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Post-traumatic stress disorder (PTSD) is a common and debilitating psychiatric disorder that may occur in individuals exposed to traumatic events such as accidents, interpersonal violence, war, combat, or natural disasters. Additionally, PTSD has been implicated in the development of a variety of chronic conditions including cardiovascular and metabolic diseases, suggesting that the biological alterations associated with the disorder can manifest themselves as chronic diseases in those suffering from PTSD. The biological underpinnings of the disorder are not well understood. Gene expression studies can illuminate the complex physiology of PTSD reflecting the embodiment of trauma, i.e., the process in which traumatic experiences in our social environments could potentially be manifested in our body by genomic mechanisms. To date, gene expression studies that examine the whole transcriptome are scarce and limited to single-timepoint assessments. Here we applied a transcriptome-wide gene expression screen with RNA-sequencing to whole blood samples from predominantly African-American participants in a community-dwelling setting to elucidate the gene expression signatures associated with the development of PTSD. The study participants (N=72, of whom 21 eventually developed PTSD) are a trauma-exposed subsample of participants enrolled in a longitudinal and prospective cohort study of adults living in Detroit, Michigan. PTSD was assessed in a structured telephone interview and whole blood samples were taken both before and after trauma exposure. We found 45 differentially expressed genes associated with PTSD development with an estimated log2 fold change > 1.5 at a nominal p-value (pPAX6, TSPAN7, PXDN, VWC2, SULF1, and NFATC4 were also ubiquitously expressed in all brain regions. Longitudinal sampling provides a promising mean to elucidate the pathophysiology underlying the embodiment of trauma.

Post-traumatic stress disorder (PTSD) is a pervasive and debilitating mental disorder with a lifetime prevalence of approximately 6.8–7.3% in the United States general population (Roberts et al., 2011; Kessler & Wang, 2008), while a higher rate on the order of 25–30% has been reported in segments of the population exposed to persistent psychological stress including combat exposed veterans, victims of assault or refugees (Breslau et al., 1998; Kessler & Wang, 2005; Roberts et al., 2011; McLaughlin et al., 2015; Fulton et al.,2015; National Institute of Mental Health, 2017). The disorder manifests itself through a range of symptoms including the recurrent and intrusive recollection of the traumatic experience, changes in arousal and reactivity, alterations in mood and cognition, avoidance of stimuli associated with traumatic event, and impairment of functioning in social and occupational contexts (American Psychiatric Association, 2013).

A range of risk factors for PTSD has been previously identified including the level of previous stress exposures, low socio-economic status, extent of social support and female sex (Yehuda & LeDoux, 2007; Brewin et al.,2000; Lowe et al., 2014; Kornfield et al., 2018). The exposure to traumatic events is the etiological component of PTSD, and exposure to such traumatic events is ubiquitous in societies around the world, although it is not deterministic as not everyone experiencing trauma develops PTSD (Resnick et al., 1993; Kessler et al. 1995, Mills et al., 2011; Kessler et al.,2017). In fact, only a small proportion of individuals exposed to traumatic events develop PTSD while the majority remains resilient sometimes even after repeated exposure (Zohar et al., 2009). Recent assessments of the World health organization's world mental health surveys spanning over 24 countries with 68,894 respondents suggest 70.4 % have experienced at least one lifetime trauma with the most frequently experienced types of traumas in the United States including interpersonal violence and sexual violence in women, while accidents or combat exposures are the most prevalent exposures in men (Benjet et al., 2016). On a global scale, natural disasters, wars, and other humanitarian emergencies account for the majority of traumatic experiences (Kessler et al., 2017).

PTSD can have a significant impact on an individual's quality of life caused by the condition itself or other medical comorbidities associated with the disorder including chronic inflammation (Speer et al., 2018), cardiovascular disease (Edmondson and Känel, 2017; Wilson et al., 2019), metabolic diseases (Talbot et al., 2015) and syndromes (Solomon et al., 2017 and Heppner et al., 2009), substance abuse and suicide (Kessler et al., 2005). Several of these

comorbidities can be mediated by a range of poor health behaviors often observed in individuals with PTSD including reduced engagement in physical activity, unhealthy diets, as well as increased tobacco and alcohol use (van den Berk-Clark et al., 2018; Wilson et al., 2019)—known factors contributing to poor metabolic and cardiovascular outcomes (Yusuf et al., 2020). The systematic implications of PTSD, negatively impacting overall health and well-being, stimulate the question of to what extent the disorder could be reflective of the embodiment of adverse or traumatic experiences. The concept of embodiment emphasizes the reciprocal nature between the mind and our body and how that relationship can shape human development, self-identity, behavior, perception, and cognition (Gibbs, 2005; Marmeleira & Santos et al., 2019). From an epidemiological perspective, embodiment can be characterized as a process in which we, as living organisms, biologically incorporate our social and ecological environment (Krieger 2005). This process is highly relevant to PTSD as many of the socio-demographic and social risk factors implicated in the disorder, such as low socio-economic status, lack of social support, and living in unsafe environments, can set the stage for frequent adverse and potentially traumatic experiences (Alegria et al., 2013) that in concert with interindividual biological variation, might jointly confer differential risk of PTSD development among individuals.

The significant individual differences in response to trauma and subsequent PTSD development might also partially be influenced by the heterogeneity at the molecular level that can impact someone's response to trauma. Consequently, identifying the molecular underpinnings influencing psychiatric risk and resilience factors in PTSD, and the mechanisms of how those molecular differences interact with psychosocial factors, has emerged as a priority in the research of the disorder. An enhanced understanding of the complex mechanisms potentially underlying a maladaptive trauma response and the subsequent development of PTSD could help to mitigate the vast morbidity and burden associated with the disorder and might also inform efforts of treatment development, interventions, and preventive strategies. Similarly, exploring the potential mechanisms of how lived traumatic experiences are embodied into our physiology and therefore, how trauma gets under the skin, can grant insights into how social adversity can give rise to psychopathology. Over the last decade, significant progress has been made in characterizing potential etiologic factors of PTSD. A large body of literature spanning across twin studies, targeted candidate gene analysis, genome, and epigenome-wide association studies, as well as methylation and gene expression studies have implicated the role of genetics

and epigenetics in the vulnerability to the development of PTSD (Logue et al., 2013; Mehta et al., 2013; Sarapas et al., 2011; Xie et al., 2013). Several risk loci associated with PTSD have been identified (Nievergelt et al., 2019; Smith et al. 2020), however, discrete diagnostic biomarkers that perhaps contribute etiology of PTSD remain to be elucidated.

Altered gene expression has been identified as an important genomic factor involved in the dysregulation of pathways involved in PTSD (Mehta et al., 2020). The assessment of gene expression can provide valuable insights into the complex pathophysiology of psychiatric disorders like PTSD. However, the majority of the few RNA-seq studies to date have been cross-sectional efforts, leaving it enigmatic whether identified transcriptional patterns precede PTSD onset, shape risk or resilience, or are merely a consequence of the disorder (Bam et al., 2016; 2015; Kuan et al., 2017; Boscarino et al., 2019; Pattinson et al., 2020). One notable exception is a study conducted by Breen and colleagues, who investigated transcriptome-wide gene expression by RNA-sequencing in the peripheral blood of 94 male U.S Marines of predominantly European ancestry pre- and post-deployment, constructing co-expression networks associated with PTSD (Breen et al., 2015). Their analysis identified modules with overexpression of genes that were enriched for processes involved with innate immunity and interferon signaling to be associated with PTSD development (Breen et al., 2015). Such longitudinal efforts can provide valuable insights into gene expression patterns associated with the development of PTSD and dissect cause and effect. Additionally, elucidating the transcriptomic underpinnings associated with PTSD development could perhaps inform future peripheral biomarkers with clinical utility or potentially reflect how traumatic experiences can be embodied on the molecular level.

To our knowledge, prospective longitudinal designs as utilized by Breen and colleagues have yet to be applied to a community-based cohort of individuals developing PTSD. The prospective transcriptomic profiling of individuals who developed PTSD following other types of traumatic events besides trauma associated with military deployment, e.g., assaultive violence experienced in the community, could reveal novel transcriptional alterations associated with PTSD development. To address this gap in knowledge, the present study aims to characterize the gene expression changes associated with the development of PTSD in trauma-exposed male and female participants in a community-dwelling, predominantly African American cohort. To this end, we explore the transcriptomic profiles in whole blood samples of trauma-exposed

individuals at baseline and a follow-up timepoint.

Materials and Methods

Study Participants and Blood Sampling

The study participants were enrolled in a five-year-long study called the Detroit Neighborhood Health Study (DNHS). The DNHS is a prospective and longitudinal cohort study of adults residing in Detroit, Michigan (Uddin et al., 2010). The initial aim of the study was to evaluate to what extent ecologic stressors including concentrated disadvantage, distribution of income, and residential segregation contribute to PTSD risk. Data collection for the DNHS began in 2008 with each wave of measurement including a phone interview over 40 minutes. The participants were compensated for their participation with $25 after each wave. Biospecimen collection was compensated with an additional $25. Data collection for the baseline wave occurred from 2009–2010 with approximately annual follow-ups until 2014. At the beginning of each interview and when a biological specimen was collected informed consent was obtained. The study protocol and the DNHS have been reviewed and approved by the Institutional Review Board of the University of Michigan and the University of North Carolina-Chapel Hill. A subset of DNHS participants consented to provide a biospecimen. The biospecimens were acquired from the participants during an in-home visit by a phlebotomist. The samples were subsequently shipped and processed at Wayne State University in Michigan (Uddin et al., 2010). From this biospecimen subset 72 trauma-exposed participants, out of which 21 developed PTSD in the follow-up wave, were identified along with 144 available whole blood samples taken both before and after trauma exposure (e.g. from wave 2 and wave 4, respectively). Of the 72 study participants, 70 experienced at least one new trauma between the baseline and the follow-up measurement. The two participants who were not exposed to a new trauma between measurements were among 69 participants who experienced at least one trauma prior to the baseline measurement, such that all 72 participants were trauma-exposed by the follow-up wave (wave 4). Total RNA was isolated from the leukocytes of the blood samples with Leukolock kits (Ambion, Austin, TX), as previously described (Logue et al., 2013).

Assessment of PTSD and Demographics

PTSD symptoms were assessed using a modified version of the PTSD Checklist (PCL-C) via a structured telephone interview (Weathers & Ford, 1996) validated against the CAPS (Uddin et al., 2010). The PCL-C is a 17-item measurement of PTSD symptoms that uses the PTSD symptom criteria from the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) (American Psychological Association [APA], 1994). The telephone interview also included a range of additional questions regarding the duration, timing, and impairment of the symptoms to identify probable cases of PTSD according to DSM-IV criteria. Additionally, a list of specific traumatic events was presented from which the participants were asked which of them were experienced in the past (Breslau et al., 1998). If all six DSM-IV criteria were met by participants at some point in their lifetime, they were considered affected by lifetime PTSD. In addition to our binary lifetime PTSD variable, a continuous variable based on post-traumatic stress (PTS) symptom severity scores was explored as a measure of traumatic stress that could potentially identify gene expression signatures in relation to PTSD development in individuals who have experienced PTS, but not to an extent consistent with a PTSD diagnosis according to all five DSM-IV criteria. Lastly, demographic variables including race, sex, and age were collected.

RNA-Sequencing and Data Preprocessing

RNA-sequencing (RNA-seq) was performed on all samples (n=144). For a detailed description of RNA isolation, library preparation, RNA-seq, quality control checks, and bioinformatics workflow refer to the RNA-seq section and Figure S1 in the supplementary methods. Following the trimming of sequencing adaptors, the reads were aligned to the reference genome. After alignment of the reads, gene expression on the gene level was quantified with the use of the *Featurecounts* function of the R-package *Subreads*, version 2.0.0 (Liao, Smyth & Shi, 2019). The resulting read count data was filtered by the number of counts and low read counts were subjected to removal prior to downstream analysis. Genes were filtered based on counts-per-million (CPM) with the use of the R-package *edgeR*, version 3.15 (Robinson, McCarthy & Smyth, 2010), retaining genes if they are expressed at CPM above 0.5 in our samples. After the filtering *edgeR* was used to calculate the normalization factors used to scale between the libraries (Robinson, McCarthy & Smyth, 2010).

Blood Cell-Type Deconvolution and Transformation of Cell Proportions

Differences in cell type proportions and composition, as well as their contributions to gene expression profiles, are important determinants of phenotypic variation capable of influencing disease states (Sharma et al., 2019). The identification of discrete cell populations is also important for the analysis of differential gene expression, as it can be substantially confounded by differences in the cell proportions in each sample. To address this issue, the deconvolution of various blood cell types was performed with *Cibersortx*, a computational framework to infer different cell types and cell type-specific expression profiles in bulk RNA samples (Newman et al., 2019). For a detailed overview of blood cell type deconvolution see supplementary methods.

Differential Gene Expression Analysis

In order to gain insights into the pathophysiology associated with PTSD development, differential gene expression analysis was performed with the differential expression for repeated measures (dream) function available in the variancePartition R package (Hoffman and Schadt, 2020), adjusting for disease status "PTSD", Sex, age, time point (wave) as well as a range of cell proportions commonly considered in transcriptomic studies of whole blood samples from individuals with mental disorders including B cells, CD4T cells, CD8T cells, and monocytes (see supplementary methods, Figure S3.1–3.3 for additional information on models).

We first analyzed cross-sectional differences in gene expression of the PTSD-affected group of participants vs those who are unaffected by PTSD using the 1) binary variable lifetime PTSD. This analysis can yield insights into the transcriptomic differences between individuals affected by PTSD vs those who are not, after the two time points. Here, the gene expression differences between cases and controls were tested first with and then without the inclusion of the time point covariate to evaluate the potential contribution of the time point to the observed variation in differential expression. Additionally, differential gene expression was assessed cross-sectionally using the 2) continuous PTS symptom severity variable.

In our main model of interest, differential gene expression between participants who developed PTSD and those who did not was assessed in a 3) longitudinal analysis. The purpose of the longitudinal analysis was to identify differentially expressed transcriptomic signatures

associated with the development of PTSD. In order to assess whether there are genes associated with the development of PTSD a time point-by-disease interaction was explored.

Differential expression in our data was evaluated at a nominally significant p-value < 0.05 with a fold change of 1.5 or greater and at an adjusted p-value < 0.05 using the Benjamini-Hochberg false discovery rate estimation (Hochberg and Benjamini, 1990).

Gene Set Enrichment Analysis (GSEA)

To gain insights into the biological processes associated with PTSD development a gene set enrichment analysis (GSEA) was performed. This process identifies classes of genes that are over-represented in our gene set of differentially expressed genes that are associated with PTSD and provides contextual information on the different pathways and functions of our genes. The R package 'clusterProfiler' was used to classify our genes into biological terms and to perform enrichment analysis of gene clusters. We have applied the commonly used Gene Ontology (GO) (Ashburner et al., 2000) that provides annotations of genes to a range of biological processes, molecular functions, and different cell components. Differentially expressed signatures at a nominally significant p-value < 0.05 were tested for enrichment.

Investigation of Differentially Expressed Signatures in the Human Brain

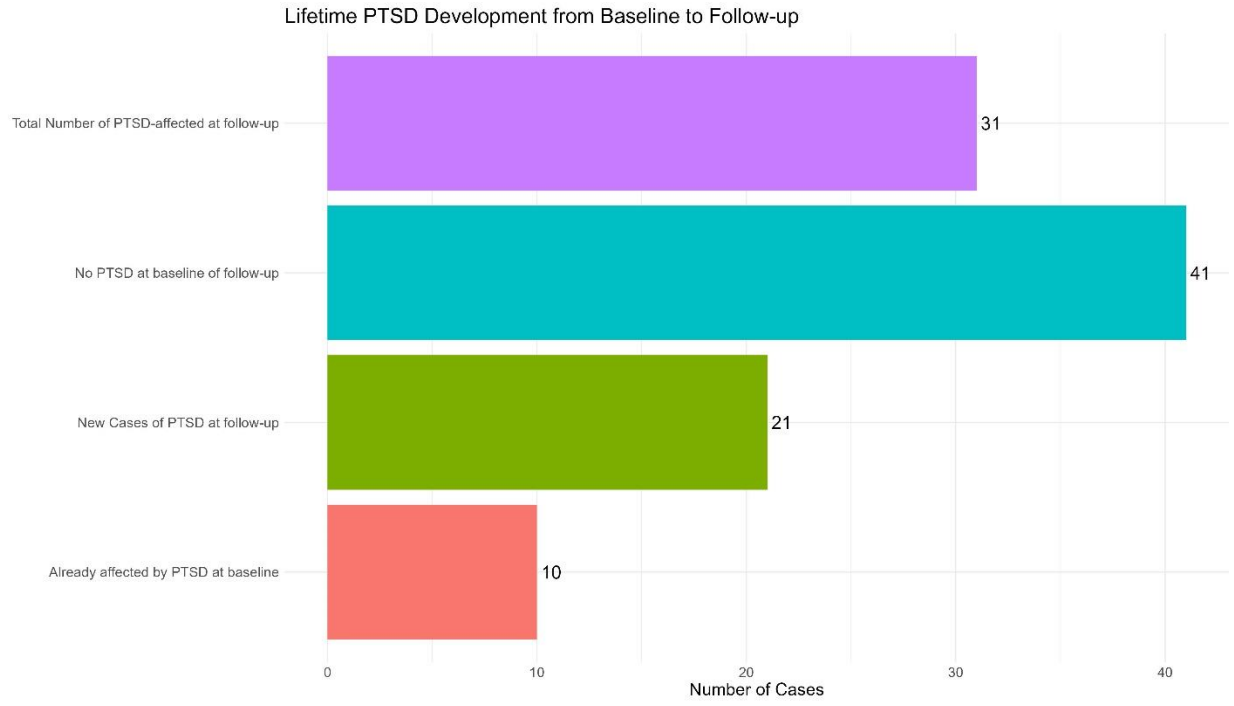
Many complex diseases involve the dysfunction of multiple tissues and organs with tissue-specific transcriptional changes mediating causal links between the genotype and disease (Cookson et al., 2009). Capturing tissue-specific gene expression patterns is an important consideration in the realm of psychiatric disorders given that the organ central to such conditions–the brain–is not directly assessable in living individuals. Characterizing the convergence between differentially expressed genes identified in peripheral blood samples and those expressed in the brain can help to evaluate how reflective blood transcriptomic profiles are of the disorders of the brain. To this end, we verified whether the differentially expressed genes found in blood are also expressed in the human brain by searching the publicly available Human Protein Atlas (HPA) database (http://www.proteinatlas.org/).

Results

Study participants

Descriptive statistics for demographic and psychosocial measures of the study participants are shown in Table 1 and illustrated in Figures 1a and 1b.

{~?~IM: insert Table 1 here.}



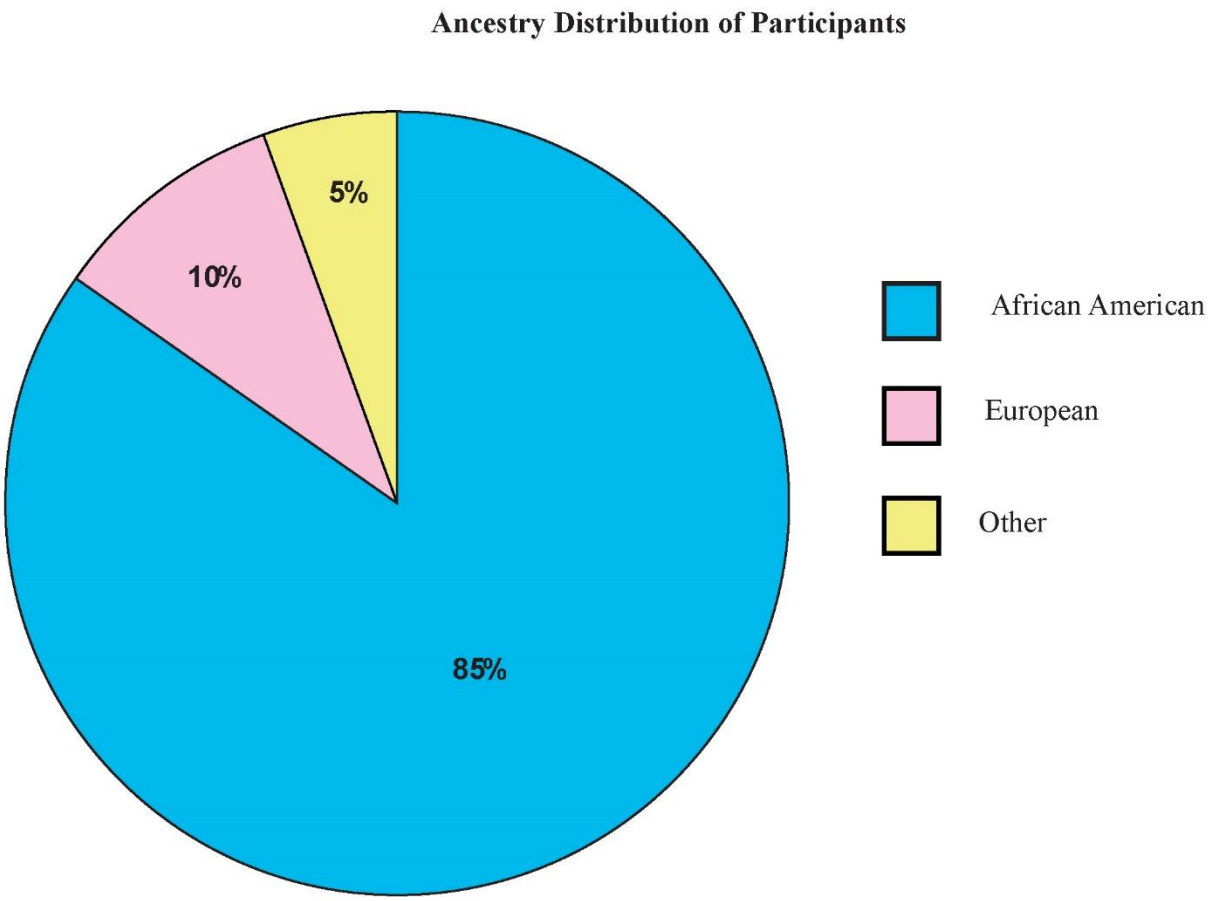


Figure 1.a. Breakdown of the number of participants with PTSD. Bar chart showing number of the 72 trauma-exposed participants and PTSD development. 10 participants already had PTSD at the baseline measure, while a total of 31 participants were affected by PTSD at the follow-up. 21 participants who did not have PTSD at the baseline measure developed PTSD by the follow-up of the study. 41 Participants did not have PTSD at the baseline measure and did not develop PTSD. **Figure 1.b.** Ancestry information of the study participants. The majority of participants (85%) were of African American ancestry, an understudied population in transcriptomic studies of PTSD.

Outcome of RNA processing, gene mapping, and gene assignment

From the original RNAseq experiment, a total of 320,197,33 reads were obtained mapping to 58,884 unique transcripts. 75% of obtained reads were successfully mapped to the GRCh38 reference genome. 87% of reads were assigned to a total of 26,904 individual genes after mapping with the use of the *FeatureCounts* function of the R-package *Subreads* (Liao, Smyth & Shi, 2019).

Estimation of the Blood Cell Type Proportions

Using quality-controlled data, Cibersortx was used to determine the cell-type composition of the heterogenous blood samples including the baseline (Figure S3) and the follow-up (Figure S4) transcriptomic measures. For both time points, the quantified proportions included B cells, CD4T, CD8T, monocytes, and NK cells for each participant. Natural killer T cell estimates were provided in the Cibersortx output but not included in the exploration of covariates among the cell proportions because they were only represented in less than 4% of the samples.

Differential Gene Expression

To gain insight into the gene expression signatures associated with PTSD development in our community-based cohort, a differential gene expression analysis was performed testing multiple coefficients of interest. Retention of power and the controlling of the false positivity rate were ensured by accounting for the repeated measures of our PTSD cases and controls with the *dream* function of the *variancePartition* R package (Hoffman et al., 2020), which enables the specification of random effects. In the dream analysis, the effects of the individual on the gene expression are modeled as a random effect for each gene with the use of a linear mixed model. Differential gene expression analyses were performed to investigate the cross-sectional transcriptomic differences between participants affected by PTSD vs those who are not, using 1) a binary PTSD variable, and subsequently, 2) a continuous PTS symptom severity measure. Additionally, differential gene expression between participants who developed PTSD and those who did not was assessed in a 3) longitudinal analysis. Correction for multiple testing was performed by applying the Benjamini and Hochberg method (Hochberg and Benjamini, 1990).

Cross-sectional analysis examining gene expression profiles of individuals who developed PTSD vs those who did not develop PTSD according to the 1) binary lifetime PTSD variable, did not identify any differentially expressed genes with a $\log^2$fold change greater than 1.5 at a nominal p-value < 0.05. Similarly, investigating differential gene expression using the 2) continuous variable PCL score measuring PTS symptom severity, did not identify any differentially expressed genes.

In contrast, our main model, 3) investigating differentially expressed genes associated with PTSD development, identified 45 differentially expressed genes with a log2fold change

greater than 1.5 at an unadjusted p-value < 0.05. The volcano plot (Figure 2) shows the global gene expression patterns according to PTSD development, indicating a larger number of upregulated genes compared to a few downregulated genes associated with PTSD development. In total, 45 genes were identified as differentially expressed at a nominal p-value < 0.05 in relation to PTSD development. However, none of the differentially expressed genes survived multiple test corrections at an adjusted p-value < 0.05.

Figure 2. Volcano plot displaying differentially expressed genes in relation to Lifetime PTSD Development. Y-axis shows the significance of differentially expressed genes in log10pavlues, X-axis shows the magnitude of the differential expression in log_2 fold change. The horizontal dashed line represents the threshold for nominal statistical significance (p-value < 0.05) while the vertical dashed lines represent the threshold for biologically significant changes in gene expression (log_2 fold change > 1.5), with upregulated genes on the right and down-regulated genes to the left. Significantly upregulated and downregulated genes are indicated in green.

{~?~IM: insert Table 2 here.}

There was a total of 37 upregulated genes associated with PTSD development at a nominal p-value < 0.05 and log_2 (fold change) above 1.5 (Table 2). Among the top differentially expressed genes with the highest fold change were *AL136317.2*, *AC013269.1*, *IGHV3–64D*, *AF130359.1*, *AL591441.1*, *TSPAN7*, *SNX18P13*, *PAX6*, *AC008543.5*, *Z69706.1* and *PXDN*. Furthermore, eight genes including *AC012501.1*, *LINC02211*, *UGT2A3*, *ACTP1*, *LINC02228*, *RPL35AP19*, *HSPB7,* and *FMOD* were identified as downregulated according to PTSD development (Table 3).

{~?~IM: insert Table 3 here.}

Gene Set Enrichment Analysis

To identify the biological processes associated with PTSD development, a gene set enrichment analysis was performed. Investigating the pathway enrichment for the 37 upregulated and eight downregulated differential expression signatures at the nominal significant p-value < 0.05 we found some gene sets to be significantly enriched. The top gene ontology terms associated with PTSD development (Figure 3) were found to be involved in cellular organization and functioning.

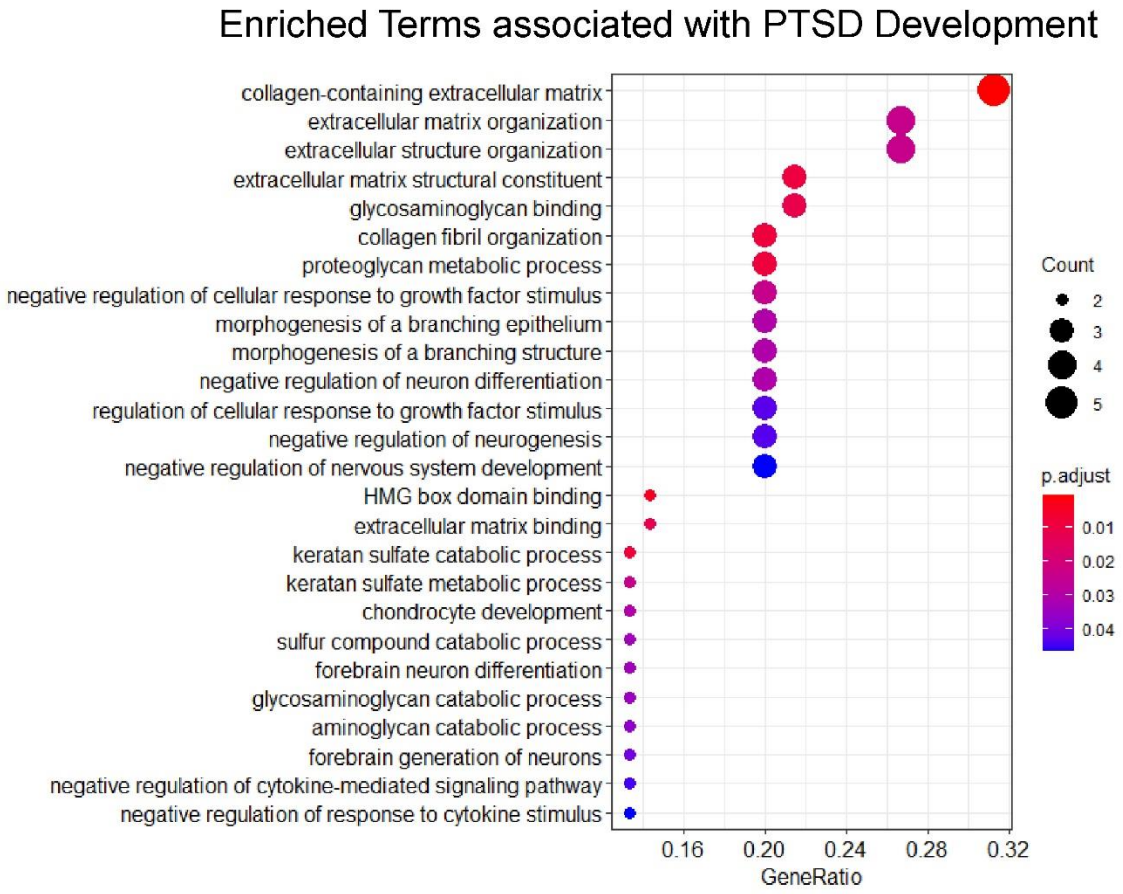


Figure 3. Top gene ontology terms associated with PTSD development. Dot plot showing the enrichment of pathways of the differentially expressed genes associated with PTSD development. Enrichment scores are depicted as colors corresponding to the adjusted p-values on the right. The annotation to the left on the Y-axis shows the name of the process or pathway in which enriched genes are involved. X-axis shows ratios of identified enriched genes that are annotated to a specific process or pathway. The top ontology terms associated with PTSD

development were involved in cellular organization and functioning.

Confirmation of blood-based differentially expressed signatures in the human brain

We leveraged the protein atlas database to assess whether our differentially expressed genes identified in the blood are also expressed in the brain, a key organ of interest in PTSD that cannot ordinarily be accessed in living individuals. Several of our differentially expressed genes identified in the blood are also expressed in the brain (Table 3). With regard to the upregulated genes, *TSPAN7, PAX6, PXDN, VWC2, SULF1, PAX3,* and *NFATC4* were also expressed in the brain. Among the downregulated genes, *UGT2A3*, *ACTP1*, *HSPB7,* and *FMOD* were also found to be expressed in the brain.

Discussion

The current study analyzed transcriptome-wide gene expression data in 72 trauma-exposed community-dwelling individuals (males and females) from the Detroit Neighborhood Health Study over a two-year period. Throughout the course of the study, 21 participants developed PTSD from the baseline to the follow-up time point. We identified 45 differentially expressed genes associated with PTSD development with an estimated $\log_2$fold change > 1.5 at a nominal p-value; however, none of these withstood correction for multiple hypothesis testing. Gene set enrichment analysis of the differentially expressed genes implicated several pathways in PTSD development. The top enriched pathways included brain functions such as forebrain neuron differentiation and the regulation of neurogenesis. Two of the top enriched pathways were involved with the negative regulation of cytokine-mediated signaling and the negative regulation of the response to cytokine stimulus, immune-related pathways that have been implicated in PTSD in previous studies (Breen et al., 2015; Bam et al., 2016; Kuan et al., 2020). PTSD has been consistently linked to increased inflammation in the body, which is in line with the findings of some of our immune-related enriched pathways (Michopolous et al., 2017; Rosen et al., 2017). Although there was no direct overlap of the identified differentially expressed genes in our community-dwelling cohort and the U.S marine cohort investigated by Breen and colleagues, our results also suggest the dysregulation of genes and pathways involved with immune functioning to be implicated in PTSD.

To assess the extent to which the in blood-identified differentially expressed genes are also expressed in the brain, we searched the Human Protein Atlas (HPA) database (http://www.proteinatlas.org/). Regarding the upregulated genes, *TSPAN7, PAX6, PXDN, VWC2, SULF1, PAX3,* and *NFATC4* were also expressed in various regions of the brain. While *TSPAN7, PAX6,* and *PXDN* were ubiquitously expressed in all regions of the brain, *PAX3* and *NFATC4* showed higher expression in the cerebellum and the white matter of the brain. *VWC2* on the other hand, has its highest expression in the cerebellum and only low expression in other areas of the brain. Out of the downregulated genes, *HSPB7* and *FMOD* were also found to be expressed in the brain. While *HSPB7* showed its highest expression in the medulla oblongata, pons, and cerebellum, *FMOD* appeared to have more expression in the cerebral cortex. *PAX6* is an important transcriptional factor for normal brain development and is involved in the regulation of the expression of several other genes coordinating cortical development (Osumi et al., 2008; Ypsilanti & Rubenstein, 2016). *TSPAN7* has been found to be differentially expressed in a previous microarray study of peripheral blood investigating the gene expression profiles of military personnel who sustained closed-head injuries from explosions during deployment (Heinzelmann et al., 2014). *TSPAN7* has also been associated with cognitive defects mediated by downstream alterations to the *TM4SF2* gene (Penzes et al., 2013). *VWC2* has been previously found to be differentially expressed (downregulated) in individuals with PTSD and mild cognitive impairment in a targeted study of World Trade Center responders (Kuan et al., 2020). *NFATC4* is among a family of five distinct nuclear factors of activated T cells genes regulating the transcriptional induction of several genes involved with immune activators and modulators including a range of adaptive immunity and pro-inflammatory cytokines (Kipanyula, Kimaro & Etet, 2016). Dysregulation of other *NFATC* loci has previously been implicated in PTSD in peripheral tissues (Breen et al. 2019; Ocean et al, 2022).

It is important to acknowledge several limitations when interpreting our findings. First, our modest sample size of 72 participants, of which 21 developed PTSD, is a major challenge in identifying differentially expressed genes. Smaller sample sizes have diminished statistical power and are unlikely to detect smaller changes in gene expression. Additionally, the difficulty to distinguish differential gene expression signals from noise, potentially mediated biological heterogeneity, or individual genomic differences, is compounded in smaller studies. RNA-seq studies employ rigorous adjustments for multiple testing often resulting in a more conservative

threshold for statistical significance, limiting the potential to identify differentially expressed genes. Thus, our only nominally significant findings did not come as a surprise. Nevertheless, even transcriptomic studies with limited sample sizes can still be valuable contributions. Small sample sizes are not uncommon in studies of minoritized communities, which are typically underrepresented in genomic studies (Sirugo et al. 2019), highlighting the need for more gene expression studies of individuals from such communities. Smaller efforts could be pooled together in meta-analysis efforts that leverage larger sample sizes to identify transcriptomic signatures underlying or accompanying the development of PTSD. Further, the implicated genes associated with PTSD development could be evaluated in future genome-wide association studies or be supplemented using multi-omics efforts including DNAm profiling.

Despite these limitations, our study has several strengths that could inform future efforts to understand PTSD. The longitudinal sampling of participants prior to and after the development of PTSD can be valuable in identifying expression signatures underlying PTSD development, as cross-sectional designs fail to inform whether the observed transcriptional alterations give rise to PTSD, or whether they are a consequence of the disease. Longitudinal efforts characterizing gene expression changes associated with the development of the disorder are scarce, with only one study of which we are aware of to date employing such a prospective design to a cohort of combat-exposed male veterans (Breen et al., 2015). Further, the community-based cohort of our study includes male and female participants of mainly African American ancestry whereas the majority of existing RNAseq studies profile gene expression in combat-exposed veterans, active-duty military, or first responders of European descent.

Importantly, the prospective profiling of gene expression may inform the broader question of how traumatic experiences become embodied on a molecular level, and perhaps how such manifestations contribute to the etiology of PTSD. Those longitudinal efforts may be particularly informative if paired with other genomic data such as DNA methylation as well as other psychosocial variables, to gain more comprehensive insights into how biological mechanisms and social factors jointly impact the conditional risk for PTSD following trauma exposure. Another important consideration regarding the embodiment of trauma is that different traumatic events may confer varying levels of conditional risk in relation to PTSD development (Kessler et al., 2017; Liu et al., 2017). Assaultive violence for example, has been identified to be of the highest conditional risk among different exposures (Breslau et al., 1998, Goldman et al.,

2011), with combat exposure and rape accounting for the highest risk in that category in men and women, respectively (Kessler et al., 2017). Interestingly, Breen and colleagues (2018) observed not only converging dysregulation of gene expression among individuals with PTSD who have experienced different modes of trauma but also that distinct transcriptional alterations define different types of traumas (Breen et al., 2018).

In our cohort of predominantly African American participants, the most frequently experienced traumas were related to exposures within the community including experience of the sudden and unexpected death of a loved one, assaultive violence or life-thretening illness whereas most existing studies have profiled PTSD gene expression profiles in combat-exposed veterans. Similarly, assessing the gene expression of individuals experiencing traumatic events in their communities may yield gene expression patterns that are distinct from those who experienced trauma during military deployment. Huckins and colleagues utilized transcriptomic imputations of brain and peripheral tissue from 29,539 PTSD-affected individuals and 166,145 controls identifying two cohort-specific genes predicted to be differentially expressed genes (Huckins et al., 2020). Their findings suggest trauma exposures experienced in military contexts could bring distinct transcriptomic signatures in comparison to traumatic events experienced in a community setting. Such identification of distinct transcriptomic profiles may also inform, or perhaps supplement, future PTSD diagnostic criteria as the *Diagnostic and Statistical Manual of Mental Disorders* does not adequately capture many psychosocial exposures (e.g., concentrated disadvantage and other social inequalities) known to influence the risk and dysfunction brought by the disorder (Harnett et al., 2022).

In summary, we applied prospective design to profile gene expression in a community-based cohort of trauma-exposed, predominantly African American individuals who developed PTSD. We identified 45 differentially expressed genes nominally associated with PTSD development. The identified dysregulation of immune-related genes and the enrichment of pathways involved in immune functioning converge with findings from an early effort. We hope that future efforts could apply a similar prospective and longitudinal design and if possible, include a larger sample size to characterize PTSD to help elucidate the complex process of how traumatic experiences are embodied into our physiology and ultimately reflected in psychopathology.

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Supplemental materials can be found at [URL TK]

Ethics Statement

The study protocol and the DNHS have been reviewed and approved by the Institutional Review Boards of the University of Michigan and the University of North Carolina-Chapel Hill. The participants provided their written informed consent to participate in this study.

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Table 1. Participant Characteristics of PTSD and Control Samples

	All participants (N = 72)		New Trauma exposure from Baseline to follow-up (N = 70)		PTSD-affected at Baseline (N = 10)		PTSD-affected at follow-up (N = 31)		New Case of PTSD (N = 21)		No PTSD by follow-up (N = 41)		PTS symptom score at Baseline ** (Min-Max:6.0–72.0)	PTS symptom score by follow-up** (Min-Max:17.0–73.0)
Characteristic	N	%	N	%	N	%	N	%	N	%	N	%		
Age*	56.66	12.78	56.27	12.55	56.98	12.80	56.49	12.94	54.38	13.91	56.97	12.10		
Female	39	54	38	54	6	60	20	64	14	67	19	46	31.27	33.25
Male	33	46	32	46	4	40	11	36	7	33	22	54	32.46	33.83
African American	61	85	60	86	9	90	24	77	15	71	37	90	32.46	33.83
European	7	10	7	10	1	10	5	16	4	19	2	5	31.16	31.13
Other	4	5	3	4	0	0	2	7	2	10	2	5	30.66	32.21

*Variables are presented by mean and standard deviation. **Variables are presented by mean.

Table 2. Upregulated Genes According to Lifetime PTSD Development

Upregulated at Nominal p-value < 0.05 in PTSD:wave (log fold change 1.5)

Ensemblid	Gene	LogFC	AveExpr	t	P.Value	Adj.P.Val	Z.std
ENSG00000274893	*AL136317.2*	2.069513	-3.80093	3.476319	0.00073	0.952575	3.37795
ENSG00000225341	*AC013269.1*	2.039292	-4.14782	3.665617	0.000393	0.952575	3.54454
ENSG00000282639	*IGHV3–64D*	1.929707	-3.0084	2.187479	0.031035	0.952575	2.156621
ENSG00000237735	*AF130359.1*	1.906208	-4.58473	3.175578	0.002008	0.952575	3.089076
ENSG00000276128	*AL591441.1*	1.844134	-4.40663	3.198495	0.00182	0.952575	3.118174
ENSG00000156298	*TSPAN7*	1.82917	-3.79308	3.170037	0.001889	0.952575	3.107136
ENSG00000230965	*SNX18P13*	1.816912	-2.16646	2.708481	0.007875	0.952575	2.657394
ENSG00000007372	*PAX6*	1.796315	-3.00469	4.301555	3.24E-05	0.877348	4.155555
ENSG00000267646	*AC008543.5*	1.782542	-3.37977	3.818061	0.000227	0.952575	3.686855
ENSG00000268836	*Z69706.1*	1.761723	-3.34508	3.357925	0.001023	0.952575	3.284242
ENSG00000130508	*PXDN*	1.754969	-1.95644	2.052409	0.042077	0.952575	2.032763
ENSG00000285079	*AL513493.1*	1.752864	-3.2731	3.208111	0.001672	0.952575	3.143115
ENSG00000184761	*PRSS40B*	1.702262	-3.44557	2.328637	0.021705	0.952575	2.295487
ENSG00000187135	*VSTM2B*	1.698038	-3.62639	2.540431	0.012212	0.952575	2.505954
ENSG00000229847	*EMX2OS*	1.681767	-1.7885	2.961896	0.003813	0.952575	2.893211
ENSG00000185736	*ADARB2*	1.676024	0.873709	2.288869	0.023651	0.952575	2.262747
ENSG00000261348	*LINC02136*	1.670426	-4.42673	2.06117	0.04176	0.952575	2.035902
ENSG00000170153	*RNF150*	1.639384	-1.0087	2.346386	0.020885	0.952575	2.310054

ENSG00000226676	*BX248123.1*	1.627546	-3.69832	3.487307	0.000746	0.952575	3.372131
ENSG00000235726	*AC010148.1*	1.597349	-1.52826	2.203848	0.029654	0.952575	2.174677
ENSG00000286099	*N/A*	1.592427	-2.53084	2.166485	0.032043	0.952575	2.143873
ENSG00000188730	*VWC2*	1.578	-4.36536	2.713234	0.007538	0.952575	2.672072
ENSG00000145147	*SLIT2*	1.571715	-0.69046	2.099633	0.03817	0.952575	2.07303
ENSG00000228314	*CYP4F29P*	1.563679	0.328895	2.503854	0.013714	0.952575	2.464657
ENSG00000233048	*LINC01722*	1.555737	-3.63935	2.646673	0.009102	0.952575	2.608181
ENSG00000137573	*SULF1*	1.553857	-4.42013	2.379621	0.019074	0.952575	2.344088
ENSG00000228971	*AL356479.1*	1.540334	-4.04196	2.219536	0.028132	0.952575	2.195446
ENSG00000228791	*THRB-AS1*	1.53563	-3.00011	2.878841	0.004867	0.952575	2.815727
ENSG00000225258	*AC009478.1*	1.529383	-4.83787	2.666998	0.008854	0.952575	2.617629
ENSG00000232006	*AC005537.1*	1.525101	-2.33867	2.087177	0.03932	0.952575	2.060819
ENSG00000276484	*AC126755.5*	1.524739	-3.30051	2.088198	0.038673	0.952575	2.067652
ENSG00000227766	*HCG4P5*	1.522675	-5.30751	2.497207	0.013944	0.952575	2.45871
ENSG00000060718	*COL11A1*	1.521923	-4.74859	2.361928	0.020029	0.952575	2.325796
ENSG00000135903	*PAX3*	1.521438	-4.30189	2.17104	0.032099	0.952575	2.143173
ENSG00000100968	*NFATC4*	1.519876	-3.85836	2.744275	0.006896	0.952575	2.701825
ENSG00000213614	*HEXA*	1.517953	-4.52251	3.040019	0.002968	0.952575	2.97099
ENSG00000168122	*ZNF355P*	1.515112	-4.04418	2.693571	0.008234	0.952575	2.642316

Table 3. Downregulated Genes According to Lifetime PTSD Development

Downregulated at Nominal p-value < 0.05 in PTSD:wave (log fold change 1.5)

Ensemblid	Gene	LogFC	AveExpr	t	P.Value	Adj.P.Val	Z.std
ENSG00000227400	*AC012501.1*	-2.33236	-2.16611	-3.41908	0.000845	0.952575	-3.33756
ENSG00000245662	*LINC02211*	-2.28962	-3.46875	-2.94785	0.00389	0.952575	-2.88695
ENSG00000135220	*UGT2A3*	-1.85419	-4.07665	-2.64266	0.009397	0.952575	-2.59725
ENSG00000229890	*ACTP1*	-1.84626	-4.4253	-2.22633	0.027948	0.952575	-2.19802
ENSG00000251273	*LINC02228*	-1.68067	-4.02054	-2.37971	0.019097	0.952575	-2.34364
ENSG00000239872	*RPL35AP19*	-1.64212	-4.78866	-3.41661	0.000874	0.952575	-3.32825
ENSG00000173641	*HSPB7*	-1.52756	-4.55159	-2.90137	0.004345	0.952575	-2.85199
ENSG00000122176	*FMOD*	-1.50034	-4.45216	-2.15001	0.033614	0.952575	-2.12468

Table 4 Differentially expressed Genes expressed in the Brain

Gene	Log_2fc	Expression in human Brain	Implicated in previous Studies of psychiatric disorders
TSPAN7	1.82917	All regions of the brain	Not implicated in psychiatric disorders
PAX6	1.796315	All regions of the brain	Important transcriptional factor for normal brain development and is involved in the regulation of the expression of several other genes coordinating cortical development (Osumi et al., 2008)
PXDN	1.754969	All regions of the brain	Differentially methylated CpG in PXDN associated with borderline personality disorder (Arranz et al., 2021)
VWC2	1.578	High expression cerebellum	Differentially expressed (downregulated) in individuals with PTSD (Kuan et al., 2020).
SULF1	1.553857	High expression in thalamus and midbrain	Differential methylation of SULF1 associated with depression during pregnancy (Viuff et al., 2018)
PAX3	1.521438	High expression in cerebellum and white matter	Not implicated in psychiatric disorders
NFATC4	1.519876	High expression in cerebellum and white matter	Dysregulation of other *NFATC* loci has previously been implicated in PTSD in peripheral tissues (Breen et al. 2019; Occean et al. 2022)

HSPB7	-1.52756	Higher expression in medulla oblongata, pons and cerebellum	Previously been associated with the development of Schizophrenia (Chaumette et al., 2019)
FMOD	-1.50034	Higher expression in cerebral cortex	Not implicated in psychiatric disorders