62) per 5-year increment in Horvath and Hannum age acceleration, respectively (Table 3). After additional adjustment for education, BMI, health behaviors, and clinical risk factors (model 2), the epigenetic age acceleration measures remained nominally associated with fatal CHD (HRs [95% CI], 1.17 [1.02–1.33] and 1.22 [1.04–1.44], respectively). Age acceleration was also nominally associated with the PAD, earlier follow-up HF, and MI (Hannum only) outcomes, with HRs per 5-year increment in age acceleration of 1.09 (Horvath) and 1.12 to 1.14 (Hannum) in fully adjusted models (model 2). Although each 5-year increment in epigenetic age acceleration was associated with CVD mortality in model 1 (HR [95% CI], 1.13 [1.04–1.22] for Horvath and 1.16 [1.06–1.26] for Hannum), these associations were largely attenuated after model 2 adjustment (HR [95% CI], 1.06 [0.98–1.14] and 1.04 [0.94–1.14], respectively). Neither measure of epigenetic age acceleration was associated with HF in the

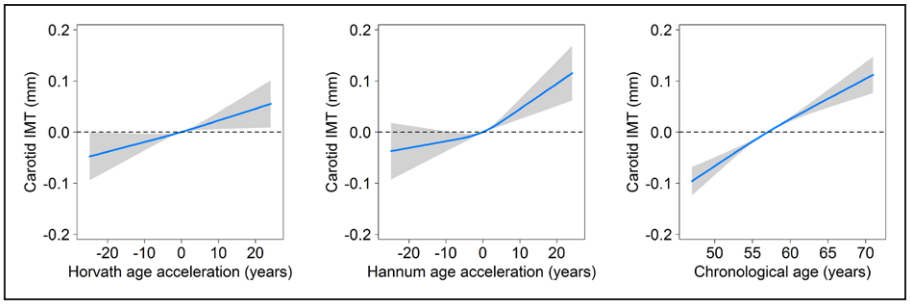


**Figure. Estimate and 95% confidence interval of the cross-sectional association of carotid intima-media thickness (IMT) in relation to epigenetic age acceleration and chronological age (modeled using restricted cubic splines with knots at the 5th, 50th, and 95th percentiles of the age measure distributions), adjusted for chronological age (except for the chronological age model), sex, visit of blood draw, and estimated leukocyte cell–type abundances, ARIC study (Atherosclerosis Risk in Communities) blacks, 1990–1995**.

later period of follow-up. By comparison, chronological age was more strongly associated with every cardiovascular outcome, with HRs per 5-year increment ranging from 1.15 for MI to 1.45 for CVD mortality in fully adjusted models. In models additionally adjusted for a quadratic age term, associations between age acceleration and cardiovascular outcomes were unchanged (results not shown).

Associations of epigenetic age acceleration with cardiovascular outcomes were generally stronger in women compared with men, although tests for interaction by sex were not statistically significant (Table I in the Data Supplement). In sensitivity analyses limited to the visit 2 subgroup, associations between epigenetic age acceleration and the cardiovascular outcomes were only modestly attenuated in models additionally adjusted for CRP and eGFR (Table II in the Data Supplement). Compared with the full sample results (Table 3), in sensitivity models limited to never smokers, associations between the aging measures and cardiovascular outcomes were similar overall but were stronger for CVD mortality and fatal CHD and weaker for PAD and early follow-up HF (Table III in the Data Supplement).

We also checked whether the 418 individual CpG probes used for estimating epigenetic age acceleration were associated with the incident cardiovascular outcomes using separate models. In fully adjusted models (model 2), 2 probes were associated with a cardiovascular event at the Bonferroni-corrected threshold (Data Supplement). Each SD increment in the Horvath probes cg20914508 (chr 3) and cg10486998 (chr 18) was associated with 1.37 (95% CI, 1.19–1.59) and 1.40 (95% CI, 1.20–1.64) times greater risk of MI and fatal CHD, respectively. Beyond fatal CHD, each SD increment of the Horvath cg10486998 probe was nominally associated ($P$<0.05) with all of the other incident cardiovascular outcomes (HRs, 1.15 [CVD mortality], 1.29 [MI], 1.20 [PAD], 1.17 [earlier follow-up HF], and 1.18 [later follow-up HF]) in fully adjusted models (model 2).

## DISCUSSION

In this population-based study of middle-aged blacks, we used whole-blood DNA methylation patterns to estimate 2 versions of epigenetic age acceleration, a marker of biological aging. Cross-sectionally, the epigenetic age acceleration measures and chronological age were positively associated with carotid IMT. Chronological age was a robust marker for future risk of CVD mortality, MI, fatal CHD, PAD, and HF (1.15–1.45 times greater incidence per 5 years of age acceleration); comparatively, the epigenetic age acceleration

**Table 2. Estimates and 95% CI From Linear Regression Models of the Cross-Sectional Difference in Carotid IMT (in mm) Per 5-y Increment in Epigenetic Age Acceleration and Chronological Age, ARIC Blacks, 1990–1995**

| | Horvath Age Acceleration | | Hannum Age Acceleration | | Chronological Age | |
|---|---|---|---|---|---|---|
| | Estimate (95% CI) | *P* Value | Estimate (95% CI) | *P* Value | Estimate (95% CI) | *P* Value |
| Model 1 | 0.010 (0.005–0.016) | <0.001 | 0.014 (0.008–0.021) | <0.001 | 0.043 (0.037–0.050) | <0.001 |
| Model 2 | 0.008 (0.002–0.013) | 0.004 | 0.010 (0.004–0.017) | 0.002 | 0.033 (0.026–0.040) | <0.001 |

Model 1: adjusted for chronological age (except for the chronological age models), sex, visit of blood draw, cell type proportion estimates, and plate number (random effect). Model 2: model 1+education, smoking (status and pack-years), drinking (status and weekly intake), sports index, body mass index, diabetes mellitus, systolic blood pressure, use of antihypertensives, total cholesterol, HDL cholesterol, use of antihyperlipidemics, and self-rated health compared with others. ARIC indicates Atherosclerosis Risk in Communities; CI, confidence interval; HDL, high-density lipoprotein; and IMT, intima–media thickness.

**Table 3. HR and 95% CI of Incident CVD Events Per 5-Y Increment in Epigenetic Age Acceleration and Chronological Age, ARIC Blacks, 1990–2013**

| Outcome | HR (95% CI) Per 5-y Increment in Each Age Measure | | |
|---|---|---|---|
| | Horvath Age Acceleration | Hannum Age Acceleration | Chronological Age |
| CVD mortality | No. of events: 410 | Person-years: 45 679 | |
| Model 1 | 1.13 (1.04–1.22) | 1.16 (1.06–1.26) | 1.62 (1.48–1.77) |
| Model 2 | 1.06 (0.98–1.14) | 1.04 (0.94–1.14) | 1.45 (1.32–1.60) |
| Myocardial infarction | No. of events: 238 | Person-years: 41 946 | |
| Model 1 | 1.02 (0.92–1.13) | 1.20 (1.07–1.34) | 1.24 (1.11–1.40) |
| Model 2 | 0.97 (0.87–1.07) | 1.12 (1.00–1.26) | 1.15 (1.01–1.30) |
| Fatal CHD | No. of events: 144 | Person-years: 43 178 | |
| Model 1 | 1.27 (1.11–1.45) | 1.39 (1.20–1.62) | 1.47 (1.27–1.70) |
| Model 2 | 1.17 (1.02–1.33) | 1.22 (1.04–1.44) | 1.35 (1.15–1.59) |
| PAD | No. of events: 310 | Person-years: 41 288 | |
| Model 1 | 1.17 (1.07–1.28) | 1.26 (1.14–1.40) | 1.38 (1.25–1.53) |
| Model 2 | 1.09 (0.99–1.19) | 1.13 (1.01–1.25) | 1.22 (1.10–1.36) |
| Heart failure (first 10 y) | No. of events: 204 | Person-years: 21 323 | |
| Model 1 | 1.19 (1.07–1.33) | 1.30 (1.15–1.47) | 1.59 (1.41–1.81) |
| Model 2 | 1.09 (0.97–1.22) | 1.14 (1.00–1.31) | 1.40 (1.22–1.61) |
| Heart failure (second 10+ y) | No. of events: 359 | Person-years: 18 915 | |
| Model 1 | 0.99 (0.92–1.08) | 1.12 (1.01–1.23) | 1.49 (1.36–1.64) |
| Model 2 | 0.94 (0.86–1.02) | 1.02 (0.92–1.12) | 1.38 (1.25–1.53) |

Model 1: Adjusted for chronological age (except for the chronological age models), sex, visit of blood draw, cell type proportion estimates, and plate number (random effect). Model 2: model 1+education, smoking (status and pack-years), drinking (status and weekly intake), sports index, body mass index, diabetes mellitus, systolic blood pressure, use of antihypertensives, total cholesterol, HDL cholesterol, use of antihyperlipidemics, and self-rated health compared with others. ARIC indicates Atherosclerosis Risk in Communities; CHD, coronary heart disease; CI, confidence interval; CVD, cardiovascular disease; HDL, high-density lipoprotein; HR, hazard ratio; and PAD, peripheral artery disease.



markers were only potentially modestly associated with most of the cardiovascular outcomes (1.09–1.22 times greater incidence per 5 years of age acceleration). These results show that blood-based epigenetic patterns of biological aging could be a potential risk marker for subclinical and clinical atherosclerotic CVD and HF outcomes independently of chronological age and traditional CVD risk factors.

The 2 versions of epigenetic age acceleration generally showed consistent associations with numerous CVD risk factors. Two previous studies involving multiethnic populations (black, non-Hispanic white, and Hispanic) assessed the associations of CVD risk factors with epigenetic age acceleration in multivariable models.[19,34] Consistent with our findings, these studies reported that female sex and lower BMI were associated with lower epigenetic age acceleration.[19,34] Also consistent with our findings, the previous studies reported that lower CRP and creatinine and higher education attainment and physical activity were associated with lower epigenetic age acceleration, but only for the Hannum version.[19,34] However, in ARIC blacks, we additionally found that current smoking and diabetes mellitus were each positively associated with both versions of epigenetic age acceleration after adjustment for other risk factors, which was generally not the case in the previous studies.[19,34] One study found that higher fasting insulin and glucose were marginally associated with higher epigenetic age acceleration, but the associations were attenuated in covariate-adjusted models.[34]

This is the first study to report on the association of epigenetic age acceleration and the risk of incident PAD and HF and adds to a growing body of literature describing the relationship between epigenetic age acceleration and incidence of aging-related diseases.[11,13,19,35–37] Similar to the findings in our study, 2 previous studies by Perna et al[12] and Dugué et al[15] generally found little evidence of an association between either age acceleration measure and risk of CVD mortality in fully adjusted models (HRs [95% CIs] per 5-year increment in age acceleration: 1.19 [0.98–1.43] and 1.00 [0.90–1.12] for Horvath; 1.00 [0.79–1.29] and 1.08 [0.97–1.21] for Hannum, respectively).

In our study, associations with incident cardiovascular outcomes were generally consistent between the 2 versions of epigenetic age acceleration, except were slightly stronger using the Hannum version, in agreement with what has been reported previously for other outcomes

such as all-cause mortality,[11,14] prevalent stroke,[38] and burden of cerebral white matter hyperintensities.[39] The difference in the strength of association may possibly be explained by the fact that the Hannum version of epigenetic age was developed using whole-blood samples and thus may be optimized for this tissue type.

Although epigenetic age acceleration was associated with subclinical atherosclerosis and CVD risk independently of chronological age and other traditional risk factors, it is important to note that chronological age was a stronger risk marker for CVD incidence compared with epigenetic age acceleration. Thus, as with most recent candidate biomarkers, it is unlikely that epigenetic age acceleration would add much incremental value in predicting future risk of CVD beyond the variables already included in established CVD risk models. Nevertheless, because DNA methylation is affected by environmental exposures, DNA methylation–based measures of biological aging may still be useful for identifying new pathophysiological mechanisms contributing to the development of CVD.[40]

To narrow the focus to specific pathophysiologic mechanisms and possibly identify potential future targets for intervention, we also looked at the CpG level to see whether any individual probes used to estimate age acceleration are important markers specifically of CVD risk. Of the 2 probes identified using a Bonferroni-corrected threshold, the Horvath cg10486998 probe was nominally associated with every outcome investigated in fully adjusted models and hence may represent a global marker for future risk of CVD. However, this finding should be validated in another study. The cg10486998 probe is located in a CpG island on chromosome 18 and is within 1500 bp upstream of the transcription start site of *GALR1* (galanin receptor 1). There is some evidence in animals that *GALR1* has cardiac involvement: previous studies have found *GALR1* to be expressed in the heart tissue of guinea pigs and rats,[41,42] and *GALR1* expression in the heart was significantly decreased in rats on exposure to stress.[42]

Both epigenetic age acceleration measures seemed to be stronger risk markers in women than in men, especially for the fatal CHD outcome. Previous meta-analyses have reported that relative risks for traditional risk factors such as diabetes mellitus and smoking are larger in women than in men.[43–45] Diabetes mellitus and smoking were strongly associated with each of the epigenetic age acceleration measures, which may in part explain the possible sex differences. Nevertheless, the sex-stratified models examining the relationship between epigenetic age acceleration and CVD were adjusted for diabetes mellitus and smoking, so other sex differences in CVD risk profiles may also be responsible for the discrepancy. In contrast to our findings, a previous investigation in women found no association between epigenetic age acceleration and CHD incidence.[19] The divergence of results may be explained by a few differences between the studies. The previous study included black, non-Hispanic white, and Hispanic women who were on average slightly older (mean age: 63 versus 57 years) and less likely to be smokers (10% versus 19% prevalence) compared with the black women in the present analysis.[19,34] Additionally, the previous study included angina and revascularization in their CHD definition,[19] whereas in this investigation, we used only hard CHD end points.

Strengths of this analysis include the relatively large sample size and number of cardiovascular events, as well as the availability of carotid IMT, a subclinical marker of atherosclerosis, and many CVD risk factors that may act as confounding variables. Limitations of this study include not having repeated measures of methylation, which may be better at capturing biological aging-related changes, and only having data for a single racial/ethnic group. Another potential limitation is the absence of directly measured white blood cell–type proportion estimates except in a small subset of participants. Because we estimated white blood cell–type abundances using methylation data, there may be some misclassification and hence the possibility of residual confounding. Although our models were adjusted for numerous CVD risk factors, it is possible that methylation levels were impacted by the presence of subclinical atherosclerosis at baseline, meaning we cannot entirely rule out the possibility of reverse causation. Finally, given that we conducted multiple testing for our primary analysis (2 epigenetic age acceleration measures and multiple cardiovascular outcomes), our results should be interpreted cautiously and require further replication in other study populations.

In conclusion, in this sample of blacks from the general population, higher epigenetic age acceleration as measured in whole blood was a potential risk marker for incident fatal CHD, PAD, and HF independently of chronological age and other traditional CVD risk factors. DNA methylation–based measures of biological aging may help to identify new pathophysiological mechanisms contributing to the development of CVD. Future work should explore whether epigenetic age acceleration can be modified, and whether slowing or reversal of epigenetic age acceleration confers a reduced risk for CVD and mortality. In particular, smoking and diabetes mellitus, and possibly also physical activity and adiposity, should be further investigated as potential modulators of epigenetic age acceleration.

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## DISCLOSURES

None.

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## FOOTNOTES

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