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Methylation, telomeres, and frailty: an epidemiological study on the interplay of epigenetic, genomic and clinical correlates of age

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Full Title:	Methylation, telomeres, and frailty: an epidemiological study on the interplay of epigenetic, genomic and clinical correlates of age
Article Type:	Research
Abstract:	Background. The epigenetic clock, in particular epigenetic pre-aging quantified by the so-called DNA methylation age acceleration, has recently been suggested to closely correlate with a variety of disease phenotypes. There remains a dearth of data, however, on its association with telomere length and frailty, which can be considered major correlates of age on the genomic and clinical level, respectively. Results. In this cross-sectional observational study on altogether 1820 subjects from the elderly general population in Germany, no correlation of epigenetic age acceleration with telomere length was found. However, there was an association of DNA methylation age acceleration with a comprehensive frailty measure, such that the accumulated deficits significantly increased with increasing age acceleration. This association was independent from a variety of confounding variables considered. Conclusions. The results of the present study suggest that epigenetic age acceleration is correlated with clinically relevant aging-related phenotypes through pathways unrelated to genomic age as assessed by telomere length. Innovative approaches like Mendelian randomization will be needed to elucidate whether epigenetic age acceleration indeed plays a causal role for the development of clinical phenotypes.

Methylation, telomeres, and frailty: an epidemiological study on the interplay of epigenetic, genomic and clinical correlates of age

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ABSTRACT

Background. The epigenetic clock, in particular epigenetic pre-aging quantified by the so-called DNA methylation age acceleration, has recently been suggested to closely correlate with a variety of disease phenotypes. There remains a dearth of data, however, on its association with telomere length and frailty, which can be considered major correlates of age on the genomic and clinical level, respectively.

Results. In this cross-sectional observational study on altogether 1820 subjects from the elderly general population in Germany, no correlation of epigenetic age acceleration with telomere length was found. However, there was an association of DNA methylation age acceleration with a comprehensive frailty measure, such that the accumulated deficits significantly increased with increasing age acceleration. This association was independent from a variety of confounding variables considered.

Conclusions. The results of the present study suggest that epigenetic age acceleration is correlated with clinically relevant aging-related phenotypes through pathways unrelated to genomic age as assessed by telomere length. Innovative approaches like Mendelian randomization will be needed to elucidate whether epigenetic age acceleration indeed plays a causal role for the development of clinical phenotypes.

Keywords: telomere length; CpG methylation; epigenetic age acceleration; frailty index; cross-sectional study; general population

BACKGROUND

DNA methylation patterns are known to change with chronological age, and multiple CpG sites with replicable associations with age have been identified [Florath 2014]. Based on regression coefficients estimated from a large number of datasets, an individual's chronological age can be predicted from DNA methylation data with high accuracy [Horvath 2013]. The difference between the thus predicted methylation age (DNAm age) and the chronological age has been termed 'age acceleration' and, intriguingly, has been found to be a substantially heritable trait in twin study datasets that furthermore shows plausible associations with several phenotypes across studies and tissues [Horvath 2013].

Frailty describes a clinical syndrome characterized by a depletion of physical and cognitive resilience and reserves, commonly associated with an accumulation of functional deficits [Saum 2014a; Kim 2015]. Frailty has received growing attention in recent years, due to pronounced associations with longevity and other aging-related phenotypes and the corresponding perception that frailty measures reflect an individual's clinically relevant biological age [Kim 2015; Jotheeswaran 2015]. Stable intra-individual differences in biological aging and accruing frailty exist [Mitnitski e2015], and DNA methylation patterns might play a role in this phenomenon [Bellizzi 2012].

Telomere length (TL) has been suggested to reflect an individual's biological age at the genomic DNA level, and associations of measures of TL with various aging- and

frailty-related phenotypes, such as sarcopenia [Marzetti 2014] and bone loss [Beakert 2005], have been reported. Whereas epigenetic age acceleration has been shown to be associated with various cancer phenotypes [Horvath 2013], its correlation with TL apparently has not been investigated to date, and seemingly only one study has addressed age acceleration and frailty [Marioni (in press)]. In a rare study analyzing both TL and DNAm age, the development of symptoms of post-traumatic stress syndrome was associated with both variables—in plausibly opposite, yet altogether unexpected directions—, but their mutual correlation apparently was not investigated [Boks 2015].

A better understanding of the interplay of epigenetic and genomic correlates of age as determinants of clinical frailty could help to elucidate novel pathways to healthy aging and longevity. In the present study, the correlation of epigenetic age acceleration with TL and frailty was investigated in two large subsets of a general population sample of community-dwelling older adults in Germany. In light of inconclusive previous findings on associations of TL with frailty [Saum 2014b], interaction analyses were conducted to evaluate whether DNAm age alters the association of TL with frailty.

RESULTS

Description of study population and main variables

Major characteristics of the study populations are shown in **Table 1**. The two ESTHER subsets analyzed in the present work—dataset 1 consisting of 1000 consecutively recruited participants of the ESTHER cohort, dataset 2 originating from a case-cohort design (see Methods section for details)—resembled one another closely. The mean age was 62.1 and 63.0 years, respectively. Methylation age and difference-based epigenetic age acceleration appeared somewhat higher in dataset 2, in which relative telomere length also tended to be lower. There was no difference with respect to the frailty index. Current smoking was prevalent in one fifth of participants in both datasets, and harmful alcohol consumption was reported by less than 10% of participants. Histograms of the main analysis variables are shown in **Supplemental Figure S1** and suggested some skewness of frailty only, whereas DNAm age acceleration and relative telomere length closely followed a normal distribution.

Age acceleration and telomere length

The results of regression analyses of relative telomere length on difference-based methylation age acceleration are shown in **Table 2**. In both ESTHER subsets and in the combined analysis, the estimated coefficients were small and not statistically significant. The results of inverse sampling probability-weighted regression (i.e., adjusting for the case-cohort nature of substudy 2; see Methods) were overall similar to the main analyses. For example, the estimate in the age-, sex- and leukocyte distribution-adjusted model was -0.0004 instead of -0.0006, the result in the model additionally adjusted for cancer history was -0.0004 instead of -0.0006 (details not shown).

Age acceleration and frailty

The regression modeling of the frailty index on epigenetic age acceleration is summarized in **Table 3**. The estimates suggested positive associations between age acceleration and FI in both datasets, with statistically significant results in the fully adjusted models in both individual datasets and the combined analysis. After adjustment for age, sex, and leukocyte distribution, FI increased by about 0.25 percent points per year of epigenetic age acceleration, which—since the FI used increases by about 2.9 percent points per deficit—translates into one additional deficit per 11.6, or roundabout half an additional deficit per 6 years of age acceleration, respectively. The adjustment for additional variables had no relevant impact, and the results were comparable when analyzing women and men separately. Weighted regression likewise produced similar results (details not shown).

Interaction analysis of methylation and telomeres on frailty

In the context of predicting frailty from relative telomere length, the additional consideration of an interaction with epigenetic age acceleration did not improve the prediction of FI (**Table 4**).

DISCUSSION

In this study of more than 1800 community-dwelling adults, there was evidence for an independent association of epigenetic age acceleration with frailty as measured by a deficit accumulation-based approach. Quantitatively, the observed association translated into roundabout half an additional deficit per 6 years—i.e., roughly 1.2 standard deviations—of age acceleration. Relative telomere length, on the other hand, was not significantly associated with age acceleration. These findings suggest that DNAm age acceleration might be correlated with clinically relevant aging-related phenotypes, in particular frailty, due to pathways unrelated to genomic age as assessed by TL.

Age acceleration is associated with a comprehensive frailty measure

The potential relationship between epigenetic age acceleration and frailty-related phenotypes apparently has been investigated only in one previous study: in an analysis of the Lothian Birth Cohort 1936 (LBC1936), significant correlation coefficients ranging from -0.05 to -0.07 were found between DNAm age acceleration and cognitive functioning, grip strength, or lung function [Marioni (in press)].

Given that the LBC1936 participants were rather strictly 70 years of age when assessed for the study of age acceleration, the present findings extend the prior evidence from the old to middle-aged-old age group, as they were based on study participants aged 50 to 75 years. Moreover, whereas age acceleration was successfully analyzed with respect to three individual healthy aging-related characteristics in LBC1936, the current work employed the frailty index, a more multi-

dimensional approach that combines parameters of multiple physiological systems and functional capacities [Kim 2015]. This very robust frailty measure features strong replicability and validity across populations and datasets [Searle 2008], fostering the relevance of the present findings and supporting a wide applicability to frailty-related research questions including study populations that may lack information on one frailty item or another.

Epigenetics have been termed an ‘attractive candidate’ for explaining frailty differences, and methylation levels of some promoter CpG islands are associated with frailty [Collerton 2014]. Findings for global DNA methylation and frailty are somewhat inconsistent, though this might be due to methodological differences [Bellizzi 2012; Collerton 2014]. Major hypotheses advanced in this context include that the activation of genes involved in a response to frailty leads to a hypomethylation of respective regulatory genomic regions, or that deficits in methylation maintenance lead to a dysregulation of gene expression and the development of frailty [Bellizzi 2012]. Intriguingly, the LBC1936 study found epigenetic age acceleration but not individual CpG methylation levels to be associated with their fitness measures [Marioni (in press)], suggesting that an ‘accelerated’ epigenetic aging may be more closely correlated to clinically relevant frailty phenotypes than any individual CpG. However, it ultimately remains unclear how the interplay of environmental factors and stochastic processes leads to the manifestation of a consistent (and possibly mechanistically relevant) epigenetic clock at specific loci in contrast to an overall inconsistent (and purely correlational) epigenetic drift [Jones e2015].

Age acceleration and telomere length are not correlated

In the absence of previous pertinent publications, the analysis of DNAm age acceleration with TL was motivated by the hypothesis that accelerated epigenetic aging could plausibly be associated also with genomic aging. The ESTHER study provided no evidence for such an association. It was furthermore hypothesized that differences in DNAm age acceleration might be responsible for the inconclusive prior reports about an association of TL with frailty-related phenotypes [Bekaert 2005; Saum 2014b; Marzetti 2014]. However, no association of TL with frailty was found in the ESTHER cohort, regardless of the level of DNAm age acceleration.

A limited number of studies have investigated DNA methylation in association with telomere length. Global hypomethylation has been suggested to be associated with decreasing TL [Wong 2014], and several individual CpGs are correlated with TL independent of chronological age [Buxton 2014]. Age-related differences in the methylation of subtelomeric regions further support a close link of epigenetics and genomic aging [Maeda 2009]. The absence of an association of TL with epigenetic age acceleration, however, seems to be in line with evidence suggesting that DNAm age does not reflect mitotic age [Horvath 2013], which is a major determinant of age-dependent telomere shortening [Koliada 2015].

Limitations and strengths

Given the observational, cross-sectional design of the present study, our findings should not be interpreted as reflecting causality. Future studies should consider repeated measurements of methylation/age acceleration as well as telomere length

and frailty in order to approach this issue. Methylation analyses were done on whole blood DNA, which constitutes a mixture of cells present in the peripheral circulation. Although methylation patterns are known to vary between tissues, DNAm age as used in the present study's main analyses features only a low correlation with cell types, presumably because it has been consciously designed as a multi-tissue predictor based on rather diverse learning data sets [Boks 2015; Horvath 2013]. Differential blood counts were not available in the present study, but our main models were adjusted for leukocyte subtype distributions estimated by the Houseman method, and this had only a minor impact on the results. Contrasting the aforementioned limitations, the large size and representative nature of the study sample, as well as the use of a thoroughly constructed multi-dimensional frailty measure, are major strengths of the present work, which featured an altogether exceptional combination of data on methylation, telomeres, and frailty.

Towards a better understanding of the human clocks

Knowledge on the interplay of genomic, epigenetic, and bioclinical aging phenomena remains surprisingly vague. Even though telomere length has been suggested to have some effects on phenotype development, current evidence altogether seems to emphasize its role as a rather innocent bystander of aging and an indicator of life-time exposures [Koliada 2015]. Aging-associated CpG sites also are not generally related to known mechanisms of aging [Marttila 2015], and it has been described as one of the chief challenges in this field 'to identify the most important genes and pathways for which altered methylation patterns contribute to age-related functional decline' [Jung 2015]. However, the causal relevance of *any* altered DNA methylation patterns—including epigenetic age acceleration—for aging-related phenotypes at

present remains speculative. The most promising way to address this issue may be genomewide association studies of frailty-associated differential CpG methylation and DNAm acceleration, which could yield instrumental variables for use in so-called Mendelian randomization studies, an analytical approach specifically designed for studying causality in observational settings [Relton 2012].

CONCLUSIONS

Confirming speculations by Horvath, the present findings suggest that epigenetic aging contains information complementary to that of the telomere clock [Horvath 2013]. The results on epigenetic age acceleration being associated with a multi-dimensional frailty phenotype appear promising, but innovative approaches like Mendelian randomization will be needed to elucidate the causal relevance of such patterns linking epigenetic, genomic, and clinical correlates of age.

METHODS

Study design and study population

The present study was based on the ESTHER epidemiological cohort study, which is an observational study of the elderly general population of Saarland, a federal state

of Germany [Raum 2007]. In brief, almost 10,000 participants aged 50 to 75 years were recruited by their general practitioner when presenting for routine health check-ups from June 2000 to December 2002. This cohort is representative for this age segment of the community-dwelling general population of Saarland [Raum 2007].

In brief, the baseline assessment forming the basis of the present investigation consisted of obtaining the data collected as part of the health check-up, drawing a blood sample that was mailed to the study center and stored at -80°C until analysis, and completing a detailed standardized questionnaire on socio-demographics, lifestyle factors, and medical history.

For the present study, only subjects with data available on epigenetic age, telomere length, and frailty (see below) were considered, and the availability of DNA methylation data was the principal limiting factor in this regard. DNA methylation measurements allowing the calculation of epigenetic age had been obtained from two subsamples of the source study: substudy 1 included 1000 consecutively recruited ESTHER participants with sufficient baseline DNA available; substudy 2 included all ESTHER participants deceased until year 8 follow-up and with sufficient DNA available (n=406 after discounting 196 individuals already included in substudy 1), plus 458 additional subjects randomly selected from ESTHER participants with sufficient DNA available and not included in substudy 1 or the deceased group. After discounting subjects with missing data on telomere length, a total of 1820 participants could be included in the present analysis (969 [97%] of substudy 1; 851 [98%] of substudy 2).

Ethics, consent and permissions. Inclusion in the ESTHER study, which complies with the Declaration of Helsinki, was conditional upon written informed consent. The study protocol and procedures were approved by the ethics committees of both the Medical Faculty of the University of Heidelberg and of the Medical Association of Saarland.

Epigenetic age and age acceleration

The DNAm age was calculated based on a predictor developed in a large study of genomewide methylation array datasets [Horvath 2013] using the R tutorial of the pertinent publication. In brief, DNAm age is calculated from methylation levels at 353 CpG sites. This measure has been suggested to reflect the 'cumulative work done by an epigenetic maintenance system' and is highly correlated with chronological age [Horvath 2013]. The so-called difference-based DNAm age acceleration can be calculated by substracting the chronological age from the predicted DNAm age [Horvath 2013].

Methylation levels were determined using the Infinium HumanMethylation450 BeadChip (Illumina, San Diego, CA) at the Genomics and Proteomics Core Facility of the German Cancer Research Center, Heidelberg, Germany. Methodological details have been published previously [Florath 2014].

Telomere length

As a measure of relative telomere length (TL), the telomere repeat copy number to number of single copy gene ratio (T/S ratio) was determined using a quantitative PCR approach [Cawthon 2002]. The assay used the single copy gene *36B4* for reference, and the PCR was done on a Lightcycler® 480 (Roche Diagnostics, Mannheim, Germany). Further details on the TL measurements, including quality control and assay validation, have been published elsewhere [Müezzinler 2015].

Frailty index

As a measure of frailty, a frailty index (FI) based on the accumulation of deficits was calculated as previously described [Saum 2014a]. In brief, the FI is defined as the proportion of deficits present, where the exact deficits considered in the construction of the index depend on the available data [Searle 2008]. In the ESTHER study population, the FI was constructed following standard recommendations [Searle 2008] and ultimately based on 34 deficits (i.e., one additional deficit increases this FI by 0.029, or 2.9 percent points), including poor self-rated general health, history of various diseases (11 items: myocardial infarction, angina pectoris, heart failure, stroke, hypertension, hyperlipidemia, diabetes, cataract, glaucoma, gout, cancer), difficulties in the activities of daily living (16 items: "vigorous activities", "climbing several flights of stairs", "climbing one flight of stairs", "walking more than one mile", "walking several blocks", "walking one block", "moderate activities, such as moving a table, pushing a vacuum cleaner, bowling, or playing golf", "lifting or carrying groceries", "bathing or dressing yourself", "bending, kneeling or stooping", "limits in normal work or activities due to pain", "accomplished less work or activities due to impaired physical health", "limits in type of work or activities due to impaired physical health", "difficulties chewing hard food", "difficulties chewing meat", "short-term

memory loss"), and various symptoms (six items: under-/overweight, pyrosis, shiver, insomnia, costiveness, aconuresis). Missing values in the variables needed for the frailty index calculation were dealt with by multiple imputation [Saum 2014a], and models including FI in the present analysis were based on 20 imputations combined by the SAS procedure MIANALYZE.

Statistical methods

The study population was first described with respect to the main analysis variables, major participant characteristics and important covariables (smoking behavior [never, former, current], alcohol consumption [none, 1-19 (women) or 1-39 (men) g/d, 20+ (women) and 40+ (men) g/d], history of cancer). Histograms were used to explore the distribution of DNAm age acceleration, TL, and FI. Subsequently, linear regression models predicting TL or FI from DNAm age acceleration were fitted with increasing adjustment sets: no covariables; age; age, sex; age, sex, and leukocyte subtype distributions (LD) estimated according to the Houseman method [Houseman 2014] (main model). Random effects were included in the models to account for methylation array and telomere assay batch effects. Subsequently, the sensitivity of the main model results to additional adjustment for smoking, alcohol or history of cancer was studied. In addition, sex-specific estimates were examined. Finally, the potential interaction of telomere length and epigenetic age acceleration on frailty was examined by fitting linear regression models predicting FI from RTL within tertiles of DNAm age acceleration.

The regression models were generally fit first to dataset 1 and dataset 2 separately, and then to the combined dataset. Additional sensitivity analyses included the use of inverse sampling probability weights (i.e., adjustment for the oversampling of deceased subjects due to the case-cohort design of substudy 2) in the combined dataset models. Statistical significance was defined as $p<0.05$. All data analyses were done using SAS 9.3.

AVAILABILTY OF DATA AND MATERIALS

Data protection standards and assurances made as part of the informed consent procedure of ESTHER preclude the publication of the source data in publicly available repositories. However, individual data access may be granted within a framework of scientific cooperation.

AUTHORS' CONTRIBUTION

LPB conceived of the study question, analyzed data, and drafted the manuscript. KUS, LP, and BS participated in data analysis. BS and BH contributed to the coordination of the study. HB conducted the ESTHER study and contributed to all aspects of this work. All authors contributed to manuscript revision and read and approved the final manuscript.

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COMPETING INTERESTS

None of the authors have competing interests.

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Table 1. Descriptives of two subsets[a] of the ESTHER study, an epidemiological study of the elderly general population in Germany.

Characteristic		Dataset 1	Dataset 2
Total	n	969	851
Age in years	μ (SD)	62.1 (6.5)	63.0 (6.7)
Methylation age in years	μ (SD)	61.7 (7.1)	64.6 (7.7)
Age acceleration in years[b]	μ (SD)	-0.5 (5.0)	1.6 (5.3)
Relative telomere length	μ (SD)	1.22 (0.31)	1.03 (0.27)
Frailty index (in %)	μ (SD)	25.0 (14.7)	25.5 (15.1)
Sex			
females	n (%)	484 (50.0)	464 (54.5)
males	n (%)	485 (50.1)	387 (45.5)
Smoking behavior[c]			
never	n (%)	455 (48.0)	371 (45.0)
former	n (%)	320 (33.7)	284 (34.5)
current	n (%)	174 (18.3)	169 (20.5)
Alcohol consumption[c]			
none	n (%)	300 (33.6)	260 (33.7)
<20 g/d (women), <40 g/d (men)	n (%)	524 (58.6)	458 (59.4)
20+ g/d (women), 40+ g/d (men)	n (%)	70 (7.8)	53 (6.9)
History of cancer[c]			
self-report negative	n (%)	882 (93.2)	742 (90.5)
self-report positive	n (%)	64 (6.8)	78 (9.5)

[a] Dataset 1: consecutively recruited subsample of the source study. Dataset 2: sampled in the context of a case-cohort study. Details see text.
[b] Difference-based age acceleration, i.e. methylation age - chronological age.
[c] Missing values (dataset 1, dataset 2) in smoking (20, 27), alcohol consumption (75, 80), and history of cancer (23, 31).

Table 2. Results of linear mixed regression models[a] predicting relative telomere length (RTL) from difference-based methylation age acceleration. Shown is the estimated change (95% confidence interval) in RTL per year of age acceleration.

Covariables	Dataset 1	Dataset 2	Overall
none	0.0013 (-0.0017, 0.0043)	-0.0016 (-0.0044, 0.0012)	0.0000 (-0.0021, 0.0021)
age	-0.0009 (-0.0039, 0.0022)	-0.0031 (-0.0060,-0.0003)	-0.0018 (-0.0039, 0.0003)
age, sex	-0.0004 (-0.0035, 0.0027)	-0.0021 (-0.0050, 0.0007)	-0.0011 (-0.0032, 0.0010)
age, sex, leucocyte distribution (LD)	0.0001 (-0.0033, 0.0034)	-0.0014 (-0.0044, 0.0016)	-0.0006 (-0.0028, 0.0017)
age, sex, LD, smoking	0.0001 (-0.0033, 0.0035)	-0.0015 (-0.0046, 0.0015)	-0.0006 (-0.0028, 0.0017)
age, sex, LD, alcohol	-0.0002 (-0.0036, 0.0033)	-0.0017 (-0.0048, 0.0015)	-0.0009 (-0.0032, 0.0015)
age, sex, LD, history of cancer	0.0001 (-0.0032, 0.0035)	-0.0017 (-0.0047, 0.0013)	-0.0006 (-0.0029, 0.0016)
age, sex, LD, interaction (age accel. with sex)			
estimate in females	-0.0020 (-0.0064, 0.0023)	-0.0014 (-0.0054, 0.0026)	-0.0011 (-0.0041, 0.0018)
estimate in males	0.0024 (-0.0022, 0.0070)	-0.0014 (-0.0056, 0.0028)	0.0001 (-0.0030, 0.0032)

[a] All models are adjusted for methylation array batch and telomere assay batch using a random effect.

Table 3. Results of linear mixed regression models[a] predicting the frailty index (FI) from difference-based methylation age acceleration. Shown is the estimated change (95% confidence interval) in FI (expressed in %) per year of age acceleration.

Covariables	Dataset 1	Dataset 2	Overall
none	-0.023 (-0.208, 0.162)	0.087 (-0.107, 0.281)	0.039 (-0.092, 0.170)
age	0.167 (-0.019, 0.353)	0.214 (0.021, 0.407)	0.183 (0.053, 0.313)
age, sex	0.183 (-0.005, 0.371)	0.242 (0.046, 0.439)	0.201 (0.069, 0.333)
age, sex, leukocyte distribution (LD)	0.250 (0.047, 0.453)	0.274 (0.068, 0.481)	0.255 (0.115, 0.396)
age, sex, LD, smoking	0.243 (0.038, 0.448)	0.282 (0.074, 0.491)	0.256 (0.113, 0.398)
age, sex, LD, alcohol	0.289 (0.080, 0.497)	0.280 (0.070, 0.490)	0.277 (0.133, 0.421)
age, sex, LD, history of cancer	0.234 (0.030, 0.437)	0.275 (0.067, 0.484)	0.250 (0.108, 0.391)
age, sex, LD, interaction (age accel. with sex)			
estimate in females	0.304 (0.036, 0.572)	0.246 (-0.026, 0.519)	0.269 (0.082, 0.455)
estimate in males	0.190 (-0.089, 0.469)	0.307 (0.013, 0.601)	0.241 (0.046, 0.436)

[a] All models are adjusted for the methylation array batch using a random effect.

Table 4. Linear regression models[a] predicting frailty from relative telomere length (RTL) within tertiles of difference-based methylation age accleration. Shown is the estimated change (95% confidence interval) of the frailty index (expressed in %) per standard deviation of RTL.

Stratum of age acceleration	Dataset 1	Dataset 2	Overall
Tertile 1 (below -1.85 years)	0.132 (-1.253, 1.517)	-0.809 (-3.548, 1.930)	-0.106 (-1.274, 1.063)
Tertile 2 (-1.85 to <2.43 years)	-0.982 (-2.635, 0.672)	-0.404 (-2.266, 1.459)	-0.506 (-1.689, 0.678)
Tertile 3 (>=2.43 years)	0.960 (-0.763, 2.682)	-0.135 (-1.881, 1.611)	0.338 (-0.829, 1.504)

[a] All models adjusted for age, sex, leukocyte distribution, and random effects of telomere and methylation array batch.

Supplementary Figure S1