THE GERONTOLOGICAL SOCIETY OF AMERICA®

Journals of Gerontology: Biological Sciences
cite as: *J Gerontol A Biol Sci Med Sci*, 2022, Vol. 77, No. 12, 2402–2412
https://doi.org/10.1093/gerona/glac133
Advance Access publication June 18, 2022

OXFORD

Original Article

DNA Methylation "GrimAge" Acceleration Mediates Sex/Gender Differences in Verbal Memory and Processing Speed: Findings From the Health and Retirement Study

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Received: March 13, 2022; Editorial Decision Date: June 3, 2022

Decision Editor: David Le Couteur, MBBS, FRACP, PhD

Abstract

Whether sex/gender differences in rates of biological aging mediate sex/gender differences in cognition in older adults has not been fully examined. The aim of the current study was to investigate this association. Data from up to 1 928 participants (mean age = 75, standard deviation = 7.04, female = 57%) who took part in the 2016 Harmonized Cognitive Assessment Protocol and Venous Blood Study; substudies of the Health and Retirement Study were included in the current study. The residuals from 4 age-adjusted epigenetic clocks (Horvath, Hannum, PhenoAge, and GrimAge) were used to measure biological age acceleration. Sex/gender differences in cognition were tested using a series of analyses of covariance. Mediation analyses tested whether the measures of age acceleration accounted for these sex/gender differences, controlling for age, education, smoking status, and white blood cell count. Women outperformed men on measures of verbal learning, verbal memory, visual scanning, and processing speed. No other significant sex/gender differences were identified. Results from mediation analyses revealed that women's slower rates of GrimAge fully accounted for their faster processing speeds and partially accounted for their better performances on verbal learning, verbal memory, and visual scanning measures. None of the other measures of age acceleration were significant mediators. Accounting for sex/gender differences in biological aging may differentiate between cognitive sex/gender differences that are driven by universal (ie, age-related) versus sex-specific mechanisms. More broadly, these findings support the growing evidence that the GrimAge clock outperforms other clocks in predicting cognitive outcomes.

Keywords: Biological age, Cognition, DNA methylation, Sex/gender differences

Sex differences in specific cognitive abilities are well-documented, with the most consistent findings showing that women outperform men on measures of verbal memory and men outperform women on visual–spatial abilities (1–3). Evidence that these differences in aspects of cognition persist across the life span supports a neurodevelopmental mechanism underlying differences. However, identifying cognitive sex differences in older cohorts is complicated by the cumulative effect of environmental/lifestyle exposure on cognition, reflected in increased cognitive heterogeneity observed in older cohorts (4). This may account for the mixed findings on sex differences in other aspects of cognition. For example, some studies have shown that women outperform men on speeded measures (5), while findings from other studies have shown the opposite (6).

Despite the extensive investigations and mixed findings on sex differences in cognition, there has been renewed interest in understanding contributing factors underlying these differences. Mounting evidence shows that cognitive impairment and dementia disproportionately affect older women compared to men (7). Alzheimer's disease (AD) is the most common cause of dementia and cognitive impairment in adults over 65, with initial symptoms typically manifesting as short-term memory loss. It is estimated that approximately two-thirds of people diagnosed with AD in the United States are women (8). Without an effective cure, identifying the earliest cognitive symptoms of AD is central to maximizing the efficacy of available treatments developed to delay symptom progression. However, identifying clinically meaningful cognitive change may be

obscured by sex differences in baseline cognitive performances. This risk is especially pertinent to women, given the evidence of their advantage on measures of verbal memory. Supported by studies on AD, women also maintain a verbal memory advantage in the early stages of the disease, despite similar levels of pathology in men, which may delay diagnosis (9).

Biological accounts of sex differences in cognitive abilities have emphasized differences in structural and functional brain Magnetic Resonance Imaging for (MRI) findings, brain metabolism (10), and circulating hormone levels (11,12). In comparison, social/environmental explanations of sex differences in cognition have emphasized the influence of gender identity (ie, male and female) in social roles and norms, which may promote or limit access to cognitively protective activities (eg, education, occupational opportunities) (13). The term "gender" refers to a socially constructed concept, defined in the *Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition* (14) (DSM-5) as "the public and usually recognized lived role as boy or girl, man or woman." While conceptually distinct gender is rooted in biological sex and acknowledged by the DSM-5 in the definition of gender as "…factors are seen as contributing in interaction with social and psychological factors to gender development." Therefore, the term sex/gender will denote this interdependency from here on in. Implied by the interdependency between sex and gender is that investigating sex/gender differences in cognitive aging as well as pathological cognitive aging (eg, dementia) requires a consideration of environmental and biological influences. From this perspective, epigenetic biomarkers of aging are well-positioned to capture this interdependent association between aspects of biology and environmental/psychosocial influences that may in part be influenced by one's sex/gender.

Several epigenetic biomarkers have been developed to capture variation in biological aging, particularly at the level of DNA methylation (DNAm). DNAm refers to the addition or removal of methyl groups across numerous Cytosine-phosphate-Guanine sites (CpGs) involved in gene regulation and expression. The rate and location of DNAm changes with advancing age (15) and can be reliably measured (16). Also known as "epigenetic clocks," these measurable DNAm patterns are perhaps the most well-studied biomarkers of age, given their high degree of accuracy in predicting chronological age and mortality (17), as well as numerous age-related diseases including diabetes, cancer, and cardiovascular disease (18). Furthermore, rates of biological aging indexed by these clocks have been shown to relate to cumulative psychosocial stress, diet, education, and early childhood adversity (19), suggesting that changes in DNAm over the life span may be one pathway through which environmental exposures can influence health outcomes in later life. Although there is currently no consensus on how to measure biological aging, DNAm is considered the most robust molecular marker of biological age (20).

Numerous epigenetic clocks have been developed in the last decade, but common among these clocks is the inclusion of a varying number of age-related methylated CpGs that are derived either from a single tissue or cell type (eg, blood) or from various tissue types (eg, skin, cortical, lung). Broadly, these can be categorized into first- and second-generation clocks. The Horvath pan tissue clock (16) and the Hannum blood-specific clock (21) are perhaps the most well-studied first-generation clocks. These clocks were developed using machine learning algorithms to identify CpGs sites that most strongly predicted chronological age and all-cause mortality. More recent refinements of these measures to predict age-related clinical outcomes (eg, cancer, cardiovascular health) have led to the emergence of what are referred to as "second-generation clocks" (22). Of these second-generation clocks, the "PhenoAge" (23) and "GrimAge" (24) clocks are the most well-studied. Unlike first-generation clocks, which were developed and trained on cross-sectional data, second-generation clocks are trained on longitudinal data sets (23,24). These measures also incorporate age-related clinical markers of physiological stress and inflammation. Rates of age acceleration can be derived by regressing the clocks on chronological age. The resulting residual can be used as a marker of biological age acceleration, with larger residuals indicating faster age acceleration (22). The suffix "AgeAccel" at the end of each clock represents this measure.

Support for the utility of these DNAm clocks in cognitive aging research has been accumulating. In one study, longitudinal associations were examined between first- and second-generation clocks with measures of physical and cognitive functioning (ie, word recall and processing speed) in a large sample of British older adults (ages 45–87). In this study, neither of the 2 first-generation clocks was associated with any of the outcome measures. In contrast, PhenoAgeAccel was associated with reduced grip strength, lung function, and slower processing speed, whereas GrimAgeAccel was associated with reduced lung function, slower processing speed, and lower word recall scores. In another study of older British adults, GrimAgeAccel was associated with decreased physical and cognitive functioning as well as smaller regional brain volumes and more white matter hyperintensities (25). Similar findings have been shown using other European samples—including The Irish Longitudinal Study on Aging (26) and the Generation Scotland: Scottish Family Health Study (27). In U.S. samples, results have been mixed, with one study showing associations between 3 second-generation clocks (ie, GrimAgeAccel, PhenoAgeAccel, and DunedinPoAmAccel) and rates of verbal memory decline. These associations were mediated by social–economic status and varied by sex/gender and race/ethnicity, such that the strongest associations were observed in White women (28). Another recent study examined whether 3 markers of age acceleration based on the Horvath and Hannum clocks were associated with cognitive decline in Black and White adults aged 30–65 and whether associations were moderated by sex/gender (29). The main findings from this study showed that the HannumAgeAccel, adjusted for immune system functioning, was associated with a faster decline in domains of attention/speed and visual memory but only in men. However, the HannumAgeAccel measure without the inclusion of immune system function was not a significant predictor of decline in either men or women. In another study of older Black Americans, faster HannumAgeAccel was associated with lower word fluency scores, independent of age, sex/gender, and education, but the Horvath clock was not (27). The authors replicated these findings in a Caucasian European sample and concluded that the Hannum clock may be better generalized to ethnically diverse samples when using cognition as the outcome. Cumulatively, these findings suggest that epigenetic clocks have the potential for increasing understanding of cognitive heterogeneity in diverse older adult populations, but continued validation of their utility as biomarkers relevant to brain aging is needed.

Regarding cognitive sex/gender differences in older adults, the possibility that differing rates of epigenetic aging may mediate the association between sex/gender and cognitive performances has not been directly examined to date. This is despite evidence that women have younger DNAm ages and less DNAm age acceleration compared to men (20). Although a few studies have indicated that the strength of associations between DNAm age acceleration and cognition may vary by sex/gender (28,29), these studies were limited by either the inclusion of only first-generation clocks and a younger

sample (29) or restricting analyses to a single measure of cognition (28). Moreover, none of the studies examined whether any of the measures of DNAm age acceleration mediated the effects of sex/gender on a given cognitive outcome. Yet epigenetic age acceleration may be best conceptualized as a mediator through which an independent variable (ie, sex/gender) exerts its effect on the dependent variable, that is, cognition. Additionally, a mediator can have both an independent and a mediation effect (30). From this perspective, sex/gender differences in aspects of cognition in older samples may reflect differences in both neurodevelopmental/genetic influences as well as the cumulative effects of lifestyle/environmental factors that may be captured in rates of biological aging.

Thus, the overarching goal of the current study was to test whether biological age acceleration would mediate putative sex/gender differences in specific cognitive abilities. We tested this using 4 of the most commonly used measures of DNAm age acceleration in studies that had a cognitive outcome measure, that is, the Hannum, Horvath, PhenoAge, and GrimAge clocks. We hypothesized that in cognitive domains where women outperformed men, this would, in part, be accounted for by slower rates of age acceleration.

Method

Participants

Data for the present study included participants enrolled in the 2016 wave of the Health and Retirement Study (HRS), who also took part in the 2016 Venous Blood Study (VBS) (31) and the Harmonized Cognitive Assessment Protocol Project (HCAP) (13). Details on the HRS study design, procedures, and substudies have been described elsewhere (33).

Data used in the current study analyses were selected by combining data from the 2016 VBS (N = 9 934), who had epigenetic clock data (n = 4 018), and who participated in the HCAP study (n = 2 292). Participants were excluded if there was an informant report from the HCAP study of a prior diagnosis of stroke (n = 159), Parkinson's disease (n = 23), or AD (n = 36). A final sample of 1 928 participants was included with full demographics, smoking status, epigenetic data, and at least a total score on the Mini-Mental State Examination (to indicate that they participated in some cognitive testing) from the HCAP study. In this final sample, participants had a mean age of 74.7 years (standard deviation [SD] = 7.04), and 56.9% were women, with ~75% of the sample identifying as non-Hispanic White. Characteristics for the total and sex/gender stratified samples are shown in Table 1.

Assessment of Biological Age

Full details of the DNA methylation "epigenetic clock" construction have been previously published (31,34). In brief, the *Horvath clock* (16) was developed from 8 000 samples from 82 Illumina to estimate the DNAm age of 51 tissue and cell types. DNAm patterns for this clock were based on 353 CpGs. The *Hannum clock* (21), a blood-based estimate of age, was derived from DNAm from 71 CpGs and selected from the Illumina 450 000 arrays (21). The *PhenoAge clock* (23) was developed from a 2-stage process and trained on 2 longitudinal studies: the U.S. National Health and Nutritional Examination Survey and Invecchiare InChianti studies. The PhenoAge clock was derived from a composite of several clinical biomarkers of 9 tissue types and immune function (ie, albumin, creatinine, serum glucose, C-reactive protein, lymphocyte percent, mean (red) cell volume, red cell distribution width, white blood cell count, and alkaline phosphate), and was based on DNAm from 513 CpGs in whole blood samples. The *GrimAge clock* (24) was also developed using a 2-step process and based on 1 030 CpGs sites. An elastic net regression model was used to predict time to death due to all-cause mortality, resulting in 7 surrogate DNAm biomarkers of plasma proteins (ie, adrenomedullin, beta-2 microglobulin, cystatin C, leptin, plasminogen activation inhibitor, tissue inhibitor metalloproteinase, and growth differentiation factor [GDF]), as well as DNAm smoking pack-years, age, and sex/gender which formed a composite estimate of mortality. This composite was linearly transformed into an age estimate to match the chronological mean age of the training data set (age 66 years), resulting in the GrimAge clock. The clock was trained on the Framingham Heart Study Offspring Cohort, a large longitudinal study (35). For more details on the GrimAge clock construction, see Supplementary Materials.

Age acceleration was assessed based on the standardized residual from regressing each of the 4 clocks onto chronological age. This method has been published elsewhere (34). Larger residuals indicate faster age acceleration.

Assessment of Cognition

Full details and references for test measures included as part of the HCAP study have been previously described (32). Further details and references for individual test measures included in the current study are described in Supplementary Materials.

In brief, *verbal learning and verbal memory* were assessed from the immediate and delayed recall trials from 3 separate measures: The 10-item word list from the Consortium to Establish a Registry for Alzheimer's Disease (CERAD-WL) and 2 short story recall tasks (i) Wechsler Memory Scale-4th edition (WMS-IV) Logical Memory I & II (LM I & II) and (ii) The Brave Man Story. After identifying sex/gender differences across the 3 verbal learning and memory measures, factor scores were generated using a principal component analysis (PCA). The first unrotated principal component was extracted based on the immediate (ie, verbal learning factor) and recall trials (ie, verbal memory factor) in separate PCAs. The verbal learning component accounted for 63% of the variance in these measures, and test loadings ranged from 0.77 to 0.83. The verbal memory component accounted for 67% of the total variance, with test loadings ranging from 0.79 to 0.84.

Visual construction and visual memory were assessed using the copy and delayed recall scores from the CERAD constructional praxis subtest.

Attention/speed of processing was assessed using the letter cancelation test (total correct letters marked within 90 seconds), a backward counting test (total number of correct numbers counted from 100 to 1 in 30 seconds), the Trail Making Test, Part A (TMT Part A: total time in seconds with higher scores indicating lower performances), and the Symbol Digit Modalities Test (SDMT: total number of correctly matched symbols within 90 seconds). *Nonverbal reasoning* was assessed with Raven's Progressive Matrices—an adapted version. The number of correct items was used as the measure of nonverbal reasoning. *Executive functioning* was assessed using the Trail Making Test Part B (TMT-B: total time in seconds), with higher scores indicating lower performances.

Assessment of Covariates and Demographic Variables

Self-reported sex assigned at birth was used to indicate sex/gender. Data on race/ethnicity (not included as a covariate), chronological

Table 1. Sample Characteristics and Descriptive Measures for DNA Methylation Epigenetic Clocks, HRS (*N* = 1 928)

Variables	Total				Women (*n* = 1 097)			Men (*n* = 831)		
			Range			Range			Range	
	N	Mean (*SD*)	Min.	Max.	Mean (*SD*)	Min.	Max.	Mean (*SD*)	Min.	Max.
Age	1 928	74.73 (7.04)	65	98	74.63 (7.00)	64	96	74.87 (7.10)	64	98
GrimAge	1 928	72.10 (7.07)	55.96	99.61	70.67 (6.71)	55.96	99.61	74.00 (7.11)	57.03	97.05
GrimAgeAccel†	1 928	0 (1.00)	−2.62	4.35	−0.29 (0.91)	−2.47	3.11	0.39 (0.99)	−2.62	4.35
PhenoAge	1 928	61.51 (8.77)	26.72	101.68	61.15 (8.85)	26.72	101.68	61.99 (8.66)	39.98	100.69
PhenoAgeAccel†	1 928	0 (1.00)	−4.11	5.28	−0.04 (1.01)	−4.11	4.51	0.06 (0.98)	−3.13	5.28
Horvath	1 928	69.68 (8.25)	37.69	114.52	69.05 (8.26)	37.69	114.52	70.51 (8.16)	38.58	105.46
HorvathAgeAccel†	1 928	0 (1.00)	−5.08	7.28	−0.08 (1.02)	−5.08	7.28	0.11 (0.96)	−4.71	4.64
Hannum	1 928	58.75 (7.66)	33.43	95.91	57.84 (7.36)	37.70	89.66	59.95 (7.89)	33.43	95.91
HannumAgeAcce†	1 928	0 (1.00)	−4.83	6.53	−0.16 (0.96)	−4.83	6.53	0.21 (1.01)	−3.33	6.38
Education	1 928	12.94 (3.07)	0	17	12.71 (3.14)	0	17	13.25 (2.94)	0	17
WBCC	1 928	6.65 (1.98)	1.70	21.8	6.65 (2.02)	2	22	6.64 (1.93)	2.30	19.4
Basophil count	1 928	0.05 (0.06)	0.00	0.30	0.06 (0.06)	0.00	0.30	0.05 (0.06)	0.00	0.30
Eosinophil count	1 928	0.22 (0.17)	0.00	3.50	0.21 (0.14)	0.00	1.30	0.24 (0.20)	0.00	3.50
Neutrophil count	1 928	3.93 (1.51)	0.60	12.70	3.89 (1.55)	0.70	11.60	3.97 (1.44)	0.60	12.70
Lymphocyte count	1 928	1.88 (0.86)	0.40	17.40	1.95 (0.82)	0.50	17.40	1.78 (0.90)	0.40	12.50
Monocyte count	1 928	0.58 (0.21)	0.10	1.80	0.55 (0.19)	0.10	1.50	0.61 (0.22)	0.10	1.80
MMSE	1 928	27.36 (2.73)	9	30	27.59 (2.3)	12	30	27.05 (2.83)	9	30
Ravens	1 913	13.01 (3.34)	0	17	12.81 (3.29)	0	17	13.26 (3.39)	0	17
CERAD-WL IR	1 920	18.26 (4.60)	0	30	19.19 (4.60)	0	30	17.02 (4.29)	4	29
CERAD-WL DR	1 923	5.5 (2.47)	0	10	5.99 (2.42)	0	10	4.85 (2.40)	0	10
LM IR	1 917	10.63 (4.86)	0	23	11.09 (4.86)	0	23	10.03 (4.79)	0	23
LM DR	1 899	8.18 (5.30)	0	22	8.72 (5.38)	0	21	7.48 (5.12)	0	22
IR Comp.	1 912	14.47 (4.06)	1.5	25	15.15 (4.06)	1.5	25	13.56 (3.89)	2.5	25.5
DR comp	1 897	6.87 (3.47)	0	15.5	7.38 (3.48)	0	15	6.18 (3.35)	0	15.5
CERAD-CP IR	1 925	8.78 (6.11)	0	97	8.39 (4.40)	0	97	9.3 (7.79)	2	97
CERAD-CP DR	1 917	6.26 (3.07)	0	11	6.17 (2.99)	0	11	6.39 (3.17)	0	11
Animal fluency	1 926	17.24 (6.22)	0	43	17.23 (6.19)	0	43	17.25 (6.28)	0	38
Letter canc.	1 871	15.58 (4.94)	0	37	16.16 (5.06)	0	37	14.82 (4.67)	0	36
Backwards ct.	1 912	31.13 (10.81)	0	99	30.32 (10.49)	0	70	32.18 (11.14)	0	99
SDMT	1 876	34.63 (12.15)	0	71	35.41 (12.50)	0	71	33.61 (11.61)	0	64
TMT-A	1 884	49.88 (28.94)	3	300	49.56 (30.39)	3	300	50.29 (26.93)	16	250
TMT-B	1 719	119.53 (55.85)	32	300	120.04 (56.67)	32	300	118.85 (54.79)	40	300
Race/ethnicity										
White	1 442	74.8%			55.2%			44.8%		
Black	261	13.5%			62.1%			37.9%		
Hispanic	185	9.6%			10.4%			8.7%		
Other ethnicity	40	2.1%			2.4%			1.7%		
Smoke, *N* (%)	220	11.4%			10.4%			12.7%		

Notes: HRS: Health and Retirement Study; *N*: number; Min.: minimum; Max.: maximum; Ravens: Raven's Standard Progressive Matrices; CERAD-WL: Consortium to Establish a Registry for Alzheimer's Disease Word List (IR: Immediate Recall; DR: Delayed Recall); LM: Wechsler Memory Scale 4th Edition Logical Memory subtest (IR: Immediate Recall; DR: Delayed Recall); CERAD-CP: Consortium to Establish a Registry for Alzheimer's Disease Constructional Praxis (IR: Immediate Recall; DR: Delayed Recall); Letter canc.: The Letter Cancelation Task; Backwards ct.: The Backwards counting task; SDMT: Symbol Digit Modalities Test; TMT-A: Trail Making Test A; TMT-B: Trail Making Test B; WBCC, white blood cell count; MMSE, Mini Mental State Examination.

†Denotes age acceleration as the standardized residual from regression the clock on chronological age.

age, years of education, and current smoking status (yes = 1, no = 0) were obtained from self-report as part of the core HRS interview. Current smoking status was included as a covariate to ensure that the effect of DNAm age acceleration was not driven by smoking status, as this has been shown to strongly relate to these measures (34). White blood cell count (10^9/L; WBCC) was included to account for current infections that could influence epigenetic clock measures (15). We limited covariates to these measures as the study aimed not to explain the variance of DNAm age acceleration but to increase the reliability that the effects revealed from our analyses were independent of education, smoking, and acute infections.

Statistical Analysis

All analyses were conducted using the Statistical Package for Social Sciences (SPSS; version 27) for Windows (IBM, Armonk, NY) (36). Descriptive statistics were used to derive the means, and *SD*s for all independent and dependent raw score variables for the whole sample and sex/gender stratified sample. For all other analyses, variables were converted to *z*-score metric to facilitate interpretation of results. Pearson's correlation coefficients between measures of age acceleration and cognitive measures are displayed in Supplementary Table S1. Correlations between sex/gender, covariates, and measures of age acceleration are shown in Supplementary Table S2. Independent-sample *t* tests were used to test differences in chronological age and years of education between men and women. An analysis of covariance (ANCOVA) was used to examine sex/gender differences in performances on cognitive scores, controlling for chronological age and years of education. Partial eta-squared (η_p^2) was used as the measure of effect size for sex/gender. To adjust for multiple testing, a Bonferroni-corrected alpha of ≤0.004 (0.05/14 tests) was interpreted as statistically significant for the results of the ANCOVA. Only measures showing a statistically significant sex/gender difference were included as dependent variables in the mediation analyses. Of note, we used the verbal learning and memory factor scores in place of the individual measures in all mediation models.

Mediation analyses were conducted to examine our main aim using the SPSS PROCESS (4.0) macro, Model 4 (37). We included education, chronological age, WBCC, and current smoking status in all mediation analyses. 10 000 bootstrapping sampling was used for all mediation analyses, and statistical significance was inferred from 99% bias-corrected bootstrap confidence intervals (CIs).

Sensitivity Analyses

Given prior evidence that sex/gender differences in immune system function may drive associations between epigenetic measures of age acceleration and cognition (29) and to facilitate interpretation of our findings, we tested whether men and women differed on measures of white blood cell types (ie, basophils, eosinophils, neutrophils, lymphocytes, and monocytes) that may not be captured in WBCC, using independent *t* tests. Given significant sex/differences in these measures (except for neutrophils), we reran the mediation analyses and replaced the WBCC variable with these individual variables to test whether this would alter our results.

Results

Descriptive Statistics

There was not a statistically significant difference in chronological age between men (M = 74.87, SD = 7.1), and women (M = 74.63, SD = 7.0), $t(1,926) = 0.738$, $p = .461$. There was a statistically significant effect of sex/gender on all 4 measures of age acceleration, with women showing less age acceleration compared to men on GrimAgeAccel, $t(1,926) = 15.52$, $p < .001$, PhenoAgeAccel, $t(1,926) = 2.12$, $p < .001$, HorvathAgeAccel, $t(1,926) = 4.28$, $p < .001$, and HannumAgeAccel, $t(1,926) = 8.01$, $p < .001$. Covariates (chronological age, education, WBCC, current smoking status) and sex/gender accounted for 31% ($p < .001$), 2% ($p < .001$), 7% ($p < .001$), and 3% ($p < .001$) of the variance in GrimAgeAccel, HorvathAgeAccel, HannumAgeAccel, and PhenoAgeAccel, respectively.

Cognitive Sex/Gender Differences

Results of the ANCOVA are displayed in Table 2. Controlling for chronological age and years of education, there was a statistically significant effect of sex/gender (ie, $p \leq .004$) on scores from the following measures: SDMT, letter cancelation, WMS-IV LM immediate and delayed recall, CERAD-WL immediate and delayed recall, and the Brave Man immediate and delayed recall, with higher scores obtained by women compared to men. The corresponding effect sizes were small for the SDMT ($\eta_p^2 = 0.02$), letter cancelation ($\eta_p^2 = 0.03$), WMS-IV LM immediate recall ($\eta_p^2 = 0.02$), delayed recall ($\eta_p^2 = 0.02$) tasks and Brave man immediate ($\eta_p^2 = 0.01$), and delayed ($\eta_p^2 = 0.01$), story recall. There was a medium effect of sex/gender on CERAD-WL immediate ($\eta_p^2 = 0.08$) and delayed ($\eta_p^2 = 0.07$) recall, with similar effects revealed for the verbal learning ($\eta_p^2 = 0.05$) and memory ($\eta_p^2 = 0.05$) factor scores. There was no statistically significant difference between men and women on the Raven's Progressive Matrices, the backward counting task, TMT Part A, TMT Part B, or the copy or delayed recall trials of the CERAD constructional praxis subtest.

Results From Mediation Analyses

GrimAgeAccel was the only measure of DNAm age acceleration that had a statistically significant mediation effect on the associations between sex/gender and cognitive outcomes. Results from mediation analyses using GrimAgeAccel are summarized in Table 3. Mediation results for all other measures of age acceleration are presented in Supplementary Table S3.

Verbal learning

Sex/gender explained 5% ($p < .001$) additional variance beyond covariates in the verbal learning factor. Controlling for covariates and sex/gender, GrimAgeAccel accounted for approximately 1% ($p < .001$) additional variance in the verbal learning factor, $F(6, 1,905) = 108.72$, $p < .001$. There was also a significant mediation effect of GrimAgeAccel ($b = 0.03$, 99% CI [0.01, 0.05]) on the sex/gender and verbal learning association. GrimAgeAccel accounted for 10% of the total effect of sex/gender on verbal learning. In sum, women's better verbal learning performances were partially explained by slower rates of GrimAgeAccel relative to men (Figure 1A).

Verbal memory

Sex/gender explained an additional 3% ($p < .001$) of the variance in verbal memory beyond covariates. The inclusion of GrimAgeAccel in the model explained an additional 1% ($p < .001$) of the variance in verbal memory performances, $F(6, 1,890) = 92.96$, $p < .001$, and there was a significant mediation effect of GrimAgeAccel ($b = 0.04$, 99% CI [0.01, 0.06]). In sum, women's higher verbal memory performances were partially explained by slower GrimAgeAccel

Table 2. ANCOVA Results Showing Sex Differences in Neuropsychological Performances

N		Women		Men				
	Outcome Variables	$M^{adj.}$	(*SE*)	$M^{adj.}$	(*SE*)	*F*	*p*	η^2
1 911	Verbal learning Factor	0.18	0.03	−0.24	0.03	107.73	<.001	0.05
1 896	Verbal Memory Factor	0.18	0.03	−0.24	0.03	101.188	<.001	0.05
1 925	CERAD-CP IR	8.45	0.18	9.23	0.21	7.75	.005	0.00
1 917	CERAD-CP DR	6.23	0.0	6.31	0.10	0.38	.547	0.00
1 913	Raven's	12.91	0.09	13.13	0.10	2.59	.108	0.00
1 913	CERAD-WL IR	19.29	0.12	16.90	0.14	162	<.001	0.08
1 923	CERAD-WL DR	6.03	0.07	4.80	0.08	146	<.001	0.07
1 917	LM IR	11.2	0.13	9.89	0.16	40.9	<.001	0.02
1 899	LM DR	8.82	0.15	7.34	0.17	44.2	<.001	0.02
1 900	Braveman IR	7.62	0.06	7.14	0.07	23.30	<.001	0.01
1 900	Braveman DR	5.73	0.09	5.03	0.11	23.57	<.001	0.01
1 871	Letter canc.	16.22	0.14	14.74	0.16	50.1	<.001	0.03
1 912	Backwards ct.	30.57	0.30	31.85	0.34	8.16	.005	0.00
1 876	SDMT	35.74	0.30	33.17	0.35	31.3	<.001	0.02
1 844	TMT-A*	48.92	0.80	51.15	0.92	3.35	.067	0.00
1 719	TMT-B*	118.9	1.62	120.35	1.86	0.34	.559	0.00

Notes: *Higher scores indicate lower performance. Statistical significance was set at $p < .004$ (Bonferroni corrected; ie, $p = .05/14$), CERAD-CP: Consortium to Establish a Registry for Alzheimer's Disease Constructional Praxis (IR: Immediate Recall; DR: Delayed Recall); Ravens: Raven's Standard Progressive Matrices; CERAD-WL: Consortium to Establish a Registry for Alzheimer's Disease Word List (IR: Immediate Recall; DR: Delayed Recall); LM: Wechsler Memory Scale 4th Edition Logical Memory subtest (IR: Immediate Recall; DR: Delayed Recall); Braveman II: The Brave man story immediate recall from the East Boston Memory Test (Braveman DR: Delayed Recall for Brave man story from the East Boston Memory Test); Letter canc.: The Letter Cancelation Task; Backwards ct.: The Backwards counting task; SDMT: Symbol Digit Modalities Test; TMT-A: Trail Making Test A; TMT-B: Trail Making Test B. $df = 1$.

Table 3. Mediation Model Results From GrimAge Acceleration

	Variable	*b* (*SE*)	*t*	LLCI	ULCI	R^2	ΔR^2
	Constant	0.00 (0.02)	−0.16				
Step 1	Sex/Gender -> LEARN factor	0.20 (0.02)	10.24	0.17	0.24	0.25***	0.04***
Step 2	Sex/Gender -> GrimAA	−0.33 (0.02)	−17.34	−0.38	−0.28	0.31***	0.11***
Step 3	Sex/Gender -> LEARN factor	0.18 (0.02)	8.35	0.12	0.23		
	GrimAA -> LEARN factor	−0.08 (0.02)	−3.23	−0.14	−0.02	0.26***	0.01***
Step 4	Indirect Effect	0.03 (0.01)		0.01	0.05		
	Constant	−0.01 (0.02)	−0.27				
Step 1	Sex/Gender -> MEM factor	0.20 (0.02)	9.94	0.16	0.24	0.22***	0.04***
Step 2	Sex/Gender -> GrimAA	−0.33 (0.02)	−17.27	−0.37	−0.29	0.31***	0.11***
Step 3	Sex/Gender -> MEM factor	0.17 (0.02)	7.69	0.13	0.21		
	GrimAA -> MEM factor	−0.11 (0.03)	−4.32	−0.17	−0.04	0.23***	0.01***
Step 4	Indirect Effect	0.04 (0.01)		0.01	0.06		
	Constant	−0.01 (0.02)	−0.66				
Step 1	Sex/Gender -> LC	0.15 (0.02)	6.99	0.09	0.20	0.18***	0.02***
Step 2	Sex/Gender -> GrimAA	−0.33 (0.02)	−17.07	−0.38	−0.28	0.30***	0.11***
Step 3	Sex/Gender -> LC	0.11 (0.02)	4.75	0.05	0.17		
	GrimAA -> LC	−0.12 (0.03)	−4.88	−0.19	−0.06	0.19***	0.01***
Step 4	Indirect Effect	0.04 (0.01)		0.02	0.06		
	Constant	−0.02 (0.02)	−1.04				
Step 1	Sex/Gender -> SDMT	0.10 (0.02)	5.49	0.05	0.15	0.35***	0.01
Step 2	Sex/Gender -> GrimAA	−0.33 (0.02)	−17.37	−0.38	−0.28	0.31***	0.11***
Step 3	Sex/Gender -> SDMT	0.05 (0.02)	2.28	−0.01	0.10		
	GrimAA -> SDMT	−0.17 (0.02)	−7.77	−0.23	−0.12	0.37***	0.20***
Step 4	Indirect Effect	0.06 (0.01)		0.04	0.08		

Notes: GrimAA: GrimAge Acceleration clock; LC: The Letter Cancelation Task; SDMT: Symbol Digit Modalities Test. Indirect effects reflect nonparametric bootstrapped confidence intervals set at 99% using 10 000 resamples, *** = p<.001.

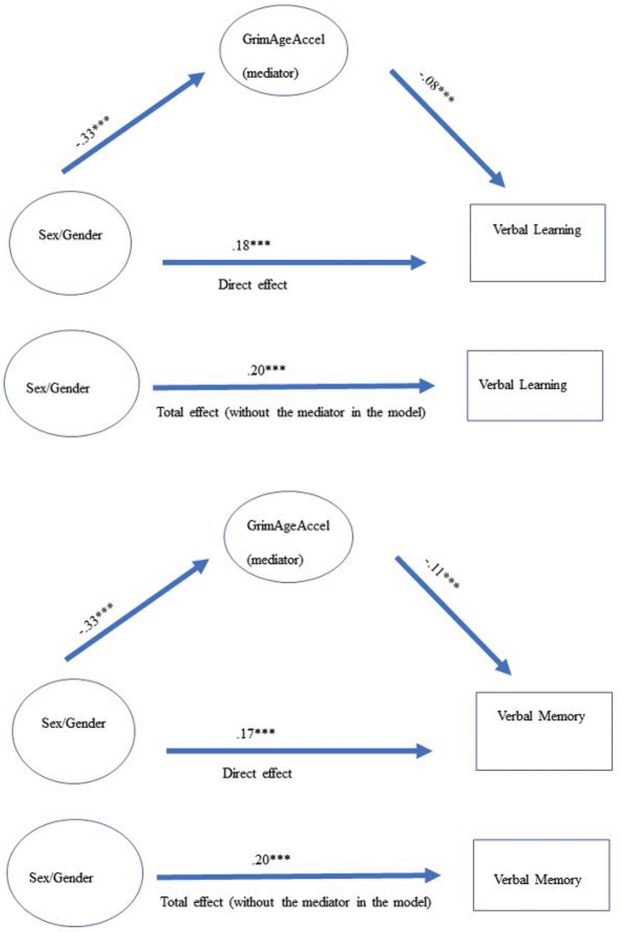

Figure 1. Associations with verbal learning. Standardized coefficients of variables, adjusted for covariates (ie, smoking status, white blood cell count, education, chronological age). Associations with verbal memory. Standardized coefficients of variables, adjusted for covariates (ie, smoking status, white blood cell count, education, chronological age, *** = p<.001.

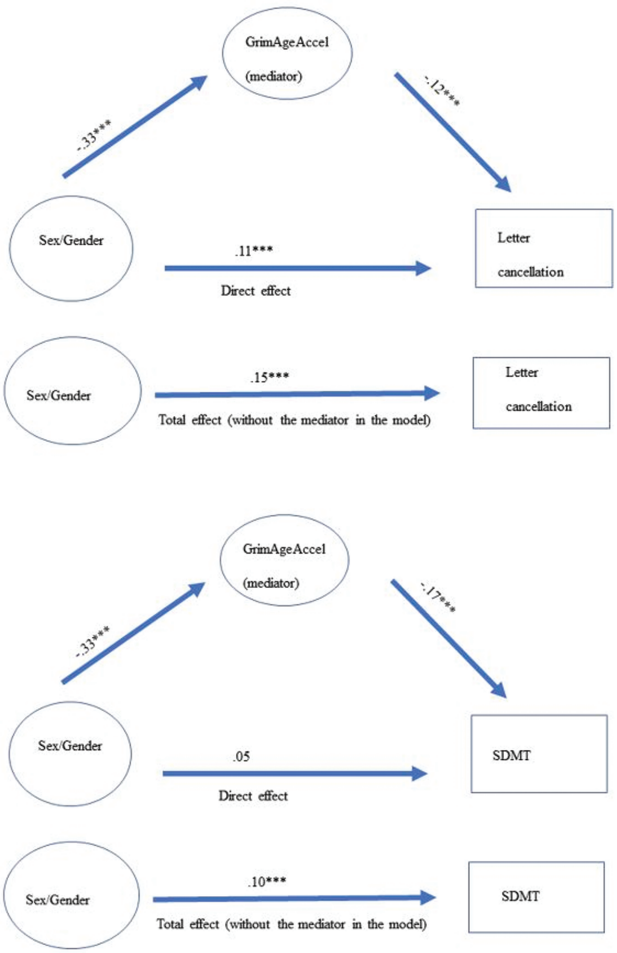

Figure 2. Associations with visual scanning/attention. Standardized coefficients of variables, adjusted for covariates (ie, smoking status, white blood cell count, education, chronological age). Associations with psychomotor processing speed. Standardized coefficients of variables, adjusted for covariates (ie, smoking status, white blood cell count, education, chronological age). SDMT = Symbol Digit Modalities Test, *** = p<.001.

and accounted for 15% of the total effect of sex/gender on verbal memory (Figure 1B).

Visual attention/scanning (letter cancelation task)

Sex/gender accounted for an additional 2% ($p < .001$) of the variance in letter cancelation scores. The addition of GrimAgeAccel explained a further 1% ($p < .001$) of the variance in scores beyond all other covariates and sex/gender, and there was a significant indirect effect of GrimAgeAccel ($b = 0.04$, 99% CI [0.02, 0.06]) that accounted for approximately 27% of the total effect of sex/gender on letter cancelation performances. Again, women's higher scores on the letter cancelation task were partially explained by slower rates of GrimAgeAccel relative to men (Figure 2A).

Psychomotor processing speed (SDMT)

Sex/gender accounted for an additional 1% of the variance beyond covariates in SDMT scores. The addition of GrimAgeAccel explained an additional 2% of the variance, $F(6, 1{,}869) = 181.63$, $p < .001$), and there was a significant indirect effect of GrimAgeAccel ($b = 0.06$, 99% CI [0.04, 0.08]). In sum, women's faster processing speeds were fully accounted for by their slower rates of GrimAgeAccel and accounted for ~50% of the total effect of sex/gender on SDMT scores (Figure 2B).

Sensitivity Analyses

Results from *t* tests (Supplementary Table S4) testing sex/gender differences in white blood cell types revealed that women had higher basophils and lymphocytes, whereas men had significantly higher eosinophils and monocytes. There was no sex/gender difference in neutrophils. Results from mediation analyses using these cell types in place of WBCC showed that none of the cell types were associated with any of the cognitive measures, and the inclusion of these measures as covariates did not alter the mediation effect of GrimAgeAccel on any of the cognitive measures. Results are shown in Supplementary Table S5.

Discussion

Consistent with prior research (1,2,5,20), the results from the current study showed that women had higher scores on measures of verbal learning, verbal memory, processing speed, and visual attention/scanning (1–3) and had slower rates of DNAm aging compared to men. Of the 4 measures of age acceleration, GrimAge was most strongly associated with cognition, again consistent with prior work (25,26). The results from testing our main aim, that is,

to investigate whether sex/gender differences in age acceleration mediated putative sex/gender differences in aspects of cognition, revealed that GrimAgeAccel was the only measure of DNAm that had a statistically significant mediation effect on these associations. Results supported our hypothesis that slower rates of DNAm aging would partly explain women's better performances in aspects of cognition. Specifically, women's faster processing speeds were fully explained by their slower rates of GrimAgeAccel relative to men. However, women's higher scores on measures of verbal learning and verbal memory were only partially explained by slower rates of GrimAgeAccel. Furthermore, the mediation effects of GrimAgeAccel were independent of the effects of chronological age, current smoking status, WBCC, and education. GrimAgeAccel also had a significant main effect on all cognitive outcomes used in the mediation models, such that increased GrimAgeAccel was associated with a linear decrease in cognitive performances.

This is the first known published study to specifically test the mediation effect of several measures of DNAm age acceleration on the associations between sex/gender and specific cognitive abilities in community-dwelling older adults. The findings from the current study suggest that sex/gender differences in aspects of cognition may reflect differences in rates of biological aging while other cognitive sex/gender differences may be truly sex-specific, or at minimum, not merely accounted for by differences in rates of biological aging. Differentiating between cognitive sex/gender effects that are age-related and sex-specific is central to other investigations seeking to understand factors associated with cognitive heterogeneity in older adults more generally. For example, interventions seeking to improve cognition in older adults via slowing rates of biological aging may expect greater improvements in processing speed compared to verbal memory. Growing evidence from clinical trials demonstrates that diet and lifestyle interventions may slow DNAm aging (38). Our findings are particularly promising for cognitive aging research, given that they converge with more well-established findings that lifestyle and environmental factors influence rates of cognitive decline as well as risk for dementia (39). Conceivably, epigenetic clocks provide a parsimonious and quantifiable estimate of cumulative environmental effects while also accounting for genetic/nonmodifiable factors. However, whether slowing DNAm age acceleration is also associated with improved cognition has not been examined to date and therefore warrants investigation.

Slowed processing speed with age is one of the most robust findings in the cognitive aging literature, with some cognitive aging experts suggesting it could be considered a biomarker of brain aging (40). Slowing of processing speed has been explained in terms of reductions in white matter integrity during aging (41), with small vessel ischemic disease (SVD) considered to play a pivotal role. Sex/gender differences in SVD are supported by a recent meta-analysis showing that men had more severe SVD compared to women with similar mean ages (ie, 67 years) (42). Drawing on these findings, women's faster speeds in the current study may reflect less SVD or greater white matter integrity compared to men as a function of younger biological ages/lesser age acceleration. This suggestion is also consistent with a prior study showing that increases in GrimAgeAccel were associated with increases in white matter hyperintensities (25). From this perspective, DNAm may represent a proxy measure of SVD. While the mechanisms underlying associations between DNAm measures and cognition are unknown, findings from other studies have suggested that some DNAm measures directly capture immune system aging processes that may account for the associations with cognition. A recent study (29) showed that HannumAgeAccel, adjusted for immune cell types, was associated with faster decline among men on measures of attention and visual memory, whereas the HannumAgeAccel measure without the immune cell adjustment did not show this effect. Although comparisons of sex/gender differences in immune cell types revealed significant differences in the current study, follow-up analyses did not reveal any significant associations with any of the cell types and cognitive measures in which there were sex/gender differences. Furthermore, the inclusion of cell types into the mediation models in place of WBCC did not alter the results suggesting that sex differences in white blood cell types were not explanatory variables in our sample. However, given the younger age in the Beydoun et al. study, it is possible that differences in immune system aging captured by DNAm markers may be offset by other mechanisms such as changes in the rate and location of methylation patterns which can markedly differ across the life span (15).

It is unclear why we found only partial mediation of GrimAgeAccel on the association between sex/gender and performances on the letter cancelation task, another speeded measure, albeit a less demanding task. It is possible that processes most pertinent to performing this task may be more strongly influenced by factors that are not necessarily a direct product of accelerated aging, such as vision quality. Furthermore, differing effects across these related but different measures were not unexpected, given evidence that sex/gender differences across speeded measures can vary across different measures of speed (5). This may also explain why there was no sex/gender difference on TMT Part A—another widely used measure of psychomotor processing speed.

Whether the partial mediation effect of GrimAgeAccel on sex/gender differences in verbal learning and memory can also be accounted for by differences in age-related decrements in more fundamental processes such as processing speed is possible but complicated by a number of factors. Specifically, more complex cognitive skills may be bolstered by compensatory and/or cognitive reserve mechanisms that are not yet well-understood. According to Stern et al.'s updated framework (39), cognitive reserve is defined as cognitive performance that is greater than expected given the degree of life-course-related brain changes or underlying disease. Lifestyle exposures, including educational attainment, occupational complexity, and lifetime engagement in leisure activities, are all purported to contribute to cognitive reserve (39). However, educational attainment is the most commonly used proxy of cognitive reserve (39). Paradoxically, evidence from population studies has shown that older female cohorts tend to have lower years of education compared to men (43), as was the case in the current study. Thus, putatively, in older cohorts, women have lower cognitive reserve compared to men and, therefore, would be expected to have lower cognitive performances compared to men. However, consistent with prior research (3), the current study showed that women still outperformed men on verbal and speeded measures of cognition, despite lower years of education. These differences are important to note in studies on cognitive reserve that rely on education proxies, as well as studies focused on cognitive sex/gender differences. Indeed, these seemingly conflicting findings have led to additional empirical support for a female verbal reserve (9), which may be better captured in other proxies of cognitive reserve such as leisure activities. However, evidence that differences in biological factors, including sex hormones, have been shown to contribute to the female verbal memory advantage (44). Conceivably, differences in sex hormone levels that change with age may be reflected in differences in aging rates among men and women.

The current study's findings are also pertinent to understanding sex/gender differences in AD, recently cited as the "gateway to precision medicine" (45). Moreover, what is considered "expected" age-related cognitive decline requires a sex-specific approach. Ultimately, if we can account for "expected" age-related cognitive decline in men and women based on their biological ages/rates of aging (vs chronological age), disease-related cognitive decline may be more easily identified. The findings from the present study support GrimAgeAccel as a potential candidate marker of biological age associated with cognitive phenotypes. Not only does this biomarker capture differing rates of biological aging between men and women, but it also explains some sex/gender differences in aspects of cognition. However, it is not clear why GrimAgeAccel was the only DNAm measure associated with cognition and the only measure to have a significant mediation effect. Other studies have also found that GrimAge was a better predictor of cognition compared to other clocks (25,26). One apparent difference between GrimAge and the other clocks is the greater number of CpG sites that it incorporates (ie, 1 030) compared to the PhenoAge (ie, 513), the Hannum (ie, 71), or Horvath (353) clocks. Conceivably, a greater number of CpGs captured by GrimAge may translate to greater sensitivity to mechanisms underscoring cognitive aging. GrimAge also includes several markers of exogenous stress (eg, cystatin C, GDF 15), among other components. Deconstructing the GrimAge clock and examining how its components relate to aspects of cognition may inform future efforts to refine these clocks and increase their utility as cognitive/brain aging biomarkers.

Limitations

There are several limitations to the present study that should be noted. Firstly, the cross-sectional design of the study is a limitation in terms of evaluating the significance of small to medium effects of GrimAgeAccel on cognitive aging outcomes (eg, rates of decline, the risk for dementia). However, even small cross-sectional effects of a given psychological phenomenon may culminate into meaningful differences in day-to-day life (46). Nevertheless, longitudinal research investigating sex/gender differences in these outcomes over time and how they relate to rates of epigenetic aging is warranted. Furthermore, the racial/ethnic diversity of the sample is a limitation, given that DNAm clocks are trained on predominantly White samples (23), which may obscure or mask clinically meaningful sources of race/ethnicity-specific heterogeneity in generating these clocks. This is not a trivial consideration given the evidence that race/ethnic minority groups may experience differential types of adverse environmental experiences (eg, systemic racism, greater barriers to care) (47), which can lead to chronic stress and negatively affect physical and cognitive outcomes (48). Aside from the suggested deconstruction of these clocks to better understand their associations with cognitive phenotypes, future research focused on refining these clocks should ensure that these biomarkers account for additional factors in their development to capture unique adverse environmental stress experienced by racial/ethnic minorities.

Conclusion

Accounting for sex/gender differences in rates of biological aging may explain some of the heterogeneity in cognitive aging in individuals with similar chronological ages while also enhancing precision medicine in clinical are. Additionally, these findings support the growing evidence that the GrimAge clock outperforms other clocks in predicting cognitive outcomes. Continued investigations into understanding the GrimAge clock and its components driving these associations would enhance research seeking to refine these measures that are applicable in cognitive aging studies.

Supplementary Material

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

Funding

The HRS is funded by the National Institute on Aging (U01 AG009740), with supplemental funding from the Social Security Administration and is conducted by the University of Michigan. The HCAP is funded by the National Institute on Aging (U01 AG058499).

Conflict of Interest

None declared.

Acknowledgments

This study was not preregistered.

Author Contributions

T.M. and G.T. contributed to the conceptualization and editing, and review of manuscript drafts. D.M.O. was the primary contributor to the manuscript's aspects, including study design, analyses, conceptualization, and editing of the manuscript.

Data Availability

The source variables used in this analysis are publicly available at the Health and Retirement Study (HRS) website (http://hrsonline.isr.umich.edu/index.php?p=shoavail&iyear=ZU).

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