**Posttraumatic Psychopathology and the Pace of the Epigenetic Clock: A Longitudinal Investigation**

**Supplementary Materials**

**Supplementary Methods**

**DNA Extraction**. A Qiagen AutoPure instrument and Qiagen reagents were used to isolate DNA and concentrations were normalized using the Quant-iT™ PicoGreen dsDNA fluorescent assay (Invitrogen). TaqMan® RNase P Detection assay (Applied Biosystems Assay, Life Technologies, Carlsbad, CA) with fluorescence detection on a 7900 Fast Real Time PCR System (Applied Biosystems, Life Technologies, Carlsbad, CA) was used per the manufacturer's protocol to determine DNA quality and quantity.

**Genotyping**. DNA was whole-genome amplified, fragmented, precipitated and resuspended prior to hybridization on Illumina HumanOmni2.5-8 beadchips for 20 hours at 48⁰C per manufacturer’s protocol (Illumina, San Diego, CA). A single-base extension followed by a multi-layered staining process was conducted following hybridization. The Illumina iScan System was used to image the beadchips, with results processed using Illumina GenomeStudio v2011.1 software containing the Genotyping v1.9.4 module.

**Ancestry PCs**. Principal components (PCs) for use as ancestry covariates were computed based on 100,000 randomly chosen common (minor allele frequency >5%) SNPs using PLINK version 1.9 (Chang, Chow et al. 2015).

**DNA Methylation Data Processing Pipeline**. Individual-level background-corrected probe data and idat files were output from GenomeStudio. DNAm data were cleaned using the CpGassoc package and the ChAMP package in R (R Development Core Team, 2008). Probes that did not meet a detection *p*-value threshold of 0.001 were set to missing. In one instance, a chip had 7 out of 8 failed samples (>10% missing). These data were discarded. We then reran these samples on a new chip and then no samples had >10% missing data. There were no samples with intensity < 50% of the experiment-wide mean or with intensity <2,000 arbitrary units (AU). Cross hybridizing probes (i.e., between autosomes and sex chromosomes) were excluded (Chen *et al.*, 2013) (*n*=44,132) as were 977 “underperforming” EPIC probes included in Illumina Product Quality Notification PQN0223 Dated 19 April, 2017.

**Calculation of Epigenetic Age**. Horvath DNAm age estimates were computed using the Horvath R script based on the 335 EPIC chip probes that were assessed on the chip and passed QC. As the Horvath method includes its own QC, Horvath age estimates were computed from the data after the removal of low-performing probes (see above), but without further normalization. For the Hannum algorithm, data were normalized using the beta mixture quantile dilation (BMIQ) method (Teschendorff *et al.*, 2013), as implemented in the wateRmelon (Touleimat and Tost, 2012; Pidsley *et al.*, 2013) R package. We corrected for batch and chip effects via an empirical Bayes batch-correction method (ComBat; Johnson *et al.*, 2007) in the Bioconductor sva package (Leek *et al.*, 2015). Missing probe data was imputed using the Bioconductor Impute package (http://www.bioconductor.org/packages/release/bioc/html/impute.html) using the K nearest neighbor method (Troyanskaya *et al.*, 2001). Of the 89 CpG sites assessed by the 450K chip used to compute Hannum methylation age estimates, 81 were also assessed by the EPIC chip, passed QC, and were used here to compute Hannum methylation age estimates.

**White Blood Cell Count Estimation**. Proportional white blood cell (WBC) counts at T1 and T2 were estimated from the methylation data itself using the R minfi package (Aryee *et al*, 2014), which has been extended to work with EPIC chip data (Fortin *et al.*, 2017). This method yields estimates of the proportion of CD4 and CD8 T-cells, natural killer (NK) cells, monocytes, and b-cells.

**Supplementary Results**

**Contribution of WBCs to the rate of DNAm age change**. None of the T1 WBCs predicted the rate of Horvath DNAm age change (smallest *p* = .07) nor the rate of Hannum DNAm age change (smallest *p* = .31). The same held true for T2 WBCs as predictors of Horvath (smallest *p* = .20) and Hannum (smallest *p* = .08) rates of DNAm age change.

**Cross-Sectional Associations between T1 Diagnoses and T1 DNAm age Residuals**

There were no significant T1 diagnostic or PTSD symptom cluster severity associations with T1 DNAm age residuals. However, in this cohort which overlaps that reported in (Wolf *et al.*, 2016), we did replicate our previously reported association between total latent PTSD severity at T1 and advanced T1 Hannum DNAm age, using the EPIC chip. Full results are reported in Table S1, below.

**Moderation of MDD, GAD, and AUD associations by Demographic Variables**. We examined potential moderation of the alcohol abuse/dependence effect by sex, race/ethnicity, marital status, age, and education and found no evidence of a significant interaction in predicting Horvath or Hannum rates of change in DNAm age.

**Moderation of PTSD effects by Demographic Variables**. We examined potential moderation of the PTSD symptom clusters by sex, race/ethnicity, age, marital status, and education on both Horvath and Hannum estimates of the rate of DNAm age change over time and found no evidence for demographic variable moderation of the association between any of the PTSD variables and the pace of the epigenetic clock over time.

**Controlling for Potential Confounds.** In follow-up analyses that included trauma exposure, PTSD symptom cluster factor scores, alcohol-use disorders, sex, and the top two PCs, we examined several potentially relevant confounds of our primary effects: lifetime count of traumatic brain injury, T1 body mass index, T1 cigarette use, and T1 use of antidepressants, anti-epileptics, sedatives, or pain medications. We found that none of these potentially confounding variables was associated with the rate of change in Horvath DNAm age estimates (smallest *p* = .25) while both alcohol-use disorder (*p* = .002) and PTSD avoidance/numbing symptoms (*p* = .036) remained statistically significant.

**Covariation of Psychopathology Change with the Rate of DNAm Age Acceleration**. Two sets of follow-up analyses evaluated the PCs, sex, trauma exposure, and change over time in psychopathology (change in PTSD, major depression, alcohol-use disorder, or generalized anxiety disorder diagnoses in one model and change in PTSD symptom cluster severity in the second model) as predictors of each DNAm age acceleration rate variable. There was no evidence that change in any psychopathology variable was associated with the rate of DNAm age change (Table S2).

Table S1

*Regression Results Predicting Cross-Sectional DNAm Age Residuals at T1*

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | Horvath | | | |  | Hannum | | | |
| Model |  | B | SE | β | *p* |  | B | SE | β | *p* |
| 1. PTSD Sx Sev |  |  |  |  |  |  |  |  |  |  |
| PC1 |  | 2.81 | 4.38 | .06 | .52 |  | -1.02 | 4.02 | -.02 | .80 |
| PC2 |  | 1.41 | 4.06 | .03 | .73 |  | -1.60 | 3.73 | -.03 | .67 |
| Sex |  | .67 | .99 | .06 | .50 |  | -.05 | .91 | .00 | .96 |
| CD8-T |  | -5.27 | 8.03 | -.05 | .51 |  | -6.92 | 7.37 | -.07 | .35 |
| CD4-T |  | -12.82 | 6.79 | -.16 | .06 |  | -12.67 | 6.23 | -.16 | .04 |
| NK |  | 6.98 | 8.61 | .07 | .42 |  | 21.13 | 7.90 | .21 | .008 |
| B cell |  | -21.99 | 14.95 | -.13 | .14 |  | -12.81 | 13.72 | -.08 | .35 |
| Monocyte |  | -12.18 | 13.92 | -.07 | .38 |  | -8.65 | 12.78 | -.05 | .50 |
| T1 trauma |  | .08 | .19 | .04 | .66 |  | -.29 | .18 | -.14 | .11 |
| **T1 PTSD sx sev** |  | .00 | .02 | .002 | .98 |  | **.03** | **.01** | **.21** | **.02** |
| 2. PTSD Sx Clusters |  |  |  |  |  |  |  |  |  |  |
| PC1 |  | 2.87 | 4.39 | .06 | .52 |  | -1.04 | 4.04 | -.02 | .80 |
| PC2 |  | .74 | 4.15 | .02 | .86 |  | -2.31 | 3.81 | -.05 | .55 |
| Sex |  | .70 | 1.00 | .06 | .48 |  | .07 | .92 | .006 | .94 |
| CD8-T |  | -5.71 | 8.07 | -.06 | .48 |  | -6.93 | 7.42 | -.07 | .35 |
| CD4-T |  | -12.01 | 6.83 | -.15 | .08 |  | -12.20 | 6.28 | -.16 | .05 |
| NK |  | 6.37 | 8.63 | .06 | .46 |  | 20.64 | 7.93 | .20 | .01 |
| B cell |  | -19.96 | 15.13 | -.11 | .19 |  | -11.23 | 13.91 | -.07 | .42 |
| Monocyte |  | -10.11 | 14.11 | -.06 | .47 |  | -6.68 | 12.97 | -.04 | .61 |
| T1 trauma |  | .12 | .20 | .05 | .56 |  | -.30 | .18 | -.14 | .10 |
| T1 reexp |  | -.07 | .11 | -.13 | .50 |  | .06 | .10 | .11 | .56 |
| T1 avd & numb |  | -.02 | .08 | -.05 | .77 |  | -.02 | .07 | -.05 | .78 |
| T1 hyperarousal |  | .10 | .10 | .17 | .32 |  | .08 | .09 | .15 | .36 |
| 3. Stress-Related Dxs |  |  |  |  |  |  |  |  |  |  |
| PC1 |  | 3.50 | 4.39 | .07 | .43 |  | -1.61 | 4.10 | -.03 | .70 |
| PC2 |  | 2.11 | 4.13 | .04 | .61 |  | -1.43 | 3.86 | -.03 | .71 |
| Sex |  | .84 | .10 | .07 | .40 |  | .14 | .93 | .01 | .88 |
| CD8-T |  | -6.30 | 8.09 | -.06 | .44 |  | -7.13 | 7.57 | -.08 | .35 |
| CD4-T |  | -13.27 | 6.82 | -.16 | .05 |  | -12.25 | 6.38 | -.16 | .06 |
| NK |  | 4.91 | 8.69 | .05 | .57 |  | 18.95 | 8.13 | .19 | .02 |
| B cell |  | -22.17 | 15.19 | -.13 | .15 |  | -12.14 | 14.19 | -.07 | .39 |
| Monocyte |  | -12.23 | 13.92 | -.07 | .38 |  | -5.99 | 13.01 | -.04 | .65 |
| T1 trauma |  | .18 | .19 | .08 | .35 |  | -.15 | .18 | -.07 | .41 |
| T1 current PTSD dx |  | -.65 | .69 | -.08 | .35 |  | .62 | .65 | .08 | .34 |
| T1 current MDD dx |  | .33 | .81 | .03 | .69 |  | .47 | .76 | .05 | .53 |
| T1 current GAD dx |  | -.44 | 1.16 | -.03 | .70 |  | -.57 | 1.08 | -.04 | .60 |
| T1 current Alc. dx |  | -1.06 | .85 | -.10 | .22 |  | -1.02 | .80 | -.10 | .20 |

*Note*. All PTSD severity variables were factor scores derived from a confirmatory factor analysis of pre-military, post-military, and current PTSD symptoms as assessed at T1. DNAm = DNA methylation; PTSD = posttraumatic stress disorder; PC = principal component; T1 = time 1; sx = symptom; dx = diagnosis; sev = severity; reexp = re-experiencing; avd = avoidance; numb = numbing; MDD = major depressive disorder; GAD = generalized anxiety disorder; Alc = alcohol.

Table S2.

*Covariation of Change in Psychopathology with the Rate of DNAm Age Acceleration*

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | Horvath | | | |  | Hannum | | | |
| Model |  | B | SE | β | *p* |  | B | SE | β | *p* |
| 1. PTSD Sx Clusters |  |  |  |  |  |  |  |  |  |  |
| PC1 |  | 2.13 | 1.82 | .092 | .24 |  | -1.77 | 1.68 | -.082 | .29 |
| PC2 |  | -.82 | 1.79 | -.036 | .65 |  | -.58 | 1.65 | -.028 | .73 |
| Sex |  | -.69 | .44 | -.13 | .12 |  | -.66 | .41 | -.13 | .11 |
| T1 trauma |  | -.017 | .078 | -.017 | .83 |  | -.004 | .072 | -.004 | .96 |
| Δ reexp sev |  | .012 | .021 | .055 | .55 |  | .000 | .019 | .001 | .99 |
| Δ avd & numb sev |  | -.017 | .016 | -.098 | .28 |  | -.017 | .015 | -.11 | .24 |
| Δ hyperarousal sev |  | -.019 | .020 | -.088 | .33 |  | -.008 | .018 | -.041 | .64 |
| 2. Stress-Related Dxs |  |  |  |  |  |  |  |  |  |  |
| PC1 |  | 2.62 | 1.83 | .11 | .15 |  | -1.61 | 1.67 | -.076 | .34 |
| PC2 |  | -.49 | 1.79 | -.021 | .79 |  | -.10 | 1.63 | -.005 | .95 |
| Sex |  | -.68 | .44 | -.12 | .13 |  | -.65 | .40 | -.13 | .11 |
| T1 trauma |  | -.042 | .079 | -.043 | .59 |  | -.011 | .072 | -.012 | .88 |
| Δ PTSD dx |  | -.27 | .30 | -.072 | .37 |  | -.30 | .27 | -.088 | .27 |
| Δ MDD dx |  | -.17 | .33 | -.040 | .61 |  | .004 | .30 | .001 | .99 |
| Δ GAD dx |  | -.15 | .41 | -.028 | .72 |  | -.13 | .37 | -.027 | .73 |
| Δ Alc. dx |  | -.38 | .33 | -.090 | .25 |  | .041 | .30 | .011 | .89 |

*Note*. DNAm = DNA methylation; PTSD = posttraumatic stress disorder; PC = principal component; T1 = time 1; sx = symptom; dx = diagnosis; sev = severity; reexp = re-experiencing; avd = avoidance; numb = numbing; MDD = major depressive disorder; GAD = generalized anxiety disorder; Alc = alcohol.

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