American Journal of Epidemiology

DOI: 10.1093/aje/kwx346

Original Contribution

Eleven Telomere, Epigenetic Clock, and Biomarker-Composite Quantifications of Biological Aging: Do They Measure the Same Thing?

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Initially submitted March 14, 2017; accepted for publication October 19, 2017.

The geroscience hypothesis posits that therapies to slow biological processes of aging can prevent disease and extend healthy years of life. To test such "geroprotective" therapies in humans, outcome measures are needed that can assess extension of disease-free life span. This need has spurred development of different methods to quantify biological aging. But different methods have not been systematically compared in the same humans. We implemented 7 methods to quantify biological aging using repeated-measures physiological and genomic data in 964 middle-aged humans in the Dunedin Study (New Zealand; persons born 1972–1973). We studied 11 measures in total: telomere-length and erosion, 3 epigenetic-clocks and their ticking rates, and 3 biomarker-composites. Contrary to expectation, we found low agreement between different measures of biological aging. We next compared associations between biological aging measures and outcomes that geroprotective therapies seek to modify: physical functioning, cognitive decline, and subjective signs of aging, including aged facial appearance. The 71–cytosine-phosphate-guanine epigenetic clock and biomarker composites were consistently related to these aging-related outcomes. However, effect sizes were modest. Results suggested that various proposed approaches to quantifying biological aging may not measure the same aspects of the aging process. Further systematic evaluation and refinement of measures of biological aging is needed to furnish outcomes for geroprotector trials.

biological aging; epigenetic clock; geroscience; telomere

Abbreviations: CpG, cytosine-phosphate-guanine; KDM, Klemera-Doubal method.

Data syntheses in biodemography and gerontology identify aging as the leading cause of human morbidity and mortality (1, 2). The so-called "geroscience hypothesis" builds on these data to posit that interventions to slow the biological processes of aging could prevent or delay many different diseases simultaneously, prolonging the healthy years of life (3). Econometric projections suggest that interventions that achieve even modest slowing of biological aging could reduce burden of disease more than curing all cancer and heart disease combined (4). Candidate interventions to slow aging are emerging from studies of animals (5, 6). The present study considered 2 issues that need to be addressed to speed human translation.

First, a barrier to translating therapies developed in animal models to help humans is that human aging is a gradual, slow-moving process that is not easily measured in clinical trials. Observing completed human life spans or even health spans (the portion of life span preceding onset of chronic disease) is time- and cost-prohibitive. In order to refine intervention targets and evaluate intervention effectiveness, surrogate endpoints are needed that can stand in as proxies for extended life spans or health spans (7). Thus, quantifications of biological aging are of growing interest in biomedical and social sciences (8, 9). Measures of biological aging are intended to provide proxy measurements of life span or health span. In contrast to chronological age, which increases at the same rate for everyone, biological aging can occur at different rates in different individuals. Various measures of biological aging have been proposed, including telomere length, algorithms applied to genome-wide DNA methylation data, and algorithms combining information on multiple clinical biomarkers (10–12). However, it is not known whether these

various approaches to quantifying biological aging measure the same or different aspects of the aging process. In addition, it is unknown whether some proposed methods are more closely associated with health span than others.

A second issue is that although biological aging measured in later life has been shown to predict disease and mortality, it is unknown whether biological aging measured in midlife can predict health span. To extend health span, "geroprotective" therapies must be delivered prior to the onset of disease and disability (i.e., in people who are still relatively young and healthy). Validation is therefore needed in this younger population to establish proof of concept that biological aging measures can serve as surrogate endpoints for health-span extension in clinical trials of geroprotective therapies.

We considered these two issues: measurement of aging within the time scale of a clinical trial and in a population of still-young, healthy individuals. We examined data from a 1-year birth cohort of 1,037 adults followed prospectively to midlife with 95% retention: the Dunedin Study (New Zealand). We analyzed repeated-measures physiological and genomic data to quantify 11 biological-aging measures in total: telomere length, telomere erosion, 3 epigenetic clocks, those clocks' longitudinal ticking rates, and 3 clinical-biomarker composite measures. Although all measures were designed to quantify the same construct—biological aging—there have not been studies to evaluate them simultaneously in the same group of humans. We tested whether the different measures quantified the same aging process. We then compared how the different methods related to the signs of aging that geroprotective interventions will aim to ameliorate: worsening physical functioning, cognitive impairment and decline, and subjective perceptions of declining health. We studied adults in their late 30s to separate processes of aging from age-related disease and to inform preventive geroprotective therapies that will target people who are still relatively young and healthy.

METHODS

Sample

Participants were members of the Dunedin Study, a longitudinal investigation of health and behavior in a complete birth cohort. Study members ($n = 1{,}037$; 91% of eligible births; 52% male) were all individuals born between April 1972 and March 1973 in Dunedin, New Zealand, who were eligible based on residence in the province and who participated in the first assessment at age 3 years. The cohort represented the full range of socioeconomic status in the general population of New Zealand's South Island. On adult health, the cohort matches the NZ National Health and Nutrition Survey (e.g., body mass index, smoking, general practitioner visits) (13). Cohort members are primarily white; fewer than 7% self-identify as having partial nonwhite ancestry, matching the South Island population (13). Assessments were carried out at birth and ages 3, 5, 7, 9, 11, 13, 15, 18, 21, 26, 32, and, most recently, 38 years, when 95% of the 1,007 of the study members still alive participated. At each assessment, each study member is brought to the research unit for a full day of interviews and examinations within 6 months of their birthday. The Otago Ethics Committee approved each phase of the study, and informed consent was obtained from all study members.

Quantification of biological aging

Biological aging measures can be discriminated along 3 axes. One axis is the technical dimension of the number of assays required (e.g., telomere length is measured with a single assay, whereas multiple assays are required for algorithms that combine different types of biomarkers). A second axis is the measurement design (i.e., a single cross-sectional measurement vs. repeated, longitudinal measurements). A third axis is the biological level at which measures are implemented (e.g., telomeres are a cellular-level measure typically implemented in a specific tissue whereas multiple-biomarker algorithms are patient-level measures that combine information from multiple organ systems). We implemented 7 methods to compute 11 measures of biological aging using data from the Dunedin Study biobank. Measures are grouped according to the 3 axes in Figure 1 and described briefly below and in Appendix 1. Detailed information on biological aging measures is included in Web Appendix 1 (available at https://academic.oup.com/aje).

Telomere length and epigenetic clocks have been proposed as cross-sectional estimates of biological aging based on a single biological measure (Figure 1, top left). We measured study members' telomere length and 3 epigenetic clocks (14–16) from blood samples taken when they were aged 38 years. We also measured study members' telomere length and epigenetic clocks from blood taken when they were aged 26 years. We calculated longitudinal telomere erosion and epigenetic ticking rates by subtracting age-26 values from age-38 values (Figure 1, top right).

Klemera-Doubal method (KDM) biological age (17) and age-related homeostatic dysregulation (18) have been proposed as cross-sectional estimates of biological aging based on multiple biological measures (Figure 1, bottom left). We calculated KDM biological age and age-related homeostatic dysregulation from data collected when study members were aged 38 years.

Pace of aging (19) is a longitudinal estimate of biological aging based on changes across repeated measurements of multiple biological measures (Figure 1, bottom right). We computed pace of aging from data collected when study members were aged 26, 32, and 38 years.

Health span–related characteristics

Using samples from when study members were aged 38 years, we measured health span–related characteristics: balance, grip strength, motor coordination, physical limitations, cognitive functioning and cognitive decline since childhood, self-rated health, and facial aging. The measures are described in Appendix 2. All health span–related characteristics were transformed to sex-specific z scores for analysis, with the exception of cognitive test scores and the facial aging measure, which are sex neutral.

Statistical analysis

We analyzed associations between quantifications of biological aging using Pearson and Spearman correlations. We analyzed associations between quantifications of biological aging and health span–related characteristics using linear regression. Models adjusted for sex.

		Measurement Design			
		Cross-Sectional	Longitudinal		
Variety of Assays	Single Assay	Telomere Length, Epigenetic Clocks	Telomere Erosion, Epigenetic Ticking	Cellular-Level Measures Assayed in Blood	Biological Level of Measurement
	Multiple-Assay Composite	KDM Biological Age, Age-Related Homeostatic Dysregulation	Pace of Aging	Patient-Level Measures Derived From Assays of Multiple Organ Systems	

Figure 1. Taxonomy of the biological aging measures for use in humans that are evaluated in this article. Epigenetic clocks are composed of dozens or hundreds of different methylation marks across the genome. We classify the clocks in the "single measure" row because genome-wide DNA methylation is measured in a single assay and reflects a single biological substrate.

For each biological aging measure, we tested associations with 3 groups of health span–related measures: First, we tested whether biological aging measures predicted deficits in physical functioning by examining study members' performance on tests of balance, grip strength, and motor coordination, and by interviewing study members about any physical limitations in carrying out activities in their daily lives. Second, we tested whether biological aging measures predicted early-onset cognitive decline by comparing study members' scores on cognitive tests taken at midlife to scores on parallel tests that they took when they were children. Third, we tested whether biological aging measures predicted subjective signs of aging, which we measured by interviewing the study members themselves and from observer ratings of the study members' aged appearance based on facial photographs.

RESULTS

Do proposed methods to quantify biological aging measure the same features of the aging process?

To test the hypothesis that the different biological aging measures quantify the same aging process, we computed correlations among the different measures (distributions in Figure 2, correlations in Figure 3, scatter plots in Web Figure 1). Epigenetic clocks were correlated with each other in the $r = 0.3$–0.5 range ($P < 0.001$ for all). Clinical biomarker algorithm measures were correlated with one another in the $r = 0.4$–0.6 range ($P < 0.001$ for all). However, telomere length was not significantly correlated with estimates from epigenetic clocks or clinical-biomarker algorithms ($r = -0.05$–0.03; $P > 0.05$ for all), and correlations of epigenetic clock measures with clinical-biomarker-algorithm measures were generally low. The 71–cytosine-phosphate-guanine (CpG) (where a cytosine nucleotide is followed by a guanine nucleotide in the sequence of bases along the 5′ to 3′ direction and the nucleotides are separated by 1 phosphate) clock was weakly correlated with the clinical biomarker measures ($r = 0.10$–0.15; $P < 0.001$ for all) and the 353- and 99-CpG clocks were also weakly correlated with KDM biological age ($r = 0.07$–0.08; $P < 0.05$ for both). Results were similar when Spearman correlations were computed to reduce the influence of extreme values (Web Tables 1 and 2) and when the analysis adjusted for sex differences (Web Table 3).

Do proposed methods to quantify biological aging predict differences in health span–related characteristics at midlife?

Telomere length was not statistically significantly associated with health span–related characteristics, with the exception of facial aging ($r = 0.07$). Likewise, the 353- and 99-CpG clocks were not associated with health span–related characteristics ($P > 0.05$ for all). However, older epigenetic age measured by the 71-CpG clock was associated with poorer health span–related characteristics in all cases except for grip strength ($0.05 \leq |r| \leq 0.16$). The 3 clinical biomarker algorithms were all associated with poorer health span–related characteristics ($0.10 \leq |r| \leq 0.20$ for most analyses), with the exception that age-related homeostatic dysregulation was not associated with grip strength. Effect sizes for health span–related characteristics are reported in Table 1 and graphed in Figure 4 and Web Figure 2.

Does change between repeated cross-sectional measures of biological aging track the aging process?

Telomere length and epigenetic clock values quantify biological aging at a cross-section. Cross-sectional measures cannot distinguish information about current rate of aging from differences already established earlier in life (20, 21). To distinguish current rate of aging from early-life exposure history, we calculated longitudinal measures of telomere erosion and epigenetic ticking: We computed difference scores by subtracting age-26 telomere length and epigenetic clock values from age-38 values. These longitudinal measures of telomere erosion and epigenetic ticking served to isolate aging-related genomic changes occurring from young adulthood to midlife from differences established prior to age 26 years. We repeated our analysis using longitudinal genomic measures and compared results to those for the pace-of-aging

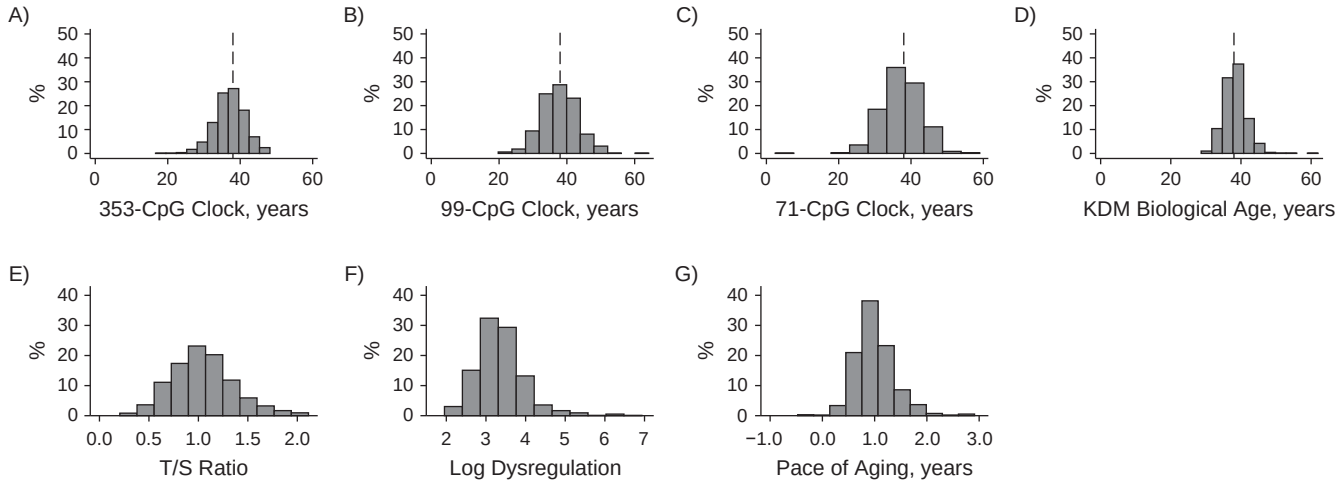

Figure 2. Distributions of cross-sectional biological aging measures and pace of aging in the Dunedin birth cohort at age 38 years (born during 1972–1973), New Zealand. Panels A through D plot biological ages estimated from DNA methylation and clinical biomarker data: A) 353–cytosine-phosphate-guanine (CpG) epigenetic clock; B) 99-CpG epigenetic clock; C) 71-CpG epigenetic clock; and D) Klemera-Doubal method (KDM) biological age algorithm. In these panels, the dashed gray line is set at age 38 years, the chronological age of the cohort at the time assays were taken. E) Telomere/single copy (T/S) ratio at chronological age 38 years. F) Age-related homeostatic dysregulation, also assayed at chronological age 38 years. G) Pace of aging, which was derived based on repeated measurements taken at ages 26, 32, and 38 years.

measure, which was also based on longitudinal changes between 26 and 38 years. Results are reported in the Web Appendix 2. Briefly, telomeres eroded from age 26 years to age 38 years, and epigenetic clocks ticked forward—by about 12 years (Web Figures 3 and 4); however, telomere erosion and epigenetic ticking were only weakly associated with the pace of aging (Pearson $r < 0.10$; Web Figure 5, Web Tables 4–6) and were mostly not associated with health span–related characteristics (Web Figures 6 and 7).

A question about biological aging measures implemented during the middle period of the life course is whether they measure processes independent of weight gain (22, 23). To address this question, we repeated all tests of association between measures of biological aging and health span–related characteristics with statistical adjustment for body mass index. We repeated analysis of age-38 telomere length, age-38 epigenetic clocks, KDM biological age, age-related homeostatic dysregulation, and pace of aging with the inclusion of age-38 body mass index as a covariate (Web Table 7). We repeated analysis of telomere erosion, epigenetic ticking, and pace of aging with the inclusion of change in body mass index from age 26 years to age 38 years as a covariate (Web Table 8). Effect sizes were essentially unchanged. We repeated this procedure to test sensitivity of findings for the slight differences in chronological age between Dunedin Study members (the standard deviation of chronological age was 3 months at the age-26 assessment and 6 months at the age-38 assessment). Again, effect sizes were essentially unchanged (Web Tables 9 and 10). We also computed associations between biological age measures and health span–related characteristics after model adjustment for 2 established health risks commonly assessed in midlife adults: smoking and socioeconomic status. Associations were modestly attenuated but generally remained of the same effect size and statistical significance (Web Tables 11 and 12).

DISCUSSION

We studied 7 proposed methods to quantify biological aging in a cohort of 964 individuals followed to midlife as part of the Dunedin Study. We quantified telomere length; telomere erosion; 353-, 99-, and 71-CpG epigenetic clocks and the clocks' longitudinal ticking rates; and 3 multiple-biomarker algorithms (KDM biological age, age-related homeostatic dysregulation, and the pace of aging). All of these measures indicated that members of the Dunedin Study, despite all being the same chronological age, varied in their biological aging. Estimates of biological aging were in line with reports about these measures; for example, epigenetic clocks varied around a mean of 38 years, matching the chronological age at which blood samples were taken. Moreover, when we compared study members' telomere and epigenetic clock measurements taken when they were aged 38 years with measurements from samples collected 12 years earlier, when they were aged 26 years, we detected the expected patterns of telomere erosion and epigenetic ticking. In fact, all 3 epigenetic clocks ticked forward by about 12 years, matching the amount of chronological time elapsed between sample collections. However, variation in different biological aging estimates did not appear to reflect a single aging process. Although epigenetic clocks correlated with one another and so did biomarker algorithms, correlations between the epigenetic clocks and biomarker algorithms were low, as were correlations of both sets of measures with telomere length. Moreover, none of the measures of biological aging were strongly associated with health span–related characteristics (balance, grip-strength, motor coordination, physical limitations, cognitive decline, self-rated health, and facial aging).

The implication of this analysis is that several methods proposed to quantify biological aging in fact appear to quantify

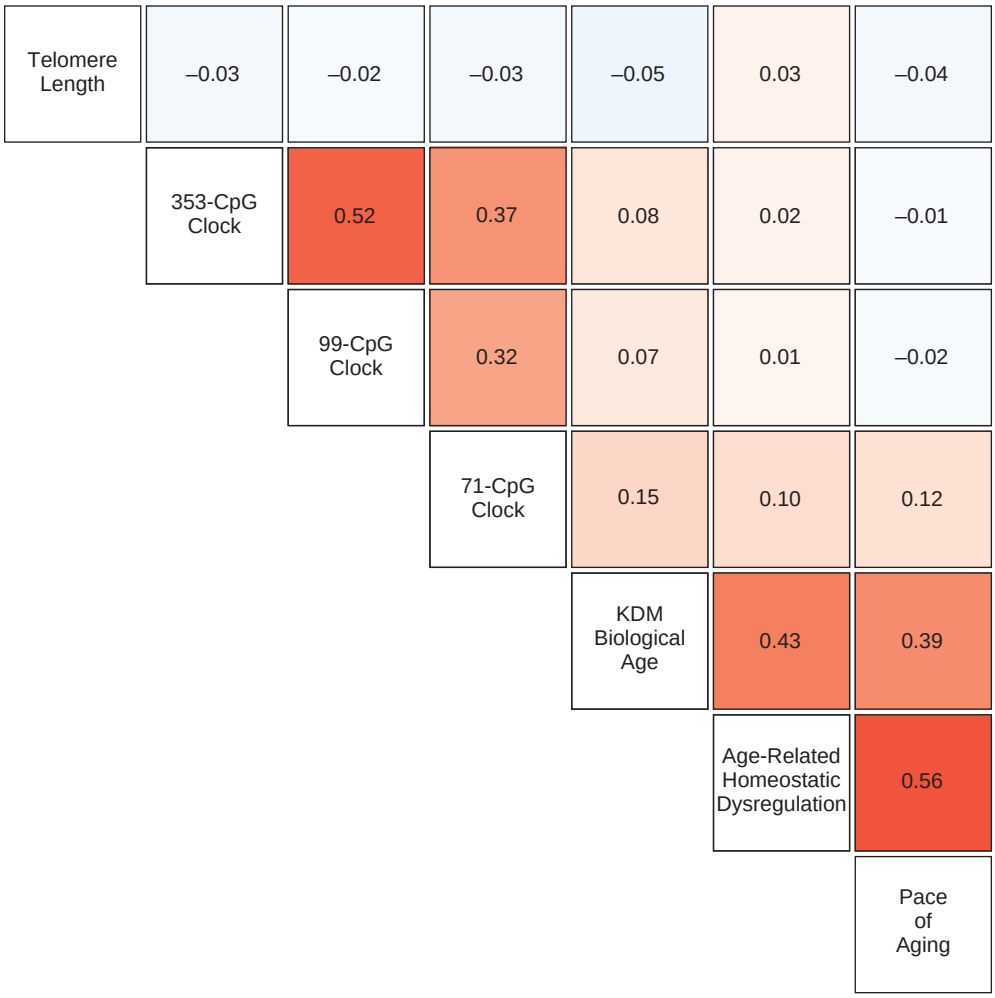

Figure 3. Correlations among 7 measures of biological aging in a birth cohort at chronological age 38 years (born during 1972–1973), New Zealand. The figure shows a matrix of correlations illustrating relationships among 7 measures of biological aging: leukocyte telomere length; 353-, 99-, and 71–cytosine-phosphate-guanine (CpG) epigenetic clocks; Klemera-Doubal method (KDM) biological age; age-related homeostatic dysregulation; and pace of aging. Data are for $n = 800$ study members with complete data on all biological aging measures. Correlations are shown above the diagonal. Values reflect Pearson correlations between the variable listed to the left and the variable listed below. Correlations of ≥0.07 are statistically significant at $P < 0.05$. Correlations between aging measures computed with adjustment for sex differences are reported in Web Table 6.

different "things." Although each of these measures has its own validation literature, our findings raise the question of whether each is measuring a distinct aspect of aging. For example, different biological aging measures may reflect different underlying "hallmarks" or "pillars" of aging (3, 24).

This study had limitations. First, we studied a single birth cohort from New Zealand that lacked ethnic minority representation. Second, our follow-up extended only through age 38 years, precluding analysis of age-related disease, disability, and mortality. Third, telomere erosion and epigenetic ticking measures were implemented using only 2 repeated measurements. Erosion and ticking measures thus could not separate measurement error from true change, as was possible with analysis of 3 repeated measures in the pace-of-aging analysis. Fourth, all molecular assays used to compute biological aging measures were implemented in samples from peripheral blood. Epigenetic clocks and telomeres may have different properties in other tissues (25). Heterogeneity in cell composition of blood samples is also a consideration. A limitation of many blood-based genomic assays is that they are typically applied to whole blood samples, and this is also true for our study. However, because whole blood is among the most available tissues, biological aging measures that can be implemented in blood samples may be most suitable for translation to clinical trials of geroprotectors. Finally, our sample lacked power to detect very small effect sizes. However, analyses were well-powered (>80%) to detect effect sizes of $r = 0.1$ and larger.

There is growing interest in methods to quantify processes of biological aging. These methods are needed for 2 purposes. One purpose is to serve as surrogate endpoints of health-span extension in clinical trials of geroprotective therapies. Geroprotective therapies aim to slow the aging process and extend years of healthy life (26). When clinical trials of such therapies are

Table 1. Associations of Cross-Sectional Biological Aging Measures and Pace of Aging With Health Span–Related Characteristics in a Birth Cohort at Chronological Age 38 Years (Born During 1972–1973), New Zealand[a]

Health Span–Related Characteristic	Telomere Shortness		353-CpG Clock		99-CpG Clock		71-CpG Clock		KDM Biological Age		Age-Related Homeostatic Dysregulation		Pace of Aging	
	r	*P* Value	*r*	*P* Value	*r*	*P* Value	*r*	*P* Value	*r*	*P* Value	*r*	*P* Value	*r*	*P* Value
Physical functioning														
Balance	0.00	0.901	−0.07	0.057	0.00	0.891	−0.08	0.020	−0.21	1.01E-10	−0.19	8.80E-09	−0.16	1.27E-06
Grip strength	−0.06	0.071	0.00	0.929	−0.05	0.141	−0.05	0.154	−0.19	6.17E-09	−0.05	0.110	−0.07	0.029
Motor coordination	−0.01	0.679	−0.01	0.681	0.03	0.336	−0.09	0.012	−0.14	2.17E-05	−0.19	3.37E-09	−0.17	1.25E-07
Physical limitations	0.03	0.400	−0.02	0.652	−0.01	0.671	−0.07	0.044	−0.13	8.74E-05	−0.14	1.47E-05	−0.12	1.30E-04
Cognitive functioning														
Cognitive function at age 38 years	−0.06	0.080	−0.02	0.557	−0.01	0.677	−0.16	1.46E-05	−0.17	3.88E-07	−0.21	1.14E-10	−0.23	1.83E-12
Cognitive decline	0.00	0.968	−0.04	0.312	−0.01	0.766	−0.09	0.016	−0.09	0.010	−0.12	0.001	−0.14	2.80E-05
Subjective aging														
Self-rated health	−0.02	0.550	−0.02	0.500	0.02	0.569	−0.08	0.031	−0.22	1.11E-11	−0.28	2.87E-18	−0.25	2.69E-15
Facial aging	−0.07	0.033	0.00	0.990	0.01	0.725	−0.12	0.001	−0.22	3.81E-11	−0.23	3.90E-12	−0.20	7.56E-10

Abbreviations: CpG, cytosine-phosphate-guanine; IQ, intelligence quotient; KDM, Klemera-Doubal method.

[a] The table shows effect sizes and *P* values for associations between the 7 measures of biological aging and health span–related characteristics. Effect sizes were estimated for 4 measures of physical functioning (balance, grip strength, motor coordination, and self-reported physical limitations), cognitive functioning (IQ score at age 38 years from the Wechsler Adult Intelligence Scale), cognitive decline (change in Wechsler-scale IQ score since childhood), and 2 measures of subjective aging (self-rated health and facial aging from assessments of facial photographs of the study members by independent raters). Effect sizes for subtests of cognitive function and cognitive decline are graphed in Web Figure 2. Health span–related characteristics were scored so that higher values indicated increased health span. Telomere length was reversed for this analysis so that higher values corresponded to shorter telomeres. Thus, the expected direction of association for all effect sizes was negative, because faster biological aging is expected to shorten health span. Standardized regression coefficients (interpretable as Pearson *r*) and their *P* values are reported. Models included sex as a covariate.

launched, the question remains: What should these trials study as outcomes? Because slowing aging in midlife may prove easier than reversing aging in late life, further research to test the effects of geroprotectors on health span and longevity will require several decades of follow-up. However, if measures of biological aging could be developed, they could be used to track the aging rate during and after administration of geroprotective therapies. Tests of change in the rate of biological aging would thus allow clinical trials to evaluate geroprotective therapies sooner (27).

A second purpose is to advance understanding of the biology of aging during the middle period of the life course. The middle period of the life course is important to aging research because this is the best opportunity for preventive geroprotective intervention (28). Age-related diseases, frailty, and death are too rare during midlife to mark the aging process. In contrast, if biological aging could be quantified for everyone, it would increase power of studies to hunt for genes, molecular processes, or psychosocial factors that influence fast, slow, or resilient aging during midlife (29).

Within this context, our study highlights progress, but also the need for a more systematic approach to development and testing of biological aging measures. Our findings do not imply that any single measure of biological aging is better than the others, or that some or all of them are entirely unhelpful. For example, although we found no relationship between telomere length or epigenetic age and health span–related characteristics, there is evidence that these measures are associated with risk of disease and death in later life (30–33). Conversely, although faster pace of aging predicted worse outcomes on the health span–related characteristics studied, its relation to mortality remains untested. To advance the geroscience agenda, biological aging research needs to address several gaps in knowledge. There are 5 main issues brought forward by our findings.

One issue is the chronological age of participants in biological aging studies. Indices of frailty already exist to quantify differences in older adults (34–36). The greatest potential value of biological aging measures is in quantifying differences in humans who do not yet have age-related disease, most of whom are still of middle age or younger. Aging is now being measured across the life span in research focused on causes and consequences of accelerated aging in children (37–39) and young to mid-life adults (40, 41), using a variety of methods. But most effort toward development and validation of biological aging measures is focused on older adults (42–45). Increased research on measuring biological aging in younger persons is needed (28).

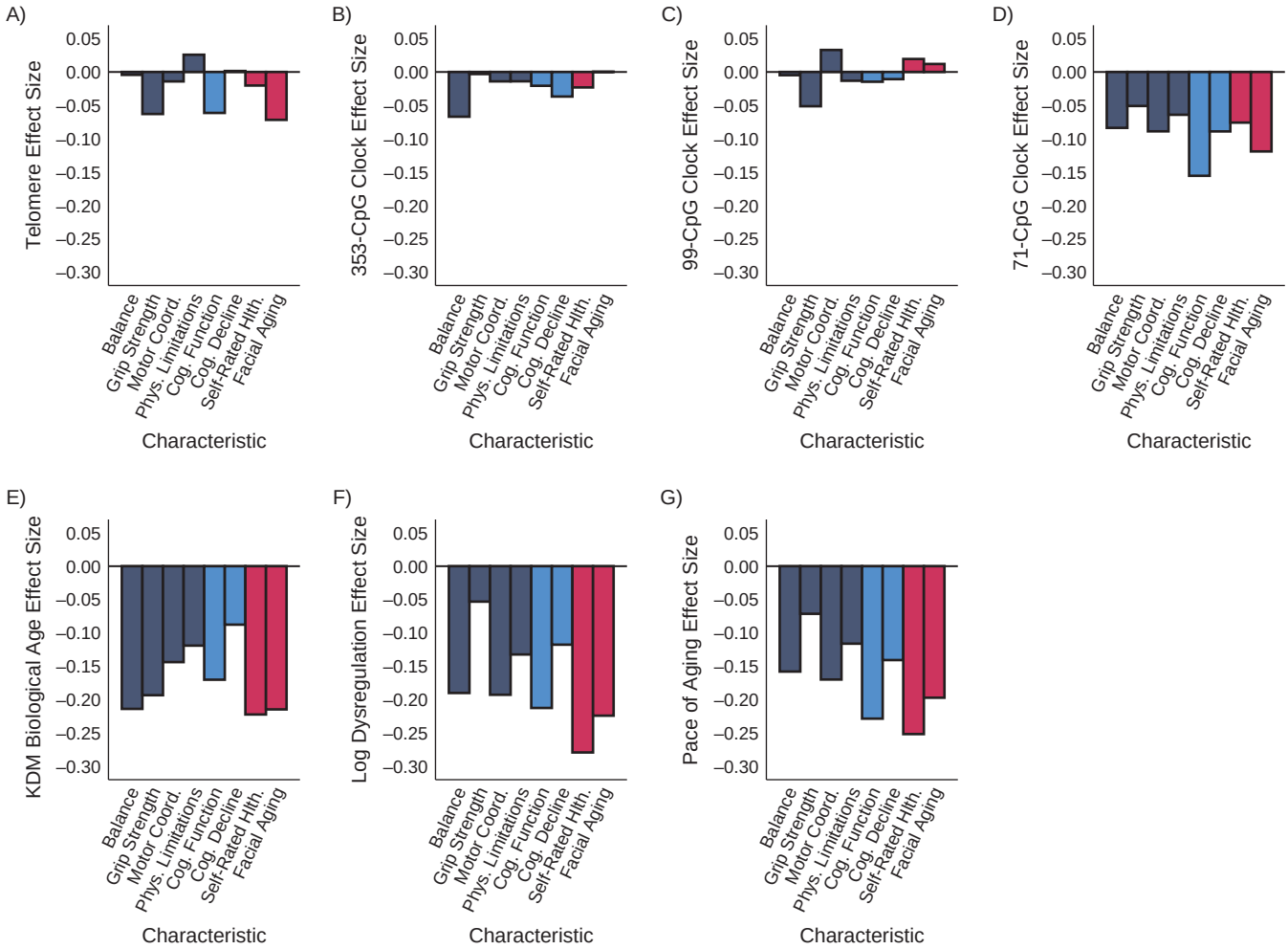

Figure 4. Associations of cross-sectional biological aging measures and pace of aging with health span–related characteristics in a birth cohort at chronological age 38 years (born during 1972–1973), New Zealand. The figure shows bar charts of effect sizes for each of the 7 measures of biological aging. Effect sizes were estimated for 4 measures of physical functioning (balance, grip strength, motor coordination, and self-reported physical limitations), cognitive functioning (intelligence-quotient score at age 38 years from the Wechsler Adult Intelligence Scale) and cognitive decline (change in Wechsler-scale intelligence-quotient score since childhood), and 2 measures of subjective aging (self-rated health and facial aging from assessments of facial photographs of the study member by independent raters). In the figure, groups of health span–related characteristics are denoted by different colors. Physical function measures are shown in dark blue. Cognitive measures are shown in light blue. Subjective aging measures are shown in red. Effect sizes for subtests of cognitive function and cognitive decline are graphed in Web Figure 2. Health span–related characteristics were scored so that higher values indicated increased health span. Telomere length was reversed for this analysis so that higher values corresponded to shorter telomeres. Thus, the expected direction of association for all effect sizes was negative—because faster biological aging is expected to shorten health span. Effect sizes are presented for the following measures: A) Telomere shortness; B) 353–cytosine-phosphate-guanine (CpG) clock; C) 99-CpG clock; D) 71-CpG clock; E) Klemera-Doubal (KDM) biological age; F) log age-related homeostatic dysregulation; and G) pace of aging.

A second issue is the need for studies that compare different approaches to quantifying biological aging. Several methods to quantify biological aging have been proposed and have shown promise. Most studies so far concentrate on a single measure of biological aging or a single type of measure (e.g., studies have measured multiple epigenetic clocks (46, 47)). Studies are needed that implement multiple methods in the same groups of humans to evaluate convergent and discriminant validity.

A third issue is the approach to validating biological age measures. The goal of geroscience is to extend health span. But validation studies of biological aging measures have focused primarily on predicting life span. Greater attention is needed to prediction of differences in the functional capacities that geroprotective therapies aim to preserve (48).

A fourth issue is how biological aging measures are developed in the first place. Chronological age is often used as the criterion standard for a biological aging measure (49). But chronological age studied in cross-sectional data does not distinguish biological processes of aging from "cohort effects"; older individuals were born and raised under historical circumstances different from those of younger ones (50). Thus, chronological age may not provide an ideal criterion standard for biological aging. A related

concern is mortality selection, the fact that comparatively fewer individuals from the earlier birth cohorts remain alive to be sampled at a given point in time (51). Consequently, cross-sectional analyses of mixed-age samples may not be optimal for development of biological aging measures. Instead, longitudinal studies of within-individual change across repeated measures provide a better platform for identification of biological changes specifically related to the aging process.

Finally, findings highlight potentially important differences between biological aging measures implemented at different "levels" of analysis, as illustrated in Figure 1. Telomere-length and epigenetic-clock methods are cellular-level measures implemented in our study in only a single tissue, blood. In contrast, the KDM biological age, age-related homeostatic dysregulation, and pace-of-aging measures draw information from multiple systems throughout the body. It is possible that composite measures of, for example, epigenetic clocks, from multiple tissues might show stronger correlation with the other measures of aging and with the health span–related characteristics we studied. Quantifications of biological aging that can be implemented at the level of a single cell are appealing because they allow for direct investigation of cellular-level mechanisms of aging. However, for purposes of measuring effectiveness of geroprotective therapies, quantifications of biological aging that draw information from multiple bodily systems may be more sensitive and specific with respect to the target outcome of health-span extension. Based on our analysis, it is possible that a geroprotective therapy might retard one measure of aging but fail to produce any health-span extension as ascertained by other measures, leaving efficacy of the therapy in question.

Methods to quantify biological aging have potential to advance efforts to elucidate the basic biology of aging and to translate emerging geroprotective therapies from animals to humans. Quantifications of biological aging may also provide clinicians with a tool to communicate complex health information to patients in a way that is easy to understand. Finally, biological age measures can provide a tool for precision medicine, helping physicians decide when a patient should begin screening for age-related conditions. To realize this promise, efforts are needed to harmonize research practices for testing proposed biological aging measures. Research on biological aging recently experienced a growth spurt. As new measures are subjected to increasingly stringent tests (52), discoveries will be tempered by caveats. Rather than discouraging further investigation, these caveats should be interpreted as signs of maturation and encourage redoubled efforts to develop measures of biological aging.

ACKNOWLEDGMENTS

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This research received support from the National Institute of Aging (grants R01AG032282, R01AG048895, 1R01AG049789, and R21AG054846), UK Medical Research Council (grant MR/P005918/1), and UK Economic and Social Research Council (grant ES/M010309/1). Additional support was provided by the National Institute of Aging (grants P30AG028716 and P30AG034424) and by the Jacobs Foundation. D.W.B. is supported by an Early-Career Research Fellowship from the Jacobs Foundation. A.A.C. is a member of the FRQS-funded Centre de recherche sur le vieillissement and Centre de recherche du CHUS, and holds a Canadian Institute for Health Research New Investigator Salary Award.

Conflict of interest: none declared.

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APPENDIX 1. MEASUREMENT DETAILS ABOUT DIFFERENT MEASURES OF BIOLOGICAL AGING

Telomere length

Leukocyte DNA was extracted from blood using standard procedures. DNA was stored at −80°C. All DNA samples were assayed for leukocyte telomere length at the same time. Leukocyte telomere length was measured using a validated quantitative polymerase chain reaction method (53), as previously described (38), which determines mean telomere length across all chromosomes for all cells sampled. The method involves 2 quantitative polymerase chain reaction analyses for each subject; one for a single-copy gene (S) and the other in the telomeric repeat region (T). All DNA samples were run in triplicate for telomere and single-copy reactions. Measurement artifacts (e.g., differences in plate conditions) may lead to spurious results when comparing leukocyte telomere length measured on the same individual at different ages. To eliminate such artifacts, we assayed DNA triplicates from the same individual from all time points, on the same plate. The coefficient of variation for triplicate cycle-threshold values was 0.81% for the telomere (T) and 0.48% for the single-copy gene (S). Age-38 telomere length was measured in $n = 829$ study members.

Telomere erosion

We measured telomere erosion by subtracting values from samples taken at age 26 years from those taken at age 38 years. Telomere erosion was measured for $n = 758$ study members with telomere data at both time points.

Epigenetic clocks

We measured 3 different epigenetic clocks based on 353–cytosine-phosphate-guanine (CpG) (14), 99-CpG (16), and 71-CpG (15) sites, respectively, from whole-genome DNA methylation assayed from peripheral-blood DNA using Illumina 450 k chips (Illumina Inc., San Diego, California). Age-38 epigenetic clocks were measured for $n = 818$ study members. Clock values were approximately normally distributed in the cohort and accurately centered on study members' chronological age (for the 353-CpG Clock, mean 37 (standard deviation (SD), 4) years; for the 99-CpG clock, mean 38 (SD, 5) years; for the 71-CpG clock, mean 37 (SD, 5) years).

Epigenetic ticking

We measured epigenetic ticking rates for the 353-, 99-, and 71-CpG epigenetic clocks by subtracting age-26 values from age-38 values. Epigenetic ticking was measured for $n = 743$ study members with epigenetic data at both time points.

Klemera-Doubal method (KDM) biological age

We measured KDM biological age from 10 blood and organ-system-function biomarkers assessed using standard assays. KDM biological age was measured for $n = 904$ study members and was approximately normally distributed in the cohort (mean 38 (SD, 3) years). We previously published on this measure as "biological age" (19). Here we refer to it as "KDM biological age" for clarity.

Age-related homeostatic dysregulation

We measured age-related homeostatic dysregulation from 18 blood and organ-system-function biomarkers assessed using standard assays. This measure quantifies deviation from a reference norm in Mahalanobis distance (54). We used the normative values for the Dunedin cohort when they were aged 26 years to form this reference. We log transformed the computed distances for analysis. Age-related homeostatic dysregulation was measured

for $n = 954$ study members and was approximately normally distributed in the cohort (mean 3.37 (SD, 0.61)).

Pace of aging

We measured pace of aging from changes in 18 blood- and organ-system-functional biomarkers assayed when study members were aged 26, 32, and 38 years (19). Pace of aging quantifies the rate of biological aging in units of years of physiological change per chronological year. Pace of aging was measured for $n = 954$ study members and was approximately normally distributed in the cohort (mean 1 (SD, 0.38)).

Age-related homeostatic dysregulation and pace-of-aging algorithms analyzed the same 18 biomarkers, and KDM biological age analyzed 7 of these in addition to 3 others. However, the algorithms, which were developed by independent research groups, take very different approaches to characterize these data (and use different numbers of repeated measures) (14–19).

APPENDIX 2. MEASUREMENT DETAILS ABOUT DIFFERENT MEASURES OF HEALTH SPAN–RELATED CHARACTERISTICS

Physical functioning

Balance. We measured balance as the maximum time achieved across 3 trials of the Unipedal Stance Test (with eyes closed) (55–57).

Grip strength. We measured grip strength with dominant hand (elbow held at 90°, upper arm held tight against the trunk) as the maximum value achieved across 3 trials using a Jamar digital dynamometer (58, 59).

Motor coordination. We measured motor functioning as the time to completion of the Grooved Pegboard Test with the dominant hand (60).

Physical limitations. Study member responses ("limited a lot," "limited a little," "not limited at all") to the 10-item Short Form Health Survey (SF-36) physical functioning scale (61) assessed their difficulty with completing various activities (e.g., climbing several flights of stairs, walking more than 1 km, participating in strenuous sports).

Cognitive functioning

Cognitive function. Intelligence quotient (IQ) is a highly reliable measure of general intellectual functioning that captures overall ability across differentiable cognitive functions. We measured IQ from the individually administered Wechsler Intelligence Scale for Children–Revised (WISC-R; averaged across ages 7, 9, 11, and 13 years) (62) and the Wechsler Adult Intelligence Scale–IV (WAIS-IV; age 38 years) (63), both with mean 100 (SD, 15).

Cognitive decline. We measured IQ decline by comparing scores from the WISC-R (in childhood) and the WAIS-IV (at age 38 years). Analyses of subtests are reported in the Web Tables 7–12.

Subjective aging

Self-rated health. Study members rated their health on a scale of 1–5 (poor, fair, good, very good, or excellent).

Facial aging. We took 2 measurements of perceived age based on facial photographs (64, 65). First, age range was assessed by an independent panel of 4 Duke University undergraduate raters. Raters were presented with standardized (nonsmiling) facial photographs of study members (taken with a Canon PowerShot G11 camera with an optical zoom; Canon Inc., Tokyo, Japan) and were kept blind to their actual age. Photos were divided into sex-segregated slideshow batches containing approximately 50 photos, viewed for 10 seconds each. Raters were randomized to viewing the slideshow batches in either forward progression or backwards progression. They used a Likert scale to categorize each study member into a 5-year age range (i.e., from ages 20–24 years to ages 65–70 years). Scores for each study member were averaged across all raters ($\alpha = 0.71$). The second measure, relative age, was assessed by a different panel of 4 Duke University undergraduate raters. The raters were told that all photos were of people aged 38 years old. Raters then used a 7-item Likert scale to assign a "relative age" to each study member (1 = "young looking" to 7 = "old looking"). Scores for each study member were averaged across all raters ($\alpha = 0.72$). Age range and relative age were highly correlated ($r = 0.73$). To derive a measure of perceived age at 38 years, we standardized and averaged both age range and relative age scores to create facial age at 38 years.

Web Material for

Eleven Telomere, Epigenetic Clock, and Biomarker-Composite Quantifications of Biological Aging: Do They Measure the Same Thing?

Web Appendix 1. Detailed Description of Biological Aging Measures

Telomere length. Telomere length was measured from leukocyte DNA collected at ages 26 and 38 years. Leukocyte DNA was extracted from blood using standard procedures (1,2). DNA was stored at -80°C. All DNA samples were assayed for leukocyte telomere length at the same time. Leukocyte telomere length was measured using a validated quantitative PCR method (3), as previously described (4), which determines mean telomere length across all chromosomes for all cells sampled. The method involves two quantitative PCR reactions for each subject; one for a single-copy gene (S) and the other in the telomeric repeat region (T). All DNA samples were run in triplicate for telomere and single-copy reactions.

Measurement artifacts (e.g., differences in plate conditions) may lead to spurious results when comparing leukocyte telomere length measured on the same individual at different ages. To eliminate such artifacts, we assayed DNA triplicates from the same individual from all time points, on the same plate. CV for triplicate Ct values was 0.81% for the telomere (T) and 0.48% for the single-copy gene (S). We computed change in telomere length as the Age-38 T/S ratio – Age-26 T/S ratio. Telomere data were available for N=829 Study members at age 38, for N=812 Study members at age 26, and for N=758 Study members at both ages of measurement.

Epigenetic Clocks. Epigenetic clocks were calculated using leukocyte DNA collected at ages 26 and 38 years. 500ng of DNA from each sample was treated with sodium bisulfite, using the EZ-96 DNA Methylation kit (Zymo Research, CA, USA). DNA methylation was quantified using the Illumina Infinium HumanMethylation450 BeadChip (Illumina Inc, CA, USA) run on an Illumina iScan System (Illumina, CA, USA) using the manufacturers' standard protocol. Briefly, these arrays simultaneously interrogate >485,000 methylation sites distributed across the genome. Samples were arranged into 96-well plates so that within-individual age-26 and -38 DNA samples were hybridized in the same row of the arrays (i.e. age 26 and 38 DNA samples from the same individual occupy array columns 1 and 2 of the same row). Array analysis was performed by the Duke University Molecular Physiology Institute Genomics Core Facility using the iScan platform (Illumina). Data quality control and normalization was carried out using the *Methylumi* Bioconductor package in the R statistical programming environment.

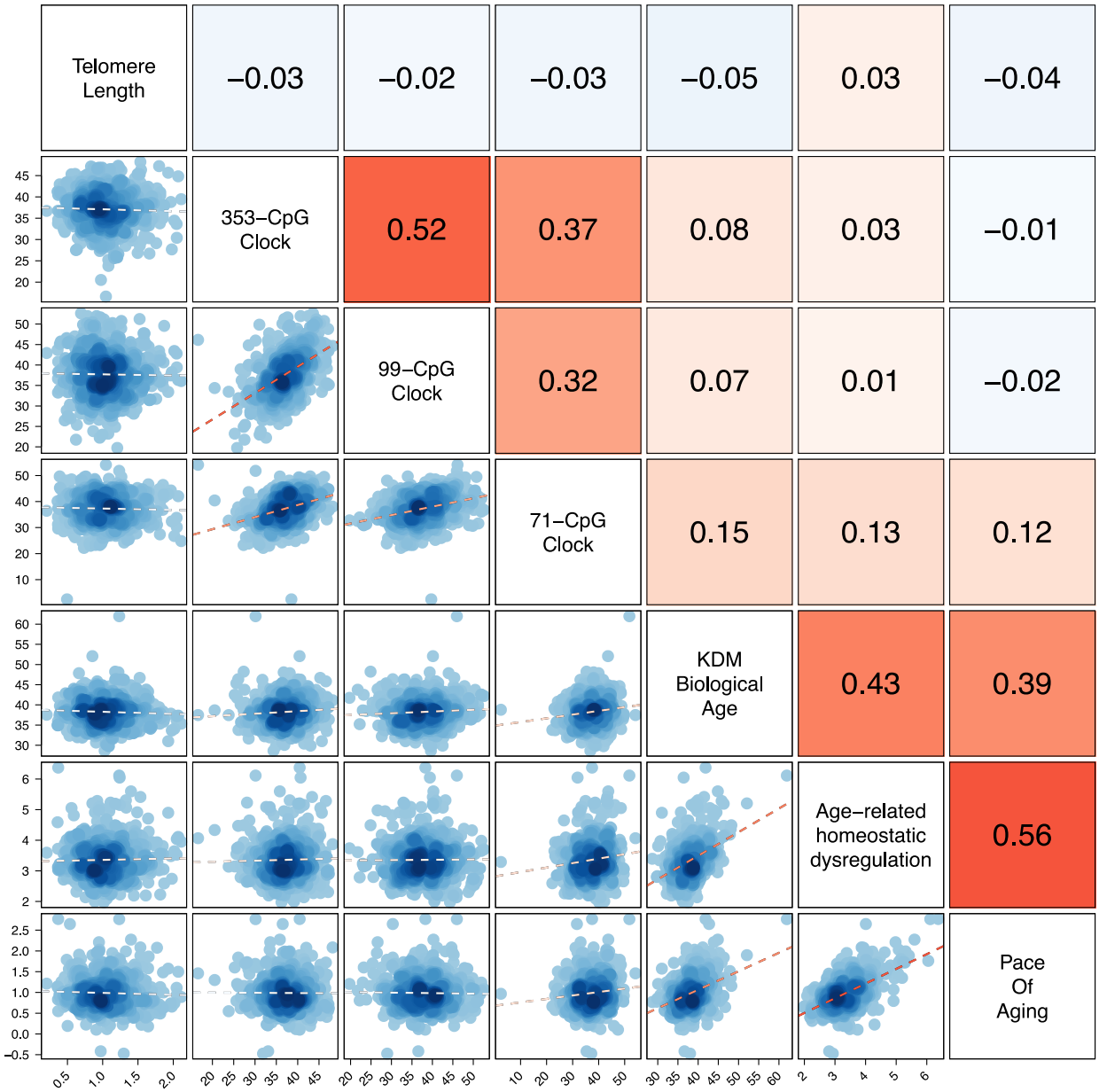
We analyzed three epigenetic clocks. The first clock, proposed by Horvath, included 353 CpG sites (5). The second clock, proposed by Hannum and colleagues, included 71 CpG sites (6). The third clock, proposed by Weidner and colleagues, included 99 CpG sites (7,8). Study members' epigenetic clock values for the 353-CpG and 71-CpG clocks were calculated using Horvath's website (https://labs.genetics.ucla.edu/horvath/dnamage/). Epigenetic clock values for the 99-CpG clock were calculated using the algorithm published by the Wagner lab (9,10). Epigenetic clock values were available for N=818 Study members at age 38, for N=821 Study members at age 26, and for N=743 Study members at both ages of measurement.

Biological Age. As described previously (11), we calculated each Study member's Biological Age at age 38 years using the Klemera-Doubal equation (12) and parameters Levine estimated from the NHANES-III dataset (13) for ten biomarkers: Glycated hemoglobin, Forced expiratory volume in one second (FEV_1), Blood pressure (systolic), Total cholesterol, C-reactive protein, Creatinine, Urea nitrogen, Albumin, Alkaline phosphatase, and Cytomegalovirus IgG. Data to calculate Biological Age data were available for N=904 Study members.

Age-Related Homeostatic Dysregulation. We measured age-related homeostatic dysregulation by applying the biomarker Mahalanobis distance method described by Cohen and colleagues (14–16) to Study members' age-38 biomarker values. The biomarker Mahalanobis distance method measures how aberrant an individual's physiology is relative to a reference norm (14). Cohen and colleagues used chronologically young individuals to form this reference norm for their calculations (15). They interpreted biomarker Mahalanobis distance from the reference as an indicator of age-related homeostatic dysregulation, a sign of biological aging. We formed our reference from the Dunedin Study members' biomarker values at age 26 years, the youngest age at which the biomarkers were measured. Thus, a Study member's biomarker Mahalanobis distance quantifies homeostatic dysregulation relative to the cohort's age-26 norm. We calculated Mahalanobis distance based on 18 biomarkers with repeated measures at ages 26 and 38 years (the same 18 biomarkers we previously used to compute Study members' Pace of Aging (11), see below). Distances were log transformed for analysis. Age-related Homeostatic Dysregulation was measured for N=954 Study members.

Pace of Aging. As described previously (11), we measured Pace of Aging with repeated assessments of a panel of 18 biomarkers taken at ages 26, 32, and 38 years. The biomarkers were: Apolipoprotein B100/A1 ratio, Blood pressure (mean arterial pressure), Body mass index (BMI) and Waist-hip ratio, C-reactive protein and white blood cell count, Cardiorespiratory fitness (VO_2Max), Creatinine clearance, Forced expiratory volume in one second (FEV_1) and Forced vital capacity ratio (FEV_1/FVC), Glycated hemoglobin, High density lipoprotein (HDL), Lipoprotein(a), Leukocyte telomere length (LTL), Periodontal disease, Total cholesterol, Triglycerides, Urea nitrogen. For each biomarker, we calculated the Study member's personal rate of change using mixed-effects growth models. We combined these rates of change into a single index scaled in years of physiological change occurring per one chronological year. The average Study member had Pace of Aging equal to one year of physiological change per one chronological year. The fastest-aging Study members experienced more than twice that rate of physiological change. The slowest-aging Study members experienced almost no change at all. Pace of Aging was measured for N=954 Study members.

Web Figure 1. Correlations among seven measures of biological aging in a birth cohort at chronological age 38 years. The figure shows a matrix of scatterplots and correlations illustrating relationships among seven measures of biological aging: Leukocyte telomere length, 353-, 99-, and 71-CpG epigenetic clocks, KDM Biological Age, Age-related Homeostatic Dysregulation, and Pace of Aging. Data are for n=800 Study members with complete data on all biological aging measures. Correlations are shown above the diagonal. (Correlations ≥0.07 are statistically significant at p<0.05.) Scatter plots are shown below the diagonal. Y-axis scales correspond to the biological aging metric listed to the right of the plot. X-axis scales correspond to the biological aging metric listed above the plot. Correlations between aging measures computed with adjustment for sex differences are reported in **Web Table 6**.



Web Table 1. Relationships among telomere length, epigenetic clocks, KDM Biological Age, Age-related Homeostatic Dysregulation, and Pace of Aging in a birth cohort at chronological age 38 years – Spearman correlations

		Spearman correlations						p-values for Spearman correlations					
		(1)	(2)	(3)	(4)	(5)	(6)	(1)	(2)	(3)	(4)	(5)	(6)
Spearman Correlations													
(1)	Telomere Length												
(2)	353-CpG Clock	-0.05						0.174					
(3)	99-CpG Clock	-0.04	0.53					0.282	5.66E-60				
(4)	71-CpG Clock	-0.04	0.41	0.34				0.276	3.06E-33	5.38E-23			
(5)	KDM Biological Age	-0.05	0.12	0.08	0.14			0.152	0.001	0.028	6.52E-05		
(6)	Age-related Homeostatic Dysregulation	0.02	0.04	0.03	0.09	0.40		0.652	0.272	0.390	0.013	8.35E-32	
(7)	Pace of Aging	-0.03	0.00	-0.01	0.12	0.36	0.48	0.351	0.905	0.711	0.001	1.48E-25	5.20E-47

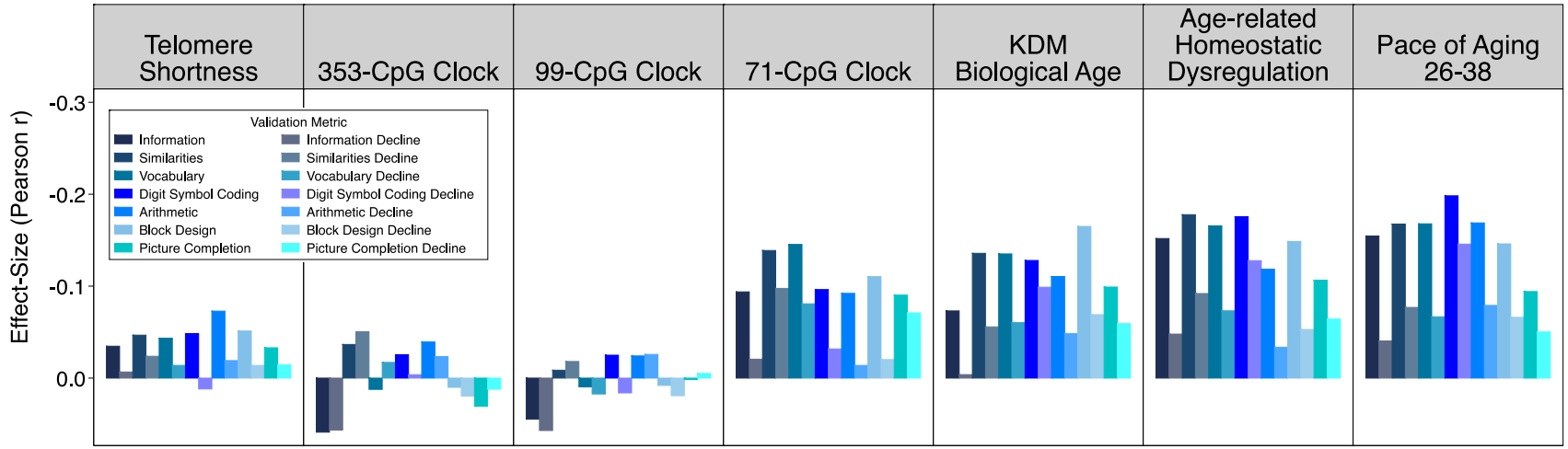
Web Table 2. Relationships among telomere length, epigenetic clocks, KDM Biological Age, Age-related Homeostatic Dysregulation, and Pace of Aging in a birth cohort at chronological age 38 years – Principal components analysis. Three principal components were estimated with eigenvalues of 1.00 or greater. Telomere length loaded most strongly on principal component three; 353- and 99-CpG epigenetic clocks loaded most strongly on principal component two and the 71-CpG clock loaded similarly on components one and two; clinical biomarker algorithm values loaded most strongly on principal component one.

	Principal Component		
	1	**2**	**3**
Eigen-value	2.05	1.71	1.00
Loadings			
Telomere Length	-0.05	-0.03	0.99
353-CpG Clock	0.32	0.53	0.03
99-CpG Clock	0.30	0.53	0.06
71-CpG Clock	0.37	0.35	0.01
KDM Biological Age	0.47	-0.23	-0.06
Age-related Homeostatic Dysregulation	0.48	-0.36	0.11
Pace of Aging	0.47	-0.37	-0.01

Web Table 3. Sex-adjusted Pearson correlations among telomere length, epigenetic clocks, KDM Biological Age, Age-related Homeostatic Dysregulation, and Pace of Aging

Sex-Adjusted Pearson Correlations		(1)	(2)	(3)	(4)	(5)	(6)
(1)	Telomere Length						
(2)	353-CpG Clock	-0.03					
(3)	99-CpG Clock	-0.02	0.52				
(4)	71-CpG Clock	-0.03	0.37	0.32			
(5)	KDM Biological Age	-0.05	0.08	0.07	0.15		
(6)	Age-related Homeostatic Dysregulation	0.03	0.02	0.00	0.10	0.43	
(7)	Pace of Aging	-0.04	-0.01	-0.02	0.12	0.39	0.57

Web Figure 2. Associations of cross-sectional biological aging measures and Pace of Aging with subtests of cognitive functioning and cognitive decline. The figure shows bar charts of effect-sizes (Pearson r) for each of the seven measures of biological aging. Effect-sizes were estimated for seven tests of cognitive function administered in parallel during childhood and age-38 assessments. The tests were subscales of the Wechsler Intelligence Tests. There were three tests of so-called "crystalized" cognitive functions (Information, Similarities, and Vocabulary), and four tests of so-called "fluid" cognitive functions (Digit Symbol Coding, Arithmetic, Block Design, and Picture Completion). All tests were scored so that higher values corresponded to indication of better cognitive functioning. Telomere length was reversed for this analysis so that higher values corresponded to shorter telomeres. Thus, the expected direction of association for all correlations was negative—because faster biological aging is expected to hasten cognitive decline. Standardized regression coefficients (interpretable as Pearson r) and their p-values are reported in the table below the figure. For each test, the graph plots the effect-size for association between biological aging and age-38 test performance first (darker shaded bars), followed by the effect-size for association between biological aging and actual decline in test performance between childhood and age 38 (lighter shaded bars).



	Telomere Shortness		353-CpG Clock		99-CpG Clock		71-CpG Clock		KDM Biological Age		Age-related Homeostatic Dysregulation		Pace of Aging	
	r / p-value													
Information														
Age 38	-0.03	0.314	0.06	0.090	0.04	0.196	-0.09	0.008	-0.07	0.027	-0.15	2.89E-06	-0.15	1.56E-06
Change from Childhood	-0.01	0.793	0.06	0.027	0.06	0.025	-0.02	0.435	0.00	0.879	-0.05	0.047	-0.04	0.093
Similarities														
Age 38	-0.05	0.181	-0.04	0.298	-0.01	0.808	-0.14	1.09E-04	-0.14	4.49E-05	-0.18	6.16E-08	-0.17	2.69E-07
Change from Childhood	-0.02	0.417	-0.05	0.087	-0.02	0.541	-0.10	0.001	-0.06	0.048	-0.09	0.001	-0.08	0.006
Vocabulary														
Age 38	-0.04	0.215	0.01	0.726	0.01	0.784	-0.15	5.25E-05	-0.14	5.07E-05	-0.17	4.92E-07	-0.17	2.71E-07
Change from Childhood	-0.01	0.589	-0.02	0.508	0.02	0.499	-0.08	0.002	-0.06	0.014	-0.07	0.003	-0.07	0.006
Digit Symbol Coding														
Age 38	-0.05	0.153	-0.03	0.456	-0.02	0.464	-0.10	0.006	-0.13	7.19E-05	-0.18	2.98E-08	-0.20	2.54E-10
Change from Childhood	0.01	0.665	0.00	0.894	0.02	0.557	-0.03	0.264	-0.10	1.40E-04	-0.13	7.23E-07	-0.15	1.15E-08
Arithmetic														
Age 38	-0.07	0.034	-0.04	0.256	-0.02	0.486	-0.09	0.009	-0.11	0.001	-0.12	2.67E-04	-0.17	1.47E-07
Change from Childhood	-0.02	0.467	-0.02	0.372	-0.03	0.327	-0.01	0.611	-0.05	0.055	-0.03	0.180	-0.08	0.001
Block Design														
Age 38	-0.05	0.137	0.01	0.772	0.01	0.817	-0.11	0.002	-0.16	5.08E-07	-0.15	5.07E-06	-0.15	6.30E-06
Change from Childhood	-0.01	0.599	0.02	0.456	0.02	0.466	-0.02	0.460	-0.07	0.006	-0.05	0.037	-0.07	0.008
Picture Completion														
Age 38	-0.03	0.345	0.03	0.383	0.00	0.962	-0.09	0.012	-0.10	0.003	-0.11	0.001	-0.09	0.004
Change from Childhood	-0.01	0.669	0.01	0.719	-0.01	0.881	-0.07	0.041	-0.06	0.068	-0.06	0.046	-0.05	0.116

Web Appendix 2. Does change between repeated cross-sectional measures of biological aging track the aging process?

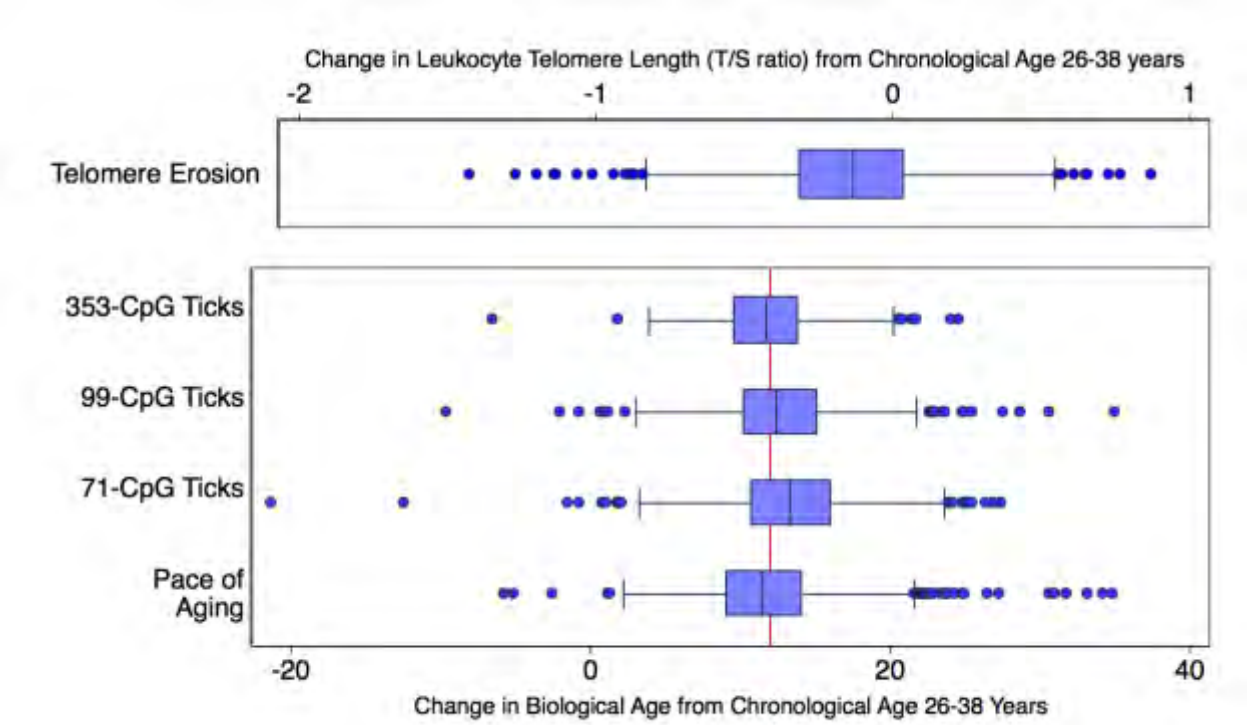
Most methods to quantify biological aging are designed for implementation using a cross-section of biomarker data. These cross-sectional methods could be used to measure changes in the rate of aging caused by geroprotective intervention if they were repeated, for example before and after administration of therapy. We were able to test if cross-sectional biological-age measures showed promise for such applications by testing within-person change in biological age estimates calculated from biological samples taken when Study members were aged 26 years and again when they were aged 38 years. We computed change scores (age-38 value – age-26 value) to test how much telomere erosion actually took place over these 12 years and how many "ticks" were registered by the epigenetic clocks. (We did not test change in the KDM Biological Age and Age-related Homeostatic Dysregulation measures because the necessary data were not available at the age-26 assessment.)

Study members experienced an average of 0.15 (SD=0.30) T/S ratio units of telomere erosion over the 12-year follow-up. This telomere erosion was equivalent to about one-half of one standard deviation of the variance in telomere length at age 38 years. Study members' epigenetic clocks ticked forward by 12-14 years (for the 353 CpG clock, M=12y, SD=3; for the 99 CpG clock, M=13y, SD=4; for the 71 CpG clock, M=14y, SD=5). This epigenetic "ticking" was equivalent to between 2 and 3 standard deviations of the variance in epigenetic clock values at age 38 years. For comparison purposes, we analyzed change in biological age as estimated by Pace of Aging. Because Pace of Aging estimates physiological-change-per-chronological-year, we multiplied each Study member's Pace of aging by 12 to estimate change in biological age between chronological ages 26 and 38 years (M=12y, SD=5). Telomere erosion, epigenetic ticking, and Pace of Aging were approximately normally distributed (**Web Figures 3 and 4**).

To test if a common aging process influenced changes in different measures of biological aging, we computed correlations among change scores. Correlations among change scores showed a pattern similar to correlations among cross-sectional measures (**Web Figure 5**). Telomere erosion was not correlated with epigenetic ticking. Epigenetic ticking was correlated across the three different clocks (r=0.17-0.42). Epigenetic ticking was weakly correlated with Pace of Aging (r=0.06-0.09). The correlation between telomere erosion and Pace of aging was relatively high (r=0.24) because telomere erosion is a component of the Pace of Aging. When telomere erosion was excluded from Pace of Aging the correlation was reduced to near zero. Results were similar when Spearman correlations were computed to reduce the influence of extreme values (**Web Table 4**).

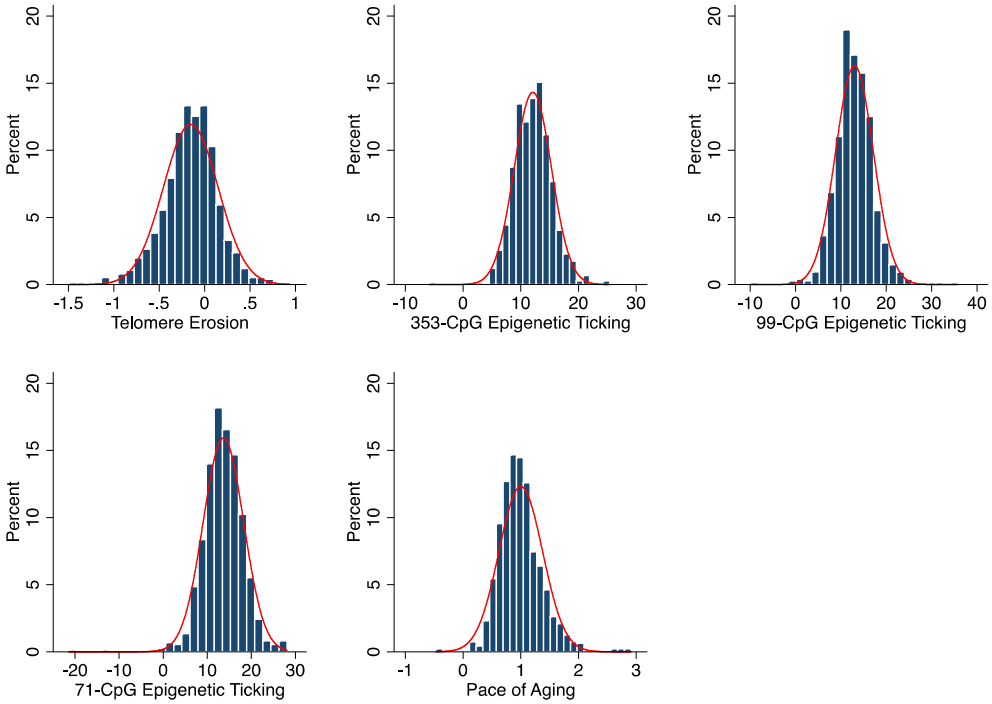
Change scores computed from repeated cross-sectional biological aging measures were not consistently associated with healthspan-related characteristics. Telomere erosion was not associated with healthspan-related characteristics (r=-0.04-0.03). Epigenetic ticking was also not associated with healthspan characteristics, with the exception of age-38 IQ score (r=0.11, p=0.003 for 353-CpG clock; r=0.09, p=0.017 for the 71-CpG clock) and self-rated health (r=-0.07, p=0.044 for the 71-CpG clock). Effect sizes are graphed in **Web Figures 6** and **7**.

Web Figure 3. Changes in cross-sectional measures of biological aging between chronological ages 26 and 38 years in the Dunedin cohort. Telomere and epigenetic clock measurements were made from DNA samples extracted from peripheral blood collected when Study members were aged 26 and 38 years. Repeated observations of each individual were assayed together on the same plate/ methylation array to reduce batch effects. Telomere erosion and epigenetic ticking were measured by subtracting age-26 values from age-38 values. For comparison purposes, Pace of Aging is plotted alongside the epigenetic clocks. Pace of Aging is estimated from three repeated measurements at ages 26, 32, and 38 years of 18 different biomarkers. Pace of Aging is scaled in years of physiological change per chronological year. For this graph, Pace of Aging was multiplied by 12 to reflect the years of biological aging estimated to have occurred between ages 26 and 38 years. The vertical red line in the bottom panel of the figure indicates a value of 12 years, the actual amount of chronological time elapsed during the measurement interval.

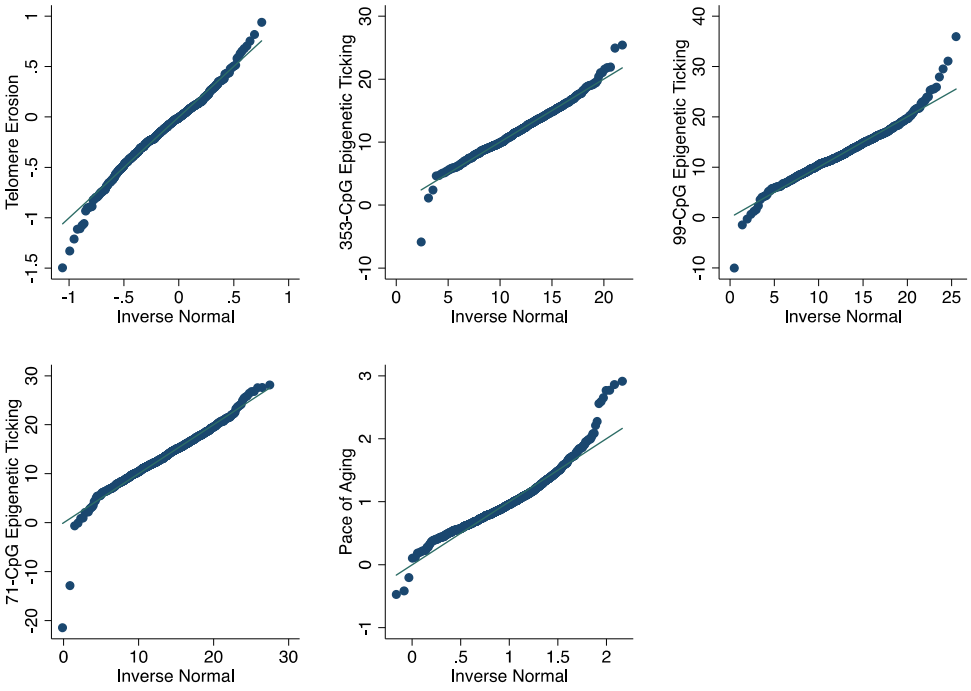


Web Figure 4. Distributions of telomere erosion, epigenetic ticking rates, and the Pace of Aging.

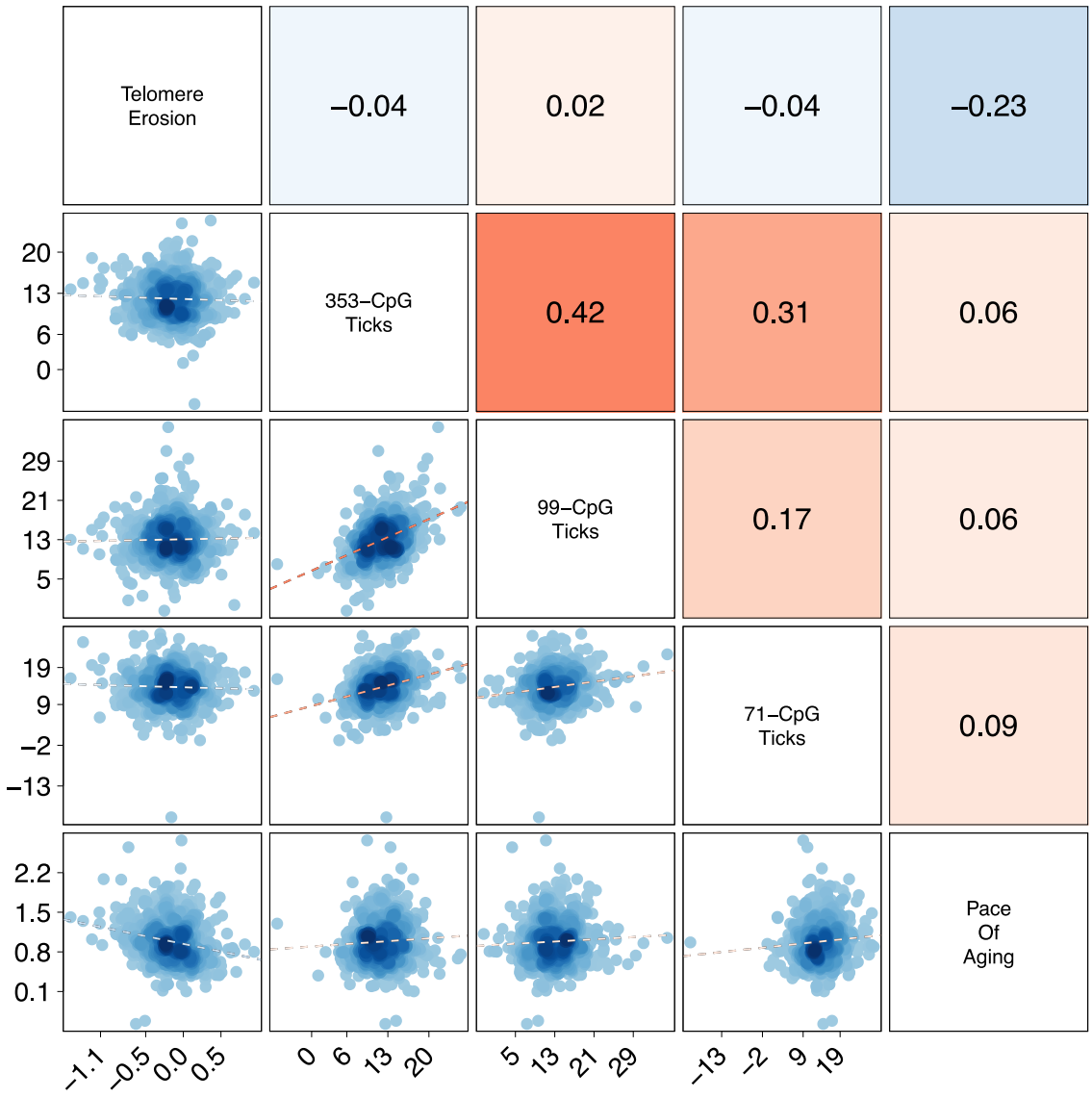
Panel A. Histograms



Panel B. Quantile plots



Web Figure 5. Correlations among longitudinal measures of biological aging. The figure shows a matrix of scatterplots and correlations illustrating relationships among 5 longitudinal measures of biological aging: telomere erosion, ticking of the 353-, 99-, and 71-CpG epigenetic clocks, and the Pace of Aging. Data are for n=733 Study members with complete data on all measures. Correlations are shown above the diagonal. (Correlations ≥0.07 are statistically significant at p<0.05.) Scatter plots are shown below the diagonal. Y-axis scales correspond to the biological aging metric listed to the left of the plot. X-axis scales correspond to the biological aging metric listed above the plot. Correlations between aging measures computed with adjustment for sex differences are reported in **Supplemental Table 5.**



Web Table 4. Relationships among telomere erosion, epigenetic ticking rates, and Pace of Aging – Spearman correlations.

		Spearman correlations				p-values for Spearman correlations			
		(1)	(2)	(3)	(4)	(1)	(2)	(3)	(4)
Spearman Correlations									
(1)	Telomere Erosion								
(2)	353-CpG Ticks	-0.04				0.336			
(3)	99-CpG Ticks	0.01	0.37			0.805	1.66E-25		
(4)	71-CpG Ticks	-0.02	0.33	0.16		0.527	8.71E-20	2.10E-05	
(5)	Pace of Aging	-0.24	0.10	0.07	0.10	8.31E-11	0.010	0.062	0.005

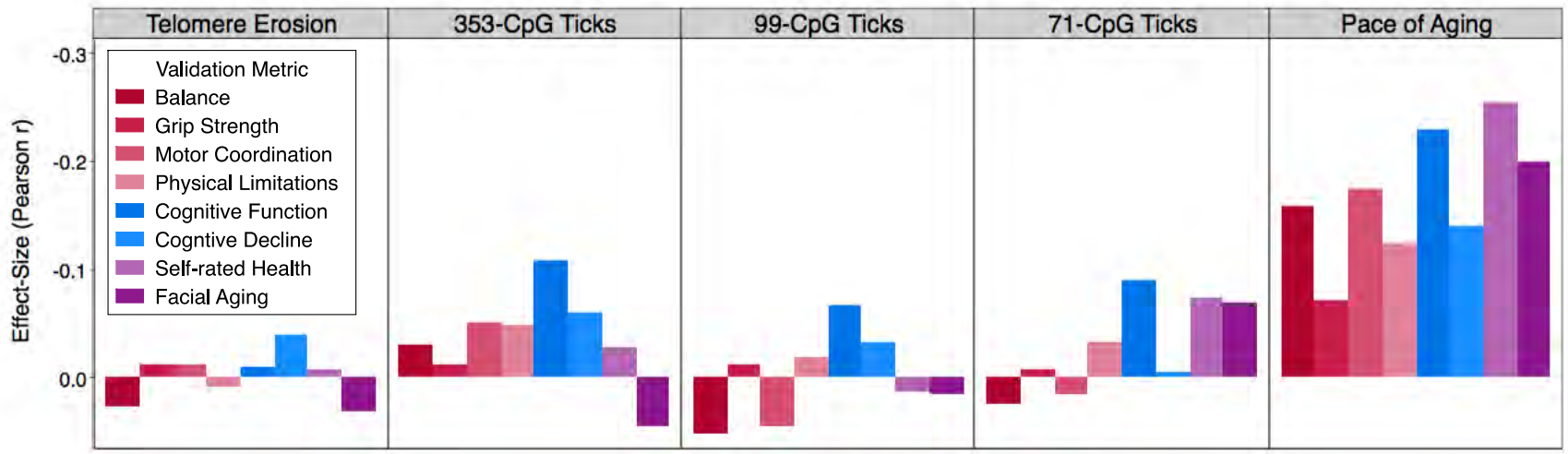
Web Table 5. Relationships among telomere erosion, epigenetic ticking rates, and Pace of Aging -- Principal components analysis.
Two principal components were estimated with eigenvalues of 1.00 or greater. Telomere erosion and the Pace of Aging loaded most strongly on the second principal component. Loadings were in the opposite direction because negative values of telomere erosion indicate faster aging whereas positive values of the Pace of Aging indicate faster aging. Co-loadings of telomere erosion and Pace of Aging on a common factor reflect the inclusion of telomere erosion in the Pace of Aging algorithm. Epigenetic clocks loaded most strongly on the first principal component.

	Principal Component	
	1	**2**
Eigen-value	1.63	1.21
Loadings		
Telomere Erosion	-0.13	0.70
353-CpG Ticking	0.63	0.16
99-CpG Ticking	0.55	0.23
71-CpG Ticking	0.48	0.02
Pace of Aging	0.22	-0.65

Web Table 6. Sex-adjusted Pearson correlations among telomere erosion, epigenetic ticking rates, and Pace of Aging

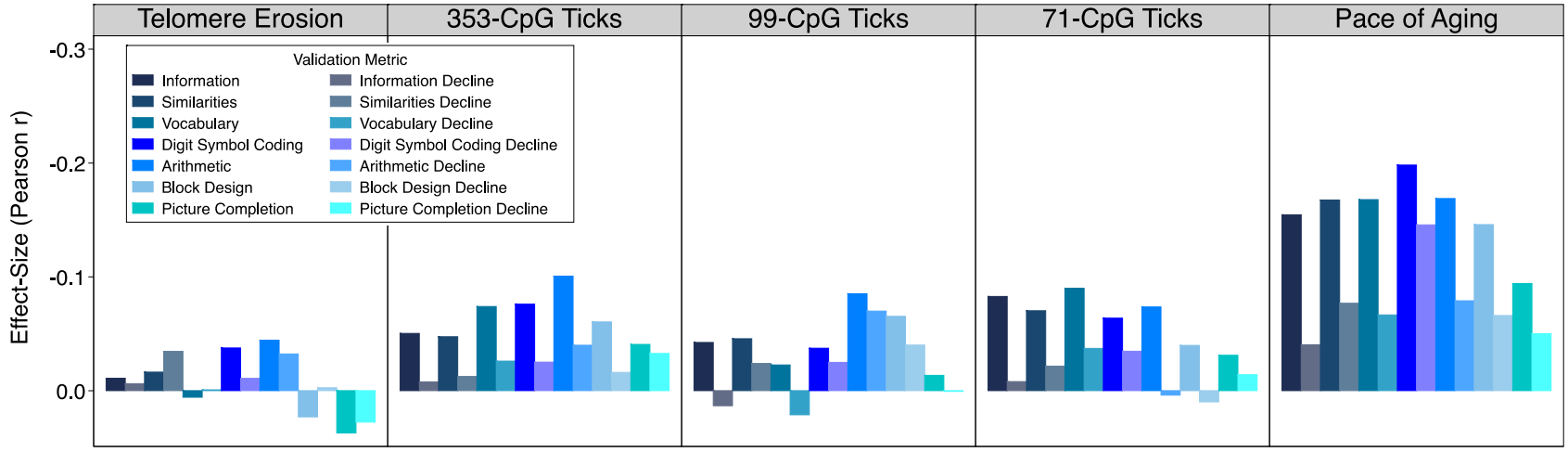
Sex-Adjusted Pearson Correlations		(1)	(2)	(3)	(4)
(1)	Telomere Erosion				
(2)	353-CpG Ticks	-0.04			
(3)	99-CpG Ticks	0.02	0.41		
(4)	71-CpG Ticks	-0.04	0.31	0.17	
(5)	Pace of Aging	-0.23	0.07	0.06	0.08

Web Figure 6. Associations of changes in cross-sectional biological aging measures and Pace of Aging with healthspan-related characteristics. The figure shows bar charts of effect-sizes for telomere erosion, ticking of 353-, 99-, and 71-CpG epigenetic clocks, and Pace of Aging. Effect-sizes were estimated for four measures of physical functioning (balance, grip strength, motor coordination, and self-reported physical limitations), cognitive functioning (IQ score at age 38 from the Wechsler Adult Intelligence Scale), cognitive decline (change in Wechsler-scale IQ score since childhood), and two measures of subjective aging (self-rated health and facial aging from assessments of facial photographs of the Study member by independent raters). Effect sizes for subtests of cognitive function and cognitive decline are graphed in **Supplemental Figure 6**. Healthspan-related characteristics were scored so that higher values indicated increased healthspan. Telomere erosion was scored for this analysis so that higher values corresponded to more telomere erosion. Thus, the expected direction of association for all correlations was negative—because faster biological aging is expected to shorten healthspan. Standardized regression coefficients (interpretable as Pearson r) and their p-values are reported in the table below the figure (next page).



Healthspan-related Characteristics	Telomere Erosion		353-CpG Ticks		99-CpG Ticks		71-CpG Ticks		Pace of Aging	
	r / p-value									
Balance	0.03	0.442	-0.03	0.424	0.05	0.147	0.02	0.498	-0.16	1.27E-06
Grip Strength	-0.01	0.742	-0.01	0.745	-0.01	0.753	-0.01	0.876	-0.07	0.029
Motor Coordination	-0.01	0.779	-0.05	0.178	0.05	0.210	0.02	0.657	-0.17	1.25E-07
Physical Limitations	0.01	0.796	-0.05	0.189	-0.02	0.637	-0.03	0.371	-0.12	1.30E-04
IQ at 38	-0.01	0.797	-0.11	0.003	-0.07	0.071	-0.09	0.017	-0.23	1.83E-12
IQ change from childhood	-0.04	0.305	-0.06	0.109	-0.03	0.402	0.00	0.907	-0.14	2.80E-05
Self-rated Health	-0.01	0.878	-0.03	0.458	0.01	0.698	-0.07	0.044	-0.25	2.69E-15
Facial Aging	0.03	0.379	0.05	0.214	0.02	0.648	-0.07	0.066	-0.20	7.56E-10

Web Figure 7. Associations of changes in cross-sectional biological aging measures and Pace of Aging with subtests of cognitive functioning and cognitive decline. The figure shows bar charts of effect-sizes (Pearson r) for telomere erosion, ticking of 353-, 99-, and 71-CpG epigenetic clocks, and Pace of Aging. Effect-sizes were estimated for seven tests of cognitive function administered in parallel during childhood and age-38 assessments. The tests were subscales of the Wechsler Intelligence Tests. There were three tests of so-called "crystalized" cognitive functions (Information, Similarities, and Vocabulary), and four tests of so-called "fluid" cognitive functions (Digit Symbol Coding, Arithmetic, Block Design, and Picture Completion). All tests were scored so that higher values corresponded to indication of better cognitive functioning. Telomere erosion was scored for this analysis so that higher values corresponded to more telomere erosion. Thus, the expected direction of association for all correlations was negative—because faster aging is expected to hasten cognitive decline. Standardized regression coefficients (interpretable as Pearson r) and their p-values are reported in the table below the figure. For each test, the graph plots the effect-size for association between biological aging and age-38 test performance first (darker shaded bars), followed by the effect-size for association between biological aging and actual decline in test performance between childhood and age 38 (lighter shaded bars). Standardized regression coefficients (interpretable as Pearson r) and their p-values are reported in the table below the figure (next page).



	Telomere Erosion		353-CpG Ticks		99-CpG Ticks		71-CpG Ticks		Pace of Aging	
	r / p-value									
Information										
Age 38	-0.01	0.762	-0.05	0.166	-0.04	0.244	-0.08	0.023	-0.15	1.56E-06
Change from	-0.01	0.819	-0.01	0.775	0.01	0.628	-0.01	0.771	-0.04	0.093
Similarities										
Age 38	-0.02	0.654	-0.05	0.197	-0.05	0.214	-0.07	0.056	-0.17	2.69E-07
Change from	-0.03	0.257	-0.01	0.686	-0.02	0.439	-0.02	0.487	-0.08	0.006
Vocabulary										
Age 38	0.01	0.875	-0.07	0.044	-0.02	0.539	-0.09	0.014	-0.17	2.71E-07
Change from	0.00	0.977	-0.03	0.334	0.02	0.431	-0.04	0.169	-0.07	0.006
Digit Symbol Coding										
Age 38	-0.04	0.287	-0.08	0.033	-0.04	0.294	-0.06	0.073	-0.20	2.54E-10
Change from	-0.01	0.710	-0.03	0.392	-0.02	0.394	-0.03	0.236	-0.15	1.15E-08
Arithmetic										
Age 38	-0.04	0.215	-0.10	0.005	-0.09	0.018	-0.07	0.042	-0.17	1.47E-07
Change from	-0.03	0.240	-0.04	0.151	-0.07	0.011	0.00	0.889	-0.08	0.001
Block Design										
Age 38	0.02	0.522	-0.06	0.096	-0.07	0.072	-0.04	0.273	-0.15	6.30E-06
Change from	0.00	0.925	-0.02	0.564	-0.04	0.150	0.01	0.723	-0.07	0.008
Picture Completion										
Age 38	0.04	0.308	-0.04	0.269	-0.01	0.714	-0.03	0.398	-0.09	0.004
Change from	0.03	0.434	-0.03	0.361	0.00	0.985	-0.01	0.695	-0.05	0.116

Web Table 7. Associations of cross-sectional biological aging measures and Pace of Aging with healthspan-related characteristics & cognitive subtests after adjustment for body-mass index. Adjustment was made by including body-mass index as a covariate in regressions.

Healthspan-related Characteristics	Telomere Shortness		353-CpG Clock		99-CpG Clock		71-CpG Clock		KDM Biological Age		Age-related Homeostatic Dysregulation		Pace of Aging	
	BMI-Adjusted r / p-value													
Balance	0.00	0.972	-0.04	0.222	0.02	0.602	-0.06	0.121	-0.15	1.42E-05	-0.14	2.34E-05	-0.09	8.16E-03
Grip Strength	-0.06	0.076	0.00	0.960	-0.06	0.119	-0.05	0.186	-0.22	3.80E-10	-0.06	0.064	-0.09	0.011
Motor Coordination	0.00	0.891	0.00	0.990	0.05	0.184	-0.08	0.037	-0.10	5.48E-03	-0.17	7.29E-07	-0.14	4.87E-05
Physical Limitations	0.03	0.454	0.00	0.938	0.00	0.918	-0.05	0.182	-0.09	1.56E-02	-0.10	3.03E-03	-0.08	1.58E-02
IQ at 38	-0.06	0.102	-0.01	0.719	-0.01	0.815	-0.15	3.16E-05	-0.15	2.04E-05	-0.19	5.73E-09	-0.22	8.04E-11
IQ change from childhood	0.00	0.964	-0.04	0.244	-0.02	0.625	-0.09	0.012	-0.09	0.012	-0.12	0.001	-0.15	1.62E-05
Self-rated Health	-0.01	0.768	0.00	0.936	0.04	0.264	-0.04	0.207	-0.16	5.07E-06	-0.23	1.60E-12	-0.20	4.32E-09
Facial Aging	-0.07	0.046	0.00	0.969	0.01	0.723	-0.12	0.001	-0.23	4.83E-11	-0.22	2.80E-11	-0.21	8.93E-10

Healthspan-related Characteristics	Telomere Shortness		353-CpG Clock		99-CpG Clock		71-CpG Clock		KDM Biological Age		Age-related Homeostatic Dysregulation		Pace of Aging	
	BMI-adjusted r / p-value													
Information														
Age 38	-0.03	0.399	0.06	0.086	0.05	0.181	-0.10	0.008	-0.07	0.044	-0.14	1.76E-05	-0.16	1.94E-06
Change from Childhood	0.00	0.875	0.05	0.035	0.05	0.039	-0.02	0.450	-0.01	0.777	-0.05	0.063	-0.05	0.071
Similarities														
Age 38	-0.04	0.214	-0.03	0.414	0.00	0.960	-0.14	1.63E-04	-0.12	7.63E-04	-0.17	8.85E-07	-0.16	3.36E-06
Change from Childhood	-0.02	0.420	-0.05	0.091	-0.02	0.613	-0.10	0.001	-0.05	0.085	-0.09	0.001	-0.08	0.007
Vocabulary														
Age 38	-0.04	0.272	0.02	0.570	0.02	0.648	-0.15	6.81E-05	-0.13	3.14E-04	-0.16	3.62E-06	-0.17	1.68E-06
Change from Childhood	-0.01	0.616	-0.01	0.590	0.02	0.476	-0.08	0.002	-0.06	0.025	-0.07	0.004	-0.07	0.011
Digit Symbol Coding														
Age 38	-0.04	0.234	-0.01	0.692	-0.02	0.631	-0.09	0.014	-0.10	2.63E-03	-0.15	2.15E-06	-0.19	1.61E-08
Change from Childhood	0.02	0.504	0.00	0.934	0.02	0.493	-0.03	0.245	-0.09	9.45E-04	-0.12	1.03E-05	-0.15	4.26E-08
Arithmetic														
Age 38	-0.07	0.052	-0.03	0.317	-0.02	0.517	-0.09	0.011	-0.09	0.007	-0.10	2.17E-03	-0.17	4.65E-07
Change from Childhood	-0.02	0.518	-0.03	0.339	-0.03	0.270	-0.02	0.565	-0.05	0.063	-0.03	0.186	-0.09	0.001
Block Design														
Age 38	-0.05	0.131	0.02	0.650	0.01	0.688	-0.10	0.004	-0.16	5.94E-06	-0.14	2.96E-05	-0.15	2.64E-05
Change from Childhood	-0.02	0.488	0.02	0.555	0.02	0.492	-0.02	0.499	-0.07	0.009	-0.05	0.050	-0.07	0.007
Picture Completion														
Age 38	-0.03	0.350	0.03	0.397	0.00	0.957	-0.09	0.018	-0.09	0.009	-0.10	0.003	-0.08	0.016
Change from Childhood	-0.01	0.661	0.01	0.765	-0.01	0.829	-0.07	0.049	-0.05	0.117	-0.06	0.081	-0.04	0.218

Web Table 8. Associations of changes in cross-sectional biological aging measures and Pace of Aging with healthspan-related characteristics and cognitive subtests after adjustment for change in body mass index. Adjustment was made by including change in body-mass index between age 26 and age 38 as a covariate in regressions.

Healthspan-related Characteristics	Telomere Erosion		353-CpG Ticks		99-CpG Ticks		71-CpG Ticks		Pace of Aging	
	BMI-adjusted r / p-value									
Balance	0.02	0.524	-0.03	0.440	0.06	0.111	0.02	0.546	-0.10	5.69E-03
Grip Strength	0.00	0.973	0.00	0.977	0.00	0.954	0.01	0.764	-0.06	0.084
Motor Coordination	-0.02	0.628	-0.05	0.156	0.05	0.217	0.01	0.806	-0.15	4.97E-05
Physical Limitations	-0.01	0.847	-0.05	0.169	-0.02	0.590	-0.04	0.320	-0.10	6.25E-03
IQ at 38	-0.01	0.818	-0.10	0.008	-0.06	0.104	-0.08	0.025	-0.23	1.91E-10
IQ change from childhood	-0.04	0.282	-0.07	0.089	-0.03	0.409	0.01	0.854	-0.16	2.07E-05
Self-rated Health	0.00	0.982	-0.01	0.766	0.01	0.698	-0.08	0.036	-0.21	8.89E-10
Facial Aging	0.02	0.583	0.05	0.166	0.02	0.649	-0.08	0.030	-0.22	1.90E-09

Healthspan-related Characteristics	Telomere Erosion		353-CpG Ticks		99-CpG Ticks		71-CpG Ticks		Pace of Aging	
	BMI-adjusted r / p-value									
Information										
Age 38	-0.01	0.766	-0.05	0.174	-0.05	0.211	-0.10	0.008	-0.16	6.13E-06
Change from Childhood	-0.01	0.705	-0.01	0.690	0.01	0.838	-0.02	0.548	-0.05	0.054
Similarities										
Age 38	-0.01	0.692	-0.05	0.225	-0.04	0.325	-0.07	0.053	-0.19	1.77E-07
Change from Childhood	-0.04	0.235	-0.01	0.648	-0.02	0.534	-0.02	0.468	-0.11	0.000
Vocabulary										
Age 38	0.01	0.741	-0.07	0.075	-0.02	0.673	-0.09	0.022	-0.18	1.11E-06
Change from Childhood	0.00	0.992	-0.02	0.413	0.03	0.319	-0.03	0.294	-0.08	0.005
Digit Symbol Coding										
Age 38	-0.05	0.198	-0.07	0.060	-0.04	0.328	-0.07	0.073	-0.18	1.39E-07
Change from Childhood	-0.02	0.562	-0.02	0.441	-0.02	0.476	-0.03	0.270	-0.15	1.06E-07
Arithmetic										
Age 38	-0.05	0.219	-0.10	0.009	-0.08	0.024	-0.06	0.091	-0.18	9.85E-07
Change from Childhood	-0.04	0.210	-0.04	0.164	-0.08	0.007	0.01	0.620	-0.09	0.002
Block Design										
Age 38	0.02	0.534	-0.05	0.168	-0.06	0.119	-0.03	0.430	-0.12	6.11E-04
Change from Childhood	0.00	0.971	-0.02	0.460	-0.04	0.166	0.02	0.565	-0.04	0.127
Picture Completion										
Age 38	0.05	0.169	-0.03	0.369	-0.02	0.640	-0.02	0.546	-0.08	0.024
Change from Childhood	0.04	0.241	-0.03	0.435	0.00	0.935	0.00	0.932	-0.04	0.240

Web Table 9. Associations of cross-sectional biological aging measures and Pace of Aging with healthspan-related characteristics & cognitive subtests after adjustment for age-in-months. Adjustment was made by including age-in-months as a covariate in regressions.

Healthspan-related Characteristics	Telomere Shortness		353-CpG Clock		99-CpG Clock		71-CpG Clock		KDM Biological Age		Age-related Homeostatic Dysregulation		Pace of Aging	
	Age-in-months-Adjusted r / p-value													
Balance	0.00	0.897	-0.07	0.061	0.00	0.931	-0.08	0.022	-0.22	6.01E-11	-0.19	1.13E-08	-0.16	1.28E-06
Grip Strength	-0.06	0.071	0.00	0.963	-0.05	0.150	-0.05	0.164	-0.20	2.44E-09	-0.05	0.109	-0.07	0.029
Motor Coordination	-0.01	0.687	-0.02	0.541	0.03	0.457	-0.10	0.006	-0.15	5.82E-06	-0.19	2.73E-09	-0.17	1.22E-07
Physical Limitations	0.03	0.401	-0.02	0.656	-0.01	0.675	-0.07	0.043	-0.13	1.63E-04	-0.14	1.51E-05	-0.12	1.36E-04
IQ at 38	-0.06	0.081	-0.02	0.517	-0.02	0.627	-0.16	9.60E-06	-0.18	1.18E-07	-0.21	1.40E-10	-0.23	1.87E-12
IQ change from childhood	0.00	0.967	-0.04	0.287	-0.01	0.727	-0.09	0.014	-0.10	0.005	-0.11	0.001	-0.14	2.88E-05
Self-rated Health	-0.02	0.542	-0.02	0.641	0.03	0.414	-0.07	0.050	-0.22	9.60E-11	-0.28	4.75E-18	-0.25	3.44E-15
Facial Aging	-0.08	0.029	0.02	0.531	0.04	0.296	-0.10	0.004	-0.19	1.40E-08	-0.22	1.11E-11	-0.19	9.17E-10

Healthspan-related Characteristics	Telomere Shortness		353-CpG Clock		99-CpG Clock		71-CpG Clock		KDM Biological Age		Age-related Homeostatic Dysregulation		Pace of Aging	
	Age-in-months-Adjusted r / p-value													
Information														
Age 38	-0.03	0.321	0.05	0.116	0.04	0.251	-0.10	0.005	-0.08	0.013	-0.15	3.50E-06	-0.15	1.57E-06
Change from Childhood	-0.01	0.798	0.05	0.046	0.05	0.046	-0.03	0.304	-0.01	0.561	-0.05	0.049	-0.04	0.095
Similarities														
Age 38	-0.05	0.188	-0.05	0.174	-0.02	0.550	-0.15	2.48E-05	-0.16	2.31E-06	-0.18	5.14E-08	-0.17	2.52E-07
Change from Childhood	-0.02	0.418	-0.06	0.042	-0.03	0.343	-0.11	0.000	-0.07	0.009	-0.09	0.001	-0.08	0.006
Vocabulary														
Age 38	-0.04	0.223	0.00	0.929	0.00	0.977	-0.16	1.35E-05	-0.15	5.63E-06	-0.16	5.00E-07	-0.17	2.64E-07
Change from Childhood	-0.01	0.590	-0.02	0.339	0.01	0.716	-0.09	0.001	-0.07	0.003	-0.07	0.003	-0.07	0.006
Digit Symbol Coding														
Age 38	-0.05	0.156	-0.03	0.416	-0.03	0.417	-0.10	0.004	-0.14	2.16E-05	-0.18	2.37E-08	-0.20	2.62E-10
Change from Childhood	0.01	0.665	0.00	0.861	0.02	0.583	-0.03	0.244	-0.11	4.12E-05	-0.13	4.82E-07	-0.15	1.24E-08
Arithmetic														
Age 38	-0.07	0.034	-0.04	0.253	-0.02	0.482	-0.09	0.009	-0.11	0.001	-0.12	2.97E-04	-0.17	1.48E-07
Change from Childhood	-0.02	0.467	-0.02	0.390	-0.03	0.344	-0.01	0.636	-0.05	0.067	-0.03	0.180	-0.08	0.001
Block Design														
Age 38	-0.05	0.136	0.01	0.727	0.01	0.764	-0.11	0.002	-0.17	5.70E-07	-0.15	6.30E-06	-0.15	6.36E-06
Change from Childhood	-0.01	0.598	0.02	0.365	0.02	0.365	-0.02	0.554	-0.07	0.009	-0.05	0.040	-0.07	0.008
Picture Completion														
Age 38	-0.03	0.332	0.04	0.258	0.01	0.740	-0.08	0.022	-0.09	0.007	-0.10	0.001	-0.09	0.004
Change from Childhood	-0.01	0.660	0.02	0.565	0.00	0.937	-0.06	0.063	-0.06	0.096	-0.06	0.044	-0.05	0.113

Web Table 10. Associations of changes in cross-sectional biological aging measures and Pace of Aging with healthspan-related characteristics and cognitive subtests after adjustment for change in age-in-months between assessments. Adjustment was made by including change in age-in-months index between the age-26 and -38 assessments as a covariate in regressions.

Healthspan-related Characteristics	Telomere Erosion		353-CpG Ticks		99-CpG Ticks		71-CpG Ticks		Pace of Aging	
	Change in age-in-months-adjusted r / p-value									
Balance	0.03	0.397	-0.03	0.381	0.05	0.156	0.02	0.525	-0.15	4.98E-06
Grip Strength	-0.01	0.778	-0.01	0.723	-0.01	0.722	-0.01	0.859	-0.07	0.046
Motor Coordination	0.00	0.938	-0.07	0.084	0.03	0.401	0.01	0.890	-0.16	8.60E-07
Physical Limitations	0.01	0.732	-0.06	0.129	-0.03	0.474	-0.04	0.292	-0.13	9.62E-05
IQ at 38	-0.01	0.836	-0.12	0.002	-0.08	0.046	-0.09	0.012	-0.22	1.86E-11
IQ change from childhood	-0.04	0.309	-0.06	0.094	-0.03	0.365	-0.01	0.884	-0.14	2.29E-05
Self-rated Health	-0.01	0.715	-0.02	0.662	0.03	0.416	-0.07	0.076	-0.25	1.07E-14
Facial Aging	0.02	0.664	0.07	0.055	0.05	0.194	-0.05	0.179	-0.19	7.09E-09

Healthspan-related Characteristics	Telomere Erosion		353-CpG Ticks		99-CpG Ticks		71-CpG Ticks		Pace of Aging	
	Change in age-in-months-adjusted r / p-value									
Information										
Age 38	-0.01	0.879	-0.06	0.099	-0.06	0.133	-0.09	0.012	-0.15	5.33E-06
Change from Childhood	0.00	0.988	-0.02	0.482	0.00	0.998	-0.02	0.524	-0.04	0.066
Similarities										
Age 38	-0.01	0.778	-0.06	0.117	-0.06	0.108	-0.08	0.031	-0.16	4.77E-07
Change from Childhood	-0.03	0.342	-0.02	0.484	-0.04	0.248	-0.03	0.343	-0.08	0.002
Vocabulary										
Age 38	0.02	0.623	-0.10	0.010	-0.05	0.197	-0.11	0.004	-0.17	3.66E-07
Change from Childhood	0.01	0.727	-0.04	0.102	0.00	0.981	-0.05	0.051	-0.08	0.002
Digit Symbol Coding										
Age 38	-0.04	0.279	-0.08	0.032	-0.04	0.297	-0.06	0.074	-0.19	2.53E-09
Change from Childhood	-0.01	0.642	-0.02	0.485	-0.02	0.507	-0.03	0.291	-0.14	6.79E-08
Arithmetic										
Age 38	-0.05	0.209	-0.11	0.004	-0.09	0.014	-0.08	0.038	-0.17	2.84E-07
Change from Childhood	-0.03	0.226	-0.04	0.144	-0.07	0.009	0.00	0.881	-0.08	0.001
Block Design										
Age 38	0.02	0.530	-0.06	0.087	-0.07	0.060	-0.04	0.261	-0.13	5.45E-05
Change from Childhood	-0.01	0.792	-0.01	0.732	-0.03	0.234	0.02	0.570	-0.05	0.040
Picture Completion										
Age 38	0.03	0.432	-0.03	0.408	0.00	0.991	-0.02	0.545	-0.09	0.007
Change from Childhood	0.02	0.599	-0.02	0.578	0.02	0.622	0.00	0.936	-0.05	0.137

Web Table 11. Associations of cross-sectional biological aging measures and Pace of Aging with healthspan-related characteristics & cognitive subtests after adjustment for smoking. Adjustment was made by including the number of cigarettes smoked per day at age 38 years (17) as a covariate in regressions.

Healthspan-related Characteristics	Telomere Shortness		353-CpG Clock		99-CpG Clock		71-CpG Clock		KDM Biological Age		Age-related Homeostatic Dysregulation		Pace of Aging	
	Smoking-Adjusted r / p-value													
Balance	0.00	0.988	-0.09	0.015	-0.04	0.295	-0.08	0.027	-0.19	3.26E-09	-0.15	1.50E-05	-0.12	6.41E-04
Grip Strength	-0.06	0.078	-0.01	0.748	-0.07	0.055	-0.05	0.171	-0.19	1.13E-08	-0.04	0.232	-0.06	0.068
Motor Coordination	-0.01	0.777	-0.03	0.374	0.00	0.892	-0.09	0.016	-0.12	2.33E-04	-0.16	2.40E-06	-0.14	4.96E-05
Physical Limitations	0.03	0.371	-0.03	0.463	-0.03	0.352	-0.07	0.050	-0.12	3.55E-04	-0.12	3.65E-04	-0.10	0.002
IQ at 38	-0.05	0.104	-0.05	0.147	-0.07	0.052	-0.15	2.09E-05	-0.14	1.86E-05	-0.15	4.17E-06	-0.17	1.86E-07
IQ change from childhood	0.01	0.864	-0.06	0.095	-0.05	0.157	-0.08	0.024	-0.06	0.053	-0.07	0.038	-0.09	0.006
Self-rated Health	-0.02	0.650	-0.05	0.140	-0.03	0.430	-0.07	0.042	-0.19	1.88E-09	-0.23	1.42E-12	-0.20	1.02E-09
Facial Aging	-0.07	0.043	-0.03	0.392	-0.04	0.239	-0.12	0.001	-0.19	6.29E-09	-0.16	8.05E-07	-0.14	3.28E-05

Healthspan-related Characteristics	Telomere Shortness		353-CpG Clock		99-CpG Clock		71-CpG Clock		KDM Biological Age		Age-related Homeostatic Dysregulation		Pace of Aging	
	Smoking-adjusted r / p-value													
Information														
Age 38	-0.03	0.385	0.03	0.327	0.00	0.979	-0.09	0.012	-0.04	0.169	-0.10	3.58E-03	-0.10	2.47E-03
Change from Childhood	-0.01	0.840	0.04	0.084	0.04	0.163	-0.02	0.468	0.01	0.724	-0.02	0.364	-0.01	0.583
Similarities														
Age 38	-0.04	0.224	-0.06	0.077	-0.05	0.133	-0.13	1.61E-04	-0.11	8.26E-04	-0.13	1.43E-04	-0.12	4.98E-04
Change from Childhood	-0.02	0.464	-0.07	0.021	-0.05	0.103	-0.09	0.002	-0.04	0.149	-0.06	0.033	-0.04	0.134
Vocabulary														
Age 38	-0.04	0.283	-0.01	0.682	-0.04	0.311	-0.14	6.86E-05	-0.11	1.27E-03	-0.11	8.60E-04	-0.11	6.62E-04
Change from Childhood	-0.01	0.647	-0.03	0.249	0.00	0.890	-0.08	0.002	-0.05	0.056	-0.05	0.058	-0.04	0.119
Digit Symbol Coding														
Age 38	-0.04	0.187	-0.05	0.181	-0.06	0.073	-0.09	0.008	-0.11	7.84E-04	-0.13	3.88E-05	-0.16	9.30E-07
Change from Childhood	0.01	0.608	-0.02	0.481	-0.01	0.692	-0.03	0.319	-0.08	1.09E-03	-0.10	2.38E-04	-0.12	1.22E-05
Arithmetic														
Age 38	-0.07	0.042	-0.06	0.097	-0.06	0.109	-0.09	0.013	-0.09	0.005	-0.08	1.46E-02	-0.14	3.79E-05
Change from Childhood	-0.02	0.485	-0.03	0.224	-0.04	0.126	-0.01	0.651	-0.04	0.104	-0.02	0.453	-0.07	0.009
Block Design														
Age 38	-0.05	0.166	-0.01	0.829	-0.02	0.499	-0.11	0.003	-0.15	5.86E-06	-0.11	5.83E-04	-0.11	6.84E-04
Change from Childhood	-0.01	0.631	0.01	0.666	0.01	0.850	-0.02	0.491	-0.06	0.014	-0.04	0.162	-0.05	0.049
Picture Completion														
Age 38	-0.03	0.396	0.02	0.637	-0.02	0.492	-0.09	0.015	-0.09	0.009	-0.08	0.017	-0.07	0.048
Change from Childhood	-0.01	0.701	0.00	0.912	-0.02	0.537	-0.07	0.046	-0.05	0.108	-0.05	0.136	-0.03	0.330

Web Table 12. Associations of cross-sectional biological aging measures and Pace of Aging with healthspan-related characteristics & cognitive subtests after adjustment for socioeconomic status. Adjustment was made by including socioeconomic status at age 38 years as a covariate in regressions. Socioeconomic status was measured using the New Zealand Socioeconomic Index (18,19).

	Telomere Shortness		353-CpG Clock		99-CpG Clock		71-CpG Clock		KDM Biological Age		Age-related Homeostatic Dysregulation		Pace of Aging	
Healthspan-related Characteristics	**Socioeconomic Status-Adjusted r / p-value**													
Balance	0.00	0.971	-0.07	0.030	-0.02	0.640	-0.06	0.073	-0.19	5.71E-09	-0.15	4.42E-06	-0.12	1.53E-04
Grip Strength	-0.06	0.079	0.00	0.904	-0.05	0.134	-0.05	0.147	-0.19	7.54E-09	-0.05	0.131	-0.07	0.037
Motor Coordination	0.00	0.888	-0.02	0.475	0.02	0.581	-0.06	0.080	-0.10	1.55E-03	-0.14	2.35E-05	-0.12	2.12E-04
Physical Limitations	0.03	0.377	-0.02	0.571	-0.02	0.593	-0.07	0.051	-0.12	3.32E-04	-0.13	6.53E-05	-0.12	5.11E-04
IQ at 38	-0.05	0.126	-0.04	0.146	-0.04	0.151	-0.11	5.20E-04	-0.10	7.31E-04	-0.10	6.54E-04	-0.14	2.77E-06
IQ change from childhood	0.01	0.861	-0.05	0.194	-0.02	0.479	-0.07	0.066	-0.06	0.081	-0.07	0.033	-0.10	0.003
Self-rated Health	-0.02	0.641	-0.03	0.310	0.01	0.823	-0.06	0.080	-0.19	2.91E-09	-0.24	8.59E-14	-0.22	1.19E-11
Facial Aging	-0.07	0.050	-0.01	0.827	0.00	0.964	-0.10	0.004	-0.19	5.07E-09	-0.19	1.45E-08	-0.16	5.22E-07

	Telomere Shortness		353-CpG Clock		99-CpG Clock		71-CpG Clock		KDM Biological Age		Age-related Homeostatic Dysregulation		Pace of Aging	
Healthspan-related Characteristics	**Socioeconomic Status-adjusted r / p-value**													
Information														
Age 38	-0.02	0.477	0.04	0.205	0.02	0.496	-0.05	0.092	-0.01	0.645	-0.06	5.16E-02	-0.08	0.009
Change from Childhood	-0.01	0.790	0.05	0.045	0.05	0.056	-0.01	0.595	0.01	0.705	-0.03	0.288	-0.03	0.296
Similarities														
Age 38	-0.04	0.254	-0.06	0.083	-0.03	0.300	-0.10	2.76E-03	-0.08	1.18E-02	-0.09	4.31E-03	-0.09	0.003
Change from Childhood	-0.02	0.407	-0.06	0.038	-0.03	0.270	-0.08	0.005	-0.04	0.172	-0.06	0.039	-0.05	0.082
Vocabulary														
Age 38	-0.03	0.340	-0.01	0.790	-0.01	0.670	-0.11	1.17E-03	-0.07	1.81E-02	-0.07	1.59E-02	-0.09	0.003
Change from Childhood	-0.01	0.573	-0.02	0.380	0.01	0.763	-0.07	0.005	-0.04	0.071	-0.05	0.051	-0.05	0.050
Digit Symbol Coding														
Age 38	-0.04	0.238	-0.04	0.214	-0.04	0.172	-0.07	0.050	-0.08	6.57E-03	-0.10	6.66E-04	-0.14	6.13E-06
Change from Childhood	0.01	0.668	-0.01	0.628	0.00	0.948	-0.02	0.487	-0.08	2.13E-03	-0.09	2.35E-04	-0.12	5.01E-06
Arithmetic														
Age 38	-0.06	0.051	-0.06	0.085	-0.05	0.161	-0.06	0.056	-0.06	0.042	-0.05	1.46E-01	-0.11	5.09E-04
Change from Childhood	-0.02	0.455	-0.03	0.264	-0.03	0.229	-0.01	0.607	-0.04	0.097	-0.03	0.299	-0.07	0.004
Block Design														
Age 38	-0.04	0.209	0.00	0.940	-0.01	0.822	-0.08	0.014	-0.13	8.41E-05	-0.09	6.86E-03	-0.09	0.003
Change from Childhood	-0.01	0.647	0.02	0.503	0.02	0.547	-0.02	0.562	-0.06	0.012	-0.04	0.121	-0.06	0.029
Picture Completion														
Age 38	-0.02	0.475	0.02	0.523	-0.01	0.806	-0.07	0.042	-0.07	0.030	-0.06	0.051	-0.06	0.068
Change from Childhood	-0.01	0.742	0.01	0.801	-0.01	0.743	-0.06	0.067	-0.05	0.145	-0.04	0.187	-0.03	0.298

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