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PTSD and epigenetic aging: a longitudinal meta-analysis

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Abstract

Background. Posttraumatic stress disorder (PTSD) has been associated with advanced epigenetic age cross-sectionally, but the association between these variables over time is unclear. This

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study conducted meta-analyses to test whether new-onset PTSD diagnosis and changes in PTSD symptom severity over time were associated with changes in two metrics of epigenetic aging over two time points.
Methods. We conducted meta-analyses of the association between change in PTSD diagnosis and symptom severity and change in epigenetic age acceleration/deceleration (age-adjusted DNA methylation age residuals as per the Horvath and GrimAge metrics) using data from 7 military and civilian cohorts participating in the Psychiatric Genomics Consortium PTSD Epigenetics Workgroup (total N = 1,367).
Results. Meta-analysis revealed that the interaction between Time 1 (T1) Horvath age residuals and new-onset PTSD over time was significantly associated with Horvath age residuals at T2 (meta β = 0.16, meta $p = 0.02$, $p\text{-}adj = 0.03$). The interaction between T1 Horvath age residuals and changes in PTSD symptom severity over time was significantly related to Horvath age residuals at T2 (meta β = 0.24, meta $p = 0.05$). No associations were observed for GrimAge residuals.
Conclusions. Results indicated that individuals who developed new-onset PTSD or showed increased PTSD symptom severity over time evidenced greater epigenetic age acceleration at follow-up than would be expected based on baseline age acceleration. This suggests that PTSD may accelerate biological aging over time and highlights the need for intervention studies to determine if PTSD treatment has a beneficial effect on the aging methylome.

Introduction

DNA methylation (DNAm)-derived epigenome-wide scores have emerged as leading biomarkers of biological age and death (e.g., Horvath, 2013; Lu et al., 2019) and are referred to as "DNAm age." Estimates of DNAm age may differ from chronological age, such that some individuals evidence advanced DNAm age relative to chronological age. Advanced DNAm age has been linked to age-related disease and adverse health outcomes, including metabolic syndrome, inflammation, neuropathology, and mortality (Chen et al., 2016; Christiansen et al., 2016; Gassen, Chrousos, Binder, & Zannas, 2017; Hillary et al., 2021; Levine et al., 2015; Marioni et al., 2016, 2015; Meier, Mitchell, Karadimas, & Faul, 2023; Miller & Sadeh, 2014; Reed, Carroll, Marsland, & Manuck, 2022), highlighting the applied significance of DNAm age as a biomarker of biological aging and its potential for identifying individuals at risk for early onset of age-related diseases.

Two of the DNAm age algorithms that have received the most attention to date are the Horvath (Horvath, 2013) marker of biological age and the Lu et al. (2019) index of time until death, referred to as "GrimAge." Both algorithms were trained through machine learning approaches to select a set of CpG sites from the epigenome that optimize prediction. The regression coefficients of the selected CpG sites are then used as weights in novel datasets to calculate the respective DNAm age estimates. Age-adjusted DNAm age is commonly defined as the residuals from regressing DNAm age on chronological age where negative values represent slowed epigenetic age and positive values represent advanced epigenetic age relative to chronological age.

Several studies have examined psychiatric diagnoses and symptoms in association with advanced DNAm age in blood using cross-sectional methods, including studies of depression (Liu et al., 2022) and alcohol use disorders (Rosen et al., 2018). Posttraumatic stress disorder (PTSD) has received particular attention in this regard, with numerous individual studies (Jovanovic et al., 2017; Katrinli et al., 2020; Roberts et al., 2017; Wolf et al., 2016; Wolf, Logue et al., 2018) and a meta-analysis (Wolf, Maniates et al., 2018) providing support for cross-sectional associations between PTSD and advanced DNAm age. Specifically, in the meta-analysis which was based on cross-sectional data from 9 cohorts contributing to the Psychiatric Genomics Consortium (5 of which contributed longitudinal data to these analyses), we previously found that the lifetime PTSD severity, but not PTSD diagnosis, and childhood trauma exposure (when measured with a consistent trauma measure across studies) were associated with increased epigenetic age (Wolf, Maniates et al., 2018). This is consistent with the hypothesis that the cumulative burden of PTSD symptom severity across the lifespan, including the chronic toll of physiological reactivity, poor sleep, anger, and arousal, may be most relevant for understanding risk for accelerated aging. Some studies have further linked PTSD-related advanced DNAm age with biomarkers of inflammation, metabolic pathology, and neuropathology (Morrison et al., 2019; Wolf et al., 2023), suggesting that individuals with PTSD may be at greater risk for developing early onset of these conditions. However, cross-sectional designs cannot address questions concerning the directionality of associations between PTSD and advanced DNAm age or the temporal stability of estimates of advanced epigenetic age over time, nor can they track how PTSD-related changes in epigenetic age accumulate and contribute to long-term health outcomes. Therefore, studying the longitudinal relationship between epigenetic aging and PTSD is crucial for understanding how trauma-related disorders contribute to premature aging and long-term health decline over time, as well as for developing targeted interventions that are matched to the pathophysiology that contributes to early onset of these health conditions.

To date, only a few studies have examined the relationship between PTSD and advanced DNAm age longitudinally, and the approach to modeling changes in DNAm age estimates over time has varied across studies, as have results. In a longitudinal cohort of 96 male Dutch soldiers assessed prior to war zone deployment and six months post-deployment, intervening combat trauma was found to be associated with an increase in raw (i.e., not accounting for chronological age) estimates of Horvath DNAm age over time (Boks et al., 2015). However, increased PTSD symptoms post-deployment were negatively associated with the change in raw DNAm age between time points (Boks et al., 2015), suggesting potential differential effects of trauma exposure versus PTSD.

Using an alternative analytic design, a study of 40 paramedicine students assessed twice (pre- and post-work-related trauma exposure) found that baseline Horvath DNAm age residuals were positively associated with PTSD severity at follow-up 12 months later (about 1–2 months following trauma exposure; Mehta et al., 2022). However, all participants reported trauma histories at baseline, raising the possibility that baseline DNAm age was advanced due to this and/or associated pre-existing psychiatric symptoms. Consistent with this possibility, the same study found that baseline PTSD severity was associated with greater Horvath and GrimAge DNAm age residuals at follow-up (Mehta et al., 2022). Most critically, the analyses did not account for baseline DNAm age acceleration, masking the extent to which DNAm age changed from baseline to follow-up. Another study (Yang et al., 2021) modeled change in DNAm age estimates through the use of correlated

change scores between GrimAge residuals and PTSD symptom severity scores among trauma-exposed male military Veterans assessed twice. Among those who had PTSD at baseline, change in PTSD severity across 3 years was correlated positively with change in GrimAge residuals, but this was based on just 26 participants. Concerns about small sample size (and associated limitations to statistical power and representativeness) apply to all the longitudinal studies of PTSD and epigenetic aging to date.

Other studies have addressed the challenges of modeling the relationships between DNAm age and time between assessments by examining the rate of change in raw DNAm age estimates relative to the time between assessments. One study (Sumner et al., 2023) followed a group of 171 children and adolescents over 2 years and found that the rate of change in DNAm age was greater (more positive) among those who experienced more negative impact from intervening stressful life events. Two other studies of the rate of change among Veteran cohorts with chronic PTSD found that baseline PTSD symptom severity and diagnosis predicted an increased pace of Horvath DNAm age over the course of approximately 2 (Wolf et al., 2019) and 5.5 (Hawn et al., 2023) years, respectively. Collectively, these studies, with sample sizes ranging from 26 to 179, highlight the challenges in modeling change over time in DNAm age and raise the possibility that different analytic strategies may be necessary to best address questions concerning how new-onset PTSD diagnoses versus chronic symptoms relate to changes in DNAm age over time.

Aims and hypotheses

This study sought to investigate the health correlates of PTSD in terms of changes in epigenetic aging over time. We examined the relationship between PTSD and future changes in epigenetic age acceleration over time using meta-analysis of longitudinal data from seven cohorts contributing to the Psychiatric Genomics Consortium (PGC) PTSD Epigenetics Workgroup (Ratanatharathorn et al., 2017). The varied methodological structures of these datasets allowed us to ask multiple questions about the relationship between PTSD and epigenetic age. Specifically, our first aim was to evaluate if the association between Time 1 (T1) and Time 2 (T2) DNAm age residuals was altered by the development of new-onset PTSD between two time points. We expected that the association between DNAm age residuals across two time points would be more positive among those with new-onset PTSD at follow-up. Our second aim was to examine whether the association between DNAm age residuals at two time points varied as a function of change in PTSD symptom severity (T2–T1). We also hypothesized that the association between DNAm age residuals at two time points would be stronger as the change in PTSD symptom severity increased. Both hypotheses were tested by modeling the association between DNAm age residuals at T2 with the T1 DNAm age residuals × new-onset PTSD and T1 DNAm age residuals × change in PTSD symptom severity interaction terms, respectively, while adjusting for their main effects and other covariates. These aims were addressed in a total of 1,367 individuals derived from 7 cohorts who were each assessed for DNAm twice. The cohorts spanned both civilian and military samples and included research methods that were focused on pre/post military deployment or pre/post trauma exposure, and those focused on chronic PTSD symptoms. The former design allowed for the examination of new-onset PTSD diagnoses over time while the latter design allowed us to examine changes in PTSD symptom severity over time in association with changes in DNAm age residuals. We were unable to examine the changing rate of epigenetic aging over time (e.g., Wolf et al., 2019) due to the structure of the contributing datasets, which included many cohorts defined by pre/post trauma exposure.

Analyses focused on Horvath age (Horvath, 2013) and GrimAge (Lu et al., 2019) as they represent the most widely used and robust metrics of biological age and time to death, respectively (sometimes referred to as first- versus second-generation clocks). In addition, these are the only DNAm age indices previously associated with PTSD in small longitudinal studies (Boks et al., 2015; Hawn et al., 2023; Mehta et al., 2022; Sumner et al., 2023; Wolf et al., 2023; Yang et al., 2021). We also sought to limit the number of tests to reduce the burden of multiple testing corrections and thus chose to limit analyses to the strongest epigenetic age metrics with prior evidence of longitudinal associations with PTSD.

Methods and materials

Participating studies

Seven participating cohorts were included in the meta-analysis. The mean time between assessments within each study ranged from 5.7 months to 5.6 years. The cohorts included: (1) The Longitudinal National Center for PTSD (NCPTSD) cohort (Wolf et al., 2023), a study of trauma-exposed Veterans (many with chronic PTSD), who were assessed twice, an average of 5.6 years apart; (2) the Translational Research Center for TBI and Stress Disorders (TRACTS) cohort (McGlinchey, Milberg, Fonda, & Fortier, 2017), which consisted of post-9/11 Veterans (many with chronic PTSD) who completed two assessments an average of 1.9 years apart; (3) the Army Study to Assess Risk and Resilience in Servicemembers (Army STARRS) (Ursano et al., 2014), a study of military service members assessed pre- and post-deployment to Afghanistan over an average of 9.6 months; (4) the Marine Resiliency Study (MRS) cohort (Baker et al., 2012; Nievergelt et al., 2015) of male US Marines assessed pre- and post-deployment to Iraq or Afghanistan over a mean interval of 12.4 months; (5) the Prospective Research in Stress-related Military Operations (PRISMO) cohort (Eekhout, Reijnen, Vermetten, & Geuze, 2016; Reijnen, Rademaker, Vermetten, & Geuze, 2015) of Dutch soldiers who were assessed pre- and post-deployment to Afghanistan over an average of 14.4 months; (6) the Detroit Neighborhood Health Study (DNHS) cohort (Goldmann et al., 2011; Uddin et al., 2010) which consisted of trauma-exposed Detroit residents (some with chronic psychiatric symptoms) who completed two assessments an average of 16.3 months apart; and (7) the Advancing Understanding of Recovery after Trauma (AURORA) Study (McLean et al., 2020), which included individuals evaluated at the emergency department following trauma exposure who were reassessed an average of 5.7 months after emergency department treatment. Table 1 lists the demographic and clinical characteristics of each cohort. Each study site obtained local IRB approval, and all participants provided written informed consent. The IRB of the VA Boston Healthcare System approved the meta-analyses of the summarized data. Individuals interested in obtaining access to the data should contact the principal investigator of each individual cohort to determine availability.

DNA and DNAm procedures

DNA was extracted from buffy coat from peripheral blood samples. DNAm was measured using Illumina Infinium EPIC BeadChip at

Table 1. Cohort characteristics

Study	Description	Total *N*[a] (% male)	IT (Months)	Mean age (SD)		Ancestry	*N* PTSD cases (%)			Mean harmonized PTSD symptom severity (*SD*)			Measure of PTSD DX and PTSD severity	
				T1	T2		T1	T2	New onset[b]	T1	T2	△[b]	T1	T2
Longitudinal NCPTSD[1]	Trauma-exposed Veterans from mixed war eras	171 (87.13)	66.95 (46.42)	53.50 (11.60)	59.15 (12.13)	EUR 71.93% AAM 16.96% LAT 1.17% OTH 9.94%	83 (48.54)	82 (47.95)	26 (15.20)	0.31 (0.19)	0.28 (0.17)	−0.03 (0.16)	CAPS[8] & CAPS–5[9]	CAPS–5[9]
TRACTS[2]	Post–9/11 Veterans assessed twice following deployment to Iraq and/or Afghanistan	404 (89.36)	23.46 (11.09)	34.54 (9.47)	36.47 (9.36)	EUR 64.85% AAM 12.13% LAT 7.43% OTH 15.59%	223 (55.20)	201 (49.75)	31 (7.67)	0.36 (0.21)	0.34 (0.22)	−0.01 (0.15)	CAPS[8] & CAPS–5[9]	CAPS[8] & CAPS–5[9]
Army STARRS[3]	US Army soldiers deployed to Afghanistan	184 (100)	9.58 (1.65)	24.47 (4.84)	25.52 (4.98)	EUR 67.93% AAM 10.87% LAT 11.41% OTH 9.79%	0 (0)	92 (50.00)	92 (50.00)	0.03 (0.05)	0.22 (0.26)	0.19 (0.25)	PCL[10c] & CIDI-SC[11]	PCL[10]
MRS[4]	US Marines deployed to Iraq or Afghanistan	127 (100)	12.44 (2.69)	22.04 (2.22)	23.07 (2.18)	EUR 69.29% AAM 3.94% LAT 11.03% OTH 15.74%	0 (0)	64 (50.39)	64 (50.39)	0.12 (0.09)	0.33 (0.24)	0.21 (0.21)	CAPS[8]	CAPS[8] & PCL–5[13e]
PRISMO[5]	Dutch Veterans deployed to Afghanistan	112 (93.75)	14.39 (1.38)	27.04 (8.96)	27.86 (8.82)	EUR 78.57% AAM 3.57% OTH 17.86%	0 (0)	32 (28.57)	32 (28.57)	0.16 (0.12)	0.30 (0.27)	0.14 (0.26)	SRIP[12]	SRIP[12]
DNHS[6]	Trauma-exposed Detroit residents	166 (37.35)	16.32 (6.22)	56.54 (14.68)	57.93 (14.79)	EUR 10.84% AAM 81.93% OTH 7.23%	11 (6.63)	4 (2.41)	1 (0.60)	0.12 (0.24)	0.19 (0.25)	0.04 (0.32)	PCL-C[10]	PCL-C[10]
AURORA[7]	Individuals who presented to the ED within 72 hours after exposure to psychological trauma	203 (25.62)	5.71 (0.32)	40.47 (14.36)	40.94 (14.36)	EUR 33.00% AAM 64.04% LAT 2.46% OTH 0.50%	57 (28.08)	58 (28.57)	19 (9.36)	0.41 (0.19)	0.29 (0.22)	−0.06 (0.23)	PCL–5[13d]	PCL–5[13]

Note: Superscripted numbers refer to the references for each cohort and PTSD measure: 1. Wolf et al. (2023); 2. McGlinchey et al. (2017); 3. Ursano et al. (2014); 4. Baker et al. (2012); Nievergelt et al. (2015); 5. Eekhout et al. (2016); Reijnen et al. (2015); 6. Goldmann et al. (2011); Uddin et al. (2010); 7. McLean et al. (2020); 8. Blake et al. (1995); 9. Weathers et al. (2018); 10. Weathers et al. (1993); 11. Kessler et al. (2013); 12. Hovens, Bramsen, and Van Der Ploeg (2002); 13. Blevins et al. (2015).

Abbreviations: NCPTSD, The National Center for PTSD Study; TRACTS, The Translational Research Center for TBI and Stress Disorders Study; Army STARRS, The Army Study to Assess Risk and Resilience in Servicemembers; MRS, The Marine Resilience Study; PRISMO, The Prospective Research in Stress-related Military Operations; DNHS, The Detroit Neighborhood Health Study; AURORA, The Advancing Understanding of RecOvery afteR traumA Study; IT, Intervening time; PTSD, posttraumatic stress disorder; DX, diagnosis; T1, time 1; T2, time 2; ED, emergency department; EUR, European ancestry; AAM, African American ancestry; LAT, Latino ancestry; OTH, Other ancestries; PCL, PTSD Checklist for DSM-IV; PCL-5, PTSD Checklist for DSM-5; PCL-C, PTSD Checklist Civilian Version; DSM, Diagnostic and statistical manual of mental disorders; CIDI-SC, Composite International Diagnostic Interview screening scales; CAPS, Clinician-Administered PTSD Scale for DSM-IV; CAPS-5, Clinician-Administered PTSD Scale for DSM-5; SRIP, Self-Report Inventory for PTSD; △ = change in harmonized PTSD symptom severity (T2-T1); SD, standard deviation.

[a]Sample sizes for computing cross-sectional and longitudinal correlations among DNAm age, DNAm age residuals, and DNAm-based cell type proportions.

[b]Sample sizes for the new-onset PTSD diagnosis analysis and change in symptom severity analysis can be found in Figures 1 and 2. Compared to the total N, the sample size for the new-onset PTSD diagnosis analysis was reduced due to exclusion of PTSD cases at baseline and missing values in PTSD diagnosis and covariates. The sample size for the change in symptom severity analysis was reduced due to missing values in PTSD symptom severity and covariates.

[c]6-item screening version of PCL.

[d]Abbreviated (six-item) civilian version of PCL-5.

[e]CAPS was used for determining PTSD DX and PCL-5 was used for assessing PTSD severity.

two time points. DNAm data were processed following a quality control (QC) pipeline developed by the PGC PTSD Epigenetics Workgroup (Ratanatharathorn et al., 2017; and its updated version at https://github.com/PGC-PTSD-EWAS/EPIC_QC). Details of QC procedures are included in the Supplementary Materials. DNAm is cell type-specific and thus DNAm levels may vary as a function of the composition of the cell types the DNA was extracted from. Given this, it is important to include proportional estimates of white blood cell types as covariates in analyses (as is standard in DNAm analyses). Proportions of six cell types (B cells, CD4+ T cells, CD8 + T cells, natural killer cells, monocytes, and neutrophils) were estimated directly from the DNAm data using a reference library of CpG sites aligned with sorted cells, as implemented in the Bioconductor package EpiDISH (Teschendorff, Breeze, Zheng, & Beck, 2017). Of these, B cells, CD4+ T cells, CD8+ T cells, natural killer cells, and monocytes were included as covariates in the analysis (neutrophils are excluded because the proportional nature of the estimates makes them colinear when all other cell types are included in the model). Because blood-based methylation is strongly influenced by smoking, we computed a DNAm-based smoking score based on 39 smoking-associated CpGs (Li et al., 2018; Supplementary Material). As our outcome of interest is epigenetic age acceleration at T2, the DNAm smoking score from T2 was included as an additional covariate in a sensitivity analysis to determine if our findings were driven by the effects of concurrent smoking, which can co-occur with PTSD.

Genotyping was conducted on various arrays. Genotype data cleaning was completed at each site according to the procedures previously described in the original publications for each study (Table 1). Ancestry was determined based on genotype data (where available) using the pipeline developed by the PGC PTSD (Nievergelt et al., 2019). Ancestry-based principal components (PCs) were computed from 100,000 randomly selected common single nucleotide polymorphisms (SNPs) with minor allele frequency (MAF) > 0.05. When genotyping data were unavailable (e.g., DNHS), methylation probes within 1 base pair of SNPs for determining ancestry were used to generate a set of DNAm-based ancestral PCs as proxies for genotype-based PCs (Barfield et al., 2014). As noted from our previous meta-analysis study, the DNAm-derived PCs are significantly correlated with genotype-based PCs (Ratanatharathorn et al., 2017; Wolf, Maniates, et al., 2018). Either the first three genotype-based PCs 1–3 or, when genotype-based PCs were unavailable, the DNAm-based PCs 2–4, were used as covariates in the analyses to account for ancestry.

Measures

PTSD measures

Current PTSD diagnosis and symptom severity were assessed using various measures as listed in Table 1. Following a previous PGC publication (Sumner et al., 2021), we harmonized PTSD symptom severity across these different measures by scaling the raw current PTSD severity score to a range from 0 to 1, representing the score as a percentage of the maximum possible score on each measure (i.e., 1 represents an individual having all symptoms at the most severe level and 0 indicates an individual having no symptoms). For this study, we examined both new-onset PTSD diagnosis (e.g., no PTSD at T1 and PTSD positive at T2 versus no PTSD at T1 and T2) and changes in PTSD symptom severity (T2–T1) using the harmonized PTSD score. For the new-onset PTSD diagnosis analyses, we only included individuals who were negative for PTSD at T1, which resulted in reductions in sample size in some of the studies that included participants with chronic PTSD (Table 1). For the change in PTSD severity analyses, we included all participants with PTSD symptom severity data across all cohorts, regardless of PTSD diagnostic status at baseline.

DNAm age indices

The Horvath DNAm age and GrimAge estimates were computed by uploading the DNAm data to Dr. Horvath's website (https://dnamage.genetics.ucla.edu/) when permitted or by running the scripts supplied by Drs. Horvath and Lu if methylation data were not allowed to be uploaded per local regulations (Horvath, 2013; Lu et al., 2019). Horvath's algorithm has its own normalization and imputation step, so raw DNAm values were used as the input for the Horvath age calculation. Values from 353 probes were used to generate Horvath age (Horvath, 2013). However, 17 (4.8%) of the 353 CpGs are missing from the EPIC chip (Dhingra et al., 2019). A small number of additional missing probes from each cohort were identified as summarized in the Supplementary Materials. GrimAge estimates were generated using normalized and imputed DNAm data. 30,084 probes were used as input for GrimAge calculation (Lu et al., 2019).

Both Horvath age and GrimAge residuals were computed by saving the unstandardized residuals from a linear model regressing the raw DNAm age on chronological age. This was done for each cohort at two time points separately, so the age residuals have a mean of 0 for each cohort at each time point. An R script was developed by the first author, tested with co-authors, and then sent to the data analyst at each participating cohort site so that identical calculation and analytic approaches would be applied in each cohort. Summary statistics from each cohort were then meta-analyzed to combine the results across studies.

Statistical analyses

We first examined the Pearson correlations between Horvath age and GrimAge with chronological age at each corresponding time point. We then assessed the correlations among Horvath age, GrimAge, Horvath age residuals, and GrimAge residuals over time. Correlations among cell types over time and between the two DNAm age residuals and cell types were also evaluated. The correlation coefficients collected from each group were meta-analyzed using the metacor (Laliberté, 2022) package in R.

We conducted two primary regression analyses. The first focused on examining how new-onset PTSD diagnosis at T2 (versus remaining negative for PTSD at both time points) alone and in interaction with T1 DNAm age residuals predicted T2 DNAm age residuals, covarying for T1 DNAm age residuals and covariates. The second analysis replaced new-onset PTSD diagnosis with change in PTSD symptom severity over time. In both analyses, we first performed a regression model without the interaction term to capture the main effects of all predictors (all main effect coefficients reported are from this initial model) and then added the interaction term into the model. The interaction term examined the extent to which the association between T1 DNAm age residuals and T2 DNAm age residuals differed as a function of change in PTSD (diagnosis or severity). A positive interaction term would indicate T2 DNAm age residuals become more extreme than what would be predicted from T1 DNAm age residuals alone, as a function of the moderating effect of changes in PTSD status. This more extreme alteration could occur at both ends of the DNAm age residuals. In the primary linear

regression models predicting T2 age residuals, the predictors in the model were DNAm age residuals at T1, new-onset PTSD diagnosis at T2 (or separately, change in PTSD symptom severity), the interaction between T1 DNAm age residuals and new-onset PTSD diagnosis (or change in PTSD severity), and the following covariates: sex (excluded if there was no variability in sex in a given sample), three ancestry PCs, and five cell type proportion estimates at T2. Significant associations were further examined in sensitivity analyses, including DNAm-based smoking scores at T2 as an additional covariate. Follow-up models evaluated the association between childhood trauma (predating T1) and changes in epigenetic age over time (Supplementary Materials).

Meta-analysis of each unstandardized parameter estimate in the regression models (except ancestry PCs) was conducted in an inverse variance-weighted random-effects model using the metafor package in R (Viechtbauer, 2010). Results for each term were corrected for multiple testing via the false discovery rate (FDR) adjustment (Benjamini & Hochberg, 1995) across the two age algorithms.

Results

Associations between chronological age, DNAm age, and DNAm age residuals

Chronological age was strongly correlated with both Horvath age and GrimAge at each time point (meta *r*s = 0.86–0.89, meta *p*s < 0.001; Supplementary Table S1). At the individual cohort level, lower correlations were observed in ArmySTARRS and MRS (Supplementary Table S1), which was likely due to the smaller variance in chronological age in these cohorts (Table 1). Raw Horvath age and GrimAge were also strongly associated with each other at each time point (meta *r*s = 0.80 and 0.78 at T1 and T2, respectively; Supplementary Table S1). However, Horvath age and GrimAge *residuals* were weakly correlated with each other at each time point (both meta *r*s = 0.09; Supplementary Table S1). Meta-analysis revealed a strong correlation between the raw Horvath age and GrimAge estimates with themselves across T1 and T2 (meta *r*s = 0.91 and 0.96, meta *p*s < 0.001; Table 2). The residuals were also consistent over time: T1 versus T2 Horvath DNAm age residuals meta-*r* = 0.65 and GrimAge residuals meta-*r* = 0.88 (Table 2).

Cell type proportions and their associations with DNAm age residuals

Estimated cell type proportions were strongly related to themselves over time. The estimated proportion of B cells showed the strongest correlation over time (meta $r = 0.75$, meta $p < 0.001$), followed by CD8+ T cells (meta $r = 0.71$, meta $p < 0.001$), natural killer cells (meta $r = 0.61$, meta $p < 0.001$), CD4+ T cells (meta $r = 0.61$, meta $p < 0.001$), and monocytes (meta $r = 0.55$, meta $p < 0.001$; Table 2). Additionally, the cell type estimates were weakly correlated with both Horvath age residuals and GrimAge residuals at each time point, with meta-correlations ranging from −0.08 to 0.11 for Horvath age residuals and from −0.21 to 0.05 for GrimAge residuals (Supplementary Table S2).

New-onset PTSD diagnosis and change in DNAm age residuals over time

We examined how T1 DNAm age residuals, new-onset PTSD diagnosis (between T1 and T2), and their interaction predicted T2 DNAm age residuals ($n = 745$). Meta-analysis revealed a significant effect of T1 age residuals on T2 age residuals for both the Horvath algorithm (meta β = 0.63, meta SE = 0.05, meta $p = 3.63 \times 10^{-37}$) and GrimAge algorithm (meta β = 0.85, meta SE = 0.02, meta $p < 2.23 \times 10^{-308}$; Table 3). New-onset PTSD diagnosis was not associated with either T2 Horvath age residuals (meta β = −0.26, meta SE = 0.24, meta $p = 0.27$) or T2 GrimAge residuals (meta β = 0.22, meta SE = 0.13, meta $p = 0.10$; Table 3). Meta-analysis also revealed that the T1 Horvath age residuals × new-onset PTSD diagnosis interaction term was significantly associated with T2 Horvath age residuals, after FDR correction for multiple testing across the two algorithms (meta β = 0.16, meta SE = 0.07, meta $p = 0.02$, $p\text{-}adj = 0.03$, $I^2 = 0$; Table 3 and Figure 1). This means that the positive association between Horvath age residuals at the two time points was greater among those with new-onset PTSD diagnosis. When additionally covarying for the smoking score, the interaction effect remained significant (meta β = 0.17, meta SE = 0.07, meta $p = 0.02$) while the smoking score was not associated with T2 Horvath age residuals (meta $p = 0.11$). This interaction association was not significant for the GrimAge algorithm (meta $p = 0.99$; Table 3). The association between the interaction term and T2

Table 2. Longitudinal correlations (T1 to T2) among DNAm age, DNAm age residuals, and cell type proportions

	DNAm age		DNAm age residuals		Cell type proportion estimates				
Cohort	Horvath	Grim	Horvath	Grim	CD8+ T	CD4+ T	NK	B Cell	Mono
Army STARRS	0.87	0.93	0.59	0.81	0.58	0.44	0.38	0.75	0.48
DNHS	0.96	0.98	0.78	0.92	0.84	0.72	0.72	0.81	0.69
Longitudinal NCPTSD	0.93	0.95	0.66	0.87	0.80	0.58	0.70	0.76	0.52
MRS	0.77	0.91	0.61	0.88	0.65	0.65	0.52	0.70	0.42
PRISMO	0.91	0.95	0.66	0.87	0.66	0.62	0.51	0.72	0.36
TRACTS	0.94	0.97	0.67	0.89	0.78	0.69	0.71	0.78	0.63
AURORA	0.91	0.98	0.48	0.89	0.58	0.51	0.64	0.71	0.65
Meta *R* 95% CI	0.91 0.87–0.94	0.96 0.94–0.98	0.65 0.57–0.71	0.88 0.85–0.90	0.71 0.62–0.79	0.61 0.53–0.68	0.61 0.51–0.70	0.75 0.72–0.78	0.55 0.45–0.63

Note: Correlations represent the Pearson correlation coefficients calculated between the same variable at T1 and T2. The 95% confidence intervals were computed for the meta-analytic correlations. All meta-analytic correlations were significantly different from zero at a *p*-value threshold of 0.001, as determined by a *t*-test.
Abbreviations: CD8+ T, CD8+ T cell; CD4+ T, CD4+ T cell; NK, natural killer cell; Mono, monocyte; CI, confidence interval; Army STARRS, The Army Study to Assess Risk and Resilience in Servicemembers; DNHS, The Detroit Neighborhood Health Study; NCPTSD, The National Center for PTSD Study; MRS, The Marine Resilience Study; PRISMO, The Prospective Research in Stress-related Military Operations; TRACTS, The Translational Research Center for TBI and Stress Disorders Study; AURORA, The Advancing Understanding of RecOvery afteR traumA Study.

Table 3. New-onset PTSD diagnosis as a predictor of T2 DNAm age residuals: main and interactive meta-analytic results

	T2 Horvath age residuals				T2 GrimAge residuals			
Variable	Beta	SE	*p*	*p-adj*	Beta	SE	*p*	*p-adj*
Intercept	1.082	0.687	0.115	NA	2.531	0.385	4.689×10^{-11}	NA
Sex	−0.604	0.854	0.479	0.959	−0.008	0.360	0.983	0.983
CD8+ T	3.549	2.664	0.183	0.183	−5.251	1.508	5.000×10^{-4}	9.940×10^{-4}
CD4+ T	−0.603	2.590	0.816	0.816	−7.485	1.430	1.670×10^{-7}	3.340×10^{-7}
NK	0.081	4.475	0.986	0.986	−2.851	3.201	0.373	0.746
B cell	−15.153	6.836	0.027	0.053	−5.771	5.099	0.258	0.258
Mono	−5.916	5.216	0.257	0.257	−5.508	3.882	0.156	0.257
T1 DNAm Age residuals	0.626	0.049	3.631×10^{-37}	3.631×10^{-37}	0.854	0.022	$<10^{-100}$	$<10^{-100}$
New-Onset PTSD DX	−0.264	0.238	0.268	0.268	0.216	0.130	0.098	0.196
T1 DNAm Age Residuals × New-Onset PTSD DX	0.164	0.068	0.016	0.033	−0.001	0.043	0.990	0.990

Note: Meta-analytic results from the individual cohort multiple regression linear models. Meta-analytic main effects are derived from the main (and covariate) effect only models in each cohort. Meta-analytic interaction effects are derived from the models with the main and interaction effects. The top three ancestry principal components from each cohort were also included in the model.

Abbreviations: CD8+ T, CD8+ T cell; CD4+ T, CD4+ T cell; NK, natural killer cell; Mono, monocyte; DX, diagnosis; SE, standard error; NA, not applicable; *p-adj*, *p*-value adjusted for multiple testing across the two age algorithms using the false discovery rate (FDR) procedure.

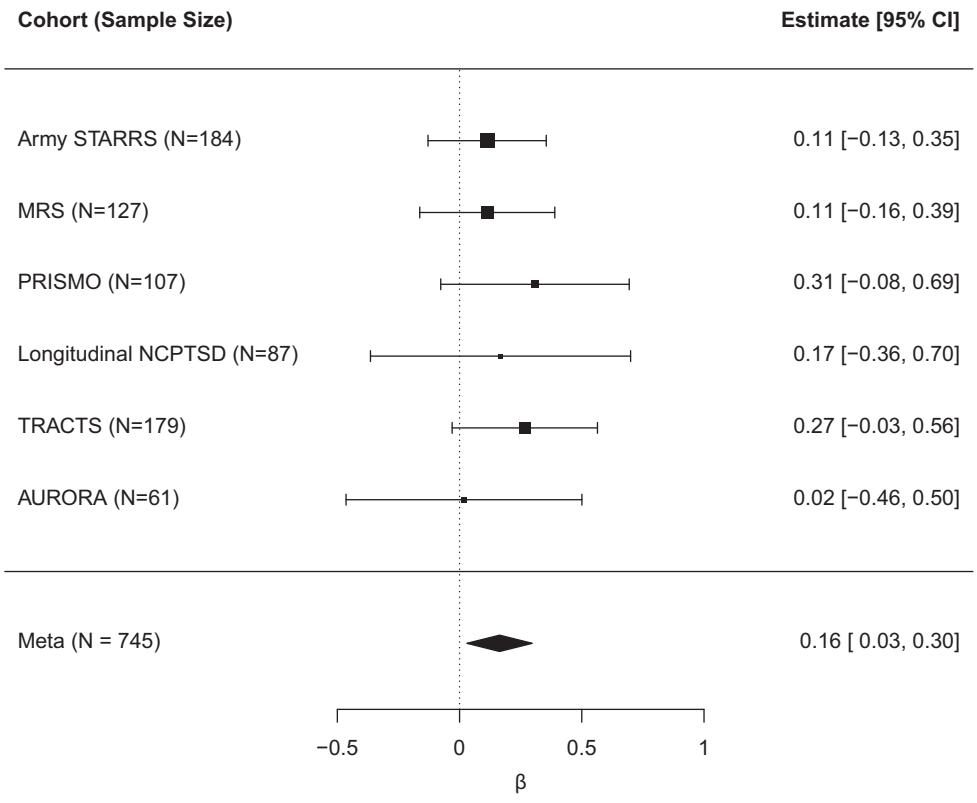

Figure 1. Forest plot for the effect of the interaction term reflecting T1 Horvath DNAm age residuals by new-onset PTSD diagnosis on T2 Horvath DNAm age residuals (controlling for all other main effects in the model).

Horvath DNAm age residuals was not driven by differences in the baseline Horvath age residuals between new-onset PTSD cases versus those who did not develop PTSD (meta $p = 0.16$; Supplementary Table S3). This implies that those who developed PTSD at T2 did not simply have higher DNAm age residuals at baseline. Full results for each individual cohort can be found in Supplementary Tables S4 and S5. Follow-up analyses revealed that childhood trauma was not related to T2 DNAm age residuals (Supplementary Materials).

Change in PTSD symptom severity and change in Horvath DNAm age residuals over time

We next examined if the relationship between Horvath DNAm age residuals over time was moderated by change in PTSD symptom severity over time ($n = 1191$) as a follow-up to the new-onset PTSD diagnosis analysis. We included the main and interactive effects of baseline Horvath age residuals and change in PTSD symptom

Table 4. Change (T2–T1) in PTSD severity as a predictor of T2 Horvath DNAm age residuals: main and interactive meta-analytic results

Variable	Beta	SE	*p*
Intercept	0.970	0.531	0.068
Sex	−0.657	0.431	0.127
CD8+ T	4.195	2.156	0.052
CD4+ T	−3.443	1.835	0.061
NK	−2.352	3.604	0.514
B cell	−11.567	4.119	0.005
Mono	−3.070	4.344	0.480
T1 Horvath DNAm age residuals	0.629	0.054	6.415×10^{-31}
△ PTSD severity	0.469	0.634	0.459
T1 Horvath DNAm age residuals × △ PTSD severity	0.243	0.124	0.049

Note: Meta-analytic results from the individual cohort multiple regression linear models. Meta-analytic main effects are derived from the main (and covariate) effect only models in each cohort. Meta-analytic interaction effects are derived from the models with the main and interaction effects. The top 3 ancestry principal components were also included in the meta-analysis. Δ = change (T2–T1).
Abbreviations: CD8+ T, CD8+ T cell; CD4+ T, CD4+ T cell; NK, natural killer cell; Mono, monocyte; SE, standard error.

severity over time as a predictor of T2 Horvath age residuals (along with covariates). The association between T1 and T2 Horvath age residuals was significant (meta β = 0.63, meta SE = 0.05, meta $p = 6.42 \times 10^{-31}$), but the change in PTSD symptom severity score was not associated with T2 Horvath age residuals (meta β = 0.47, meta SE = 0.63, meta $p = 0.46$). The interaction between T1 Horvath age residuals and change in PTSD symptom severity over time was significantly associated with T2 Horvath DNAm age residuals (meta β = 0.24, meta SE = 0.12, meta $p = 0.05$, $I^2 = 0$; Table 4 and Figure 2). The relationship between DNAm age residuals over time became stronger (a steeper slope) among those with the greatest increase in PTSD symptom severity from T1 to T2. The interaction effect remained significant when further adjusting for the smoking score (meta β = 0.25, meta SE = 0.12, meta $p = 0.04$), while the smoking score was not associated with T2 Horvath age residuals (meta $p = 0.28$). The association between the interaction term and T2 Horvath age residuals was not accounted for by baseline differences in T1 Horvath age residuals as a function of change in PTSD symptom severity (meta $p = 0.41$; Supplementary Table S3). Full results for each individual study are listed in Supplementary Table S6. Although we did not find a significant interaction between GrimAge residuals and new-onset PTSD diagnosis, we conducted the change in PTSD severity analysis for GrimAge residuals for completeness and report the (null) result in the Supplementary Materials.

Discussion

The major goals of this study were to first test if individuals who developed new-onset PTSD between two measurements showed greater epigenetic aging than would be expected based on their epigenetic aging at baseline from a time at which they did not meet criteria for PTSD. The second aim was to examine if changes in PTSD symptom severity moderated the strength of the association between epigenetic aging at two time points among individuals with and without PTSD. We expected that individuals with new-onset PTSD and those with increased PTSD symptom severity over time

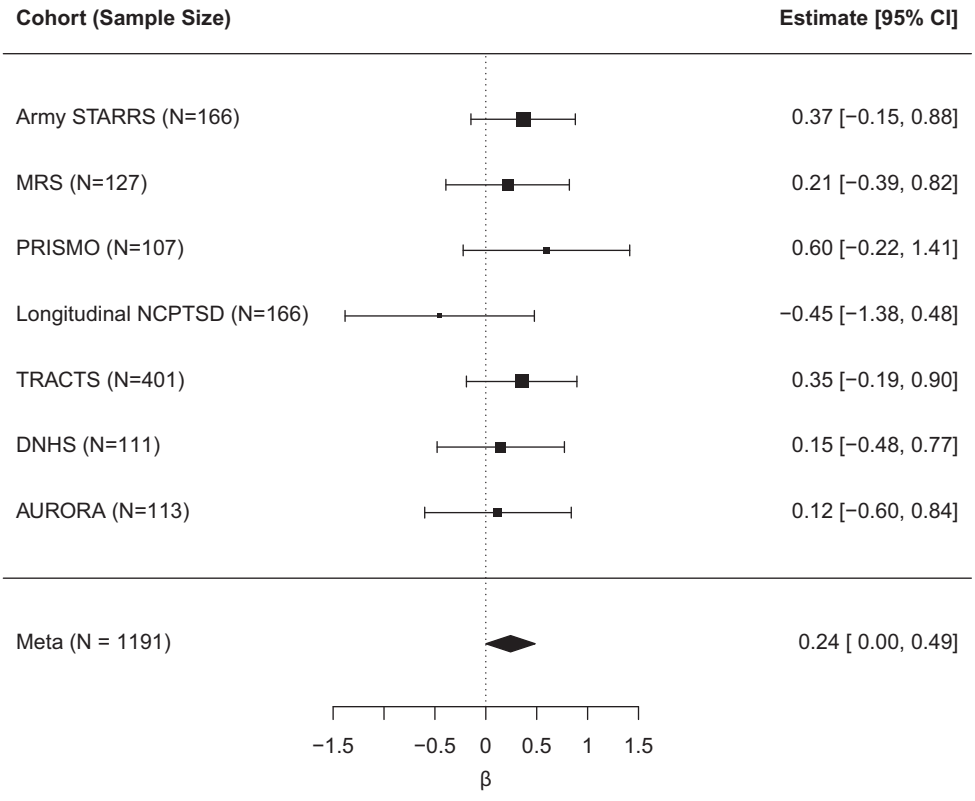

Figure 2. Forest plot for the effect of the interaction term reflecting T1 Horvath DNAm age residuals by change in harmonized PTSD symptom severity on T2 Horvath DNAm age residuals (controlling for all other main effects in the model).

would show higher DNAm age residuals at follow-up than would be expected based on DNAm age residuals at baseline. We evaluated this question via meta-analysis of 7 cohorts. We found that, for every one-year of advanced Horvath DNAm age at baseline, new-onset PTSD cases evidenced an additional 0.16 years of epigenetic aging per the Horvath algorithm at T2 compared to longitudinal controls who did not develop PTSD at T2. In other words, those with new-onset PTSD aged faster by an extra 1.9 months over the time interval, which was on average approximately 18.9 months. Furthermore, an individual experiencing a maximum possible symptom severity change (from having no symptoms at all to having all PTSD symptoms at the most severe level) would have an additional 0.24 years of Horvath age acceleration at T2 compared to an individual with no change in symptom severity score. This implies that this group aged faster by an extra 2.9 months over the time interval, which was on average approximately 21.3 months for the symptom severity analyses.

To further facilitate the interpretation of our findings, we also estimated the pooled standard deviation of the change in harmonized PTSD symptom severity scores across cohorts (SD_{pooled} = 0.23). This allows for the following interpretation of the interaction term: for every one-year increase in Horvath age residuals at baseline, an individual experiencing a pooled SD (23%) increase in PTSD symptom severity at T2 would have an extra 0.68 months of Horvath age acceleration over the average time interval of 21.3 months compared to an individual with no change in symptom severity across the two time points. Although the observed effect sizes were relatively small, the cumulative impact of PTSD on epigenetic aging could become substantial over the course of a lifespan given the inclusion of a large number of young Veterans in their early 30s in this meta-analysis (e.g., ArmySTARRS, MRS, and TRACTS cohorts).

These results are consistent with our hypotheses that age acceleration is increased among individuals with new-onset PTSD and increased symptom severity. No prior studies have examined interactions between new-onset PTSD (or change in symptom severity) by baseline age acceleration, however the structure of these data, with numerous cohorts defined by pre- and post-trauma exposure, made it critical to ask questions regarding how new-onset diagnoses would impact change in metrics of advanced DNAm age. It would not be possible in these cohorts to use baseline PTSD diagnoses to predict change in DNAm age residuals (or the rate of DNAm age change per intervening year) as most individuals were negative for PTSD at the pre-exposure timepoint.

Prior studies have found that PTSD symptom change is associated with changes in DNAm loci that also contribute to Horvath DNAm age and could partially relate to the changes in Horvath DNAm age observed in this study. Specifically, Katrinli et al. (2022) modeled the association between post-deployment DNAm and change in PTSD symptom severity (PTSS) conditioned on pre-deployment DNAm and found 15 differentially methylated regions (DMRs) associated with change in PTSS. The guanine nucleotide-binding protein, alpha-stimulating activity polypeptide (*GNAS*) complex locus was one of 15 DMRs associated with PTSD symptom changes and that locus includes a CpG site, cg14597908, which also contributes to the Horvath age calculation (Horvath, 2013). Differential methylation at *GNAS* has been linked with maternal stress (Vangeel et al., 2015) and anxiety (Alisch et al., 2014, 2017), suggesting that it may be sensitive to traumatic stress as well. In a pig model, *GNAS* was also associated with cellular senescence (Jeon et al., 2012). Thus, PTSD-related alterations in *GNAS* DNAm over time could potentially influence the broader changes in advanced epigenetic age observed in this study. Katrinli *et al.* also reported that PTSS was associated with change in DNAm in genes involved in immune processes and oxidative stress (Katrinli et al., 2022). Alterations in these biological pathways, which also have known associations with accelerated aging (Cevenini, Monti, & Franceschi, 2013), could impact the aging process and potentially contribute to the effects observed in this study.

Our primary interaction effects suggested PTSD modulated the slope of age acceleration over time, raising the question of whether PTSD treatment could slow cellular aging and reduce the risk of early onset of age-related disease. However, important challenges exist when attempting to address this question. First, the strongest predictor of follow-up DNAm age acceleration was baseline DNAm age acceleration, so after accounting for this effect, there may be only a small amount of variance remaining to be predicted by treatment. This would suggest that effects of PTSD treatment on cellular aging would be small and sample sizes would need to be large (possibly larger than most intervention trials) to observe an effect. Second, DNAm age estimates may not be sensitive enough to reflect changes in cellular age accurately over the course of a typical 12- or 18-week trial. The study would need to have sufficiently long follow-up periods to observe reliable changes in the DNA methylome. That said, pharmacological studies suggest some promise for effects of treatment on epigenetic aging. For example, a study of 30 individuals with bipolar disorder and 30 healthy controls indicated an association between the use of combination mood stabilizers (lithium carbonate, sodium valproate, and carbamazepine), in contrast to either monotherapy or no medication, with decreased Horvath age acceleration (Okazaki et al., 2020). In human neuroblastoma cells, lithium, valproate, and carbamazepine induced hypermethylation at 377, 70, and 66 CpG sites, respectively, and hypomethylation at 145, 37, and 14 CpG sites, respectively (Asai et al., 2013). Similar studies are needed in PTSD samples to explore the effect of PTSD treatments and their potential to alter the aging methylome.

Our study also reported high correlations between DNAm age estimates and age residuals with themselves over time, suggesting the robustness of the two age calculators. Both GrimAge and GrimAge residuals were more strongly correlated with themselves over time than were those for the Horvath algorithm. This could be due to its unique 2-step calculation, the inclusion of age and sex in the model (Lu et al., 2019), or that the thousands of GrimAge probes are collectively more stable than the smaller set of probes included in the Horvath model. The stronger correlation between GrimAge residuals over time may be one reason we did not observe a significant interaction between T1 GrimAge residuals and PTSD. There simply may be little variance remaining in the outcome after adjusting for the T1 GrimAge main effect. In addition, the two models may capture different elements of the aging process reflected by methylation and have differential relevance for PTSD-related pathology.

This study also provided the opportunity to test the stability of estimated white blood cell types over time. We found that the cell type proportion estimates were strongly correlated with themselves over time, particularly CD8+ T and B cells. This highlights the robustness of the cell-type estimation algorithm and its value in studying aging-related changes in cell-type composition.

Study limitations

Our results should be considered with several limitations in mind. We did not adjust for intervening time in any analysis because there

was little to no variability in time between assessments in the pre- and post-deployment military cohorts. This makes it impossible to determine how correlated measures of epigenetic age are over shorter versus longer periods of time and how factors such as severe, chronic PTSD (i.e., more symptoms over a longer period of time) might alter accelerated aging. Data were only available at two time points so we were unable to test if the pattern of change in epigenetic age is constant or nonlinear over time. Given the absence of baseline PTSD cases in the pre/post deployment samples, it was impossible to explore the association between epigenetic aging and baseline diagnosis and severity.

We did not assess intervening trauma or life stress across the two time points across all cohorts so we could not adjust for new trauma exposure/life stressors or substitute it for new-onset PTSD in our analyses. Thus, we were not able to distinguish the effects of PTSD from trauma or life stress as these variables are often correlated, which is a common challenge. However, a number of prior studies have suggested that PTSD, rather than trauma exposure alone (which is near universal across populations), is more strongly linked to advanced epigenetic age (Wolf et al., 2016, 2019; Wolf, Logue et al., 2018). We suspect that it is the ongoing chronic psychological and physiological stress associated with PTSD (e.g., startle, arousal, anger, and poor sleep) that may have a more direct and sustained influence on physiological processes, including those linked to epigenetic aging.

As information on participants' medical and pharmacy records were not available, we were unable to identify their associations with epigenetic aging or adjust for them in analyses. We were also unable to adjust for lifestyle factors like body mass index and substance misuse given that these data were not consistently available across cohorts. However, sensitivity analyses revealed that smoking did not account for our primary associations of interest. The error associated with the Horvath age algorithm (3.6-year median absolute difference between the estimated and actual age in the original test data; Horvath, 2013) was greater than the mean time between assessments in some studies (e.g., the AURORA study with a 5.7-month mean interval). It is possible that analyses in cohorts with smaller intervals were more susceptible to measurement error as the algorithm may not be sufficiently sensitive to detect minor changes in methylation over a small period of time. We chose not to analyze the recently developed DunedinPACE metric (Belsky et al., 2022), which uses DNAm data from a single time point to predict pace of aging because we wanted to calculate the observed DNAm age at two timepoints and measure the change in association between them. Due to the limited sample size of civilian participants, we were not able to address whether or how the military versus civilian nature of the samples might influence the findings of this study. While the participants included in this study are diverse in terms of sex, race, ethnicity, military versus civilian status, and geography, they may still not fully represent any particular population, potentially limiting the generalizability of our findings. Finally, although our data are longitudinal, we can make no claims as to a causal association between PTSD and changes in epigenetic age.

Conclusions

This was the first meta-analysis and largest study to date of the associations between PTSD and changes in DNAm age over time. We found meta-analytic evidence across seven cohorts spanning both military and civilian samples that new-onset PTSD diagnosis and increases in PTSD symptom severity were associated with greater age acceleration per the Horvath metric than would be expected based on the baseline measure of age acceleration. This adds to the growing body of evidence suggesting that stress-related disorders may accelerate cellular aging, potentially contributing to the association between traumatic stress and early onset of age-related diseases, such as cardiovascular conditions (Vidal et al., 2018) and dementia (Yaffe et al., 2010). This highlights the importance of integrating our understanding of mental and physical health even at the cellular level and underscores the tremendous personal costs associated with traumatic stress.

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