PTSD is associated with increased DNA methylation across regions of *HLA-DPB1* and *SPATC1L*

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Abstract

Posttraumatic stress disorder (PTSD) is characterized by intrusive thoughts, avoidance, negative alterations in cognitions and mood, and arousal symptoms that adversely affect mental and physical health. Recent evidence links changes in DNA methylation of CpG cites to PTSD. Since clusters of proximal CpGs share similar methylation signatures, identification of PTSD-associated differentially methylated regions (DMRs) may elucidate the pathways defining differential risk and resilience of PTSD. Here we aimed to identify epigenetic differences associated with PTSD. DNA methylation data profiled from blood samples using the MethylationEPIC BeadChip were used to perform a DMR analysis in 187 PTSD cases and 367 trauma-exposed controls from the Grady Trauma Project (GTP). DMRs were assessed with R package bumphunter. We identified two regions that associate with PTSD after multiple test correction. These regions were in the gene body of *HLA-DPB1* and in the promoter of *SPATC1L*. The DMR in *HLA-DPB1* was associated with PTSD in an independent cohort. Both DMRs included CpGs whose methylation associated with nearby sequence variation (meQTL) and that associated with expression of their respective genes (eQTM). This study supports an emerging literature linking PTSD risk to genetic and epigenetic variation in the *HLA* region.

Keywords

EWAS; epigenetics; DNA methylation; eQTM; meQTL

1. Introduction

Posttraumatic stress disorder (PTSD) is a debilitating psychiatric disorder characterized by re-experiencing, avoidance and hyperarousal symptoms that cause negative alterations in cognition, mood and physiologic health (American Psychiatric Association, 2013). Among other psychiatric disorders, PTSD is unique as it requires a trauma exposure to develop. Although 60 to 90% of the population (Benjet et al., 2016; Breslau et al., 1998; Kessler et al., 1995) are exposed to at least one traumatic event during their lifespan, the prevalence of PTSD is 46.2% in highly traumatized US population (Gillespie et al., 2009) and only 3.9% globally (Koenen et al., 2017). This notable difference in prevalence between trauma exposure and PTSD development suggests that some individuals are more vulnerable to trauma and are at higher risk of developing PTSD. Recent evidence suggests that this differential susceptibility may arise from distinct epigenetic changes resulting from trauma (Daskalakis et al., 2018; Morrison et al., 2019; Sheerin et al., 2017).

Epigenetic factors – structural modifications to DNA that regulate gene expression without changing the DNA sequence – link environmental circumstances and experiences (e.g. stress or trauma exposure) to biological response. Identification of epigenetic alterations related to PTSD is a crucial step in characterization of the pathways defining differential risk and resilience of PTSD. Multiple reviews summarize the link between trauma exposure, PTSD and epigenetic alterations (Daskalakis et al., 2018; Kim et al., 2018; Vinkers et al., 2015; Zannas et al., 2015).

To date, a number of epigenome-wide association studies (EWAS) conducted on individual cohorts and meta-analyses reported PTSD-associated differentially methylated CpG sites in genes involved in neurotransmission, neural development and immune pathways (Kuan et al., 2017; Mehta et al., 2013; Smith et al., 2011; Smith et al., 2019; Uddin et al., 2010; Uddin et al., 2018). Since clusters of proximal CpGs often share similar methylation profiles (Martorell-Marugan et al., 2019), joint analysis of multiple CpGs in regions, called differentially methylated regions (DMRs), are more robust with respect to statistical power (Michels et al., 2013) and correlate well with gene expression (Docherty et al., 2014). Therefore, identification of PTSD-associated DMRs may advance our understanding of the molecular mechanisms underlying the disorder. For instance, a recent study conducted on 73 traumatized police officers reported consistently higher methylation of a DMR located at the *PAX8* gene (Krzyzewska et al., 2018). A longitudinal study investigating the effect of changes in DNA methylation between postdeployment and pre-deployment samples showed PTSD-associated DMRs in multiple genes, including *ZFP57* and *SPATC1L* (Rutten et al., 2018). A follow-up prospective study reported significant changes in DNA methylation at the DMR located in *ZFP57*, after successful treatment of PTSD (Vinkers et al., 2019). Recently, a meta-analysis of three longitudinal military cohorts identified 12 novel DMRs associated with PTSD, including *MAD1L1* and *HLA-DPB1*, genes that have previously been implicated in PTSD (Snijders et al., 2020). The SNP rs10235664 in *MAD1L1* was reported in the PTSD GWAS of the Million Veteran Program (MVP) (Gelernter et al., 2019), and our group recently identified *HLA-DPB1*17:01* as a PTSD risk allele (Katrinli et al., 2019).

In the current study, we performed an EWAS to evaluate the association of current PTSD diagnosis with differentially methylated positions (DMPs) and DMRs. To provide functional context to our findings, we leveraged expression quantitative trait methylation (eQTM) and methylation quantitative trait locus (meQTL) data available for the Grady Trauma Project (GTP) cohort, and also tested the association between the significant DMRs and PTSD in an independent study. Identification of these DMRs may advance our understanding of the genes undergoing regulation in those with PTSD and may provide insights into diagnostic or treatment strategies.

2. Methods

2.1. Grady Trauma Project (GTP)

Participants were recruited as part of the GTP, which investigates the influence of genetic and environmental factors on responses to stressful life events in a predominantly African American, urban population of low socioeconomic status (Gillespie et al., 2009). Briefly, participants were approached in waiting rooms of the general medical clinics of a large, urban, public hospital in Atlanta, GA. Subjects willing to participate provided written informed consent and participated in a verbal interview and blood draw. All procedures in this study were approved by the Institutional Review Board of Emory University School of Medicine and the Grady Health Systems Research Oversight Committee.

Participants provided self-reported demographic information including subject age, sex, and ancestry. For the current study, cases were defined as having current PTSD, and controls

were exposed to trauma but had no current or lifetime history of the disorder. PTSD diagnosis was mainly assessed by the Clinician-Administered Post-traumatic Stress Disorder Scale (CAPS) for DSM-IV, a structured diagnostic instrument measuring PTSD that has been demonstrated to have excellent psychometric properties (Blake et al., 1995; Weathers et al., 2001). The CAPS provides a diagnostic status of lifetime and current PTSD. Participants were identified as having PTSD if they met DSM-IV PTSD criteria for current PTSD from the CAPS interview. This resulted in 158 PTSD cases and 332 controls. The CAPS was administered on the same day as the blood draw. For 64 (12%) participants with missing CAPS information, the modified PTSD Symptomatic Scale (PSS) was used as a measure of PTSD symptoms and identified an additional 29 PTSD cases and 35 controls (Foa and Tolin, 2000). PSS frequency items were used to obtain a continuous measure, with values of above 20 considered as clinically significant PTSD symptoms (Falsetti et al., 1993). In addition, to be diagnosed with PTSD, individuals needed to have clinically significant symptoms in three clusters, representing intrusive (B), avoidance/numbing (C), and hyperarousal (C) symptoms on the PSS (Jovanovic et al., 2010). The PSS was only used if it occurred within 30 days of the blood draw (mean = 15, median = 14). Using this PTSD diagnosis algorithm, we defined 187 individuals as PTSD cases and 367 as trauma-exposed controls.

Lifetime exposure to traumatic events was assessed using the Traumatic Events Inventory (TEI) (Bradley et al., 2008; Schwartz et al., 2005), which measures lifetime (childhood and adulthood) exposure to trauma such as natural disaster, serious accident or injury, and physical or sexual assault.

2.2. DNA methylation

DNA was extracted from whole blood using the E.Z.N.A. Blood DNA Midi Kit (Omega Bio-tek, Norcross, GA) and interrogated using the MethylationEPIC BeadChip (Illumina) according to manufacturer's instructions. Raw methylation beta values were determined via GenomeStudio (Illumina). Internal Illumina controls were used to assess the quality of staining, extension, hybridization, bisulfite conversion, and specificity. Samples with probe detection call rates <90% and those with an average intensity value of either <50% of the experiment-wide sample mean or <2,000 arbitrary units (AU) were removed using R package CpGassoc (Barfield et al., 2012). Probes with detection p-values >0.01 set to missing. CpG sites that cross hybridize between autosomes and sex chromosomes were removed (McCartney et al., 2016). A total of 819,380 probes passed QC and were used in subsequent analyses. Single-sample Noob (ssNoob) normalization method implemented in R package *minfi* was used for dye bias equalization (Fortin et al., 2017). Following normalization, the ComBat procedure in the R package SVA was used to remove chip and positional batch effects, controlling for age and PTSD status (Leek et al., 2012). Because of the heteroskedastic nature of beta values, logit transformed beta values (M-values) were used for subsequent analysis (Du et al., 2010).

2.3. Analysis of differentially methylated positions

Since DNA methylation varies by cell type, cell proportions can be important confounding factors in DNA methylation analysis of whole blood samples (Houseman et al., 2015). The proportions of CD8+T, CD4+T, natural killer (NK), B cells, monocytes (mono) and

neutrophils were estimated using publicly available reference data (GSE110554) and Robust Partial Correlation (RPC) method implemented in R package Epidish (Teschendorff et al., 2017). Estimated proportions of CD8+T, CD4+T, NK, B cells and mono were included as covariates in all analyses.

Logit transformed β values (M-values) were modeled by linear regression as a function of PTSD, adjusting for age, CD8+T, CD4+T, NK, B cell, and monocyte cell proportions, and ancestry using first three principal components (PCs) calculated from GWAS. P-values were adjusted for multiple-testing using the Benjamini-Hochberg FDR procedure at 5% FDR level (Benjamini and Hochberg, 1995).

2.4. Analysis of differentially methylated regions

Identification of DMRs was conducted using bump-hunting method, implemented in R package *minfi* (Aryee et al., 2014; Jaffe et al., 2012). Probes separated by at least 1000 bp was used to define clusters. For each probe, we estimated the difference in the average logit of beta values (defined as M-values) between PTSD cases and controls, adjusting for sex, age and first three principal components, and smoothed these estimated differences using loess (Cleveland, 1979). For all data, an empirical distribution was created by performing 1000 bootstrap samples. Candidate regions with estimate M-value difference >0.1 and smoothed estimated differences >95% quantile of the empirical distribution were selected. Consistent with the approach of Rhead et al. (Rhead et al., 2018), regions with family-wise error (FWER) <0.2 were identified as significant PTSD-associated DMRs, as controlling the FWER (the probability of making at least one type I error) is more conservative than controlling the false discovery rate (controls the proportion of type I errors). All positions and regions were in reference to GRCh37/hg19.

2.5. Translational Research Center for TBI and Stress Disorders (TRACTS)

Association between PTSD and DMRs identified in the GTP discovery cohort was also tested in an independent replication cohort, using 445 blood samples (309 cases and 136 controls) interrogated using the MethylationEPIC BeadChip (Illumina) from Translational Research Center for TBI and Stress Disorders (TRACTS) cohort (Logue et al., 2020). PTSD was assessed using CAPS and number of different types of trauma was measured by Traumatic Life Events Questionnaire (TLEQ). Cleaning and processing of the methylation data for TRACTS data has been described in detail elsewhere (Logue et al., 2020), using a consortium-based pipeline for processing, similar to that used in the discovery data (Ratanatharathorn et al., 2017).

Bumphunter was used to analyze PTSD in the TRACTS cohort using the same parameters as described above. Analyses included age, sex and cell counts (CD8+T, CD4+T, NK, B cells, and monocytes) as covariates. Any significant region reported in the primary analysis were reported. Based on concerns about the null simulations performed internally by Bumphunter to generate significance values, and to validate the observed evidence of replication, we performed our own null simulations of the DMRs at chr6: 33047185 – 33049505 and chr21: 47604052 – 47605174. As genome-wide null simulations are computationally demanding, we analyzed a reduced set of CpGs in the regions above +/– 50000 bp. First, we tested for

association with PTSD as described above in these reduced set of CpGs. Then, we randomly permuted the affection status of TRACTS participants 1000 times, and analyzed the reduced set of CpGs around the two DMRs to generate an empirical p-value distribution of each region under the null. As Bumphunter only evaluates significance of a region when it meets certain criteria, replicates in which Bumphunter did not generate a p-value were recorded as p=1. Our empirical p values for these two regions were then estimated as the percentile of the observed reduced-set p-values in the corresponding region's null p-value distribution.

2.6. Correlation between genetic effects, gene expression and methylation

Correlation between methylation of CpGs in the DMRs and expression of its annotated gene was evaluated using previously generated expression quantitative trait methylation (eQTM) data from GTP (Kennedy et al., 2018). These eQTMs were identified through analysis of methylation data generated by HumanMethylation450 BeadChip and expression data generated via Illumina HT12v3 and HT12v4 expression BeadChips (Illumina).

To evaluate whether the CpGs in the PTSD-associated DMRs associate by nearby genetic polymorphisms, we have used previously generated meQTL data on 780 subjects from GTP (data not shown). Briefly, 5,971,966 genomic variants (genotyped with Illumina HumanOmni1-Quad, HumanOmniExpress, or Multi-Ethnic Global arrays) were tested for association with 608,245 CpG sites (interrogated with MethylationEPIC BeadChip) using a linear mixed model implemented in matrixeQTL (Shabalin, 2012). The model was adjusted for age, gender, estimated cell proportions and 3 genotype-based principal components (PCs), and treated ethylation (CpG) as the outcome and SNP allele count as an explanatory variable. A stringent Bonferroni threshold of $p < 8.21E-14$ was used to identify meQTLs.

We also tested the association between 24 CpGs in the *HLA-DPB1* DMR and HLA-*DPB1*17:01* allele, which independently associates with PTSD (Katrinli et al., 2019). To do so, we modeled each CpG as a function of the *HLA-DPB1*17:01* allele under a dominant model, adding sex, age and cell proportions as covariates. P-values were adjusted for multiple-testing using a Bonferroni correction for 24 tests.

2.7. Correlation of CpGs in DMRs between blood and brain tissues

The degree of correlation between blood and brain tissue methylation of CpGs in the DMRs were assessed using data from IMAGE-CpG (Iowa Methylation Array Graphing for Experimental Comparison of Peripheral tissue & Gray matter (Brain)) (Braun et al., 2019). This data provides spearman correlation coefficients (rho) and associated p-values for the association of the methylation status of individual CpG sites assayed with MethylationEPIC in blood and the brain.

3. Results

3.1. Demographic characteristics of cohorts

The demographics of the cohorts are presented in Table 1. The GTP cohort (discovery) was predominantly African American (94%) and female (73%). Of 554 participants, 34% had a current PTSD diagnosis, assessed by CAPS and PSS. We observed a moderate correlation

between PSS and current CAPS in this dataset (Pearson r = 0.53, p < 2.2E-16). Cases and controls did not differ significantly in terms of age. However, cases were more likely to be female (p = 2.92E-6) and were exposed to more traumatic events as compared to controls (p = 1.10E-15).

The TRACTS cohort (replication), which included 309 cases and 136 controls, was predominantly male (92%) and Caucasian (91%), unlike GTP. However, like GTP, PTSD cases were more likely to be female and had a higher trauma burden relative to controls (Table 1).

3.2. Identification of PTSD-associated DMPs and DMRs

A total of 819,380 CpG sites were evaluated. Of these, 39,597 were nominally associated with PTSD, but no individual DMPs were significant after correction for multiple testing, FDR < 0.05 (Table S1).

Next, we sought to identify DMRs (Lin et al., 2016). In the GTP discovery cohort, 622 potential DMRs with a M-value cut-off of 0.1 were identified and tested for association with PTSD (Table S2). Of these, 110 regions had a p < 0.05, where p is the probability under null distribution, but only two regions remained differentially methylated following correction for multiple comparisons (Table 2). PTSD cases had increased methylation in i) a region (chr6: 33047185 – 33049505) that is annotated to both the promoter of *HLA-DPA1* and gene body of *HLA-DPB1* and ii) the promoter region of *SPATC1L* (chr21: 47604052 – 47605174) (Figure 1).

To evaluate whether DMRs that associated with PTSD in the GTP also associate with PTSD in an independent and demographically different cohort, we performed a DMR analysis in TRACTS cohort. We initially observed nominal associations of the DMRs at chr6: 33047185 – 33050741 (*HLA-DPB1* region; p = 2.89E-05) and chr21: 47604052 – 47605174 (*SPATC1* region; p = 0.001), but only the DMR at chr6: 33047185 – 33050741 remained associated after generation of an empirical (simulation based) p-value (p = 0.006; Table 2). The *HLA-DPB1* DMR identified in TRACTS was wider than that of GTP, and the direction of the effect in TRACTS was opposite of what was observed in GTP, such that the DMR had lower methylation in PTSD cases compared to controls (Mean M = −0.16).

3.3. Gene expression and genetic effects

We evaluated the degree of correlation between methylation of CpGs in DMRs and gene expression, using previously generated eQTM data on GTP cohort. For the 24 CpGs in the DMR that are annotated to both the promoter of *HLA-DPA1* and the gene body of *HLA-DPB1* we evaluated expression with both genes in GTP. Each of the 18 CpGs in this DMR present on both the HumanMethylation450 array and MethylationEPIC arrays negatively correlated with *HLA-DPB1* expression (Table S3) though none correlated with *HLA-DPA1* expression. Thus, subsequent analyses focused on *HLA-DPB1*. Of the eight CpGs in the *SPATC1L* DMR interrogated on both arrays, each negatively correlated with *SPATC1L* expression (Table S4).

Next, we investigated genetic-epigenetic correlations in the PTSD-associated DMRs. For the *HLA-DPB1* DMR, four of 24 CpGs were associated with 380 unique SNPs (Table S5). It is important to note that the HLA-DPB1*17:01 allele, which independently associated with PTSD (Katrinli et al., 2019), also associated with two CpGs in the *HLA-DPB1* DMR (Table S6). For the *SPATC1L* DMR, methylation of six out of 10 CpGs were associated with 123 unique SNPs (Table S7). None of the meQTL SNPs associated independently with PTSD in the recent Psychiatric Genetics Consortium (PGC) – PTSD meta-analysis (Nievergelt et al., 2019).

3.4. Correlation of DMRs between blood and brain tissues

Using the IMAGE-CpG database, the correlation of CpGs in *HLA-DPB1* and *SPATC1L* DMRs were compared in blood-derived and brain-derived samples (Table S8). The DMR in *HLA-DPB1* includes 24 CpGs, but only 21 of those CpGs are available in the IMAGE-CpG database. Out of 21 CpGs tested, five CpGs are correlated between blood and brain tissues (rho > 0.4, p < 0.05). The DMR in *SPATC1L* includes 10 CpGs, and all CpGs are correlated between blood and brain tissues (rho > 0.88, p < 5e-5).

4. Discussion

Understanding the biological characteristics that distinguish individuals with versus without PTSD is crucial for developing new prevention and intervention strategies following a traumatic event. In the current study, we aimed to identify epigenetic loci associated with PTSD in a predominantly African American cohort of traumatized civilians. We identified two regions, located in *HLA-DPB1* and *SPATC1L*, that were differentially methylated in PTSD. Both DMRs showed increased methylation in PTSD cases compared to trauma-exposed controls in the GTP discovery cohort.

The DMR in *HLA-DPB1* supports the increasing evidence of the link between PTSD and immune system dysregulation (Eraly et al., 2014; Michopoulos et al., 2017). Interestingly, *HLA-DPB1* region associated with PTSD in a recent longitudinal meta-analysis conducted on three military cohorts (Snijders et al., 2020). This study reported DMRs in *HLA-DPB1* body (chr6: 33043976 - 33054001) and CpG island (chr6: 33048416 - 33048814), suggesting that methylation changes throughout HLA locus might be associated with PTSD. However, in this longitudinal study of Snijders et al. (2020), PTSD was associated with decreased *HLA-DPB1* DMR methylation. This difference in directions may be based on methodological differences, since their methods reported wider regions than what we identified in our study, or difference in cohort characteristics (e.g. the TRACTS study included military cohorts that are predominantly Caucasian and male, but the GTP cohort consist of predominantly African-American and women civilians). It is important to note that we also observed lower methylation at the *HLA-DPB1* DMR in the PTSD cases of TRACTS cohort, which is the opposite of what we observed in GTP. Since our discovery cohort (GTP) is predominantly African American and TRACTS is Caucasian, we hypothesize the opposite direction of association of the DMR in *HLA-DPB1* may reflect differences in underlying genetic effects; 96% of the *HLA-DPB1* meQTL SNPs differ in minor allele frequencies (MAFs) by more than 40% between those of African versus

European decent. The region includes 4 CpGs (out of 24 CpGs) with meQTLs in which the allele frequencies of the underlying SNPs vary by ancestry. For example, the A allele frequency of rs1042117 is 48% in African Americans and 9% in Europeans. Thus, the differences in the direction of association the *HLA-DPB1* DMR and PTSD may be attributable to sequence-dependent DNA methylation patterns that are ancestry-specific. Examples of allele-specific methylation, which can vary by ancestry, are well-documented (Hellman and Chess, 2010; Onuchic et al., 2018; Smith et al., 2014; Zhang et al., 2009). Moreover, this DMR may play a role in regulation of *HLA-DPB1* gene expression, as 75% of the CpGs in the DMR (18 out of 24) were negatively correlated with *HLA-DPB1* expression.

The HLA locus is one of the key elements of the individual's immunogenetic background, which plays an important role in shaping their response to stress or trauma exposure. *HLA-DPB1* belongs to highly polymorphic HLA Class II genes, which are a combination of different SNPs (i.e. haplotypes) that results in functionally distinct proteins. According to the HLA PheWAS database, various HLA alleles associate with neuropsychiatric phenotypes, including substance abuse, schizophrenia, bipolar disorder, anxiety disorder and PTSD (Karnes et al., 2017). In our recent study, we showed that HLA-DPB1*17:01 allele was more frequent in PTSD cases compared to trauma-exposed controls in African Americans (Katrinli et al., 2019). Here, we observed that two CpGs out of 24 CpGs (8%) on *HLA-DPB1* DMR were associated with *HLA-DPB1*17:01* allele (Bonferroni-adjusted p < 0.05). Interestingly, this PTSD-associated HLA-DPB1*17:01 allele seems to be more prevalent in African Americans, as the HLA-DPB1*17:01 allele frequency is 7 times lower in Caucasians compared to African Americans (0.9% vs 7%, respectively) (Gonzalez-Galarza et al., 2020). Thus, the association between PTSD and the *HLA-DPB1* DMR in African Americans may reflect indirect effects of the HLA-DPB1*17:01 allele.

Notably, the DMR in *SPATC1L* associated with PTSD in a recent longitudinal study in a Caucasian male military cohort (Rutten et al., 2018), where the increased PTSD symptom scores over time were associated with decreased DNA methylation levels at the DMR. The difference in directions between this study and ours may be based on cohort characteristics, since the cohort analyzed in the study of Rutten et al. (2018) was a Caucasian male military cohort, while GTP is a predominantly women and African American civilian cohort, or methodological differences (e.g. Rutten et al. (2018) used continuous PTSD symptom scores and conducted a longitudinal analysis, whereas we used categorical PTSD diagnosis variable and conducted a cross-sectional analysis). It is important to note that *SPATC1L* DMR did not replicate in TRACTS cohort. This region is under genetic control, with 60% CpGs in the *SPATC1L* DMR are associated with SNPs, and 78% of those meQTL SNPs differ in MAFs by more than 20% between those of African versus European decent, which again indicating a sequence-dependent allele-specific methylation. Hence, we can speculate that this region may be ancestry specific. We also evaluated if the CpGs in *SPATC1L* DMR differ by sex, through regressing methylation levels of those CpGs on sex, but we did not find any evidence that this region might be sex-specific. We also demonstrated that *SPATC1L* DMR has functional implications, as 80% of the CpGs in the DMR (eight out of 10) were negatively correlated with *cis* gene expression. Therefore, increased methylation on the

SPATC1L promoter would be expected to result in decreased *SPATC1L* expression in PTSD cases.

SPATC1L protects cells from cell death induced by DNA-damaging alkylating agents (Fry et al., 2008), which are byproducts of normal cellular metabolism as well as from oxidative stress and chronic inflammation (Sharma et al., 2017). Increased methylation of *SPATC1L* promoter in PTSD cases may result in decreased *SPATC1L* expression, leading to increased sensitivity to DNA alkylating agents (Fry et al., 2008) and cell death, which produces reactive oxygen species. Notably, clinical evidence supports significant differences in blood antioxidant enzyme concentrations and oxidative stress-related gene expression between PTSD cases and controls and supports involvement of oxidative stress in the physiopathology of PTSD (Atli et al., 2016; Borovac Stefanovic et al., 2015; Tylee et al., 2015; Zieker et al., 2007). Moreover, a study conducted on HEK293 cells showed translocation of *SPATC1L* at cell junctions after neurokinin-2 (NK2) receptor activation, by the neurotransmitter, neurokinin A (NKA) (Lecat et al., 2015). NKA is a member of the tachykinin family of neuropeptides that functions as a neurotransmitter and contributes to inflammation (Onaga, 2014). Animal studies show increased NKA expression following exposure to inflammatory stimuli. For example, administration of bacterial Lipopolysaccharide (LPS) led to increased NKA expression in mouse spinal cord (Bret-Dibat et al., 1994), as well as inflammatory cytokines IL-1 (Kalra et al., 1994) and IL-6 (De Laurentiis et al., 2003) increased NKA expression in rat hypothalamus. Interestingly, a recent study reported increased blood NKA levels in PTSD cases (Oglodek, 2017). In line with the inflammatory physiopathology of PTSD, NK2 receptor antagonists were suggested as novel therapies in the treatment of depression and stress-related disorders. Results from animal trials were promising for NK2 receptor antagonist SR48968 (Saredutant), which has been shown to block NK2 receptors in brain and reduce depressive-like behaviors in mice that undergo forced swimming test, and in guinea pig pups that go through maternal separation (Steinberg et al., 2001). Even though the exact role of *SPATC1L* in this mechanism is still unknown, it is clear that regulation of *SPATC1L* expression is important to maintain homeostasis against oxidative stress and inflammation, which are commonly observed in PTSD patients.

Our findings should be considered alongside a number of study limitations. First, our study was limited to CpG sites included on the MethylationEPIC BeadChip, which captures only a fraction of the CpG sites in the genome. Thus, we are likely missing some PTSD-associated CpG sites that can be captured through sequencing methods. However, this array-based technology allowed us to replicate our findings in independent groups of subjects. Second, as this is a cross-sectional study, we cannot ascertain whether the identified epigenetic changes in PTSD cases are a consequence of the disorder or a part of its etiology. Third, our discovery cohort consists of blood samples, which is not the ideal proxy to study psychiatric disorders as brain tissue. However, all CpGs in the *SPATC1L* DMR and 21% of the CpGs (five out of 24) in the *HLA-DPB1* DMR are correlated between blood and brain tissues. Fourth, we used two different PTSD measures (CAPS and PSS), but two measures were moderately correlated in the cohort. Finally, some preliminary simulations by our group reported higher false positive rates while using bumphunter. Hence, we performed null simulations of *HLA* and *SPATC1L* to generate emprical p values for replication analysis

performed on TRACTS cohort. These simulations supported the association with the *HLA-DPB1* DMR, but did not indicate sufficient evidence to conclude that *SPATC1L* was assocaited in the TRACTS cohort.

Taken together, the findings of the current study support the need for further study of epigenetic regulation of *HLA-DPB1* and *SPATC1L* with regards to PTSD. Longitudinal and functional studies are required to elucidate whether the identified DMRs are consistently and causally associated with PTSD or its treatment.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgement

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Highlights

Methylation in *HLA-DPB1* and *SPATC1L* associate with PTSD in African American civilians.

Methylation in *HLA-DPB1* replicates in a Caucasian military cohort.

This study links PTSD risk to genetic and epigenetic variation in the *HLA* region.
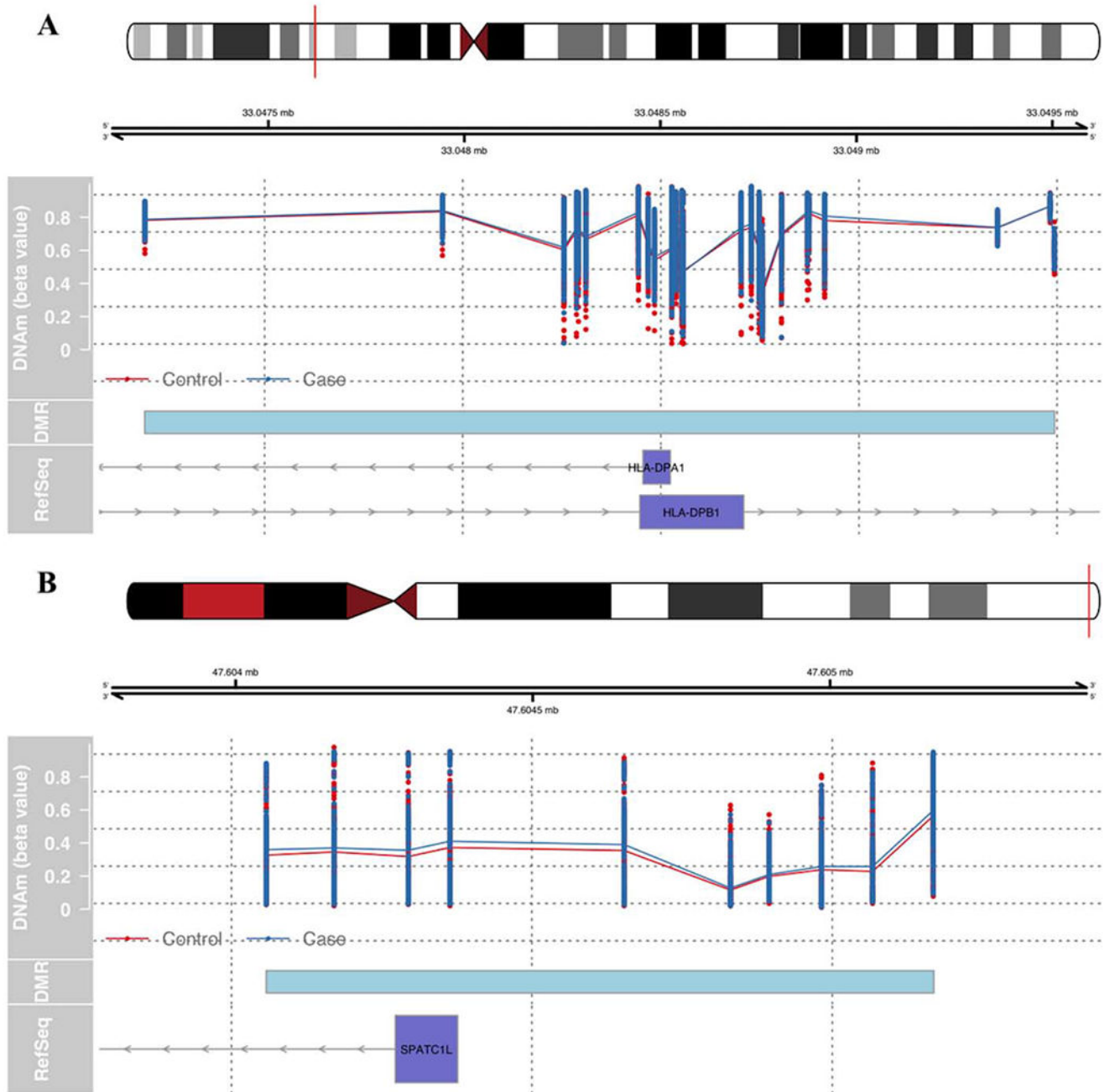
Fig 1: PTSD-associated DMR regions in the discovery cohort.
Unadjusted methylation values for the control (red) and PTSD case (blue) groups. Cyan box represents DMR region. The blue axis represents the known RefSeq genes downloaded from UCSC browser: (A) region near *HLA-DPA1* and *HLA-DPB1L*, (B) region near *SPATC1*.

Table 1:

Clinical and demographic characteristics of the subjects

	Discovery Study (GTP – EPIC)			Replication Study (TRACTS)		
	Cases (N=187)	Controls (N=367)	P-value	Cases (N=309)	Controls (N=136)	P-value
Age, mean ± SD	41.58 ± 11.45	42.04 ± 12.86	0.668	33.10 ± 8.80	32.58 ± 9.96	0.58
Female (%)	159 (85.0%)	245 (66.8%)	2.92E-06	31 (10.03%)	4 (2.94%)	0.02
African American, N (%)	175 (93.6%)	346 (94.3%)	1.00	25 (8.09%)	14[b] (10.37%)	0.55
Trauma burden[a], mean ± SD	6.94 ± 3.25	4.44 ± 2.77	1.10E-15	2.42 ± 1.93[c]	0.89 ± 1.11[d]	3.02E-16

Abbreviations: PTSD, post-traumatic stress disorder. The p-values were computed from t-test (for Age, PTSD and Trauma burden) and Fisher's exact test (for sex, ancestry and childhood trauma) comparing PTSD cases to trauma exposed controls.

[a]Based on Traumatic Events Inventory (TEI) measure for GTP and Traumatic Life Events Questionnaire (TLEQ) for TRACTS

[b]Calculated after removing 1 missing data

[c]Calculated after removing 49 missing data

[d]Calculated after removing 32 missing data

Table 2:

DMRs in discovery cohort with an average M-value difference > 0.1 and FWER < 0.2.

Nearest Gene		GTP (Discovery)					TRACTS (Replication)					
	Chr	Start	End	Mean M	nCpGs	p-value	Chr	Start	End	Mean M	nCpGs	p-value	P_{perm}
HLA-DPA1, HLA-DPB1	chr6	33047185	33049505	0.13	24	2.47E-04	chr6	33047185	33050741	−0.16	24	2.89E-05*	0.006
SPATC1L	chr21	47604052	47605174	0.36	10	6.11E-05	chr21	47604052	47605174	0.18	10	0.001	0.24

Abbreviations: nCpGs, number of CpGs in the DMR; FWER, family-wise error rate; pperm, permutation p-value.