Age-Associated Genetic and Environmental Contributions to Epigenetic Aging Across Adolescence and Emerging Adulthood

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Abstract

Background

Epigenetic aging estimators commonly track chronological and biological aging, quantifying its accumulation (i.e., epigenetic age acceleration) or its speed (i.e., epigenetic aging pace). These estimates reflect a combination of inherent biological programming and the impact of environmental factors, which are suggested to vary at different stages of life. The transition from adolescence to adulthood is an important period in this regard, marked by an increasing

and, then, stabilizing epigenetic aging variance. Whether this pattern arises from environmental influences or genetic factors remains uncertain.

This study delves into understanding the age-associated genetic and environmental contributions to differences both between and within individuals across these developmental stages. For this purpose, we analyzed four differently developed epigenetic aging estimators, namely, Horvath Acceleration, GrimAge Acceleration, PedBE Acceleration, and DunedinPACE, which were collected from 976 twins aged 8 to 31 years (*M*=16.0, *SD*=6.05) measured twice, two years apart.

Results

Approximately half (33-76%) of the between-individual differences in epigenetic aging and most (70-99%) of the variance in within-individual changes across the two years were attributable to unique environmental factors. The contribution of these factors to the variance of epigenetic estimators trained on chronological age notably increased with time (Horvath Acceleration: from 80% to 90%; PedBE Acceleration: from 70% to 99%).

Genetic contributions were more pronounced in the between-individual differences than in the variance of within-individual changes. For epigenetic aging estimators trained on chronological age, both additive genetic factors (8%-39%) and shared environmental influences (13%-49%) contributed to the variance. For epigenetic estimators trained on biological indicators, either additive (GrimAge Acceleration) or additive and non-additive genetic (DunedinPACE) factors were relevant. The variance of three estimators, initially developed in adult samples, demonstrated increasing contributions of genetic factors to the variance across adolescence and emerging adulthood (Horvath Acceleration: from 18% to 39%; GrimAge Acceleration: from 24% to 43%; DunedinPACE: from 42% to 57%).

Conclusions

Our findings suggest that increasing between-individual differences in epigenetic aging during adolescence and emerging adulthood are the result of both unique life experiences and genetic underpinnings, whereas variance in within-individual changes is primarily attributable to unique life experiences.

Keywords Epigenetic aging, Age-moderation, Adolescence, Gene-environment interaction, the Horvath clock, the PedBE clock, GrimAge, DunedinPACE.

Background

Estimators of epigenetic aging offer insights into how individuals age over time and across age groups [20]. They are predictive of age-related diseases and mortality in adults [34, 45], worse cognitive functioning [46] and socioeconomic disadvantage [47] in adolescents and children. By measuring DNA methylation (DNAm) levels at specific DNA loci, epigenetic estimators address universal and specific aspects of aging processes. For example, the Horvath clock, which was trained to predict chronological age in samples with a large age range, considers differences between chronological and epigenetic age to indicate accelerated or decelerated aging across the whole life span [19]. Similarly, in buccal samples of children and adolescents, the pediatric-buccal-epigenetic (PedBE) clock illuminates the specific aging processes in younger age groups [37]. Alternatively, epigenetic aging estimators have been developed on the basis of links between aging and biological health indicators. Examples of such measures include the biomarker of human mortality risk GrimAge [31, 32] and the biomarkers of the pace of aging, DunedinPoAm and DunedinPACE [3, 4]. These measures account for DNAm at locus-specific sites associated, for example, with plasma proteins and smoking (GrimAge) or biomarkers of organ systems integrity, such as blood pressure or serum leptin levels (Dunedin). In addition, Dunedin measures are trained on longitudinal data and represent, in contrast to the degree of epigenetic aging, the pace of epigenetic aging [3, 4].

The strength of the prediction by epigenetic aging measures stems from their ability to represent both genetically driven aging processes and the cumulative effects of the environment on DNAm [27]. The contributions of genetic factors to the variance in epigenetic aging can be reflected in the narrow- and broad-sense *heritability*, the proportion of the phenotypic variance attributable to genetic differences at the time and age of measurement in a certain population. Narrow-sense heritability quantifies only the contribution of additive genetic factors, while broad-sense heritability also includes the interactions between genetic factors such as allelic dominance (i.e., interactions within specific gene loci of chromosomes) and epistasis (i.e., interactions across genetic variants between gene loci).

Figure 1 and Additional file 1: Table S1, summarizes previously reported estimates of SNP- [1], pedigree-[2], and twin-based[3] heritability for the aforementioned epigenetic measures. As shown in Figure 1, the narrow-sense heritability of the Horvath clock is not constant across particular ages. According to previous studies, heritability estimates vary from 39% to 61% in older adults, which is on average lower than the estimates obtained for young adults, ranging from 62% to 74% [19, 22, 25, 52]. In adolescents, pedigree- and SNP-based research revealed a lower heritability of the Horvath clock than in young adult samples, with a range from 37% to 43% [34, 53]. The possibility of extrapolating this reverse U-shaped trend in the heritability of the Horvath clock to other epigenetic aging measures and various DNAm resources remains less explored. For example, comparisons of heritability between epigenetic aging estimators are mainly confined to emerging adults where the Horvath clock, GrimAge, and pace of aging demonstrated similar heritabilities of 62-73% [26]. Knowledge on the comparability of heritability between tissues is rather limited, as most studies have focused on blood tissue.

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[1] Variance explained by all single nucleotide polymorphisms used in a genome-wide association study in conventionally unrelated individuals [64].
[2] Variance explained by the patterns of genetic resemblance among not-too-distantly related family members [17].
[3] Variance explained by the patterns of genetic resemblance between monozygotic and dizygotic twins.

Studying the age-dependent patterns of heritability across a broader spectrum of epigenetic aging measures based on noninvasive saliva samples could elucidate universal trends or tissue- and measure-specific differences in epigenetic aging.

The trend of epigenetic aging heritability across particular ages may arise from non-genetic environmental sources and their interaction with genetic factors. A design of twins reared together, for example, allows to disentangle environmental factors shared by twins, which increase twin siblings' similarity, from non-shared or individually unique environmental factors, which increase twin siblings' dissimilarity. Particularly in younger age, when twins live together in one household, shared environments may be important. These environments can encompass prenatal factors, social backgrounds, common parental and peer influences, and shared neighborhoods. Research on complex traits suggests that shared environmental and genetic factors may interact, particularly in younger people. With respect to the variance in epigenetic aging, the role of shared environmental factors was found to increase before adulthood when twins lived together [29]. Individually unique environmental factors are presented across the whole life span and can obtain greater importance when twins live in different households and have more separate life experiences. These environments can include stochastic changes and individual-specific experiences, such as different peer groups or differences in experiences of objectively shared environments. Unique environmental factors have been shown to moderate the influence of genetic factors on DNAm [15] and possibly have the same effect on epigenetic aging during adulthood [22]. These shifts in genetic and environmental contributions to the variance of epigenetic aging over the life course suggest the possible link between life stage processes and gene□environment interplay in epigenetic aging.

Adolescence and the transition to adulthood, so-called emerging adulthood, is potentially one of the periods of dynamic genetic and environmental contributions to the variance of epigenetic aging. The time before transition is a period of growth and development with a faster

change rate in DNAm and epigenetic age [1, 20]. Studies have shown, for instance, that this change rate peaks in infancy, decreases nonlinearly until approximately age 20, and then stabilizes [19, 20]. Variance in epigenetic aging can also undergo changes. Starting small at birth, the variance in the epigenetic age of the Horvath clock increases with chronological age until adulthood onset, and then stabilizes [29]. It remains unclear whether these trends of later stabilization in change rates correspond to trends in the relative contributions of genetic and non-genetic factors and which sources, genetic or environmental, are responsible for the growing and stabilizing variance of epigenetic aging during adolescence and the transition to adulthood.

To address this gap in the epigenetic literature, this study aimed to investigate the trends in genetic and environmental contributions to the variance in epigenetic aging across adolescence and emerging adulthood. We focused on between- and within-individual differences. With between-individual differences, we can examine how genetic and environmental sources contribute to the variance in epigenetic aging and how these sources shift across age groups. Analyzing within-individual differences would reveal, in addition, the genetic and environmental contributions to the variance in individual changes in epigenetic aging (approximately two years in this study), which allows us to investigate individual deviations from the common trends of epigenetic aging for different developmental stages. We examined four epigenetic aging estimates measured twice, two years apart. DNAm was obtained from DNA extracted from saliva samples of early (*M(age)* = 10.7 years) and late adolescents (*M(age)* = 16.6 years), emerging adults (*M(age)* = 22.7 years), and young adult twins (*M(age)* = 28.8 years). The epigenetic aging estimates included two acceleration measures trained on chronological age, the Horvath epigenetic clock [19] and the PedBE epigenetic clock [37], and two epigenetic aging measures trained on biological indicators of aging, one of which, GrimAge [31], represents acceleration and the other, DunedinPACE, represents the pace of aging [4]. Comparing these epigenetic aging estimates allowed us to (a) assess the age-associated trends in

the genetic and environmental contributions to between-individual differences and within-individual changes across adolescence and emerging adulthood, (b) compare the genetic and environmental contributions to the variance in differently developed epigenetic aging estimators, and (c) examine whether heritability estimates of epigenetic aging assessed based on saliva are similar to those that have previously been observed in other studies of blood samples (see Figure 1 and Additional file 1: Table S1).

Figure 1

The SNP-, pedigree-, and twin-based heritability of epigenetic aging estimated in previous studies

Note. The estimates of SNP-, pedigree-, and twin-based heritability are presented for Horvath Acceleration (purple), GrimAge Acceleration (green), and epigenetic aging pace measures, DunedinPoAm and DunedinPACE (yellow).

Methods

Sample

The research questions were examined in a sample of 976 twins from the TwinLife Epigenetics Change Satellite (TECS) project, which is a subsample of the German Twin Family Panel (TwinLife). TwinLife is a longitudinal study of four cohorts of same-sex twin pairs and their families recruited from the general population of Germany [11]. The study focuses on longitudinal measures of social inequality and the potential underlying biopsychosocial mechanisms [18]. The current analysis sample consisted of 263 monozygotic (MZ) and 225 dizygotic (DZ) complete twin pairs aged 8 to 29 years at the time of the first epigenetic measurement (M = 16.0) and aged 10 to 31 years at the second measurement (M = 18.3). At the time of assessment, members of the four cohorts were in the ages of early adolescence, late

adolescence, emerging adulthood, and young adulthood, respectively (for more details see Table 1).

DNA methylation

Saliva sampling and DNA extraction

Saliva sampling for DNA extraction was conducted as a part of two TwinLife satellite projects, namely TwinSNPs and TECS. Twins and their family members were invited to participate in the molecular genetic analysis during TwinLife face-to-face interviews carried out in 2018–2020. During face-to-face interviews, interviewers offered to participate in the molecular genetic studies, which involved genotyping and epigenetic profiling. A short video explaining the study goals and procedures of the saliva sampling accompanied the recruitment procedure. The second saliva collection took place in 2021. This time, participants were contacted by mail. In addition to the invitation to participate, respondents received consent forms, saliva collection toolkits, and corresponding instructions. For all participants who provided valid consent, saliva samples were sent to the researchers at the University Hospital Bonn, Germany. The samples were collected using Oragene® saliva self-collection kits (OG-500 and OG-600; DNA Genotek, Canada) and DNA was extracted following the manufacturer's protocol.

DNA methylation assessment

The assessment of DNA methylation profiles was conducted in a set of 2 128 samples, of which the present sample constituted a subset. The samples were randomized by age, sex, zygosity, and complete versus single twin pair across 23 plates (266 arrays) using the R package "Omixer" (version 1.4.0; [54]). The family identifier was used as a blocking variable to ensure that all samples and timepoints of a twin pair ran on the same batch/array. Bisulfite-conversion

of 500 ng of DNA was performed using the D5033 EZ-96 DNA Methylation-Lightning Kit (Deep-Well; Zymo Research Corp., Irvine, USA), and methylation profiles were assessed using the Infinium MethylationEPIC BeadChip v1.0 (Illumina, San Diego, CA, USA).

Preprocessing of the methylation samples (*n* = 2 128) was performed following a standard pipeline by Maksimovic et al. [33] using the R package “minfi” (version 1.40.0; [2]). The following sample exclusion criteria were applied: a mean detection *p* value > .05 (*n* = 3) and sex mismatches between estimated sex from methylation data and confirmed phenotypic sex (*n* = 14). To normalize the beta values, we applied stratified quantile normalization [58], followed by BMIQ [56]. In further analysis, we removed probes containing SNPs (*n* = 24 038), X or Y chromosome probes (*n* = 16 263), cross-hybridizing probes (*n* = 46 867) and polymorphic probes (*n* = 297) according to Chen et al. [8] and McCartney et al. [36], and probes with a detection *p* value > .01 in at least one sample (*n* = 79 922). The batch effects were corrected for transformed M-values using Combat [28]. For this purpose, we examined the strength of associations between three possible batches (plate, slide, array) and the first five principal components via principal component analysis. The strongest batch effects were iteratively removed. Sample mix-ups were checked with MixupMapper [60]. The comparison of beta values with the existing genotype data (assessed using Global Screening Arrays [GSA+MD-24v3.0-Psych-24v1.1, Illumina, San Diego, CA, USA]; for further details see [9]) revealed four mix-ups. For these four cases, both samples (first and second measurements) were removed (total of *n* = 8 individual samples). The resulting DNA methylation dataset comprises 2 102 samples of *n* = 1 055 participants (among which *n* = 488 complete twin pairs or 976 individuals with two timepoints of DNAm data) and 698 472 probes.

The cell type composition was estimated using the R package “EpiDish” (version 2.10; [57]) and the epidish() function. Because saliva consists of three (highly correlated) cell type

estimates (leukocytes, epithelial cells, fibroblasts), we obtained the first principal component at each occasion separately to control for cell type composition in the following analyses.

Calculating epigenetic age, epigenetic age acceleration, and pace of epigenetic aging

Four measures of epigenetic aging were calculated based on the normalized batch-corrected beta values: the Horvath clock [19], the PedBE clock [37], GrimAge2 (an improved version of the original GrimAge; [31,32]), and DunedinPACE estimate ([4]; Additional file 2: Figure S1). The Horvath and PedBE epigenetic age were calculated using the R package "methylclock" (version 1.01; [42]). A total of 334 (94.6%) of the original 353 Horvath clock CpG sites and all 94 CpG sites of the PedBE clock were present in the dataset. Version 2 of GrimAge was computed based on 1 029 CpG sites present in our dataset (out of 1 030 mentioned in the publication) using analysis code from Lu et al. [31] provided by A. Lu and S. Horvath via personal correspondence. The DunedinPACE values were calculated with the DunedinPACE R package (https://github.com/danbelsky/DunedinPACE/) using all of 173 CpG sites.

The four epigenetic age estimates were significantly positively correlated with chronological age (r(Horvath epigenetic age) = .80 (p < .001), r(PedBE epigenetic age) = .77 (p < .001), r(GrimAge) = .80 (p < .001), and r(DunedinPACE) = .08 (p < .001); Additional file 2: Figure S2). For the Horvath epigenetic age, the Median Absolute Error (MAE) estimates ranged from 2.75 to 3.70 (3.26 to 3.65 for early adolescents, from 2.75 to 2.93 for late adolescents, from 3.33 to 3.34 for emerging adults, and from 2.89 to 3.71 for young adults). For the PedBE epigenetic age, the MAE estimates ranged from 1.07 to 12.72 (2.22 to 2.91 for early adolescents, from 1.08 to 2.01 for late adolescents, from 6.49 to 7.51 for emerging adults, and from 11.61 to 12.72 for young adults). For GrimAge, the MAEs ranged from 19.38 to 25.60 (22.96 to 24.38 for early adolescents, from 25.42 to 25.60 for late adolescents, from 21.84 to 22.57 for emerging adults, and from 19.39 to 20.25 for young adults; Additional file 2: Figure S3).

Acceleration estimates for the Horvath, PedBE, and GrimAge epigenetic ages were obtained as follows: epigenetic age was regressed on chronological age (in years and decimal months), biological sex, and cell-type composition within each measurement occasion. In addition, the Horvath and PedBE epigenetic ages were regressed on smoking exposure, which was estimated based on hypomethylation of the AHRR probe [43]. The resulting residuals (i.e., Horvath Acceleration, PedBE Acceleration, and GrimAge Acceleration), defined as "age acceleration" (positive score) or "age deceleration" (negative score) according to the direction of the deviation, were used in our analyses. The DunedinPACE values were controlled for smoking probe, biological sex, and cell type composition. For DunedinPACE, a value of one reflects a pace of aging in line with 1 year of chronological aging, with a value > 1 reflecting an increased pace and values < 1 reflecting a decreased pace of aging.

Analytical strategy

To examine the role of genetic and environmental factors in epigenetic aging, biometrical twin variance decomposition in structural equation modeling was applied [39]. MZ twins share close to 100% of their genetic makeup, while DZ twins share, on average, 50% of genetic variants that differ among humans. As a consequence, the correlation of genetic factors is approximately 1 for MZ twins, whereas it is assumed to approach .5 for additive genetic factors and .25 for non-additive genetic factors due to allelic dominance deviation for DZ twins. Therefore, a greater correlation within MZ twin pairs than in DZ twin pairs signals the presence of genetic influences on the variance in a specific phenotype, such as epigenetic aging. Furthermore, both MZ and DZ twins are assumed to share some prenatal and familial factors [6] that act to increase their resemblance beyond genetic factors to the same extent. Therefore, these shared environmental factors contribute to the similarity within MZ twin pairs to the same degree as within DZ twins. Only individual-specific unique environmental factors are assumed to

increase the dissimilarity between monozygotic twin siblings. These assumptions lie in the essence of the classical biometrical model, which allows for a decomposition of the observable variance into variance attributable to genetic factors (*A* – additive genetic and *D* – non-additive genetic effects) and environmental influences (*C* – shared by twins and *E* – unique environment; [6]). For identification purposes, only three sources of variance can be estimated simultaneously in the classical twin model. Depending on the pattern of the correlations within the MZ and DZ twins, either *D* (if rMZ < 2 × rDZ) or *C* (if rMZ > 2 × rDZ) components have to be fixed to zero, resulting in the specification of either ACE or ADE models [12].

First, we started with the analysis of the between-individual differences and specified univariate variance decomposition models for each epigenetic aging estimator. Four age groups with two measures each produced eight unique timepoints analyzed in one model. We set the variance of all latent variables (A, C/D, E) to 1 for identification purposes and ensured that the statistical conditions specified for twin modeling, namely nonsignificant differences in means and variances across twin order and zygosity, were met in our sample. Then, we decided whether to use ACE or ADE models. After that, the model that fit to the data at best for each of the four epigenetic aging measures was identified, and the standardized coefficients with 90% confidence intervals were calculated. We have chosen among the ACE, AE, CE, and E models or, alternatively, among the ADE, AE, and E models. Among the two models that did not differ significantly based on the chi-square test, the model with a lower Akaike information criterion (AIC) was considered to fit the data better. Among the two models that differed significantly based on the chi-square test, the model with the higher number of parameters was considered as the model that best fit the data [14].

In a second step, we tested whether the age of participants moderated genetic and environmental contributions to the variance in epigenetic aging to unveil possible gene□environment interaction [44]. Since gene□environment interaction effects cannot be

directly assessed in classical twin models, the estimates of genetic and environmental contributions could be confounded with interaction effects of these two [44]. In the case of interactions between additive genetic factors (*A*) and environmental factors that are shared by siblings reared together (*C*), interaction effects would be confounded with estimates of additive genetic effects. Potential interaction effects between genetic factors (*A*) and environmental factors not shared by siblings reared together (*E*) would be confounded with estimates of nonshared environmental effects. While the first interaction pattern is more plausible in younger people, when twins are still living in a common household, the latter is more plausible in adult years, when twins are living in different households and having more separate life experiences. As a consequence, higher estimates of genetic contributions to the variance in epigenetic aging are plausible in adolescence and emerging adulthood with declining trends across adulthood due to accumulating effects of individually unique experiences that may or may not interact with genetic factors.

Adding age as a moderator within the estimated structural equation models partitioned the variance of ACE/ADE components into 3 paths of non-moderated ACE/ADE components, 3 paths of the moderated ACE/ADE components, age moderating effect on means, and independent linear and quadratic effects of age, producing in total a model with 9 parameters. The model was still identified as the saturated model had in total 10 measured parameters. We started with the full models (with 9 estimated parameters) and then dropped nonsignificant paths to obtain more parsimonious models with more degrees of freedom. We excluded parameters until we reached the model that fit the data best, taking model parsimony into account (i.e., a model with a lower AIC that did not fit the data significantly worse based on the chi-square test). The variance components, confidence intervals, and standardized estimates were then derived.

To investigate the role of genetic and environmental sources of variance in within-individual two-year changes, we first calculated the individual change in epigenetic aging

measures, obtained as the difference between the second and first measurements of epigenetic aging (referred to as Δ in the following). The means and standard deviations for two-year changes in four epigenetic aging measures within each age group were calculated. Then, we repeated the statistical analysis described above. Similar to the analysis of between-individual differences, we started with univariate variance decomposition models for each epigenetic aging estimator and then examined the moderation of age. This resulted in four estimates of the genetic and environmental factors across ages from 8 to 31 for each epigenetic aging estimator.

For sensitivity analysis, we scrutinized the models with age moderation further not controlling the epigenetic aging measures either for sex, smoking probe, or cell type composition. We repeated the age moderation analysis, first, for all chosen epigenetic aging measures unadjusted for sex, second, for three epigenetic aging measures unadjusted for smoking, and, finally, for four epigenetic aging measures unadjusted for cell type composition.

The analyses were performed in R studio 2023.06.2 with R version 4.2.3. For data preparation, we used the R packages “tidyverse” [61], “ltm” [49], “dlyr” [16], and “psych” [48]. For the variance decomposition, we used “OpenMX” [40].

Prior power analysis

For the estimation of power to detect model misspecification, we applied the functions acePow() and powerValue() proposed by Verhulst [59] to univariate ACE models. For that purpose, we formulated two possible expectations regarding genetic factors (*A*) based on estimates obtained in larger sample studies. Previous research suggested a contribution of *A* 13% over the life course [29] and 37% at age 15 (combination with middle-aged mothers; [53]). Our expectation regarding shared environmental factors (*C*) was based on plots for MZ and DZ twins in Li et al. ([29]; page 8, Figure 4, cohabitation-dependent CE model). Here, we identified approximated estimates of *C* for our sample’s mean age of about 35% (for age 17.1). A power

calculation demonstrated that 976 pairs (526 MZ and 450 DZ twin pairs) in the between-individual difference analysis and 488 pairs (263 MZ and 225 DZ twin pairs) in the within-individual change analysis were sufficient to detect the genetic contributions mentioned in the literature (13% or 37%), given that 35% of the variance was attributable to shared environmental factors. The power for the analysis of between-individual differences was equal to .99 for both expected genetic variance components. For the analysis of within-individual changes, the power was good for higher estimates of genetic contributions to the variance (.96 given 37% of additive genetic factors) and still acceptable for lower estimates of genetic contributions (.90 given 13% of additive genetic factors).

Results

Descriptive analysis

The means and standard deviations for the epigenetic aging measures across age groups are presented in Table 1, the twin correlations of epigenetic aging measures for MZ and DZ twins for each timepoint are given in Table 2, and within-individual and within-twin pair rank-order stability estimates for MZ and DZ twins are shown in Additional file 3: Table S2.

(please add Table 1 here)

(please add Table 2 here)

Trained on the chronological age, Horvath and PedBE Acceleration demonstrated acceleration in the groups of late adolescents (M = 16.6) and emerging adults (M = 22.7) and deceleration in two other groups of early adolescents (M = 10.7) and young adults (M = 28.8; see Table 1). GrimAge Acceleration was higher in late adolescents with a mean age of 17.75, emerging adults (aged from 20 to 29), and at the onset of adolescence. The estimates of DunedinPACE showed a faster pace during age from 26 to 31 compared to other age periods. For the two-year changes in epigenetic aging measures, we observed a greater positive change

for the period of late adolescence (change from 14 to 18 years of age) and a higher negative change in the groups of early adolescents (change from 8 to 12 years of age) and young adults (change from 26 to 31 years of age), which potentially reflects the growing within-individual differences during these age periods.

Plotting the spread and locality of four epigenetic aging estimates across age groups demonstrated patterns of increased variance (Table 1 and Additional file 3: Figure S4). Two measures trained on chronological age, Horvath and PedBE Acceleration also showed a slightly decreasing trend during young adulthood. In the eldest age group (M = 29.92) compared to the youngest age group (M = 9.51), the standard deviation was 60% greater for Horvath Acceleration, 43% greater for PedBE Acceleration, 29% greater for both GrimAge Acceleration and DunedinPACE. For the individual two-year change in epigenetic aging, increasing standard deviation was also observed for Δ Horvath (30%) and Δ PedBE (50%) Acceleration. The standard deviation in Δ GrimAge Acceleration decreased by 9% and remained relatively stable for Δ DunedinPACE.

Twin correlations for Horvath and PedBE Acceleration demonstrated substantial similarity for adolescent twins, with only marginal differences between MZ and DZ twins (Table 2), indicating contributions of shared environmental influences to variance during this period of life. In groups of emerging adults and later, the correlations for MZ twins were greater than the correlations for DZ twins, indicating genetic influences on variance in epigenetic aging in adulthood. In contrast, Spearman correlations for GrimAge Acceleration and DunedinPACE were greater for MZ twins than for DZ twins across almost all age groups.

The within-individual rank-order stability across two measurement points was approximately .50 with no differences between the MZ and DZ twin groups for all measures except GrimAge Acceleration, where within-individual rank-order stability was lower in younger age groups. MZ within-twin pair rank-order stability estimates tended to be greater than those of

DZ twins in adulthood (Additional file 3: Table S2), indicating declining contributions of shared environmental factors and increasing genetic contributions to rank-order stability with age. Finally, the twin correlations for two-year individual change in epigenetic aging were much lower than the correlations regarding individual differences within measurement occasions for different ages, suggesting either proneness to measurement error in the prediction of chronological and biological age or the greater role of unique environmental factors contributing to variance in within-individual changes than to between-individual differences.

Univariate twin model analysis of between-individual differences in epigenetic aging

To perform univariate variance decomposition analysis of the between-individual differences in epigenetic aging measures, we first examined whether the statistical conditions specified for twin modeling were met (Additional file 3: Table S3). We observed small differences in means across twin siblings' order for Horvath Acceleration and DunedinPACE, but the models with the expected means and variances to be equal across twin siblings' order and zygosity did not fit the data significantly worse. For Horvath, PedBE and GrimAge Acceleration, cross-cohort MZ twin correlations were not more than twice as strong as DZ twin correlations, indicating that shared environmental factors (*C*) were more likely to contribute to the variance than non-additive genetic factors: rMZ = .51 < 2 × rDZ = .74 in the case of Horvath Acceleration, rMZ= .45 < 2 × rDZ = .96 in the case of PedBE Acceleration and rMZ = .32 < 2 × rDZ = .34 in the case of GrimAge Acceleration (see Table 2). The correlational patterns for MZ twins and DZ twins for DunedinPACE indicated, besides additive genetic factors, the presence of non-additive genetic factors (*D*): rMZ = .48 > 2 × rDZ = .38. Therefore, we focused on ACE variance decomposition in the following analysis for Horvath, PedBE, and GrimAge Acceleration and on ADE variance decomposition for DunedinPACE.

The results of the univariate twin model analyses of the epigenetic aging measures are presented in Figure 2 and Additional file 3: Tables S4-5. For Horvath Acceleration, we observed a better fit of the full model (ACE), indicating that the variance in Horvath Acceleration was best explained by additive genetic as well as unique and shared environmental factors. PedBE Acceleration, GrimAge Acceleration, and DunedinPACE estimates in the univariate analysis were better represented by restricted models (the CE model for PedBE Acceleration and the AE models for GrimAge Acceleration and DunedinPACE). The variation in PedBE acceleration was thus best explained by shared and unique environmental factors, whereas the variance in GrimAge Acceleration and DunedinPACE was best explained by additive genetic and unique environmental factors.

Figure 2

Cross-cohort standardized genetic and environmental variance components of epigenetic aging measures

Note. The estimates are derived from the best-fitting models in the univariate twin model analysis for between-individual differences (A) and variance in two-year changes (B) in epigenetic aging measures.

Age moderation of genetic and environmental between-individual differences in epigenetic aging

Then, we explored whether the relative contributions of the *A*, *C* (or *D*), and *E* components to the between-individual differences of epigenetic aging differed across age groups (Table 3, Figure 3, Additional file 3: Tables S6-7, Figure S5). Here, we only present the models that fit the data best (the model fit statistics can be found in Additional file 3: Table S6).

When moderation by age in the between-individual differences models was allowed, moderation was observed for all epigenetic aging measures. For acceleration measures trained on chronological age, Horvath and PedBE Acceleration, we observed the moderation of the unique environmental component (*E*), which increased for both estimators. The proportion of the unique environmental factors in the between-individual variance of the Horvath and PedBE Acceleration increased from 47% to 49% and from 33% to 68% respectively. Moderation of the variance attributable to genetic components, which increased from early adolescence to adulthood, was found for Horvath and GrimAge Acceleration (increasing additive genetic component) and for DunedinPACE (increasing non-additive genetic component). The narrow-sense heritability increased from 18% to 39% for Horvath Acceleration and from 24% to 43% for GrimAge Acceleration. The broad-sense heritability (*A* + *D*) of DunedinPACE increased from 42% to 57%.

(please add Table 3 here)

Figure 3

Unstandardized and standardized variance components derived from age-moderated models of between-individual differences

Note. The estimates are derived from the best-fitting univariate models of age moderation of genetic and environmental components of between-individual differences in the four epigenetic aging measures. Unstandardized variance components moderated by age are depicted as curves with filled areas.

Univariate twin model analysis of variance in two-year changes of epigenetic aging

In a further step, we decomposed the variance in two-year within-individual changes, quantified as the difference in epigenetic aging measures across two timepoints (Figure 2, Additional file 3: Tables S8-9). The models with the expected means and variances to be equal

across twin order and zygosity did not fit the data significantly worse than did the saturated models (Additional file 3: Table S3) confirming that the statistical conditions specified for twin modeling were met. MZ correlations were not more than twice as large as DZ correlations indicating the possible contributions of shared environmental factors (*C*) rather than non-additive genetic factors (*D*) to the variance in the four epigenetic aging measures.

The results showed that the variance of two-year within-individual changes in the epigenetic acceleration measures, Δ Horvath, Δ GrimAge, and Δ PedBE Acceleration, was best explained by shared and unique environmental factors (CE model), whereas the variation in two-year changes of DunedinPACE was best explained by additive genetic and unique environmental factors (AE model). For all epigenetic aging measures, however, unique environmental factors not shared by twins were found to be the primary source of differences in changes in epigenetic aging (Additional file 3: Table S9). It is important to note that, in contrast to the a priori power analysis, we did not have enough power to distinguish between the CE and AE models and between the CE and E models for Δ Horvath and Δ GrimAge Acceleration.

Age moderation of genetic and environmental variance in two-year changes in epigenetic aging

In the final step, we investigated whether the genetic and environmental contributions to the variance in the two-year changes in epigenetic aging estimates varied with chronological age (Table 4, Figure 4, Additional file 3: Tables S10-11, Figure S6). For three epigenetic acceleration measures, Δ Horvath, Δ PedBE, and Δ GrimAge, the models with age moderation of the non-genetic component demonstrated a better fit to the data. It was the AE model with the moderation of the unique environment component (*E*; increasing trend) for Δ Horvath Acceleration, the CE model including moderation of the shared environmental component (*C*; decreasing trend) and the unique environment component (*E*; increasing trend) for Δ PedBE

Acceleration, and the CE model including moderation of the unique environment component (*E*; decreasing trend) for Δ GrimAge Acceleration. In two models (Δ Horvath and Δ PedBE Acceleration), we had sufficient power to identify the presence of the moderation of unique environmental contributions. For Δ DunedinPACE, the CE model without moderation of the genetic and environmental factors showed no worse fit to the data, possibly reflecting the difference in the estimations of epigenetic age acceleration and pace of epigenetic aging.

(please add Table 4 here)

Figure 4

Unstandardized and standardized variance components derived from age-moderated models of the two-year changes

Note. The estimates are derived from the best-fitting univariate models of age moderation of genetic and environmental components of two-year changes in the four epigenetic aging measures. Unstandardized variance components moderated by age are depicted as curves with filled areas.

Sensitivity analysis

For sensitivity analysis, we performed three separate analyses of age moderation of between-individual differences and variance in within-individual changes in epigenetic aging measures unadjusted either for biological sex, a smoking probe, or cell-type composition. The model's goodness-of-fit statistics and the estimates of the squared standardized coefficients are presented in the Additional file 4. Noncontrolling for sex or smoking did not affect the conclusions of the observed moderation in the main analysis, with the exclusion of two models for within-individual changes in epigenetic aging where the power to distinguish between models was limited (Δ PedBE Acceleration and Δ DunedinPACE, Additional file 4: Tables S12-19). Not

controlling for cell type composition obscured some of the main analysis findings (Additional file 4: Tables S20-23). While the results were robust for Horvath Acceleration, not accounting for cell type composition in between-individual differences introduced moderation of the unique environment component for other epigenetic aging measures, eliminating the moderation of genetic components for GrimAge Acceleration and DunedinPACE. The analysis of the within-individual differences not controlled for cell type composition confirmed the moderation of unique environmental contributions to variance in Δ PedBE Acceleration, introduced it to DunedinPACE variance, and eliminated it in Δ GrimAge Acceleration. The analysis of epigenetic aging measures unadjusted for cell type composition did not reach sufficient power to distinguish between models with or without moderation. The observed pattern is potentially explained by the fact that different tissues have heterogeneous cell type compositions [55], which, in turn, can have a strong independent association with age [62]. Therefore, the unadjusted for cell-type composition measure of epigenetic aging reflects, in addition to the genuine age-associated signal of DNAm, heterogeneity in different cell types regarding DNAm, and the link between age and cell-type composition.

Discussion

In this study, we investigated age-associated differences in genetic and environmental contributions to the variance of epigenetic aging across adolescence and emerging adulthood. Two main results can be reported. First, common trends of increasing between-individual differences in epigenetic aging from adolescence to young adulthood were driven by both environmental and genetic influences, while individual deviations from this general trend of variance expansion were the result of unique environmental factors. Second, the proportions of genetic and environmental contributions to the variance varied for different epigenetic aging estimators.

Unique environmental contributions to age-associated differences in epigenetic estimators tracking chronological age and biological aging.

The results of the analysis suggest that during adolescence and emerging adulthood between-individual differences and variance in within-individual changes in epigenetic aging estimators trained on chronological age are driven by unique experiences. One explanation for these findings is the accumulated effect of unique life experiences. Childhood and adolescence are a sequence of sensitive and critical periods in development, during which the effects of positive and negative factors can be particularly influential. Experiences of violence in children or institutional caring [21], for example, have already been mentioned to predict greater epigenetic age, affecting ongoing brain and emotional regulation development [51]. An alternative explanation for our findings might be the *epigenetic drift*, stochastic changes in DNAm. These random changes in DNAm have been shown to contribute to progressively increasing epigenetic differences between genetically identical MZ twins over time [13]. When epigenetic drift happens in epigenetic estimators' locus-specific DNAm, the increase in unique environmental factors estimated in twin modeling would partly reflect epigenetic drift. This would also explain the greater power of epigenetic age to predict chronological age at the onset of life compared to childhood and adolescence [37, 53].

While we observed an increasing unique environmental contribution to the variance of the epigenetic aging measures tracking chronological age, unique environmental contributions did not increase in the epigenetic aging estimators trained on biological health indicators. For GrimAge Acceleration, we did not observe moderation of the unique environmental component in between-individual differences, while the environmentally driven variance in individual two-year changes demonstrated a decreasing trend. Consistently, no age-moderation of unique environmental factors was found for the pace of epigenetic aging DunedinPACE in either of the

two analyses. In contrast to epigenetic aging estimators trained on chronological age, the two later measures were developed in (exclusively adult) blood samples and rely on intrinsic biological functioning. In particular, GrimAge is linked to the level of plasma proteins [31] and DunedinPACE reflects among other factors, the level of glycated hemoglobin, leptin, and serum C-reactive proteins [4]. Because they are also intrinsically driven, these processes may be more elusive and less sensitive to trends in unique environmental contributions when they are measured in saliva.

The absence of age-moderation of unique environmental factors in DunedinPACE could also signal the difference between training designs across epigenetic aging estimators. While GrimAge, Horvath, and PedBE clocks were developed by analyzing cross-sectional data, DunedinPACE estimate was trained on longitudinal data, accounting for the within-individual trends of aging. Representing the speed of aging based on 12 months, DunedinPACE does not directly relate to chronological age and the accumulation of aging processes, which are commonly represented by acceleration measures. This could also explain the observed trends in the variances in epigenetic aging across age groups. In our analysis, late adolescents and emerging adults both demonstrated increasing variance in epigenetic age acceleration measures. In particular, the between-individual differences increased up to the ages 20-24 for Horvath and PedBE Acceleration and continued to do so in the older age group for GrimAge Acceleration. A similar pattern was found for Δ Horvath and Δ PedBE Acceleration. In contrast, for DunedinPACE, we observed, if at all, only a slight increase in variance. This was in line with the previous findings of the remarkable stability of DunedinPoAm, the predecessor of DunedinPACE, for the age period from 7 to 20 [41]. The smaller changes in variance and the absence of moderation in DunedinPACE suggest that the roles of unique environmental factors in the pace of epigenetic aging are potentially more stable than the environmental contributions to epigenetic acceleration measures.

The genetic contribution to age-associated between-individual differences in epigenetic acceleration and the pace of aging

Three out of four estimators, Horvath Acceleration, GrimAge Acceleration, and DunedinPACE, trained in adults, demonstrated an increase in the variance of between-individual differences across age due to genetic (additive or non-additive) factors. First, this pattern is consistent with longitudinal studies of the SNP-heritability of epigenetic aging and studies of complex phenotypes. SNP-heritability in children and their parents demonstrated that, starting at 0% during the onset of life, it increases up to 43% later in life [34, 53]. Similarly, genetic contributions to the variance in complex phenotypes, such as intelligence, depressive symptoms, and personality traits, demonstrate increasing heritability from the transition to adolescence and adulthood [5, 24, 38].

The observed phenomenon of the increased role of genetic factors during this age is commonly explained by active and evocative gene environment correlation in the presence of gene environment interaction, "…when people become more and more autonomous and self-directed during adolescence. They have increasing opportunities to pave their own way, evoke, select and create environments that match their heritable tendencies. These environments in turn can provide experiences that have the potential to reinforce the pre-existing tendencies" ([24]; p. 238). One illustrative example of an active gene environment correlation is the selection of the suitable environments by people that fit their genetic predispositions that prevail over the passive gene environment correlation effects created by the genetic background of parents [5, 23]. This would happen when, for instance, individuals with genetic predispositions for a slower epigenetic aging rather develop healthy habits and search for communities of similar individuals, eventually supporting and exacerbating this genetic tendency. As the increasing importance of active gene environment correlation would come along with an increase of genetic differences,

while a declining role of passive gene environment correlation would be reflected by decreasing effects of shared environmental influences [7, 44], our findings can be considered in line with this interpretation.

Alternatively, the gene environment interplay underlying estimates of genetic factors can reflect the interaction of genetic and shared environmental factors [24, 44], which is more plausible for twins reared together and, thus, more probable for them during adolescence, when they still share a common family household. Previous studies illuminating the role of shared environmental factors [30] prioritized the role of the prenatal environment and the driving role of cohabitation in epigenetic aging, implying that the longer relatives live together, the more similar they were epigenetically [29]. From adolescence to adulthood, the role of these factors seems to be steadily replaced by genetic and unique environmental forces as well as by interactions between them. As cohabiting is more common before adulthood, our findings extend previous conclusions, implying an increasing role of active gene environment correlation in the presence of interactions between genetic and environmental factors, when relatives, on average, transit to adulthood and tend to start living separately.

The transition to adulthood could explain why an increasing trend of genetic moderation was observed for all epigenetic aging measures but not for PedBE Acceleration. The age composition of the initial training samples in the chosen epigenetic estimators differed. In contrast to the PedBE clock, which was initially developed in a sample of individuals up to age 20, the Horvath clock, GrimAge, and DunedinPACE were trained in adults. Therefore, the increasing genetic contributions we observed for these three epigenetic measures might potentially characterize the intrinsic epigenetic processes that are activated later in life. These processes can include the trajectories of the physical development, for example, the transition from puberty to the maintenance of the biological systems and the high organism functionality. Alternatively, these processes can reflect the trajectories in gene environment interaction and

the relationships between individuals and their immediate environments. This would include, for instance, the transition from the sorting into fitting environments to the active creation and modification of environments or finding a balance between person environment fitting and adjustment [23]. Because these processes are less pronounced during adolescence, they may be not fully reflected in the genetic contributions to the variance in PedBE Acceleration.

The heritability of epigenetic aging based on saliva samples

The age-specific pattern of genetic contributions to the variance in epigenetic aging in saliva obtained in our analysis was rather consistent with the assessments reported in blood. However, our heritability estimates were, on average, lower than those reported in studies based on blood tissue samples. For example, previous research reported that genetic differences accounted for 69%, 73%, and 68% of the variance in Horvath Acceleration, GrimAge Acceleration, and DunedinPACE in 22-year-olds, respectively [26]. The broad-sense heritability for 21.5-year old twins in our analysis was substantially lower (32%, 35%, and 51% respectively) (see Additional file 3: Figure S7). This difference in results could be related to variations in the age compositions of the studies' samples, the presence of age moderation, or the genuine differences in epigenetic aging across tissues [63]. Therefore, further studies are required to investigate whether the observed pattern of the lower heritability of epigenetic aging based on saliva can be replicated.

Limitations and future research

Among the strengths of our study are the relatively large sample size, the focus on both epigenetic age acceleration and the pace of epigenetic aging measures, the examination of additive and non-additive genetic sources, and, finally, the consideration of age differences during the second and third decades of life. Nevertheless, our study is not without limitations.

First, despite the advantage of a comparatively large sample size with epigenetic data and usage of both measurement points in the biometrical variance decomposition, the post hoc power analysis demonstrated that the differentiation between several nested models with age as a moderator was limited, as confidence intervals overlapped. Earlier, power analyses in twin studies have suggested a minimum sample size of 600 twin pairs for univariate analysis and larger samples for detecting possible non-additive genetic factors, which, unfortunately, could not be reached [35, 50]. Nevertheless, we had enough power to detect the presence of genetic and environmental age moderation in the between-individual differences in epigenetic measures and the moderation of unique environmental factors contributing to the variance in within-individual two-year changes for epigenetic age acceleration.

Second, differences in the performance of epigenetic aging measures across different ages and tissues could have introduced additional sources of measurement error. The Horvath clock, for example, is less precise in younger samples, while the PedBE clock could be more biased in adults [37]. Similarly, our study exclusively utilized saliva samples, but not all applied epigenetic aging estimators were trained on saliva-based methylation profiles. While the Horvath clock is a pantissue epigenetic clock and the PedBE clock was developed in buccal cells, DunedinPACE and GrimAge were trained in blood. Although the pace of aging can be assessed based on saliva [46, 47] and the second version of GrimAge demonstrated good performance in saliva methylation data [31], applying these epigenetic estimators in a tissue different from the initial design may affect the estimates. The Horvath clock, at the same time, being a pantissue clock, could lose some precision when applied to any tissue.

Third, the utilization of measurements from two timepoints in four age groups could cause additional sources of variance. Different age groups, for instance, might introduce cohort effects that cannot be entirely disentangled from age effects. In addition, the second measurement occurred during the COVID-19 pandemic. While other research groups have not

confirmed the effect of the COVID-19 pandemic on epigenetic aging in adolescents [10], we assume that changes in health status, lockdowns, and restrictions are potential new influential environmental sources. Hence, the role of these factors in epigenetic aging dynamics should be clarified further.

Fourth, classical twin analysis is an efficient way to estimate genetic and environmental sources of variance but at the cost of several assumptions. One assumption is that environmental experiences shared by twins contribute to the similarity within MZ twin pairs to the same degree as within DZ twin pairs. One study showed that this assumption might not hold true for epigenetic aging, as MZ and DZ pairs can possibly share their preadult environmental effects to different extents [29]. In addition, the classical twin design does not allow, so far, the estimation of shared environmental (*C*) and non-additive genetic (*D*) contributions in one model, while both sources of variance can be relevant for variance in epigenetic aging at the same or during different developmental stages. These aspects should be addressed in further investigations.

Conclusion

Altogether, the present study demonstrated that the role of genetic and environmental sources of variance in epigenetic aging is not constant across adolescence and emerging adulthood. Increasing between-individual differences in epigenetic aging during the transition to adulthood were rather driven by increasing contributions of genetic and individually unique environmental factors. The individual trajectories of epigenetic aging were mostly independent of genetic sources and primarily driven by unique individual experiences. These findings contribute to a better understanding of the epigenetic aging process during the transition to adulthood.

Declarations

Ethics approval and consent to participate

The TwinLife study was reviewed and approved by the German Psychological Society (Deutsche Gesellschaft für Psychologie; protocol number: RR 11.2009). The TwinSNPs project received ethical approval from the Ethical Committee of Bielefeld University (No. 2020-180) and the TwinSNPs and TECS projects received ethical approval from the Ethical Committee of the Medical Faculty of the University of Bonn (Lfd. Nr. 113/18). All participants provided written informed consent. Participation was voluntary and participants could withhold participation at any timepoint. The personal data collection and analysis complied with the corresponding data protection regulations. The statistical analysis was performed on pseudonymized data from the servers of Bielefeld University.

Availability of data and materials

The TwinLife data are archived in the GESIS data catalog: https://search.gesis.org/research_data/ZA6701. The data are released for academic research and teaching after the data depositor's written authorization. The epigenetic data are available for collaborators via TwinLife data management.

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Competing interests

The authors report that there are no competing interests to declare.

Authors' contributions

DVK & BM were involved in the study conception and design. DVK analyzed the data and interpreted the results. DVK, YL, AMS, and CK drafted the manuscript. DVK, YL, AMS, DC, JI, CKLP, MMN, EBB, MD, AJF, CK, and BM provided valuable feedback for the manuscript revision. MD & MMN were responsible for obtaining ethical approval. MD, BM, MMN, AJF, CKLP, FMS, EBB, DC, and CK collected the data. AMS, DC, DVK, and CKLP assessed DNA methylation and generated the epigenetic aging scores. BM supervised the study. All the authors have read and approved the final manuscript.

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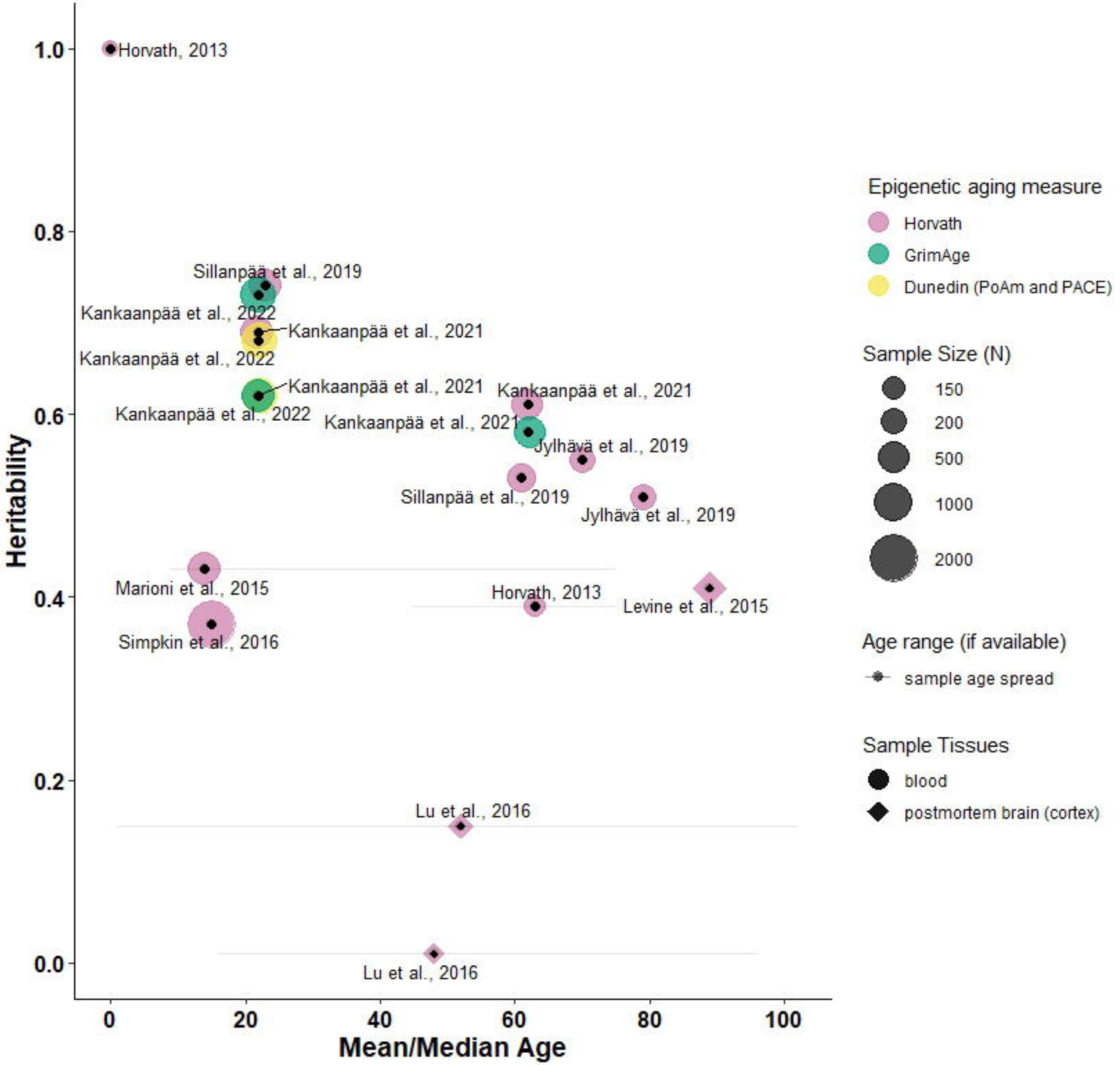
Additional files

Additional file 1. Previously published estimates of SNP-, pedigree-, and twin-based heritability of the analyzed epigenetic aging measures.

Additional file 2. Performance characteristics of epigenetic aging measures. The figures and tables represent CpG sites overlap between the epigenetic aging measures, Median Absolute Errors and mean deviations for three epigenetic age estimators.

Additional file 3. Supplementary Tables and Figures.

Additional file 4. Sensitivity analysis results. Tables S12-23 report goodness-of-fit statistics and standardized variance components for univariate age-moderated twin models of epigenetic aging measures unadjusted for biological sex, smoking probe, or cell type composition.


Horvath, 2013
Sillanpää et al., 2019
Kankaanpää et al., 2022
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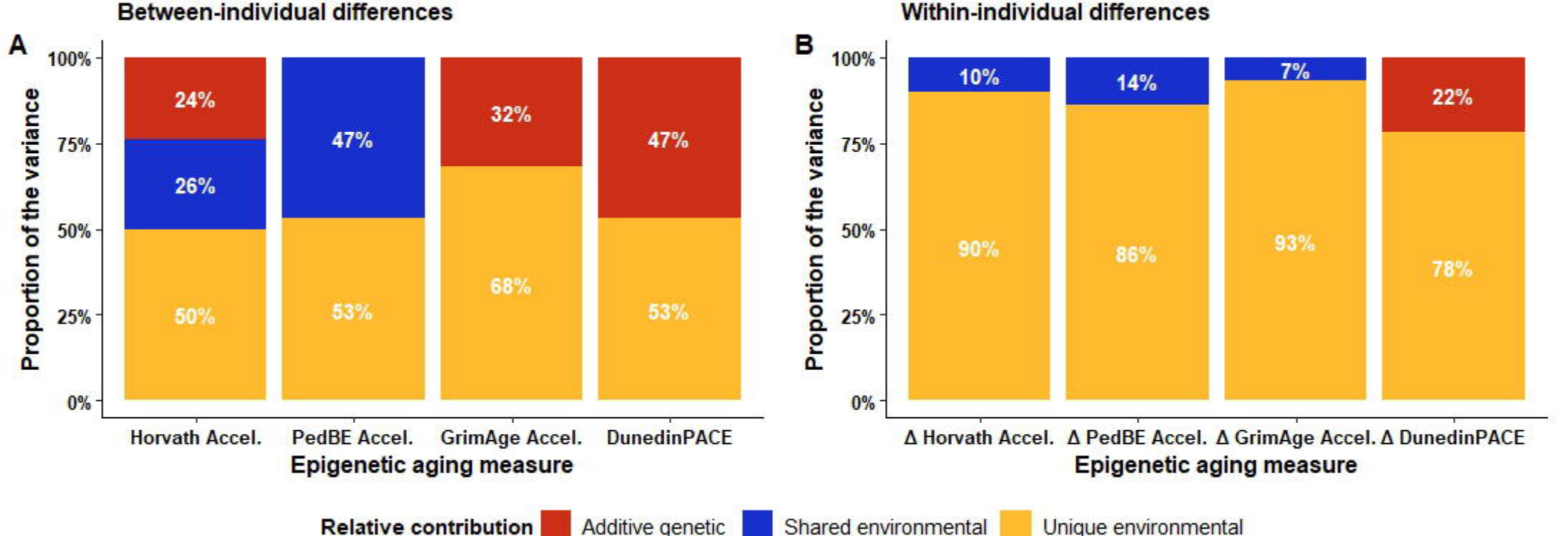
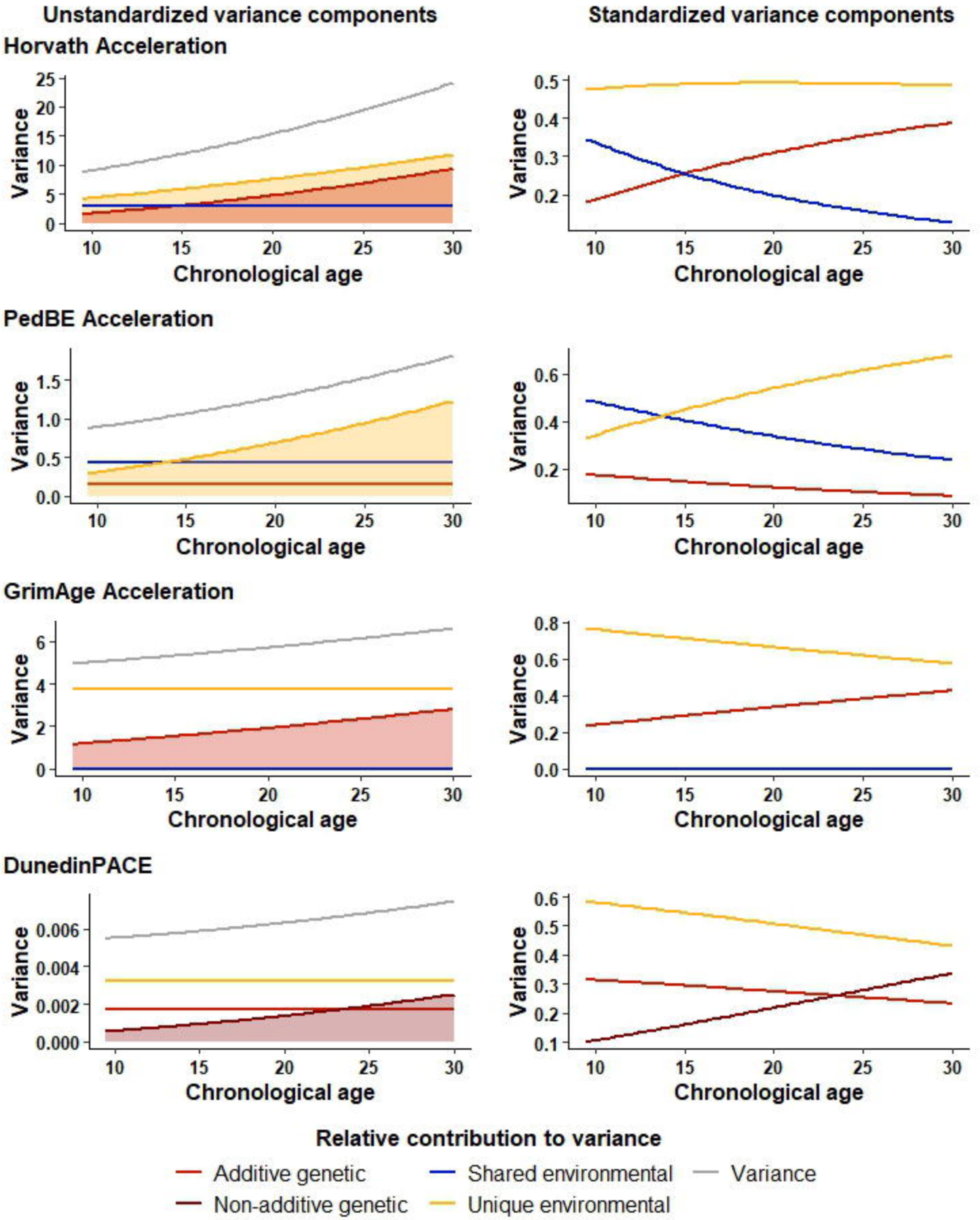
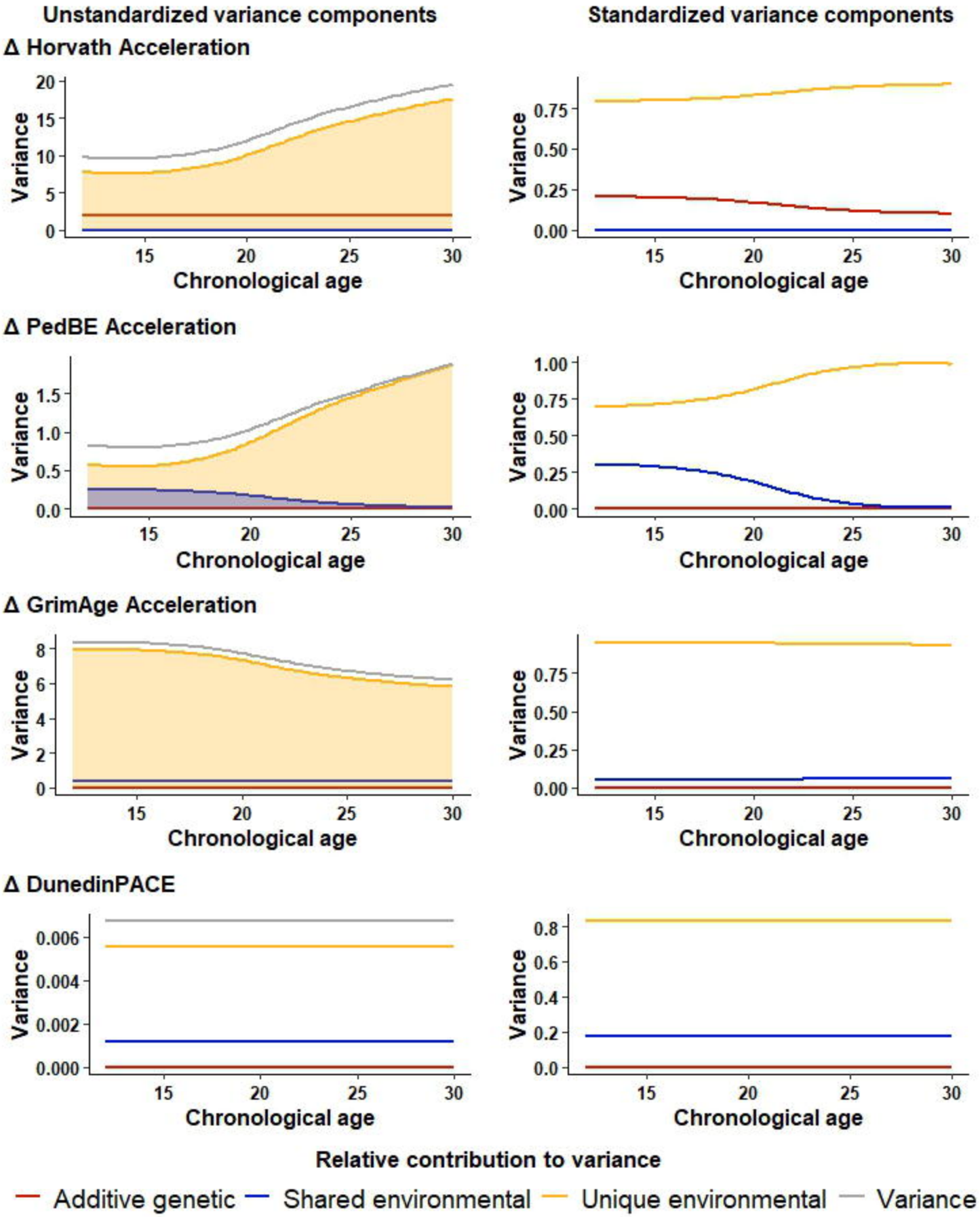
Kankaanpää et al., 2022
Kankaanpää et al., 2021
Kankaanpää et al., 2021
Jylhävä et al., 2019
Sillanpää et al., 2019
Jylhävä et al., 2019
Marioni et al., 2015
Horvath, 2013
Levine et al., 2015
Simpkin et al., 2016
Lu et al., 2016
Lu et al., 2016
Heritability
1.0
0.8
0.6
0.4
0.2
0.0
0
20
40
60
80
100
Mean/Median Age
Epigenetic aging measure
Horvath
GrimAge
Dunedin (PoAm and PACE)
Sample Size (N)
150
200
500
1000
2000
Age range (if available)
sample age spread
Sample Tissues
blood
postmortem brain (cortex)


Between-individual differences
A
100%
75%
50%
25%
0%
Proportion of the variance
24%
26%
50%
47%
53%
32%
68%
47%
53%
Horvath Accel.
PedBE Accel.
GrimAge Accel.
DunedinPACE
Epigenetic aging measure
Within-individual differences
B
10%
90%
14%
86%
7%
93%
22%
78%
Δ Horvath Accel.
Δ PedBE Accel.
Δ GrimAge Accel.
Δ DunedinPACE
Epigenetic aging measure
Relative contribution
Additive genetic
Shared environmental
Unique environmental


Unstandardized variance components
Standardized variance components
Horvath Acceleration
PedBE Acceleration
GrimAge Acceleration
DunedinPACE
Variance
Chronological age
Relative contribution to variance
Additive genetic
Non-additive genetic
Shared environmental
Unique environmental
Variance


Unstandardized variance components
Standardized variance components
Δ Horvath Acceleration
Δ PedBE Acceleration
Δ GrimAge Acceleration
Δ DunedinPACE
Variance
Chronological age
Relative contribution to variance
Additive genetic
Shared environmental
Unique environmental
Variance