Concept Paper

Provisional Paper Title: Association of blood-based DNA methylation estimates of pace of aging with brain integrity during midlife and aging in three cohorts

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Please describe your proposal in 2-3 pages with sufficient detail for helpful review.

Objective of the study:

Aging is a gradual process of degradation and accumulated damage across body systems. Crucially, this process does not occur at the same rate for everyone. Individuals of the same *chronological* age often vary greatly in their *biological* age. *Biological* age is of great clinical relevance because it is more closely related to mortality and age-related disease. Despite its importance, there still is no agreed upon measure for biological age. One such method includes building machine learning algorithms trained on DNA methylation to predict biological age. This project tests the associations between measures of structural brain integrity and five leading measures of aging based on DNA methylation.

DNA methylation is an epigenetic process where specific points of the genome are modified (methylated) to affect gene regulation. Blood DNA methylation is a process that is sensitive to age-related change (Horvath & Raj, 2018), and thus a good candidate aging biomarker. Algorithms to estimate biological age from blood DNA methylation have been developed in three generations. First generation methylation "clocks" were trained on chronological age. Thus, if someone's clock looks like a chronologically older person, they are thought to be biologically older. This includes the Horvath and Hannum clocks. Second generation clocks are trained on measures of current physiology to estimate current health and predict future mortality. This generation includes PhenoAge and GrimAge. Finally, a third-generation clock known as DunedinPACE is trained on the Dunedin Study cohort's rate of physiological change in 19 biomarkers over two decades. Our group has recently demonstrated in two independent datasets (The Alzheimer's Disease Neuroimaging Initiative [ADNI], and the Framingham Offspring Study) that DunedinPACE is more closely associated with cognitive decline and dementia than first- and second-generation methylation clocks (Sugden et al., 2022). Here we extend this work to include several measures of brain structure in these same datasets as well as in the Dunedin cohort itself.

Brain degeneration during aging affects a wide variety of phenotypes that can be measured using magnetic resonance imaging (MRI). Neurodegeneration is associated with decline in overall gray matter (specifically cortical thickness and cortical surface area) as well as decline in the volume of the entire brain. It is also associated with declines in the structural integrity of white matter, which appear as white matter hyperintensities in MR images. Additionally, certain brain structures such as the hippocampus show strong age-related decline in

their size. In addition, hippocampal degradation is thought to be involved in the pathogenesis of dementia. Finally, our group has previously used machine learning prediction algorithms to combine these measures to generate a summary estimate of 'brain age' (Elliott et al., 2021).

Aging is also associated with brain phenotypes measured using positron emission tomography (PET). Briefly, PET imaging works by 'tagging' certain molecules and monitoring their movement in the brain to get a sense of the various chemical processes occurring in the brain. For example, tagging glucose molecules and measuring their uptake can provide a measure of brain metabolism. Measurement of brain metabolism by tagging glucose is known as fluorodeoxyglucose (FDG) PET imaging. PET can also be used to measure the accumulation of amyloid-beta and pTau proteins, both of which are thought to be implicated in the pathogenesis of dementia. Concentrations of these proteins can also be measured by sampling cerebrospinal fluid obtained through a lumbar puncture, although this is much more invasive.

All the above brain measures are robustly related to age-related decline, but their association with blood-based DNA methylation clocks is not clear. This project will use the data from the Dunedin Study, Framingham Offspring Study, and ADNI to test associations with structural brain measures, PET-derived imaging measures, and cerebrospinal fluid biospecimen measures from lumbar puncture.

Question 1:
Which methylation clocks show the strongest cross-sectional associations with aging-related brain measures?

We will first test for cross-sectional associations between structural brain measures and estimates of accelerated aging derived from the five methylation clocks listed above. This analysis will be completed in all three datasets (Dunedin Phase 45, Framingham, and ADNI). These three datasets have both shared and unique imaging phenotypes, allowing us to test a large variety of brain measures. In Dunedin we will test associations with total brain volume, hippocampal volume, white matter hyperintensity volume, mean cortical thickness, and an algorithmically estimated measure of brain aging (brainAGE; Elliott et al., 2021). In the Framingham study, we will again test all these measures excluding brainAGE, which is not currently available. In ADNI we will again test associations with total brain volume, hippocampal volume, and white matter hyperintensity volume as well as measures of brain metabolism and amyloid beta and pTau accumulation derived from PET imaging and cerebrospinal fluid samples.

Question 2:
Can blood DNA methylation predict future decline in the brain?

Next, we will leverage the longitudinal structure of ADNI to test whether baseline estimations of accelerated aging from all five DNA methylation algorithms can predict subsequent decline in brain structure, metabolism, and accumulation of amyloid beta and pTau. To do this, we will estimate growth curve models for repeated observations in ADNI to derive individual rates of change for each imaging or biospecimen measure. We will then test whether estimates of accelerated aging from the first methylation observation are related to these rates of subsequent change.

Variables needed at which ages:
Dunedin:
Pace of Aging
DNA methylation clocks at age 45 (Horvath, Hannum, PhenoAge, GrimAge, & DunedinPACE)
Mean cortical thickness at age 45
Total cortical surface area at age 45
Total brain volume at age 45
Hippocampal volume at age 45
brainAGE at age 45
White matter hyperintensity volume at age 45

ADNI:
All DNA methylation clocks (Horvath, Hannum, PhenoAge, GrimAge, & DunedinPACE) in 649 participants included in Sugden et al. (2022).
All observations of total brain volume in 649 participants included in Sugden et al. (2022).
All observations of hippocampal volume in 649 participants included in Sugden et al. (2022).
All observations of white matter hyperintensity volume in 649 participants included in Sugden et al. (2022).
All observations of fluorodeoxyglucose (FDG) PET in 649 participants included in Sugden et al. (2022).
All observations of amyloid/PIB PET in 649 participants included in Sugden et al. (2022).
All observations of tau PET in 649 participants included in Sugden et al. (2022).
All observations of amyloid-beta concentration from lumbar punctures in 649 participants included in Sugden et al. (2022).
All observations of pTau concentration from lumbar punctures in 649 participants included in Sugden et al. (2022).
All FreeSurfer segmentation outputs for 649 participants included in Sugden et al. (2022) to estimate brain age.

Framingham Offspring Cohort:
Total brain volume measured during Offspring 8th follow-up visit
Hippocampal volume measured during Offspring 8th follow-up visit
Mean thickness measured during Offspring 8th follow-up visit
White matter hypointensity volume measured during Offspring 8th follow-up visit

Significance of the study:

This analysis will provide an important validation of prior work showing that DNA methylation clocks are associated with clinical observation of cognitive decline and dementia. In doing so, we will further test the performance of DNA methylation clocks to detect relevant age-related change of the central nervous system using three large, independent datasets.

References:

Elliott, Maxwell L., Daniel W. Belsky, Annchen R. Knodt, David Ireland, Tracy R. Melzer, Richie Poulton, Sandhya Ramrakha, Avshalom Caspi, Terrie E. Moffitt, and Ahmad R. Hariri. (2019). “Brain-Age in Midlife Is Associated with Accelerated Biological Aging and Cognitive Decline in a Longitudinal Birth Cohort.” *Molecular Psychiatry* 26 (8): 3829–38.

Horvath, Steve, and Kenneth Raj. (2018). "DNA Methylation-Based Biomarkers and the Epigenetic Clock Theory of Ageing." *Nature Reviews. Genetics* 19 (6): 371–84.

Sugden, Karen, Avshalom Caspi, Maxwell L. Elliott, Kyle J. Bourassa, Kartik Chamarti, David L. Corcoran, Ahmad R. Hariri, et al. (2022). "Association of Pace of Aging Measured by Blood-Based DNA Methylation With Age-Related Cognitive Impairment and Dementia." *Neurology*, July. https://doi.org/10.1212/WNL.0000000000200898.