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RESEARCH ARTICLE

Epigenetic age acceleration and cognitive performance over time in older adults

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Abstract

INTRODUCTION: This study investigated whether epigenetic age acceleration (AA) is associated with the change in cognitive function and the risk of incident dementia over 9 years, separately in males and females.

METHODS: Six epigenetic AA measures, including GrimAge, were estimated in baseline blood samples from 560 Australians aged ≥70 years (50.7% female). Cognitive assessments included global function, episodic memory, executive function, and psychomotor speed. Composite cognitive scores were also generated. Dementia (Diagnostic and Statistical Manual for Mental Disorders – IV [DSM-IV] criteria) was adjudicated by international experts.

RESULTS: Associations between epigenetic AA and cognitive performance over-time varied by sex. In females only, GrimAA/Grim2AA was associated with worse delayed recall, composite cognition, and composite memory (adjusted-beta ranged from −0.1372 to −0.2034). In males only, GrimAA/Grim2AA was associated with slower processing speed (adjusted-beta, −0.3049) and increased dementia risk (adjusted hazard ratios [HRs], 1.78 and 2.00, respectively).

DISCUSSION: Epigenetic AA is associated with cognitive deterioration in later life but with evidence of sex-specific associations.

KEYWORDS
cognitive performance, dementia, epigenetic age acceleration, older adults, sex differences

Highlights

- Epigenetic age acceleration was associated with cognitive deterioration over time.
- However, these associations differed by sex.
- In females, accelerated GrimAge appeared to be a better marker of decline in memory.
- In males, accelerated GrimAge was associated with slower processing speed over time.
- Association between accelerated GrimAge and dementia risk was found only in males.

1 | BACKGROUND

Decline in cognitive function becomes increasingly common with advanced age and is an important concern for many people in older age.[1,2] However, the extent of cognitive decline can vary among older individuals.[1–3] Some older individuals can retain good cognitive ability into advanced age with only minor changes, while others may experience more severe cognitive deterioration with age.[1–3] For some older individuals, the degree of cognitive decline is substantial and impacts the ability to complete everyday tasks and eventually results in a diagnosis of dementia.[1–3] The reasons for these individual variabilities in cognitive aging are complex and multifaceted, extending beyond chronological age and established modifiable factors such as education. Differences in biological aging could help account for these variations.[4]

Epigenetic age provides an estimate of an individual's biological age determined by age-related changes in DNA methylation (DNAm) levels at specific sites across the genome.[5–12] Epigenetic mechanisms, like DNAm, regulate gene expression in response to lifestyle, environmental, and genetic factors.[13,14] Epigenetic age may therefore provide a more accurate measure of how well an individual's body is functioning at the molecular level compared to chronological age.[12] Epigenetic age acceleration (AA) refers to an older epigenetic age relative to chronological age and is a promising aging biomarker.[6–10] Evidence has shown that epigenetic AA was associated with an increased risk of decline in physical function, frailty, age-related diseases, and all-cause mortality.[6–10,15,16] However, it remains unclear to what extent epigenetic AA is associated with cognitive ageing across different domains of function, and there is inconsistent evidence regarding the association between epigenetic AA and dementia risk.[13] Investigating the relationship between epigenetic AA and changes in cognitive function and risk of dementia may provide insight into the validity of these as biomarkers of healthy ageing and inform a better understanding of the mechanisms underlying age-related cognitive decline. Moreover, further research is needed to explore potential sex differences in these associations,[13] as emerging evidence shows sex disparities in the relationships between epigenetic AA and risk factors for worse general health.[15,16]

Therefore, this exploratory study aimed to examine whether different epigenetic AA scores are associated with change in cognitive function over 9 years, and the 9-year risk of incident dementia, separately in males and females.

2 | METHODS

2.1 | Study population

This study sub-sampled 560 older Australians aged ≥70 years from the ASPREE (ASPirin in Reducing Events in the Elderly) study, who provided blood samples at baseline through the ASPREE Healthy Ageing Biobank.[15,17] A total of 97.3% of the ASPREE Australian participants were self-identified as White/Caucasian.[18] Full details regarding the ASPREE sampling procedure and methods have been reported elsewhere.[17,18] Briefly, in Australia, ASPREE participants were mainly recruited through partnerships with general practitioners from March 2010 and December 2014 and followed prospectively. At study enrollment, participants were free of known dementia, cardiovascular disease (CVD), or other major life-limiting diseases, and had a Modified Mini-Mental State Examination (3MS) score of ≥78. The ASPREE study complies with the Declaration of Helsinki and was approved by multiple Institutional Review Boards (www.aspree.org). All participants provided written informed consent for participation before enrollment. The present sub-study was approved by the Monash University Human Research Ethics Committee (MHREC 30734).

2.2 | Epigenetic age

DNAm from baseline peripheral blood samples (buffy coat), was extracted using the Qiagen DNeasy Blood & Tissue Kits (https://www.qiagen.com/au).[15,16,19] Epigenome-wide DNAm was measured using the Illumina Infinium Methylation EPIC BeadChip (EPIC) and run at the Australian Genome Research Facility, Melbourne, Victoria.[15,16,19] Preprocessing of data was performed using R version 4.1.3 with R package minfi and normalization of the EPIC data was undertaken using the preprocessNoob method.[15,16,19,20] DNAm-derived epigenetic age, namely HorvathAge,[6] HannumAge,[7] PhenoAge,[8] GrimAge,[9] and GrimAge2[10],

and their AA scores were estimated using the online DNAm age calculator https://dnamage.genetics.ucla.edu/new and https://dnamage.clockfoundation.org/. The principal component (PC)-trained version of the available DNAm-derived epigenetic ages (HorvathAge, HannumAge, PhenoAge, and GrimAge), and their AA scores were also estimated with codes available on GitHub from the Levine Lab https://github.com/MorganLevineLab/PC-Clocks.[21] AA scores are residuals resulting from the regression of each DNAm-derived epigenetic age on chronological age and a positive (negative) value of AA indicates an older (younger) epigenetic age relative to chronological age.[6–10] DunedinPACE, a DNAm-based biomarker of the Pace of Aging, was estimated in R version 4.1.3 with the codes described in Belsky et al.[11] Higher DunedinPACE value indicates an accelerated rate of aging.[11]

2.3 | Cognitive performance

Cognitive function assessments were conducted at baseline and over follow-up (year 1, year 3, year 9) visits. The numbers of participants who completed cognitive function assessments at each visit were shown in Tables S1–S7. Cognitive tests included (1) the Modified Mini-Mental State Examination (3MS) to measure global cognition,[22] (2) the Hopkins Verbal Learning Test-Revised (HVLT-R) delayed recall task to measure episodic memory,[23,24] (3) the Controlled Oral Word Association Test (COWAT—-single letter F version) to measure phonemic verbal fluency,[25,26] and (4) the Symbol Digit Modalities Test (SDMT) to measure psychomotor speed.[27,28] The procedures of these cognitive function assessments were provided in supplementary materials Text-S1. These four measures of cognitive functioning are the validated tools for assessing their respective domains of cognitive function, with high reliability and internal consistency.[22–28] They have demonstrated sensitive changes in their relative cognitive domains, making them useful and reliable for monitoring cognitive decline in both patients and general populations.[22–28]

The composite cognitive score was calculated to estimate overall cognitive performance by summing the *z*-scores of all four tests.[29,30] The composite executive/psychomotor functioning score was estimated by summing the z-scores of SDMT, COWAT, and the similarities subscales of the 3MS.[30] The composite memory score was calculated by summing the *z*-scores of HVLT-R delayed recall and the memory subscales of the 3MS.[30]

2.4 | Incident dementia

Dementia was a secondary endpoint in the ASPREE study, and full details of the dementia trigger and a description of the dementia adjudication process in ASPREE have been reported previously.[29] In brief, three dementia triggers (people with a suspected dementia diagnosis) were identified: participants with a 3MS score < 78 or a drop of >10.15 points from their predicted score based on their own baseline 3MS score with adjustment for age and education; a report of memory concerns or other cognitive problems; or a medical diagnosis of dementia or prescription of acetylcholinesterase inhibitors. To reduce the possibility of delirium, further assessments were performed at least 6 weeks after the initial dementia trigger.[29] Further assessments include Alzheimer's Disease Assessment Scale–Cognitive subscale, Color Trails, Lurian overlapping figures, and the Alzheimer's Disease Cooperative Study Activities of Daily Living scale.[29] When available, other documents such as results of laboratory and blood tests, brain computed tomography (CT) scans, or magnetic resonance imaging (MRI) reports, detailed medical records, and clinical case notes were gathered from their healthcare providers. All available information was reviewed by the dementia adjudication committee composed of neurologists, neuropsychologists, and geriatricians from Australia and the United States. Dementia diagnosis was adjudicated based on the Diagnostic and Statistical Manual for Mental Disorders – IV (DSM-IV), American Psychiatric Association, criteria.[31] For confirmed dementia cases by the adjudication committee, the date of the dementia trigger was used as the date of dementia diagnosis.

RESEARCH IN CONTEXT

1. **Systematic review**: A systematic review was conducted through MEDLINE, Embase, and PsycINFO. Findings highlighted the extent to which epigenetic age acceleration (AA) measures are associated with cognitive performance across different domains remains unclear, and the associations between epigenetic AA and dementia risk have been inconsistently found. Additionally, further research should explore potential sex differences in these associations.
2. **Interpretation**: Our findings showed sex differences in the associations between epigenetic AA and worse cognitive performance over time. Epigenetic AA, particularly measured by GrimAA/Grim2AA, was a better marker of decline in memory for females and processing speed for males. The association between GrimAA/Grim2AA and incident dementia was only found in males.
3. **Future directions**: Our finding highlights the importance of considering sex differences in addressing age-related health disparities. Further research is warranted to investigate the reasons underlying these differential associations.

2.5 | Statistical analysis

The statistical analyses were undertaken separately in males and females. First, the correlation between epigenetic AA markers and different measures of baseline cognitive performance (3MS,

HVLT-R, COWAT, SDMT, and composite scores—cognitive, executive/psychomotor functioning, and memory) was examined using Pearson's correlation, with pairwise deletion. Next, linear mixed models were used to determine the association between AA and cognitive function over time. The AA scores were standardized so results could be interpreted as a 1-standard deviation (SD) increase in AA. Each model included a participant-specific random intercept and random slope, and an AA by time interaction to assess the effect of baseline epigenetic AA on cognitive performance over time. The multivariable models were adjusted for chronological age; education (<12 years or ≥12 years); socioeconomic status (SES; very low, low, middle, high, and very high) estimated through the Socio-Economic Indices for Areas-Index of Relative Socioeconomic Advantage and Disadvantage (SEIFA-IRSAD)[32]; smoking (never, former, and current); number of chronic conditions (which considered hypertension, diabetes, dyslipidemia, chronic kidney disease, obesity, and depression); and batch effect. Furthermore, it is known that cellular heterogeneity in the blood can greatly affect DNAm-derived epigenetic age, and adjusting the proportions of blood cells in the analysis can mitigate these impacts and produce more accurate and reliable results.[33,34] Therefore, we also included adjustments for the online DNAm age calculator https://dnamage.genetics.ucla.edu/new estimated cells counts (natural killer cells [NK], monocytes [Mono], granulocytes [Gran], Plasmablasts, CD4+ T lymphocytes, CD8pCD28nCD45RAn [exhausted cytotoxic T cells] and CD8 naive) in our models.

In addition, the analysis was repeated using binary groups defined as decelerated aging (≤0) and accelerated aging (>0), for HorvathAA, HannumAA, PhenoAA, GrimAA, and Grim2AA and low (≤0.97) and high (>0.97) for DunedinPACE. Next, the associations between baseline AA measures and incident dementia were examined using the Cox proportional hazards regression models, adjusted using the covariates reported above.

Finally, we also examined the associations between available PC-trained epigenetic AA measures and cognitive performance over time, and dementia risk as additional analyses to ensure associations remained consistent with the version of epigenetic AA measures which are trained on PC for more robust measures.[21] All analyses were performed using Stata 17 (StataCorp).

Furthermore, using the *p*-values from modeling the associations between different epigenetic clocks and each cognitive function score over time or dementia risk, we applied the Hochberg method[35,36] through the online calculator available at www.multipletesting.com and checked whether our investigated associations were consistent after the adjustment of *p*-values for multiple tests.

3 | RESULTS

3.1 | Participants' characteristics

The baseline characteristics of the study participants, with 97.3% being White Australians, are shown in Table 1. Approximately half were female (51%), and the majority of participants had ≥12 years of education (59.8% in males and 56.7% in females, Table 1). The mean chronological age for males and females was 74.5 (4.2). GrimAge2 (75.4 [5.5] in males and 72.8 [4.8] in females) was similar to the mean chronological age, whereas HorvathAge and GrimAge (approximately 70 years in both males and females) were slightly younger than chronological age. Males compared to females showed statistically higher epigenetic aging and lower cognitive performance scores. The positive values found in the AA scores of males showed that their epigenetic ages were relatively older than chronological age, indicating epigenetic AA. In contrast, the negative values of females' AA scores demonstrated that they were epigenetically aging at a rate slower than their chronological age.

3.2 | Sex-specific correlations between epigenetic AA scores and cognitive performance at baseline

Correlations between AA scores and cognitive performance were more commonly found in females (Figure 1), with all epigenetic AA scores except for HorvathAA negatively correlated with multiple domains of baseline cognitive performance (r ranged from −0.12 to −0.34, Figure 1). DunedinPACE showed the strongest correlations with cognitive function. In males, correlations were found between PhenoAA and worse cognitive performance on delayed recall for episodic memory ($r = -0.13$), and between DunedinPACE and worse performance on all domains except for COWAT (r ranged from −0.13 to −0.19).

3.3 | Epigenetic AA scores and change in cognitive performance over an average of 7 years of follow-up

Sex differences were found in the associations between epigenetic AA scores (on a continuous scale and categorical groups) and the change in cognitive performance over time (Figure 2, Table 2, Tables S8–S14). Accelerated GrimAge and GrimAge2 were associated with worse cognitive performance on delayed recall for episodic memory (HVLT-R) over time in females (adjusted-beta = −0.1732 and −0.2034, respectively, $p < 0.05$, Table 2), but not in males. Likewise, accelerated GrimAge2 was only associated with worse performance over time for composite cognitive score (adjusted beta = −0.1933, $p = 0.04$) and composite memory score (adjusted-beta = −0.1372, $p = 0.01$, Table 2) in females. The association between accelerated GrimAge and worse performance on the speed of cognitive processing (SDMT) was found only in males (adjusted-beta = −0.3049, $p = 0.03$, Table 2). These associations remained consistent when we repeated the models by excluding cognitive function scores in years 4, 8, and 9 where the number of cognitive assessment completions is lower compared to other follow-up visits. Similar results were also found when additionally adjusting for chronological age in the fully adjusted models (data not shown) and when we used PC-trained epigenetic AA scores in the additional analyses (Tables S15 and S16). The associations between accelerated GrimAge/GrimAge2 and HVLT-R (delayed recall) and

TABLE 1 Characteristics of participants at baseline, separately in males and females

Characteristic	Males ($n = 276$, 49.3%) Mean (SD)	Females ($n = 284$, 50.7%) Mean (SD)	*p*-Value
Age in years	74.5 (4.2)	74.5 (4.2)	0.98
Epigenetic age in years			
HorvathAge	71.0 (6.1)	70.1 (6.0)	0.06
HannumAge	60.4 (6.1)	58.6 (6.0)	<0.001
PhenoAge	59.7 (7.7)	58.1 (7.6)	0.01
GrimAge	71.5 (5.1)	68.6 (4.5)	<0.001
GrimAge2	75.4 (5.5)	72.8 (4.8)	<0.001
DunedinPACE	0.99 (0.11)	0.95 (0.11)	<0.001
Epigenetic AgeAccels (AA) in years			
HorvathAA	0.49 (4.96)	−0.31 (4.92)	0.05
HannumAA	0.79 (5.04)	−0.64 (4.54)	<0.001
PhenoAA	0.76 (6.46)	−0.55 (6.21)	0.01
GrimAA	1.35 (3.34)	−1.27 (3.19)	<0.001
Grim2AA	1.16 (3.99)	−1.08 (3.78)	<0.001
PC-trained epigenetic age in years			
HorvathAge	69.3 (6.2)	67.3 (5.8)	<0.001
HannumAge	74.1 (6.3)	71.9 (6.0)	<0.001
PhenoAge	69.6 (7.6)	66.9 (7.6)	<0.001
GrimAge	81.4 (4.7)	78.8 (4.7)	<0.001
PC-trained epigenetic AgeAccels (AA) in years			
HorvathAA	0.96 (5.12)	−0.81 (4.77)	<0.001
HannumAA	1.03 (5.13)	−0.87 (4.90)	<0.001
PhenoAA	1.27 (6.42)	−1.06 (6.43)	<0.001
GrimAA	1.20 (3.14)	−1.07 (3.15)	<0.001
Cognitive function scores			
3MS	92.9 (4.7)	94.0 (4.1)	0.005
HVLT-R (Delayed recall)	7.6 (2.6)	8.4 (2.4)	<0.001
COWAT (Verbal fluency)	12.1 (4.3)	13.2 (4.4)	0.003
SDMT (Processing speed)	37.0 (8.7)	38.8 (9.2)	0.01
Composite scores			
Composite cognitive	−0.13 (2.55)	0.80 (2.34)	<0.001
Composite executive/psychomotor functioning	0.06 (1.96)	0.60 (1.84)	<0.001
Composite memory	−0.19 (1.64)	0.29 (1.41)	<0.001
	***n* (%)**	***n* (%)**	***p*-value**
Ethno-racial group			
White Australian	267 (96.7)	278 (97.9)	0.40
Hispanic/Latino/Asiatic/Other[a]	9 (3.3)	6 (2.1)	
Years of education			
<12 years	111 (40.2)	123 (43.3)	0.46
≥12 years	165 (59.8)	161 (56.7)	

(Continues)

TABLE 1 (Continued)

	n (%)	*n* (%)	*p*-value
Socioeconomic status (SES)			
Very low	23 (8.4)	25 (8.8)	0.94
Low	19 (6.9)	25 (8.8)	
Middle	43 (15.6)	42 (14.8)	
High	61 (22.2)	60 (21.1)	
Very high	129 (46.9)	132 (46.5)	
Smoking			
Never	129 (46.7)	183 (64.4)	<0.001
Former	135 (48.9)	92 (32.4)	
Current	12 (4.4)	9 (3.2)	
Number of chronic conditions[b]			
<2 comorbidities	110 (39.9)	96 (33.8)	0.14
≥2 comorbidities	166 (60.1)	188 (66.2)	

Notes: SES was estimated through the Socio-Economic Indices for Areas-Index of Relative Socioeconomic Advantage and Disadvantage (SEIFA-IRSAD) based on the information from the 2011 Australian Census using the residential postcodes of participants; All *p*-values are from chi-squared or *t*-test comparison of males versus females.

Abbreviation: 3MS, Modified Mini-Mental State Examination; COWAT, Controlled Oral Word Association Test; HVLT-R, Hopkins Verbal Learning Test-Revised; SD, standard deviation; SDMT, Symbol Digit Modalities Test.

[a]Other included Aboriginal and Torres Strait Islander, other Pacific Islander, Maori, or more than one race.

[b]Chronic conditions included hypertension, diabetes, dyslipidemia, chronic kidney disease, obesity, and depression.

composite memory scores, found in females, remained significant after adjustment of *p*-values for multiple comparisons.

3.4 | Epigenetic AA scores and incident dementia

Over an average of 7 years of follow-up to capture dementia diagnosis, 37 males (19.61 events per 1000-persons-year) and 50 females (25.48 events per 1000-persons-year), developed incident dementia, respectively. Associations between epigenetic AA scores and incident dementia are shown in Tables 3 and S17. A 1-SD increase in GrimAA/Grim2AA was associated with up to two-fold increased risk of dementia in males (Table 3), but not in females. The results were the same when considering the competing risk of death in these multivariable Cox models (adjusted HRs in males: 1.77 (95% CI 1.02–3.07) for GrimAA and 1.95 (95% CI 1.20–3.15) for Grim2AA). The association between Grim2AA and increased risk of dementia in males remained significant even after adjustment of *p*-values for multiple comparisons. In the additional analyses using PC-trained epigenetic AA scores, a 1-SD increase in GrimAA in males was associated with a 49% increased risk of dementia, but this association did not reach statistical significance (Tables S18 and S19).

4 | DISCUSSION

In this study of 560 initially healthy community-dwelling older people aged 70 years and over, we found moderate cross-sectional correlations between epigenetic AA and baseline cognitive function, particularly for GrimAA, Grim2AA, and DunedinPACE measures, which were more commonly observed in females. Furthermore, there were sex differences in the associations between epigenetic AA and cognitive change over time. In females, over an average of 7 years of follow-up, GrimAA/Grim2AA was associated with worse episodic memory, composite memory, and composite cognitive function. In contrast, the association between GrimAA and worse performance on processing speed over time was observed only in males. Furthermore, the association between GrimAA/Grim2AA and incident dementia was only observed in males.

Few studies have investigated the association between epigenetic aging and cognitive performance in older people aged ≥65 years, and the findings have been inconsistent across studies.[13] Further, most prior studies have been cross-sectional while others focused on a single epigenetic age measure and cognitive performance at one time point, rather than examining changes in cognitive performance over time.[13] Our study thus contributes important new information demonstrating that certain AA measures, most notably GrimAA, were most strongly associated with worse cognitive performance over time, but with clear sex-specific associations. Epigenetic AA was not associated with global cognitive function or verbal fluency in either sex but appeared to be a better marker of decline in memory (for females) and processing speed (for males). Aligned with these findings, memory and processing speed are domains which appear more susceptible to decline in older age. Memory loss is an early sign of cognitive aging. The hippocampus, important for the storage and retrieval of episodic memory, is particularly susceptible to structural and

Males	3MS	HVLT-R (Delayed recall)	COWAT (Verbal fluency	SDMT (Processing speed)	Composite Cognitive	Composite executive/ psychomotor functioning	Composite Memory
HorvathAA	0	-0.11	0	-0.12	-0.08	-0.05	-0.09
HannumAA	0.01	-0.06	0.02	-0.11	-0.04	-0.03	-0.01
PhenoAA	-0.03	-0.13 *	0.1	-0.06	-0.04	0.01	-0.08
GrimAA	-0.04	-0.06	-0.03	-0.07	-0.07	-0.11	-0.04
Grim2AA	-0.05	-0.08	-0.03	-0.06	-0.08	-0.11	-0.04
DunedinPACE	-0.14 *	-0.17 *	-0.08	-0.13 *	-0.19 **	-0.14 *	-0.14 *

Females	3MS	HVLT-R (Delayed recall)	COWAT (Verbal fluency	SDMT (Processing speed)	Composite Cognitive	Composite executive/ psychomotor functioning	Composite Memory
HorvathAA	-0.06	-0.05	-0.02	-0.03	-0.06	-0.03	-0.03
HannumAA	-0.13 *	-0.13 *	-0.03	-0.05	-0.13 *	-0.05	-0.1
PhenoAA	-0.11	-0.09	-0.01	-0.17 **	-0.14 *	-0.14 *	-0.07
GrimAA	-0.08	-0.05	-0.08	-0.28 ***	-0.19 **	-0.24 ***	-0.04
Grim2AA	-0.12 *	-0.08	-0.08	-0.29 ***	-0.22 ***	-0.26 ***	-0.09
DunedinPACE	-0.14 *	-0.14 *	-0.24 ***	-0.24 ***	-0.29 ***	-0.34 ***	-0.1

*p-value < 0.05; **p-value < 0.01; ***p-value < 0.001

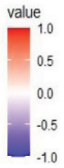

FIGURE 1 Correlations between epigenetic age acceleration (AA) and cognitive function scores at baseline, separately in males ($n = 276$) and females ($n = 284$).

functional age-related changes.[37] The more evident memory decline in females may be partially due to their smaller hippocampal volumes and lower tissue densities compared to males,[38] which may make them less resilient against cognitive aging. The function of processing speed is primarily linked to the integrity of white matter tracts,[39] where myelinated axons enable rapid neurotransmission and efficient cognitive processing.[40] Similarly, microstructural changes have also been observed in these tracts with age,[41] echoing the prevalent psychomotor slowing observed previously.[42] Although the mechanisms of sex differences in psychomotor slowing remain inconclusive, the stronger association between AA and processing speed decline in males may be driven by our prior finding showing males compared to females have lower performance in processing speed and are more susceptible to age-related declines in this domain.[28] In addition, our findings that certain AA measures were more consistently associated with cognitive performance likely reflect the way in which these AA measures were trained and developed.[6–11] The earliest generation of AA measures, HorvathAA and HannumAA, were trained to predict chronological

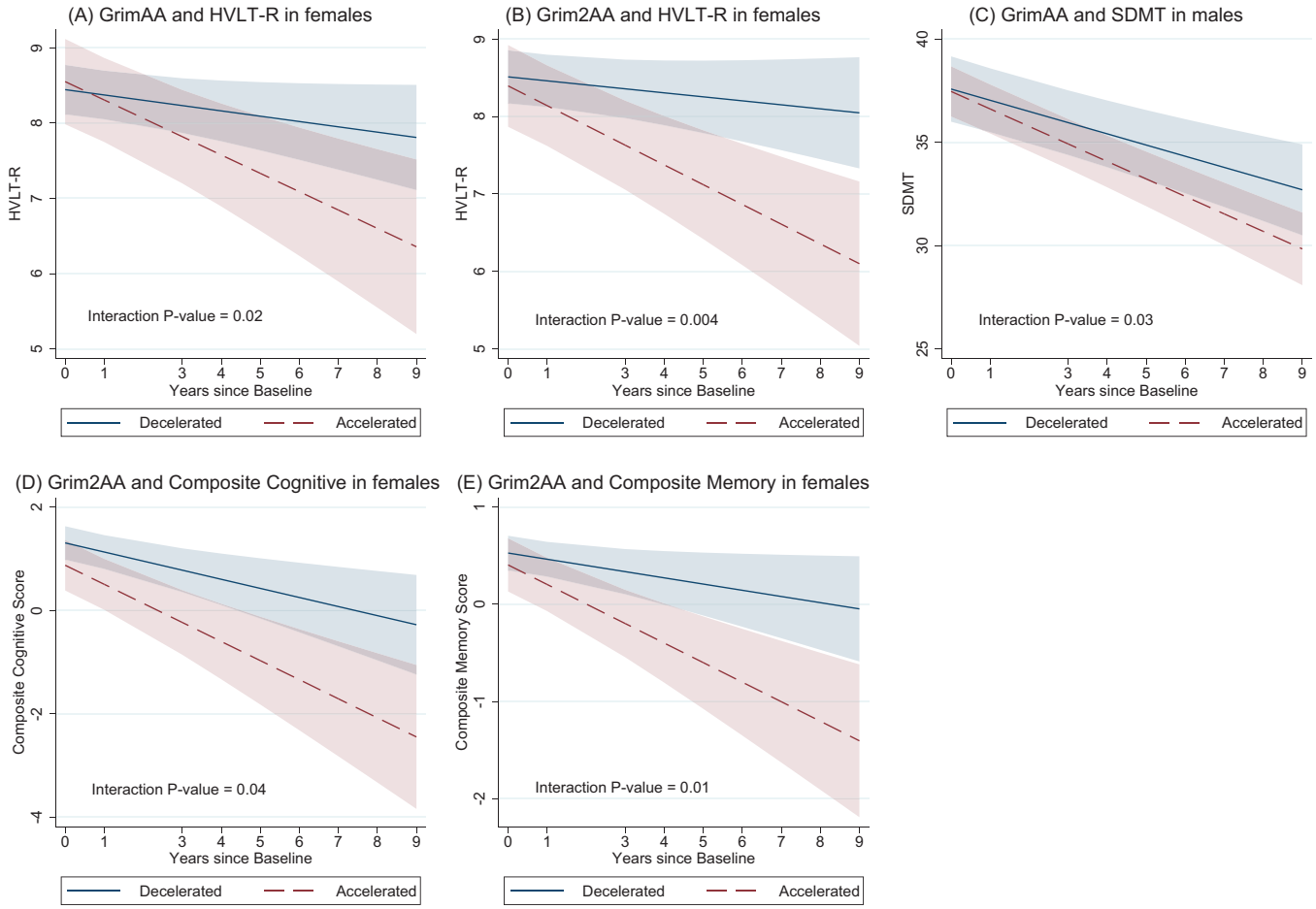

FIGURE 2 Estimated marginal means of cognitive performance score over time by categorical epigenetic AA.

age[6,7]; however, the later generations, such as GrimAA/Grim2AA[9,10] and DunedinPACE,[11] incorporate additional health biomarkers alongside chronological age, to better reflect age-related chronic health conditions and mortality. Therefore, such markers designed to predict chronic diseases and mortality may also, to some extent, capture late-life cognitive deterioration due to common underlying biological aging processes as well as shared pathophysiological mechanisms such as protein misfolding, inflammation, and vascular problems.[43]

The exploration of sex differences in the association between AA and health outcomes in older individuals has been seldom investigated. We have previously shown evidence of a sex difference in the cross-sectional associations between AA scores and chronic diseases, including hypertension, obesity, and depression,[15] and AA scores were more strongly associated with frailty burden over time in females than males.[16] Building upon these findings, the present study indicates clear sex differences in the associations between AA scores and cognitive decline over time, suggesting that these AA measures may better capture cognitive aging in females than males. Although the exact reason behind this finding remains unclear, it may be partly driven by the sex-specific biological phenotypes and hormonal influences along with a higher burden of comorbidities and frailty in females. For example, frailty and numbers of chronic disease conditions have been shown to be more strongly associated with AA scores in females than in males[9,10,15,16] and are also associated with cognitive deterioration through common pathological mechanisms such as chronic inflammation, cardiovascular risk, and endocrine dysregulation.[44] This interplay relationship underscores the need for further investigations on how comorbidities and frailty mediate the association between AA scores and cognitive function, separately in males and females. Moreover, it is known that the female sex hormone estrogen regulates biological functions such as metabolic regulation and epigenetic modifications.[45,46] Estrogen also plays a multifaceted role in the neurobiology of cognitive processing, providing neuroprotective effects and enhancing cognitive function.[47] So, menopausal hormone changes (i.e., declining estrogen levels) somewhat compromise these neuroprotective effects, detrimentally affecting cognitive performance.[47] Therefore, differences in biological phenotypes, genetic constitutions, and hormonal fluctuations between males and females may contribute to sex differences in the associations between epigeneticAA and cognitive aging. Further research is warranted to explore the underlying mechanisms that cause these relationships to differ between males and females.

In recent years, a few studies have investigated the longitudinal association between epigenetic AA meaures and incident dementia. However, the findings on this relationship have been inconsistent

TABLE 2 Association between baseline GrimAA/Grim2AA and change in cognitive performance over follow-up, separately in males and females.

	Males ($n = 275$)		Females ($n = 284$)	
Mixed model analysis with interactions	**Adjusted Beta[c]**	***p*-value**	**Adjusted beta[c]**	***p*-Value**
3MS				
GrimAA				
GrimAA[a]×time	−0.0756	0.34	−0.1984	0.14
Accelerated_GrimAA[b]×time	−0.1488	0.31	−0.5383	0.05
Grim2AA				
Grim2AA[a]×time	−0.0597	0.44	−0.1668	0.20
Accelerated_Grim2AA[b]×time	−0.0042	0.98	−0.3565	0.17
HVLT-R (delayed recall)				
GrimAA				
GrimAA[a]×time	−0.0203	0.52	−0.0686	0.07
Accelerated_GrimAA[b]×time	0.0073	0.90	**−0.1732**	**0.02**
Grim2AA				
Grim2AA[a]×time	−0.0105	0.73	**−0.0808**	**0.03**
Accelerated_Grim2AA[b]×time	0.0560	0.33	**−0.2034**	**0.004**
COWAT (verbal fluency)				
GrimAA				
GrimAA[a]×time	−0.0345	0.38	−0.0389	0.39
Accelerated_GrimAA[b]×time	0.0002	1.00	−0.0378	0.67
Grim2AA				
Grim2AA[a]×time	−0.0332	0.39	−0.0304	0.48
Accelerated_Grim2AA[b]×time	0.0044	0.95	−0.0428	0.61
SDMT (processing speed)				
GrimAA				
GrimAA[a]×time	−0.1332	0.08	−0.0835	0.35
Accelerated_GrimAA[b]×time	**−0.3049**	**0.03**	0.0417	0.81
Grim2AA				
Grim2AA[a]×time	−0.1430	0.06	−0.0497	0.56
Accelerated_Grim2AA[b]×time	−0.1378	0.33	0.1536	0.35
Composite cognitive score				
GrimAA				
GrimAA[a]×time	−0.0400	0.22	−0.0858	0.09
Accelerated_GrimAA[b]×time	−0.0524	0.39	−0.1406	0.16
Grim2AA				
Grim2AA[a]×time	−0.0273	0.40	−0.0930	0.05
Accelerated_Grim2AA[b]×time	0.0114	0.85	**−0.1933**	**0.04**
Composite executive/psychomotor functioning score				
GrimAA				
GrimAA[a]×time	−0.0271	0.17	−0.0313	0.14
Accelerated_GrimAA[b]×time	−0.0482	0.19	−0.0162	0.70
Grim2AA				
Grim2AA[a]×time	−0.0243	0.21	−0.0294	0.15
Accelerated_Grim2AA[b]×time	0.0002	1.00	−0.0098	0.81

(Continues)

TABLE 2 (Continued)

Mixed model analysis with interactions	Males ($n = 275$) Adjusted Beta[c]	*p*-value	Females ($n = 284$) Adjusted beta[c]	*p*-Value
Composite memory score				
GrimAA				
GrimAA[a]×time	−0.0128	0.53	−0.0339	0.24
Accelerated_GrimAA[b]×time	−0.0076	0.84	−0.0786	0.17
Grim2AA				
Grim2AA[a]×time	−0.0092	0.65	−0.0415	0.13
Accelerated_Grim2AA[b]×time	0.0241	0.52	**−0.1372**	**0.01**

Notes: For SDMT and composite executive/psychomotor functioning score, males ($n = 274$) and females ($n = 284$); for composite cognitive score, males ($n = 274$) and females ($n = 283$). Bold text indicates statistically significant associations.
Abbreviations: 3MS, Modified Mini-Mental State Examination; COWAT, Controlled Oral Word Association Test; HVLT-R, Hopkins Verbal Learning Test-Revised; SDMT, Symbol Digit Modalities Test.
[a]On a continuous scale, per 1 standard deviation increase.
[b]Decelerated epigenetic age acceleration was the reference group.
[c]Adjusted for years of education, socioeconomic status, smoking, numbers of chronic conditions, batch effect, and cells counts.

TABLE 3 Associations between epigenetic AA measures and risk of incident dementia, separately in males and females.

Parameter	Males ($n = 275$) Adjusted hazard ratio	95% CI	Females ($n = 284$) Adjusted hazard ratio	95% CI
Epigenetic AA measures per 1-SD increase				
HorvathAA	0.89	(0.58–1.35)	1.18	(0.86–1.63)
HannumAA	1.29	(0.79–2.10)	0.92	(0.62–1.37)
PhenoAA	0.93	(0.58–1.49)	1.05	(0.71–1.54)
GrimAA	**1.78**	**(1.04–3.04)**	0.96	(0.60–1.55)
Grim2AA	**2.00**	**(1.15–3.48)**	0.96	(0.60–1.53)
DunedinPACE	0.85	(0.51–1.40)	0.74	(0.52–1.06)

Note: Adjusted for chronological age, years of education, SES, smoking, numbers of chronic conditions, batch effect, and cells counts. Bold text indicates statistically significant associations.
Abbreviations: AA, age acceleration; CI confidence iterval; SD, standard deviation.

across studies,[13,48–50] with some studies revealing epigenetic AA can predict dementia risk[48,49] whereas others showed null results.[13,50] In addition, knowledge about sex differences in these relationships remains limited despite our well-known understanding of sex differences in dementia risk.[13] Thus, the present study employing the sex-stratified analysis advances the field by showing associations between GrimAA/Grim2AA and incident dementia risk only in males. Our preliminary finding of sex differences in these associations underscores the importance of investigating sex-specific biological pathways in dementia development, highlighting the need for further investigation into this area.

This study had notable strengths and limitations. First, our strength includes the availability of longitudinal cognitive assessments across different domains (global cognition, episodic memory, verbal fluency, and psychomotor processing speed) over an average of 7 years, undertaken by rigorously trained and qualified ASPREE staff, ensuring the reliability and high-quality of the data. Almost half of our study participants were females; thus, this sex-balanced cohort allowed us to explore possible sex differences in these investigated associations. Limitations are that ASPREE participants were free of diagnosed CVD, dementia, or known major life-limiting diseases at recruitment,[17] and 97.3% of our current study cohort were White/Caucasian Australians; thus, our findings may be somewhat less generalizable to less healthy populations and other specific ethnic-racial groups. Further, it is noted that we assessed cognitive functions across global and three major domains in this study, which could be a limited subset of the range of cognitive functions. Thus, our study will be limited to capturing potential associations with other cognitive domains which we did not explore. In addition, we have previously shown that most participants maintained their cognitive performance over time, with only a small proportion experiencing a substantial decline in global cognition and episodic memory, reflecting a healthy cohort.[42] Therefore, an average follow-up of 7 years may be relatively short to capture a substantial decline in cognition and incident dementia.

In conclusion, our study demonstrated the associations between accelerated epigenetic aging (particularly in more recent iterations of epigenetic AA measures) and worse cognitive performance over time, even in relatively healthy older people. These associations varied between males and females. Over an average follow-up of 7 years, accelerated epigenetic age (GrimAA/Grim2AA) is associated with cognitive decline in overall cognition, delayed recall, and memory functioning in females, and the decline in cognitive processing speed in males. The associations between GrimAA/Grim2AA and increased incident dementia risk were found only in males. Our comprehensive findings highlight the importance of considering sex differences in the aging process, demonstrating the need for sex-specific interventions and healthcare strategies in addressing age-related health disparities.

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CONFLICT OF INTEREST STATEMENT

The authors declare no competing interests. Author disclosures are available in the supporting information.

CONSENT STATEMENT

The data of the present secondary data analysis study were from a 5-year ASPREE clinical trial—Trial Registration: International Standard Randomized Controlled Trial Number Register (ISRCTN 83772183) and clinical trials.gov (NCT 01038583). The ASPREE trial in Australia was approved by multiple Institutional Review Boards with primary ethics approval granted by the Monash University Human Research Ethics Committee (CF07/3730-2006/745MC). In Australia, the ASPREE-XT study received ethics approval from the Human Research Ethics Committee of Alfred Health (HREC/17/Alfred/198); The University of Tasmania (H0017149); Monash University (12771); ACT Health (ETH.3.18.037E); and The University of Adelaide (32802). The current study was approved by the Monash University Human Research Ethics Committee (MHREC 30734). All individual participants of the ASPREE and ASPREE-XT studies including the ASPREE Biobank sub-study signed informed consent on participation.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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