

UC San Diego

UC San Diego Previously Published Works

Title

Epigenome-wide meta-analysis of PTSD symptom severity in three military cohorts implicates DNA methylation changes in genes involved in immune system and oxidative stress

Permalink

<https://escholarship.org/uc/item/2r09g7r7>

Journal

Molecular Psychiatry, 27(3)

ISSN

1359-4184

Authors

Katrinli, Seyma
Maihofer, Adam X
Wani, Agaz H
et al.

Publication Date

2022-03-01

DOI

10.1038/s41380-021-01398-2

Peer reviewed



Published in final edited form as:

Mol Psychiatry. 2022 March ; 27(3): 1720–1728. doi:10.1038/s41380-021-01398-2.

Epigenome-wide meta-analysis of PTSD symptom severity in three military cohorts implicates DNA methylation changes in genes involved in immune system and oxidative stress

Seyma Katrinli¹, Adam X. Maihofer², Agaz Wani³, John R. Pfeiffer^{4,5}, Elizabeth Ketema², Andrew Ratanatharathorn⁶, Dewleen G. Baker^{2,7,8}, Marco P. Boks⁹, Elbert Geuze^{9,10}, Ronald C. Kessler¹¹, Victoria B. Risbrough^{2,8}, Bart P. F. Rutten¹², Murray B. Stein^{2,7,13}, Robert J. Ursano¹⁴, Eric Vermetten^{9,15,16,17}, Mark W. Logue^{18,19,20,21}, Caroline M. Nievergelt^{2,7,8}, Alicia K. Smith^{1,22}, Monica Uddin^{3,*}

¹Department of Gynecology and Obstetrics, Emory University, Atlanta, GA 30322, USA

²Department of Psychiatry, University of California San Diego, La Jolla, CA 92093, USA

³College of Public Health, University of South Florida, Tampa, FL 33620, USA

⁴Department of Psychology, University of Illinois at Urbana-Champaign, Urbana, IL 61820, USA

⁵Carl R. Woese Institute for Genomic Biology, Urbana, IL 61801, USA

⁶Department of Epidemiology, Columbia University, New York, NY 10027, USA

⁷Veterans Affairs San Diego Healthcare System, San Diego, CA 92161, USA

⁸Veterans Affairs Center of Excellence for Stress and Mental Health, San Diego, CA 92161, USA

⁹Brain Center Rudolf Magnus, University Medical Center Utrecht, 3584 CG Utrecht, The Netherlands

¹⁰Brain Research and Innovation Centre, Netherlands Ministry of Defence, 3509 AA Utrecht, The Netherlands

¹¹Department of Health Care Policy, Harvard Medical School, Boston, MA 02115, USA

¹²Department of Psychiatry and Neuropsychology, Maastricht University Medical Centre, School for Mental Health and Neuroscience, 6229 HX Maastricht, The Netherlands

¹³School of Public Health, University of California San Diego, La Jolla, CA 92093, USA

Users may view, print, copy, and download text and data-mine the content in such documents, for the purposes of academic research, subject always to the full Conditions of use: <https://www.springernature.com/gp/open-research/policies/accepted-manuscript-terms>

*Corresponding author: Monica Uddin, PhD, Professor, Genomics Program, College of Public Health, University of South Florida, 3720 Spectrum Blvd., Ste. 304, Tampa, FL 33612, Ph. 813-974-9765, monica43@usf.edu.

Contributions

Interpreted results, writing, and editing the manuscript: S.K., A.K.S., M.U. Conceptualization and supervision of project: A.K.S., M.W.L., M.U., C.M.N. Sample and Metadata Collection: D.G.B., M.P.B., E.G., R.C.K., V.B.R., B.P.F.R., M.B.S., R.J.U., E.V., M.W.L., C.M.N. Sample Preparation: J.R.P. Formal Data Analysis: S.K., A.X.M., A.W., E.K., A.R.

Conflict of Interest

Dr. Uddin was a paid consultant for System Analytic. In the past 3 years, Dr. Kessler was a consultant for Datastat, Inc., Holmusk, RallyPoint Networks, Inc., and Sage Pharmaceuticals. He has stock options in Mirah, PYM, and Roga Sciences. No other author declares any conflict of interest.

¹⁴Center for the Study of Traumatic Stress, Department of Psychiatry, Uniformed Services University School of Medicine, Bethesda, MD 20814, USA

¹⁵Department of Psychiatry, Leiden University Medical Center, 2333 ZA Leiden, The Netherlands

¹⁶Research Center, Netherlands Defense Department, 3509 AA Utrecht, UT, The Netherlands

¹⁷Arq Psychotrauma Expert Group, 1112 XE Diemen, The Netherlands

¹⁸National Center for PTSD, Behavioral Science Division at VA Boston Healthcare System, Boston, MA 02130, USA

¹⁹Department of Psychiatry, Boston University School of Medicine, Boston, MA 02118, USA

²⁰Department of Medicine (Biomedical Genetics), Boston University School of Medicine, Boston, MA 02118, USA

²¹Department of Biostatistics, Boston University School of Public Health, Boston, MA 02118, USA

²²Department of Psychiatry and Behavioral Sciences, Emory University, Atlanta, GA 30322, USA

Abstract

Epigenetic factors modify the effects of environmental factors on biological outcomes. Identification of epigenetic changes that associate with PTSD is therefore a crucial step in deciphering mechanisms of risk and resilience. In this study, our goal is to identify epigenetic signatures associated with PTSD symptom severity (PTSS) and changes in PTSS over time, using whole blood DNA methylation (DNAm) data (MethylationEPIC BeadChip) of military personnel prior to and following combat deployment. A total of 429 subjects (858 samples across 2 time points) from three male military cohorts were included in the analyses. We conducted two different meta-analyses to answer two different scientific questions: one to identify a DNAm profile of PTSS using a random effects model including both time points for each subject, and the other to identify a DNAm profile of change in PTSS conditioned on pre-deployment DNAm. Four CpGs near four genes (*F2R*, *CNPY2*, *BAIAP2L1* and *TBXAS1*) and 88 differentially methylated regions (DMRs) were associated with PTSS. Change in PTSS after deployment was associated with 15 DMRs, of those 2 DMRs near *OTUD5* and *ELF4* were also associated with PTSS. Notably, three PTSS-associated CpGs near *F2R*, *BAIAP2L1* and *TBXAS1* also showed nominal evidence of association with change in PTSS. This study, which identifies PTSD-associated changes in genes involved in oxidative stress and immune system, provides novel evidence that epigenetic differences are associated with PTSS.

Keywords

EWAS; Longitudinal; DNAm; DMR; Meta-analysis; PTSD; Epigenetics

Introduction

Posttraumatic stress disorder (PTSD) can develop in some people following trauma and results in severe symptoms including intrusive thoughts, avoidance of trauma-related stimuli, negative cognitive and mood changes, and hyperarousal that disturb mental and physical

wellbeing¹. Although a vast majority of the population experiences trauma to at some point in their life², PTSD prevalence is only 6.8% among US population³. Because only a fraction of people who experience trauma go on to develop PTSD, it is important to understand the factors that increase risk for the disorder or contribute to its symptom severity. DNA methylation (DNAm), an epigenetic modification, is one such factor involved in adaptation to traumatic stress⁴⁻⁶.

Epigenome-wide association studies (EWASs) of PTSD have discovered differentially methylated CpGs in genes related to neuronal and immune pathways⁷⁻¹⁴. The majority of these studies have a cross-sectional design; DNAm being examined at a single time-point. In addition to previously identified, cross-sectional associations, understanding whether DNAm changes as individuals develop PTSD or experience changes in PTSD symptom severity (PTSS) is crucial. Recently, two longitudinal studies reported DNAm changes associated with PTSD development in individuals exposed to combat trauma^{11, 12}. Both studies used modest sample sizes of 93 and 266 subjects, respectively, and the HumanMethylation450 BeadChip to identify CpGs and differentially methylated regions (DMRs) associated with PTSD development. Rutten et al. observed lower DNAm levels in PTSS at genomic regions in *ZFP57*, *RNF39* and *HIST1H2APS2*¹¹. Snijder et al. reported contributions from the immune system through the HLA locus, *HEXDC* and *MAD1L1* in development of PTSD, using different subjects of the three military cohorts that participated in this study¹².

Building on the prior work of Snijder et al.¹², this study features a larger sample size, a denser and more comprehensive array, and additional statistical models to gain more insight into the epigenetics of PTSD. We first performed a meta-analysis in 858 samples (429 subjects with pre- and post- deployment samples) to identify CpGs and DMRs that associate with PTSS. Then, we conducted a second meta-analysis in 429 subjects to identify associations between DNAm and change in PTSS pre- to post-deployment. Finally, we evaluated CpGs identified in previously published Psychiatric Genomics Consortium PTSD Workgroup (PGC-PTSD) EWAS¹⁰⁻¹⁴ in targeted longitudinal analyses. We focus on PTSS in order to overcome case-control selection bias, as some participants had elevated PTSD symptom scores before deployment, and to gain statistical power through the use of continuous variables¹⁵.

Methods

Cohorts

This study includes 429 subjects from three military cohorts that are presented in Table 1: Marine Resilience Study (MRS), Army Study to Assess Risk and Resilience in Servicemembers (Army STARRS), and Prospective Research in Stress-related Military Operations (PRISMO). Details of each cohort are in the Supplement. PTSS was measured by each individual study pre- and post- deployment. All participants gave informed consent, and all studies were approved by respective institutional review boards.

Quality Control (QC) Procedures

Whole blood DNAm was measured using the Illumina MethylationEPIC BeadChip. The same QC pipeline was applied separately to each of the cohorts. We used the R package *CpGassoc* to filter out samples with probe detection call rates <90% and an average intensity value of either <50% of the experiment-wide sample mean or <2000 arbitrary units (AU)¹⁶. We set low quality probes (detection p-values >0.01) as missing. We filtered out probes that were missing for >10% of samples within studies. We removed cross hybridizing probes¹⁷. A total of 820 498 probes passed QC in all cohorts and were included in our analyses. We performed single-sample Noob (ssNoob) normalization using R package *minfi*¹⁸. To remove chip and positional batch effects, we applied *ComBat*, protecting age and PTSD status¹⁹. We used logit transformed beta values (M-values) in our analyses²⁰.

For each sample, cellular heterogeneity (i.e. the proportion of CD8+T, CD4+T, natural killer (NK), B cells, monocytes and neutrophils) was predicted using the Robust Partial Correlation (RPC) method implemented in *Epidish*²¹ using the reference data reported by Salas and colleagues²². Ancestry principal components (PCs) were generated from DNAm, following the method described by Barfield et al.²³, as previously implemented²⁴. The components that correlate most with self-reported race/ethnicity (PCs 2–3) were used to adjust for ancestry (Supplementary Figure 1)^{23, 24}. DNAm data was used to estimate smoking information as previously described²⁵. Computation of ancestry PCs and smoking scores are described in the Supplement.

Statistical analysis

Since different measures of PTSS were used across studies and timepoints, heterogeneity was minimized by rescaling PTSS using a min-max normalization method to scale the range in [0, 1]. To identify CpGs associated with PTSS (Meta-Analysis 1), we used a linear mixed model with DNAm values at both time points as the dependent variable, PTSS at both time points as a main effect, and a random intercept for subject. Age, CD8+T, CD4+T, NK, B cell, and monocyte cell proportions, and ancestry PCs derived from DNAm data were included as covariates. To identify CpGs associated with *change* in PTSS (Meta-Analysis 2), we conducted a longitudinal analysis using a linear regression model, where post-deployment DNAm was modeled as a function of change in PTSS while adjusting for pre-deployment DNAm, PCs for ancestry, and changes in age (i.e. time passed between pre- and post-deployment data collection), CD8T+, CD4T+, NK, B cell, and monocyte cell proportions.

Meta-analysis of cohorts was performed using weighted sum of z-scores method, as Cochran's Q test did not show substantial heterogeneity^{26–28}. To control for multiple testing, we used the epigenome-wide significance threshold proposed for the MethylationEPIC BeadChip ($p < 9.0E-08$)²⁹. Post-hoc sensitivity analysis explored the possible confounding effects of smoking (Meta-Analysis 1) and changes in smoking status (Meta-Analysis 2) by including DNAm derived smoking scores as a covariate. A post-hoc sensitivity analysis also examined the impact of early life trauma on PTSS-related DNAm changes by including early life trauma burden as a covariate in the models. To assess the variability of the PTSS-associated CpGs over time, a third post-hoc analysis in which PTSS was excluded

from the model was performed. Post-hoc power analyses were performed as described in the Supplement.

In addition to our two primary meta-analyses, we also performed DMR analyses to identify i) DMRs associated with PTSS (i.e. using the same framework as Meta-Analysis 1), and ii) DMRs associated with change in PTSS by conditioning post-deployment DNAm on baseline DNAm (i.e. using the same framework as Meta-Analysis 2). We used *DMRcate* to calculate the significance of regions (at least 2 probes within 1kb of each other) based on EWAS summary statistics³⁰. This included at least one strongly associated CpG site ($p < 0.0001$). DMRs with a Stouffer transformed false-discovery rate (FDR) of 5% across the region were considered significant.

Finally, to provide additional insight into earlier findings, we evaluated the PTSD-associated CpGs from previous PGC-PTSD EWAS¹⁰⁻¹⁴ via targeted analyses using the framework for Meta-Analysis 1 described above. Bonferroni correction was used to account for multiple comparisons.

Blood-Brain Correlations

Correlation between blood and brain DNAm of associated CpGs was examined using the IMAGE-CpG database³¹. Specifically, this database maintains Spearman correlation coefficients (ρ) and associated p-values for CpGs from 27 individuals with paired blood and live brain samples.

Genetic influence of CpGs associated with PTSS

To evaluate the effect of nearby polymorphisms on DNAm levels of CpGs associated with PTSS, we leveraged cis-methylation quantitative trait locus (cis-meQTL) data from BIOS QTL browser³². Cis-meQTL, here was described as the correlation between a CpG and a SNP within 250 kb with a CpG-level FDR threshold of 5% ($p = 1.38E-04$)³². To evaluate whether the meQTLs from BIOS QTL browser had similar effects in our study, we tested the associations between post-deployment methylation levels of CpGs and their respective meQTL SNPs in all three cohorts, using linear regression models that adjust for the cohorts. For CpGs with identified meQTLs, we performed post-hoc sensitivity analyses by adding their respective meQTL SNP as a covariate to Meta-Analysis 2.

Pathway enrichment analysis

We conducted exploratory gene ontology (GO) pathway enrichment analyses using MissMethyl³³ and including variably methylated probes (VMPs) from any of the 3 cohorts that nominally associate with PTSS (Meta-Analysis 1) or change in PTSS (Meta-Analysis 2). FDR of 5% was used to define significant pathways. To identify VMPs, we first calculated longitudinal DNAm differences for all CpG sites ($\beta = \beta_{\text{post}} - \beta_{\text{pre}}$); then we computed the median value of absolute DNAm differences ($|\text{median}(\beta)|$), defining those with >1% difference from the median ($|\text{median}(\beta)| > 0.01$) as variable.

Code availability

The scripts generated to perform the Meta-Analysis 1, Meta-Analysis 2, and DMR analysis are available in <https://github.com/PGC-PTSD-EWAS/PGC-PTSD-Longitudinal-Analysis>.

Results

Demographics of the cohorts

Demographic and clinical information of participants from all studies (total N subjects = 429) are summarized in Table 1. All participants were male and were primarily of European ancestry (N = 330, 77%). Age and smoking did not differ between PTSD cases and trauma-exposed controls. Eventual cases had higher pre-deployment PTSS, compared to controls in all three cohorts, potentially due to higher rates of early life trauma (Table 1).

Meta-Analysis 1: Evaluating CpGs associated with PTSS

We identified four significant CpGs (Table 2, Figure 1A, Supplementary Table 1). These sites were located near the coagulation factor II thrombin receptor (*F2R*), canopy FGF signaling regulator 2 (*CNPY2*), Brain-specific angiogenesis inhibitor 1-associated protein 2-like protein 1 (*BAIAP2L1*), and thromboxane A synthase 1 (*TBXAS1*) genes. For all sites, lower DNAm levels associated with higher PTSS (Supplementary Figures 2–4). These observed associations were not due to ageing (Supplementary Table 1). All four sites remained significant with the same direction of association in our sensitivity analysis adjusted for smoking score (Supplementary Table 1). Only the CpG in *CNPY2* did not exceed the genome-wide significance threshold when we adjusted for early life trauma (Supplementary Table 1), indicating that the PTSS-associated DNAm changes were largely uninfluenced by early life trauma burden.

Of the four CpGs, blood DNAm levels of cg00277769 in *BAIAP2L1* and cg03604364 in *TBXAS1* were correlated with brain DNAm levels (Supplementary Table 2).

In addition, we identified 88 DMRs that were significantly associated with PTSS (Stouffer $p < 0.05$; Supplementary Table 3). All DMRs except two were still significant with the same direction of association in the sensitivity analysis adjusted for smoking score (Supplementary Table 3).

Meta-Analysis 2: Evaluating CpGs associated with change in PTSS

We identified 47 660 CpGs where post-deployment methylation was nominally ($p < 0.05$) associated with change in PTSS; however, none exceeded the genome-wide significance threshold (Figure 1B). Of the four significant CpGs from Meta-Analysis 1, three associated with *change* in PTSS after deployment at the $p < 0.05$ level, all with the same direction of association (cg11627632 in *F2R*, cg00277769 in *BAIAP2L1*, cg03604364 in *TBXAS1*; Table 2).

In addition, we identified 15 DMRs whose post-deployment DNAm significantly differed from pre-deployment DNAm based on their change in PTSS (Stouffer $p < 0.05$; Supplementary Table 4). All DMRs were still significant with the same direction of effect

in the sensitivity analysis that accounted for smoking (Supplementary Table 4). Of those 15 DMRs that were associated with *change* in PTSS, two DMRs located on genes *OTUD5* and *ELF4* were also associated with PTSS, which was not more than would be expected by chance (Fisher's exact test $p = 0.10$; Table 3, Supplementary Figure 5).

Genetic effects of CpGs associated with PTSS

Out of four CpGs associated with PTSD symptom severity, three (cg11627632 in *F2R*, cg00277769 in *BAIAP2L1*, cg03604364 in *TBXAS1*) correlated with at least one nearby SNP within 250 kb of the CpG, according to BIOS QTL browser (Supplementary Table 5). However, in our study, only cg03604364 in *TBXAS1* was associated with its meQTL SNP (rs3779130) from BIOS QTL browser (Supplementary Table 5). Controlling for genotypes in the Meta-Analysis 1 to evaluate the effect of SNPs from BIOS QTL browser did not substantially affect the observed results, and all three CpGs maintained genome-wide significance (Note that *BAIAP2L1* does not reach genome-wide significance for one of the two SNPs; Supplementary Table 6).

Evaluation of CpGs from previously published PGC-PTSD EWAS

We compared our results to previous PGC-PTSD EWAS results^{10–14} to evaluate published genome-wide significant CpGs (Table 4). Out of 31 CpGs from five studies, we observed nominal evidence of association for three CpGs: cg05575921 in *AHRR* (Meta-Analysis 1), cg26703534 in *AHRR* (Meta-Analysis 1), and cg19534438 on *GOS2* (in both Meta-Analysis 1 and 2). The direction of association was the same as that reported in the original studies for cg05575921 and cg26703534, but opposite for cg19534438 (Table 4).

It is important to note that 41 subjects of the PRISMO cohort who participated this study were also included in some of the previous studies^{11, 12, 14}. To perform an independent analysis for the *AHRR* CpGs, cg05575921 and cg26703534, we repeated our targeted meta-analysis by removing these 41 subjects. The two CpGs were still significant and showed a same direction of effect (cg05575921, $p = 0.002$, $z = -3.15$; cg26703534, $p = 0.004$, $z = -2.88$). Of note, cg05575921 remained significant after multiple test correction for 31 genome-wide significant CpGs identified in previous PGC-PTSD EWASs.

Pathway enrichment analysis

We identified 157 809 VMPs, of which 16 974 associated with PTSS and 8569 with change in PTSS. GO enrichment analysis revealed 173 biological processes enriched in CpGs that associate with PTSS and 9 with change in PTSS (FDR < 0.05). Many processes relate to immune function (Supplementary Table 7), and 7 processes, including leukocyte migration, are enriched in both PTSS and change in PTSS analyses.

Discussion

It is unclear why some develop PTSD after trauma while others do not^{34–36}. A likely underlying mechanism is epigenetic alteration, which links environmental circumstances and experiences to biological response. Here, we employed two study designs: i) to identify CpGs that are associated with PTSS measured at pre- and post-deployment (Meta-Analysis

1), and ii) to investigate associations of DNAm with change in PTSS pre-to-post deployment (Meta-Analysis 2).

The first meta-analysis showed that increased PTSS is associated with lower methylation levels at four CpGs located in *F2R*, *CNPY2*, *BAIAP2L1* and *TBXAS1*. The CpGs in *F2R*, *BAIAP2L1* and *TBXAS1* were also nominally associated with change in PTSS, and therefore show only a small change in pre- to post-deployment DNAm. *F2R* participates thrombotic response regulation, and is involve in mediating the cross-talk between coagulation and inflammation³⁷. A study that investigated gene-expression levels in peripheral blood samples reported lower *F2R* expression in PTSD cases³⁸. Together, this information suggests that transcriptional regulation of *F2R* may contribute to PTSD, conceivably by modulating immune response. *CNPY2* functions in the endoplasmic reticulum (ER) and plays a key role in the transitioning from the non-stressed to the stressed state³⁹. In addition, *CNPY2* contributes to central nervous system development by stimulating neurite outgrowth⁴⁰. This evidence suggests the possible role of *CNPY2* in oxidative stress and central nervous system development⁴⁰, which are critical pathways in PTSD⁴¹. *BAIAP2L1* promotes cell proliferation by stimulating the EGFR-ERK pathway⁴² and regulating short actin bundles during cell movement⁴³. A recent study that investigated alterations in brain transcriptomics associated with intergenerational stress transmission reported upregulation of *BAIAP2L1* in neonatal and adult mice⁴⁴. In addition, methylation levels of cg00277769 in *BAIAP2L1* correlated in blood and brain tissues of human subjects as reported in IMAGE-CpG³¹. Hence, *BAIAP2L1* expression might be regulated in response to stress and trauma.

Of particular interest in relation to PTSD are the findings relating to *TBXAS1*. The ER membrane protein TBXAS1 metabolizes Prostaglandin H2 (PGH2), which regulates the dilation of blood vessels⁴⁵ to i) Thromboxane A2 (TXA2), which is critical during inflammation^{46, 47}, ii) 12-Hydroxyheptadecatrienoic acid (12-HHT), which may participate in monocyte- and neutrophil-based inflammation^{48–50}, and iii) Malonyldialdehyde, a marker for oxidative stress⁵¹. This metabolic reaction may be regulated by DNAm, as a study conducted in endothelial cells reported that *TBXAS1* demethylation resulted increased thromboxane B2 (TXB2), the product of TXA2 breakdown⁵². TXA2 promotes platelet aggregation by binding to thromboxane receptor (TP)⁵³. In addition, TXA2–TP signaling has been suggested to amplify dopamine overflow from the striatum⁵⁴. Altered striatal dopamine function has been linked to early life and adulthood adversity, as well as psychiatric disorders, such as schizophrenia and PTSD^{55–59}. In addition, SNPs in *TBXAS1* have been reported to predict gray matter volume changes of left collateral sulcus of visual cortex (hOC3vL) in schizophrenia⁶⁰. Interestingly, the SNP in *TBXAS1* that associates with cg03604364 methylation levels (rs3779130) was nominally associated with PTSS in the recent PGC-PTSD meta-analysis ($z = 2.00$, $p = 0.045$)⁶¹. According to the BIOS meQTL browser and PGC-PTSD meta-analysis, carriers of the rs3779130 T allele have lower methylation levels and higher PTSS. This aligns with our own findings that individuals with higher PTSS have lower cg03604364 methylation levels. Moreover, cg03604364 showed the strongest within-individual correlation of methylation measured in blood and brain among the four CpGs associated with PTSS. Together, these results suggest that alterations within

TBXAS1 associate with traumatic events and could contribute to psychiatric disorders, possibly through inflammatory and/or oxidative stress pathways.

In the second meta-analysis, no CpGs associated with the change in PTSS. Nevertheless, in our region-based analyses, we identified 15 DMRs associated with change in PTSS. For all 15 DMRs, direction of effect was positive, indicating increased DNAm with increased post-deployment PTSS, conditional on baseline DNAm. Interestingly, three DMRs were located on the X chromosome, which is largely overlooked or excluded in EWAS due to sex-dimorphic distribution⁶². Since this study only included males, we could interpret X chromosome results. The three X chromosome DMRs are located on ovarian tumor deubiquitinase 5 (*OTUD5*), 74 like ETS transcription factor 4 (*ELF4*) and RB binding protein 7, chromatin remodeling factor (*RBBP7*). Notably, *OTUD5*, *ELF4* and *RBBP7* expression has previously been implicated in PTSS development in women upon trauma⁶³. DMRs near *OTUD5* and *ELF4* were also associated with PTSS in our first DMR analysis that uses the same framework as Meta-Analysis 1. The other notable DMR findings are adenylate cyclase 6 (*ADCY6*) and the GNAS Complex Locus (*GNAS*), which have previously been implicated in PTSD. *ADCY6* expression was altered in the amygdala of a PTSD-like mouse model⁶⁴. The *GNAS* locus is known for its complex imprinted expression pattern and produces multiple transcripts due to promoter DMRs⁶⁵. Differential methylation of *GNAS* has been shown to be associated with schizophrenia in multiple studies^{66–68}. In addition, *GNAS* was differentially expressed in blood, hemibrain and spleen of a PTSD mouse model⁶⁹.

Finally, we assessed whether 31 CpGs from previous PGC-PTSD EWAS results^{10–14} were also significant in our study. Only three (cg19534438 in *GOS2*, cg05575921 and cg26703534 in *AHRR*) were nominally significant with the same direction in Meta-Analysis 1. The inconsistency of the remaining CpGs might be due to previous overestimation of the effect, or to the operationalization of the PTSD phenotype, as all previous studies used dichotomous case-control phenotype, whereas we used symptom scores. We did not attempt to evaluate DMR findings due to methodological challenges related to the DMR analysis, that is, DMR results may vary by methylation array type and the DMR analysis methodology.

Our study is not without limitations. First, the MethylationEPIC BeadChip captures only ~3% of the human methylome and, thus, we do not interrogate all CpGs that may associate with PTSS or change in PTSS⁷⁰. Second, in Meta-Analysis 1, we used a linear mixed model to identify CpGs that are associated with PTSS. Hence, that analysis does not distinguish whether the identified epigenetic differences are a cause or consequence of the symptomatology. Third, the methylation data was generated from blood. Though this approach captures PTSD-related DNAm changes and is informative to discover biomarkers of PTSD, it may not reflect DNAm patterns in brain. However, two out of four significant CpGs are moderately correlated between blood and brain tissues, suggesting a similar PTSD-related DNAm pattern for these CpGs in brain. Finally, the cohorts participating in this study are comprised of male and predominantly European participants who were exposed to military trauma. Hence, it is not clear how these findings will translate to females, civilians, or other ancestry groups.

Despite these limitations, this work represents the largest longitudinal study of epigenetics in relation to traumatic stress to date. Our results support a role for epigenetic mechanisms in PTSD severity and implicate genes that are involved in immune system and oxidative stress, and they align with previous studies that support the role of inflammatory processes in PTSD through HPA-axis reactivity, co-morbid metabolic conditions, and behavioral changes common in individuals with PTSD⁷¹. Our findings also support the need to fully understand the regulation of biologically significant genes, such as *CNPY2*, *BAIAP2L1*, *TBXAS1*, *ADCY6* and *GNAS*, with regards to PTSD, particularly through functional studies that can delineate directionality in PTSD development, symptom severity, or treatment.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

This work was supported by the National Institute of Mental Health (NIMH; 2R01MH108826 and 2R01MH106595). The Marine Corps, Navy Bureau of Medicine and Surgery (BUMED) and VA Health Research and Development (HSR&D) provided funding for MRS data collection. Acknowledged are Mark A. Geyer (UCSD), Daniel T. O'Connor (UCSD), and all MRS investigators, as well as the MRS administrative core and data collection staff. Data collection of PRISMO was funded by the Dutch Ministry of Defence, and DNAm analyses were funded by the VID1 Award fellowship from the Netherlands Organization for Scientific Research (NWO, grant number 917.18.336 to B.P.F. Rutten). Army STARRS was sponsored by the Department of the Army and funded under cooperative agreement number U01MH087981 (2009–2015) with the National Institutes of Health, National Institute of Mental Health (NIH/NIMH).

Data Availability

The main summary statistics data that support the findings of this study are available within Supplementary Data. Owing to military cohort data sharing restrictions, data from MRS, Army STARRS, and PRISMO cannot be publicly posted. Individual-level data from the cohorts or cohort-level summary statistics will be made available to researchers following an approved analysis proposal through the PGC Post-traumatic Stress Disorder group with agreement of the cohort PIs. For additional information on access to these data, including PI contact information for the contributing cohorts, please contact the corresponding author.

References

1. American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders. 2013.
2. Benjet C, Bromet E, Karam EG, Kessler RC, McLaughlin KA, Ruscio AM et al. The epidemiology of traumatic event exposure worldwide: results from the World Mental Health Survey Consortium. *Psychol Med* 2016; 46(2): 327–343. [PubMed: 26511595]
3. Kessler RC, Berglund P, Demler O, Jin R, Merikangas KR, Walters EE. Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the National Comorbidity Survey Replication. *Arch Gen Psychiatry* 2005; 62(6): 593–602. [PubMed: 15939837]
4. Daskalakis NP, Rijal CM, King C, Huckins LM, Ressler KJ. Recent Genetics and Epigenetics Approaches to PTSD. *Curr Psychiatry Rep* 2018; 20(5): 30. [PubMed: 29623448]
5. Morrison FG, Miller MW, Logue MW, Assef M, Wolf EJ. DNA methylation correlates of PTSD: Recent findings and technical challenges. *Prog Neuropsychopharmacol Biol Psychiatry* 2019; 90: 223–234. [PubMed: 30503303]
6. Sheerin CM, Lind MJ, Bountress KE, Nugent NR, Amstadter AB. The genetics and epigenetics of PTSD: overview, recent advances, and future directions. *Curr Opin Psychol* 2017; 14: 5–11.

7. Uddin M, Aiello AE, Wildman DE, Koenen KC, Pawelec G, de Los Santos R et al. Epigenetic and immune function profiles associated with posttraumatic stress disorder. *Proc Natl Acad Sci U S A* 2010; 107(20): 9470–9475. [PubMed: 20439746]
8. Smith AK, Conneely KN, Kilaru V, Mercer KB, Weiss TE, Bradley B et al. Differential immune system DNA methylation and cytokine regulation in post-traumatic stress disorder. *Am J Med Genet B Neuropsychiatr Genet* 2011; 156B(6): 700–708. [PubMed: 21714072]
9. Kuan PF, Waszczuk MA, Kotov R, Marsit CJ, Guffanti G, Gonzalez A et al. An epigenome-wide DNA methylation study of PTSD and depression in World Trade Center responders. *Transl Psychiatry* 2017; 7(6): e1158. [PubMed: 28654093]
10. Uddin M, Ratanatharathorn A, Armstrong D, Kuan PF, Aiello AE, Bromet EJ et al. Epigenetic meta-analysis across three civilian cohorts identifies NRG1 and HGS as blood-based biomarkers for post-traumatic stress disorder. *Epigenomics* 2018; 10(12): 1585–1601. [PubMed: 30456986]
11. Rutten BPF, Vermetten E, Vinkers CH, Ursini G, Daskalakis NP, Pishva E et al. Longitudinal analyses of the DNA methylome in deployed military servicemen identify susceptibility loci for post-traumatic stress disorder. *Mol Psychiatry* 2018; 23(5): 1145–1156. [PubMed: 28630453]
12. Snijders C, Maihofer AX, Ratanatharathorn A, Baker DG, Boks MP, Geuze E et al. Longitudinal epigenome-wide association studies of three male military cohorts reveal multiple CpG sites associated with post-traumatic stress disorder. *Clin Epigenetics* 2020; 12(1): 11. [PubMed: 31931860]
13. Logue MW, Miller MW, Wolf EJ, Huber BR, Morrison FG, Zhou Z et al. An epigenome-wide association study of posttraumatic stress disorder in US veterans implicates several new DNA methylation loci. *Clin Epigenetics* 2020; 12(1): 46. [PubMed: 32171335]
14. Smith AK, Ratanatharathorn A, Maihofer AX, Naviaux RK, Aiello AE, Amstadter AB et al. Epigenome-wide meta-analysis of PTSD across 10 military and civilian cohorts identifies methylation changes in AHRH. *Nat Commun* 2020; 11(1): 5965. [PubMed: 33235198]
15. Verhulst B, Neale MC. Best Practices for Binary and Ordinal Data Analyses. *Behav Genet* 2021.
16. Barfield RT, Kilaru V, Smith AK, Conneely KN. CpGassoc: an R function for analysis of DNA methylation microarray data. *Bioinformatics* 2012; 28(9): 1280–1281. [PubMed: 22451269]
17. McCartney DL, Walker RM, Morris SW, McIntosh AM, Porteous DJ, Evans KL. Identification of polymorphic and off-target probe binding sites on the Illumina Infinium MethylationEPIC BeadChip. *Genom Data* 2016; 9: 22–24. [PubMed: 27330998]
18. Fortin JP, Triche TJ Jr., Hansen KD. Preprocessing, normalization and integration of the Illumina HumanMethylationEPIC array with minfi. *Bioinformatics* 2017; 33(4): 558–560. [PubMed: 28035024]
19. Leek JT, Johnson WE, Parker HS, Jaffe AE, Storey JD. The sva package for removing batch effects and other unwanted variation in high-throughput experiments. *Bioinformatics* 2012; 28(6): 882–883. [PubMed: 22257669]
20. Du P, Zhang X, Huang CC, Jafari N, Kibbe WA, Hou L et al. Comparison of Beta-value and M-value methods for quantifying methylation levels by microarray analysis. *BMC Bioinformatics* 2010; 11: 587. [PubMed: 21118553]
21. Teschendorff AE, Breeze CE, Zheng SC, Beck S. A comparison of reference-based algorithms for correcting cell-type heterogeneity in Epigenome-Wide Association Studies. *BMC Bioinformatics* 2017; 18(1): 105. [PubMed: 28193155]
22. Salas LA, Koestler DC, Butler RA, Hansen HM, Wiencke JK, Kelsey KT et al. An optimized library for reference-based deconvolution of whole-blood biospecimens assayed using the Illumina HumanMethylationEPIC BeadArray. *Genome Biol* 2018; 19(1): 64. [PubMed: 29843789]
23. Barfield RT, Almli LM, Kilaru V, Smith AK, Mercer KB, Duncan R et al. Accounting for population stratification in DNA methylation studies. *Genet Epidemiol* 2014; 38(3): 231–241. [PubMed: 24478250]
24. Ratanatharathorn A, Boks MP, Maihofer AX, Aiello AE, Amstadter AB, Ashley-Koch AE et al. Epigenome-wide association of PTSD from heterogeneous cohorts with a common multi-site analysis pipeline. *Am J Med Genet B Neuropsychiatr Genet* 2017; 174(6): 619–630. [PubMed: 28691784]

25. Logue MW, Miller MW, Wolf EJ, Huber BR, Morrison FG, Zhou Z et al. An epigenome-wide association study of posttraumatic stress disorder in US veterans implicates several new DNA methylation loci. *Clinical Epigenetics* 2020; IN PRESS.
26. de Bakker PI, Ferreira MA, Jia X, Neale BM, Raychaudhuri S, Voight BF. Practical aspects of imputation-driven meta-analysis of genome-wide association studies. *Hum Mol Genet* 2008; 17(R2): R122–128. [PubMed: 18852200]
27. Lee CH, Cook S, Lee JS, Han B. Comparison of Two Meta-Analysis Methods: Inverse-Variance-Weighted Average and Weighted Sum of Z-Scores. *Genomics Inform* 2016; 14(4): 173–180. [PubMed: 28154508]
28. Cochran WG. Some Methods for Strengthening the Common χ^2 Tests. *Biometrics* 1954; 10(4): 417–451.
29. Mansell G, Gorrie-Stone TJ, Bao Y, Kumari M, Schalkwyk LS, Mill J et al. Guidance for DNA methylation studies: statistical insights from the Illumina EPIC array. *BMC Genomics* 2019; 20(1): 366. [PubMed: 31088362]
30. Peters TJ, Buckley MJ, Statham AL, Pidsley R, Samaras K, R VL et al. De novo identification of differentially methylated regions in the human genome. *Epigenetics Chromatin* 2015; 8: 6. [PubMed: 25972926]
31. Braun PR, Han S, Hing B, Nagahama Y, Gaul LN, Heinzman JT et al. Genome-wide DNA methylation comparison between live human brain and peripheral tissues within individuals. *Transl Psychiatry* 2019; 9(1): 47. [PubMed: 30705257]
32. Bonder MJ, Luijk R, Zhernakova DV, Moed M, Deelen P, Vermaat M et al. Disease variants alter transcription factor levels and methylation of their binding sites. *Nat Genet* 2017; 49(1): 131–138. [PubMed: 27918535]
33. Phipson B, Maksimovic J, Oshlack A. missMethyl: an R package for analyzing data from Illumina's HumanMethylation450 platform. *Bioinformatics* 2016; 32(2): 286–288. [PubMed: 26424855]
34. Nemeroff CB, Bremner JD, Foa EB, Mayberg HS, North CS, Stein MB. Posttraumatic stress disorder: a state-of-the-science review. *J Psychiatr Res* 2006; 40(1): 1–21. [PubMed: 16242154]
35. Yehuda R. Risk and resilience in posttraumatic stress disorder. *J Clin Psychiatry* 2004; 65 Suppl 1: 29–36.
36. Brewin CR, Holmes EA. Psychological theories of posttraumatic stress disorder. *Clin Psychol Rev* 2003; 23(3): 339–376. [PubMed: 12729677]
37. Jose RJ, Williams AE, Chambers RC. Proteinase-activated receptors in fibroproliferative lung disease. *Thorax* 2014; 69(2): 190–192. [PubMed: 24186921]
38. Tylee DS, Chandler SD, Nievergelt CM, Liu X, Pazol J, Woelk CH et al. Blood-based gene-expression biomarkers of post-traumatic stress disorder among deployed marines: A pilot study. *Psychoneuroendocrinology* 2015; 51: 472–494. [PubMed: 25311155]
39. Hong F, Liu B, Wu BX, Morreall J, Roth B, Davies C et al. CNPY2 is a key initiator of the PERK-CHOP pathway of the unfolded protein response. *Nat Struct Mol Biol* 2017; 24(10): 834–839. [PubMed: 28869608]
40. Bornhauser BC, Olsson PA, Lindholm D. MSAP is a novel MIR-interacting protein that enhances neurite outgrowth and increases myosin regulatory light chain. *J Biol Chem* 2003; 278(37): 35412–35420. [PubMed: 12826659]
41. Miller MW, Lin AP, Wolf EJ, Miller DR. Oxidative Stress, Inflammation, and Neuroprogression in Chronic PTSD. *Harv Rev Psychiatry* 2018; 26(2): 57–69. [PubMed: 29016379]
42. Wang YP, Huang LY, Sun WM, Zhang ZZ, Fang JZ, Wei BF et al. Insulin receptor tyrosine kinase substrate activates EGFR/ERK signalling pathway and promotes cell proliferation of hepatocellular carcinoma. *Cancer Lett* 2013; 337(1): 96–106. [PubMed: 23693078]
43. Millard TH, Dawson J, Machesky LM. Characterisation of IRTKS, a novel IRSp53/MIM family actin regulator with distinct filament bundling properties. *J Cell Sci* 2007; 120(Pt 9): 1663–1672. [PubMed: 17430976]
44. Alhassen S, Chen S, Alhassen L, Phan A, Khoudari M, De Silva A et al. Intergenerational Stress Transmission is Associated with Brain Metabotranscriptome Remodeling and Mitochondrial Dysfunction. *bioRxiv* 2021: 2021.2004.2009.438868.

45. Woodward DF, Jones RL, Narumiya S. International Union of Basic and Clinical Pharmacology. LXXXIII: classification of prostanoid receptors, updating 15 years of progress. *Pharmacol Rev* 2011; 63(3): 471–538. [PubMed: 21752876]
46. Offermanns S Activation of platelet function through G protein-coupled receptors. *Circ Res* 2006; 99(12): 1293–1304. [PubMed: 17158345]
47. Smyth EM. Thromboxane and the thromboxane receptor in cardiovascular disease. *Clin Lipidol* 2010; 5(2): 209–219. [PubMed: 20543887]
48. Sadowitz PD, Setty BN, Stuart M. The platelet cyclooxygenase metabolite 12-L-hydroxy-5, 8, 10-hepta-decatrienoic acid (HHT) may modulate primary hemostasis by stimulating prostacyclin production. *Prostaglandins* 1987; 34(5): 749–763. [PubMed: 3124219]
49. Campbell PB, Tolson TA. Modulation of human monocyte leukotactic responsiveness by thromboxane A2 and 12-hydroxyheptadecatrienoic acid (12-HHT). *J Leukoc Biol* 1988; 43(2): 117–124. [PubMed: 3422086]
50. Hecker M, Ullrich V. 12(S)-Hydroxy-5,8,10 (Z,E,E)-heptadecatrienoic acid (HHT) is preferentially metabolized to its 12-keto derivative by human erythrocytes in vitro. *Eicosanoids* 1988; 1(1): 19–25. [PubMed: 3272822]
51. Del Rio D, Stewart AJ, Pellegrini N. A review of recent studies on malondialdehyde as toxic molecule and biological marker of oxidative stress. *Nutr Metab Cardiovasc Dis* 2005; 15(4): 316–328. [PubMed: 16054557]
52. Xue SS, He JL, Zhang X, Liu YJ, Xue FX, Wang CJ et al. Metabolomic analysis revealed the role of DNA methylation in the balance of arachidonic acid metabolism and endothelial activation. *Biochim Biophys Acta* 2015; 1851(10): 1317–1326. [PubMed: 26170200]
53. Narumiya S, Sugimoto Y, Ushikubi F. Prostanoid receptors: structures, properties, and functions. *Physiol Rev* 1999; 79(4): 1193–1226. [PubMed: 10508233]
54. Mitsumori T, Furuyashiki T, Momiyama T, Nishi A, Shuto T, Hayakawa T et al. Thromboxane receptor activation enhances striatal dopamine release, leading to suppression of GABAergic transmission and enhanced sugar intake. *Eur J Neurosci* 2011; 34(4): 594–604. [PubMed: 21749493]
55. Dahoun T, Nour MM, McCutcheon RA, Adams RA, Bloomfield MAP, Howes OD. The relationship between childhood trauma, dopamine release and dexamphetamine-induced positive psychotic symptoms: a [(11)C]-(+)-PHNO PET study. *Transl Psychiatry* 2019; 9(1): 287. [PubMed: 31712556]
56. Bloomfield MA, McCutcheon RA, Kempton M, Freeman TP, Howes O. The effects of psychosocial stress on dopaminergic function and the acute stress response. *Elife* 2019; 8.
57. Howes OD, Montgomery AJ, Asselin MC, Murray RM, Valli I, Tabraham P et al. Elevated striatal dopamine function linked to prodromal signs of schizophrenia. *Arch Gen Psychiatry* 2009; 66(1): 13–20. [PubMed: 19124684]
58. Avram M, Brandl F, Cabello J, Leucht C, Scherr M, Mustafa M et al. Reduced striatal dopamine synthesis capacity in patients with schizophrenia during remission of positive symptoms. *Brain* 2019; 142(6): 1813–1826. [PubMed: 31135051]
59. Boukezzi S, Baunez C, Rousseau PF, Warrot D, Silva C, Guyon V et al. Posttraumatic Stress Disorder is associated with altered reward mechanisms during the anticipation and the outcome of monetary incentive cues. *Neuroimage Clin* 2020; 25: 102073. [PubMed: 31794925]
60. Wang Q, Xiang B, Deng W, Wu J, Li M, Ma X et al. Genome-wide association analysis with gray matter volume as a quantitative phenotype in first-episode treatment-naive patients with schizophrenia. *PLoS One* 2013; 8(9): e75083. [PubMed: 24086445]
61. Maihofer AX, Choi KW, Coleman JRI, Daskalakis NP, Denckla CA, Ketema E et al. Enhancing discovery of genetic variants for PTSD through integration of quantitative phenotypes and trauma exposure information. *Biological Psychiatry* 2021.
62. Lindsay S, Monk M, Holliday R, Huschtscha L, Davies KE, Riggs AD et al. Differences in methylation on the active and inactive human X chromosomes. *Ann Hum Genet* 1985; 49(2): 115–127. [PubMed: 3000273]
63. Yu S, Chen C, Pan Y, Kurz MC, Datner E, Hendry PL et al. Genes known to escape X chromosome inactivation predict co-morbid chronic musculoskeletal pain and posttraumatic stress symptom

- development in women following trauma exposure. *Am J Med Genet B Neuropsychiatr Genet* 2019; 180(6): 415–427. [PubMed: 30537437]
64. Tanaka M, Li H, Zhang X, Singh J, Dalgard CL, Wilkerson M et al. Region- and time-dependent gene regulation in the amygdala and anterior cingulate cortex of a PTSD-like mouse model. *Mol Brain* 2019; 12(1): 25. [PubMed: 30922409]
65. Grybek V, Aubry L, Maupetit-Mehouas S, Le Stunff C, Denis C, Girard M et al. Methylation and transcripts expression at the imprinted GNAS locus in human embryonic and induced pluripotent stem cells and their derivatives. *Stem Cell Reports* 2014; 3(3): 432–443. [PubMed: 25241742]
66. Plongthongkum N, van Eijk KR, de Jong S, Wang T, Sul JH, Boks MP et al. Characterization of genome-methylome interactions in 22 nuclear pedigrees. *PLoS One* 2014; 9(7): e99313. [PubMed: 25019935]
67. Castellani CA, Laufer BI, Melka MG, Diehl EJ, O'Reilly RL, Singh SM. DNA methylation differences in monozygotic twin pairs discordant for schizophrenia identifies psychosis related genes and networks. *BMC Med Genomics* 2015; 8: 17. [PubMed: 25943100]
68. Sokolowski M, Wasserman J, Wasserman D. Gene-level associations in suicide attempter families show overrepresentation of synaptic genes and genes differentially expressed in brain development. *Am J Med Genet B Neuropsychiatr Genet* 2018; 177(8): 774–784. [PubMed: 30381879]
69. Muhie S, Gautam A, Chakraborty N, Hoke A, Meyerhoff J, Hammamieh R et al. Molecular indicators of stress-induced neuroinflammation in a mouse model simulating features of post-traumatic stress disorder. *Transl Psychiatry* 2017; 7(5): e1135. [PubMed: 28534873]
70. Shu C, Zhang X, Aouizerat BE, Xu K. Comparison of methylation capture sequencing and Infinium MethylationEPIC array in peripheral blood mononuclear cells. *Epigenetics Chromatin* 2020; 13(1): 51. [PubMed: 33228774]
71. Michopoulos V, Powers A, Gillespie CF, Ressler KJ, Jovanovic T. Inflammation in Fear- and Anxiety-Based Disorders: PTSD, GAD, and Beyond. *Neuropsychopharmacology* 2017; 42(1): 254–270. [PubMed: 27510423]

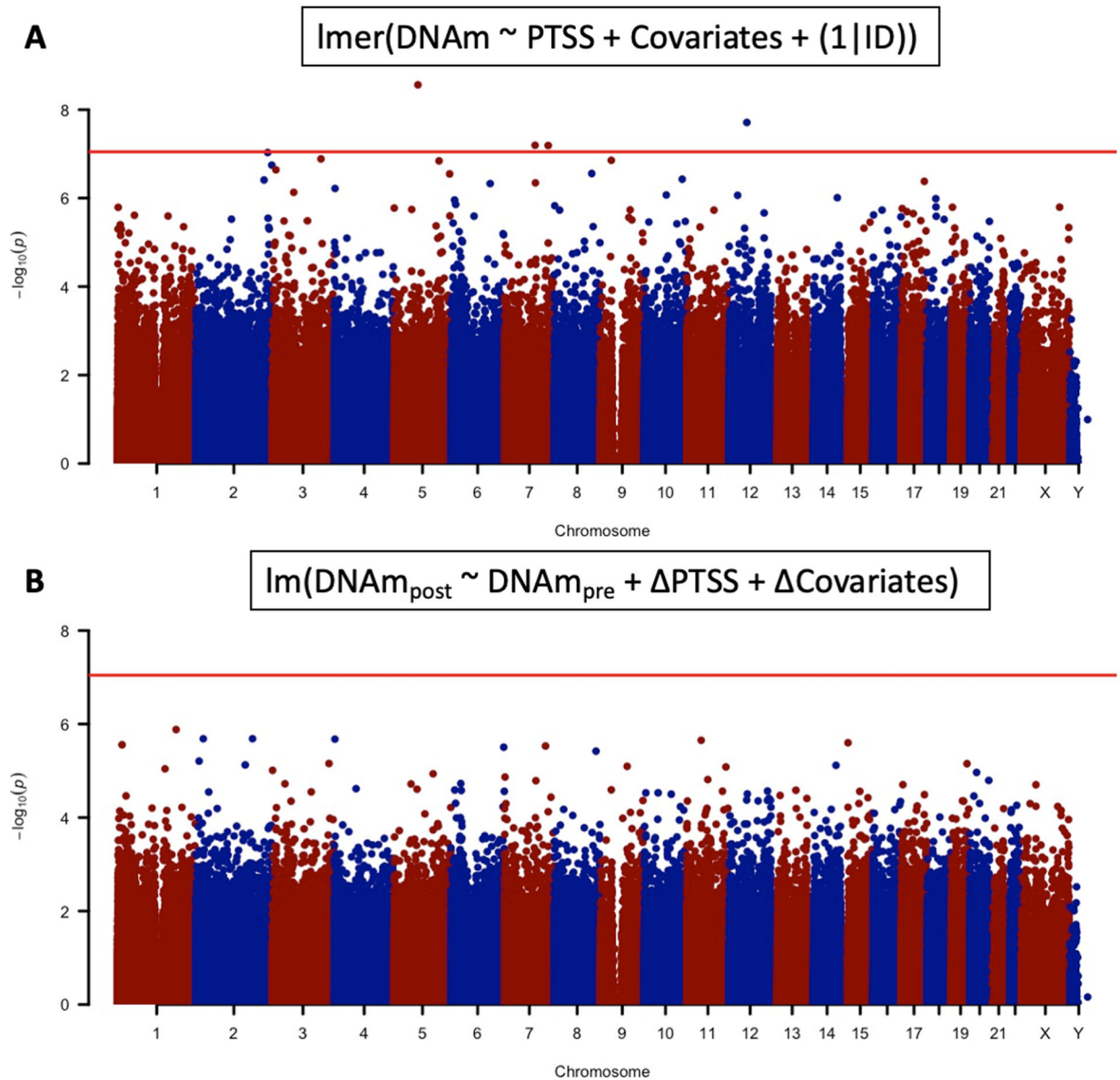


Figure 1. PTSS associates with DNAm across the genome.

A) Manhattan plot for Meta-Analysis 1 across 3 cohorts (N samples = 858). Association analyses of each cohort are based on a random intercept model with a random effect of subject. **B)** Manhattan plot for Meta-Analysis 2 across 3 cohorts (N subjects = 429). Association analyses of each cohort are conducted by conditioning post-deployment methylation on baseline DNAm. The x-axis is the chromosomal location of each site across the genome. The y-axis is the $-\log_{10}$ of the unadjusted p-value for the association with PTSD symptom severity. The red line indicates genome-wide EWAS statistical significance at $p < 9.0\text{E-}8$.

Table 1:
Demographic and clinical characteristics of MRS, Army STARRS, and PRISMO

	Cases	Controls	<i>p</i> value	Total
Number				
MRS	64	63	-	127
Army STARRS	92	92	-	184
PRISMO	43	75	-	118
Age, mean (SD)				
MRS	22.16 (2.35)	21.97 (2.12)	0.64	22.06 (2.24)
Army STARRS	24.41 (4.85)	24.52 (4.86)	0.88	24.47 (4.84)
PRISMO	27.84 (9.69)	27.07 (8.74)	0.67	27.35 (9.06)
PTSD symptoms pre-deployment, mean (SD)				
MRS, PCL-17	24.88 (8.69)	20.00 (4.54)	0.0001	22.46 (7.34)
Army STARRS, PCL-6	6.92 (1.34)	6.48 (0.95)	0.01	6.7 (1.18)
PRISMO, SRIP	29.00 (4.14)	26.72 (4.09)	0.005	27.55 (4.24)
PTSD symptoms post-deployment, mean (SD)				
MRS, PCL-17	49.23 (11.17)	22.03 (6.06)	< 2.2e-16	35.74 (16.34)
Army STARRS, PCL-17	43.83 (16.04)	20.74 (3.77)	< 2.2e-16	31.18 (16.01)
PRISMO, SRIP	42.14 (4.67)	27.11 (4.76)	< 2.2e-16	32.56 (8.66)
Self-reported Race/Ethnicity, N (%)				
MRS			0.98	
European	43 (67.2)	45 (71.4)	-	88 (69.3)
African American	3 (4.8)	2 (3.2)	-	5 (3.9)
Latino	9 (14.0)	7 (11.1)	-	16 (12.6)
East Asian	1 (1.5)	1 (1.6)	-	2 (1.6)
Other	8 (12.5)	8 (12.7)	-	16 (12.6)
Army STARRS			0.90	
European	61 (66.3)	63 (68.5)	-	124 (67.4)
African American	10 (10.9)	11 (12.0)	-	21 (11.4)
Other	21 (22.8)	18 (19.5)	-	39 (21.2)
PRISMO			1.00	
European	43 (100)	75 (100)	-	118 (100)
Smoking Score pre-deployment, mean (SD)				
MRS	-7.69 (14.14)	-8.20 (13.89)	0.84	-7.94 (13.96)
Army STARRS	-6.30 (18.98)	-10.26 (16.43)	0.13	-8.28 (17.81)
PRISMO	2.42 (21.08)	2.96 (24.42)	0.90	2.77 (23.16)
Smoking Score post-deployment, mean (SD)				
MRS	-7.21 (16.40)	-9.33 (14.43)	0.44	-8.26 (15.43)
Army STARRS	-4.78 (18.33)	-9.60 (16.64)	0.063	-7.19 (17.62)
PRISMO	4.17 (21.68)	3.53 (25.35)	0.88	3.76 (23.98)
Early life trauma, mean (SD)				

	Cases	Controls	<i>p</i> value	Total
MRS, CTQ ^a	41.55 (13.08)	36.85 (11.27)	0.047	39.22 (12.39)
Army Stars, NSS	6.76 (2.80)	6.21 (2.05)	0.13	6.48 (2.46)
PRISMO, ETI ^b	5.09 (3.29)	3.37 (3.23)	0.007	4.01 (3.35)

Each study used different scales for PTSD, the corresponding scales are included in the row names: CTQ, Childhood Trauma Questionnaire; ETI, Early Trauma Inventory; NSS, The Army STARRS New Soldier Survey; PCL-17, 17-item PTSD Checklist; PCL-6, 6-item PTSD Checklist; SRIP, Self-Report Inventory for PTSD. SD: standard deviation. Smoking Score was estimated using DNAm data. The p-values were computed using *t*-test (for continuous variables) and Fisher's exact test (for categorical variables) for comparison of PTSD case and control groups.

^aMissing data for 18 subjects.

^bMissing data for 2 subjects.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Table 2:Genome-wide significant CpG sites ($p < 9.0E-08$) associated with PTSS

CpG	Location	Gene	Features	Z	p	Z ₂	p ₂
cg11627632	chr5:76011698	<i>F2R</i>	TSS200	-5.95	2.75E-09	-2.38	0.02*
cg12961546	chr12:56709730	<i>CNPY2</i>	5'UTR; 1stExon	-5.62	1.95E-08	-1.78	0.07
cg00277769	chr7:97922759	<i>BALAP2L1</i>	3'UTR	-5.41	6.39E-08	-2.55	0.01*
cg03604364	chr7:139705703	<i>TBXAS1</i>	Body	-5.41	6.47E-08	-2.66	0.01*

Genome-wide significant results of the EWAS meta-analysis 1 of three cohorts (N samples = 858). Association analyses of each cohort are based on a random intercept model with a random effect of subject. Z₂ and p₂ represents the statistics of meta-analysis 2. The sites that were nominally significant in meta-analysis 2 were indicated by an asterisk (*) in column p₂.

Differentially methylated regions (DMRs) associated with both PTSS and change in PTSS

Table 3:

DMR analysis with Meta-Analysis 1 Framework		DMR analysis with Meta-Analysis 2 Framework						
Overlapping Genes	Position	N CpGs	p-value	Direction ^a	Position	N CpGs	p-value	Direction ^b
<i>OTUD5</i>	chrX:48814580-48814955	8	2.46E-05	+	chrX:48814205-48815125	13	9.53E-09	+
<i>ELF4</i>	chrX:129244725-129244742	3	1.63E-02	+	chrX:129244725-129245245	9	8.50E-07	+

Chr: chromosome, Start/End: start and end position of DMR (hg19), N CpG: the number of CpGs measured within the DMR, Overlapping Genes: genes that span the DMR. p-value: Stouffer p value of the DMR. Direction: direction of the relation between change in PTSD symptom severity and DNAm levels.

^a +: increased methylation with increase in PTSS.

^b +: increased methylation with increased change in PTSS.

Table 4:

Evaluation of CpGs identified in previously published PGC-PTSD EWAS

CPG	Reference	Tissue	Gene	Meta-Analysis 1		Meta-Analysis 2	
				<i>z</i>	<i>p</i>	<i>z</i>	<i>p</i>
cg19534438	Logue et al., 2020	Blood	<i>G0S2</i>	-3.10^a	0.002	-2.37^a	0.018
cg04130728	Logue et al., 2020	Brain	<i>CHST11</i>	-1.63	0.103	-1.95	0.051
cg14911689	Rutten et al., 2018	Blood	<i>NINJ2</i>	-0.98	0.33	0.78	0.44
cg24406898	Rutten et al., 2018	Blood	<i>COL1A2</i>	-0.75	0.45	-0.76	0.45
cg01516881	Rutten et al., 2018	Blood	<i>DUSP22</i>	-0.66	0.51	0.12	0.90
cg11763394	Rutten et al., 2018	Blood	<i>PAX8</i>	-0.60	0.55	0.61	0.54
cg11235426	Rutten et al., 2018	Blood	<i>DUSP22</i>	-0.55	0.58	0.27	0.79
cg06417478	Rutten et al., 2018	Blood	<i>HOOK2</i>	-0.49	0.62	-1.07	0.29
cg04657146	Rutten et al., 2018	Blood	<i>HOOK2</i>	-0.33	0.74	1.37	0.17
cg26654770	Rutten et al., 2018	Blood	<i>NINJ2</i>	-0.31	0.75	-0.36	0.72
cg18110333	Rutten et al., 2018	Blood	<i>DUSP22</i>	-0.30	0.77	0.12	0.90
cg21548813	Rutten et al., 2018	Blood	<i>DUSP22</i>	-0.21	0.83	0.81	0.42
cg05785424	Rutten et al., 2018	Blood	<i>intergenic</i>	-0.13	0.89	-0.19	0.85
cg07249765	Rutten et al., 2018	Blood	<i>SDK1</i>	-0.10	0.92	-0.55	0.58
cg10075506	Rutten et al., 2018	Blood	<i>MYTIL</i>	-0.09	0.92	0.07	0.94
cg03517284	Rutten et al., 2018	Blood	<i>HIST1H2APS2</i>	0.06	0.95	0.47	0.64
cg11738485	Rutten et al., 2018	Blood	<i>HOOK2</i>	-0.04	0.97	0.35	0.73
cg03395511	Rutten et al., 2018	Blood	<i>DUSP22</i>	-0.03	0.98	1.12	0.26
cg04657146	Rutten et al., 2018	Blood	<i>intergenic</i>	0.00	1.00	0.25	0.80
cg05575921	Smith et al., 2020	Blood	<i>AHRR</i>	-3.36	0.001^{b,c}	-1.30	0.194
cg26703534	Smith et al., 2020	Blood	<i>AHRR</i>	-2.51	0.012^c	-0.50	0.614
cg25648203	Smith et al., 2020	Blood	<i>AHRR</i>	1.56	0.120	0.55	0.579
cg21161138	Smith et al., 2020	Blood	<i>AHRR</i>	-1.04	0.298	-1.20	0.231
cg05901543	Snijders et al., 2020	Blood	<i>CDH15</i>	-1.39	0.165	-0.84	0.399
cg18917957	Snijders et al., 2020	Blood	<i>CTRC</i>	1.05	0.292	0.56	0.574
cg12169700	Snijders et al., 2020	Blood	<i>MAD1L1</i>	0.70	0.484	0.19	0.852
cg05656210	Snijders et al., 2020	Blood	<i>intergenic</i>	0.49	0.627	0.11	0.915
cg16956686	Snijders et al., 2020	Blood	<i>SDK1</i>	0.38	0.706	0.06	0.953
cg20756026	Snijders et al., 2020	Blood	<i>HEXDC</i>	0.23	0.816	1.21	0.225
cg19577098	Uddin et al., 2018	Blood	<i>HGS</i>	1.62	0.106	1.10	0.272
cg23637605	Uddin et al., 2018	Blood	<i>NRG1</i>	0.04	0.969	-0.60	0.550

CpGs with nominal significance of association in our study are shown in bold.

^aOpposite direction of effect from the original study.^bSignificant after Bonferonni correction for 31 CpGs (0.05/31 = 0.0016).^cSignificant when 82 duplicate samples (41 subjects) from PRISMO cohort were removed.