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Original Article

Epigenetic Age Acceleration and Chronological Age: Associations With Cognitive Performance in Daily Life

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Abstract

DNA methylation-derived epigenetic clocks offer the opportunity to examine aspects of age acceleration (ie, the difference between an individual's biological age and chronological age), which vary among individuals and may better account for age-related changes in cognitive function than chronological age. Leveraging existing ambulatory cognitive assessments in daily life from a genetically diverse sample of 142 adults in midlife, we examined associations between 5 measures of epigenetic age acceleration and performance on tasks of processing speed and working memory. Covarying for chronological age, we used multilevel models to examine associations of epigenetic age acceleration (Horvath 1, Horvath 2, Hannum, PhenoAge, and GrimAge clocks) with both average level and variability of cognitive performance. Positive age acceleration (ie, epigenetic age greater than chronological age) was associated with poorer mean processing speed (Horvath 1 and 2) and working memory (GrimAge). Higher chronological age was also associated with poorer mean processing speed and working memory performance. Further, positive age acceleration was generally associated with greater intraindividual variability in working memory and processing speed tasks, whereas being chronologically older was associated with less intraindividual variability. Although further work is needed, our results indicate age acceleration effects have comparable or greater size as those for chronological age differences, suggesting that epigenetic age acceleration may account for additional risk and interindividual variation in cognitive performance above chronological age.

Keywords: DNA methylation, Epigenetic clock, Ecological momentary assessment, Processing speed, Working memory

Performance in domains such as processing speed and working memory tends to decline over the adult lifespan (1). Although chronological age is widely used for these comparisons, there remains considerable unexplained variance among similarly aged individuals; that is, individuals of the same age can have vastly different levels of cognitive performance. This is likely because the simple passage of time—which is what chronological age reflects—does not fully capture individual differences in accumulations of biological and environmental influences on cognition (2). In contrast, certain biomarkers of biological age may better explain differences in disease, mortality, and cognitive decline among individuals who are the same chronological age (3). Epigenetic clocks as determined by levels of DNA methylation (DNAm) at key sites in the genome that covary with age have emerged as promising predictors of lifespan, mortality, and neurodegenerative diseases such as Alzheimer's (4–6). These epigenetic clocks provide the opportunity to assess a component of biological aging, and the difference between an individual's biological age and chronological age can be used to produce estimates of "age acceleration." Positive age acceleration, in which a person's epigenetic age is older than their chronological age, has been linked with the risk of disease and mortality (5,7). In sum, age acceleration could represent a more robust predictor for cognition than chronological age. The present study examined age acceleration as a predictor of cognitive function in daily life (eg, outside of laboratory).

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Biomarkers, Epigenetic Clocks, and Epigenetic Age Acceleration

Although all cells of an organism contain the same basic genomic sequence, the chromatin structure can be changed at a tissue or even cell-specific level with epigenetic modifications (8). The most well-studied epigenetic modification found in vertebrates and plants is DNAm, where, predominantly at CpG dinucleotides, the cytosine possesses an additional methyl group (9). Variations in levels of DNAm at particular sites in the genome are thought to alter gene expression, with increased methylation commonly associated with lower gene expression. While DNAm can be heritable, it is subject to variation throughout the lifetime, and there are certain sites in the genome that show close associations (both negative and positive, linear and log) with chronological age, regardless of tissue (5,8,10). With genomic technology (both array hybridization and next-generation sequencing) now allowing the characterization of levels of methylation at millions of sites in the genome simultaneously, researchers have used such patterns to develop "epigenetic clocks" (4,5,8,10). By combining information from only a hundred or so CpG sites, these clocks have a remarkable ability to estimate someone's age within just a few years of accuracy.

The first generation of epigenetic clocks (Horvath 1 (8), Horvath 2 (11), and Hannum (10)) were trained to simply predict chronological age. However, second-generation clocks, such as PhenoAge (5) and GrimAge (4), have been developed using clinical data to predict outcomes such as mortality, and hence can capture both the process of aging beyond chronological age and function as markers of biological aging. Broadly, deviations between epigenetic and chronological ages are thought to represent variation in the rate of biological aging among individuals, with positive deviations representing accelerated biological (relative to chronological) aging and negative deviations representing decelerated biological aging. As such, 2 individuals who have the same chronological age could show differences in cognitive performance that may be explained by differences in their epigenetic clock and their associated age acceleration.

Mean Level and Intraindividual Variability in Cognition

Crystalized cognitive abilities, defined as acquired skills and knowledge (eg, vocabulary), often remain stable until late in life; conversely, fluid abilities, defined as reasoning and problem-solving ability (eg, processing speed, working memory), decline on average as people age chronologically (12). Slower processing speed is a core feature of cognitive aging (13), and processing speed and working memory are the most sensitive cognitive domains related to successful aging (through the absence of disease and disability) (14). Processing speed is a measure of time required to respond to information in one's environment (15). In performance-based assessments of processing speed, items require a simple decision to be made as quickly as possible. Importantly, given unlimited time, most people would complete all items correctly. Working memory refers to the ability to maintain information in active memory while simultaneously performing interfering or distracting activities (16). In performance-based tasks, participants store, maintain, and subsequently retrieve information over brief periods of time. For example, temporarily storing an image or word while performing mental math. While remaining accurate, chronologically older adults tend to perform more slowly in processing speed and more poorly in working memory tasks than those who are chronologically younger (12). This indicates that processing speed and working memory may be important domains for understanding cognitive functioning and decline with age.

To understand age-related decline, it is possible to consider different metrics of performance—for example, what might be considered a person's typical (ie, mean) performance as well as their instability (ie, variability). Although cross-sectional studies have traditionally relied on single or average (ie, mean level) scores, it is possible to consider intraindividual variability along different timescales (eg, trial-to-trial, block-to-block, day-to-day, week-to-week) using repeated trials or assessments across longer intervals of time. Lower single or average scores may be indicative of poorer cognitive performance, whereas greater intraindividual variability is proposed to be an early indicator of poor cognitive outcomes (17,18). Specifically, greater intraindividual variability on cognitive tasks has been associated with cognitive decline (19) (trial-to-trial), mild cognitive impairment classification (20,21) (day-to-day, trial-to-trial), cognitively impaired-not-dementia classifications (22) (trial-to-trial), and dementia (23) (occasion-to-occasion). Prior work shows older adults tend to have higher variability in their performances than younger adults (24–26); however, this finding may be dependent on the timescale examined (eg, trial, occasion, day) (27). In sum, examining individuals' mean performance and variability in performance may better profile their overall cognitive function and provide sensitive indicators of early cognitive problems than mean performance alone.

Epigenetic Age Acceleration and Cognitive Performance

The literature on age acceleration and cognitive performance in processing speed and working memory tasks is limited, yet growing. In models controlling for chronological age, prior studies have found associations between second-generation clocks and lab-based cognitive performance, but not first-generation clocks (28–30). For instance, in a sample of 45- to 87-year-old individuals, greater age acceleration in second-generation PhenoAge and GrimAge clocks is related to slower processing speed (31). This pattern was also found for GrimAge in a sample of individuals over 50 years of age (7). In longitudinal mediation models using the Health and Retirement Study sample of 65- to 98-year-old individuals, women's slower age acceleration rates in GrimAge fully accounted for their faster processing speed (32). In sum, second-generation clocks that were trained on health-relevant phenotypic outcomes are promising for understanding differences in performance in common lab-based assessments.

The aforementioned studies use single scores, means, or composite scores from lab-based processing speed or working memory tasks. Laboratory settings, however, may not always be congruent with real-world settings where people typically perform more complex cognitive demanding tasks (33). In contrast, ambulatory assessment designs, in which

repeated assessments occur over multiple days, allow for data collection to occur in individuals' daily life (eg, natural environments outside of the laboratory that vary in location and activity contexts). Whereas contextual factors are normally controlled in laboratory settings, ambulatory assessments capture moments in everyday life that vary from day to day (eg, social company, location, and mood states) (34)—potentially revealing how cognitive performance varies across different times of days, situations, or activities across adulthood. Further, repeated assessments across multiple days of data collection improve reliability for measuring individual differences, as estimates are derived from many observations (35). Finally, intensive repeated measures allow for the examination of patterning of an individual's performance in terms of intraindividual variability, which has been implicated in cognitive decline. For these reasons, the present study focused on mean level as well as moment-to-moment intraindividual variability.

Present Study

The present study tested associations between individual differences in established epigenetic age acceleration markers with cognitive performance in daily life in terms of mean level of performance and intraindividual variability, while controlling for chronological age differences. We leveraged existing 14-day ambulatory cognitive data to examine the association between age acceleration and performance on processing speed and working memory. Prior work in this data set demonstrated that these ambulatory cognitive tasks are both valid and reliable measures of conventional lab-based processing speed and working memory constructs (33), and reflect the expected pattern of cross-sectional differences associated with chronological age (36).

Based on previous research, we predicted that individuals with greater age acceleration would have slower processing speed and poorer mean levels of working memory in daily life. Based on links between greater intraindividual trial-to-trial variability in performance and impairment, we predicted that individuals with greater age acceleration would show greater moment-to-moment variability in their cognitive performance on each task. We predicted that these effects would be stronger for age acceleration than for chronological age due to studies showing epigenetic age as a more robust indicator for disease and mortality than chronological age. We did not make specific predictions regarding which clocks would be the strongest predictors of cognitive performance, nor possible differences in patterns across tasks due to the lack of prior work on epigenetic age acceleration and cognitive performance in daily life. This study was preregistered (https://osf.io/7rv6t/?view_only=749ea15f48f347c9863b9bd97e8edba1).

Method

Participants

This secondary data analysis draws from the first wave of the Effects of Cognitive Aging, Physiology, and Emotion study (37). Out of 265 study participants who provided blood samples at the first wave of data collection, the present study examined age acceleration in a subset of 142 individuals who had samples with sufficient blood volume to extract DNA and conduct methylation array typing and low-coverage whole genome sequencing (see Supplementary Figure 1). This analytic subsample did not differ from the full sample of participants in age, education, income, self-reported race and ethnicity, or current smoking status (see Supplementary Table 1). Individual ages ranged from 25 to 65 years old (M = 47.20 years, standard deviation [SD] = 11.12; see Supplementary Figure 2), were majority female (65%), and the median annual household income was between $40 000 and $59 000. This sample consisted of 58.45% non-Hispanic Black, 21.13% Hispanic White, 10.56% non-Hispanic White, 5.63% Hispanic Black, 3.52% other, and 0.70% Asian participants, as determined by self-reported race and ethnicity. The average number of cognitive assessments completed among participants is reported in Table 1. On average, participants completed 59.15 cognitive assessments (range = 6–84 assessments). This reflects an average compliance score of approximately 83% (ie, completing ambulatory assessments when prompted to do so).

Procedure

Baseline demographics and ambulatory protocol

Data were collected by the Einstein College of Medicine between 2012 and 2016. Participants were recruited from the Bronx, NY, through systematic probability sampling of the New York Registered Voter Lists. Individuals were mailed introductory letters and received a phone call to establish eligibility (ie, 18 years or older, fluent in English, free of visual impairment). Eligible and interested participants were mailed a packet of self-report surveys to complete at home before visiting the research office. During the office visit, participants received training in the use of study smartphones that were preloaded with an application that administered cognitive assessments to measure cognitive performance in daily life.

Participants carried smartphones that prompted them to complete cognitive assessments at 5 quasi-random times (every 2–3 hours) per day for 14 days (for a total of 70 assessments per task). Some individuals, however, completed more assessments (if they accessed the survey when they were not prompted to do so) or fewer assessments (if they missed prompted assessments). Each cognitive assessment consisted

Table 1. Means, Standard Deviations, and Range From Variables of Interest in the Analytic Sample

	M (*SD*)/%(*n*)	Min	Max
Total cognitive assessments completed	59.15 (13.22)	6	84
Chronological age	47.20 (11.25)	25	65
Epigenetic age acceleration			
Horvath 1	−0.01 (4.11)	−8.55	8.69
Horvath 2	0.008 (4.77)	−10.04	11.45
Hannum	0.02 (3.82)	−9.35	9.03
PhenoAge	0.02 (5.39)	−14.8	18.14
GrimAge	0.01 (3.73)	−9	11.38
Cognitive performance			
Dot memory	3.61 (1.86)	0.13	8.09
N-back	85.36 (28.34)	32.36	156.07
Symbol search	24.03 (5.84)	11.68	45.56

Note: N = 142. SD = standard deviation.

of three brief (~1 minute) validated cognitive assessments (33) presented in the following order: symbol search, dot memory, and N-back (see Supplementary Figure 3). At the end of the 14-day period, participants returned to the research office to return the study smartphone and undergo a blood draw, to which participants consented to future blood and genetic analysis. Thus, blood collection occurred after the 14-day cognitive assessment period. Participants were compensated $160 (receiving additional bonuses for reaching compliance thresholds).

Blood collection and DNA extraction

Drawn blood plasma was collected in yellow top tubes, stored in acid citrate dextrose solution A and frozen at −80°C for long-term storage. Individual extractions were done from 400 µL of blood using the DNeasy Blood and Tissue kit (Qiagen) following the manufacturer's recommendations. Genomic DNA was quantified using the Qubit 3.0 Fluorometer using the dsDNA HS assay kit. Approximately 1 µg of genomic DNA for each specimen ($n = 144$) was sent to Beijing Genomics Institute (BGI, Hong Kong) for low-pass whole genome resequencing using BGI's proprietary sequencing platform and approximately 2 µg per specimen of genomic DNA was sent for genome-wide methylation screening on the Illumina Infinium MethylationEPIC BreadChip that targeted ~850 000 known CpG sites at the Center for Genome Technology (Miami, FL).

Low-pass resequencing and imputation

One submitted sample failed BGI's quality control for library preparation. The remaining 143 samples underwent low coverage (mean ~1.25×) via their DNBSEQ platform with paired end 100 bp reads. Downstream processing to finally imputed variant calls was performed by BGI via Gencove. Mapping to the human genome build GR37 was performed using bwa mem (38), variant calling by GATK (39), and imputation (40) via loimpute-v0.1.5, resulting in diploid calls at ~8 million single nucleotide polymorphisms (SNPs).

Genome-wide DNA methylation data processing and quality control

Average DNAm for all 144 blood samples was assayed at over 800 000 CpG sites throughout the genome using the Illumina MethylationEPIC array. Raw intensity files were processed according to standard protocols using the R packages *minfi* and *wateRmelon*, including sample filtering, CpG probe filtering, and signal correction and normalization. From the raw intensity data, we calculated the median methylation and unmethylated signal for each sample, and estimated each individual's biological sex. All samples had adequate median signal and did not have excessive numbers of CpG probes failing the detection p value cutoff of 10%. We then removed poor-quality CpG probes, checking for those that failed typing in an excessive proportion of our sample or had been determined to be cross-reactive (41). We also excluded CpG probes that mapped to the sex chromosomes from downstream analyses. We then performed color and background correction using *noob*, between-array functional normalization, and within-array Type 1 and 2 probe scaling using beta-mixture quantile normalization. Finally, we set any observed beta values with a poor detection p value (>.05) to missing.

Principal component analysis

Genotype calls were merged with the 1 000 genome populations (42), all missing SNPs were removed, a minor allele frequency cutoff of 1% was imposed, linkage disequilibrium filtering was performed in PLINK (43) using the --indep-pairwise 50 5 0.2 command, and the resulting SNP set thinned by 10% to a final size of ~200k SNPs. Principal component analysis (PCA) was then performed using smartpca (43) with no outliers removed. PC1 and PC2 were extracted to control for population genetic structure in downstream analysis.

Measures

Person-level variables

Chronological age

Chronological age (since birth) was measured from self-reported birth date in the baseline survey.

Age acceleration

Methylation β values were used to estimate epigenetic age and age acceleration for each individual using 3 first-generation DNAm epigenetic clocks: Horvath 1 (8), Horvath 2 (11), Hannum (10) and 2 second-generation DNAm epigenetic clocks: PhenoAge (5), GrimAge (4). Age acceleration represented the extent to which a person's biological age exceeds their chronological age at the time of measurement. We estimated these ages using the approach of Higgins and colleagues (44) via the software PC-clocks, which reduces variance in estimates of epigenetic age that result from technical noise at individual CpG sites by levering other sites sharing similar signals identified from PCA. We could not estimate epigenetic age for 2 samples due to high levels of site missingness, resulting in 142 samples for which we had both epigenetic age and genetic ancestry estimates.

Within-person outcomes

Processing speed

Participants were presented with 3 pairs of symbols at the top of the screen and 2 pairs of symbols at the bottom of the screen (see Supplementary Figure 3). Participants touched the symbol pair on the bottom of the screen that matched one of the symbol pairs at the top of the screen as quickly as possible followed by a 200-millisecond interval for a total of 12 trials. The outcome for this task was the total number of correct responses per minute (metric for speed) and lower scores indicated poorer performance.

Working memory: Dot memory task

During a 3-second encoding phase, participants saw 3 red dots in a 5 × 5 grid for 3 seconds (see Supplementary Figure 3). Next, participants were instructed to touch all the "Fs" in an array of "Es" and "Fs" for 8 seconds. Finally, the original 5 × 5 grid reappeared (now blank), and participants were instructed to touch the 3 cells that contained the red dots from the encoding phase. The outcome for this task was Euclidean error distance summed across trials for each assessment (2 trials each). Higher scores indicated poorer performance (ie, greater distance from the original location).

Working memory: N-back task

This task consisted of 2 phases with 12 trials. In Phase 1, participants were presented with 3 face-up playing cards (see

Supplementary Figure 3). Participants were asked whether the target card (leftmost card) matched the test card (rightmost card). After a 500-millisecond delay, the cards shifted 1 position to the left (ie, the test card in the leftmost box moved offscreen, the middle card became the new test card, the rightmost card moved to the middle, and a new card became the new target card). In Phase 2, the leftmost and middle cards flipped face down to increase the memory demand. Participants indicated whether the face-up target card matched the face-down test card. On incorrect responses, all cards flipped face up briefly as a reminder before returning face down. The outcome for this task was proportion correct per minute (metric for accuracy), with lower scores indicating poorer performance.

Covariates

Self-reported demographic variables (ie, gender, education, current smoking status) were included as covariates to account for demographic characteristics. To account for the effects of variability in genetic ancestry, PC1 and PC2 from the PCA of the imputed genotype data alongside 1 000 genomes were also included as 2 separate continuous covariates (45).

Statistical Approach for Mean Level and Intraindividual Variability Models

To test the effects of age acceleration and chronological age on mean level cognitive performance, we used multilevel models (MLMs) using PROC Mixed in SAS (46) to account for the nested data structure (ie, moments nested within days, nested within participants). Separate, parallel MLMs were tested for each cognitive outcome (ie, dot memory, N-back, symbol search); to avoid collinearity and age acceleration effects, each of the 5 epigenetic clocks was tested separately. This resulted in a total of 5 models for each outcome (ie, 1 model for each clock). These models tested whether chronological age (*ChronAge*) and age acceleration (*AccelAge*) predicted mean performance (see Supplementary Statistical Methods 1 for equations). For interpretation, chronological age was centered at age 45 years old; epigenetic clocks have an interpretable 0 which represents individuals whose chronological and epigenetic age are identical. Thus, the regression coefficient for chronological age represents the difference in average performance per 1-year-older chronological age. The regression coefficient for age acceleration represents the difference in average performance per 1 accelerated year (the effect of being 1 year biologically older than one's chronological age). Both age acceleration and chronological age values represent differences on the same scale (ie, differences in years) and are in the same model. Thus, it is possible to compare the effect sizes directly to each other. Model comparisons used for effect size are described in Supplementary Statistical Methods 2. We included linear and quadratic effects at Level 1 (momentary) to account for trends related to practice with the tasks; a random linear slope was included but the model would not converge for a random quadratic effect, thus it was not included in the models. We included covariates at Level 3 (person) to account for individual differences related to these characteristics.

To test the effects of age acceleration and chronological age on intraindividual variability in cognitive performance, we tested for individual differences in Level 1 variance. Conventional MLMs assume homoscedasticity—that the Level 1 variance (ie, the residuals that represent intraindividual variation) is constant across individuals. This assumption, however, can be tested and individual differences in intraindividual variation can be modeled. We allowed for heterogeneous variances (47) and tested whether chronological age (*ChronAge*) or epigenetic age acceleration (*AgeAccel*) was associated with greater within-person variability in cognitive performance, defined as individuals whose performance from assessment to assessment varied more (48). See Supplementary Statistical Methods 1 for equations.

Results

Descriptive Statistics

Table 1 shows the means, *SD*s, and ranges for variables of interest in the analytic sample. The average age acceleration among all 5 clocks was close to zero indicating that the sample was not, on average, biologically younger or older than their chronological age. The ranges, however, indicated that some individuals in the sample were 8–10 or more years epigenetically older or younger than their chronological age. Figure 1 displays PC1 on the x-axis and PC2 on the y-axis with self-reported race-ethnicity overlayed (see Supplementary Figure 4 for a color version). This figure demonstrates the diverse genetic ancestry in our sample, even among groups of individuals who declare the same self-identified ethnicity, and thus the need to control for this diversity via PCA in our downstream modeling (though we note this does not negate self-identified ethnicity as a correlated but distinct covariate).

Do Age Acceleration and Chronological Age Predict Mean Cognitive Performance?

Processing speed

Symbol search

Table 2 (and Supplementary Table 2) shows that age acceleration derived from either Horvath 1 ($B = -0.25$, standard error [*SE*] = 0.11, $p < .05$) or Horvath 2 ($B = -0.20$, $SE = 0.09$, $p < .05$) indicated that being 1 year epigenetically older than one's chronological age was associated with lower average number of correct responses per minute (regardless of chronological age). Older chronological age was also a significant predictor of poorer performance; for

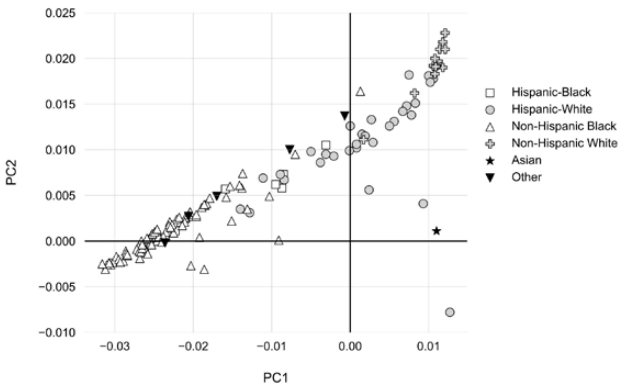

Figure 1. PC1 and PC2 overlayed by self-reported ethnicity. Principal component analysis (PCA) was applied to genotype data to calculate 1000G PC1 and 1000G PC2. PC1 and PC2 are continuous axes of variation that reflect genetic variation due to genetic ancestry in the sample. Each symbol represents a different participant, coded to reflect their self-reported racial—ethnic group. For easier readability, we provide a color-coded version of Figure 1 in Supplementary Figure 2.

Table 2. Mean-Level Results for Dot Memory, N-back, and Symbol Search Tasks

		Dot Memory (Working Memory)	N-Back (Working Memory)	Symbol Search (Processing Speed)
Model	Predictor	Estimate (*SE*)	Estimate (*SE*)	Estimate (*SE*)
#1	GrimAge Accel	0.09* (0.04)	−0.070 (0.60)	−0.08 (0.12)
	Chronological age	0.04** (0.01)	−1.28*** (0.18)	−0.21*** (0.04)
#2	PhenoAge Accel	0.05 (0.03)	−0.28 (0.41)	−0.01 (0.08)
	Chronological age	0.04** (0.01)	−1.32*** (0.20)	−0.22*** (0.04)
#3	Hannum Age Accel	0.05 (0.04)	−0.19 (0.59)	−0.15 (0.11)
	Chronological age	0.04** (0.01)	−1.32*** (0.20)	−0.22*** (0.04)
#4	Horvath 1 Age Accel	0.06 (0.04)	−0.36 (0.52)	−0.25* (0.11)
	Chronological age	0.04** (0.01)	−1.32*** (0.18)	−0.22*** (0.04)
#5	Horvath 2 Age Accel	0.04 (0.03)	−0.26 (0.46)	−0.20* (0.09)
	Chronological age	0.04** (0.01)	−1.32*** (0.18)	−0.21*** (0.04)

Notes: Accel = acceleration; *SE* = standard error. Models used method = ML. GrimAge Accel and PhenoAge Accel are second-generation clocks. Hannum Age Accel, Horvath 1 Accel, and Horvath 2 Accel are first-generation clocks. Except for GrimAge Accel models, in which we excluded current smoking status, all models controlled for gender, education, current smoking status, linear practice effects, quadratic practice effects, PC1, and PC2. The full set of results for these models, including fixed effect estimates for covariates and random effects, can be found in Supplementary Tables 2–4.
***$p < .001$. **$p < .01$. *$p < .05$.

each year beyond the sample's chronological average age of 45, individuals had lower average numbers of correct responses per minute in the symbol search task. Coefficient effect size estimate tests showed that epigenetic clock coefficients did not differ significantly from chronological age (Horvath 1: $p = .80$; Horvath 2: $p = .90$), indicating that age acceleration effects were similar in size to chronological age differences.

Working memory

Dot memory

GrimAge was a significant predictor for worse mean performance (Figure 2). As estimated by the GrimAge clock, being 1 year epigenetically older than one's chronological age resulted in a greater average Euclidean error distance ($B = 0.09$, $SE = 0.05$, $p < .05$). Chronological age was also significant; for each year chronologically older than the sample's average age of 45, individuals had greater average Euclidean error distance (Table 2 and Supplementary Table 3). Coefficient effect size estimate tests for GrimAge and chronological age did not differ significantly from each other ($p = .20$), indicating that GrimAge effects were similar in size to chronological age differences.

N-back

Age acceleration across all clocks was nonsignificant predictor for mean N-back performance. As with the other cognitive outcomes, older chronological age was associated with lower proportion of correct answers per minute on the N-back task (Table 2 and Supplementary Table 4).

Do Age Acceleration and Chronological Age Predict Intraindividual Variability in Cognitive Performance?

Processing speed

Symbol search

Age acceleration from 2 clocks and chronological age were significant predictors of intraindividual variability in processing speed (Table 3 and Supplementary Table 5). Intraindividual variability was larger with positive Horvath 1 age acceleration; specifically, the residual variance (on log scale) for individuals who were 1 year biologically older than their chronological age was 0.01 units higher ($SE = 0.004$, $p < .01$). For PhenoAge, however, this finding was in the opposite direction (ie, less variability with positive age acceleration; $B = -0.01$, $SE = 0.003$). No significant associations emerged for the other clocks. For each year beyond the sample's chronological age, individuals showed less intraindividual variability. Likelihood ratio tests compared the effect size between chronological age and age acceleration. For both Horvath 1 [chisqdiff(1) = 58, $p < .001$] and PhenoAge [chisqdiff(1) = 21.4, $p < .001$] models, age acceleration had a larger effect size than chronological age.

Working memory

Dot memory

Age acceleration from all 5 clocks and chronological age were significant predictors of intraindividual variability in processing speed (Table 3 and Supplementary Table 6). Intraindividual variability was larger with positive age acceleration for all 5 clocks. In contrast, for each year older than the chronological average of 45, individuals showed less intraindividual variability. Likelihood ratio tests compared the effect size between chronological age and age acceleration within each model. For all models, age acceleration showed larger effects than chronological age [GrimAge: chisqdiff(1) = 29.6, $p < .001$; PhenoAge: chisqdiff(1) = 74.5, $p < .001$; Hannum: chisqdiff(1) = 56, $p < .001$; Horvath 1: chisqdiff(1) = 58.3, $p < .001$; Horvath 2: chisqdiff(1) = 55.5, $p < .001$].

N-back

Age acceleration from 1 clock and chronological age were significant predictors of intraindividual variability in processing speed (Table 3 and Supplementary Table 7). Only Horvath 1 age acceleration emerged as a significant predictor of variability, where intraindividual variability was larger with positive age acceleration (residual variance = 0.009, $SE = 0.004$, $p < .01$; Table 3). In contrast, older chronological age was

associated with less intraindividual variability. Likelihood ratio tests showed that age acceleration, as estimated by Horvath 1, had a larger effect size than chronological age [chisqdiff(1) = 58, $p < .001$].

Discussion

Our study is among the first to examine associations between chronological age and markers of epigenetic age acceleration on daily cognitive performance. We replicated prior work in this sample (33,36) which had supported theoretical predictions for chronological age-related declines in working memory and processing speed (1,49). Specifically, chronologically older adults showed poorer mean-level performance in processing speed (ie, symbol search) and working memory (ie, dot memory, N-back). Importantly, in a diverse sample of middle-aged urban dwelling adults, we applied 5 DNAm-based epigenetic clocks using 3 first-generation (Horvath 1 (8), Horvath 2 (11), Hannum (10)) and 2 second-generation (PhenoAge (5), GrimAge (4)) algorithms and tested whether epigenetic age acceleration predicted differences in both mean and within-person variability, while accounting for the established chronological age differences. Below, we interpret the age acceleration results from different clocks.

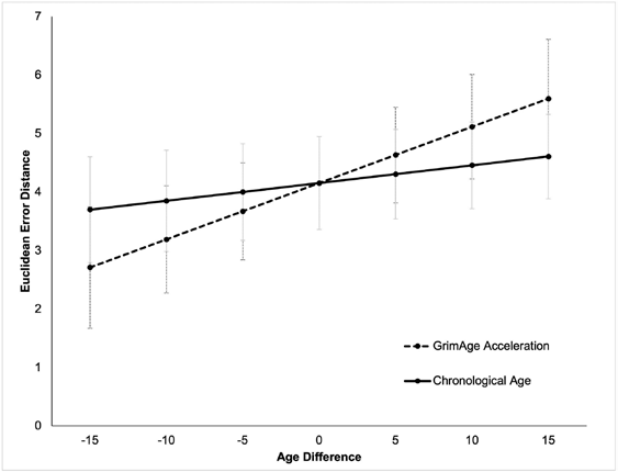

Figure 2. Estimated mean-level performance in dot memory for GrimAge acceleration and chronological age across negative and positive difference scores. The x-axis represents an age difference score spanning from −15 to +15. Zero represents alignment with one's epigenetic age and chronological age (for GrimAge) and a 45-year-old individual (for chronological age). An age difference score for someone +5 years in GrimAge acceleration represents a positive 5-year difference score between their chronological age and GrimAge, thus would represent accelerated aging. An age difference score for someone +5 years in chronological age represents a positive 5-year difference score from the samples average chronological age of 45 years old; thus, someone who is 50 years old. Similar patterns would hold for negative difference scores.

Positive accelerated aging was associated with worse mean performance for several clocks (ie, both Horvath clocks predicted worse performance in the processing speed task and GrimAge predicted worse performance in 1 working memory task); all these effects were similar in size to those of chronological age differences. A unique aspect of our study utilized ambulatory measures of cognitive performance. Our age acceleration findings are broadly consistent with the limited past literature utilizing in-lab cognitive tasks. Previous studies found associations between processing speed and second-generation clocks (7,31), but not first-generation clocks. Our results for processing speed, however, were the reverse; only age acceleration from first-generation clocks (ie, Horvath 1 and 2) predicted mean symbol search performance. To our knowledge, only 1 study has examined the relationship between age acceleration in first-generation epigenetic clocks and working memory, in which no significant associations were found (29). For working memory tasks, our results showed that only GrimAge, a second-generation clock, significantly predicted mean dot memory performance; no significant associations were found for mean N-back. It is possible that no significant associations were observed between mean-level performance in the N-back task and first- or second-generation clocks due to the lack of variability in overall performance in this task.

Table 3. Intraindividual Variability Results for Dot Memory, N-Back, and Symbol Search

		Dot Memory (Working Memory)	N-Back (Working Memory)	Symbol Search (Processing Speed)
Model	Predictor	Estimate (*SE*)	Estimate (*SE*)	Estimate (*SE*)
#1	GrimAge Accel	0.02*** (0.004)	−0.0006 (0.005)	0.005 (0.004)
	Chronological age	−0.004* (0.002)	−0.04*** (0.002)	−0.03*** (0.002)
#2	PhenoAge Accel	0.03*** (0.003)	−0.0009 (0.003)	−0.01** (0.003)
	Chronological age	−0.005** (0.002)	0.05*** (0.002)	−0.03*** (0.002)
#3	Hannum Accel	0.03*** (0.005)	−0.0005 (0.004)	−0.008 (0.005)
	Chronological age	−0.005** (0.002)	−0.05*** (0.002)	−0.03*** (0.002)
#4	Horvath 1 Accel	0.03*** (0.004)	0.009* (0.004)	0.01* (0.004)
	Chronological age	−0.004** (0.01)	−0.05*** (0.002)	−0.03*** (0.002)
#5	Horvath 2 Accel	0.02*** (0.004)	0.004 (0.004)	0.005 (0.004)
	Chronological age	−0.004** (0.31)	−0.05*** (0.002)	−0.03*** (0.002)

Note. Accel = acceleration; *SE* = standard error. Models used method = REML. GrimAge Accel and PhenoAge Accel are nd generation clocks. Hannum Age Accel, Horvath 1 Accel, and Horvath 2 Accel are 1st generation clocks. Negative values represent less variability, whereas positive values represent more variability with increasing chronological age or epigenetic age acceleration. Except for GrimAge Accel models, in which we excluded current smoking status, all models controlled for gender, education, current smoking status, linear practice effects, quadratic practice effects, PC1, and PC2. The full set of results for these models, including estimates for covariates and random effects, can be found in Supplementary Materials: eTables 5-7. ***p < .001. **p < .01. *p < .05.

The associations between first-generation clocks (and processing speed) and second-generation clocks (and working memory) may be partially explained by how the epigenetic clocks were trained. First-generation clocks were designed to predict chronological age, whereas second-generation clocks included clinical biomarkers in their estimations. Prior work has shown that chronological age may be a stronger predictor of processing speed, whereas physical health parameters (eg, morbidity) may be a stronger predictor of working memory (14). This may explain why we saw associations between processing speed and first-generation epigenetic clocks that were trained on chronological age, and between working memory and second-generation epigenetic clocks that were additionally trained on clinical biomarkers.

Assessing ambulatory working memory and processing speed allowed us to test for unique associations between chronological age and age acceleration on within-person moment-to-moment variability. For the processing speed task and both working memory tasks, older chronological age was associated with lower intraindividual variability. Given the associations between within-person variability and disease, one might expect in a mixed-age sample that chronological age would be associated with greater intraindividual variability. However, our results align with prior studies in which chronologically older adults (compared to chronologically younger adults) had less day-to-day variability on cognitive tasks (27). Indeed, this pattern highlights the distinction between chronological aging (the passage of time) and biological aging (degradation of biological systems), and the potential utility of age acceleration for understanding cognitive function in adulthood. Consistent with our hypotheses, for all 5 clocks, positive age acceleration was associated with greater intraindividual variability in performance on the dot memory task, which evaluates working memory. This effect was also observed on the other working memory task (ie, N-back) for the Horvath 1 clock and for the processing speed task (ie, symbol search) for the Horvath 1 clocks. Further, in all models in which epigenetic clocks were significant predictors, age acceleration had larger effect sizes than chronological age, indicating that age acceleration may be a more robust predictor for within-person variability.

The opposite direction of associations that were observed, between measures of biological or chronological age and intraindividual variability, highlights that chronological age may reflect an individual's nonbiological influences (eg, developmental, cohort, and period effects), whereas epigenetic age may reflect an individual's biological influences (eg, epigenetic changes); the latter may be affected by broad environmental influences (eg, neighborhood disadvantage, incarceration exposure, discrimination experiences) that chronological age does not capture. Overall, our results suggest that there is utility in examining age acceleration in conjunction with chronological age. Further research is needed to disentangle what specific aspects of biological aging these epigenetic biomarkers are capturing, given that systematic reviews have found insufficient evidence to draw causative conclusions between environmental, lifestyle, and health factors in predicting age acceleration (9,50).

Strengths, Limitations, and Future Directions

There are many strengths of the present study, including diversity in self-reported race and ethnicity (as well as genetic ancestry) of the sample, our novel research questions accessing mean level and intraindividual variability of cognitive performance, and sampling in midlife to counteract cohort selection bias (eg, individuals with lower inherent mortality rates are likely to survive to older ages than their peers with high mortality rates, and thereby are more likely to be observed at older ages). We also note several limitations. First, although age acceleration was estimated from 5 different clocks, each clock was produced from blood samples drawn at the study's baseline. Similarly, although the ambulatory cognitive assessments provide intensive repeated measures over 2 weeks, this provides a profile of the individual at baseline but cannot speak to patterns of longitudinal cognitive change over years. Future work should include repeated assessments of age acceleration to evaluate how acceleration or deceleration in epigenetic age across time is related to longitudinal changes in cognitive performance. Second, our study did not consider immediate environmental influences (eg, unpredictable life demands) on cognitive performance variability at different timescales. Importantly, our cross-sectional results make it impossible to determine whether age acceleration was the cause of worse mean-level performance and greater intraindividual variability or if having worse mean level and more variable cognitive performance leads to age acceleration through an unknown mechanism (eg, age-related disease). Given the cross-sectional design, we frame this study as an examination of contemporaneous individual differences in biological aging and cognitive performance. Although there is not strong evidence that an individual would change in their age acceleration in such a short amount of time (ie, 2 weeks), it is important to acknowledge that the 2 weeks of ambulatory cognitive assessment preceded the blood draw. Additionally, as a secondary data analysis, our analytic data set was limited to the existing data in terms of sample size and cognitive assessments available; future studies may benefit from examining associations with other cognitive outcomes beyond processing speed and working memory. Finally, as technology continues to advance, other epigenetic clocks are being developed that may be more useful for predicting cognitive outcomes.

Conclusion

Chronological aging is associated with a progressive loss in processing speed and working memory. Yet, chronological age alone cannot explain the heterogeneity in age-related disease and decline. The current study explored cross-sectional associations between chronological age and epigenetic age acceleration on cognitive performance in daily life. Our findings suggest that markers of biological aging may provide insight into cognitive performance during midlife, not only in terms of how people differ from each other, but also in how individuals' cognitive function varies from 1 moment to the next.

Supplementary Material

Supplementary data are available at *The Journals of Gerontology, Series A: Biological Sciences and Medical Sciences* online.

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Conflict of Interest

None.

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Author Contributions

D.V.Z. and S.B.S designed the study and conducted the statistical analyses. J.E.G., C.E., and M.J.S. led the development of the ESCAPE study that generated the data used in this study. K.V., K.R., N.D., and S.G. led the methods and analysis of the epigenetic clocks. All authors critically revised the manuscript and contributed important intellectual content. D.V.Z. holds all data files and analysis files to enable replication of the findings.

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