



## Short communication

## No association between frailty index and epigenetic clocks in Italian semi-supercentenarians

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## ARTICLE INFO

## Keywords:

Frailty index  
Epigenetic clocks  
Ageing  
Centenarians

## ABSTRACT

Centenarians experience successful ageing, although they still present high heterogeneity in their health status. The frailty index is a biomarker of biological age, able to capture such heterogeneity, even at extreme old age. At the same time, other biomarkers (e.g., epigenetic clocks) may be informative the biological age of the individual and potentially describe the ageing status in centenarians. In this article, we explore the relationship between epigenetic clocks and frailty index in a cohort of Italian centenarians. No association was reported, suggesting that these two approaches may describe different aspects of the same ageing process.

The study of centenarians, that nowadays represent the fastest-growing group among older persons, is crucial for understanding the mechanisms that regulate ageing and age-related conditions. Centenarians are characterized by notable heterogeneity in their health status (Evert et al., 2003), as a result of the intricate interplay between genetic background and life-long exposure to environmental stressors (Ostan et al., 2019; Salvioli et al., 2009).

Recently, it has been demonstrated that this heterogeneity can be captured by the Frailty Index (FI) (Arosio et al., 2019). The FI estimates the biological ageing through a quantitative approach to the age-related accumulation of health deficits, allowing the discrimination of different

degrees of frailty (Rockwood and Mitnitski, 2007). It has been shown that centenarians present a higher level and heterogeneity of the FI compared to persons aged 65–99 years (Arosio et al., 2019). Furthermore, the FI predicts mortality and health conditions, as demonstrated in a large cohort of Chinese centenarians, suggesting that it can discriminate different degrees of frailty even at very advanced age (Arosio et al., 2019; Gu and Feng, 2015).

Another approach to measure the individual's biological age is represented by the epigenetic clock, based on the DNA methylation (DNAm) levels found in sets of CpG sites across the genome (Horvath and Raj, 2018; Jylhävä et al., 2017). Centenarians have an epigenetic age

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<https://doi.org/10.1016/j.mad.2021.111514>

Received 11 March 2021; Received in revised form 20 May 2021; Accepted 24 May 2021

Available online 4 June 2021

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**Table 1**  
Characteristics of the cohort.

Age	mean (yrs)	105.2
	range (yrs)	104–109
	SD	1.15
	mean (yrs)	0.49
FI	range (yrs)	0.31–0.65
	SD	0.08
	mean (yrs)	93.90
	range (yrs)	60.10–110.01
DNAm age according to Horvath's clock (DNAmAge)	SD	8.88
	mean (yrs)	93.98
	range (yrs)	58.64–110.71
	SD	9.63
DNAm age according to Hannum's clock (DNAmHannum)	mean (yrs)	86.35
	range (yrs)	61.34–95.82
	SD	6.35
	mean (yrs)	86.07
DNAm age according to Skin&BloodClock (DNAmAgeSkinBloodClock)	range (yrs)	50.57–105.14
	SD	10.58
	mean (yrs)	98.58
	range (yrs)	87.61–108.54
DNAm age according to PhenoAge (DNAmPhenoAge)	SD	4.35
	mean (yrs)	98.58
	range (yrs)	87.61–108.54
	SD	4.35

younger than expected and a high variability in the predicted epigenetic age (Armstrong et al., 2017; Gutman et al., 2020; Horvath et al., 2015).

Some studies have investigated the relationship between the FI and epigenetic clocks. Interestingly, two papers reported that the FI outperforms epigenetic clocks in predicting mortality (Kim et al., 2017; Zhang et al., 2018). At the same time, whereas Li et al. have observed only a weak association between FI and epigenetic age (Li et al., 2020), others have reported positive associations (Breitling et al., 2016; Gale et al., 2018). In this context, we decided to analyse the association between the FI and epigenetic clocks in a cohort of well-characterized semi-supercentenarians (older than 105 years).

A standardized, structured questionnaire was administered to all subjects referring to three Italian research centers (Milan, Bologna, and Cosenza) (Bucci et al., 2014). The FI was computed following the standardization criteria described by Searle et al. (Searle et al., 2008), and calculated as the ratio between the number of health deficits presented by the individual and the total number of health deficits considered in a comprehensive evaluation (Arosio et al., 2020, 2019). Genome-wide DNAm was measured in peripheral blood mononuclear cells using the Infinium Human Methylation 450 BeadChip (Illumina). As previously described (Horvath et al., 2015) unsupervised hierarchical clustering on inter-array correlation was used to identify outliers, and accordingly 7 centenarians samples were removed. DNAm data were uploaded in the freely available software available at: <https://dnamage.genetics.ucla.edu/new> (New Methylation Age Calculator, Advanced Analysis). The software returns a series of DNAm estimates, including: the multi-tissue Horvath's clock (Horvath, 2013), Hannum's clock, that is specific for blood (Hannum et al., 2013), the skin & blood clock, developed for studies on cell cultures (Horvath et al., 2018), the PhenoAge, that incorporates composite clinical measures related to differences in lifespan and healthspan (Levine et al., 2018), and the GrimAge, based on the estimation of the plasma levels of 7 proteins (adrenomedullin, beta-2-microglobulin, cystatin-C, growth differentiation factor 15,

**Table 2**

Results of robust linear regression models (adjusted for sex and study site) reporting the association of the independent variables (individually considered in the analysis) and chronological age.  $\beta$ -values ( $\beta$ ), standard error (SE), and BH-corrected p-value are reported.

Independent Variables	$\beta \pm SE$	BH-corrected p-value
FI	$-0.01 \pm 0.01$	1
DNAm age according to Horvath's clock (DNAmAge)	$0.41 \pm 1.32$	1
DNAm age according to Hannum's clock (DNAmHannum)	$-1.1 \pm 0.77$	0.73
DNAm age according to Skin&BloodClock (DNAmAgeSkinBloodClock)	$0.39 \pm 0.91$	1
DNAm age according to PhenoAge (DNAmPhenoAge)	$0.85 \pm 1.46$	1
DNAm age according to GrimAge (DNAmGrimAge)	$0.67 \pm 0.69$	1
Difference between chronological age and DNAmAge	$-0.59 \pm 1.32$	1
Difference between chronological age and DNAmAgeHannum	$-2.1 \pm 0.77$	0.27
Difference between chronological age and DNAmAgeSkinBloodClock	$-0.61 \pm 0.91$	1
Difference between chronological age and DNAmPhenoAge	$-0.15 \pm 1.46$	1
Difference between chronological age and DNAmGrimAge	$-0.33 \pm 0.69$	1
Predicted levels of adrenomedullin	$4.31 \pm 3.31$	0.82
Predicted levels of beta-2-microglobulin	$9166.57 \pm 12422.54$	1
Predicted levels of cystatin-C	$2829.09 \pm 4238.21$	1
Predicted levels of growth differentiation factor 15	$-9.51 \pm 17.81$	1
Predicted levels of leptin	$544.49 \pm 483.38$	0.97
Predicted levels of plasminogen activator inhibitor 1	$736.54 \pm 500.91$	0.73
Predicted levels of tissue Inhibitor Metalloproteinases 1	$72.13 \pm 107.02$	1
Predicted telomere length	$0 \pm 0.04$	1

leptin, plasminogen activator inhibitor 1, tissue Inhibitor Metalloproteinases 1) and of smoking pack-years, an estimated parameters that is strongly associated with mortality (Lu et al., 2019).

Statistical analyses were performed on 31 semi-supercentenarians for which both the FI and the DNAm data were available. The cohort had a mean age of 105.2 years (SD: 1.16; age range: 104–109), and included 21 women and 10 men. The mean value of the FI was 0.49 (SD: 0.08; range: 0.31–0.65). As previously reported (Horvath et al., 2015), semi-supercentenarians were epigenetically younger than expected (Table 1).

We focused our attention on the variables reported in Tables 2 and 3. First of all, we evaluated the association between each parameter representing the biological age of the individual (i.e., FI and DNAm estimates) with his/her chronological age, using robust regression. The statistical models were corrected for sex and recruitment center and p-values were adjusted for multiple testing with BH method (Table 2).

Neither the FI nor the DNAm estimators were associated with age in centenarians. This result might be explained by the heterogeneous health status of the centenarians compressed within a narrow range of chronological age.

Table 3 shows the association between DNAm estimators and FI, using robust linear regression and adjusting for chronological age, sex, and recruitment center. Also in this case, p-values were corrected for multiple testing (BH method).

The FI was not associated with any DNAm age estimators nor with DNAm age acceleration values. These results differ from those reported by Breitling et al. and Gale et al. (Breitling et al., 2016; Gale et al., 2018). Compared to these studies, the limited size of our cohort could prevent

**Table 3**

Results of robust linear regression models (adjusted for chronological age, sex, and study site) reporting the association of the independent variables (individually considered in the analysis) and FI.  $\beta$ -values ( $\beta$ ), standard error (SE) and BH-corrected p-value are reported.

Independent Variables	$\beta \pm SE$	BH-corrected p-value
DNAm age according to Horvath's clock (DNAmAge)	$-29.03 \pm 14.71$	0.4
DNAm age according to Hannum's clock (DNAmHannum)	$0.87 \pm 10.74$	0.94
DNAm age according to Skin&BloodClock (DNAmAgeSkinBloodClock)	$-8.05 \pm 11.21$	0.81
DNAm age according to PhenoAge (DNAmPhenoAge)	$-7.4 \pm 19.71$	0.88
DNAm age according to GrimAge (DNAmGrimAge)	$5.96 \pm 9.36$	0.81
Difference between chronological age and DNAmAge	$-29.03 \pm 14.71$	0.4
Difference between chronological age and DNAmAgeHannum	$0.87 \pm 10.74$	0.94
Difference between chronological age and DNAmAgeSkinBloodClock	$-8.05 \pm 11.21$	0.81
Difference between chronological age and DNAmPhenoAge	$-7.4 \pm 19.71$	0.88
Difference between chronological age and DNAmGrimAge	$5.96 \pm 9.36$	0.81
Predicted levels of adrenomedullin	$47.24 \pm 42.48$	0.81
Predicted levels of beta-2-microglobulin	$63741.76 \pm 173872.32$	0.88
Predicted levels of cystatin-C	$-8890.21 \pm 58997.65$	0.94
Predicted levels of growth differentiation factor 15	$-287.47 \pm 242.26$	0.81
Predicted levels of leptin	$14640.21 \pm 6552.56$	0.4
Predicted levels of plasminogen activator inhibitor 1	$5526.12 \pm 6770.28$	0.81
Predicted levels of tissue Inhibitor Metalloproteinases 1	$1162.71 \pm 1447.02$	0.81
Predicted telomere length	$-0.34 \pm 0.51$	0.81

us from finding significant associations. On the other hand, our results are in agreement with those by Li et al. (Li et al., 2020), suggesting that the FI and the epigenetic clocks describe different aspects of the ageing process (Li et al., 2020; McCrory et al., 2020). Indeed, while FI provides a multidimensional measure of clinical phenotypes relative to deficits measured in the clinical setting, epigenetic clocks provide an estimate of molecular changes that are not necessarily of clinical relevance. Therefore these markers estimate ageing from different points of view. Likely, the differences in the domains grasped by the two are even more pronounced in centenarians, whose phenotype is highly heterogeneous and for which the tools that are commonly used to evaluate ageing may not provide consistent results (e.g. Mini-Mental State Examination) (Arosio et al., 2017).

In conclusion, our results indicate that currently available epigenetic clocks are not optimized to disentangle the heterogeneity that characterizes centenarians as assessed by a multidimensional clinical biomarker like FI.

## Funding

Data generation was supported by the Italian Ministry of University and Research (Project PRIN 2009). Data analysis was supported by the European Union (EU)'S H2020 Project "PROPAG-AGEING" (grant agreement 634821) and by the EU JPNP "ADAGE".

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