Epigenetics Communications

RESEARCH

Open Access

# Connections between cross-tissue and intra-tissue biomarkers of aging biology in older adults

R. Waziry[1*], Y. Gu[1,2,3,4], O. Williams[1] and S. Hägg[5]

## Abstract

**Background** Saliva measures are generally more accessible than blood, especially in vulnerable populations. However, connections between aging biology biomarkers in different body tissues remain unknown.

**Methods** The present study included individuals ($N=2406$) who consented for saliva and blood draw in the Health and Retirement Telomere length study in 2008 and the Venous blood study in 2016 who had complete data for both tissues. We assessed biological aging based on telomere length in saliva and DNA methylation and physiology measures in blood. DNA methylation clocks combine information from CpGs to produce the aging measures representative of epigenetic aging in humans. We analyzed DNA methylation clocks proposed by Horvath (353 CpG sites), Hannum (71 CpG sites), Levine or PhenoAge, (513 CpG sites), GrimAge, (epigenetic surrogate markers for select plasma proteins), Horvath skin and blood (391 CpG sites), Lin (99 CpG sites), Weidner (3 CpG sites), and VidalBralo (8 CpG sites). Physiology measures (referred to as phenotypic age) included albumin, creatinine, glucose, [log] C-reactive protein, lymphocyte percent, mean cell volume, red blood cell distribution width, alkaline phosphatase, and white blood cell count. The phenotypic age algorithm is based on parametrization of Gompertz proportional hazard models. Average telomere length was assayed using quantitative PCR (qPCR) by comparing the telomere sequence copy number in each patient's sample (T) to a single-copy gene copy number (S). The resulting T/S ratio was proportional to telomere length, mean. Within individual, relationships between aging biology measures in blood and saliva and variations according to sex were assessed.

**Results** Saliva-based telomere length showed inverse associations with both physiology-based and DNA methylation-based aging biology biomarkers in blood. Longer saliva-based telomere length was associated with 1 to 4 years slower biological aging based on blood-based biomarkers with the highest magnitude being Weidner ($\beta=-3.97$, $P=0.005$), GrimAge ($\beta=-3.33$, $P<0.001$), and Lin ($\beta=-3.45$, $P=0.008$) biomarkers of DNA methylation.

**Conclusions** There are strong connections between aging biology biomarkers in saliva and blood in older adults. Changes in telomere length vary with changes in DNA methylation and physiology biomarkers of aging biology. We observed variations in the relationship between each body system represented by physiology biomarkers and biological aging, particularly at the DNA methylation level. These observations provide novel opportunities for integration of both blood-based and saliva-based biomarkers in clinical care of vulnerable and clinically difficult to reach populations where either or both tissues would be accessible for clinical monitoring purposes.

*Correspondence:
R. Waziry
rw2872@cumc.columbia.edu; reemwaziry@gmail.com
Full list of author information is available at the end of the article

© The Author(s) 2023. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated in a credit line to the data.

## Introduction

Aging is hypothesized to be a key driver of major age-related pathologies and vascular disease [1–3]. Measures of aging biology have been proposed as a proxy to the global aging status of an individual [4]. Different tissues are commonly used, including saliva and blood derivatives, to measure biological aging. Blood tissues used include venous versus capillary and whole blood versus point of care and other blood products such as plasma [5]. Saliva is considered one of the most accessible body tissues and have been effectively used in clinically difficult populations, among whom obtaining blood access is not feasible [6]. Clinical uses of saliva include monitoring drug doses of cardiovascular and neurological disorders such as diabetes mellitus, multiple sclerosis, and epilepsy [6]. It has proven economic efficacy due to its easier access and the need for less materials and time to obtain, ship, and store samples [7, 8].

There are variations in concentration and abundance of molecular analytes between saliva and blood [9]. These established differences would prompt the use of either tissue according to the targeted investigation, age group, or endpoint being assessed [10]. Differences in abundance may therefore directly affect the precision of what is measured and may translate in differences in predicting risk of complications [10–12]. Similarly, differences between blood and saliva include variations according to age, sex, type and size of salivary gland, blood type, and physiological status, which are more likely to influence salivary tissue content [13, 14].

The precise differences between those measures would provide opportunities for more effective diagnostic and prognostic tools in clinical medicine and would open avenues for interventions at a whole population scale as cost-effective screening and monitoring tools become available in the near future [14]. In the present study, we aimed to assess connections between cross-tissue (i.e., saliva-blood) and intra-tissue (i.e., blood-blood) aging biomarkers to evaluate whether saliva-based telomere length biomarkers of aging biology reflect changes in blood-based DNA methylation and physiology biomarkers in older adults.

## Methods

### Study population

The Health and Retirement Study (HRS) is a nationally representative longitudinal survey that recruited more than 37,000 individuals aged 50 and older in the USA. The survey has been conducted every 2 years since 1992 with a focus on issues related to changes in health and economic circumstances in aging at both the individual and population levels [15]. HRS data are linked to records from Social Security, Medicare, Veteran's Administration, the National Death Index, and employer-provided pension plan information. Genetic ancestry in HRS is identified through PC analysis on genome-wide SNPs [15]. HRS is coordinated by the Institute for Social Research at the University of Michigan. The present study included individuals who consented for saliva and blood draw in the health and retirement telomere length study in 2008 and the venous blood study in 2016 respectively and who had complete data for both tissues ($N=2406$) [15–17]. Individuals without data on telomere length, physiology, and DNA methylation measures were not included in the present investigation to allow for direct individual comparisons using blood-based biomarkers and saliva-based biomarkers. The included sample had similar overall age and sex distribution to the full sample with weighted mean age equals 67 and 54% females. The Health and Retirement Study is reviewed and approved by the University of Michigan's Health Sciences IRB. The present study has been conducted in accordance with STROBE guidelines for observational studies (https://www.strobe-statement.org/).

### Biological aging biomarkers

We assessed biological aging based on (A) telomere length, (B) DNA methylation, and (C) physiology measures.

#### *(A) Telomere length measurement*

Telomere length data were available from 5808 HRS respondents who consented for saliva sample draw during the 2008 interview wave. Assays were performed by Telome Health (Telomere Diagnostics, http://www.telomehealth.com/). Average telomere length was assayed using quantitative PCR (qPCR) by comparing telomere sequence copy number in each patient's sample (T) to a single-copy gene copy number (S). The resulting T/S ratio was proportional to telomere length, mean. The HRS *2008 Telomere* data set is sponsored by the National Institute on Aging (NIH NIAU01AG009740 and RC4 AG039029) and was conducted by the University of Michigan [17].

*The venous blood substudy (VBS) and assay protocol in 2016 Health and Retirement Study* All respondents, with the exception of proxy and nursing home respondents, who completed the HRS interview in 2016 (visit 13) were asked to consent for blood draw (78.5% of participants interviewed through September 5, 2017) [16]. Physiology-based biomarkers were assessed on the whole sample of participants who consented for blood draw, while DNA methylation was assessed in a subsample randomly selected and fully representative of the whole sample [16]. Blood samples were centrifuged and

shipped overnight to CLIA-certified Advanced Research and Diagnostic Laboratory at the University of Minnesota. Tube processing was done within 24 h of arrival at the lab (within 48 h of collection). All assays were done at the University of MN Advanced Research and Diagnostic Laboratory (ARDL) under the direction of Bharat Thyagarajan.

#### *(B) DNA methylation*

DNA methylation assays were done by Infinium Methylation EPIC BeadChip at the University of Minnesota following the manufacturer's instruction. The minfi package in R was used in data processing, and DNA methylation measures were provided through HRS to the research community [18, 19]. Epigenetic clocks are commonly based on portions of the genome where methylation changes are related to chronological age and, more recently, health outcomes [18]. The clocks combine information from CpGs to produce the aging measure that represents an indicator of epigenetic aging [18]. DNA methylation assessment included the following clocks: Horvath [20, 21] and Hannum et al. [22], Levine et al. or PhenoAge [23], Lu et al. (referred to as GrimAge) [24], Horvath skin and blood [25], Lin [26–28], Weidner [28], and VidalBralo [29]. The Horvath epigenetic clock predicts age using 353 CpG sites in the DNA methylation profile and incorporates 51 healthy tissues and cell types [20, 21]. The Hannum et al. clock comprises 71 CpG sites selected from the Illumina 450 k array that capture changes in chronological age. It was developed in whole blood of humans at ages 19 to 101 [22]. DNAm PhenoAge based on 513 CpGs predicted phenotypic age in whole blood from the same sample [23]. GrimAge is a mortality predictor and was constructed based on surrogate markers for select plasma proteins ((adrenomedullin, β-2-microglobulin, CD56, ceruloplasmin, cystatin C, EGF fibulin-like ECM protein 1, growth differentiation factor 15, leptin, myoglobin, plasminogen activator inhibitor 1, serum paraoxonase/arylesterase 1, and tissue inhibitor metalloproteinases 1) and smoking pack-years in a two-stage procedure [24].GrimAge is suggested to have predictive ability for time to death, coronary heart disease, cancer, and age-related conditions [24]. Skin and blood was developed as a novel and highly robust DNAm age estimator (based on 391 CpGs) for human fibroblasts, keratinocytes, buccal cells, endothelial cells, lymphoblastoid cells, skin, blood, and saliva samples [25]. The clock is suggested to have high age correlations in sorted neurons, glia, brain, liver, and bone samples [25]. The skin and blood clock shares 45 CpGs with blood-based Hannum and 60 CpGs with Horvath pan-tissue clock [21, 22]. Lin was developed using a 99-CpG aging model derived in DNAm profiles of normal blood samples and trained on life expectancy [26–28].Weidner was developed based on three age-related CpGs located in the genes ITGA2B, ASPA, and PDE4C to estimate epigenetic aging in blood [28]. VidalBralo was developed in whole blood of 8 CpG sites that were selected as the most informative CpGs in a training dataset of 390 healthy subjects and validated in three datasets [29].

The chronological classification of the clocks can be summarized as follows: the first-generation clocks were developed using machine learning to predict chronological age. These clocks demonstrated two important proofs of concept; they recorded increases in clock age within individuals as they grew older [30, 31], and more advanced clock-age estimates (i.e., clock ages older than chronological age) were associated with increased mortality risk among individuals of the same chronological age [23]. Second-generation DNAm clocks were developed from analysis of mortality risk, incorporating information from DNAm prediction of physiological parameters [23, 24]. These second-generation clocks are more predictive of morbidity and mortality [32] and are proposed to have improved potential for testing impacts of interventions to slow aging [33].

#### *(C) Physiology measures*

Among available and validated measures of biological aging based on physiologic parameters, we opted to use physiology-based phenotypic age described in detail by Liu et al. [34]. This clock uses the following nine biomarkers assessed in blood representing the different body systems: albumin, creatinine, glucose, [log], C-reactive protein [CRP], lymphocyte percent, mean cell volume, red blood cell distribution width, alkaline phosphatase, and white blood cell count [35]. These biomarkers were selected using a Cox proportional hazard elastic net model for mortality. The phenotypic age algorithm is based on parametrization of two Gompertz proportional hazard models, one including the 10 selected variables and the other including only chronological age [34, 36].

### Statistical analysis

Summary statistics were assessed as median (IQR) for continuous variables and percentages for categorical variables. Survey weights were used in descriptive analysis [37, 38]. Correlation coefficients (Pearson) between cross-tissue (saliva-blood) and intra-tissue (blood-blood) measures were assessed for magnitude, direction, and statistical significance with correction for multiple comparison [39, 40]. We have also assessed correlations between each physiological biomarker with telomere length and DNA methylation biological aging measures. Linear regression models were conducted with DNA methylation or physiology-based biological aging

measure as the dependent variable. Blood measures were used in the raw form to allow for direct comparisons. Telomere length was logged and included in the models as tertiles [41]. Tertiles were used to allow for interpretable change per unit increase in telomere length. Models were adjusted for sex, sex and telomere length interaction, and chronological age difference in years between time at saliva and blood draws. These covariates were determined a priori with emphasis on sex and chronological age for their established biological relevance and data completeness [35, 42]. Inclusion of sex and telomere length interaction term was determined a priori based on hypothesized sex-based differences in telomeres [42–45]. Sex-stratified models adjusted for chronological age difference in years between time at saliva and blood draws were also conducted. Sensitivity analysis was conducted to assess the models after exclusion of telomere length T/S ratio greater than 2.0 since greater values are more likely to be artificial in salivary samples [46]. Models were also assessed without sex and telomere length interaction term. All analyses were conducted using Stata SE V.16.0.

## Results

### Population characteristics

A total of 2406 individuals had complete data on salivary telomere length and physiology-based measurements, and 1029 had complete data on salivary telomere length, physiology-based, and DNA-based measurements. Median age for the study sample at the time when saliva was drawn was 66 years (*IQR* 59, 72), median age at time of blood draw was 74 (*IQR* 67, 80), the majority were females (60%), and African Americans comprised 11% of the sample, while the majority were Whites (77%). In terms of self-reported health, respondents who reported "very good" and "good" represented 34% and 31%, respectively. Median time difference between saliva and blood draw was 8 years (*IQR* 8, 8.3) (Table 1, Supplementary Table 1). For saliva-based telomere length, median telomere length was 1.30 (*IQR* 1.14 and 1.50). For blood-based measures of DNA methylation, median biological ages were as follows: Horvath 69.00, Hannum 57.80, Levine 60.70, skin and blood 73.54, Lin 61.60, Weidner 67.43, VidalBralo 65.15, and GrimAge 71.30. Median age for physiology-based biological aging, phenotypic age, was 74.41 (Table 1). Weighted summary for variables included is described in Supplementary Table 1.

**Table 1** Detailed sample characteristics

| Characteristic | Summary (unweighted) |
|---|---|
| *N* | 2406 |
| Chronological age (years), median (IQR)[a] | 66 (59, 72) |
| Female, % | 1449 (60) |
| Time difference, median (IQR)[b] | 8 (8, 8.3) |
| Race | |
| African American, *N* (%) | 270 (11) |
| Self-reported health | |
| Excellent | 350 (15) |
| Very good | 815 (34) |
| Good | 732 (31) |
| Fair | 359 (15) |
| Poor | 98 (4) |
| **Biomarkers**[c] | |
| Saliva based | |
| Telomere length, median (IQR) | 1.30 (1.14, 1.50) |
| Blood-based physiological measure | |
| Phenotypic age, median (IQR) | 74.41 (65.6, 83.55) |
| Blood-based DNA methylation measures[d] | |
| Chronological age (years), median (IQR)[e] | 74 (67, 80) |
| Horvath, median (IQR) | 69.00 (63.00, 75.00) |
| Hannum, median (IQR) | 57.80 (52.06, 63.55) |
| Levine, median (IQR) | 60.70 (54.41, 66.84) |
| Skin & blood, median (IQR) | 73.54 (67.70, 78.25) |
| Lin, median (IQR) | 61.61 (55.09, 68.85) |
| Weidner, median (IQR) | 67.43 (60.90, 76.82) |
| VidalBralo, median (IQR) | 65.15 (61.67, 69.00) |
| GrimAge, median (IQR) | 71.30 (65.64, 77.25) |

[a] Chronological age at time of telomere assessment. [b]Time difference represents time years between saliva and blood draw. [c]Biological aging measures are scaled per 1-year change; [d]$n = 1,029$ had complete data on salivary telomere length, physiology based, and DNA methylation measures. [e]Chronological age at time of blood draw assessment

### Connections between cross-tissue (saliva–blood) biomarkers of biological aging

There was an inverse relationship between saliva-based telomere length and all blood-based measures, such that longer telomeres reflected younger biological aging. Measures that showed statistically significant correlation coefficients included Lin (−0.067, $P = 0.031$) and Weidner (−0.064, $P = 0.037$) (Table 2).

In tertile analysis of saliva-based telomere length, compared to the lowest tertile, longer saliva-based telomere length was associated with 1 to 4 years slower biological aging based on blood-based biomarkers with the highest magnitude being Weidner ($\beta = -3.97$, $P = 0.005$), GrimAge ($\beta = -3.33$, $P < 0.001$), and Lin ($\beta = -3.45$, $P = 0.008$) biomarkers of DNA methylation. Models were adjusted for sex, sex and telomere length interaction, and time difference between saliva and blood draw. Similar results were observed after exclusion of telomere length T/S ratio greater than 2 (Table 3, Supplementary Table 2). Models without the sex-telomere length interaction term showed highest magnitude with Lin (reference lowest tertile, $\beta = -3.69$, $P < 0.001$) and phenotypic age ($\beta = -3.14$, $P < 0.001$). Sex-stratified models showed

**Table 2** Correlation coefficients of the relationship between (1) cross-tissue biomarkers of biological aging and (2) intra-tissue biomarkers of biological aging

| | Cross-tissue biomarkers | | Intra-tissue biomarkers | |
|---|---|---|---|---|
| | Telomere length (saliva based) | | Phenotypic age (blood based) | |
| Biomarkers (blood based) | Correlation coefficient | *p*-value | Correlation coefficient | *p*-value |
| Phenotypic age | −0.0264 | 0.1959 | 1.00 | – |
| Horvath | −0.0452 | 0.1478 | 0.5776 | < 0.001* |
| Hannum | −0.0318 | 0.3082 | 0.6896 | < 0.001* |
| Levine | −0.0320 | 0.3056 | 0.6525 | < 0.001* |
| Skin & blood | −0.0367 | 0.2391 | 0.6640 | < 0.001* |
| Lin | −0.0670 | 0.0317 | 0.5839 | < 0.001* |
| Weidner | −0.0647 | 0.0379 | 0.3229 | < 0.001* |
| VidalBralo | −0.0529 | 0.0897 | 0.5204 | < 0.001* |
| GrimAge | −0.0438 | 0.1608 | 0.7498 | < 0.001* |

* Denotes a significant *P*-value with Bonferroni correction [40]

variations in effect estimates across measures of biological aging in blood between males and females with every tertile increase in telomere length (Fig. 1).

Estimates varied widely across the nine physiological biomarkers with DNA methylation measures with the highest correlation observed with creatinine and lymphocyte percent (Supplementary Tables 3 and 4).

### Connections between intra-tissue (blood–blood) biomarkers of biological aging

In a secondary analysis, all blood-based biomarkers of DNA methylation showed directly proportional and strong statistically significant relationships with blood-based physiology measures of biological aging. Among blood-based DNA methylation biomarkers, GrimAge showed the strongest correlation with blood-based physiological measure, phenotypic age (correlation coefficient = 0.75, $P < 0.001$) followed by Hannum (0.68, $P < 0.001$), and Horvath skin and blood measure (0.66, $P < 0.001$) (Table 2).

## Discussion

In the present study, we found that increases in telomere length measured in saliva was reflected in younger biological age based on DNA methylation and physiology measured in blood ($P < 0.001$) 8 years later. Such that one tertile increase in telomere length in saliva translated to approximately 4 years younger biological age in blood measures, with some variations between males and females, in a representative population of older adults, despite relatively weak correlations between cross-tissue measures compared to intra-tissue measures in blood. We also observed correlations between physiological biomarkers, creatinine, and lymphocyte percent in particular, with DNA methylation-based biological aging, suggesting potentially prominent role of kidney and immunity functions on accelerating biological aging. In contrast, alkaline phosphatase showed little correlation with biological aging measures.

Common biomarkers used in aging investigations include telomere length, physiological measures, and epigenetic clocks [4, 47, 48]. At the molecular level, biological aging changes have been translated to several domains including telomere attrition, epigenetic alterations, genomic instability, mitochondrial dysfunction,

**Table 3** Cross-tissue comparisons between blood-based biomarkers of biological aging (outcome variable) and (log) saliva-based telomere length (with lowest tertile as reference)

| Biomarkers[a] | β | 95% *CI* | *p*-value |
|---|---|---|---|
| Physiological measure, phenotypic age | – | | |
| 2nd tertile | −2.30 | −4.30, −0.30 | 0.024 |
| 3rd tertile | −2.38 | −4.37, −0.38 | 0.019 |
| Horvath | | | |
| 2nd tertile | −1.10 | −3.27, 1.07 | 0.320 |
| 3rd tertile | −1.55 | −3.74, 0.63 | 0.163 |
| Hannum | | | |
| 2nd tertile | −1.61 | −3.59, 0.36 | 0.109 |
| 3rd tertile | −2.40 | −4.39, −0.40 | 0.018 |
| Levine | | | |
| 2nd tertile | −1.10 | −3.35, 1.14 | 0.335 |
| 3rd tertile | −2.45 | −4.71, −0.19 | 0.033 |
| Skin & blood | | | |
| 2nd tertile | −0.99 | −2.88, 0.88 | 0.298 |
| 3rd tertile | −1.91 | −3.81, −0.02 | 0.047 |
| Lin | | | |
| 2nd tertile | 0.12 | −2.40, 2.65 | 0.921 |
| 3rd tertile | −3.45 | −6.00, −0.91 | 0.008 |
| Weidner | | | |
| 2nd tertile | −1.27 | −4.05, 1.50 | 0.369 |
| 3rd tertile | −3.97 | −6.76, −1.17 | 0.005 |
| VidalBralo | | | |
| 2nd tertile | −1.49 | −2.93, −0.06 | 0.040 |
| 3rd tertile | −2.11 | −3.55, −0.67 | 0.004 |
| GrimAge | | | |
| 2nd tertile | −1.75 | −3.56, 0.05 | 0.057 |
| 3rd tertile | −3.33 | −5.15, −1.51 | < 0.001 |

[a] Measures per tertile increase in telomere length with lowest tertile as reference; models were adjusted for sex, sex and telomere length interaction, and time difference between saliva and blood draw

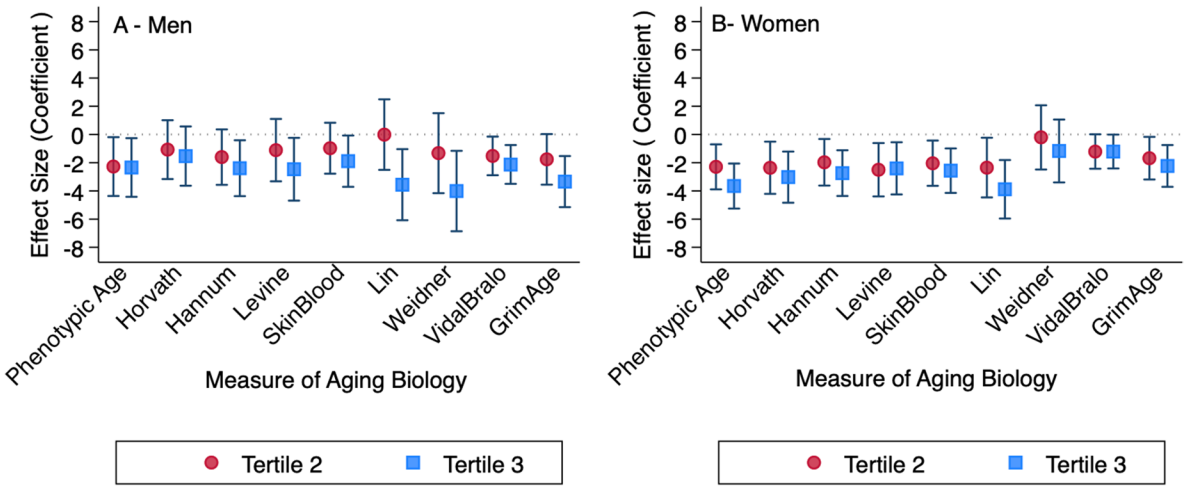


**Fig. 1** Relationship between biological age measures in blood and telomere length (tertiles)

cellular senescence, stem cell exhaustion, and altered intercellular communication [4, 49]. An important goal of measuring aging is to identify both modifiable targets and informative surrogate endpoints that can be used to track effects of aging interventions in humans [50, 51]. There are several qualities that have to be recognized in aging measures to allow optimal function and replication [50]. Reliability, sensitivity to changes in aging, and feasibility in terms of access and costs are among the most important qualities that make an aging metric widely implemented and accordingly optimize its development and generalizability [50, 52]. Existing evidence supports associations between telomere length and global DNA methylation in youth, which are suggested to affect genome stability and disease risk susceptability [53]. In addition, DNA methylation-based estimator of telomere length is suggested to be more strongly related to age compared to measured telomere length [54].

Saliva-based biomarkers are likely to provide real-time reflections of the individual's health at the time of collection [14]. In addition to their highly economic advantages and accessibility, saliva is generally a preferred means for populations and age groups among whom a blood draw is not accessible and in clinically difficult situations [6]. The ease of access however should be considered alongside factors that directly affect the composition and molecular abundance in saliva tissue. Saliva is secreted in response to sympathetic and parasympathetic stimuli, and nervous stimuli variations directly affect saliva characteristics in terms of volume, viscosity, protein, and mucin concentrations. There are suggestions that telomere length may not be the ultimate biological aging measure; in addition, qPCR may be less optimal compared to other methods [3, 55, 56]. For DNA methylation measured in saliva, recent evidence suggests that variations seen across cell types, compared to within a cell type, are likely due to variations in immune cell contamination [33, 57]. Moreover, saliva composition could be affected by medications use, age, circadian rhythms among other individual-specific exposures, and conditions such as diabetes, multiple sclerosis, liver conditions, and infectious diseases [6, 58, 59]. Therefore, it is important to consider tissue type and method of measurement in biological aging investigations. Recent data have also shown the utility of measuring DNA methylation in saliva and buccal cells with amounts as low as 10 ng of genomic DNA producing reproducible results [60–62].

In terms of blood-based biomarkers, in the present study, we used DNA methylation and physiology-based measures of aging biology. DNA methylation is the mechanism by which a methyl (CH3) group is added to DNA resulting in modification of genetic function without changes to DNA sequence. This process regulates gene expression and therefore plays an important role in human development and disease [63]. DNA methylation is suggested to be both tissue and disease specific. Different tissue types have been observed to show strikingly varying epigenetic profiles [64]. Commonly used tissues include blood and skin as illustrated in the present investigation [25]. In addition, differences in DNA methylation are suggested to be also tissue-state specific. For example, genome-wide analysis of DNA methylation in the right coronary artery has shown differences in DNA methylation patterns in areas with advanced atherosclerosis compared to areas that are atherosclerotic resistant and the great saphenous vein obtained from same patients [65]. Tissue-based differences in DNA methylation could

provide opportunities for novel therapeutic targets and tissue areas related to variations in response to treatment [64, 65]. However, DNA methylation remains to be challenged by cost-related barriers and limited consistency in the measurements as there is no gold standard to define the optimal genome sites and methods of measurements in humans [66, 67]. Therefore, comprehensive comparisons provide a rigorous and reproducible way to capture biological aging effects. Prior evidence suggests that a substantial proportion of data points do not yield the equivalent value when re-quantified from the same DNA sample; furthermore, repeated measures are crucial to uncover consistent replicable signals of DNA methylation dynamics across important variables including time, between populations, and between exposures [68]. That said, cross-tissue variability in DNA methylation profiles has been suggested to be more concordant across tissues than gene expression changes across tissues with age [21, 28]. Physiology-based measures largely represent composites of blood-based analytes that represent the various body domains that change with aging [1]. In the present study, we opted to use phenotypic age, representing parameters that have been validated as predictors of aging in comparable settings including albumin, creatinine, glucose, [log] C-reactive protein [CRP], lymphocyte percent, mean cell volume, red blood cell distribution width, alkaline phosphatase, and white blood cell count [34]. A core motivation to using phenotypes of physiological biomarkers is that one biomarker might not be sufficient to delineate the underlying pathogenesis; therefore, combinations of biomarkers provide an added value over single biomarkers and hence more powerful diagnostic and prognostic tools [69, 70].

The wide variations in the relationships between each physiology biomarker individually with DNA methylation-based biological aging measures suggest differences in how each body system represented by physiology biomarkers interacts with biological aging at the DNA methylation level. For example, creatinine, a biomarker of kidney function, and lymphocyte percent, a blood and immunity biomarker, showed strong positive and negative correlations respectively with some of the DNA methylation-based biological aging measures that exceeded their correlation with chronological age in the same sample [71–75]. These strong correlations suggest potent role of the renal and immunity systems in accelerating biological aging.

There are several limitations in the present study. The biomarkers were measured at two different time points. This time difference could potentially introduce imprecision through impacting the aging profiles across time and power; however, time differences were adjusted for across all the models. In addition, measures of biological aging other than telomere length were not available in saliva; however, the sample was restricted to individuals with complete information for the three measures and therefore allowed for within-individual comparisons. Moreover, biological aging measures are likely complementary to each other rather than alternative exclusive tools since they capture different aspects of the aging process [4, 49]. Our study was also limited in the number of measurements on each individual; therefore, we were not able to assess changes in biomarkers over a lengthy period of time. Repeated measures however would mainly serve as a confirmatory step and might not be feasible to assess over long durations for the same population. Lastly, age ranged between 59 and 72 in the present investigation, and therefore, the results cannot be extrapolated to other age groups and younger individuals who joined HRS after 2008 [15].

Collectively, differences in methods of measurement, cost, accessibility, and generalizability between saliva and blood-based measures determine their applicability in large-scale investigations and the feasibility of their integration in clinical practice [14, 76, 77]. It is also important to consider that blood and saliva secretions are interconnected in many ways, presumably a drug circulating in blood passes through capillary wall, the basement membrane, and glandular epithelial cells prior to being secreted into the salivary duct. This plasma-salivary interchange is bound by active and passive processes and an ultrafiltration step. Drug- or compound-specific characteristics also play a role in this process [6, 14]. Therefore, uncovering connections between both tissues represent an important step towards optimization of accessibility for integration in clinical practice and wide-scale investigations.

## Conclusions

Results of the present investigation suggest strong connections between aging biology biomarkers in saliva and blood in older adults. We observed variations in the relationship between each body system represented by physiology biomarkers and biological aging, particularly at the DNA methylation level. These observations could provide novel opportunities for integration of both blood-based and saliva-based biomarkers in clinical care of vulnerable and clinically difficult to reach populations where either or both tissues would be accessible for clinical monitoring purposes.

## Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s43682-023-00022-4.

**Additional file 1: Supplementary Table 1.** Weighted population characteristics. **Supplementary Table 2.** Cross-tissue comparisons between blood-based biomarkers of biological aging (outcome variable) and (log) saliva-based telomere length (with lowest tertile as reference and after exclusion of Telomere Length greater than 2). **Supplementary Table 3.** Summary of Physiology Biomarkers. **Supplementary Table 4.** Correlation coefficients of the relationship between physiology, saliva-based telomere length and DNA methylation biomarkers.

**Acknowledgements**
The HRS 2008 telomere and HRS venous blood studies are sponsored by the National Institute on Aging (grant number NIA U01AG009740) and were conducted by the University of Michigan. We thank Dr. Caleb H. Miles for the review and feedback while preparing the manuscript.

**Authors' contributions**
RW, conception, funding, data acquisition and approvals, statistical analysis, and drafting the article; SH, statistical approach, review of the manuscript drafts, and critical appraisal. YG, OW, and SH, review of the final drafts and critical appraisal.

**Funding**
Clinical Translational Scholarship in Cognitive Aging and Age-Related Memory Loss funded by the McKnight Brain Research Foundation through the American Brain Foundation, in collaboration with the American Academy of Neurology, Grant K99 AG075196, and a Pilot grant from the Gertrude H. Sergievsky Center at the Department of Neurology of Vagelos College of Physicians and Surgeons at Columbia University.

**Availability of data and materials**
Data was extracted from the Health and Retirement Study. More details in https://hrs.isr.umich.edu/data-products/access-to-public-data.

## Declarations

**Ethics approval and consent to participate**
The Health and Retirement Study is reviewed and approved by the University of Michigan's Health Sciences IRB. The present study has been conducted in accordance with STROBE guidelines for observational studies (https://www.strobe-statement.org/).

**Consent for publication**
NA.

**Competing interests**
The authors declare that they have no competing interests.

**Author details**
[1]Department of Neurology, Columbia University Irving Medical Center, Vagelos College of Physicians and Surgeons, Columbia University, New York, NY 10032, USA. [2]The Taub Institute for Research in Alzheimer's Disease and the Aging Brain, Columbia University, New York, NY, USA. [3]G.H. Sergievsky Center, Vagelos College of Physicians and Surgeons, Columbia University, New York, New York, USA. [4]The Department of Epidemiology, Joseph P. Mailman School of Public Health, Columbia University, New York, NY, USA. [5]Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Solna, Sweden.

Received: 22 February 2023 Accepted: 28 September 2023
Published online: 12 October 2023

**References**

1. Ferrucci L, Levine ME, Kuo P-L, Simonsick EM. Time and the metrics of aging. Circ Res. 2018;123:740–4.
2. Codd V, Nelson CP, Albrecht E, et al. Identification of seven loci affecting mean telomere length and their association with disease. Nat Genet. 2013;45:422–7.
3. Jylhävä J, Pedersen NL, Hägg S. Biological age predictors EBioMedicine. 2017;21:29–36.
4. López-Otín C, Blasco MA, Partridge L, Serrano M, Kroemer G. The hallmarks of aging. Cell. 2013;153:1194–217.
5. Rao LV, Moiles D, Snyder M. Finger-stick complete blood counts: comparison between venous and capillary blood. Point of Care. 2011;10:120–2.
6. Aps JK, Martens LC. The physiology of saliva and transfer of drugs into saliva. Forensic Sci Int. 2005;150:119–31.
7. Gorodischer R, Burtin P, Hwang P, Levine M, Koren G. Saliva versus blood sampling for therapeutic drug monitoring in children: patient and parental preferences and an economic analysis. Ther Drug Monit. 1994;16:437–43.
8. Patsalos PN, Berry DJ. Therapeutic drug monitoring of antiepileptic drugs by use of saliva. Ther Drug Monit. 2013;35:4–29.
9. Abraham JE, Maranian MJ, Spiteri I, et al. Saliva samples are a viable alternative to blood samples as a source of DNA for high throughput genotyping. BMC Med Genomics. 2012;5:1–6.
10. Kupke IR, Kather B, Zeugner S. On the composition of capillary and venous blood serum. Clin Chim Acta. 1981;112:177–85.
11. Ansari S, Abdel-Malek M, Kenkre J, et al. The use of whole blood capillary samples to measure 15 analytes for a home-collect biochemistry service during the SARS-CoV-2 pandemic: a proposed model from North West London Pathology. Ann Clin Biochem. 2021;58:411–21.
12. Colagiuri S, Sandbaek A, Carstensen B, et al. Comparability of venous and capillary glucose measurements in blood. Diabet Med. 2003;20:953–6.
13. Schipper RG, Silletti E, Vingerhoeds MH. Saliva as research material: biochemical, physicochemical and practical aspects. Arch Oral Biol. 2007;52:1114–35.
14. Pfaffe T, Cooper-White J, Beyerlein P, Kostner K, Punyadeera C. Diagnostic potential of saliva: current state and future applications. Clin Chem. 2011;57:675–87.
15. Sonnega A, Faul JD, Ofstedal MB, Langa KM, Phillips JW, Weir DR. Cohort profile: the health and retirement study (HRS). Int J Epidemiol. 2014;43:576–85.
16. Crimmins EM, Faul JD, Thyagarajan B, Weir D. Venous Blood Collection and Assay Protocol in the 2016 Health and Retirement Study. Ann Arbor: Survey Research Center, Institute for Social Research, University of Michigan; 2017.
17. Data HaRSHT. 2008 telomere data [online]. Available at: https://hrsdata.isr.umich.edu/data-products/2008-telomere-data. Accessed 10/25/2022.
18. Crimmins E, Kim J, Fisher J, Faul J. HRS Epigenetic clocks–release 1. Ann Arbor, MI: Survey Research Center, Institute for Social Research, University of Michigan; 2020.
19. Crimmins EM, Thyagarajan B, Levine ME, Weir DR, Faul J. Associations of age, sex, race/ethnicity, and education with 13 epigenetic clocks in a nationally representative US sample: the Health and Retirement Study. The Journals of Gerontology: Series A. 2021;76:1117–23.
20. Horvath S, Raj K. DNA methylation-based biomarkers and the epigenetic clock theory of ageing. Nat Rev Genet. 2018;19:371.
21. Horvath S. DNA methylation age of human tissues and cell types. Genome Biol. 2013;14:1–20.
22. Hannum G, Guinney J, Zhao L, et al. Genome-wide methylation profiles reveal quantitative views of human aging rates. Mol Cell. 2013;49:359–67.
23. Levine ME, Lu AT, Quach A, et al. An epigenetic biomarker of aging for lifespan and healthspan. Aging (Albany NY). 2018;10:573.
24. Lu AT, Quach A, Wilson JG, et al. DNA methylation GrimAge strongly predicts lifespan and healthspan. Aging (Albany NY). 2019;11:303.
25. Horvath S, Oshima J, Martin GM, et al. Epigenetic clock for skin and blood cells applied to Hutchinson Gilford progeria syndrome and ex vivo studies. Aging (Albany NY). 2018;10:1758.
26. Lin Q, Wagner W. Epigenetic aging signatures are coherently modified in cancer. PLoS Genet. 2015;11: e1005334.
27. Lin Q, Weidner CI, Costa IG, et al. DNA methylation levels at individual age-associated CpG sites can be indicative for life expectancy. Aging (Albany NY). 2016;8:394.
28. Weidner CI, Lin Q, Koch CM, et al. Aging of blood can be tracked by DNA methylation changes at just three CpG sites. Genome Biol. 2014;15:1–12.

29. Vidal-Bralo L, Lopez-Golan Y, Gonzalez A. Simplified assay for epigenetic age estimation in whole blood of adults. Front Genet. 2016;7:126.
30. Belsky DW, Moffitt TE, Cohen AA, et al. Eleven telomere, epigenetic clock, and biomarker-composite quantifications of biological aging: do they measure the same thing? Am J Epidemiol. 2018;187:1220–30.
31. Marioni RE, Suderman M, Chen BH, et al. Tracking the epigenetic clock across the human life course: a meta-analysis of longitudinal cohort data. The Journals of Gerontology: Series A. 2019;74:57–61.
32. Levine ME. Assessment of epigenetic clocks as biomarkers of aging in basic and population research. USA: Oxford University Press; 2020.
33. Fahy GM, Brooke RT, Watson JP, et al. Reversal of epigenetic aging and immunosenescent trends in humans. Aging Cell. 2019;18: e13028.
34. Liu Z, Kuo P-L, Horvath S, Crimmins E, Ferrucci L, Levine M. A new aging measure captures morbidity and mortality risk across diverse subpopulations from NHANES IV: a cohort study. PLoS Med. 2018;15: e1002718.
35. Levine ME. Modeling the rate of senescence: can estimated biological age predict mortality more accurately than chronological age? The Journals of Gerontology: Series A. 2012;68:667–74.
36. Kwon D, Belsky DW. A toolkit for quantification of biological age from blood chemistry and organ function test data: BioAge. GeroScience. 2021;43:2795–808.
37. Health, Study R. Sampling weights revised for tracker 2.0 and beyond. Ann Arbor: Survey Research Center, University of Michigan; 2002.
38. Bollen KA, Biemer PP, Karr AF, Tueller S, Berzofsky ME. Are survey weights needed? A review of diagnostic tests in regression analysis. Annual Review of Statistics and Its Application. 2016;3:375–92.
39. Lee Rodgers J, Nicewander WA. Thirteen ways to look at the correlation coefficient. Am Stat. 1988;42:59–66.
40. Sedgwick P. Multiple significance tests: the Bonferroni correction. Bmj. 2012;344:e509–e509.
41. Niedzwiedz CL, Katikireddi SV, Pell JP, Smith DJ. Sex differences in the association between salivary telomere length and multimorbidity within the US Health & Retirement Study. Age Ageing. 2019;48:703–10.
42. Barrett EL, Richardson DS. Sex differences in telomeres and lifespan. Aging Cell. 2011;10:913–21.
43. Gardner M, Bann D, Wiley L, et al. Gender and telomere length: systematic review and meta-analysis. Exp Gerontol. 2014;51:15–27.
44. Aviv A. Telomeres, sex, reactive oxygen species, and human cardiovascular aging. J Mol Med. 2002;80:689–95.
45. Martens DS, Van Der Stukken C, Derom C, Thiery E, Bijnens EM, Nawrot TS. Newborn telomere length predicts later life telomere length: tracking telomere length from birth to child-and adulthood. EBioMedicine. 2021;63:103164.
46. Puterman E, Gemmill A, Karasek D, et al. Lifespan adversity and later adulthood telomere length in the nationally representative US Health and Retirement Study. Proc Natl Acad Sci. 2016;113:E6335–42.
47. Cui C, Qu Y, Sia JEV, et al. Assessment of aging-related function variations of P-gp transporter in old-elderly Chinese CHF patients based on modeling and simulation. Clin Pharmacokinet. 2022;61(12):1789–800.
48. Hou Y, Dan X, Babbar M, et al. Ageing as a risk factor for neurodegenerative disease. Nat Rev Neurol. 2019;15:565–81.
49. López-Otín C, Blasco MA, Partridge L, Serrano M, Kroemer G. Hallmarks of aging: an expanding universe. Cell. 2023;186:243–78.
50. Justice J, Miller JD, Newman JC, et al. Frameworks for proof-of-concept clinical trials of interventions that target fundamental aging processes. Journals of Gerontology Series A: Biomedical Sciences and Medical Sciences. 2016;71:1415–23.
51. Newman JC, Milman S, Hashmi SK, et al. Strategies and challenges in clinical trials targeting human aging. J Gerontol A Biol Sci Med Sci. 2016;71:1424–34.
52. Kirkland JL. Translating the science of aging into therapeutic interventions. Cold Spring Harb Perspect Med. 2016;6: a025908.
53. Dong Y, Huang Y, Gutin B, Raed A, Dong Y, Zhu H. Associations between global DNA methylation and telomere length in healthy adolescents. Sci Rep. 2017;7:4210.
54. Lu AT, Seeboth A, Tsai PC, et al. DNA methylation-based estimator of telomere length. Aging (Albany NY). 2019;11:5895–923.
55. Lai T-P, Wright WE, Shay JW. Comparison of telomere length measurement methods. Philosophical Transactions of the Royal Society B: Biological Sciences. 2018;373:20160451.
56. Pearce EE, Horvath S, Katta S, et al. DNA-methylation-based telomere length estimator: comparisons with measurements from flow FISH and qPCR. Aging (Albany NY). 2021;13:14675.
57. Zheng SC, Webster AP, Dong D, et al. A novel cell-type deconvolution algorithm reveals substantial contamination by immune cells in saliva, buccal and cervix. Epigenomics. 2018;10:925–40.
58. Cui Y, Zhang H, Wang S, et al. Obtaining a reliable diagnostic biomarker for diabetes mellitus by standardizing salivary glucose measurements. Biomolecules. 2022;12(10):1335.
59. Zhu C, Yuan C, Wei F, Sun X, Zheng S. Characterising salivary peptidome across diurnal dynamics and variations induced by sampling procedures. Clin Oral Investig. 2023;27(1):285–98.
60. Ambroa-Conde A, Girón-Santamaría L, Mosquera-Miguel A, et al. Epigenetic age estimation in saliva and in buccal cells. Forensic Sci Int Genet. 2022;61: 102770.
61. Hong SR, Jung S-E, Lee EH, Shin K-J, Yang WI, Lee HY. DNA methylation-based age prediction from saliva: high age predictability by combination of 7 CpG markers. Forensic Sci Int Genet. 2017;29:118–25.
62. Aliferi A, Ballard D, Gallidabino MD, Thurtle H, Barron L, Syndercombe CD. DNA methylation-based age prediction using massively parallel sequencing data and multiple machine learning models. Forensic Sci Int Genet. 2018;37:215–26.
63. Schübeler D. Function and information content of DNA methylation. Nature. 2015;517:321–6.
64. Christensen BC, Houseman EA, Marsit CJ, et al. Aging and environmental exposures alter tissue-specific DNA methylation dependent upon CpG island context. PLoS Genet. 2009;5: e1000602.
65. Nazarenko MS, Markov AV, Lebedev IN, et al. A comparison of genome-wide DNA methylation patterns between different vascular tissues from patients with coronary heart disease. PLoS ONE. 2015;10: e0122601.
66. Dugué PA, English DR, MacInnis RJ, et al. Reliability of DNA methylation measures from dried blood spots and mononuclear cells using the humanmethylation450k beadarray. Sci Rep. 2016;6:30317.
67. Bose M, Wu C, Pankow JS, et al. Evaluation of microarray-based DNA methylation measurement using technical replicates: the Atherosclerosis Risk in Communities (ARIC) study. BMC Bioinformatics. 2014;15:312.
68. Sugden K, Hannon EJ, Arseneault L, et al. Patterns of reliability: assessing the reproducibility and integrity of DNA methylation measurement. Patterns. 2020;1: 100014.
69. Waziry R, Gras L, Sedaghat S, et al. Quantification of biological age as a determinant of age-related diseases in the Rotterdam Study: a structural equation modeling approach. Eur J Epidemiol. 2019;34:793–9.
70. Waziry R, Hofman A, Ghanbari M, et al. Biological aging for risk prediction of first-ever intracerebral hemorrhage and cerebral infarction in advanced age. J Stroke Cerebrovasc Dis. 2022;31: 106568.
71. Globerson A, Effros RB. Ageing of lymphocytes and lymphocytes in the aged. Immunol Today. 2000;21:515–21.
72. Cooper MD. B lymphocytes. N Engl J Med. 1987;317:1452–6.
73. Fabbri M, Smart C, Pardi R. T lymphocytes. Int J Biochem Cell Biol. 2003;35:1004–8.
74. Kashani K, Rosner MH, Ostermann M. Creatinine: from physiology to clinical application. Eur J Intern Med. 2020;72:9–14.
75. Levey AS, Perrone RD, Madias NE. Serum creatinine and renal function. Annu Rev Med. 1988;39:465–90.
76. Streckfus CF, Bigler LR. Saliva as a diagnostic fluid. Oral Dis. 2002;8:69–76.
77. Lee YH, Wong DT. Saliva: an emerging biofluid for early detection of diseases. Am J Dent. 2009;22:241–8.


## Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.