**PTSD and Epigenetic Aging: A Longitudinal Meta-analysis**

**Supplementary Materials**

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**Supplementary Methods**

**Cohort Description**

Army STARRS

The Army Study to Assess Risk and Resilience in Servicemembers (Army STARRS) is a multicomponent prospective study that investigates risk and resilience factors for suicidality and related psychopathologies among US Army personnel (Ursano et al., 2014). Participants completed the Composite International Diagnostic Interview screening scales (CIDI-SC) and a 6-item PTSD Checklist (PCL-6) for DSM-IV approximately 6 weeks before deployment to Afghanistan, followed by the PCL for DSM-IV at 1-, 2-, and 6-months post-deployment. Trauma exposure was measured using responses to questions on childhood, adulthood, civilian, and military traumatic events. PTSD diagnosis was determined using multiple imputation methods based on PCL and CIDI-SC data (Kessler et al., 2013). Early life trauma was assessed at pre-deployment using 15 items from The Army STARRS New Soldier Survey (NSS) as previously described (Stein et al., 2018). Whole blood for methylation assays was collected from 92 cases and 92 trauma-exposed controls about 6 weeks before deployment and 1-month post-deployment. None of the individuals had been diagnosed with PTSD before their deployment. PTSD cases were identified based on their PTSD diagnosis at 6 months post-deployment. Controls, who did not have PTSD, were matched with cases based on age, deployment-related stress, and childhood adversity. The procedures for recruitment, consent, human subject, and data protection were approved by the Human Subjects Committees of the Uniformed Services University of the Health Sciences for the Henry M. Jackson Foundation (the primary grantee), the Institute for Social Research at the University of Michigan (the organization collecting the data), and all other collaborating organizations. All participants provided informed consent.

MRS

The Marine Resiliency Study (MRS; Baker et al., 2012; Nievergelt et al., 2015) is a prospective cohort study focusing on PTSD among Marines and accompanying Navy personnel deployed to Iraq or Afghanistan. PTSD symptoms were evaluated up to 3 times, once before deployment and at 3 and/or 6 months after deployment, using the Clinician Administered PTSD Scale (CAPS) and the PTSD Checklist (PCL) for DSM-IV. Whole blood samples were collected during each PTSD symptom assessment. Early life trauma was assessed prior to deployment using the Childhood Trauma Questionnaire (D. P. Bernstein & Fink, 1998). All participants included in this study were exposed to combat trauma, and none of them had a current PTSD diagnosis at the time of deployment (CAPS scores ≤ 25). In this study, a subset of 64 cases and 63 combat trauma-exposed controls were included. PTSD cases were selected from the post-deployment visits (3 or 6 months) with the highest CAPS score, following an approximate 7-month deployment. Combat-exposed controls who had low to no PTSD-symptoms were selected from post-deployment visits and matched by age, ancestry, and timing of the post-deployment visit. The study was approved by the institutional review boards of the University of California San Diego, VA San Diego Research Service, and Naval Health Research Center. All participants provided informed consent.

PRISMO

The Prospective Research in Stress-related Military Operations (PRISMO) is a large prospective study of Dutch military soldiers scheduled for deployments of at least four months to Afghanistan with follow-up assessments over time (Eekhout, Reijnen, Vermetten, & Geuze, 2016; Reijnen, Rademaker, Vermetten, & Geuze, 2015). Current PTSD symptoms were measured using the Self-Report Inventory for PTSD (SRIP;Hovens, Bramsen, & Van Der Ploeg, 2002), and blood samples were collected approximately 1 month before deployment and at 1 and 6 months after deployment. Deployment-related traumatic stress exposure was evaluated with a 19-item checklist of deployment experiences (Van Zuiden et al., 2011). Early life trauma was assessed at pre-deployment using the Early Trauma Inventory (ETI; Bremner, Vermetten, & Mazure, 2000). PTSD cases were selected from assessments conducted 6 months post-deployment. Controls were combat-exposed with low PTSD symptoms (Rutten et al., 2018). The study was approved by the Institutional Review Board of the University Medical Center Utrecht (Utrecht, the Netherlands) and conducted in accordance with the Declaration of Helsinki. All participants provided written informed consent.

Longitudinal NCPTSD

This cohort (Wolf et al., 2023) consisted of male and female veterans who screened positive for PTSD via phone and were later comprehensively assessed for PTSD using the Clinician Administered PTSD Scale (CAPS) for DSM-IV (Blake et al., 1995) or the CAPS-5 (for DSM-5; Weathers et al., 2018), depending on the current DSM version at the time of study enrollment. Participants were recruited from flyers posted throughout the VA medical center and from a database of veterans who previously expressed interest in participating in studies at the NCPTSD. The cohort included 221 veterans at baseline and 178 completed the follow-up portion at T2, approximately 5 years later. The interviews were videotaped and approximately 25% of them were reviewed by a second rater for determining diagnostic reliability. This yielded mean (across the two timepoints) inter-rater reliability statistics of intraclass correlation coefficient = .91 for PTSD symptom severity and kappa = .83 for PTSD diagnostic determinations. Trauma exposure was assessed with the Traumatic Life Events Questionnaire (Kubany et al., 2000) and childhood traumatic events occurring before the age of 18 were summed for supplementary analyses. The DNAm beadchips were balanced for sex, PTSD diagnostic status, and timepoint such that DNA samples from the same participant at two timepoints were always run on the same chip. Written informed consent was obtained from all participants and the study was approved by the local IRB.

TRACTS

This cohort (McGlinchey, Milberg, Fonda, & Fortier, 2017) is comprised of post-9/11 veterans who deployed to Iraq or Afghanistan. Veterans were excluded from the study if they had neurological or cognitive disorders other than those related to traumatic brain injury or if there was a concern of acute substance intoxication or psychotic or safety issues. Veterans were assessed for PTSD with the Clinician Administered PTSD Scale (CAPS) for DSM-IV (Blake et al., 1995) and these were audio recorded; 23 of the recordings were reviewed by an independent rater for inter-rater reliability purposes and analysis of the secondary ratings revealed intraclass correlation coefficient of r = .92 for PTSD symptom severity and kappa = .68 for PTSD diagnoses. Trauma exposure was assessed with the Traumatic Life Events Questionnaire (Kubany et al., 2000) as above. This is an ongoing study and participants return approximately every two years for reevaluation. The EPIC DNAm beadchips were counterbalanced for sex, PTSD diagnosis, and timepoint. The study was approved by the local IRB and written informed consent was obtained from all participants prior to study enrollment and at each successive follow-up portion of the study.

AURORA

The Advancing Understanding of RecOvery afteR traumA (AURORA) study is a large multi-ancestry cohort study of women and men presenting to the emergency department (ED) within 72 hours after exposure to psychological trauma (McLean et al., 2020). Trauma exposures qualifying for study enrollment included motor vehicle collision, physical assault, sexual assault, fall greater than 10 feet, or mass casualty incidents. Other exposures qualified if participants reported experiencing the event as involving actual or threatened serious injury, sexual violence, or death (through direct exposure, witnessing, or learning about it) and if the research assistant agreed that the exposure was a plausible qualifying event. Exclusion criteria included administration of general anesthesia, long bone fractures, laceration with significant hemorrhage, solid organ injury graded above the American Association for the Surgery of Trauma Grade 1, not alert and oriented at the time of enrollment, not fluent in written or spoken English, visual or auditory impairment that precluded completing neurocognitive evaluations and/or telephone follow-ups, self-inflicted or occupational injury, prisoners, pregnancy or breastfeeding, ongoing domestic violence, and high-dose morphine use (> 20 mg or equivalent per day). Eligible patients also required an iOS or Android-compatible smartphone with internet access and a regularly used email address. PTSD diagnosis at 6 months was determined using the PTSD Checklist for DSM-5 (PCL-5; Blevins, Weathers, Davis, Witte, & Domino, 2015; Bovin et al., 2016; Kessler et al., 2021). Childhood trauma history was evaluated using a modified, 11-item survey based on the short version of the Childhood Trauma Questionnaire (CTQ; Bernstein et al., 2003) including two items each from the physical neglect, emotional neglect, emotional abuse, and physical abuse subscales and 3 items from the sexual abuse subscales, with frequency rated on a 0-4 scale. The present study is focused on a subset of the AURORA cohort with available phenotypic and DNA methylation data at the ED and 6-month follow-up after trauma exposure.

DNHS

The Detroit Neighborhood Health Study (DNHS) is a prospective longitudinal cohort study of predominantly African American adults residing in Detroit, Michigan (Goldmann et al., 2011; Uddin et al., 2010). The primary goal is to investigate how genetic variation, lifetime experience of stress and trauma, and neighborhood environmental factors contribute to psychopathological and behavioral outcomes. Participants completed a 40-minute, structured telephone interview annually between 2008 and 2012 to assess perceptions of participants' neighborhoods, mental and physical health, social support, exposure to traumatic events, and alcohol and tobacco use. PTSD was assessed using the civilian version of the PTSD checklist (PCL-C; F. Weathers, Litz, Herman, Huska, & Keane, 1993) All survey participants were invited to provide a specimen (venipuncture, blood spot, or saliva) for immune and inflammatory marker testing as well as genetic testing of DNA. Informed consent was obtained at the beginning of each interview and again before specimen collection. The study protocol was reviewed and approved by the Institutional Review Board of the University of Michigan. A subset of the DNHS cohort, with available longitudinal data on methylation, PTSD, and other related phenotypes, was included in this study (Wani et al., 2021).

**Methylation Data Cleaning**

Methylation probes were first screened using the 17 Illumina type control metrics (https://support.illumina.com/content/dam/illumina-support/documents/documentation/chemistry\_documentation/infinium\_assays/infinium\_hd\_methylation/beadarray-controls-reporter-user-guide-1000000004009-00.pdf). Then, outliers and duplications identified by the SNPs measured on the DNAm chip were flagged. Noob background correction was then performed using the minfi package to normalize the probes (Aryee et al., 2014). Sex predicted by methylation values was checked against the genotyped sex if available, or the self-reported sex, and mismatches were identified and dropped. Individual probe values that did not meet a detection *p*-value of 0.01 were set to missing. Samples which had more than 10% missing data or that were outside the bounds of the probe intensity threshold (< 50% of the experiment-wide mean or with intensity <2,000 arbitrary units) were removed. Cross-hybridized probes were also excluded. A ComBat adjustment was then performed to remove chip and position effects while preserving variation of age, sex, and PTSD diagnosis using the Bioconductor package sva (Leek, Johnson, Parker, Jaffe, & Storey, 2012). Imputation was implemented using k-nearest neighbor method via Bioconductor impute package (Hastie T, Tibshirani R, Narasimhan B, & Chu G, 2023). A DNAm-based smoking score was computed by the product of the methylation M values at 39 smoking-associated CpG sites and the effect size estimates of their association with smoking pack years (Li et al., 2018).

**Supplementary Results**

**Missing Probes**

We summarized probes that were missing in the calculation of Horvath age for each cohort. Among the 353 CpG sites used for Horvath age calculation, 17 (4.8%) were missing from the EPIC chip. In addition, the number of missing probes was 1 (0.3%) for PRISMO, 2 (0.6%) for ArmySTARRS, MRS, DNHS, TRACTS, and longitudinal NCPTSD, and 18 (5.1%) for AURORA. This information was not available for GrimAge algorithm as the specific probes used for its calculation were not published.

**Age Range and the Correlations between Chronological Age and Epigenetic Age**

The lower correlations observed between chronological age with Horvath age and GrimAge in ArmySTARRS and MRS (Table S1) were likely due to the smaller variance in chronological age in these cohorts (age ranges for these studies are 18-45 and 18-33, respectively), which limits the maximum magnitude of the coefficient. This same observation has been reported in many prior studies (such as birth cohorts) with limited range in chronological age (e.g., Bozack et al., 2023).

**Change in PTSD Symptom Severity and Change in GrimAge Residuals Over Time**

For completeness, we also examined the association between change in PTSD symptom severity and change in GrimAge residuals. Consistent with the new onset PTSD diagnosis findings, the interaction between change in PTSD symptom severity and T1 GrimAge residuals was not significantly associated with T2 GrimAge residuals (meta-p = 0.642). Full results for each individual study are listed in Table S7.

**Childhood Trauma and Change in DNAm Age Residuals Over Time**

We conducted an additional follow-up analysis replacing the PTSD variable with childhood trauma as assessed at T1 in n = 1317 participants spanning 7 cohorts. This analysis modeled T2 DNAm age residuals as a function of the interaction between T1 DNAm age residuals and childhood trauma measured at T1 while adjusting for their main effects and covariates (consistent with our main analyses for PTSD). The analysis revealed no significant main effect of childhood trauma on T2 Horvath age residuals (meta-p = 0.48) or T2 GrimAge residuals (meta-p = 0.07). The interaction term showed no significant association with T2 Horvath age residuals (meta-p = 0.56) or T2 GrimAge residuals (meta-p = 0.41) either, suggesting that childhood trauma did not alter the nature of the associations between epigenetic age residuals over time. This analysis is fundamentally different than our main analysis focused on new onset PTSD and changing PTSD symptoms in that this analysis examined childhood trauma which predated the T1 DNAm assessment while our main analysis is focused on changing PTSD diagnoses and symptom severity between T1 and T2.

**Supplementary Tables**

Table S1.

*Pearson Correlations between Chronological Age, DNAm Age, and DNAm Age Residuals*

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Cohort | Age vs DNAm age | | | | Horvath Age vs GrimAge | | Horvath Age Residuals vs  GrimAge Residuals | |
|  | Horvath Age | | GrimAge | |
|  | T1 | T2 | T1 | T2 | T1 | T2 | T1 | T2 |
| Army STARRS | 0.81 | 0.82 | 0.81 | 0.82 | 0.68 | 0.69 | 0.07 | 0.05 |
| DNHS | 0.93 | 0.91 | 0.89 | 0.90 | 0.87 | 0.85 | 0.26 | 0.18 |
| Longitudinal NCPTSD | 0.94 | 0.92 | 0.92 | 0.91 | 0.87 | 0.86 | 0.06 | 0.16 |
| MRS | 0.63 | 0.67 | 0.65 | 0.53 | 0.35 | 0.26 | -0.09 | -0.14 |
| PRISMO | 0.91 | 0.90 | 0.88 | 0.89 | 0.81 | 0.80 | 0.02 | -0.01 |
| TRACTS | 0.92 | 0.91 | 0.90 | 0.88 | 0.84 | 0.82 | 0.06 | 0.07 |
| AURORA | 0.92 | 0.91 | 0.92 | 0.92 | 0.88 | 0.88 | 0.23 | 0.24 |
| Meta  95% CI | 0.89 \*\*\*  0.82-0.93 | 0.88 \*\*\*  0.82-0.92 | 0.87 \*\*\*  0.81-0.92 | 0.86 \*\*\*  0.78-0.92 | 0.80 \*\*\*  0.67-0.88 | 0.78 \*\*\*  0.64-0.87 | 0.09 \*  0.01-0.18 | 0.09  -0.003-0.18 |

*Note*. 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; CI = confidence interval. The 95% confidence intervals were computed for the meta-analytic correlations. A t-test was conducted to test if each meta-analytic correlation is zero, with significance levels indicated as \* *p* < 0.05 and \*\*\* *p* < 0.001.

Table S2.

*Pearson Correlations between Cell Type Proportion Estimates and DNAm Age Residuals*

1. **Horvath Age Residuals**

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Cohort | CD8+T | | CD4+T | | NK | | B Cell | | Mono | |
|  | T1 | T2 | T1 | T2 | T1 | T2 | T1 | T2 | T1 | T2 |
| Army STARRS | -.09 | .13 | .01 | .10 | -.04 | .09 | .09 | .04 | -.02 | -.02 |
| DNHS | .19 | .22 | -.20 | -.06 | .04 | .04 | -.10 | -.09 | -.04 | .03 |
| Longitudinal NCPTSD | .18 | .09 | -.08 | -.12 | .11 | .05 | -.01 | -.06 | -.03 | -.01 |
| MRS | -.03 | -.03 | .05 | -.05 | -.06 | -.02 | .07 | -.10 | .06 | -.01 |
| PRISMO | .07 | .21 | -.001 | -.03 | .11 | .03 | .06 | .05 | -.02 | -.11 |
| TRACTS | .06 | .06 | -.08 | -.12 | -.001 | -.01 | -.07 | -.13 | -.06 | .002 |
| AURORA | -.01 | .12 | -.04 | .01 | .003 | -.05 | -.08 | -.14 | -.02 | -.06 |
| Meta  95% CI | .05  -.02, .13 | .11 \*\*  .05, .16 | -.06 \*  -.11, -.01 | -.05  -.11, .02 | .02  -.04, .07 | .01  -.04, .06 | -.02  -.08, .04 | -.08 \*\*  -.13, -.02 | -.03  -.08, .02 | -.02  -.07, .03 |

1. **GrimAge Residuals**

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Cohort | CD8+T | | CD4+T | | NK | | B Cell | | Mono | |
|  | T1 | T2 | T1 | T2 | T1 | T2 | T1 | T2 | T1 | T2 |
| Army STARRS | -.23 | -.28 | -.27 | -.37 | -.19 | -.23 | -.22 | -.22 | -.07 | -.09 |
| DNHS | .03 | -.09 | -.14 | -.20 | -.11 | -.10 | -.09 | -.14 | -.01 | .13 |
| Longitudinal NCPTSD | -.10 | -.11 | -.16 | -.19 | -.31 | -.31 | -.09 | -.14 | -.15 | -.11 |
| MRS | -.27 | -.30 | -.24 | -.27 | -.13 | -.18 | -.28 | -.19 | .02 | .09 |
| PRISMO | -.16 | -.07 | -.07 | -.12 | -.33 | -.04 | -.10 | -.13 | .05 | .09 |
| TRACTS | -.22 | -.22 | -.11 | -.16 | -.26 | -.19 | -.13 | -.19 | .02 | -.01 |
| AURORA | -.002 | .04 | -.11 | -.15 | -.01 | -.02 | .06 | .03 | .16 | .23 |
| Meta  95% CI | -.14 \*\*  -.22, -.05 | -.15 \*\*  -.24, -.06 | -.15 \*\*\*  -.20, -.10 | -.21 \*\*\*  -.27, -.14 | -.19 \*\*\*  -.28, -.11 | -.16 \*\*\*  -.23, -.08 | -.12 \*\*  -.20, -.04 | -.14 \*\*\*  -.21, -.08 | .002  -.07, .08 | .05  -.05, .14 |

*Note*. CD8+T = CD8+ T cell; CD4+T = CD4+ T cell; NK = natural killer cell; Mono = monocyte; 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; CI = confidence interval. The 95% confidence intervals were computed for the meta-analytic correlations. A t-test was conducted to test if each meta-analytic correlation is zero, with significance levels indicated as \* *p* < 0.05, \*\* *p* < 0.01, and \*\*\* *p* < 0.001.

Table S3.

*Associations between Baseline Horvath DNAm Age Residuals with New-Onset PTSD Diagnosis and Change in PTSD Symptom Severity*

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Cohort | New-onset PTSD DX | | | Change in the harmonized PTSD  symptom severity (T2-T1) | | |
|  | Beta | SE | *p* | Beta | SE | *p* |
| Army STARRS | 0.092 | 0.528 | 0.862 | -0.404 | 1.096 | 0.713 |
| MRS | -0.600 | 0.625 | 0.339 | -1.205 | 1.454 | 0.409 |
| PRISMO | -2.683 | 0.772 | 0.001 | -3.354 | 1.389 | 0.018 |
| Longitudinal NCPTSD | -1.128 | 0.873 | 0.200 | 2.046 | 1.722 | 0.237 |
| TRACTS | 0.750 | 0.720 | 0.299 | 0.614 | 1.186 | 0.605 |
| DNHS | NA | NA | NA | 0.644 | 1.357 | 0.636 |
| AURORA | -1.139 | 1.199 | 0.347 | -2.456 | 1.852 | 0.188 |
| Meta | -0.700 | 0.501 | 0.162 | -0.538 | 0.647 | 0.406 |

*Note*. Both models were adjusted for sex (if applicable), three ancestry PCs, and proportion estimates of the five cell types (CD8+ T cells, CD4+ T cells, B cells, natural killer cells, and monocytes) at baseline. 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; DX = diagnosis; SE = standard error.

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