

Associations Between DNA Methylation Age Acceleration, Depressive Symptoms, and Cardiometabolic Traits in African American Mothers From the InterGEN Study

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ABSTRACT

BACKGROUND: African American women (AAW) have a high risk of both cardiometabolic (CM) illness and depressive symptoms. Depressive symptoms co-occur in individuals with CM illness at higher rates than the general population, and accelerated aging may explain this. In this secondary analysis, we examined associations between age acceleration; depressive symptoms; and CM traits (hypertension, diabetes mellitus [DM], and obesity) in a cohort of AAW.

METHODS: Genomic and clinical data from the InterGEN cohort ($n = 227$) were used. Age acceleration was based on the Horvath method of DNA methylation (DNAm) age estimation. Accordingly, DNAm age acceleration (DNAm AA) was defined as the residuals from a linear regression of DNAm age on chronological age. Spearman's correlations, linear and logistic regression examined associations between DNAm AA, depressive symptoms, and CM traits.

RESULTS: DNAm AA did not associate with total depressive symptom scores. DNAm AA correlated with specific symptoms including self-disgust/self-hate (-0.13 , 95% CI -0.26 , -0.01); difficulty with making decisions (-0.15 , 95% CI -0.28 , -0.02); and worry over physical health (0.15 , 95% CI 0.02 , 0.28), but were not statistically significant after multiple comparison correction. DNAm AA associated with obesity (0.08 , 95% CI 1.02 , 1.16), hypertension (0.08 , 95% CI 1.01 , 1.17), and DM (0.20 , 95% CI 1.09 , 1.40), after adjustment for potential confounders.

CONCLUSIONS: Associations between age acceleration and depressive symptoms may be highly nuanced and dependent on study design contexts. Factors other than age acceleration may explain the connection between depressive symptoms and CM traits. AAW with CM traits may be at increased risk of accelerated aging.

KEYWORDS: Depressive symptoms, DNA methylation, African American women, accelerated aging, chronic medical conditions

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Background

Cardiometabolic (CM) illnesses and depressive symptoms are increasingly common problems, each with profound personal and public health consequences.^{1–4} African American women (AAW) bear a disproportionate CM burden, with higher rates of diabetes mellitus (DM), hypertension, and obesity—presenting at earlier ages—than other groups in the USA.^{5,6} AAW also experience a greater proportion of depression that is considered chronic or severe compared to other racial/ethnic groups.^{7–9} However, AAW are often poorly represented in studies aimed to elucidate biological correlates in CM illness and depressive symptoms.

Depressive presentations are heterogeneous and include an array of symptoms spanning affective, cognitive, and somatic domains. This heterogeneity combined with a lack of diagnostic biomarkers remains a barrier to the development of precision health strategies. Consequently, the National Institute of Mental Health's (NIMH) Research Domain Criteria (RDoC) has shifted focus away from the sole use of diagnostic labels, encouraging examination of biomarkers in relation to continuous symptoms or domains.¹⁰ Depressive symptoms include depressed mood, loss of interest in previously enjoyed activities; disruption in sleep patterns; changes in appetite; loss of energy; poor concentration and recall; overwhelming feelings of guilt,



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hopelessness, or worthlessness; psychomotor slowing; and thoughts of death or suicide.¹¹ These symptoms not only diminish quality of life but are also associated with increased all-cause mortality, suggesting a quantitative impact on longevity.^{12,13} Specific symptoms or symptom profiles (eg, atypical symptoms including hypersomnia, hyperphagia, interpersonal rejection sensitivity) may associate more or less strongly with CM or aging indicators than classic melancholic presentations,¹⁴ highlighting the strength of the RDoC approaches.

Adults with CM illness, when compared to those with other chronic conditions, are disproportionately affected by depressive symptoms—occurring in 55% of hypertension cases and 35% of DM cases.¹³ Further, 44% of adults with depression meet the criteria for obesity (ie, body mass index [BMI] ≥ 30).¹⁵ In AAW specifically, depressive symptoms have been associated with increased blood pressure.¹⁶ Links between CM traits and depressive symptoms are characterized as bidirectional and mutually reinforcing through behavioral, biological, and genomic factors, but these connections are not well understood or sufficiently explored in AAW—a population most likely to be negatively affected.^{15,17,18}

A plausible and testable explanation for the cross-over between CM conditions—often associated with aging—and depressive symptoms is aging itself, or more specifically, accelerated aging.^{19–21} Accelerated aging refers to being biologically older than expected for years lived,^{19,22} and depressive symptoms are associated with several estimates of accelerated aging, including inflammatory cytokines, products of oxidative stress, and telomere length.^{20,21,23} DNA methylation (DNAm) age is a reliable and parsimonious estimate of biological age based on patterns of DNAm and is highly predictive of all-cause mortality and independent of other aforementioned measures.^{24,25} DNAm age is calculated and then contrasted with chronological age to determine the degree of DNAm age *acceleration* (DNAm AA).²⁵ Many DNAm age “clocks” (ie, algorithms) now exist, each with distinctions in tissue selection, training data populations, and DNAm sites selected.²⁶

Methodologies to calculate DNAm age are relatively new, and their application to exploring correlates of depressive symptoms and CM traits is nascent. Positive associations between DNAm AA and depression have been observed, but in these studies, AAW are not well represented and included CM indices are limited.^{27,28} In a study of over 4000 adults, individuals with depression exhibited significantly greater DNAm AA than age-matched controls, an association partly mediated by BMI.²⁸ In another large case-control study, Han et al²⁷ demonstrated significant DNAm AA in individuals with depression and replicated this association in postmortem brain tissue. Pathway analysis of postmortem brain tissue, from a separate replication sample in this study, further demonstrated overlap of depression and several DNAm age CpG sites linked to neurogenesis, neuron differentiation, and regulation

of neuron death, indicating several pathways relevant to depression were enriched in this DNAm indicator of cell aging.²⁷

Premature aging may be a plausible common factor in depressive symptoms and other stress-related and aging-related conditions including CM illness. DNAm AA has been shown to associate with hypertension, cardiovascular disease,^{29,30} BMI,³¹ and blood glucose,³² but not consistently across diverse populations. This variability warrants further study, especially because links between DNAm AA and lifetime cumulative stress/early exposure to racial discrimination exist among African Americans,^{33,34} positioning DNAm AA as a possible connection between stressors and clinical outcomes.

Some studies have demonstrated associations between DNAm AA and specific depressive symptoms—as opposed to composite scores or diagnostic labels alone.^{35–37} Notably, in one of these studies, DNAm AA did not associate with total depressive symptom scores but did positively correlate with reduced positive affect,³⁵ underscoring the importance of considering symptoms rather than diagnostic labels or clinical cut-offs which yield heterogeneous cases.

Taken together, these findings suggest that examination of depressive symptoms and DNAm AA is enhanced when related factors such as specific symptoms and CM indices are considered. Such examinations may help to illuminate pathophysiological connections between highly comorbid presentations (ie, CM traits and depression) and result in a more nuanced description of depressive phenotypes to distinguish which depressive features in what context correlate to biological aging. To our knowledge, no studies to date have used DNAm age to examine associations between premature aging, depressive symptoms, and CM traits specifically in AAW. Here, we aim to address this knowledge gap by examining potential associations between DNAm AA, depressive symptoms, and CM traits in a population that bears a disproportionate risk. Specifically, we hypothesize that greater DNAm AA will associate with higher depressive symptom scores generally and especially in those with CM traits.

Methods

Study design and participants

The Intergenerational Impact of Genetic and Psychological Factors on Blood Pressure (InterGEN) study is a multidisciplinary, longitudinal investigation of genomic, environmental, and psychosocial factors influencing blood pressure among African American mother-child dyads.^{38,39} The 250 dyads were recruited from early care and education (ECE) centers in Southwest and Central Connecticut, and data was collected from 2015 to 2020.^{38,39} Eligible participants included women who were (i) English-speaking, (ii) ≥ 21 years old, (iii) African American or Black (self-identified), (iv) mothers to their biological children (ages between 3 and 5 years), and (v) without evidence of psychiatric or cognitive impairment that could interfere with reliable reporting of information as determined by Mini-Mental Status Examination (MMSE).³⁹ Yale

University IRB (approval #1311012986) approved procedures for this study.

Comprehensive study procedures have been previously described.^{38,39} Briefly, trained InterGEN staff measured blood pressure, height, and weight following national guidelines and collected DNA samples (saliva) using Oragene Format tubes.³⁸ Audio self-assisted interviewing software was used to collect sociodemographic, health history, and psychosocial data.³⁹ Researchers collected clinical and psychosocial data every 6 months over 2 years. The present analysis includes data collected at the first visit, as DNAm data was only collected at the first time point.

Instruments and measures

Beck depression inventory. The Beck et al Depression Inventory (BDI-I) measured depressive symptoms spanning affective, cognitive, and somatic domains including sadness, guilt, psychomotor agitation, sleep disturbance, and appetite loss. The tool is highly reliable and valid for assessing both intensity of depressive symptoms and identifying undiagnosed persons who would meet criteria for a depressive disorder.⁴⁰ The BDI consists of 21 items that are individually scored from 0 to 3 according to severity. The sum of item scores represents the overall depressive symptom severity which ranges from normal ups and downs (0-10) and mild and moderate symptoms (11-30) to severe and extreme depression (over 30).⁴⁰ The BDI demonstrates high internal consistency in low-income African American outpatients and is well-validated in populations with chronic medical conditions.^{40,41}

Cardiometabolic traits. Health history and physiologic measures gauged the presence of CM traits. BMI (weight in kg/height in m²) was examined continuously and categorically according to the standards set by the World Health Organization. Accordingly, obesity was defined as BMI ≥ 30 kg/m². Prior diagnoses (self-reported) of hypertension and DM were assessed via a health history questionnaire.³⁹

Sociodemographic. Sociodemographic variables included age, race, ethnicity, income, education, and employment. Although all participants identified as Black/AA, additional racial identities were assessed. The parent study categorized race as American Indian, Asian, Black or African American, Pacific Islander, White, and Other (participants select all that apply) and classified ethnicity as Hispanic (yes/no). Annual household income levels were categorized as less than \$5000, \$5000 to \$9999, \$10 000 to \$14 9999, . . . through \$100 000, and above. Highest level of education ordinal responses included less than high school, high school diploma or GED, some college, Associate's degree, Bachelor's degree, Master's degree, and Doctorate. Employment (yes/no) was defined as any paid employment in the past 12 months including self-employment.

DNA methylation data and assessment of accelerated aging

Calculation of DNAm age in the cohort has previously been described.³¹

Briefly, the Illumina Infinium Methylation EPIC (850K) BeadChip was used to perform the DNAm at over 850 000 CpG dinucleotides, providing near-exhaustive genomic coverage.³⁹ Confirmation of DNAm was achieved by methylation-specific PRC and bisulfite sequencing.³⁹ Fluorescent signals from methylated and unmethylated probes indicated methylation level at each CpG site ($\beta = \max[M, 0/|U| + |M| + 100]$).^{31,38} Sites with missing genotype cell rates over 10% were excluded using a detection *P*-value threshold ($>.001$) for each CpG site.³¹

DNAm age for each participant was calculated using the Horvath²⁵ multi-tissue epigenetic clock and DNAm data from saliva specimens.³¹ The Horvath clock was selected over other DNAm age clocks because of its reliability across tissue and cell types (including saliva), and up until the time of this analysis, other clocks were based on DNAm in blood.²⁶ It is worth noting that other blood-based clocks such as PhenoAge and GrimAge have performed well even in other tissues^{42,43}; however, the phenotypic characteristics and plasma proteins required for the calculation of these clocks were not available in this dataset. Further, as this was a secondary analysis of existing data generated from DNAm data, rather than the reanalysis of raw DNAm data, determination of biological age by other clocks was not feasible.

The Horvath epigenetic clock is based upon an elastic net regression analysis from over 8000 samples from 82 publicly available Illumina datasets. β values from 353 CpG sites specified by the Horvath model were plugged into the following formula to calculate DNAm age.

$$\text{DNAm Age} = \text{inverse.F}(b_0 + b_1\text{CpG}_1 + \dots + b_{353}\text{CpG}_{353})$$

DNAm AA, an indicator of more rapid aging, was defined as the residual term when DNAm age is regressed on chronological age,^{25,31} a distinction from Δ Age (ie, the difference between DNAm age and chronological age by subtraction).

Statistical analysis

Power analysis estimated that 191 participants were needed to detect a minimal effect size (correlation coefficient = .2) at 80% power and an alpha level of .05. Effect size was estimated from prior studies that examined associations between DNAm AA and depressive symptoms showing an effect size range between 0.18 and 0.27.^{27,35,44} The final sample included 227 African American mothers, exceeding the 191 participants necessary for 80% power. Individuals lacking depressive symptom data or saliva samples insufficient to calculate DNAm age ($n=23$) were not eligible for inclusion in the present analysis.

Normality assumptions were tested with histograms, scatterplots, and Shapiro-Wilks testing. Since the questionnaires have a risk for social desirability bias, patterns of missingness (completely missing at random, missing at random, and not missing at random) were assessed.

Less than 10 observations were missing across sociodemographic and BDI items, and these participants were excluded from the corresponding analysis. Missing data from these questionnaires revealed a non-monotone pattern of missingness and no evidence of non-ignorable missingness as there were no significant associations between cases with missing data and the values of the other observed variables.

Spearman's correlations and linear regression examined associations between DNAm AA and BDI scores in the total sample and subsamples with versus without one or more CM traits. Logistic regression examined associations between DNAm AA and categorical variables including CM traits. Variables that demonstrated significant association (ie, CM traits) with DNAm AA in unadjusted models were assessed again in models adjusted for smoking, income, education, marital status, ethnicity, and depression as covariates.^{45,46} Wilcoxon signed-rank tests examined differences in BDI scores between participants with or without a CM trait and those with positive versus negative age acceleration. Chi-squared tests examined associations between age acceleration (binary) and categorical sociodemographic and clinical variables (eg, marital status, smoking). All analyses for this study were performed in R studio with R version 4.0.0. Descriptive and inferential statistics were performed utilizing [psych] and [hmisc] packages, and missingness analysis was conducted using the R packages [naniar], [finalfit], [ggplot], and [dplyr].⁴⁷⁻⁵²

Results

Descriptive statistics

The sample for this study included 227 African American women with complete data for DNAm age and recorded depressive symptom data. Chronological age was correlated strongly with DNAm age ($r = .918$, $P < .001$) (Figure 1). Table 1 presents sample characteristics and DNAm AA results generated from regression analysis of DNAm age on chronological age. Overall, this sample demonstrated a younger median DNAm age (28.13 years) than chronological age (31.23). Over half the sample (54.19%) had at least 1 CM trait including hypertension (20.96%), DM (6.17%), or obesity as defined as a BMI of 30 or above (44.93%). Depressive symptom scores were heavily right-skewed (4, [IQR 1-11]), and 72.25% scored within a range indicative of normal ups and downs. A total of 52 participants identified as current smokers (22.91%).

Spearman's correlations indicated a significant positive association between DNAm AA and BMI (P -value $< .001$; Table 2). Contrary to our hypothesis, no significant associations were observed between overall depressive symptom severity (BDI total score) or symptom domain scores (affective, cognitive, and somatic depressive symptoms scores) and DNAm AA. No significant associations between depressive symptoms and DNAm AA were observed in subsamples with and without a CM trait (Table 3). In further exploratory analysis of BDI items (Supplemental Table 1), DNAm AA

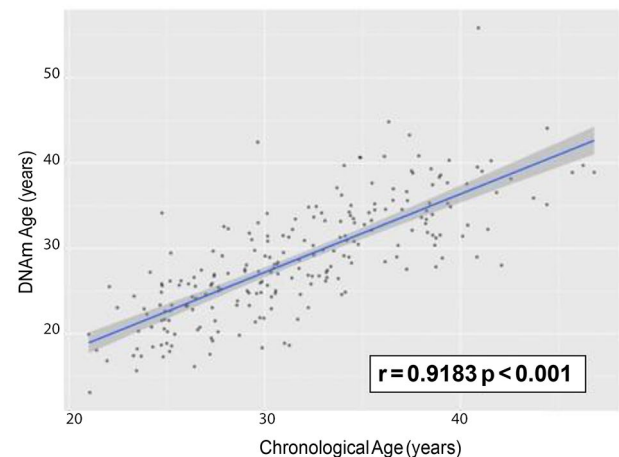


Figure 1. Linear regression calculating DNA methylation age acceleration. Significant correlation between chronological age and DNAm age in 227 African American women from the InterGEN study. The blue line is the regression line and the surrounding gray area indicates confidence interval. Points above or below the regression line indicate positive age acceleration (ie, individuals biologically older than chronological age) and age deceleration (ie, individuals biologically younger than chronological age) respectively. DNAm age acceleration refers to the residuals between each observation and the regression line.

demonstrated negative correlations with specific depressive symptoms scores including disappointment, disgust, or hatred toward self (BDI item 7), frequency of crying (BDI item 10), and a positive association with BDI item 20, worry about physical health. However, the application of a Bonferroni correction reduced the alpha significance threshold to a value of $P \leq .0024$. No symptom remained significantly associated after this correction for multiple comparisons. In subsequent regression analysis (Table 4), none of the aforementioned symptoms significantly associated with DNAm AA.

Logistic regression analysis demonstrated significant positive associations between DNAm AA and CM traits (Table 5)—specifically hypertension, DM, and obesity, in unadjusted and adjusted models which included smoking and sociodemographic factors as covariates. Wilcoxon signed-rank tests detected no significant differences between depressive symptom severity scores between subsamples with or without at least 1 CM trait (binary) or subsamples divided according to positive versus negative DNAm AA (binary). DNAm AA was not significantly associated with depression clinical cut-offs ($\text{BDI} \geq 10$), or sociodemographic variables (ie, marital status, multiracial status, ethnicity, education, income, or employment) in Chi-squared analysis.

Discussion

Refuting our initial hypothesis, no association between DNAm AA—as determined by the Horvath epigenetic clock—and total depressive symptoms was detected among the AAW sampled from the InterGEN study. There was also no association between DNAm AA and specific depressive symptom domain subscores nor were there associations between DNAm AA and depressive

Table 1. Summary statistics of sample characteristics.

CHARACTERISTICS	MEDIAN/N	(IQR)/(%)
Chronological age (years)	31.23	(27.32, 35.62)
DNA methylation age (years)	28.81	(24.36, 32.47)
Δ Age (years)	-3.19	(-5.93, -0.43)
DNAm AA (years)	-0.12	(-2.98, 2.58)
Age acceleration (binary)		
No	175	(77.09%)
Yes	52	(22.91%)
Hispanic/Latina		
No	208	(91.63%)
Yes	19	(8.37%)
Multiracial		
No	221	(97.36%)
Yes	16	(7.05%)
Marital status		
Married	54	(23.79%)
Single	149	(65.64%)
Other	24	(10.57%)
Education		
Less than high school	13	(5.73%)
High school/GED	78	(34.36%)
Some college	76	(33.48%)
Associate's degree	26	(11.45%)
Bachelor's degree	25	(11.01%)
Master's degree	7	(3.08%)
Doctorate	2	(0.88%)
Annual income		
<\$5000	51	(22.47%)
\$5000-\$9999	30	(13.22%)
\$10000-\$14999	26	(11.45%)
\$15000-\$19999	18	(7.93%)
\$20000-\$24999	22	(9.69%)
\$25000-\$34999	30	(13.22%)
\$35000-\$49999	27	(11.89%)
\$50000-\$74999	12	(5.29%)
\$75000-\$99999	7	(3.08%)
\$100000 or higher	4	(1.76%)

(Continued)

Table 1. (Continued)

CHARACTERISTICS	MEDIAN/N	(IQR)/(%)
Employed		
No	70	(30.84%)
Yes	155	(68.28%)
Depression score (total)	4	(1, 11)
Affective subscore	0	(0, 2.25)
Cognitive subscore	1	(0, 3)
Somatic subscore	2.5	(0, 5)
Possible MDD		
No: BDI < 10	164	(72.25%)
Yes: BDI \geq 10	63	(27.75%)
Ever diagnosed HTN		
No	181	(79.74%)
Yes	46	(20.26%)
Systolic BP, mmHg	113.33	(105.67, 120.00)
Diastolic BP, mmHg	72	(64.67, 79.33)
Ever diagnosed DM		
No	213	(93.83%)
Yes	14	(6.17%)
BMI, kg/m ²	28.67	(23.53, 34.21)
Obese (BMI \geq 30)		
No	125	55.07%
Yes	102	(44.93%)
Any CM trait		
No	104	(45.81%)
Yes	123	(54.19%)
Smoking (current)		
No	175	(77.09%)
Yes	52	(22.91%)

Abbreviations: BMI, body mass index; BP, blood pressure; CM, cardiometabolic; DM, diabetes mellitus; HTN, hypertension; MDD, major depressive disorder.

DNA methylation age refers to the biological age calculated by the Horvath epigenetic clock; Δ Age is the difference between DNA methylation age and chronological age; DNAm AA is (DNA methylation age acceleration) is the value of the residual of DNA methylation age regressed on chronological age in a linear model.

symptoms in subsamples with versus without CM traits. Irrespective of depressive symptoms, significantly higher DNAm AA was associated with participants with DM, hypertension, and obesity. In this sample, we observed negative DNAm AA (ie, participants were biologically younger than chronological

Table 2. Associations between DNAm AA and depressive symptoms and cardiometabolic indicators as determined by Spearman's correlations in the sample of African American mothers from the InterGEN study (n=227).

VARIABLE	DNAm AA
Depression score	−0.02
Affective subscore	−0.13†
Cognitive subscore	−0.05
Somatic subscore	0.02
Disappointment, disgust, or hatred of oneself	−0.13*
Frequency of crying	−0.08
Difficulty making decisions	−0.15*
Worry/preoccupation with physical health	0.15*
Systolic BP	0.12
Diastolic BP	0.12†
BMI	0.25***

* $P < .05$. *** $P < .001$. † $P \leq .06$.

Table 3. Results from Spearman's correlations between age acceleration variables, depressive symptoms, and cardiometabolic indicators in the CM trait subsample (n=123) and no CM trait subsample (n=104).

VARIABLE	CM TRAIT (N=123)	NO CM TRAIT (N=104)
Depression score	0.03	−0.11
Disappointment, disgust, or hatred of oneself	−0.13	−0.10
Frequency of crying	−0.03	−0.11
Difficulty making decisions	−0.16	−0.16
Worry/preoccupation with physical health	0.16†	0.04

* $P < .05$. ** $P < .01$. *** $P < .001$. † $P \leq .06$.

age), an unexpected finding in light of the known risk factors typical of the target population. These findings, however, are consistent with a prior analysis in the InterGEN cohort³¹ and with reported slower rates of biological aging among AAW compared to men and White women.⁵³ The possibility exists that AAW may not have been well represented in the training data used to generate the estimate of biological age, and concern for racial bias has been raised in similar DNAm age clocks as many methylation sites included in these algorithms are related to genetic ancestry as well as aging.⁵⁴ Alternatively, this finding could represent resiliency—perhaps more characteristic of healthier self-selected volunteers than the target population in general—warranting further exploration of this paradoxical discrepancy between biological and chronological age.

Table 4. Results from linear regression of DNAm AA and depressive symptoms.

VARIABLE	β	(95% CI)
Depression score	−.01	(−0.53, 1.19)
Affective subscore	−.19	(−0.45, 0.064)
Disappointment, disgust, or hatred of oneself	.33	(−0.53, 1.19)
Frequency of crying	−.43	(1.23, 0.39)
Difficulty making decisions	−1.01†	(−2.16, 0.19)
Worry/preoccupation with physical health	.73†	(−0.07, 1.54)

† $P \leq .06$.

Table 5. Results from logistic regression models of DNAm AA and cardiometabolic traits.

CARDIOMETABOLIC TRAIT	UNADJUSTED		ADJUSTED MODEL**	
	β	(95% CI)	β	(95% CI)
Hypertension	.08	(1.00, 1.17)	.08	(1.01, 1.17)
Diabetes	.18	(1.01, 1.35)	.20	(1.09, 1.40)
Obesity	.07	(1.01, 1.15)	.08	(1.02, 1.16)

**The adjusted model includes smoking, income, education, marital status, ethnicity, and total depression score as covariates.

Consistency of findings with similar studies

Overall depressive symptom severity. Although several lines of evidence suggest that depression and depressive symptoms are associated with age-dependent molecular markers and age-related pathologies,^{22,27,28,34} we observed no evidence of association between depressive symptoms and DNAm AA, similar to studies prior studies with more representation of African Americans.^{33,55} Crawford et al⁵⁵ did not detect an association between participants with a history of depression and Horvath-estimated DNAm AA; however, they did observe significantly decreased telomere lengths in these individuals. In contrast, Whalley et al²⁸ reported significant DNAm AA with a small effect size (0.20year) estimated by the Horvath (but not Hannum) clock among subjects with depression in a study of 1219 depression cases and 3833 healthy controls. Transcriptomic and age-dependent gene expression methods have detected positive associations between age acceleration and depressive symptoms.^{22,34} Collectively, these findings suggest that the associations between age acceleration and depressive symptoms may be highly nuanced and susceptible to reduced detectability in certain samples and study design contexts. This nuance is plausible given that the aforementioned studies that detected associations based their DNAm analysis on blood or brain tissue, a distinction from this study which used saliva samples. Distinctions in sample characteristics between this study and prior studies may have further reduced comparability and/or

detectability of any effect in this sample of AAW. For example, all but two^{33,34} of the aforementioned studies included samples of predominately White males and females—a large proportion of whom had depressive scores near or exceeding the clinical threshold for diagnosis of major depressive disorder.^{27,28,35,44} In contrast, the InterGEN cohort is comprised exclusively of Black/African American mothers with predominately low levels of depression symptoms, typical of self-selected volunteers.

Specific depressive symptoms. Although no significant association was found concerning total depressive symptom severity scores and DNAm AA, the findings of this secondary analysis do not preclude the possibility of depressive symptom associations with biological aging. Another study that examined DNAm AA in a sample of 3720 men and women found that although DNAm AA (Horvath) did not significantly associate with total depressive symptom scores, increased DNAm AA was associated with reduced positive affect, even after adjusting for multiple comparisons,³⁵ in contrast to the current study.

Several of the specific depressive symptoms that demonstrated a correlation (both positive and negative), before correction for multiple comparisons, with DNAm AA in the current study (ie, self-hate/disgust, difficulty with decision making, and worry/preoccupation with health), have been identified in relation to age acceleration in prior studies.⁵⁶⁻⁵⁸ The concept of self-hate may have particular relevance to the population of AAW,⁵⁹ but this has yet to be fully explored in relation to aging. Self-hate/disgust and difficulty with decision-making are prominent depressive symptoms among persons with chronic conditions, including CM illnesses,^{57,58} which makes the negative correlation between DNAm AA and self-hate/ self-disgust, observed in this sample unexpected and worthy of validation and additional follow-up. Increased health worry, anxiety, and/or cumulative stress had been associated with increased DNAm AA,³³ and limitations in cognitive functioning, including decision-making difficulty, have demonstrated associations with increases in Horvath-estimated DNAm AA.^{60,61} The initial findings of the current study that these symptoms varied in correspondence with DNAm AA are not inconsistent with the notion of heterogeneity in depression; however, multiple comparison correction yielded non-significant findings, which may be resultant in part from reduced detectability in a sample whose overall depression scores were low.

Age acceleration and cardiometabolic illness. With respect to age acceleration associations in CM illness, the findings of this study are consistent with prior analysis showing that DNAm AA associates with hypertension,^{31,62} but in contrast with earlier studies less representative of AAW,^{29,30} suggesting that this finding is particularly salient for this population. Increased DNAm AA has also been demonstrated in cardiovascular disease and hypertensive target organ damage.³⁰ Therefore, detectability of age acceleration in cardiac disease may be

threshold dependent, relative to disease stage, and variable across samples.

The results of this analysis are consistent with findings from a prior analysis by the InterGEN study team that demonstrated a strong positive relationship between BMI and obesity-related traits and DNAm AA.³¹ The current study adds to the growing body of literature concerning the relationship between DM and accelerated aging.²⁰ Among women with DM, increased Horvath-estimated DNAm AA has been associated with increases in BMI, waist circumference, and fasting blood glucose,³² and recently GrimAge accelerated aging has demonstrated associations with fasting insulin in African Americans⁶². The current secondary analysis further demonstrates significant associations between DM/obesity and DNAm AA, specifically in AAW, a noteworthy finding considering the population's high degree of CM risk.

Other factors. Although we examined depressive symptoms in relation to DNAm AA in this study, additional factors including discrimination and racism could also play a role in DNAm AA. Mounting evidence confirms the damaging effects of perceived discrimination and other components of racism on health,^{63,64} particularly CM health in AAW.⁶⁵⁻⁶⁷ In line with the notion of biological weathering^{68,69}—described as the early deterioration of health as a physiologic result of socioeconomic disadvantage—several studies show associations between measures of racial discrimination and socioeconomic stress with accelerated aging and DNA methylation among AAW.⁷⁰⁻⁷³ Further, experiences of discrimination may also affect children of AAW, through effects on parenting stress, highlighting the intergenerational impact of discrimination on health.^{71,74} Future studies should consider this major stressor among AAW when examining DNAm AA.

Study strengths and limitations

Although a target sample size was determined through power analysis centered on the small to moderate effect size reported in prior studies,^{27,28,35,44} this study may have been underpowered to detect small, but meaningful, associations between depressive symptom severity scores and DNAm AA. Generalizability may be affected by the relationship between fertility and biological aging as well as the role that menopause plays in accelerating biological aging.^{75,76} Because the participants in this sample were all mothers with a median chronological age under 35 years and low BDI scores, the results of this study should not be generalized to significantly depressed, nulliparous, or post-menopausal women. Currently, there remains a dearth of available datasets with both quality genomic data and highly valid depressive symptom measures with samples of AAW—even fewer with data reflecting medical comorbidities. This lack of information underscores the uniqueness and importance of the InterGEN cohort data, which provided a rare, yet urgently needed, opportunity to examine accelerated aging and depressive symptoms in an understudied population with

disproportionate risk. Because of the cross-sectional nature of the study design, there can be no distinction of cause from consequence concerning the noted correlates of DNAm AA.

This study has several notable strengths. First, the rigor and richness of the parent study data allowed for the exploration of the primary research question in a population with disproportionate risk. In addition to the use of a well-validated measure of depressive symptoms, this study also examined domains of depressive symptoms including cognitive, somatic, and affective domains and specific depressive symptoms, in light of the heterogeneity of the phenotype. Additionally, a standard, accessible, and multi-tissue DNAm marker of epigenetic aging was used, enhancing the replicability of these findings for future studies. The current analysis adds to the nascent literature demonstrating associations between DM/obesity and DNAm AA among AAW, a group with disproportionately high rates of morbidity and mortality compared to other groups, including African American men.

Implications for clinical practice and future research

DNA methylation patterns, unlike gene sequences, can be influenced by many environmental behavioral, and lifestyle factors (eg, diet, smoking, stress) throughout the life course. Determinants such as diets high in plants and lean meats and physical activity have been linked to slower epigenetic aging, confirming current wisdom surrounding the relationship between lifestyle and longevity.⁷⁷ Patient education regarding these modifiable risk factors should remain a mainstay of prevention and management for a variety of age-related conditions. The finding that DNAm AA is strongly associated with DM and obesity in this sample underscores the importance and urgency of diabetes and prediabetes prevention and management among AAW. Clinicians must redouble their health promotion and screening efforts—starting at younger ages in this highest-risk population. These evidence-based efforts include monitoring for the development of DM annually, referring patients with prediabetes to intensive lifestyle behavior change programs modeled after the Diabetes Prevention Program that include nutritional counseling, and encouraging weekly targets of at least 150 minutes of moderate-intense physical activity.⁷⁸ Currently, metformin therapy is recommended in those with prediabetes, especially those over age 60 or with BMI ≥ 35 .⁷⁸ In light of both its anti-diabetes and potential anti-aging effects,^{79,80} metformin regimens could conceivably be justified for use earlier within the course of disease development in this population, although further research is needed. Diabetes prevention efforts must also be supported at the systems level, through initiatives aimed at improving access and affordability of care and increasing high-quality patient-centered care environments equipped with coordinated care teams adept at assessing and addressing psychological concerns and social determinants of health (eg, food insecurity and homelessness) known to have detrimental effects on diabetes outcomes.⁸¹

There are several important implications for future studies, specifically the need for larger samples that include wider age ranges, a full array of severity of depressive symptoms, and women across the adult lifespan. Additional studies with larger samples of AAW, particularly genomic studies, which address considerations outlined by the American Heart Association,⁸² are needed to better understand factors related to DNAm AA in AAW. Focus on samples of women with 1 CM illness, and increasing distinctions between groups with and without depressive symptoms may address the need for increased power in future studies by reducing variance and increasing the magnitude of group differences in depressive symptoms. Longitudinal designs could offer an opportunity to assess temporal relationships between epigenetic age acceleration and clinical outcomes. The additional inclusion of environmental, sociodemographic, and stress-related variables, particularly lifetime/cumulative stress and early childhood experiences—as these have been significant in prior studies examining DNAm AA—would further enhance this objective.^{33,83,84} Future studies should also consider the use of more than one method of biological age estimation, as several have been developed and further refined since the completion of this study.

Conclusions

This study aimed to address several substantial gaps in the current literature surrounding depressive symptoms and accelerated aging in AAW with or at risk for CM traits. Despite the noted limitations, this secondary analysis adds to the literature by posing this question in an understudied population with disproportionate risk and approaching inquiry with a focus on symptoms rather than psychiatric diagnostic labels. The notion that epigenetic aging estimated by DNAm markers may be associated with specific depressive symptoms, rather than total depressive symptom scores reinforces the need for the RDoC approach, as suggested by NIMH, to examine potential biomarkers alongside continuous symptoms or domains—as opposed to sole reliance on diagnostic criteria that frequently yields heterogeneous cases and lacks biological validity.¹⁰ This study also demonstrated links between CM traits and accelerated aging, independent of depressive symptoms. Further research with larger, well-defined samples is warranted to clarify these findings and better inform clinical practice.

Authors Contributions

NBP- conceptualization, formal analysis, visualization, writing original draft, writing review and editing, project administration.

GEM- conceptualization, methodology, supervision, writing review and editing.

AAW- conceptualization, methodology, supervision, writing review and editing.


FW- methodology, writing review and editing.

SG- methodology, writing review and editing.

GY- conceptualization, methodology, formal analysis, writing review and editing .

YVS- formal analysis, writing review and editing.
CAC- funding acquisition, writing review and editing.
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Supplemental Material

Supplemental material for this article is available online.

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