

Supplementary Methods

Telomere Length Measurement

Each assay (measuring the single copy gene 36B4 and measuring telomere repeats) was run three times and triplicate values were generated. One average value from each assay was then calculated for further analysis. If more than 2% discordance was found among triplicates, the data was removed from analyses. The T/S ratio value for all experimental samples was compared to the T/S ratio of five pooled genomic DNA reference samples, derived from normal human subjects of mixed age and sex. The relative T/S ratio (-ddCt) was determined by subtracting the reference sample T/S from the unknown sample T/S ratio, and then exponentiating (2^{-ddCt}). A modified version of the qPCR telomere assay was performed in a 384-well format with a 7900HT PCR System (Life Technologies, Carlsbad, CA). 5ng of buffy-coat derived genomic DNA was dried down in a 384-well plate and resuspended in 10 μ L of either the telomere or 36B4 reaction mixture and stored at 4°C up to 6 hours. The telomere reaction mixture consists of 1x Quantitect SYBR Green Master Mix (Qiagen, Venlo, Netherlands), 2.0mM of DTT, 270nM of Tel-1 primer-(GGTTTTTGAGGGTGAGGGTGAGGGTGAGGGTGAGGGT), and 900nM of Tel-2 primer-(TCCCGACTATCCCTATCCCTATCCCTATCCCTATCCCTATCCCTA). The reaction proceeded for 1 cycle at 95°C for 10 min, followed by 40 cycles at 95°C for 15 sec, and 54°C for 2 min. The 36B4 reaction consists of 1x Quantitect SYBR Green Master Mix, 300nM of 36B4U primer-(CAGCAAGTGGGAAGGTGTAATCC), and 500nM of 36B4D primer-(CCCATCTATCATCAACGGGTACAA). The 36B4 reaction will proceed for 1 cycle at 95°C for 10 min, followed by 40 cycles at 95°C for 15 sec, and 58°C for 1 min 10 sec. Samples for both the telomere and 36B4 reactions were performed in triplicate on different plates. Each 384-

well plate contained a 6-point standard curve from 0.625ng to 20ng to assess PCR efficiency. A slope of $-3.33 \pm 10\%$ for the standard curve of both the telomere and 36B4 reactions was deemed acceptable. Two quality controls were plated and run along with the primary study samples in every plate to measure inter- and intra- plate variability of Ct values. Each quality control sample was comprised of 2-4 different pooled DNA samples of mixed age and sex. The inter-plate % coefficient of variation (CV) for both the telomere Ct and 36B4 Ct values were consistently under 0.75% in previous projects performed at this facility and the CV for the T/S ratio of quality control samples was 14.3% in this sample. No threshold was set for critically short telomeres; all samples amplified within the expected range of cycle. The telomere T/S ratio ranged from 0.2-1.2.

Laboratory and Statistical Procedures for DNAm and DNA

Genotype. Full details on the genotyping techniques and data cleaning procedures are available in Logue et al. (2013) and are summarized here. DNA was isolated from peripheral blood samples on a Qiagen AutoPure instrument with Qiagen reagents; DNA samples were whole-genome amplified, fragmented, precipitated and resuspended before hybridization on Illumina HumanOmni2.5-8 beadchips per manufacturer's protocol (Illumina, San Diego, CA). Samples with call rates less than 95% were excluded. We screened for cryptic relatedness and mismatches between sex and X-chromosome heterozygosity.

Methylation. The integrity and quantity of the DNA samples were determined by 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) according to the manufacturer's protocol. Samples were bisulfite-modified using Zymo EZ-96 DNA Methylation Kits (D5004). The bisulfite-mediated

conversion efficiency was determined by PCR with DAPK1 primers (Zymo) and gel electrophoresis of PCR-products. Samples were hybridized to Illumina HumanMethylation 450 beadchips for scanning using the Illumina iScan System. Internal quality control and performance was examined with GenomeStudio v2011.1 software using Methylation module v1.9.0 (see Wolf et al., in press).

Background-corrected methylation-probe data were available for 489 white non-Hispanic samples. Of these, 20 duplicates and three previously identified sample swaps were excluded, leaving 466 samples for analysis. Of these, two were missing chronological age and 11 were missing other key variables (primarily TL estimates), yielding a final sample size of 453. The pipeline established by the Psychiatric Genomics Consortium PTSD Workgroup for the processing, quality control, and cleaning of DNAm was utilized (see Ratanatharathorn et al. 2017). DNAm data were cleaned using the CpGassoc package Bioconductor package in R (R Development Core Team, 2008). All samples passed intensity thresholds (intensity > 50% of the experiment-wide and > 20,000 arbitrary units). We set singleton probe values with detection-p greater than 0.001 to missing. Probes and subjects with > 10% missing values were excluded. Probe normalization was performed using beta mixture quantile dilation (BMIQ) method (Teschendorff et al., 2013) which was implemented in the watermelon package (Pidsley et al., 2013; Touleimat & Tost, 2012). The Bayes batch-correction method (ComBat; Johnson & Rabinovic, 2007) in the Bioconductor sva package (Leek et al., 2013) was used to remove chip and position effects. Missing data were imputed using a k nearest neighbor method (KNN; Hastie et al., 1999) via the Bioconductor impute package (<http://www.bioconductor.org/packages/release/bioc/html/impute.html>). This approach uses the

methylation patterns for similarly behaving genes (Troyanskaya et al., 2001) to generate values for missing data.

DNAm age Estimates. The Horvath DNAm age estimate was calculated using the statistical software program R, as detailed by Dr. Horvath (<http://labs.genetics.ucla.edu/horvath/dnamage/>). We calculated Hannum DNAm age estimates from beta values (proportion of methylated DNA) after filtering quality control procedures. Of the 89 probes specified for use in the Hannum et al. (2013) “all data” algorithm, two loci (cg25428494 and ch.13.39564907R) did not survive cleaning and quality control procedures and were excluded. Of the 87 remaining probes, 15 had some missing data and imputed data were used for the Hannum DNAm age calculation; there was no more than 3.24% missing data for any probe. Hannum methylation age estimates were computed via linear multiplication of the beta values from the 87 available probes and the coefficients reported by Hannum et al. in the R statistical analysis package.

Ancestry. Self-reported ancestry was compared against DNA data using a Bayesian clustering analysis of single nucleotide polymorphisms via the program STRUCTURE (Falush, Stephens, & Pritchard, 2003; Pritchard, Stephens, & Donnelly, 2000). This process was used to identify the White non-Hispanic sample that would undergo DNA methylation analysis. Within this sample, principal components (PC) were created to explore the potential confounding effect of population substructure (e.g., differences within the white non-Hispanic cohort). These PCs were generated with EIGENSTRAT (Shadick et al., 2006) based on 100,000 randomly chosen SNPs.

White blood cells. White blood cell counts (CD4 and CD8 T-cells, natural killer cells, B cells, and monocytes) were estimated from the DNAm data as there is variability in the type of

white blood cell that the DNA was extracted from. This was conducted in the R minfi package using the procedures described by Houseman et al. (2012) and Jaffe and Irizarry (2014).

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Supplementary Table 1.

Prevalence of Somatic Comorbidities in Subset of Study Sample (BMI n= 133, cigarette smoking n= 309, all other variables n= 219)

	M	SD	%
BMI	31.02	5.95	
Type 2 diabetes			10.0
Hyperlipidemia			32.9
Hypertension			32.4
Cardiovascular disease			13.2
History of cigarette smoking			78.1

Note. BMI= body mass index.

Supplementary Table 2.

Regression Examining Estimated White Blood Cell Count Proportions, Population Substructure, Demographic Variables, Trauma, and Lifetime PTSD Symptoms as Predictors of TL

	Std. β	ΔR^2
Step 1		.104***
Age	-.252***	
Sex	-.082	
Num. diff. traumas	.043	
PTSD symptoms	-.019	
CD4 T-cells	-.040	
CD8 T-cells	-.071	
NK cells	-.086	
B cells	-.039	
Monocytes	-.118*	
PC1	-.030	
PC2	-.047	
Step 2		.025***
Age x PTSD symptoms	-.161**	

Note. TL = telomere length; Unstd. *B* = unstandardized beta; SE = standard error; Std. β = standardized beta; Num. Diff. Traumas = number of different traumas as measured by the Traumatic Life Events Questionnaire (TLEQ); PTSD = posttraumatic stress disorder. PTSD symptoms = lifetime symptom score on the Clinician Administered PTSD scale (CAPS). NK Cells = Natural killer cells. PC = principal component (reflecting ancestry from genome wide genotype data). Age and PTSD symptoms were centered on their means.

* $p < .05$, ** $p < .01$, *** $p < .001$.

Supplementary Table 3.

Results of Multiple Regressions predicting Estimated White Blood Cell Proportions

Outcome	CD4 T-Cells	CD8 T-Cells	NK Cells	B Cells	Monocytes
	Std. β	Std. β	Std. β	Std. β	Std. β
Age	-.049	-.281***	.213***	-.018	.089
Sex	-.153**	-.209***	.109*	-.047	.291***
Num. Diff. Traumas	-.022	.055	.091	.033	-.076
PTSD symptoms	.077	.024	-.076	.034	.047
Age X PTSD symptoms	-.023	.018	.019	.072	-.084

Note. NK Cells = Natural killer cells; Num. Diff. Traumas = number of different traumas as measured by the Traumatic Life Events Questionnaire (TLEQ); PTSD = posttraumatic stress disorder. PTSD symptoms= lifetime symptom score on the Clinician Administered PTSD scale (CAPS). Age and PTSD symptoms were centered on their means.

CD4 T Cells Model $R^2 = .029$; CD8 T Cells Model $R^2 = .135$; NK Cells Model $R^2 = .069$; B Cells Model $R^2 = .011$; Monocytes Model $R^2 = .107$.

* $p < .05$, ** $p < .01$, *** $p < .001$.

See Supplementary Table 2 for a regression predicting to TL while controlling for estimated white blood cell proportions.

Supplementary Table 4.

Regression Examining Somatic Comorbidities as Predictors of TL

	Std. β	ΔR^2
Step 1		.104***
Age	-.271***	
Sex	-.159*	
Step 2		.008***
Type 2 diabetes	.004	
Hyperlipidemia	-.069	
Hypertension	.003	
Cardiovascular disease	.081	

Note. TL = telomere length; BMI = body mass index. In a separate regression examining a larger subset of the sample (n= 309), history of cigarette use was not a significant predictor of TL, controlling for age and sex ($\beta = .010, p = .856$). No significant associations were found between body mass index and TL when controlling for age and sex within a smaller subset of the sample for which we had this data available (n= 133; $\beta = -.129, p = .131$).

* $p < .05$, ** $p < .01$, *** $p < .001$.