

Epigenome-wide association studies identify novel DNA methylation sites associated with PTSD: A meta-analysis of 23 military and civilian cohorts

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Abstract

Background: The occurrence of post-traumatic stress disorder (PTSD) following a traumatic event is associated with biological differences that can represent the susceptibility to PTSD, the impact of trauma, or the sequelae of PTSD itself. These effects include differences in DNA methylation (DNAm), an important form of epigenetic gene regulation, at multiple CpG loci across the genome. Moreover, these effects can be shared or specific to both central and peripheral tissues. Here, we aim to identify blood DNAm differences associated with PTSD and characterize the underlying biological mechanisms by examining the extent to which they mirror associations across multiple brain regions.

Methods: As the Psychiatric Genomics Consortium (PGC) PTSD Epigenetics Workgroup, we conducted the largest cross-sectional meta-analysis of epigenome-wide association studies (EWASs) of PTSD to date, involving 5077 participants (2156 PTSD cases and 2921 trauma-exposed controls) from 23 civilian and military studies. PTSD diagnosis assessments were harmonized following the standardized guidelines established by the PGC-PTSD Workgroup. DNAm was assayed from blood using either Illumina HumanMethylation450 or MethylationEPIC (850K) BeadChips. A common QC pipeline was applied. Within each cohort, DNA methylation was regressed on PTSD, sex (if applicable), age, blood cell proportions, and ancestry. An inverse variance-weighted meta-analysis was performed. We conducted replication analyses in tissue from multiple brain regions, neuronal nuclei, and a cellular model of prolonged stress.

Results: We identified 11 CpG sites associated with PTSD in the overall meta-analysis ($1.44e-09 < p < 5.30e-08$), as well as 14 associated in analyses of specific strata (military vs civilian cohort, sex, and ancestry), including CpGs in *AHRR* and *CDC42BPB*. Many of these loci exhibit blood-

brain correlation in methylation levels and cross-tissue associations with PTSD in multiple brain regions. Methylation at most CpGs correlated with their annotated gene expression levels.

Conclusions: This study identifies 11 PTSD-associated CpGs, also leverages data from postmortem brain samples, GWAS, and genome-wide expression data to interpret the biology underlying these associations and prioritize genes whose regulation differs in those with PTSD.

Keywords: PTSD, trauma, DNA methylation, postmortem brain, GWAS, gene expression

Introduction

Posttraumatic stress disorder (PTSD) is a serious psychiatric disorder characterized by intrusive memories of the traumatic event(s), avoidance of or numbing to situations that trigger those memories, and hyperarousal symptoms that can disturb mental and physical health (1). These symptoms are associated with lower levels of self-care, lower compliance with medical treatment, and higher rates of substance use (2, 3). Thus, it is not surprising that PTSD increases the risk for chronic medical conditions, such as cardiovascular disorders, independent of lifestyle factors (e.g., substance use and sleep quality)(4, 5). Although most individuals experience at least one traumatic event, only a small fraction develop PTSD(6). Genetic and environmental factors contribute to this differential susceptibility in PTSD development upon trauma exposure (7, 8).

Genome-wide association studies (GWAS) of PTSD demonstrated remarkable success at identifying relevant genes, many of which are involved in the stress response or immune function (see reviews(9, 10)). The recent Psychiatric Genomics Consortium PTSD Workgroup (PGC-PTSD) Freeze 3 GWAS identified 95 genomic loci associated with PTSD, implicating genes involved in stress, immune, fear, and threat-related processes (11). Nonetheless, genetic differences do not fully account for an individual's susceptibility to PTSD. Trauma exposure has been shown to alter epigenetic patterns in both animal and human studies, prompting the need to conduct epigenetic studies of PTSD in addition to genetic studies (12, 13). Epigenetic mechanisms are chemical modifications that can dictate the timing and magnitude of gene expression without altering the DNA sequence (14). The most widely studied epigenetic mechanism is DNA methylation (DNAm), which is defined as the addition of a methyl group to

cytosine bases, particularly at cytosine-guanine dinucleotides (CpG sites). DNAm patterns respond to changes in the environment, are potentially reversible, and can be targeted for disease therapies (15, 16). Environmental influences on DNAm are apparent across the life span and may provide insight into the biological response to trauma(17).

Which specific DNAm sites differ across individuals and how they correlate with exposures and gene expression can vary across tissues (18). DNAm in human brain tissue, which is most relevant to the study of psychiatric disorders, is not easily accessible in living patients and hence is not a viable PTSD biomarker for clinical use. However, correlation has been observed between peripheral tissues (e.g., blood) and brain DNAm levels at specific genomic loci, and hence blood DNAm can potentially serve as a robust biomarker for implementing early intervention and developing improved preventative or therapeutic strategies for PTSD (19, 20). Moreover, PTSD symptoms have been linked to the components of the peripheral immune system (21, 22) that can be readily assessed in blood DNAm. Multiple peripheral epigenome-wide association studies (EWASs) of PTSD identified CpGs in genes related to the immune system and neurotransmission(23-28). While prior EWASs of PTSD have reported promising results, the small sample sizes and variability of analysis methods across studies make it difficult to combine and interpret the findings effectively. Recent meta-analyses led by the PGC-PTSD Epigenetics Workgroup minimized these issues by increasing sample size, increasing sample diversity, and using a common quality control and analysis pipeline(29-33). These meta-analyses identified multiple new loci associated with PTSD, including *NRG1*, *AHRR*, *MAD1L1*, and *TBXAS1*, implicating immune dysregulation in those with PTSD (30-33).

Building on the prior work by Smith et al. (31), which conducted an EWAS meta-analysis in 1896 participants from 10 cohorts, this study includes 13 additional cohorts with a denser and more comprehensive DNAm array, bringing the sample up to 5077 participants from 23 civilian and military cohorts. Our current investigation replicated the findings of the initial PGC-PTSD epigenome-wide meta-analysis, reporting lower *AHRR* methylation in those with PTSD, and identified 8 new (11 total) PTSD-associated loci. We also leveraged data from postmortem brain samples, a cellular model of prolonged stress, GWAS, and genome-wide gene expression studies to interpret the biology underlying these associations and prioritize genes whose regulation differs in those with PTSD.

Methods

Cohorts and post-traumatic stress disorder assessments

The study includes 2156 current PTSD cases and 2921 trauma-exposed controls from 9 civilian cohorts: BEAR, DNHS, DCHS, GTP, NIU, Shared Roots, AURORA, H3A_Rwanda, WTC; and 9 military cohorts: GMRFQUT, MRS, PRISMO, Army STARRS, PROGrESS, NCPTSD/TRACTS, INTRuST, and VA cohorts (VA-M-AA & VA-M-EA). For DNHS, GTP, MRS, PRISMO, and Army STARRS, two different datasets were available based on the DNAm array. Two different datasets for these five cohorts did not have any overlapping samples and were treated as independent studies. Sample characteristics for the 23 studies that participated in the meta-analysis are summarized in Table 1.

The sample is heterogeneous in terms of sex (41% female), ancestry (46% European, 43% African, and 11% of other ancestries), and cohort type (56% military cohort). Civilian

cohorts skew towards being more female (67%) and African (70%), whereas military cohorts are predominantly male (80%) and European (64%).

All participants were exposed to a traumatic event and 42% met the criteria for current PTSD. The current PTSD diagnosis was assessed by each study following the standardized guidelines established by the PGC-PTSD Workgroup (7). Briefly, current PTSD diagnosis was determined based on the specific criteria set by the principal investigator of each study. All control subjects had experienced trauma. Subjects without a current PTSD but with a prior history of PTSD (i.e., remitted PTSD), were excluded. Detailed descriptions of cohorts and PTSD assessments are provided in the Supplement. All participants in these studies gave informed consent. The institutional review boards of each respective institution approved these studies.

DNA methylation

Whole blood DNAm was measured using the Illumina MethylationEPIC BeadChip in 14 studies, and the HumanMethylation450 BeadChip in 9 studies (Table 1). All studies used a standardized consortium-developed quality control (QC) pipeline that differed somewhat depending on which chip was used. The 450K array pipeline (29, 31) is described in eMethods in the Supplement.

The EPIC pipeline (available at https://github.com/PGC-PTSD-EWAS/EPIC_QC) was similar to the 450K array pipeline. Samples with probe detection call rates lower than 90% and average intensity values that were either less than 50% of the overall sample mean or below 2000 arbitrary units (AU) were excluded. Probes with detection p -values > 0.01 were considered low quality and treated as missing. Probes that were missing in $> 10\%$ of the samples within the

studies and were cross-hybridizing were removed (34). Data was normalized using single-sample Noob (ssNoob) implemented in R package *minfi* (35). *ComBat* was used to account for batch effects of chip and position while preserving PTSD, age, and sex effects (if applicable) (36). Blood-cell composition (i.e., the proportion of CD8+T, CD4+T, natural killer (NK), B cells, monocytes, and neutrophils) was estimated using the Robust Partial Correlation (RPC) method in *Epidish* (37) with a reference data specific to EPIC array (38). For studies without genome-wide genotype data (DNHS, NIU, Shared Roots, AURORA, H3A_Rwanda, GMRFQUT, PROGRESS), we estimated ancestry principal components (PCs) from DNAm data, using the method developed by Barfield et al. (39), as previously described (33). PCs 2 and 3, which were the components that correlate most with self-reported ancestry, were included as covariates (33, 39). In cohorts with available genome-wide genotype data, PCs 1-3 from GWAS were used to adjust for ancestry. We used R package *bacon* to control inflation, only if doing so results in the lambda being closer to 1 (40). To predict smoking status, a DNAm-based smoking score was calculated, as previously described (27) for cohorts with EPIC array data. A detailed description of DNAm-based ancestry PCs and smoking score calculation is provided in eMethods in the Supplement.

Epigenome-wide association analysis

The association between PTSD and DNAm was tested using multivariable linear regression models within cohorts with balanced plate designs. For studies in which plate layouts were not balanced (Shared Roots, H3A_Rwanda, GMRFQUT), we conducted mixed-effect regression models, including chip as a random effect term. R package *CpGassoc* was used to fit

the models (41). The models were adjusted for age, sex (if applicable), blood cell composition (i.e., CD8T, CD4T, NK, B cell, and monocyte cell proportions), and ancestry PCs. A post-hoc sensitivity analysis was performed by including a covariate for smoking: DNAm-based smoking score in studies with EPIC data and current smoking status for studies with 450K data. Furthermore, we conducted stratified analyses for both sexes, ancestry (European and African ancestry), and cohort type (civilian and military cohorts).

To combine results across studies, we performed inverse-variance weighting (IVW) meta-analysis in *meta* (42). Meta-analysis tested 411,786 CpGs common to 450K and EPIC arrays (23 studies), and 404,794 EPIC array specific CpGs (14 studies). Epigenome-wide significance threshold recommended for the MethylationEPIC BeadChip ($p < 9.0E-08$) was used to determine statistical significance (43). Gene Ontology (GO) enrichment analyses were conducted using the top 1000 CpGs in *missMethyl* (44). An FDR threshold of 5% was used to identify significant GO terms.

Cross-tissue association analyses

Blood – Brain Correlations. The Blood Brain DNA Methylation Comparison Tool (19) was used to assess the correlations between methylation in blood and prefrontal cortex (PFC), entorhinal cortex (EC), superior temporal gyrus (STG), and cerebellum.

Postmortem brain DNAm. DNAm measured from post-mortem brains was obtained from two studies, each of which examined a unique but not necessarily distinct set of brain regions and cohorts: the National Center for PTSD Brain Bank cohort (NCPTSD-BB (45)) and the PsychENCODE Consortium for PTSD (PEC-PTSD) Brainomics cohort (46) (see eMethods in the

Supplement for details), both of which were sourced from the Lieber Institute for Brain Development.

Methylation at PTSD-associated CpGs from the EWAS was tested for association with PTSD in DNA extracted from postmortem dorsolateral prefrontal cortex (dlPFC, BA9/46), ventromedial prefrontal cortex (vmPFC, BA12/32), amygdala, and dentate gyrus (DG). DNAm in the post-mortem tissue was measured using the EPIC array. We examined the associations with PTSD in dlPFC and vmPFC of 42 PTSD cases and 30 controls from the NCPTSD-BB. The associations between DNAm and PTSD in amygdala and DG were tested in 77 PTSD cases and 77 controls from the PEC-PTSD.

Neuronal nuclei. We examined cross-tissue association from neuronal nuclei isolation from the orbitofrontal cortex (OFC) of 25 PTSD cases and 13 healthy controls collected at the VA's NCPTSD-BB (47). Fluorescence-Activated Nuclei Sorting (FANS) protocol was employed to isolate NeuN+ cells and the nuclei underwent reduced representation oxidative bisulfite-sequencing (RRoxBS), as previously described (48). We examined whether there was differential methylation and hydroxymethylation within 500 bp of CpGs from the epigenome-wide association analyses. Eight CpG sites match between the EPIC array and RRoxBS, two evaluating the same position (cg21566642 and cg25691167), and six within 500 bp (cg05575921, cg15148933, cg19558029, cg21161138, cg23576855, and cg26599989).

Cellular model of prolonged stress. We explored the associations between each of the PTSD-associated CpGs resulting from epigenome-wide association analyses and a cellular model of prolonged stress in which fibroblasts were subjected to physiological stress hormone (cortisol) levels for a prolonged period (51 days) as previously described (49, 50).

Gene Regulation

Correlations between PTSD-associated CpG sites' DNAm levels and expression levels of the corresponding gene (as determined by the EPIC array annotation) were tested in whole-blood RNA-sequencing (RNA-seq) data from participants in the BEAR (n=127), AURORA (n=173), NCPTSD Merit (n=204), and MRS cohorts (n=128 with multiple visits totaling 357 samples). The results were meta-analyzed using the IVW method using a Bonferroni correction for the number of CpGs examined. Detailed information about cohort-specific RNA-seq data generation is described in eMethods in the Supplement.

Genetic effects

To evaluate the effect of nearby (<1 MB) polymorphisms on DNAm levels of CpGs associated with PTSD, we used cis-methylation quantitative trait locus (cis-meQTL) data from GoDMC(51) and meQTL EPIC(52) databases. For both databases, their default multiple testing adjustment was utilized: an FDR threshold of 5% in meQTL EPIC and $p < 1e-08$ in GoDMC. We checked the associations between the identified cis-SNPs and PTSD in the recent Freeze 3 GWAS from PGC-PTSD(11). Finally, we evaluated genetic associations between the genes with PTSD-associated DNAm changes and PTSD, using the gene-based test results from the recent PGC-PTSD Freeze 3 GWAS(11) using a Bonferroni correction for the 11 genes examined.

Results

Epigenome-wide association meta-analysis

We identified 11 PTSD-associated CpGs that passed the epigenome-wide significance threshold ($p < 9e-08$, Table 2, Figure 1, eFigure 1 in the Supplement). All CpG sites, except one site (cg21161138) near *AHRR*, remained nominally significant ($1.52e-03 < p < 2.10e-07$) with the same direction of association in the sensitivity analysis adjusted for smoking score (eTable 1 and eFigure 1 in the Supplement).

Stratified analyses for sex, ancestry, and cohort type

The directions of associations of the 11 significant CpGs were consistent at nominal significance ($p < 0.05$) when stratified by both sex, cohort type (civilian and military), and European ancestry. However, in the African ancestry stratified analysis, only 8 CpGs remained associated ($p < 0.05$) with PTSD (eTable 2 in the Supplement). We identified 1 epigenome-wide significant CpG site associated with PTSD in the female-stratified analysis, 1 in the male-stratified analysis, 5 in European ancestry-stratified analysis, 1 in the African ancestry-stratified analysis, 1 in the analysis for civilian-cohort analysis, and 5 in the military-cohort analysis (Table 3, eFigures 2-8 in the Supplement). Of note, some CpGs identified in the stratified analyses were specific to their stratum and were not associated with PTSD ($p > 0.05$) in the other stratum. For instance, cg25691167 in *FERD3L* was associated with PTSD in females ($p = 4.24E-08$), but not in males ($p = 0.56$) or the primarily male military cohorts ($p = 0.13$).

Gene enrichment analysis

We identified 3 GO term enrichments in the female-stratified analysis (FDR < 0.05), including nervous system development (eTable 3 in the Supplement). We did not identify any significant enrichments in the main analysis or other strata.

Cross-tissue associations

Of the 19 significant CpGs from the primary and stratified analyses, 3 were not available in the Blood Brain DNA Methylation Comparison Tool database. Methylation at 9 of the remaining 16 CpGs was correlated between blood and at least one brain region (Figure 2, eTable4 in the Supplement). The strongest correlation was observed between the blood and PFC ($r = 0.91$) for cg23576855 (*AHRR*).

We next evaluated whether the PTSD-associated CpGs from blood were also associated with PTSD in the brain regions of dlPFC, vmPFC, amygdala, and DG, as well as neuronal nuclei. Out of 19 CpGs from the primary and stratified analyses in bulk tissue, 9 were nominally associated with PTSD in at least one brain region or the neuronal nuclei ($p < 0.05$, Figure 2, eTables 5 and 6 in the Supplement). For instance, PTSD cases had higher cg04987734 (*CDC42BPB*) methylation both in the blood ($p = 3.26e-8$) and the dlPFC ($p = 3.9e-3$). In addition, 7 CpGs exhibited nominally significant methylation changes (5 CpGs with the same direction of association) in fibroblasts when subjected to physiological stress hormone levels in a cellular model of prolonged stress ($0.024 < \Delta\text{DNAm} < 0.225$, $p < 0.05$, Figure 2, eTable 7 in the Supplement)(49). Notably, the CpG sites (cg21161138 [*AHRR*], cg25320328 [*GFI*], cg19719391

[*intergenic*], cg25691167 [*FERD3L*]) that were consistently associated with PTSD in blood and postmortem brain also demonstrated a significant change in this cell model.

Gene Regulation

Of the 11 PTSD-associated CpGs annotated to a gene, 10 correlated with their gene expression in at least one of the 4 RNA-seq cohorts (Figure 2, eTable 8 in the Supplement). Meta-analysis across cohorts identified 4 CpGs whose methylation levels were negatively correlated with their annotated gene expression after multiple test corrections: cg05575921, cg21161138, and cg23576855 with *AHRR*, and cg27541344 with *BCL11B* ($p < 4.5e-03$, $-16.69 \leq z \leq -4.86$ eTable 8 in the Supplement).

Genetic regulation of PTSD-associated CpGs

Of the 19 CpGs from the primary and stratified analyses, 12 CpGs were associated with at least one nearby SNP within 1 MB according to GoDMC(51) and meQTL EPIC(53) databases (eTable 9 in the Supplement). In total, we identified 41 lead meQTLs, of which 8 were nominally associated with PTSD ($p < 0.05$, albeit in the opposite direction) in the PGC-PTSD Freeze 3 GWAS(11) (eTable 9 in the Supplement). For instance, lower methylation at the intergenic cg14753356 site is associated with PTSD ($z = -5.72$, $p = 1.09E-08$, Table 2) and higher methylation at cg14753356 is associated with rs28986310 T allele (beta = 0.31, $p < 5e-324$), which increases the risk of PTSD ($z = 6.73$, $p = 1.68e-11$, eTable 9 in the Supplement).

In addition, of the 11 genes harboring PTSD-associated CpGs, *AHRR*, *CDC42BPB*, and *BCL11B* were implicated in the recent PGC-PTSD gene-based analysis ($3.48e-03 < p < 5.40e-05$,

eTable 10 in the Supplement). All genes are still significant after Bonferroni correction for 11 tests ($p < 4.5e-03$).

Discussion

In the present epigenome-wide meta-analysis of blood DNAm levels, we identified 11 CpG sites associated with PTSD. Many of these are also associated with PTSD in multiple brain regions and a cellular model of prolonged stress exposure (49). Many of the PTSD-associated CpGs were associated with the expression of their respective genes. An examination of the most recent PGC-PTSD GWAS (11) indicated that many SNPs near significant CpGs were associated with both DNAm levels and PTSD diagnosis. Moreover, many of the EWAS-implicated genes were significant in gene-based tests from the GWAS. Thus, the GWAS results represent further evidence supporting the role of these genes in PTSD pathogenesis. However, the lower significance of the SNPs in association with PTSD relative to the corresponding CpGs (eTable 10 in the Supplement) indicates that the association between PTSD and the CpGs is likely not simply a byproduct of PTSD-associated SNPs.

Our *CDC42BPB* (CDC42 binding protein kinase beta) findings are of particular interest. *CDC42BPB* is involved in the regulation of cytoskeletal rearrangement, cell migration, and neurodevelopment(54). In the current study, increased *CDC42BPB* methylation at cg04987734 was associated with PTSD in blood and the dlPFC. Methylation at this site was also positively correlated with *CDC42BPB* expression in blood. Notably, higher methylation at cg04987734 has been associated with depressive symptoms (55) and increased C-reactive protein (CRP) levels (56, 57), which is perhaps not surprising given the bi-directional genetic association between

PTSD and CRP levels (58). Multiple studies reported increased CRP levels and other inflammatory markers in those with PTSD, suggesting inflammation as an important component of PTSD (59-61). Future studies are warranted to investigate *CDC42BPB* methylation as a potential mediator of the relationship between PTSD and inflammation.

We observed 3 CpGs (cg05575921, cg21161138, and cg23576855) in *AHRR* (aryl-hydrocarbon receptor repressor) that were identified in the initial PGC-PTSD EWAS (31) and an independent study of US veterans (45). The aryl hydrocarbon receptor (AhR) plays a role in immunomodulation, including the regulation of T lymphocytes, B cell maturation, and the activity of macrophages, dendritic cells, and neutrophils (62), supporting the link between the immune system and PTSD. Although methylation at the *AHRR* CpGs is known to be influenced by smoking (63), cg05575921 and cg23576855 remained nominally significant ($p = 1.52E-03$ and $p = 7.59E-04$, respectively) in our sensitivity analysis adjusting for DNAm-based smoking scores. Notably, cg21161138 DNAm was also associated with PTSD in postmortem dlPFC and neuronal nuclei and demonstrated a significant change in the cellular model of prolonged stress (49), supporting the notion that there is an association between methylation at this locus and PTSD which is independent of the effects of smoking.

The stratified analyses identified DNAm-PTSD associations specific to sex, ancestry, and cohort type. The PTSD-associated site cg25691167 (*FERD3L*) in females ($p = 4.24e-08$) was not associated with PTSD in males ($p = 0.57$), suggesting that DNAm changes in cg25691167 might be sex-specific. Similarly, cg27541344 (*BCL11B*) was associated with PTSD in the civilian ($p = 7.21e-08$), but not the military cohorts ($p = 0.38$), whereas 3 out of 5 PTSD-associated CpGs in the military cohorts were not significant in the civilian cohorts ($p > 0.05$). We speculate that

some of these DNAm changes specific to sex, ancestry, or cohort type might be due to unique characteristics of those strata, such as hormonal factors and environmental exposures.

Strengths and Limitations

To our knowledge, this is the largest EWAS of PTSD to date. Our sample is diverse in terms of sex, ancestry, and cohort type. We leveraged data from postmortem brain samples, a cellular model of prolonged stress, GWAS, and genome-wide expression data to support our findings.

However, the study is not without limitations. First, methylation BeadChips only assess a subset of CpG sites in the genome; therefore, we may not capture all PTSD-associated CpG sites. Second, this is a cross-sectional study of participants with prior exposure to a traumatic event; thus, we were not able to assess whether the differences in DNAm between individuals with and without PTSD are a cause or consequence of PTSD or both. Third, our primary meta-analysis was performed using measures of blood DNAm. While this strategy provided valuable insights for future research on biomarkers of PTSD, it might not accurately represent the DNAm patterns within other tissues that are likely the most relevant to PTSD. However, the majority of the PTSD-associated CpG sites' methylation levels were correlated between blood and at least one brain region. In addition, most PTSD-associated CpGs in blood were also associated with PTSD in one or more brain regions. Fourth, we used bulk tissue and adjusted for cellular heterogeneity, which might have obscured some signals, given the alterations in cell composition in those with PTSD(64, 65). Additionally, the brain regions examined varied between the online databases used to examine the blood-brain correlation of methylation values and the gene expression

differences associated with PTSD, which can complicate interpretation. Finally, most cohorts that participated in the meta-analysis did not have detailed physical or psychiatric information on participants, including detailed information on trauma type and timing, PTSD symptom course, and treatment, making it challenging to evaluate and adjust for potential confounders, including substance use, comorbidities, or medication use.

Conclusions

Taken together, this study replicates our previous findings and identifies novel PTSD-associated CpGs. Supporting data from multiple sources suggest that epigenetic mechanisms, particularly methylation in *AHRR* and *CDC42BPB*, may contribute to the complex relationship between the immune system and PTSD.

List of abbreviations

CpG	Cytosine-guanine dinucleotides
DG	Dentate gyrus
dIPFC	Dorsolateral prefrontal cortex
DNAm	DNA methylation
EWAS	Epigenome-wide association study
FANS	Fluorescence-Activated Nuclei Sorting
GWAS	Genome-wide association study
meQTL	Methylation quantitative trait locus
NK	Natural killer

OFC	Orbitofrontal cortex
PC	Principal component
PGC-PTSD	Psychiatric Genomics Consortium PTSD Workgroup
PTSD	Posttraumatic stress disorder
RPC	Robust Partial Correlation
RRoxBS	Reduced representation oxidative bisulfite-sequencing
vmPFC	Ventromedial prefrontal cortex

Declarations

Ethics approval and consent to participate

All participants in these studies gave informed consent. The institutional review boards of each respective institution approved these studies.

Consent for publication

Not applicable.

Availability of data and materials

The main summary statistics data that support the findings of this study will be available within Supplementary Data upon publication. 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-PTSD Epigenetics Workgroup with agreement of the cohort PIs. The raw data for the GTP cohort is available in the Gene Expression Omnibus database with the

accession code GSE132203. Owing to military cohort data sharing restrictions, data from the VA, VA MIRECC, MRS, Army STARRS, PRISMO, PROGrESS, and NCPTSD/TRACTS cannot be publicly posted. However, such data can be provided in de-identified form from a data repository through a data use agreement following applicable guidelines on data sharing and privacy protection. For additional information on access to these data, including PI contact information for the contributing cohorts, please contact the corresponding author.

Competing interests

CYC is an employee of Biogen. NPD has served on scientific advisory boards for BioVie Pharma, Circular Genomics and Sentio Solutions for unrelated work. NRN serves as an unpaid member of the Illumina advisory board. SAMR receives support from the Wounded Warrior Project (WWP), Department of Veterans Affairs (VA), National Institute of Health (NIH), McCormick Foundation, Tonix Pharmaceuticals, Woodruff Foundation, and Department of Defense (DOD). Dr. Rauch receives royalties from Oxford University Press and American. KJR serves as a consultant for Acer, Bionomics, and Jazz Pharma; SABs for Sage, Boehringer Ingelheim, and Senseye. DJS has received consultancy honoraria from Discovery Vitality, Johnson & Johnson, Kanna, L'Oreal, Lundbeck, Orion, Sanofi, Servier, Takeda and Vistagen. MBS has in the past 3 years received consulting income from Acadia Pharmaceuticals, Aptinyx, Atai Life Sciences, BigHealth, Biogen, Bionomics, BioXcel Therapeutics, Boehringer Ingelheim, Clexio, Delix Therapeutics, Eisai, EmpowerPharm, Engrail Therapeutics, Janssen, Jazz Pharmaceuticals, NeuroTrauma Sciences, PureTech Health, Sage Therapeutics, Sumitomo Pharma, and Roche/Genentech. MBS has stock options in Oxeia Biopharmaceuticals and EpiVario. MBS has been paid for his editorial work on

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Table 1. Overview of the studies

Cohort	Array	N	Cases N (%)	Controls N (%)	Female N (%)	European N (%)	African N (%)	Age mean (SD)
Civilian								
BEAR	EPIC	162	36 (22%)	126 (78%)	119 (73%)	112 (69%)	3 (2%)	15.16 (1.45)
DNHS-1	EPIC	423	26 (6%)	397 (94%)	255 (60%)	23 (5%)	384 (91%)	54.54 (16.87)
DCHS	EPIC	95	46 (48%)	49 (52%)	95 (100%)	0 (0%)	54 (57%)	26.81 (5.2)
GTP-1	EPIC	479	158 (33%)	321 (67%)	340 (71%)	12 (3%)	448 (94%)	42.22 (12.25)
NIU	EPIC	140	18 (13%)	122 (87%)	140 (100%)	110 (79%)	19 (14%)	26.01 (1.74)
Shared Roots	EPIC	120	61 (51%)	59 (49%)	85 (71%)	0 (0%)	120 (100%)	43.15 (10.77)
AURORA	EPIC	206	57 (28%)	149 (72%)	154 (75%)	67 (33%)	131 (64%)	39.24 (14.17)
H3A_Rwanda	EPIC	73	32 (44%)	41 (56%)	73 (100%)	0 (0%)	73 (100%)	45.54 (7.29)
DNHS-2	450K	100	40 (40%)	60 (60%)	60 (60%)	13 (13%)	87 (87%)	53.6 (14.01)
GTP-2	450K	265	74 (28%)	191 (72%)	187 (71%)	16 (6%)	249 (94%)	41.95 (12.37)
WTC	450K	180	84 (47%)	96 (53%)	0 (0%)	138 (77%)	7 (4%)	49.72 (8.25)
<i>Civilian Total</i>		<i>2243</i>	<i>632 (28%)</i>	<i>1611 (72%)</i>	<i>1508 (67%)</i>	<i>491 (22%)</i>	<i>1575 (70%)</i>	<i>41.89 (16.27)</i>
Military								
GMRFQUT	EPIC	96	48 (50%)	48 (50%)	0 (0%)	96 (100%)	0 (0%)	68.67 (4.36)
MRS-1	EPIC	127	64 (50%)	63 (50%)	0 (0%)	88 (69%)	5 (4%)	23.07 (2.18)
PRISMO-1	EPIC	89	24 (27%)	65 (73%)	9 (10%)	74 (83%)	3 (3%)	27.51 (8.63)
Army STARRS-1	EPIC	216	106 (49%)	110 (51%)	0 (0%)	149 (69%)	22 (10%)	25.13 (4.82)
PROGrESS	EPIC	140	112 (80%)	28 (20%)	14 (10%)	89 (64%)	40 (29%)	34.77 (8.33)
NCPTSD/TRACTS	EPIC	1028	638 (62%)	390 (38%)	231 (22%)	706 (69%)	123 (12%)	44.06 (13.7)
MRS-2	450K	126	63 (50%)	63 (50%)	0 (0%)	72 (57%)	10 (8%)	22.2 (3.04)
PRISMO-2	450K	62	32 (52%)	30 (48%)	0 (0%)	62 (100%)	0 (0%)	27.1 (9.23)
Army STARRS-2	450K	102	51 (50%)	51 (50%)	0 (0%)	102 (100%)	0 (0%)	23.79 (4.25)
INTRuST	450K	303	116 (38%)	187 (62%)	102 (34%)	206 (68%)	58 (19%)	34.09 (11.68)
VA-M-AA	450K	369	183 (50%)	186 (50%)	184 (50%)	0 (0%)	369 (100%)	38.36 (9.36)
VA-M-EA	450K	176	87 (49%)	89 (51%)	38 (22%)	176 (100%)	0 (0%)	34.87 (9.89)
<i>Military Total</i>		<i>2834</i>	<i>1524 (54%)</i>	<i>1310 (46%)</i>	<i>578 (20%)</i>	<i>1820 (64%)</i>	<i>630 (22%)</i>	<i>37.08 (14.33)</i>
TOTAL		5077	2156 (42%)	2921 (58%)	2086 (41%)	2311 (46%)	2205 (43%)	39.2 (15.4)

Participating civilian cohorts: Biomarkers, social, and affective predictors of suicidal thoughts and behaviors in adolescents (BEAR), Detroit Neighborhood Health Study (DNHS), Drakenstein Child Health Study (DCHS), Grady Trauma Project (GTP), Northern Illinois University Trauma Study (NIU), Shared Roots, Advancing Understanding of Recovery after trauma (AURORA), Human Heredity and Health in Africa, Rwanda (H3A_Rwanda), World Trade Center 9/11 Responders (WTC). Participating military cohorts: Gallipoli Medical Research Foundation Queensland University of Technology (GMRFQUT), Marine Resiliency Study (MRS), Prospective Research in Stress-related Military Operations (PRISMO), Army Study to Assess Risk and Resilience in Servicemembers (Army STARRS), PROlonged ExpoSure and Sertraline Trial

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Table 2. CpG sites associated with current PTSD in the primary meta-analysis

CpG	Position	Gene	z	p-value
cg05575921	chr5:373378	<i>AHRR</i>	-6.05	1.44E-09
cg21161138	chr5:399360	<i>AHRR</i>	-5.96	2.50E-09
cg04583842	chr16:88103117	<i>BANP</i>	5.81	6.29E-09
cg14753356	chr6:30720108	<i>Intergenic</i>	-5.72	1.09E-08
cg25320328	chr1:92953037	<i>GFI1</i>	-5.65	1.64E-08
cg26599989	chr11:1297087	<i>TOLLIP</i>	5.58	2.35E-08
cg19719391	chr4:26789915	<i>Intergenic</i>	5.58	2.45E-08
cg16758086	chr1: 6173356	<i>CHD5</i>	5.55	2.85E-08
cg04987734	chr14:103415873	<i>CDC42BPB</i>	5.53	3.26E-08
cg23576855	chr5:373299	<i>AHRR</i>	-5.52	3.44E-08
cg09822192	chr14:24801191	<i>ADCY4</i>	5.44	5.30E-08

Position is based on hg19.

Table 3. CpG sites associated with current PTSD in the stratified analyses

CpG	Position	Gene	z	p-value
<i>Stratified analysis for females</i>				
cg25691167	chr7:19184961	<i>FERD3L</i>	5.48	4.24E-08
<i>Stratified analysis for males</i>				
cg05575921	chr5:373378	<i>AHRR</i>	-6.12	9.30E-10*
<i>Stratified analysis for European ancestry</i>				
cg05575921	chr5:373378	<i>AHRR</i>	-6.85	7.24E-12*
cg11256214	chr12:110211642	<i>MGC14436</i>	5.76	8.17E-09
cg21161138	chr5:399360	<i>AHRR</i>	-5.62	1.95E-08*
cg15977432	chr19:56709655	<i>Intergenic</i>	-5.46	4.68E-08
cg04583842	chr16:88103117	<i>BANP</i>	5.43	5.66E-08*
<i>Stratified analysis for African ancestry</i>				
cg02003183	chr14:103415882	<i>CDC42BPB</i>	5.48	4.26E-08
<i>Stratified analysis for civilian trauma</i>				
cg27541344	chr14:99650422	<i>BCL11B</i>	5.39	7.2073E-08
<i>Stratified analysis for military trauma</i>				
cg05575921	chr5:373378	<i>AHRR</i>	-6.22	4.92E-10*
cg00774777	chr11:76478902	<i>RP11-21L23.4</i>	5.55	2.79E-08
cg14753356	chr6:30720108	<i>Intergenic</i>	-5.47	4.53E-08*
cg21566642	chr2:233284661	<i>Intergenic</i>	-5.41	6.36E-08
cg03329539	chr2:233283329	<i>Intergenic</i>	-5.36	8.53E-08

Position is based on hg19. The sites that were also epigenome-wide significant ($p < 9e-08$) in the primary meta-analysis were indicated by an asterisk (*).

Figures

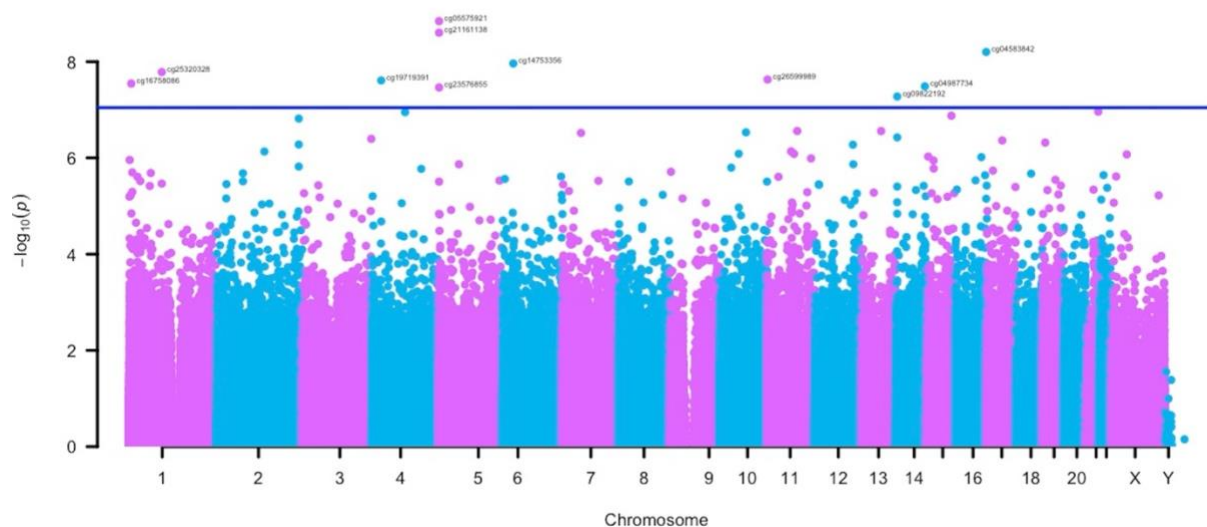


Figure 1. Manhattan Plot of the epigenome-wide association meta-analyses. The x-axis depicts chromosomes and the location of each CpG site across the genome. The y-axis depicts the $-\log_{10}$ of the unadjusted p -value for the association with current PTSD. Each dot represents a CpG site. The solid blue line indicates the epigenome-wide statistical significance at $p < 9.0e-8$.

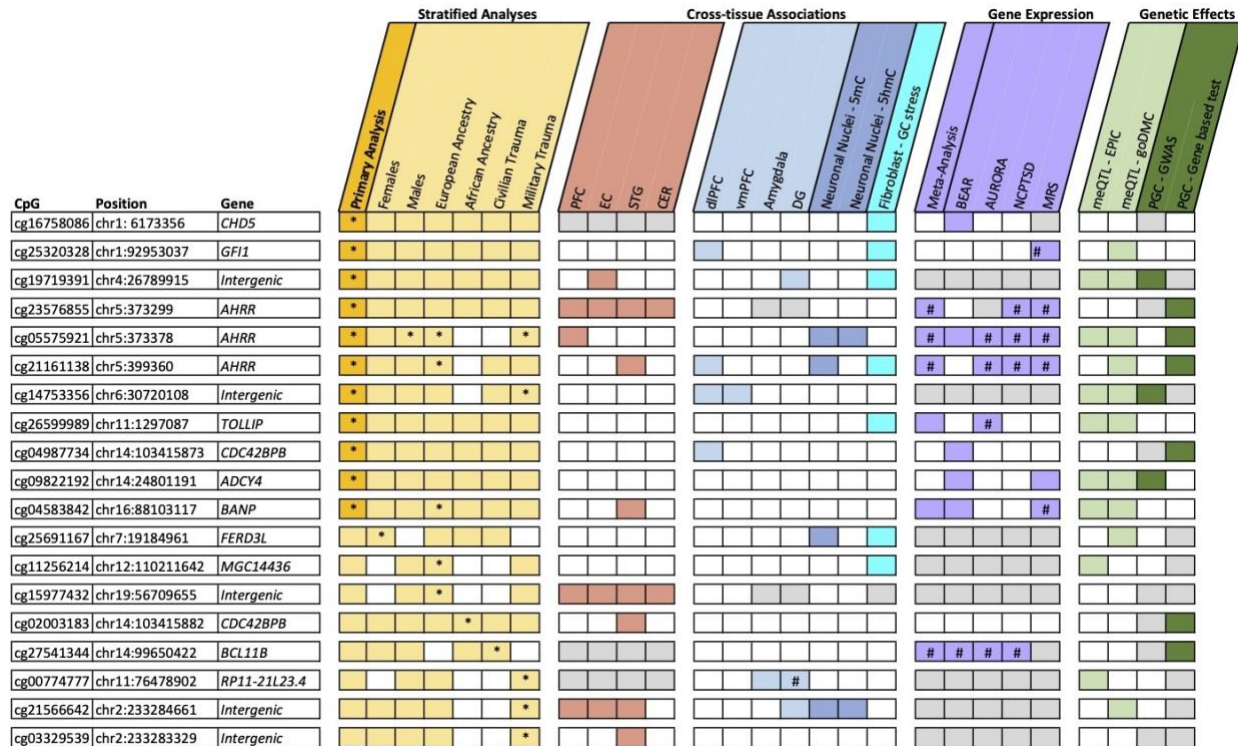


Fig 2. Summary of all analyses and findings. Figure combines CpGs from the main analysis (gold) and stratified analyses for sex, ancestry, and trauma type (light gold); and summarizes the results of blood and brain correlations (rose); cross-tissue associations for multiple brain regions (light blue), neuronal nuclei (blue), and a fibroblast model of prolonged stress (aqua), gene expression (purple); and genetic effects, including methylation quantitative trait loci (meQTL) analyses (light green) and genetic associations from the recent PGC-PTSD GWAS (dark green). Positive findings ($p < 0.05$) are indicated with the specific color of the respective category. Asterisk (*) indicates epigenome-wide significance ($p < 9e-8$). Gray represents the CpGs or genes were not present in the respective datasets. PFC: prefrontal cortex. EC: entorhinal cortex. STG: superior temporal gyrus. CER: cerebellum. dIPFC: dorsolateral prefrontal cortex. vmPFC: ventromedial prefrontal cortex. DG: dentate gyrus. 5mC: 5-Methylcytosine. 5hmC: 5-Hydroxymethylcytosine. GC: glucocorticoid.

References

1. American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders. 2013.
2. Zen AL, Whooley MA, Zhao S, Cohen BE. Post-traumatic stress disorder is associated with poor health behaviors: findings from the heart and soul study. *Health Psychol.* 2012;31(2):194-201.
3. Jacobsen LK, Southwick SM, Kosten TR. Substance use disorders in patients with posttraumatic stress disorder: a review of the literature. *Am J Psychiatry.* 2001;158(8):1184-90.
4. O'Donovan A, Cohen BE, Seal KH, Bertenthal D, Margaretten M, Nishimi K, et al. Elevated risk for autoimmune disorders in iraq and afghanistan veterans with posttraumatic stress disorder. *Biol Psychiatry.* 2015;77(4):365-74.
5. Ryder AL, Azcarate PM, Cohen BE. PTSD and Physical Health. *Curr Psychiatry Rep.* 2018;20(12):116.
6. 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):327-43.
7. Logue MW, Amstadter AB, Baker DG, Duncan L, Koenen KC, Liberzon I, et al. The Psychiatric Genomics Consortium Posttraumatic Stress Disorder Workgroup: Posttraumatic Stress Disorder Enters the Age of Large-Scale Genomic Collaboration. *Neuropsychopharmacology.* 2015;40(10):2287-97.

8. Nievergelt CM, Ashley-Koch AE, Dalvie S, Hauser MA, Morey RA, Smith AK, et al. Genomic Approaches to Posttraumatic Stress Disorder: The Psychiatric Genomic Consortium Initiative. *Biol Psychiatry*. 2018;83(10):831-9.
9. Polimanti R, Wendt FR. Posttraumatic stress disorder: from gene discovery to disease biology. *Psychol Med*. 2021;51(13):2178-88.
10. Duncan LE, Cooper BN, Shen H. Robust Findings From 25 Years of PTSD Genetics Research. *Curr Psychiatry Rep*. 2018;20(12):115.
11. Nievergelt CM, Maihofer AX, Atkinson EG, Chen C-Y, Choi KW, Coleman JR, et al. Discovery of 95 PTSD loci provides insight into genetic architecture and neurobiology of trauma and stress-related disorders. *medRxiv*. 2023:2023.08.31.23294915.
12. Daskalakis NP, Rijal CM, King C, Huckins LM, Ressler KJ. Recent Genetics and Epigenetics Approaches to PTSD. *Curr Psychiatry Rep*. 2018;20(5):30.
13. Zannas AS, Provencal N, Binder EB. Epigenetics of Posttraumatic Stress Disorder: Current Evidence, Challenges, and Future Directions. *Biol Psychiatry*. 2015;78(5):327-35.
14. Bonasio R, Tu S, Reinberg D. Molecular signals of epigenetic states. *Science*. 2010;330(6004):612-6.
15. Baker-Andresen D, Ratnu VS, Bredy TW. Dynamic DNA methylation: a prime candidate for genomic metaplasticity and behavioral adaptation. *Trends Neurosci*. 2013;36(1):3-13.
16. Rasmi Y, Shokati A, Hassan A, Aziz SG, Bastani S, Jalali L, et al. The role of DNA methylation in progression of neurological disorders and neurodegenerative diseases as well as the prospect of using DNA methylation inhibitors as therapeutic agents for such disorders. *IBRO Neurosci Rep*. 2023;14:28-37.

17. Murgatroyd C, Wu Y, Bockmuhl Y, Spengler D. Genes learn from stress: how infantile trauma programs us for depression. *Epigenetics*. 2010;5(3):194-9.
18. Lolk K, Modhukur V, Rajashekar B, Martens K, Magi R, Kolde R, et al. DNA methylome profiling of human tissues identifies global and tissue-specific methylation patterns. *Genome Biol*. 2014;15(4):r54.
19. Hannon E, Lunnon K, Schalkwyk L, Mill J. Interindividual methylomic variation across blood, cortex, and cerebellum: implications for epigenetic studies of neurological and neuropsychiatric phenotypes. *Epigenetics*. 2015;10(11):1024-32.
20. Daskalakis NP, Cohen H, Nievergelt CM, Baker DG, Buxbaum JD, Russo SJ, et al. New translational perspectives for blood-based biomarkers of PTSD: From glucocorticoid to immune mediators of stress susceptibility. *Exp Neurol*. 2016;284(Pt B):133-40.
21. 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;90:223-34.
22. Katrinli S, Oliveira NCS, Felger JC, Michopoulos V, Smith AK. The role of the immune system in posttraumatic stress disorder. *Transl Psychiatry*. 2022;12(1):313.
23. 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):9470-5.
24. 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):700-8.

25. 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.
26. 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):1145-56.
27. 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.
28. Hammamieh R, Chakraborty N, Gautam A, Muhie S, Yang R, Donohue D, et al. Whole-genome DNA methylation status associated with clinical PTSD measures of OIF/OEF veterans. *Transl Psychiatry*. 2017;7(7):e1169.
29. 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):619-30.
30. 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):1585-601.
31. 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 AHRR. *Nat Commun*. 2020;11(1):5965.

32. 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.
33. Katrinli S, Maihofer AX, Wani AH, Pfeiffer JR, Ketema E, Ratanatharathorn A, et al. 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. *Mol Psychiatry*. 2022;27(3):1720-8.
34. 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;9:22-4.
35. Fortin JP, Triche TJ, Jr., Hansen KD. Preprocessing, normalization and integration of the Illumina HumanMethylationEPIC array with minfi. *Bioinformatics*. 2017;33(4):558-60.
36. 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):882-3.
37. 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.
38. 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.

39. Barfield RT, Almlı LM, Kilaru V, Smith AK, Mercer KB, Duncan R, et al. Accounting for population stratification in DNA methylation studies. *Genet Epidemiol.* 2014;38(3):231-41.
40. van Iterson M, van Zwet EW, Consortium B, Heijmans BT. Controlling bias and inflation in epigenome- and transcriptome-wide association studies using the empirical null distribution. *Genome Biol.* 2017;18(1):19.
41. Barfield RT, Kilaru V, Smith AK, Conneely KN. CpGassoc: an R function for analysis of DNA methylation microarray data. *Bioinformatics.* 2012;28(9):1280-1.
42. Balduzzi S, Rucker G, Schwarzer G. How to perform a meta-analysis with R: a practical tutorial. *Evid Based Ment Health.* 2019;22(4):153-60.
43. 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.
44. Phipson B, Maksimovic J, Oshlack A. missMethyl: an R package for analyzing data from Illumina's HumanMethylation450 platform. *Bioinformatics.* 2016;32(2):286-8.
45. 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.
46. Daskalakis NP, Iatrou A, Chatzinakos C, Jajoo A, Snijders C, DiPietro CP, et al. Systems Biology Dissection of PTSD and MDD across brain regions, cell-types and blood. *Science.* In Revision.

47. Friedman MJ, Huber BR, Brady CB, Ursano RJ, Benedek DM, Kowall NW, et al. VA's National PTSD Brain Bank: a National Resource for Research. *Curr Psychiatry Rep.* 2017;19(10):73.
48. Rompala G, Nagamatsu ST, Martinez-Magana JJ, Nunez-Rios DL, Wang J, Girgenti MJ, et al. Profiling neuronal methylome and hydroxymethylome of opioid use disorder in the human orbitofrontal cortex. *Nat Commun.* 2023;14(1):4544.
49. Zannas AS. Naturalistic Stress Hormone Levels Drive Cumulative Epigenomic Changes along the Cellular Lifespan. *Int J Mol Sci.* 2021;22(16).
50. Leung CS, Kosyk O, Welter EM, Dietrich N, Archer TK, Zannas AS. Chronic stress-driven glucocorticoid receptor activation programs key cell phenotypes and functional epigenomic patterns in human fibroblasts. *iScience.* 2022;25(9):104960.
51. Min JL, Hemani G, Hannon E, Dekkers KF, Castillo-Fernandez J, Luijk R, et al. Genomic and phenotypic insights from an atlas of genetic effects on DNA methylation. *Nat Genet.* 2021;53(9):1311-21.
52. Villicana S, Castillo-Fernandez J, Hannon E, Christiansen C, Tsai PC, Maddock J, et al. Genetic impacts on DNA methylation help elucidate regulatory genomic processes. *Genome Biol.* 2023;24(1):176.
53. Villicaña S, Castillo-Fernandez J, Hannon E, Christiansen C, Tsai P-C, Maddock J, et al. Genetic impacts on DNA methylation help elucidate regulatory genomic processes. *bioRxiv.* 2023:2023.03.31.535045.

54. Chen XQ, Tan I, Leung T, Lim L. The myotonic dystrophy kinase-related Cdc42-binding kinase is involved in the regulation of neurite outgrowth in PC12 cells. *J Biol Chem*. 1999;274(28):19901-5.
55. Story Jovanova O, Nedeljkovic I, Spieler D, Walker RM, Liu C, Luciano M, et al. DNA Methylation Signatures of Depressive Symptoms in Middle-aged and Elderly Persons: Meta-analysis of Multiethnic Epigenome-wide Studies. *JAMA Psychiatry*. 2018;75(9):949-59.
56. Ligthart S, Marzi C, Aslibekyan S, Mendelson MM, Conneely KN, Tanaka T, et al. DNA methylation signatures of chronic low-grade inflammation are associated with complex diseases. *Genome Biol*. 2016;17(1):255.
57. Wielscher M, Mandaviya PR, Kuehnel B, Joehanes R, Mustafa R, Robinson O, et al. DNA methylation signature of chronic low-grade inflammation and its role in cardio-respiratory diseases. *Nat Commun*. 2022;13(1):2408.
58. Muniz Carvalho C, Wendt FR, Maihofer AX, Stein DJ, Stein MB, Sumner JA, et al. Dissecting the genetic association of C-reactive protein with PTSD, traumatic events, and social support. *Neuropsychopharmacology*. 2021;46(6):1071-7.
59. Eraly SA, Nievergelt CM, Maihofer AX, Barkauskas DA, Biswas N, Agorastos A, et al. Assessment of plasma C-reactive protein as a biomarker of posttraumatic stress disorder risk. *JAMA Psychiatry*. 2014;71(4):423-31.
60. Yang JJ, Jiang W. Immune biomarkers alterations in post-traumatic stress disorder: A systematic review and meta-analysis. *J Affect Disord*. 2020;268:39-46.

61. Passos IC, Vasconcelos-Moreno MP, Costa LG, Kunz M, Brietzke E, Quevedo J, et al. Inflammatory markers in post-traumatic stress disorder: a systematic review, meta-analysis, and meta-regression. *Lancet Psychiatry*. 2015;2(11):1002-12.
62. Hao N, Whitelaw ML. The emerging roles of AhR in physiology and immunity. *Biochem Pharmacol*. 2013;86(5):561-70.
63. Wilson R, Wahl S, Pfeiffer L, Ward-Caviness CK, Kunze S, Kretschmer A, et al. The dynamics of smoking-related disturbed methylation: a two time-point study of methylation change in smokers, non-smokers and former smokers. *BMC Genomics*. 2017;18(1):805.
64. Katrinli S, Smith AK. Immune system regulation and role of the human leukocyte antigen in posttraumatic stress disorder. *Neurobiol Stress*. 2021;15:100366.
65. Kim GS, Smith AK, Xue F, Michopoulos V, Lori A, Armstrong DL, et al. Methylomic profiles reveal sex-specific differences in leukocyte composition associated with post-traumatic stress disorder. *Brain Behav Immun*. 2019;81:280-91.