

Associations of Systems-Based DNA Methylation Clocks with Cognitive Impairment, Plasma Biomarkers of Alzheimer’s Pathology, and MRI biomarkers of ADRD

Steve Nguyen¹, PhD; Bowei Zhang¹, MS; Raghav Sehgal¹², PhD; Michael J. LaMonte³, PhD; Steve Horvath⁴, PhD; Mark A. Espeland⁵, PhD; Stephen R. Rapp⁶, PhD; Adam X. Maihofer^{7, 8}, PhD; Caroline M. Nievergelt^{7, 8}, PhD; Andrea Z. LaCroix¹, PhD; Linda K. McEvoy⁹, PhD; Ramon Casanova¹⁰, PhD; Eric Hyde¹, PhD; Susan M. Resnick¹¹, PhD; Aladdin H. Shadyab^{1,12}, PhD

¹ Herbert Wertheim School of Public Health and Human Longevity Science, University of California San Diego, La Jolla, CA, USA; ² Department of Psychiatry, Yale University School of Medicine, New Haven, Connecticut, USA; ³ Department of Epidemiology and Environmental Health, State University of New York at Buffalo; ⁴ Department of Medicine, Division of Geriatrics, David Geffen School of Medicine, University of California, Los Angeles, CA, USA; ⁵ Division of Gerontology and Geriatric Medicine, School of Medicine, Wake Forest University, Winston-Salem, North Carolina, USA; ⁶ Department of Psychiatry and Behavioral Medicine, Wake Forest University School of Medicine, Winston-Salem, NC, USA; ⁷ Department of Psychiatry, University of California San Diego, La Jolla, CA, USA; ⁸ Research Service, Veterans Affairs San Diego Healthcare System, San Diego, CA, USA; ⁹ Kaiser Permanente Washington Health Research Institute, Seattle, WA, USA; ¹⁰ Department of Biostatistics and Data Science, Wake Forest University School of Medicine, Winston-Salem, North Carolina; ¹¹ University of Pennsylvania, Philadelphia, PA, USA; ¹² Division of Geriatrics, Gerontology, and Palliative Care, Department of Medicine, University of California San Diego

Introduction

- DNA methylation (DNAm) Systems Age was recently developed to quantify aging across 11 distinct physiological systems¹
- DNAm Systems Age has not been examined in relation to incident MCI and dementia, blood-based biomarkers of Alzheimer’s Disease (AD), and MRI biomarkers of AD and related dementias (ADRD).

Objective

- Examine the associations of DNAm Systems Age and its individual systems with (1) incident MCI and dementia risk, (2) longitudinal changes in plasma ADRD biomarkers over time, and (3) MRI biomarkers of ADRD measured ~8 years later.

Methods

- 6026 women in the Women’s Health Initiative (WHI) Memory Study (WHIMS) who were free of mild cognitive impairment or probable dementia at baseline in 1996-1999².
- DNAm Systems Age consists of 11 distinct physiological systems: Heart, Lung, Kidney, Liver, Brain, Immune, Inflammatory, Blood, Musculoskeletal, Hormone, and Metabolic¹. We calculated epigenetic age acceleration (EAA) for DNAm Systems Age and each of its systems as the residual from a linear regression of each measure on age.
- Women completed global cognitive function tests annually from baseline through 2021 ³. Women who scored below age and education adjusted cutoffs underwent additional neuropsychological testing. MCI and dementia were centrally adjudicated with a panel of experts^{2, 3}.
- Plasma phosphorylated tau at threonine-217 (p-tau217), p-tau181, glial fibrillary acidic protein (GFAP), neurofilament light chain (NfL), and amyloid beta (Aβ)42:40 at baseline and ~15 years later were measured using Simoa assays⁴.
- MRI measures consisted of AD Pattern Similarity (AD-PS) scores⁵. We modeled AD-PS scores as ln(AD-PS + 1) given its non-normal distribution⁴.
- Multivariable Cox proportional hazards models estimated hazard ratios (HR) and 95% confidence intervals (CI) for DNAm Systems Age and each of its 11 systems adjusting for age, education, race/ethnicity, smoking, hormone therapy trial arm, physical activity, body mass index, and estimated white blood cell counts.
- To examine the associations of baseline DNAm Systems Age with changes in plasma ADRD biomarkers over 15 years, we fitted linear mixed effects models for DNAm Systems Age and individual systems that were associated with incident MCI/dementia.
- To examine the associations of baseline DNAm Systems Age with MRI measures, we fitted linear regression models for DNAm Systems Age and individual systems that were associated with incident MCI/dementia.

References

1. Sehgal R, Markov Y, Qin C, Meer M, Hadley C, Shadyab AH, Casanova R, Manson JE, Bhatti P, Moore AD, Crimmins EM, Hagg S, Assimes TL, Whitset EA, Higgins-Chen AT, Levine M. Systems Age: a single blood methylation test to quantify aging heterogeneity across 11 physiological systems. *Nat Aging*. 2025 Sep;5(9):1880-1896. doi: 10.1038/s43587-025-00958-3. Epub 2025 Sep 15. PMID: 40994326; PMCID: PMC13222069.

2. Shumaker SA, Reboussin BA, Espeland MA, et al. The Women’s Health Initiative Memory Study (WHIMS): a trial of the effect of estrogen therapy in preventing and slowing the progression of dementia. *Control Clin Trials*. 1998;19(6):604-621. doi:10.1016/s0197-2456(98)00038-5

3. Espeland MA, Rapp SR, Manson JE, et al. Long-term Effects on Cognitive Trajectories of Postmenopausal Hormone Therapy in Two Age Groups. *J Gerontol A Biol Sci Med Sci*. 2017;72(6):838-845. doi:10.1093/gerona/glw156

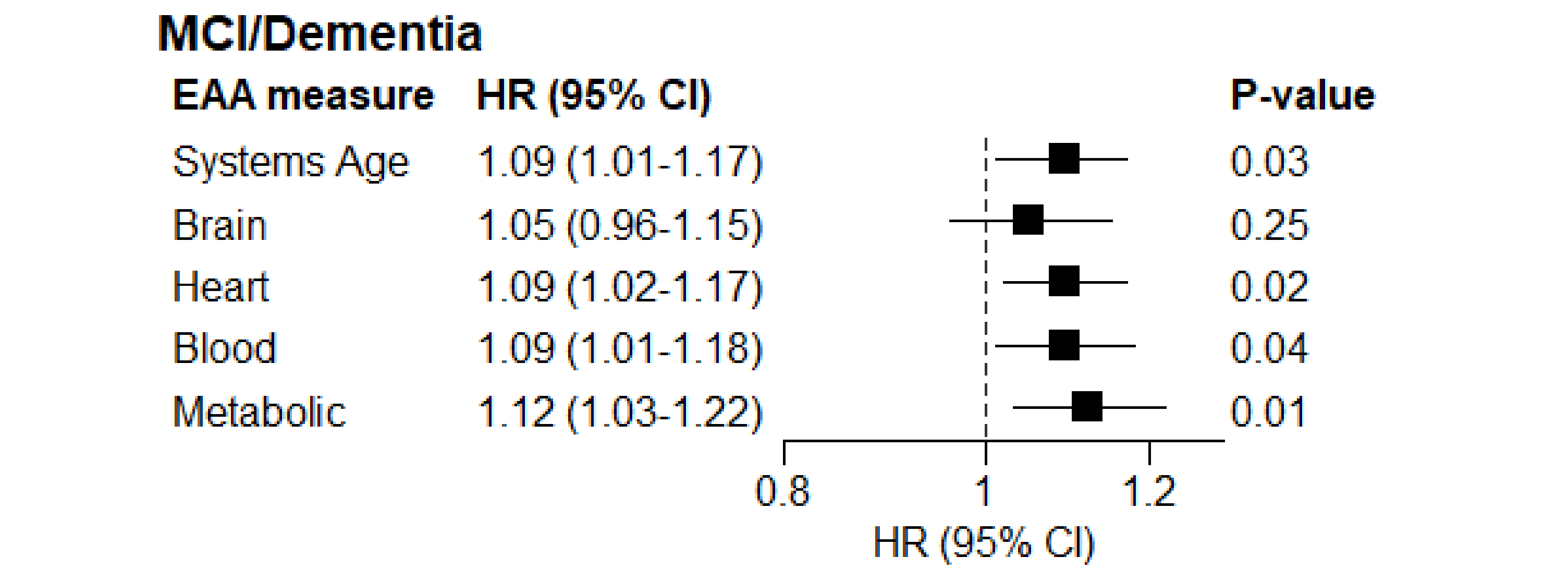
4. Zhang B, McEvoy LK, Nguyen S, Espeland MA, Rapp SR, Horvath S, Lu AT, LaCroix AZ, Nievergelt CM, Maihofer AX, Resnick SM, Mielke MM, Beckman K, Li D, Silver B, Manson JE, Ferrucci L, Shadyab AH. Epigenetic clocks and longitudinal plasma biomarkers of Alzheimer’s disease. *Alzheimers Dement*. 2025 Dec;21(12):e70983. doi: 10.1002/alz.70983. PMID: 41399190; PMCID: PMC12706121.

5. Casanova R, Hsu FC, Sink KM, Rapp SR, Williamson JD, Resnick SM, Espeland MA; Alzheimer’s Disease Neuroimaging Initiative. Alzheimer’s disease risk assessment using large-scale machine-learning methods. *PLoS One*. 2013 Nov 8;8(11):e77949. doi: 10.1371/journal.pone.0077949. PMID: 24250789; PMCID: PMC3826736.

Results

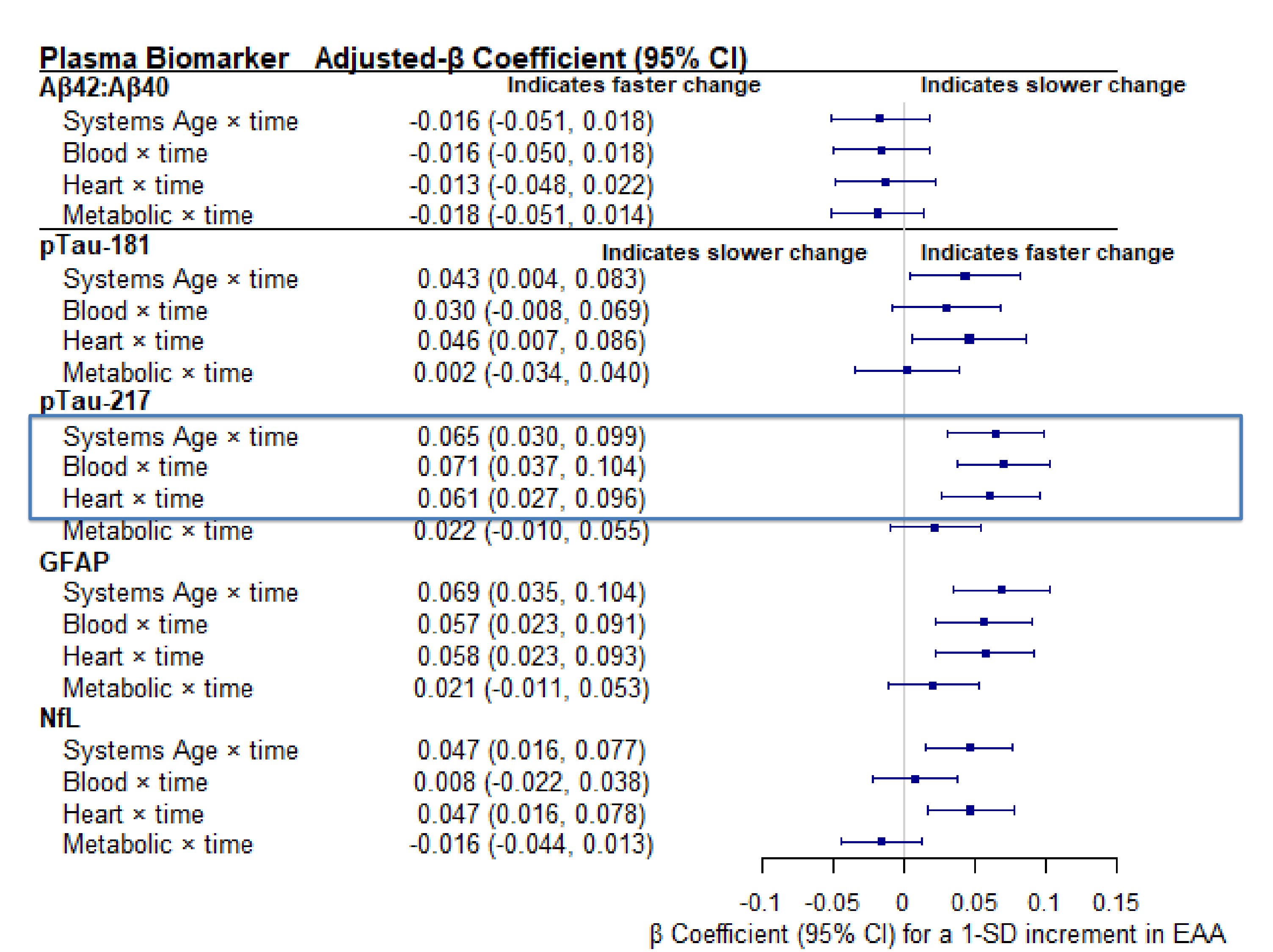
- There were 1301 incident MCI or dementia events (797 MCI and 782 dementia events separately) over a median follow-up of 9.3 (interquartile range = 6.1-16.1) years.

Figure 1. Higher DNAm Systems Age, and Heart, Blood, and Metabolic age acceleration, but not other individual systems, were associated with higher risk of incident MCI/dementia.



Abbreviations: MCI = mild cognitive impairment; EAA = epigenetic age acceleration; HR = hazard ratio; CI = confidence interval. HR (95% CI) are for each 1-standard deviation increment in epigenetic age measure. The standard deviation was 14.1 for DNAm Systems Age acceleration, 11.8 for Blood acceleration, 13.9 for Heart acceleration, and 14.3 for Metabolic acceleration.

Figure 2. Higher DNAm Systems Age, and Heart and Blood age acceleration, were associated with faster increases in plasma ADRD biomarkers over an average of 15 years (n=2,403)



Abbreviations: Aβ = amyloid beta; pTau-181 = phosphorylated tau at threonine 181; pTau-217 = phosphorylated tau at threonine 217; GFAP = glial fibrillary acidic protein; NfL = neurofilament light chain; SD = standard deviation. Associations between baseline DNAm Systems Age and its systems and rates of change per decade in plasma biomarkers were calculated from linear mixed effects regression models. Results are changes in standardized log₂-transformed plasma biomarker per decade for each 1-standard deviation increment in epigenetic age acceleration. The standard deviation of log₂-transformed was 0.32 for Aβ42:Aβ40 ratio, 0.57 for pTau-181, 0.83 for pTau-217, 0.58 for NfL, and 0.63 for GFAP. Models adjusted for age, time since baseline in decades, race, ethnicity, education, hormone therapy trial arm, smoking, physical activity, body mass index, diabetes, cardiovascular disease, hypertension, total cholesterol, high-density lipoprotein cholesterol, estimated glomerular filtration rate, and estimated white blood cell counts (CD8T, CD4T, natural killer cells, B cells, monocytes, and neutrophils).

Contact

Steve Nguyen: stn013@health.ucsd.edu

Aladdin Shadyab: aladdinshadyab@health.ucsd.edu

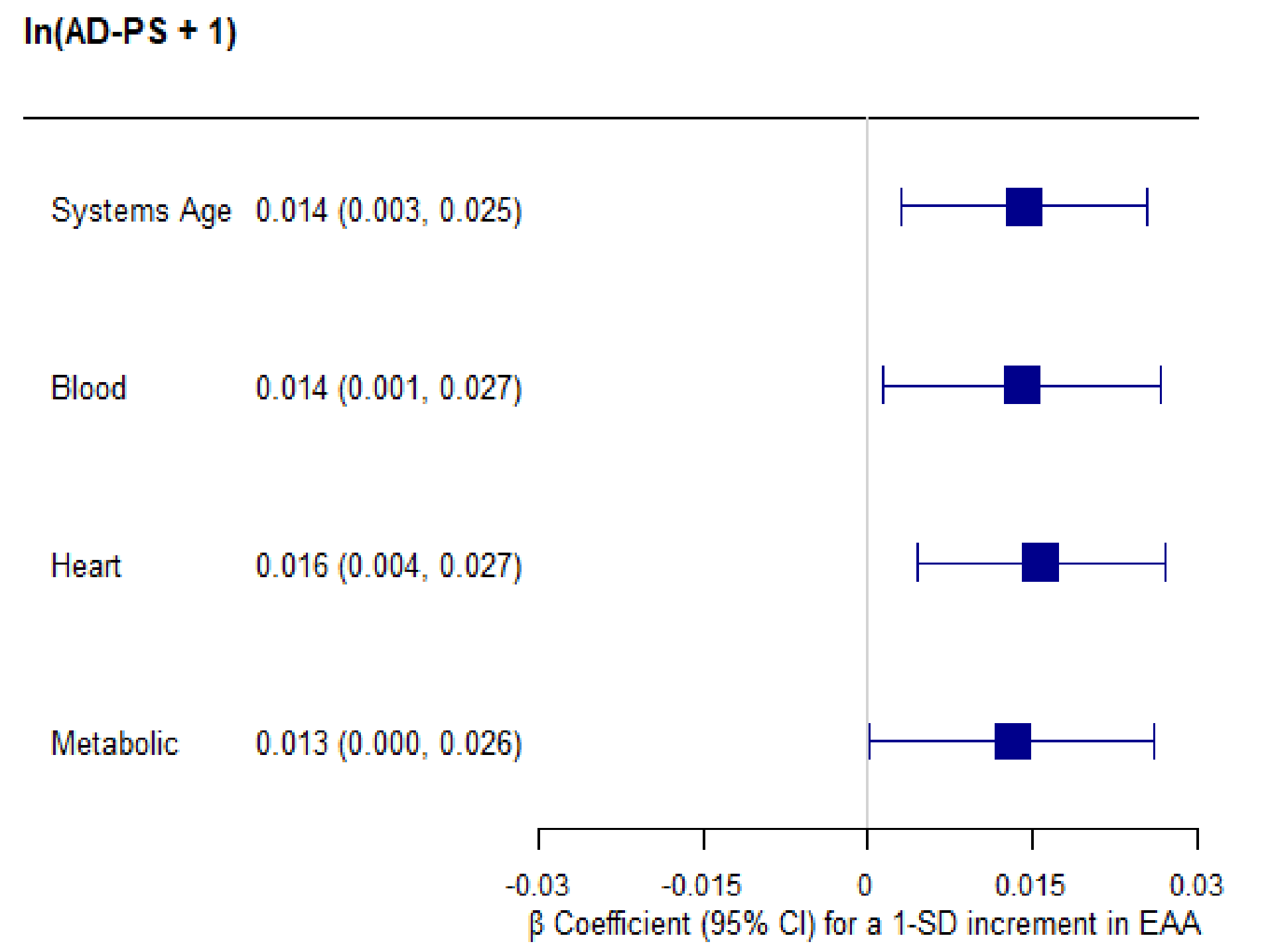


Table 1. WHIMS cohort characteristics (n=6026)

Characteristic	Total
Age, mean (SD)	70.0 (3.8)
College graduate or higher	1891 (31.5)
White	5365 (89)
Hispanic or Latina	159 (2.6)
Current smoker, n (%)	411 (6.9)
Body mass index; kg/m ² , mean (SD)	28.5 (5.7)
Total energy expenditure from recreational physical activity; MET-hours/week, mean (SD)	11.4 (13.2)
Diabetes, n (%)	376 (6.2)
Hypertension, n (%)	4199 (69.9)
Total cholesterol; mg/dL, mean (SD)	234 (39.9)
HDL cholesterol; mg/dL, mean (SD)	53.5 (12.6)
eGFR; mL/min/1.73m ² ; mean (SD)	84.2 (13.4)
APOE ε4 carriage	1292 (25.2)

Abbreviations: SD=standard deviation; MET=metabolic equivalent of task

Figure 3. Higher DNAm Systems Age acceleration and Heart age acceleration were associated with higher AD-PS scores (n=1196)



Models adjusted for the same covariates as in the Cox models plus intracranial volume.

Summary and Conclusions

Higher DNAm Systems Age acceleration was associated with higher risk of incident MCI/dementia, faster increases in plasma ADRD biomarkers over time including p-Tau217, and higher AD-PS scores.

There was a similar pattern of associations for Heart and Blood age acceleration, suggesting that vascular aging is an important contributor to dementia risk.