JACC: BASIC TO TRANSLATIONAL SCIENCE VOL. 9, NO. 5, 2024
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ORIGINAL RESEARCH - CLINICAL

Associations of Epigenetic Age Acceleration With CVD Risks Across the Lifespan

The Bogalusa Heart Study

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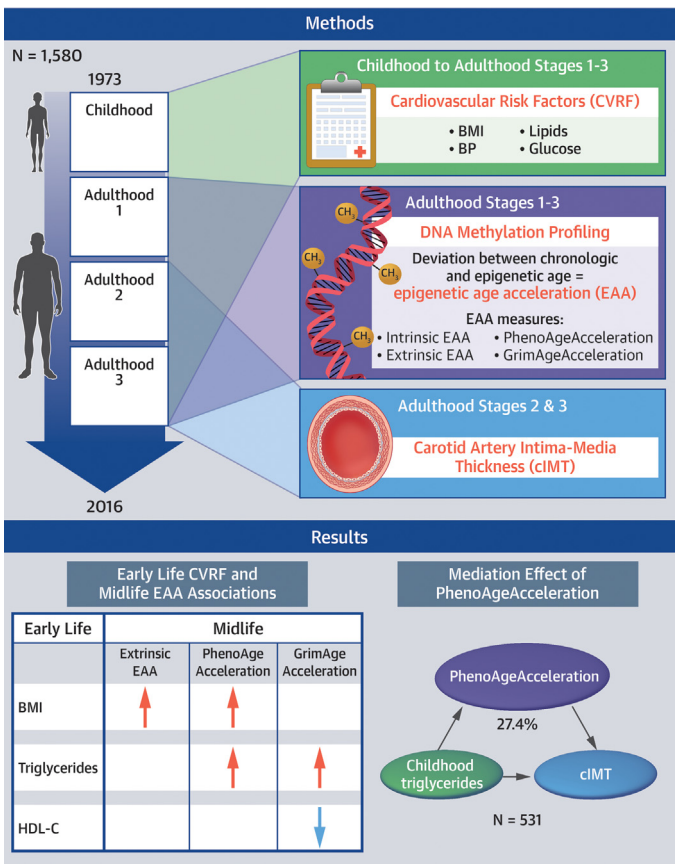
VISUAL ABSTRACT



Sun X, et al. J Am Coll Cardiol Basic Trans Science. 2024;9(5):577-590.

HIGHLIGHTS

- **Prospective associations between individual CVD risk factors in early life and EAA in midlife suggest that childhood BMI and TGs may affect adulthood EAA, pointing to potential precision strategies to decelerate the biological aging process.**
- **Simultaneously measured CVD risk factors and EAA provide temporal evidence that CVD risk factors such as BMI, TGs, and HDL-C act as upstream determinants rather than consequences of EAA.**
- **The mediating effect of EAA in the association between childhood CVD risk factors and subclinical atherosclerosis implicates EAA as a potential molecular link between early life CVD risk and the development of subclinical disease in adulthood.**

ISSN 2452-302X https://doi.org/10.1016/j.jacbts.2024.01.018

JACC: BASIC TO TRANSLATIONAL SCIENCE VOL. 9, NO. 5, 2024
MAY 2024:577–590

ABBREVIATIONS AND ACRONYMS

AUC = area under the curve

BMI = body mass index

BP = blood pressure

cIMT = carotid intima-media thickness

CVD = cardiovascular disease

CVH = cardiovascular health

DBP = diastolic blood pressure

DNAm = DNA methylation

EAA = epigenetic age acceleration

EEAA = extrinsic epigenetic age acceleration

HDL-C = high-density lipoprotein cholesterol

IEAA = intrinsic epigenetic age acceleration

GrimAgeAccel = GrimAge acceleration

LDL-C = low-density lipoprotein cholesterol

PhenoAgeAccel = PhenoAge acceleration

SBP = systolic blood pressure

TG = triglyceride

SUMMARY

Although epigenetic age acceleration (EAA) might serve as a molecular signature of childhood cardiovascular disease (CVD) risk factors and further promote midlife subclinical CVD, few studies have comprehensively examined these life course associations. This study sought to test whether childhood CVD risk factors predict EAA in adulthood and whether EAA mediates the association between childhood CVD risks and midlife subclinical disease. Among 1,580 Bogalusa Heart Study participants, we estimated extrinsic EAA, intrinsic EAA, PhenoAge acceleration (PhenoAgeAccel), and GrimAge acceleration (GrimAgeAccel) during adulthood. We tested prospective associations of longitudinal childhood body mass index (BMI), blood pressure, lipids, and glucose with EAAs using linear mixed effects models. After confirming EAAs with midlife carotid intima-media thickness and carotid plaque, structural equation models examined mediating effects of EAAs on associations of childhood CVD risk factors with subclinical CVD measures. After stringent multiple testing corrections, each SD increase in childhood BMI was significantly associated with 0.6-, 0.9-, and 0.5-year increases in extrinsic EAA, PhenoAgeAccel, and GrimAgeAccel, respectively ($P < 0.001$ for all 3 associations). Likewise, each SD increase in childhood log-triglycerides was associated with 0.5- and 0.4-year increases in PhenoAgeAccel and GrimAgeAccel ($P < 0.001$ for both), respectively, whereas each SD increase in childhood high-density lipoprotein cholesterol was associated with a 0.3-year decrease in GrimAgeAccel ($P = 0.002$). Our findings indicate that PhenoAgeAccel mediates an estimated 27.4% of the association between childhood log-triglycerides and midlife carotid intima-media thickness ($P = 0.022$). Our data demonstrate that early life CVD risk factors may accelerate biological aging and promote subclinical atherosclerosis. (J Am Coll Cardiol Basic Trans Science 2024;9:577–590) © 2024 The Authors. Published by Elsevier on behalf of the American College of Cardiology Foundation. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Cardiovascular disease (CVD) remains the leading cause of mortality globally,[1] with major risk factors including high blood pressure (BP), adverse lipid profile, high fasting plasma glucose, and high body mass index (BMI) contributing heavily to its global disease burden.[2,3] Evidence suggests that CVD events are the consequence of a lifelong atherosclerotic process, starting with the development of related risk factors during early life.[4] Carotid intima-media thickness (cIMT) is a noninvasive measurement of subclinical atherosclerosis,[5,6] which has been reproducibly linked to early life CVD risk factors and shown to predict the development of clinical CVD events. Although the associations of CVD risk factors with subclinical CVD have been well established, the molecular mechanisms underlying these relations remain an area of active investigation. Improved mechanistic understanding is needed for the development of novel therapeutic strategies that might delay or even reverse the lifelong atherosclerotic process. Furthermore, work in this area could enhance early detection efforts.

Unlike the human genome, the methylome can be influenced by environmental factors[7-9] in addition to genetics, making it an attractive target for disease prevention research. The past decade has given rise to an abundance of "epigenetic clocks," which are composed of DNA methylation (DNAm) sites that predict chronological age with remarkable

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The authors attest they are in compliance with human studies committees and animal welfare regulations of the authors' institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the Author Center.

Manuscript received July 6, 2023; revised manuscript received January 9, 2024, accepted January 9, 2024.

accuracy.[10-14] Deviation of chronological age from epigenetic clock age, termed epigenetic age acceleration (EAA), has been identified as a powerful biomarker of aging-related disease and mortality.[15,16] Most commonly, EAA has been estimated based on clocks derived from Horvath (Horvath DNAmAge),[11] Hannum et al (Hannum DNAmAge),[12] Levine et al (PhenoAge),[16] and Lu et al (GrimAge),[17] with each EAA measure composed of distinct methylation sites that reflect both unique and common aging-related processes. For example, intrinsic epigenetic age acceleration (IEAA) represents aging independent of blood immune cell composition,[18] while extrinsic epigenetic age acceleration (EEAA) estimates aging taking into account blood immune cell-type composition. PhenoAge acceleration (PhenoAgeAccel) and GrimAge acceleration (GrimAgeAccel) were developed to correlate with aging-related physiological dysregulation[16] and mortality,[17] respectively. A growing body of published data has reported associations between EAA measures and CVD,[19-21] along with its risk factors.[20,22] Despite their potential clinical significance, existing works are predominantly based on cross-sectional associations and lack temporal clarity. Recent studies leveraging longitudinal data have reported prospective associations of EAA with CVD and overall cardiovascular health (CVH).[19,23] However, longitudinal associations of EAA with individual CVD risk factors remain unknown, and whether EAA could mediate associations between individual CVD risk factors and CVD has not been studied.

Here, we report the results of our investigation into the associations between early life CVD risk factors, EAA measures, and subclinical CVD among a biracial sample of participants from the on-going BHS (Bogalusa Heart Study). Our unique study design leveraged multiple measures of individual CVD risk factors and EAA collected across the life course to examine longitudinal and temporal relationships between these variables. Furthermore, we confirmed associations of EAA with subclinical CVD in the BHS and further explored the mediating effects of EAA on the associations between early life CVD risk factors and the development of subclinical CVD.

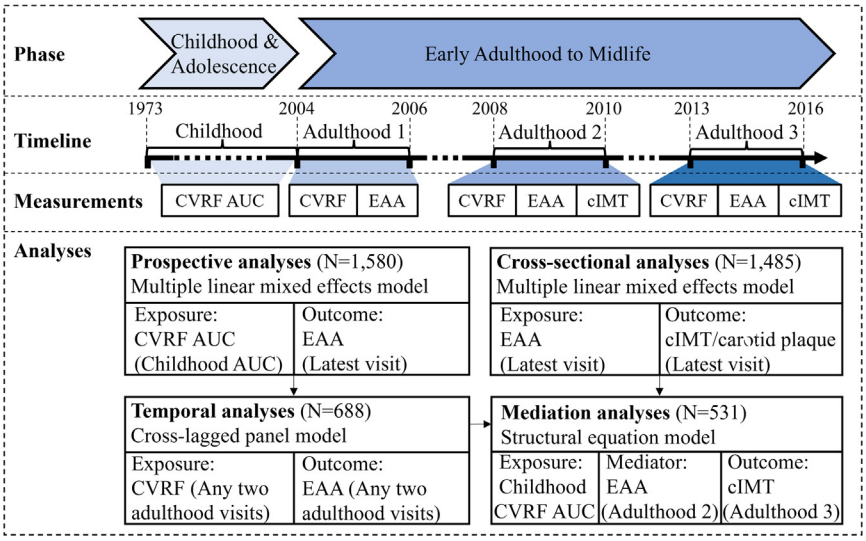
METHODS

STUDY PARTICIPANTS. The BHS is a population-based long-term study examining the natural history of CVD and its risk factors from childhood to adulthood among residents of Bogalusa, Louisiana. From 1973 to today, 9 surveys were conducted in children and adolescents aged 4-17 years, and 11 surveys were conducted among adults aged 18-51 years who had been examined previously as children. Detailed description of the BHS design and methods have been reported previously.[24] This study included a total of 1,580 participants from the BHS who had at least 1 measure of clinical CVD risk factors, including BMI, systolic blood pressure (SBP), diastolic blood pressure (DBP), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglycerides (TGs), and glucose in childhood and at least 1 assessment of genome-wide DNAm in adulthood. Among these participants, we examined the prospective relationship of childhood CVD risk factors with adulthood EAA. Of the 1,580 participants included in the prospective analyses, 688 had at least 2 simultaneously collected measures of identified clinical CVD risk factors and DNAm spanning young adulthood through midlife, contributing to cross-lagged panel analyses that were employed to support causal inference. A total of 1,485 participants had available EAA and subclinical atherosclerosis measurements for cross-sectional analyses aimed to confirm these associations in the BHS, and among them, 531 participants had temporally appropriate CVD risk factor, EAA, and subclinical CVD risk factor data for mediation analyses. **Figure 1** provides a visual schematic of our unique study design.

Informed consents were obtained from all the BHS participants after detailed explanation of the study. The study was approved by the Institutional Review Board at Tulane University.

MEASUREMENT OF EAA. DNAm profiling was conducted using whole blood samples obtained in up to 3 BHS visit cycles, including the 2004-2006, 2008-2010, and 2013-2016 visits, using methods detailed in a previous report.[18,25] In brief, DNA was extracted from whole blood using the PureLink Pro 96 Genomic DNA Kit (Life Technologies, Thermo Fisher Scientific) following the manufacturer's instructions. Following DNA extraction, the Infinium HumanMethylation450 BeadChip (Illumina) was used for whole genome DNAm quantification. Samples were processed at the Microarray Core Facility, University of Texas Southwestern Medical Center at Dallas. Generated raw data were processed and normalized using Illumina's GenomeStudio Methylation Module software to generate a final matrix of beta estimates for each cytosine-phosphate-guanine site. Following epigenetic profiling and normalization, EEAA, IEAA, PhenoAgeAccel, and GrimAgeAccel at each study visit were calculated using Horvath's online DNA Methylation Age Calculator.[26] This calculator incorporates an internal normalization procedure to further control batch effects using a modified beta mixture

FIGURE 1 Study Design



In the BHS (Bogalusa Heart Study), 1,580 participants were included in the analyses to detect prospective association between childhood cardiovascular risk factors (CVRFs) and 4 forms of midlife epigenetic age accelerations (EAAs). The childhood CVRFs were aggregated using area under curve (AUC) method. Temporal relationships between factors with significant associations were further investigated among 688 participants using the cross-lagged panel analysis with simultaneously measured CVRFs and EAAs at the 2004-2006, 2008-2010, and 2013-2016 visits. A total of 1,485 participants were used to access the cross-sectional association between carotid intima media thickness (cIMT) or presence of carotid plaque and EAA. Mediation effects of identified EAA measurements were then evaluated among 531 participants.

quantile dilation normalization method.[27] Prior to conduct of statistical analyses, the normality of EAA variable distributions was confirmed.

MEASUREMENT OF CVD RISK FACTORS AND COVARIATES. In the ongoing BHS, demographic characteristics such as age, sex, race, and lifestyle risk factors including smoking and drinking status, as well as medical history are collected using standardized questionnaires at each visit. Smoking and drinking status were categorized as never or ever. During a physical examination, anthropometric measures were obtained with participants in light clothing without shoes. At each visit, body weight and height were measured twice to the nearest 0.1 kg and 0.1 cm, respectively. BMI was calculated by dividing mean body weight in kilograms by mean height in meters squared. In childhood, BP was measured in duplicate from the right arm using a mercury sphygmomanometer while participants were in a relaxed, sitting position. In adulthood, BP was measured in triplicate on the right arm of participants using the Omron HEM 907XL digital BP device after 5 minutes in the sitting position. For 12 hours prior to the study visit, participants were advised to avoid eating, smoking, intake of caffeine and alcohol, and physical activity.[28,29] Fasting blood samples are collected by venipuncture by trained personnel. Plasma glucose and serum total cholesterol, HDL-C, and TGs are measured by standard enzymatic procedures. LDL-C is estimated using the Friedewald equation.[30]

MEASUREMENT OF SUBCLINICAL ATHEROSCLEROSIS. Carotid ultrasonography was performed on a subset of BHS participants by trained personnel at the 2008-2010 and 2013-2016 study visits. Ultrasound measurements included maximum cIMT at diastole from the far walls of the common carotid artery, carotid bulb, and internal carotid artery segments bilaterally. The mean of the maximum cIMT test reading from the 3 left and 3 right far walls of the common, bulb, and internal segments were used for the analysis. Carotid plaque was defined as a cIMT ≥1.5 mm at any of the 6 measured sites.[5]

STATISTICAL ANALYSIS. Characteristics table description. As shown in Table 1, patient characteristics by visit are presented using the mean ± SD or median (Q1, Q3) for continuous variables and count (percentage) for categorical variables.

Early life AUC calculations. To leverage repeated childhood measurements of CVD risk factors, early life BMI, SBP, DBP, LDL-C, HDL-C, log-transformed TGs, and fasting plasma glucose were individually summarized as an area under the curve (AUC)

TABLE 1 Characteristics of 1,580 BHS Participants Who Participated at the Baseline Visit and Underwent Epigenetics Profiling in at Least 1 of 3 Most Recent Study Visits

	Baseline (N = 1,580)	2004-2006 Visit (n = 1,114)	2008-2010 Visit (n = 888)	2013-2016 Visit (n = 1,281)
Age, y	9.7 ± 3.9	39.5 ± 4.4	43.5 ± 4.5	48.2 ± 5.3
Male	43.8	43.2	42.7	41.3
African American	32.9	29.3	31.6	34.4
High school or less	–	39.7	42.2	50.9
Drinker	–	60.4	63.0	56.0
Ever smoker	–	56.7	56.2	50.2
BMI, kg/m^2	17.6 ± 3.6	30.4 ± 7.6	30.9 ± 7.7	31.4 ± 7.8
SBP, mm Hg	99.4 ± 10.0	117.8 ± 15.4	118.6 ± 15.7	123.6 ± 17.2
DBP, mm Hg	61.3 ± 8.7	79.4 ± 10.4	82.1 ± 9.9	78.7 ± 11.6
LDL-C, mg/dL	89.8 ± 24.3	126.4 ± 35.5	124.7 ± 34.1	114.8 ± 35.5
HDL-C, mg/dL	65.6 ± 20.7	48.7 ± 13.5	46.8 ± 14.7	51.5 ± 16.3
Triglyceride, mg/dL	61.0 (46.0, 80.0)	110.0 (75.0, 163.0)	109.0 (74.0, 164.0)	109.0 (78.0, 158.0)
Glucose, mg/dL	82.8 ± 9.1	90.5 ± 22.3	91.8 ± 18.9	107.5 ± 38.3
IEAA	–	−0.17 ± 4.6	4.70×10^{-2} ± 4.0	-2.47×10^{-17} ± 4.0
EEAA	–	−0.15 ± 4.9	4.09×10^{-2} ± 4.7	1.54×10^{-16} ± 5.2
PhenoAgeAccel	–	−0.33 ± 4.9	8.96×10^{-2} ± 5.3	-2.81×10^{-16} ± 5.6
GrimAgeAccel	–	−0.31 ± 5.1	8.61×10^{-2} ± 5.2	1.99×10^{-16} ± 4.9
cIMT, mm	–	0.84 ± 0.19	0.66 ± 0.15	0.94 ± 0.32
Carotid plaque	–	5.8	3.7	31.9

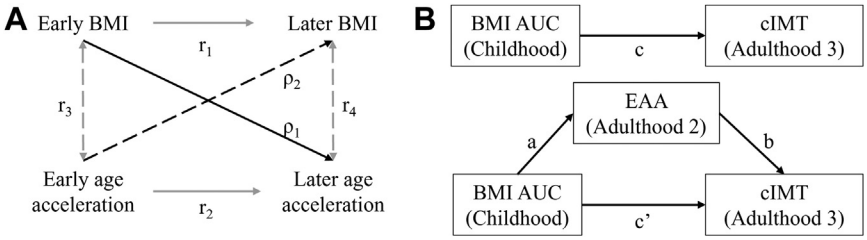
Values are mean ± SD, %, or median (Q1, Q3).

BHS = Bogalusa Heart Study; BMI = body mass index; cIMT = carotid intima-media thickness; DBP = diastolic blood pressure; EEAA = extrinsic epigenetic age acceleration; GrimAgeAccel = GrimAge acceleration; HDL-C = high-density lipoprotein cholesterol; IEAA = intrinsic epigenetic age acceleration; LDL-C = low-density lipoprotein cholesterol; PhenoAgeAccel = PhenoAge acceleration; SBP = systolic blood pressure.

estimate for each participant using methods developed specifically for the BHS and employed extensively in this cohort.[31-33] In brief, growth curves of CVD risk factors measured multiple times during childhood (when a participant was <18 years of age) were constructed using a random effects model in SAS (version 9.4, SAS Institute, Inc). The mixed model regresses the continuous risk factor on fixed and random effects of age and its higher order terms. The model allows the intercept, linear, and nonlinear parameters to vary from individual to individual, with the random effect coefficients representing the difference between the fixed parameters and the observed values for each individual. To avoid collinearity of age with its higher order terms, age was centered to the mean age of BHS participants in childhood. Quadratic growth curves were fitted for all factors in race-sex groups. The AUCs were calculated as the integral of the curve parameters during the follow-up period for each participant.

Prospective associations of early life CVD risk factors with adulthood EAA. Associations of the early life CVD risk factors (as continuous AUC values) with EAA were tested using multiple linear mixed effects models that leveraged up to 3 repeated adulthood measures of EAA. An autoregressive correlation matrix was used to account for the repeated measures within individuals. Multiple covariates measured simultaneously with EAA were adjusted. Model 1 was adjusted for age, sex, and race. Model 2 additionally accounted for smoking and drinking status. A Bonferroni correction to account for testing each EAA measure for association with 7 CVD risk factors was employed, with a P value threshold of 7.1×10^{-3} used for determining statistical significance. The results of mixed effects models are presented as regression coefficient (beta) and SE. To further support temporal inference for identified associations between clinical CVD risk factors and EAA, simultaneously collected measures of CVD risk factors and EAA at 2 time points, spanning young adulthood through midlife, were leveraged to conduct cross-lagged panel analyses.[34] Specifically, participants who attended at least 2 study visits where CVD risk factors and EAA were concurrently measured were included in this analysis. If data were available for more than 2 visits, data from the first and last available visits were used. Figure 2A provides a schematic of the conceptual model underlying the cross-lagged analysis approach using BMI as an example. This analysis examines reciprocal, longitudinal relationships between CVD risk factors and EAA and was conducted using the R package Lavaan (R Foundation).[35] Two sensitivity analyses were

FIGURE 2 Illustration of Cross-lagged Panel Analysis Model and Mediation Analysis Model



(A) Cross-lagged panel analysis model of body mass index (BMI) and DNA methylation age acceleration: ρ_1 and ρ_2 are 2 cross-lagged coefficients; r_1 and r_2 are correlations between 2 instances of the same variable; and r_3 and r_4 are 2 synchronous correlations. The detailed result is shown in Table 2. (B) Mediation analyses of EAA on repeatedly measured childhood BMI with midlife cIMT. Mediator EAAs were measured at the 2008-2010 visit and cIMT was measured at the 2013-2016 visit. *c* is total effect; *c'* is direct effect; and $a \times b$ is indirect effect. The mediation effect of EAA is calculated as $(a \times b / c) \times 100\%$. The detailed result is shown in Figure 4 and Supplemental Table 6. Abbreviations as in Figure 1.

performed to test whether differences in time intervals between exams influenced results of the cross-lagged analysis. We conducted the first sensitivity analysis among a subset of the cross-lagged analysis participants who attended the 2 most recent study visits between 2008-2010 and 2013-2016. Our second sensitivity analysis utilized data from all participants included in the original cross-lagged modelling approach, additionally including time interval between visits as a covariable in the analysis.

Associations of EAAs with subclinical atherosclerosis. Cross-sectional associations between EAA and the continuous cIMT, as well as the discrete carotid plaque phenotypes, were examined using multiple linear and logistic regression models, respectively. Two multivariable models were utilized: model 1 adjusted for age, sex, and race; and model 2 adjusted for covariates in model 1, along with smoking and drinking status. These analyses were conducted based on data obtained from the most recently completed study visit for each participant.

Mediation effects of EAA. To assess whether identified EAA measures (longitudinally associated with a CVD risk factor and cross-associated with cIMT) mediated the associations between early life CVD risk factors and midlife cIMT, we conducted mediation analyses based on the path diagrams illustrated in Figure 2B, again using BMI as an example. For these analyses, measurement of cIMT at the 2013-2016 visit was assessed as the outcome, the EAA measurement in a visit prior to the cIMT measurement was assessed as the mediator, and the AUC of childhood CVD risk factors, measured when participants were 18 years of age or less, were assessed as the exposure. Here, the beta coefficient for the association between the childhood CVD risk factor AUC and midlife cIMT is defined as total effect (denoted as *c* in Figure 2B). The indirect effect is estimated as the product of coefficients *a* and *b* derived from 2 regression models, the first regressing EAA on BMI AUC and adjusting for covariables and the latter regressing of cIMT on EAA after controlling for BMI AUC and covariables. Two sets of covariables measured simultaneously with EAA were included in the mediation analyses. Covariables in model 1 included age, sex, race, and model 2 further included smoking and drinking. Prior to conducting the mediation analyses, continuous variables were standardized using *Z*-transformation. The mediation effect was estimated as the percentage of the contribution of the indirect effect to the total effect, with statistical significance determined using the bootstrap method with 1,000 bootstrap iterations.[36] The analysis was conducted using the R package Mediation.[37]

RESULTS

DESCRIPTION OF PARTICIPANTS. Table 1 describes the characteristics of the 1,580 BHS participants at their baseline and most recently completed study visits. Among this biracial cohort, 43.8% were male and 32.9% were African American. With a median follow-up of 38.8 (Q1, Q3: 34.9, 40.8) years, the mean ages at baseline, the 2004-2006, 2008-2010, and 2013-2016 study visits were 9.7, 39.5, 43.5, and 48.2 years, respectively. As expected, BMI, SBP, DBP, LDL-C, TGs, and glucose were higher in the 3 midlife visits compared to the baseline visit. In this population, EAA variability was substantial, with each SD increase corresponding to an absolute increase of 4-5 years in accelerated aging across measures of midlife visits. On average, cIMT were 0.8 mm, 0.7 mm, and 0.9 mm,

with 5.8%, 3.7%, and 31.9% of participants demonstrating carotid plaque at the 2004-2006, 2008-2010, and 2013-2016 study visits, respectively.

ASSOCIATION OF EARLY LIFE CVD RISK FACTORS AND EAA. Associations between childhood CVD risk factor AUCs and adulthood EAA are shown in **Figure 3** and Supplemental Table 1. Childhood BMI demonstrated significant or nominally significant associations with all measures of EAA. For example, in the fully adjusted model (model 2), each SD increase in early life BMI AUC was associated with 0.27 ($P = 5.402 \times 10^{-3}$), 0.63 ($P = 2.624 \times 10^{-7}$), 0.88 ($P = 6.062 \times 10^{-11}$), and 0.52 ($P = 3.196 \times 10^{-7}$) years increased IEAA, EEAA, PhenoAgeAccel, and GrimAgeAccel, respectively. Early life log-transformed TG AUC consistently associated with both PhenoAgeAccel and GrimAgeAccel across models, with each SD increment increase conferring a respective 0.50 ($P = 5.506 \times 10^{-4}$) and 0.43 ($P = 9.269 \times 10^{-5}$) year increase in the EAA measures. Likewise, early SBP AUC was associated with IEAA (0.27; $P = 6.498 \times 10^{-3}$) and PhenoAgeAccel (0.52; $P = 1.216 \times 10^{-4}$), and each SD increase in early HDL-C was associated with 0.32 ($P = 2.125 \times 10^{-3}$) years decreased GrimAgeAccel in the fully adjusted models. There were no consistent associations of early life DBP, LDL-C, or glucose with adulthood EAA.

To further discern the temporal relationships of BMI, SBP, TGs, and HDL-C with EAA, we carried out cross-lagged panel analyses utilizing simultaneous measurements of these variables collected at the 2 most distant time points in young adulthood through midlife. The path coefficients between selected CVD risk factors and EAA are presented in **Table 2**. In the fully adjusted model, path coefficients from baseline BMI to follow-up EEAA, PhenoAgeAccel, and GrimAgeAccel were statistically significant ($P_{\text{BMI} \rightarrow \text{EEAA}} = 2.795 \times 10^{-3}$, $P_{\text{BMI} \rightarrow \text{PhenoAgeAccel}} = 1.675 \times 10^{-2}$, and $P_{\text{BMI} \rightarrow \text{GrimAgeAccel}} = 1.202 \times 10^{-3}$). Likewise, path coefficients from baseline TGs to follow-up PhenoAgeAccel and GrimAgeAccel were statistically significant ($P_{\text{logTG} \rightarrow \text{PhenoAgeAccel}} = 1.930 \times 10^{-3}$, and $P_{\text{logTG} \rightarrow \text{GrimAgeAccel}} = 4.051 \times 10^{-3}$) and path coefficients from baseline HDL-C to follow-up GrimAgeAccel was statistically significant ($P_{\text{HDL-C} \rightarrow \text{GrimAgeAccel}} = 1.527 \times 10^{-2}$). In contrast, path coefficients examining temporal associations of baseline EAA to follow-up BMI, TGs, and HDL-C were not statistically significant in either of the models tested, providing no evidence of EAA temporally preceding these measures. No sex-based differences were present (data not shown). Findings of sensitivity analyses restricting the cross-lagged panel analyses to only those with EAA and CVD risk factor measures at the 2 most recent study visits (Supplemental Table 2) and adjusting for time interval between study visits (Supplemental Table 3) were consistent with those of the main analysis.

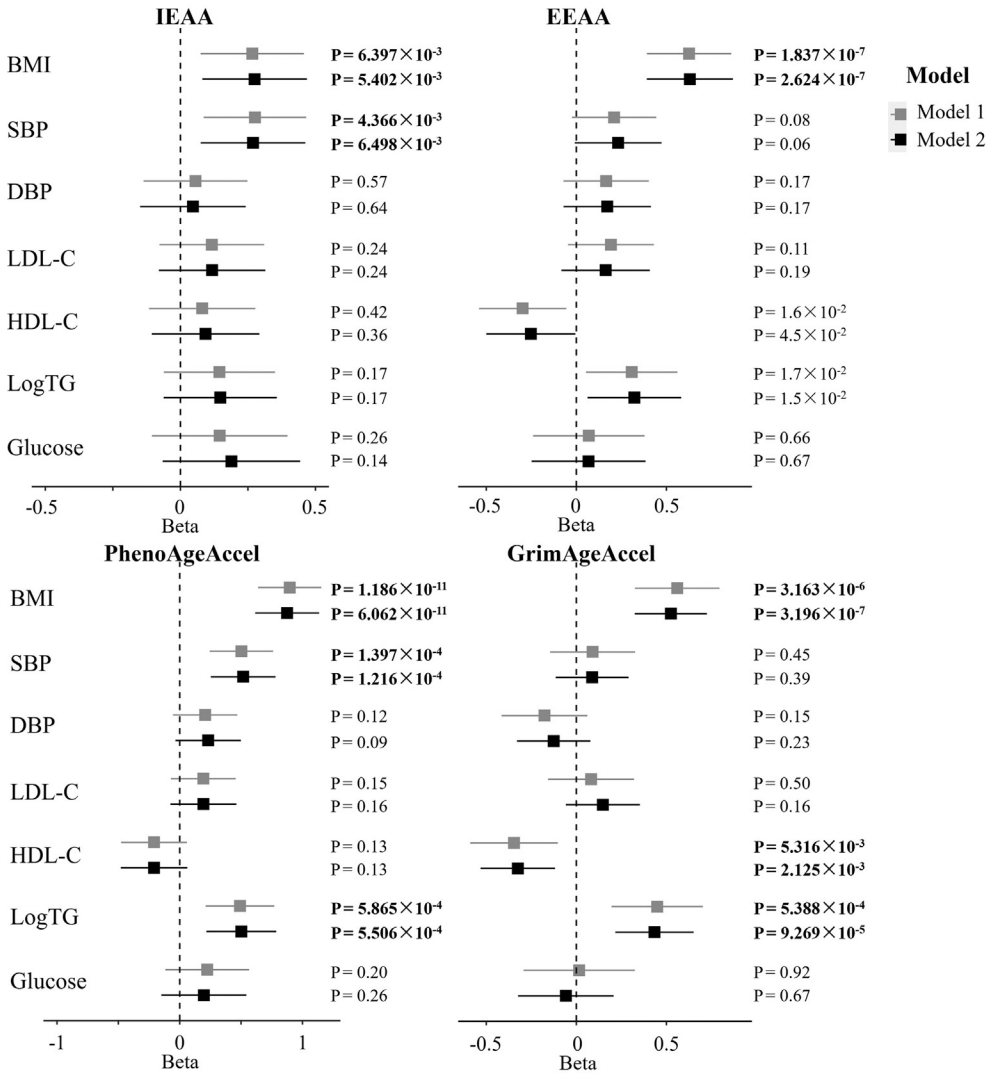
CROSS-SECTIONAL ANALYSES EXAMINING ASSOCIATIONS OF EAA WITH cIMT AND CAROTID PLAQUE. Associations of EAA with the continuous and discrete measures of subclinical atherosclerosis are shown in Supplemental Table 4. Our results demonstrated strong and consistent associations of EEAA, PhenoAgeAccel, and GrimAgeAccel with both cIMT and carotid plaque in the BHS participants. For example, each SD increase in EEAA, PhenoAgeAccel, and GrimAgeAccel was cross-sectionally associated with a 0.034 mm ($P = 1.660 \times 10^{-5}$), 0.034 mm ($P = 1.443 \times 10^{-5}$), and 0.046 mm ($P = 1.031 \times 10^{-6}$) increase in cIMT, respectively, in the fully adjusted model (model 2). Similarly, each SD increase in in EEAA, PhenoAgeAccel, and GrimAgeAccel was cross-sectionally associated with 1.26-fold ($P = 3.173 \times 10^{-4}$), 1.39-fold ($P = 2.540 \times 10^{-7}$), and 1.49-fold ($P = 6.902 \times 10^{-8}$) higher odds of carotid plaque in the fully adjusted model. There was no evidence of associations between IEAA and either cIMT or carotid plaque.

MEDIATION ANALYSES. Prior to mediation analyses, we confirmed results of previous studies,[38-42] demonstrating significant associations of childhood BMI, BP, lipids, and glucose AUC with adulthood cIMT in the BHS (Supplemental Table 5). The results of analyses investigating the potentially mediating effects of identified EAA measures on known associations of childhood BMI, TGs, and HDL-C with cIMT are shown in **Figure 4** and Supplemental Table 6. In model 1, PhenoAgeAccel and GrimAgeAccel had significant mediation effects ($P = 0.014$ and $P = 0.016$, respectively) on the associations between childhood BMI and cIMT, with trends that were similar and marginally significant in model 2. PhenoAgeAccel was identified as a significant mediator of the association between childhood TGs and cIMT ($P = 0.006$) in model 1 and remained significant ($P = 0.022$) in model 2 (Supplemental Table 6). Mediation effects of EEAA on BMI-cIMT associations of GrimAgeAccel on TG-cIMT associations and of GrimAgeAccel on HDL-C-cIMT were not observed in this small subsample.

DISCUSSION

In the current study examining the relation of individual early life CVD risk factors with multiple measures of epigenetic aging, we found that increased childhood BMI, SBP, TGs, and HDL-C predicted accelerated epigenetic aging in adulthood. Furthermore, through the implementation of discrete time

FIGURE 3 Associations of Childhood CVD Risk Factors With Adulthood EAA



Model 1 adjusts for age, sex, and race. Model 2 adjusts for all variables in model 1 + smoking and drinking. Childhood cardiovascular disease (CVD) risk factors were summarized as an area under the curve estimate for each participant. Beta coefficients represent the change in EAA per SD increase in the CVD risk factor measure. The *P* values smaller than the significant threshold of 7.1×10^{-3} after Bonferroni correction are boldface. DBP = diastolic blood pressure; EEAA = extrinsic epigenetic age acceleration; GrimAgeAccel = GrimAge acceleration; HDL-C = high-density lipoprotein cholesterol; IEAA = intrinsic epigenetic age acceleration; LDL-C = low-density lipoprotein cholesterol; PhenoAgeAccel = PhenoAge acceleration; SBP = systolic blood pressure; TG = triglyceride; other abbreviations as in Figures 1 and 2.

structural equation models, our findings support increased BMI, TGs, and HDL-C as upstream determinants, rather than downstream consequences of accelerated epigenetic aging. As expected, EAA measures were cross-sectionally associated with subclinical atherosclerosis in the midlife BHS cohort. Further analyses demonstrated that mediating effects of PhenoAgeAccel on the association of childhood TGs and BMI with subclinical atherosclerosis in midlife. In total, these findings provide temporal evidence of EAA as a molecular footprint of adverse childhood CVH, while further implicating EAA as a mechanism linking early life CVD risk factors to later life subclinical atherosclerosis.

Our work not only supports but expands on recent findings from the CARDIA (Coronary Artery Risk Development in Young Adults) study.[23] In CARDIA, Joyce et al [23] identified prospective associations

TABLE 2 Results of Cross-lagged Panel Analyses Assessing Temporal Relations Between Identified CVD Risk Factors and EAA in a Subsample With Simultaneously Collected Repeated Measures of CVD Risk Factors and EAA (n = 688)

	Model 1[a]						Model 2[b]					
	EAA_{BL} → $Risk\ Factor_{FU}$			$Risk\ Factor_{BL}$ → EAA_{FU}			EAA_{BL} → $Risk\ Factor_{FU}$			EAA_{BL} → $Risk\ Factor_{FU}$		
	Beta	SE	*P* Value	Beta	SE	*P* Value	Beta	SE	*P* Value	Beta	SE	*P* Value
BMI (n = 688)												
IEAA	−0.013	0.030	0.67	−0.01	0.016	0.52	−0.018	0.041	0.66	−0.021	0.018	0.23
EEAA	−0.028	0.032	0.39	0.052	0.019	7.634×10^{-3}	−0.001	0.044	0.99	0.066	0.022	2.795×10^{-3}
PhenoAgeAccel	0.046	0.026	0.075	0.052	0.02	9.831×10^{-3}	-0.024	0.033	0.46	0.058	0.024	1.675×10^{-2}
GrimAgeAccel	−0.068	0.027	0.01	−0.012	0.014	0.39	0.042	0.035	0.24	0.054	0.017	1.202×10^{-3}
SBP (n = 688)												
IEAA	0.206	0.124	0.098	−0.001	0.008	0.87	0.332	0.173	0.055	−0.012	0.009	0.21
PhenoAgeAccel	0.079	0.106	0.46	0.012	0.01	0.23	0.061	0.126	0.63	0.000	0.011	1.00
Log triglyceride (n = 678)												
PhenoAgeAccel	0.007	0.003	0.039	1.126	0.237	1.957×10^{-6}	0.003	0.004	0.37	0.896	0.289	1.930×10^{-3}
GrimAgeAccel	0.014	0.004	4.587×10^{-5}	0.783	0.174	6.627×10^{-6}	0.007	0.004	0.067	0.631	0.219	4.051×10^{-3}
HDL-C (n = 688)												
GrimAgeAccel	−0.135	0.104	0.19	−0.016	0.01	0.12	−0.08	0.109	0.46	−0.023	0.009	1.527×10^{-2}

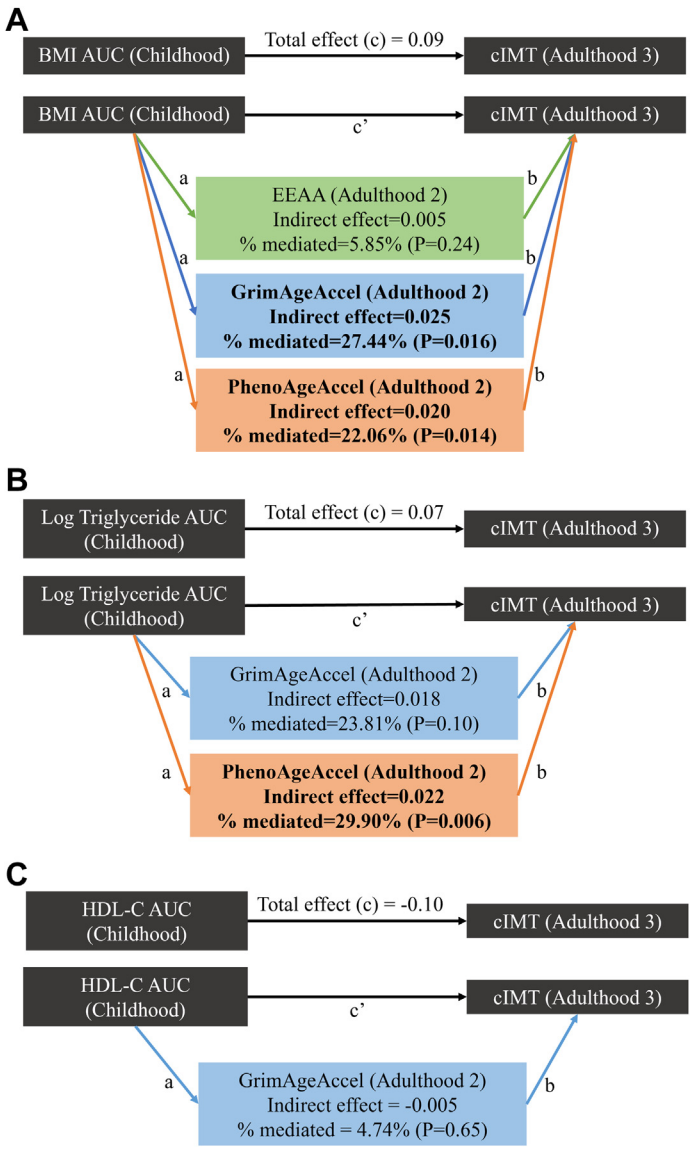
[a]Model 1 adjusts for age, sex, and race. [b]Model 2 adjusts for all variables in model 1 + smoking and drinking status simultaneously measured with tested factor.
BL = baseline; CVD = cardiovascular disease; EAA = epigenetic age acceleration; FU = follow-up; other abbreviations as in Table 1.

between a composite CVH score during young adulthood and GrimAgeAccel, with further evidence that GrimAgeAccel mediated associations of CVH score with subclinical atherosclerosis, as measured by coronary artery calcification. We extend CARDIA's findings to demonstrate that prospective associations of clinical CVD risk factors with adulthood EAA can be observed even earlier, starting in childhood. Furthermore, rather than examining a composite CVH score, our study looked at individual CVD risk factors to pinpoint the clinical measures that might be driving CVH-EAA associations. Our analyses suggest that early life BMI and TGs, but not BP and glucose, may be most relevant in accelerating epigenetic aging across the life course. Furthermore, our cross-lagged panel analyses, which leveraged repeated simultaneously measured CVD risk factors and EAA, provides temporal evidence supporting CVD risk factors as upstream determinants rather than consequences of EAA. Like CARDIA, EAA measures in the BHS were also associated with subclinical atherosclerosis, here measured as cIMT and the discrete carotid plaque endpoint. Whereas power was somewhat limited for our mediation analyses, our findings suggest that measures of EAA may, in part, link childhood BMI and TGs to subclinical atherosclerosis in adulthood. In total, these findings continue to highlight the relevance of early life CVD risk factors in subclinical atherosclerosis, while pointing out specific risk factors that might be pivotal in decelerating epigenetic aging for the prevention of CVD.

Although we are among the first to identify prospective and temporal associations between early life BMI and accelerated epigenetic aging in midlife, several studies have reported cross-sectional associations of these 2 variables.[43-47] For example, Quach et al[49] identified associations of EEAA and IEAA with higher BMI among participants of the WHI (Women's Health Initiative). In one of the few longitudinal studies in this area, Quach et al[49] further examined whether BMI at baseline predicted EEAA and IEAA after 2.7 years follow-up among a small subsample of participants of the InCHianti study (N = 239). In contrast to our findings, no associations were observed.[48] Given the small sample size and limited follow-up time, it is unclear whether this study was sufficiently powered for such analyses. Consistent with our findings that suggest increased EAA as a downstream consequence but not upstream determinant of increased BMI, Simpkin et al[49] found no associations between EAA (measured at age 7) and change in BMI through 17 years of age using models with or without adjustment for cell counts. Overall, our findings contribute additional information to the growing evidence of an association between obesity and epigenetic aging, providing compelling new data that implicate obesity as precursor to this phenomenon.

Our study also identified prospective and temporal associations of early life TGs and HDL-C with EAA. Like BMI, TGs have been associated with measures of

FIGURE 4 Results of Mediation Analyses



Mediation effects of EAAs on the (A) BMI-cIMT, (B) triglyceride-cIMT, and (C) HDL-C-cIMT associations in childhood and adulthood, respectively, are shown. The examined EAA measurements were selected based on our prospective and temporal analyses. Total effect, direct effect, and indirect effect are indicated as *c*, *c'*, and $a \times b$, respectively. Abbreviations as in Figures 1 to 3.

EAA in several cross-sectional studies,[16,18,20,21,48,50] including the work of Quach et al[48] in the WHI. Furthermore, in a recent CARDIA study, Gao et al[51] examined associations of early adulthood lipid levels with midlife GrimAgeAccel. Like us, they identified strong associations between early life TGs and HDL-C with midlife GrimAgeAccel, with their analyses focused on young adulthood TG levels compared to our focus on childhood.

As expected, increased EAA was associated with subclinical atherosclerosis in our cross-sectional analyses of BHS participants, including both cIMT and carotid plaque. Numerous reports have identified associations between EAA and measures of both subclinical and clinical CVD.[19,20,23,52-55] For example, a 2018 report by Roetker et al[19] identified baseline cross-sectional associations between EAA and cIMT among African American participants of the ARIC (Atherosclerosis Risk in Communities) study. Likewise, analyses by Joyce et al[23] suggested an association (and mediating effect) of GrimAgeAccel on coronary artery calcification in CARDIA participants. In one of the only studies to investigate prospective associations of EAA with clinical CVD events, the ARIC study went on to demonstrate that each 5-year increase in IEAA and EEAA was associated with, respectively, 17% and 22% increased risks of fatal coronary heart disease.[19] In aggregate, we confirm previous associations and present new evidence for a role of EAA in subclinical CVD in a rural cohort.

We identified a significant mediating effect of EAA on the relationship of increased early life TGs and BMI with midlife subclinical atherosclerosis. We reported the significant mediation effects of both PhenoAgeAccel and GrimAgeAccel on the associations between childhood BMI and midlife cIMT. To our knowledge, only the previous study by Gao et al,[51] which focused specifically on TGs and the GrimAgeAccel measure alone, has investigated mediation of EAA in CVD risk factor-subclinical (or -clinical) CVD associations. In CARDIA, Gao et al[51] showed that GrimAgeAccel mediated 17% of the association between early life TGs and subclinical atherosclerosis. Although this mediation was not significant in the current study, it is worth noting that the estimated effect size was similar to that reported in CARDIA, with a mediation effect of 16.8% in our most comparable model. Given the smaller sample size of the BHS, we may have been underpowered to detect this signal. PhenoAgeAccel was identified as a significant mediator, explaining 27.4% of the association between childhood TGs with midlife cIMT in the BHS. In total, our findings implicate EAA as a potential molecular link between early life CVD risk and the development of subclinical disease in adulthood. Given the small sample size for this analysis and generally marginally significant signals, despite rather large mediation effect sizes, more research in this area is warranted.

The associations of accelerated epigenetic aging with CVD risk factors and subclinical CVD were not

homogeneous across measures. For instance, SBP showed a significant association with IEAA and PhenoAgeAccel, but no association was found with EEAA or GrimAgeAccel. On the other hand, BMI exhibited strong associations with all 4 EAAs. This was not all together unexpected given a general lack of overlap of methylation sites across the 4 different EAA measures.[14,56-58] Furthermore, recent work by Liu et al[56] demonstrated both similarities and differences in the aging processes reflected across various measures of EAA. For example, gene coexpression analyses identified consistent enrichment across epigenetic clocks for biological pathways,[56] including immunity and inflammation,[59] chromatin modification,[60] and autophagy.[61] However, correlations with gene coexpression for these shared biological pathways varied in magnitude across clocks.[56] Furthermore, in vitro experiments identified strong associations of PhenoAge but not other epigenetic clocks with mitochondrial dysfunction and cellular senescence,[56] molecular mechanisms that may play key roles in the development of CVD.[62-68] In total, these findings provide impetus for future work to integrate EAA measures with other multiomics data, which might reveal further molecular insights into the relationships observed here.

Our study has several important strengths. The unique longitudinal design of the BHS enabled a unique investigation into the influence of childhood CVD risk factors on EAA, as well as the potentially mediating effect of this molecular footprint on subclinical disease. By leveraging an average of 3-4 measures of CVD risk factors in childhood and up to 3 adulthood measures of EAA, our study was able to powerfully and precisely investigate associations between early life CVD risk factors and epigenetic aging in a biracial, rural cohort. Furthermore, the BHS offered simultaneously collected measures of CVD risk factors and EAA to more clearly articulate the temporal relationship between these variables for the first time.

STUDY LIMITATIONS. Blood samples from childhood were not available in BHS participants, so we could not adjust for baseline EAA in our investigation of the association between early life CVD risk factors and midlife EAA. Likewise, whereas apolipoprotein B or direct LDL measurements would offer greater accuracy in our analyses compared to relying on the Friedewald equation for estimating LDL-C, particularly in cases of hypertriglyceridemia, these data are not available in the BHS.[69] Furthermore, we note caution in drawing conclusions regarding associations between HDL-C and subclinical CVD given null associations observed in randomized clinical trials and Mendelian randomization studies.[70] Because only smaller subsamples had appropriate data available for the cross-lagged panel and mediation analyses, nonsignificant findings do not clearly suggest a lack of association and could instead reflect a lack of statistical power. Further research to investigate the promising marginally significant findings identified here may be warranted.

CONCLUSIONS

Our study provides compelling early evidence that accelerated epigenetic aging in adulthood is influenced by childhood BMI and TG levels. Furthermore, our data suggest that EAA may mediate the associations of these childhood CVD risk factors and the development of subclinical disease. In total, our findings provide information on unique strategies that might decelerate the biological aging process early in life, while pointing to potentially modifiable molecular mechanisms that could be targeted for precision disease prevention across the life span.

ACKNOWLEDGMENTS The authors are grateful for the contribution of all staff members who were involved in conducting the Bogalusa Heart Study. The authors extend their gratitude to the participants of Bogalusa Heart Study, many of whom have diligently participated since they were children.

FUNDING SUPPORT AND AUTHOR DISCLOSURES

The Bogalusa Heart Study was supported by the National Institute on Aging of the National Institutes of Health (awards R01AG041200, R01AG062309, R01AG077000, and R33AG057983) and the National Institute of General Medical Sciences of the National Institutes of Health (award P20GM109036). The authors have reported that they have no relationships relevant to the contents of this paper to disclose.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: CVD remains the leading cause of mortality globally. Evidence suggests that CVD events are the consequence of a lifelong atherosclerotic process, starting with the development of related risk factors during early life. Although the associations of CVD risk factors with subclinical CVD have been well established, the molecular mechanisms underlying these relations remain an area of active investigation. Improved mechanistic understanding is needed for the development of novel therapeutic strategies that might delay or even reverse the lifelong atherosclerotic process. The past decade has given rise to an abundance of epigenetic clocks, which are composed of DNAm sites that accurately predict chronological age. Deviation of chronological age from epigenetic clock age, termed EAA, has been identified as a powerful biomarker of aging-related disease and mortality. Although EAA might serve as a molecular signature of childhood CVD risk factors and further promote midlife subclinical CVD, few studies have comprehensively examined these life course associations. The current report comprehensively examines these associations, providing compelling early evidence that accelerated epigenetic aging in adulthood is influenced by childhood BMI and TG levels and might further mediate the associations of these childhood CVD risk factors with the development of subclinical disease.

TRANSLATIONAL OUTLOOK: This study highlights epigenetic age acceleration as a molecular signature of suboptimal early life CVH that might promote midlife subclinical disease. Further research is warranted to explore the promising translational potential of this research, which might include assessing interventions targeting EAA to mitigate the risk of accelerated biological aging and subsequent development of subclinical atherosclerosis. Furthermore, investigating the downstream proteins and metabolites that influence EAA could uncover additional novel therapeutic targets for preventing or delaying the onset of CVD in later life.

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KEY WORDS biological aging, cardiovascular disease risk factors, epigenetic age acceleration, life span, subclinical atherosclerosis

APPENDIX For supplemental tables, please see the online version of this paper.