Provisional Paper Title	. An epigenetic signature to quantify the longitudinal Pace of Aging from a single blood sample
Proposing Author	. DW Belsky
P.I. Sponsor	TE Moffitt & A Caspi
Other Contributors	A Caspi, TE Moffitt, J Mill, D Corcoran, E Hannon, R Houts, J Prinz, K Sugden, B Williams, R Poulton, B Kraus, K Huffman, C Peiper
Today's Date	. 12-28-17

Objective of the study: To develop an epigenetic measure to quantify the longitudinal pace of aging from a single blood sample.

Data analysis methods:

We will use elastic net regression [2] to derive an epigenetic signature of the measured Pace of Aging. Elastic net regression is a machine learning approach that builds a predictive model through iterative analysis of a dataset. The method is designed to identify a parsimonious predictive model in settings where the number of variables exceeds the number of observations. Elastic net regression is the method used to develop the famous epigenetic clocks [3,4]. In those analyses, elastic net regression was used to fit DNA methylation data to chronological age variation in mixed-age cohort. In our analysis, we will use elastic net regression to fit DNA methylation to variation in the rate of biological aging in a birth cohort, in which all members are the same chronological age.

To measure the rate of biological aging in the Dunedin cohort, we will use the Pace of Aging measure, which we published in 2015 [1]. The Pace of Aging measures the rate of aging-related decline in system integrity. The measure was derived from longitudinal analysis of18 biomarkers tracking integrity of organ systems throughout the body. The original Pace of Aging analysis included data from three repeated measurements taken when Study members were aged 26, 32, and 38 years. In our elastic net analysis, we will analyze DNA methylation collected when Study members were aged 38 years. We will use these data to model the Pace of Aging retrospectively (because the DNA methylation data is collected after the changes measured by the Pace of Aging have occurred).

The resulting epigenetic measure will reflect the rate of aging over the 12 years prior to DNA collection.

We will "train" our elastic net model in Dunedin data. We will then "project" the model derived from analysis of Dunedin data into a new dataset from the CALERIE randomized trial. We will use the CALERIE dataset to validate the elastic net model trained in the Dunedin data.

CALERIE is the first-ever human trial of long-term (2y) caloric restriction, an intervention long-established to slow the rate of aging in worms, flies, and mice [5], and recently shown to extend healthy lifespan in rhesus monkeys [6,7]. CALERIE collected blood samples at 3 time points (baseline, 12mo, and 24mo) in 220 participants randomized to either ad libitum (as usual) diet or 25% caloric restriction [8]. We previously conducted analysis to test if caloric restriction slowed the rate of biological aging in CALERIE participants. Our analysis showed that participants in the caloric restriction arm of the trial experienced a slower rate of biological aging from baseline to follow-up as measured by two different clinical biomarker algorithms [9]. We subsequently obtained pilot funding from CALERIE to generate whole-genome DNA methylation data from blood samples taken at baseline. We propose to use these baseline DNA methylation data to validate an epigenetic measure of the Pace of Aging.

Specifically, we will test the hypothesis that an epigenetic measure of the Pace of Aging computed at CALERIE baseline will correlate with the rate of change in biological age over the subsequent 2 years within the ad libitum (normal diet) arm of the trial, but not in the caloric restriction arm of the trial. The logic of our hypothesis is that the rate of aging over the recent past should be predictive of the rate of aging in the future. Thus, in the ad libitum (normal diet) arm of the CALERIE trial, the epigenetic signature of the Pace of Aging at baseline, which measures the rate of aging in the recent past, should predict the rate of aging over the next 2 years. In contrast, in the caloric restriction arm of the trial, intervention slowing biological aging should disrupt the continuity between the past rate of aging and the rate of aging going forward. This should attenuate toward zero the association between the epigenetic signature of the Pace of Aging at baseline and the measured rate of aging during the 2 years of CALERIE follow-up.

Variables needed at which ages:

The Pace of Aging (Belsky et al. 2015 PNAS)
DNA methylation at ages 26 and 38y

Significance of the Study (for theory, research methods or clinical practice):

Quantification of biological aging is of increasing interest in studies of aging and longevity as well as studies of social determinants of health, developmental origins of health and disease, and the long-term outcomes of early-life adversity [10–14]. Biological aging is an appealing construct in these diverse fields because it quantifies damage to physiological integrity that accrues prior to the onset of disease. Moreover, because processes of aging are thought to mediate much disease and disability [15], changes to biological aging can index effects of prevention therapies. A challenge is that measurement of biological aging is assay-intensive. The best-performing measures require information about the functioning of multiple organ systems, including some measures that cannot be derived from banked biological samples [16,17]. Moreover, to measure the rate of aging, it is necessary to take these measurements at multiple time points. Therefore, a critical need is a measurement that can be derived from a single banked bio-sample that reflects patterns of longitudinal change in the functioning of multiple organ systems. Our study proposes to develop such a measure from DNA methylation data. Many studies are now adding DNA methylation databases and costs of generating methylation data are falling due to the advent of low cost sequencing. Our measure will thus be readily applicable in any study with stored DNA.

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Data Security Agreement

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Please keep one copy for your records and return one to the PI Sponsor

Please initial your agreement

X	I am current on Human Subjects Training (CITI (www.citiprogram.org) or equivalent)
X	My project is covered by Duke or Otago ethics committee OR I have /will obtain ethical approval from my home institution.
X	I will treat all data as "restricted" and store in a secure fashion. My computer or laptop is: a) encrypted (recommended programs are FileVault2 for Macs, and Bitlocker for Windows machines) b) password-protected c) configured to lock-out after 15 minutes of inactivity AND d) has an antivirus client installed as well as being patched regularly.
X	I will not "sync" the data to a mobile device.
x	In the event that my laptop with data on it is lost, stolen or hacked, I will immediately contact Professor Moffitt or Caspi. (919-684-6758, tem11@duke.edu, ac115@duke.edu)
x	I will not share the data with anyone, including my students or other collaborators not specifically listed on this concept paper.
x	I will not post data online or submit the data file to a journal for them to post. *Some journals are now requesting the data file as part of the manuscript submission process. The Dunedin Study Members have not given informed consent for unrestricted open access, so we have a managed-access process. Speak to Terrie or Avshalom for strategies for achieving compliance with data-sharing policies of journals.*
x	I will delete all data files from my computer after the project is complete. Collaborators and trainees may not take a data file away from the office. The data remains the property of the Study and cannot be used for further analyses without an approved concept paper for new analyses.

Signature: _____

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B. To be completed by potential co-authors:

Comments:

Please check your contribution(s) for authorship:

	Approved
	Not Approved
	Let's discuss, I have concerns
.	Conceptualizing and designing the longitudinal study
	.Conceptualizing and collecting one or more variables
	Data collection
	.Conceptualizing and designing this specific paper project
.	.Statistical analyses
	Writing
	Reviewing manuscript drafts
.	Final approval before submission for publication
	Acknowledgment only, I will not be a co-author

Signature:

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