A. OVERALL COVER PAGE

Project Title: Longitudinal genome-wide transcriptome study of PTSD symptom change in WTC responders	
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Program Director/Principal Investigator Information: PEIFEN KUAN , PHD **Phone Number:** 6316321419 **Email:** peifen.kuan@stonybrook.edu	**Recipient Organization:** STATE UNIVERSITY NEW YORK STONY BROOK STATE UNIVERSITY NEW YORK STONY BROOK The Office of Sponsored Programs STONY BROOK, NY 117943362 **DUNS:** 804878247 **EIN:** 1141368361A1 **RECIPIENT ID:**
Change of Contact PD/PI: NA	
Administrative Official: SABRINA CEREZO W5510 Melville Library Stony Brook University Stony Brook, NY 117943362 **Phone number:** 6316329840 **Email:** sabrina.cerezo@stonybrook.edu	**Signing Official:** SABRINA CEREZO W5510 Melville Library Stony Brook University Stony Brook, NY 117943362 **Phone number:** 6316329840 **Email:** sabrina.cerezo@stonybrook.edu
Human Subjects: NA	**Vertebrate Animals:** NA
hESC: No	**Inventions/Patents:** No

B. OVERALL ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

The major goals/specific aims of this project are to:
1. Investigate the association between changes in gene expression and changes in PTSD symptoms across an 18-month period. Specifically, we will study: (1) the association between genome-wide changes of expression at gene, isoform and splice variant levels with change in PTSD symptoms, including allele specific expression; (2) genetic pathways and networks implicated in these changes, that will provide insight into biological processes relevant to change in PTSD symptoms; and (3) using machine learning approaches, identify the Gene Expression Signature (GES) associated with change in PTSD symptoms.
2. Evaluate whether the GES identified in Aim 1 is associated with change in each PTSD symptom dimension (re-experiencing, avoidance, numbing, hyperarousal). We expect to find both common and distinct genes/pathways regulating the change in each PTSD symptom dimension.
3. Test the directionality of prospective associations between the GES score and PTSD symptom severity, i.e., whether the GES at the first time point predicts PTSD severity 18 months later, and vice versa.
4. Evaluate whether change in GES identified in Aim 1 is associated with change in LRS. We hypothesize that any association observed between the GES and LRS will be mediated by change in PTSD symptom severity, indicating shared biological mechanisms underpinning PTSD-LRS comorbidity.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

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B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

For this reporting period, is there one or more Revision/Supplement associated with this award for which reporting is required?

No

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

NOTHING TO REPORT

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

NOTHING TO REPORT

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

Not Applicable

Final Progress Report

Title Page

PI: Dr. Pei Fen Kuan
Position/Title: Associate Professor
Organization Name: The Research Foundation for The State University of New York
Department: Applied Math and Statistics
Address: 106 Mathematics Building, Stony Brook University, Stony Brook, NY 11794-3600
Phone Number: 631-632-1419
Email: peifen.kuan@stonybrook.edu

Project Title: Longitudinal genome-wide transcriptome study of PTSD symptom change in WTC responders
Co-Investigators: Dr. Evelyn Bromet
Dr. Benjamin Luft
Dr. Monika Waszczuk
Project Director: Dr. James Yiin
Sponsor: CDC/NIOSH
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Project End Date: 06/30/2021 (NCE)
Date of Final Report Completion: Aug 27, 2021

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Abstract

The 9/11 World Trade Center terrorist attack was a massive disaster, resulting in long-term physical and psychological trauma to the responders, in particular PTSD and lower respiratory symptoms (LRS), with 10-20% of responders experiencing symptoms consistent with the diagnosis of PTSD a decade later. Our group found that PTSD in WTC responders is closely linked to their respiratory diseases, and PTSD mediated the relationship between WTC exposures and LRS. Genetic vulnerability and gene-environment interactions have been implicated in the etiology of PTSD. In this study, we aim to evaluate association between changes in gene expression (gene, isoform and alternative splicing (AS)) with changes in PTSD symptom severity, and PTSD symptom dimension (re-experiencing, avoidance, numbing, hyperarousal). We also aim to identify the genetic pathways implicated by these changes; and identify the Gene Expression Signature (GES) associated with change in PTSD symptoms. An in-depth understanding of the biological processes underpinning PTSD, and identification of its GES carries clinical significance by identifying processes that maintain PTSD and can be targeted for intervention, and will inform treatment development for WTC responders as well as other trauma-exposed populations. Our results indicated that avoidance differed the most from other dimensions with respect to DE genes and AS events. Gene set enrichment analysis (GSEA) identified pathways involved in inflammatory and metabolic processes, which may have implications in the treatment of PTSD. Overall, the findings shed a novel light on the wide range of transcriptomic alterations associated with PTSD at the gene and AS levels. The results of DE analysis associated with PTSD dimensions highlights the importance of studying PTSD symptom heterogeneity.

Section 1 of the Final Progress Report (2-page limit)

Significant or Key Findings

Aim 1. Investigate the association between changes in gene expression and changes in PTSD symptoms and identify the gene expression signature (GES) associated with change in PTSD symptoms.

Key Findings: This study identified both shared and specific differential expression patterns at the gene and alternative splicing (AS) levels associated with total PTSD and its dimensions. Genes involved in inflammation, as well as small nucleolar RNA genes were found to be associated with changes in PTSD symptoms. Among the differentially expressed (DE) genes, many showed a nonlinear association with changes in PTSD symptoms, suggesting that future research on gene expression and quantitative measurements of psychopathology (i.e., continuous) could potentially gain power by exploring nonlinear patterns. Analysis at individual time point indicated that the avoidance dimension differed the most from other dimensions with respect to DE genes and AS events. Skipping exons constituted the largest number of AS events, whereas the proportion of alternative first exons (AF) was higher among the DE AS events, and the proportion of skipping exons (SE) was lower as compared with transcriptome-wide proportions. Gene set enrichment analysis (GSEA) identified pathways involved in inflammatory and metabolic processes, which may have implications in the treatment of PTSD. DE analysis associated with each dimension offers complementary findings, emphasizing the importance of studying the homogeneous components of PTSD. The GES was constructed using the top 5000 DE genes associated PTSD symptoms as candidate features and achieved moderate association with changes in PTSD symptoms ($r = 0.547$).

Aim 2. Evaluate whether the GES identified in Aim 1 is associated with change in each PTSD symptom dimension.

Key Findings: The GES achieved highest strength of associations with re-experiencing ($r = 0.554$), followed by hyperarousal ($r = 0.520$), numbing ($r = 0.503$) and lowest with avoidance ($r = 0.302$).

Aim 3. Test the directionality of prospective associations between the GES score and PTSD symptom severity.

Key Findings: The GES trained at base line (T1) achieved moderately lower correlation in predicting PTSD symptom severity at follow-up (T2) ($r = 0.384$) and the strength of correlation was comparable to the correlation between GES trained at T2 and PTSD symptom severity at T1 ($r = 0.378$), i.e., supporting a bidirectional prediction between GES and PTSD symptoms.

Aim 4. Evaluate whether change in GES is associated with change in LRS.

Key Findings: The GES trained at base line (T1) achieved moderate association with LRS ($r = 0.425$, $\beta = 0.538$, $p < 0.001$). After including PTSD symptoms in the model, the association became insignificant ($\beta = 0.009$, $p = 0.945$), i.e., PTSD symptoms fully accounted for the association between the GES and LRS.

Translation of Findings

First, this study took a step forward toward identification of prospective biomarkers for PTSD as well as LRS, which revealed novel mechanisms that maintain PTSD and can be targeted for intervention. Second, the gene expression signature can aid in predicting exacerbations in PTSD symptoms, which may prove helpful for clinical prediction in the future. Furthermore, characterizing the transcriptomic patterns and other gene-regulated pathways underlying PTSD symptoms provided invaluable insights into the biological processes of PTSD, and as such can

help identify drug-discovery targets. Third, the dimension-specific gene expression pathways offered insights into future treatment development work by subdividing the PTSD cases into more homogeneous subgroups clinically and target biological processes specific to each subgroup. Finally, the study explicated the biological mechanisms underlying persistent PTSD-LRS comorbidity in WTC responders. This may inform development of treatments aimed at preventing the exacerbation of physical symptoms by intervening at the level of the etiological pathway. Such treatment would have a profound impact on the everyday lives of responders, as it could reduce the severity of the co-occurring social and occupational impairments intrinsic to PTSD.

Research Outcomes/Impact

The study considerably advanced basic science, clarifying the role of gene expression in PTSD, with no previous studies in the gene expression field investigating longitudinal associations, individual PTSD dimensions, and physical symptom comorbidities. Other important contributions included the potential clinical implications for screening and treatment of PTSD. Taken together, by integrating psychiatric epidemiology and gene expression, this study offers a cutting-edge translational approach to inform clinical practice and improve the health of WTC responders.

Potential outcomes from the findings of this study include:
(a) Increased awareness of OSH issues
Study objectives and approaches were presented as a poster at the at the Society of the Biological Psychiatry 2018 Annual Meeting, New York, NY

(b) Basic/Etiologic only: Other researchers use knowledge as conceptual basis for additional basic or applied research
Our publications were cited by epigenetics researchers in psychiatric disorders, thus we anticipate that the findings and gene expression signature developed in this study can be tailored to other psychiatric disorders and trauma exposed cohorts.

Section 2 of the Final Progress Report

Scientific Report

Aim 1. Investigate the association between changes in gene expression and changes in PTSD symptoms and identify the gene expression signature (GES) associated with change in PTSD symptoms.

PTSD assessment

PTSD symptom severity (total and four dimensions, namely re-experiencing, avoidance, numbing, and hyperarousal) was measured using the Post-traumatic Stress Disorder Checklist-Specific Version (PCL-17) (1), a 17-item self-report questionnaire modified to assess the severity of DSM-IV WTC-related PTSD symptoms over the past month on a scale of 1 (never bothered by) to 5 (extremely bothered by) (Cronbach's $\alpha = 0.96$). Our research team has previously validated the four-dimensional model in WTC responders and found it to be more informative than the alternatives (2, 3).

RNA-Seq data preprocessing

Method. The whole transcriptome libraries were prepared using KAPA HyperPrep Kit with RiboErase (HMR) kit (Roche Sequencing Solutions) and sequenced on Illumina NovaSeq 6000 sequencer at a sequencing depth of over 100 million paired end reads (100 bp) per sample. Raw reads that passed the quality filter from Illumina RTA were pre-processed using fastqc (4) for sequencing base quality control and cutadapt (5) to remove adapter sequences if applicable. Alignment was performed with the TopHat2 software (6, 7), utilizing Bowtie2 (http://bowtie-bio.sourceforge.net/bowtie2/index.shtml) in the RefSeq (NCBI Reference Sequence Database) annotation database (8) and the human reference genome (GrCh37-hg19 version). Other genomic-related data were obtained using the UCSC genome repository (9, 10). A second round of QC using RSeQC was applied to the mapped bam files to identify potential RNA-Seq library preparation problems. From the mapping results, the number of reads aligned to each gene was calculated using HTSeq (11). The raw count data were transformed into fragments per kilobase of transcript per million mapped reads (FPKM) to account for library size differences across the samples. Additional details are described in our publication (12).

Isoform-level and alternative splicing quantification

Method. Isoform-level quantification was performed with the Salmon software (13), whereas the SUPPA software (14) was used to quantify the different AS events, namely skipping exons, alternative 5'/3' splice sites, mutually exclusive exons, retained introns, and alternative first/last exons. Each AS event was represented as a PSI_{ori} value, defined as the ratio of abundance of transcripts that include the exon over the abundance of transcripts that include or skip the exon. $PSI = \log(PSI_{ori}/(1\text{-}PSI_{ori}))$ was used for AS quantification in our analysis. Genes and isoforms with low read counts were filtered out prior to statistical analysis.

Estimation of batch effects

Method. The potential batch effect was estimated using the surrogate variable analysis approach for sequencing data (svaseq) (15). Proportions of CD4T, CD8T, monocytes, natural killer (NK), B-cells, macrophage, dendritic, mast cells, eosinophils and neutrophils were estimated using the CIBERSORT software (16). The correlations between the estimated proportions of cell types and PCL were compared using Pearson correlation coefficients. The

estimated surrogate variables and proportion of cell types were included as covariates in the DE analysis.

Differential expression analysis

Method. Our main analysis was restricted to n = 226 male samples who have complete data (PTSD symptoms and the dimensions across both time points). Samples which were excluded from main analysis including female samples were included in sensitivity analysis, because < 10% of the samples were female and females showed notably different gene expression patterns from males (17). For each gene and isoform, we fitted construct a linear model on $\log_2[(\text{FPKM}_{T2}+1)/((\text{FPKM}_{T1}+1)]$ versus PCL_{T2}-PCL_{T1}, adjusting for age at T1, gender, race, batch effect, difference in cell type proportions and surrogate variables. To evaluate the potential nonlinear associations between gene expression and PTSD symptoms, we extended the linear model to spline model, where the degrees of freedom were estimated via generalized cross-validation. Statistically significant genes were identified at FDR < 0.05. Analyses were repeated for isoform and AS events. GSEA (18) was conducted on the entire list of genes ranked by negative log p-values. Both the gene ontology (GO) (19) and KEGG canonical pathway (20) gene sets were tested. The minimum and maximum gene set sizes were 15 and 500. FDR < 0.05 was used to identify statistically significant gene sets for each comparison.

Results. The distributions of the differences in PTSD symptoms and the dimensions (T2-T1) were shown in Figure 1, whereas the differences in the estimated cell proportions were shown in Figure 2. At FDR < 0.05, 25 genes were significantly associated with changes in PTSD symptoms (PCL diff) using the spline model, whereas no gene was detected using the linear model, indicating that most of the genes showed a nonlinear association with PCL diff. This result suggests that future research on gene expression and quantitative measurements of psychopathology (i.e., continuous) could potentially gain power by exploring nonlinear patterns. Examples of the top genes were provided in Figure 3. Both GTF3C6 and PANX1 are protein coding genes which were previously implicated in hippocampal gene expression of depressed patients (21) and prefrontal cortex of mice induced by chronic social defeat stress (22), respectively. PANX1 also mediates the inflammation processes (23). Additionally, 15 out of these 25 genes were small nucleolar RNA (small noncoding RNAs). This result is particularly interesting because emerging evidence suggested that noncoding RNAs may prove to be useful biomarkers to facilitate personalized medicines in PTSD and traumatic brain injury (24, 25).

Analysis at isoform level identified 41 isoforms to be significantly associated with PCL diff using the spline model, whereas no gene was detected using the linear model, indicating that most of the isoforms showed a nonlinear association with PCL diff. Among these 41 isoforms, 14 were small nucleolar RNA (small noncoding RNAs). GSEA identified 12 GO terms and 20 canonical pathways associated with PCL diff (Table 1). The significant terms include several neuronal related pathways such as substantia nigra, neural nucleus and nervous system development. These pathways may be important for PTSD, for example substantia nigra is a midbrain dopaminergic nucleus which has a critical role in modulating motor movement and reward functions as part of the basal ganglia circuitry (26) and chronic stress has been shown to reduce the activity of neurons in the substantia nigra (27). We also found gene sets associated with telomere regulation to be enriched among the GO terms. Telomeres consist of regions of repetitive nucleotide sequences at the end of chromosomes and have been shown to influence cell fate and aging by adjusting the cellular response to stress and growth stimulation (28). This finding motivated us to perform a related computational study by

investigating whether PTSD is associated with accelerated transcriptional aging, as described in Study A below.

Figure 1. Distribution of differences in PTSD symptoms (PCL diff), re-experiencing (PCL_R diff), avoidance (PCL_A diff), numbing (PCL_N diff) and hyperarousal (PCL_H diff) between T2 and T1

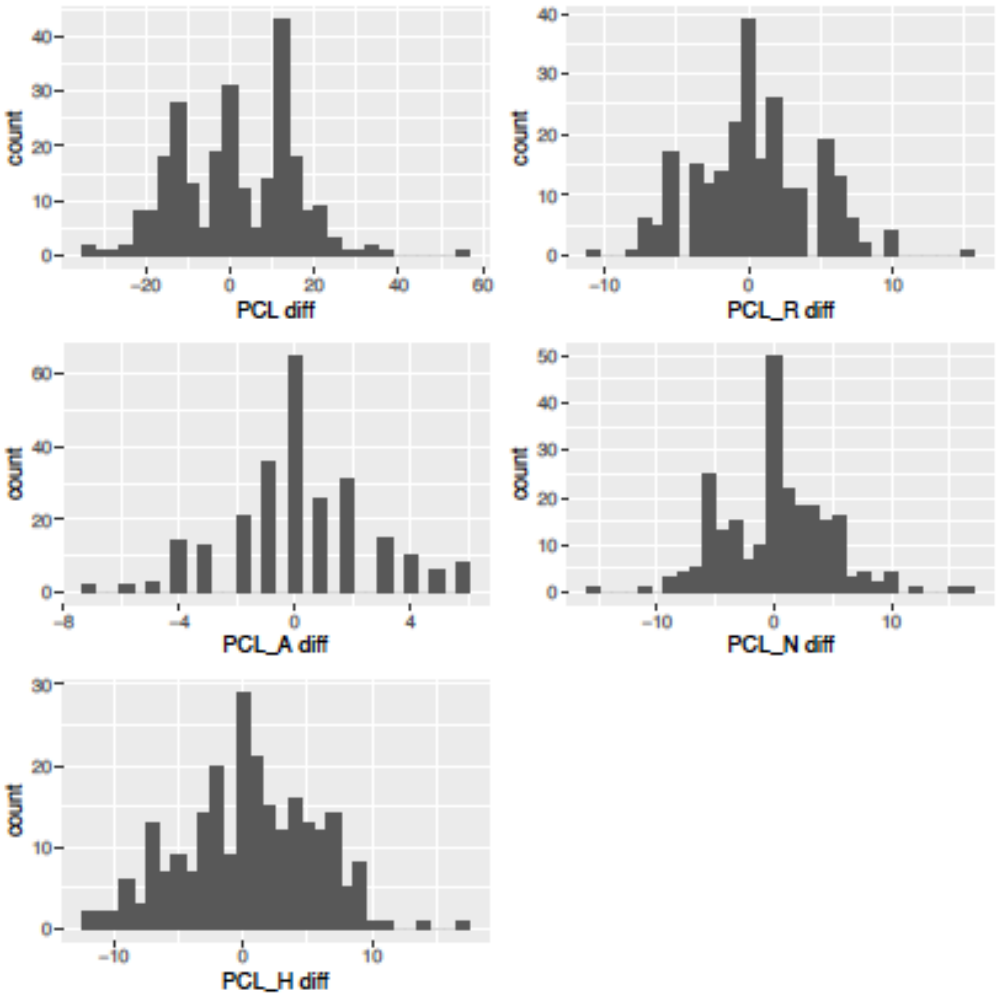


Figure 2. Distribution of differences in estimated cell proportions between T2 and T1

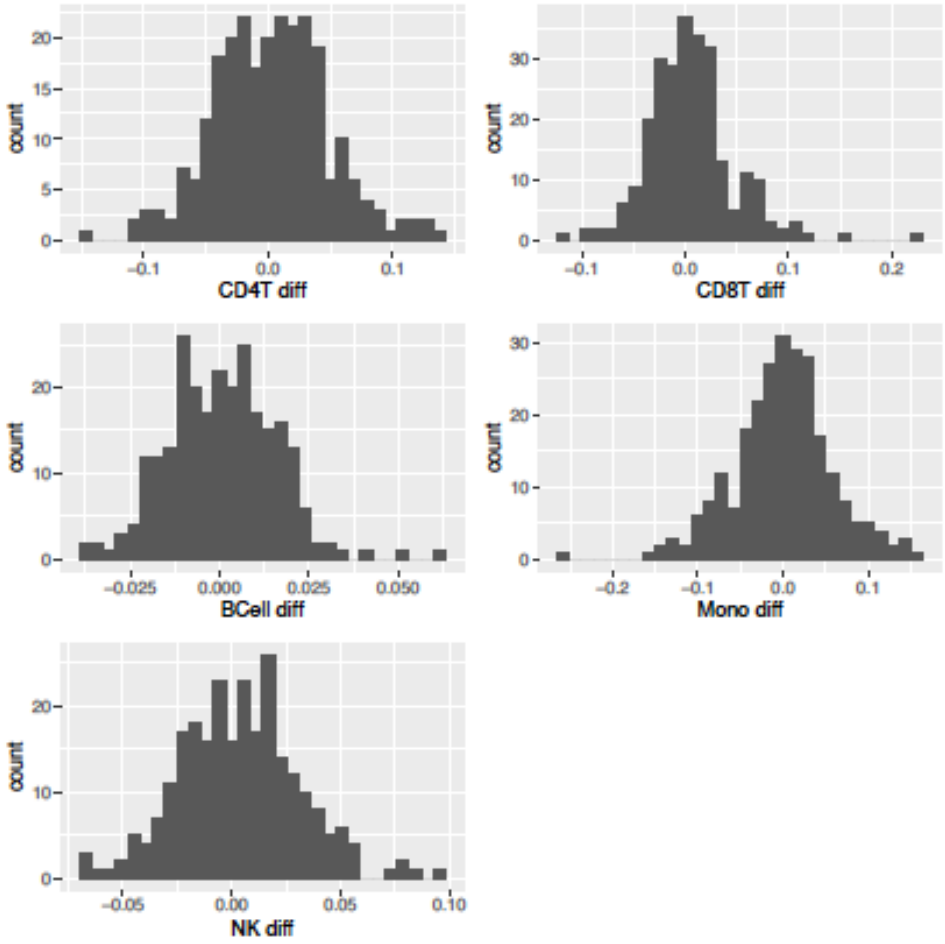


Figure 3. Top genes associated with PCL diff from the spline model.

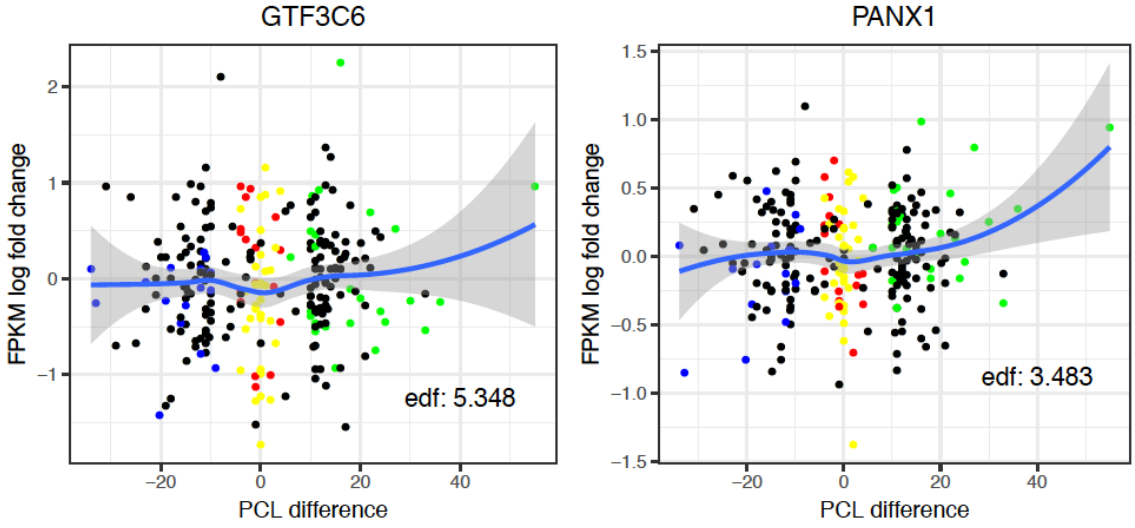


Table 1. List of GO and canonical pathway gene sets from GSEA at FDR < 0.05.

Significant GO categories at FDR < 0.05	Significant canonical pathways at FDR < 0.05
Chaperone complex Binding of sperms to zona pellucida Atapse regulator activity Sperm egg recognition Unfolded protein binding Substantia nigra development Positive regulation of telomerase to RNA localization to cajal body Neural nucleus development Cellular carbohydrate catabolic process Protein folding Positive regulation of telomerase maintenance via telomere lengthening Establishment of protein localization to chromosome	Cooperation of prefoldin and tric cct in actin and tubulin folding Pathogenic escherichia coli infection Formation of tubulin folding intermediates by cct tric Eph ephrin signaling Ephb mediated forward signaling Rho gtpases activate iqgaps Gap junction trafficking and regulation Nervous system development Processing of capped intron containing pre mrna Mrna splicing Hsp90 chaperone cycle for steroid hormone receptors shr Gap junction assembly Cilium assembly Response to elevated platelet cytosolic ca2 Translocation of slc2a4 glut4 to the plasma membrane Rho gtpase effectors Sealing of the nuclear envelope ne by escrt iii Spliceosome Semaphorin interactions Activation of ampk downstream of nmdars

Limitations and alternative strategies. Although the two time points were measured 18 months apart and we oversampled for subjects with PCL diff > 10, we did not find statistically significant genes or isoforms associated with changes in each PTSD symptom dimension. As an alternative strategy, we repeated the analysis at individual time points to better understand time specific gene expression regulation. We found remarkable results when analyzing the gene expression at T2 which we summarized below.

At individual time point analysis, we capitalized on the actual data generating mechanism of the RNA-Seq data, which is based on over-dispersed count data. From our earlier analysis which showed that many of the genes exhibit nonlinear association between changes in gene expression and PCL diff, we conducted the DE analysis of gene-level count data using NBAMSeq (29) to identify genes associated with total PCL and each dimension, following adjustment for age, race, cell proportions (CD4T, CD8T, monocytes, NK and B-cells), and potential surrogate variables. NBAMSeq is a recently developed method by our group for RNA-Seq analysis based on a flexible generalized additive model that enables the detection of both linear and nonlinear associations between gene expression and the phenotype of interest (29).

DE analysis identified 1, 48, 15, and 49 significant genes for re-experiencing, avoidance, numbing, and hyperarousal, respectively, at an FDR < 0.05. More than 83% of the DE genes showed a nonlinear association with the phenotypes, with an estimated edf ranging

from 1.6 to 7.1 (mean edf: 2.7). All the genes identified by re-experiencing, numbing, and hyperarousal had p-value < 0.1 in the total PCL analysis. On the other hand, 21 out of the 48 genes associated with avoidance had p-value > 0.1 in the total PCL analysis. The number of overlapping genes among the four dimensions was reported in Figure 4A. Pearson correlation coefficients computed for the estimated negative log p-values from NBAMSeq to summarize the strength of gene expression association among re-experiencing, avoidance, numbing, and hyperarousal were given in Figure 4B, which indicate that DE analysis of avoidance had the lowest correlation as compared with that of the other dimensions. A total of 23 and 2 genes were unique to avoidance and hyperarousal DE analysis, respectively, whereas no genes were unique to re-experiencing or numbing (Table 2). Across the p-value thresholds, hyperarousal and re-experiencing had the most and least number of significant genes, respectively (Figure 4C).

12,071 AS events were detected in 6,528 genes using SUPPA and were divided into 7 types: A3: Alternative 3' splice sites; A5: Alternative 5' splice sites; AF: Alternative first exons; AL: Alternative last exons; MX: Mutually exclusive exons; RI: Retained introns; and SE: Skipping exons. Skipping exons constituted the largest number of AS events, at 5,250. The relative proportions of each event were given in Figure 5. The *UTY* gene contained the largest number of AS events in our dataset, consistent with previous findings that this gene has a huge splicing frequency (30). At FDR < 0.05, 103 AS events were associated with total PCL. A total of 101 out of the 103 identified events had nonlinear associations with total PCL. The relative proportions of the 7 event types were shown in Figure 5, which suggest that the proportion of alternative first exons (AF) was higher, whereas the proportion of skipping exons (SE) was lower as compared with transcriptome-wide proportions (chi-square test $p < 0.05$ after combining AL, MX, and RI).

GSEA identified 45 GO terms and 26 canonical pathways associated with total PCL. The top GO terms included interleukin-17 production and response to type I interferon, whereas the top canonical pathways included nervous system development and interferon-α/β signaling. On the other hand, GSEA at PTSD dimension analyses identified 4, 5, 9, and 26 canonical pathways associated with re-experiencing, avoidance, numbing, and hyperarousal, respectively. All the canonical pathways identified by numbing and hyperarousal had p-value < 0.1 in the total PCL analysis. On the other hand, 1 out of the 4 pathways associated with re-experiencing and 2 out of the 5 pathways associated with avoidance had p-value > 0.1 in the total PCL analysis. Comparison of the GSEA results of the four dimensions identified 1, 1, 1, and 2 canonical pathways unique to re-experiencing, avoidance, numbing, and hyperarousal, respectively (Table 3). Global results at the GSEA level are consistent with those at the gene and isoform levels, where GSEA of avoidance had the lowest correlation as compared with the other dimensions for both GO terms and canonical pathways. Additionally, among the 23 genes unique to avoidance, the hemoglobin chaperone pathway (Biocarta), acetylation, and cytosol GO terms were significant at FDR < 0.1.

Our results demonstrate a pattern of distinct yet intercorrelated DE associated with each of the four dimensions, adding to the growing body of evidence that unique biological pathways underpin the disorder subtypes. Specifically, the largest gene expression difference was between the avoidance dimension and the other PCL dimensions. The 23 DE genes unique to avoidance were enriched in the hemoglobin chaperone pathway, acetylation, and cytosol GO terms. Furthermore, three DE genes unique to avoidance, namely *AHSP*, *ALAS,* and *HMBS,* were involved in the hemoglobin chaperone pathway. Hemoglobin is responsible for delivering oxygen to tissues, and *AHSP* is a molecular chaperone that prevents its precipitation, thereby acting as a balancing component of hemoglobin (31). The biological mechanism underlying hemoglobin regulation in PTSD is unclear; however, some studies have established an association between depression/anxiety disorders and hemoglobin levels and anemia (32, 33).

GSEA identified energy-dependent regulation of *mTOR* by the *LKB1–AMPK* pathway unique to avoidance. Both the *AMPK* and *mTOR* serine/threonine kinases were involved in growth control, cell proliferation, and metabolism (34). Additionally, several of the GO terms unique to avoidance were involved in metabolic processes. This observation is interesting given that PTSD has been found to be a risk factor for metabolic syndromes (35, 36), suggesting that further investigation of the molecular and cellular mechanisms associated with the avoidance dimension may provide important insights into metabolic problems in PTSD. This study has been published (12).

Figure 4. A. Venn diagram comparing the overlap among genes associated with re-experiencing, avoidance, numbing, and hyperarousal. B. Pearson correlation coefficients comparing the negative log p-values among re-experiencing, avoidance, numbing, and hyperarousal

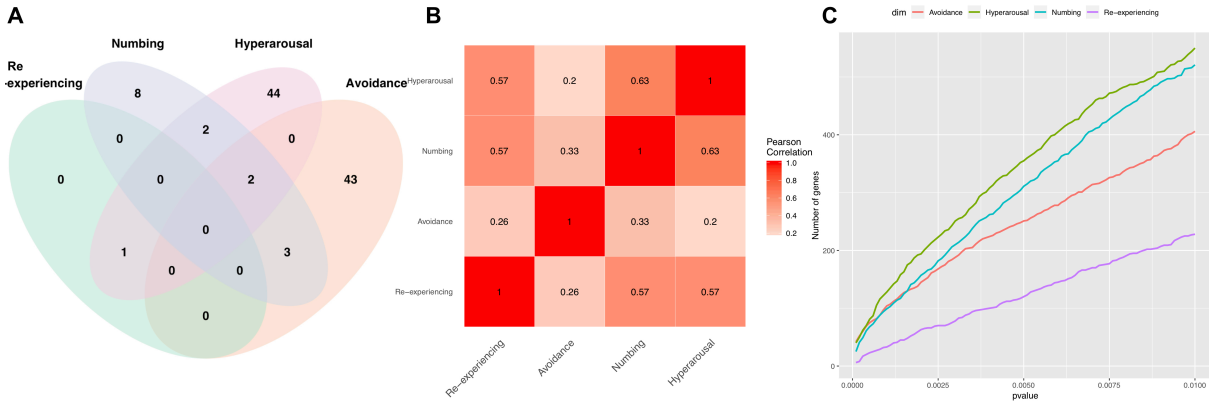


Figure 5. Bar graph comparing the proportions of significant AS events associated with total PCL to the transcriptome-wide proportions of AS events detected by SUPPA. A3: Alternative 3' splice sites; A5: Alternative 5' splice sites; AF: Alternative first exons;

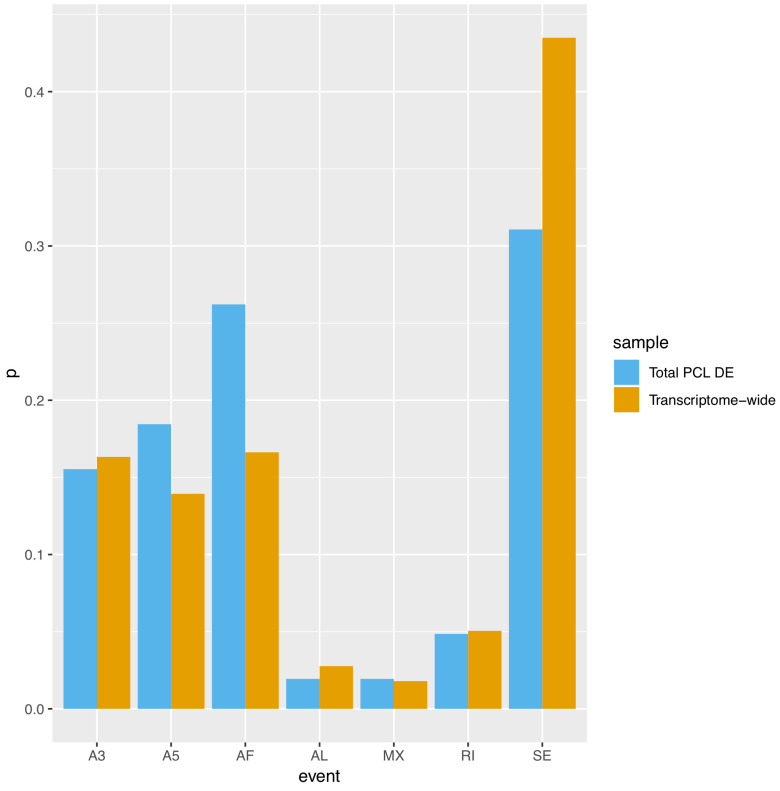


Table 2. List of DE genes and AS events unique to each PCL dimension analysis at T2.

Dimension	Unique DE genes	Unique AS events
Re-experiencing	None	*SIGLEC10*:A3, *UPF2*:AF, *POLD3*:SE
Avoidance	*AHSP*, *ALAS2*, *CA1*, *CCNDBP1*, *CMAS*, *CREG1*, *CTNNAL1*, *DPCD*, *GABARAPL2*, *GLRX5*, *H2AFJ*, *HBD*, *HMBS*, *PAGE2B*, *PCTP*, *PITHD1*, *POLR1D*, *PRDX2*, *RGCC*, *RRAGA*, *SLC22A4*, *TERF2IP*, *TFRC*	None
Numbing	None	*ATP2A3*:A3, *HAUS4*:A5, *RNPS1*:AF, *RAD51B*:AL
Hyperarousal	*LOC728743*, *STMN3*	None

Table 3. List of GO and canonical pathway gene sets unique to each PCL dimension analysis

Dimension	Unique GO gene sets	Unique canonical pathways
Re-experiencing	Positive regulation of chemotaxis Positive regulation of cyclin dependent protein kinase activity Positive regulation of dephosphorylation Regulation of chemotaxis	Matrisome
Avoidance	Antibiotic metabolic process Antioxidant activity Autophagosome Cellular detoxification Cofactor catabolic process Cofactor metabolic process Hydrogen peroxide metabolic process Organelle disassembly Oxidoreductase activity acting on peroxide acceptors Regulation of TOR signaling Response to starvation Tetrapyrrole biosynthetic process Tetrapyrrole metabolic process TOR signaling Transcription coactivator activity	Energy-dependent regulation of *mTOR* by *LKB1-AMPK*
Numbing	Catalytic step 2 spliceosome Protein targeting to mitochondrion Regulation of response to cytokine stimulus Spliceosomal complex U2 type spliceosomal complex	Spliceosome

Hyperarousal	Cytosolic transport Ribosomal RNA binding Polysomal ribosome miRNA metabolic process	Phosphoinositide pathway RAS pathway

Construction of gene expression signatures (GES)

Method. The 226 samples were divided into training (60%, n = 136) and test (40%, n = 90) set. To evaluate the utility of transcriptome in identifying changes in PTSD symptoms, the elastic net (37) algorithm was applied to the training set using $log_2[(FPKM_{T2} + 1)/((FPKM_{T1} + 1)]$. The elastic net was based on a regularized logistic regression model which automatically selected non-redundant informative genes in high-dimensional data to create a polygenic expression score, i.e., composite of genes that are most informative and predictive of PTSD status. The top 5000 genes ranked by the p-values from the spline regression differential expression analysis in the training set were used as candidate feature set in the elastic net algorithm. The optimal tuning parameters were determined via a tenfold cross-validation. Pearson correlation coefficients were computed between the predicted scores and PTSD symptoms on the test set as metrics for performance evaluation.

Pathway and gene ontology analyses were carried out using the over-representation via the Bioconductor package clusterProfiler (38) on the top 500 genes retained in the final model ranked by the estimated coefficients using the functions enrichGO and enrichKEGG. In total, 3216 gene ontologies (GO) including biological processes, molecular functions, and cellular components and 221 KEGG pathways (the range of genes per gene set was 15-500) which overlap with the 500 genes were tested. Statistically significant gene sets corresponded to those with FDR < 0.05 from over-representation analyses.

Results. The final elastic net model retained 4891 genes in the model. The GES achieved moderate association with changes in PTSD symptoms ($r = 0.547$, $p < 0.001$) in the test set. Pathway and gene ontology analyses identified 11 GO categories listed in Table 4. No KEGG pathway was identified at FDR < 0.05. We listed the KEGG pathways at $p < 0.05$ in Table 4. Metabolism related pathways ranked among the top KEGG pathways.

Table 4. List of GO and KEGG pathways for the top 500 genes from elastic net algorithm

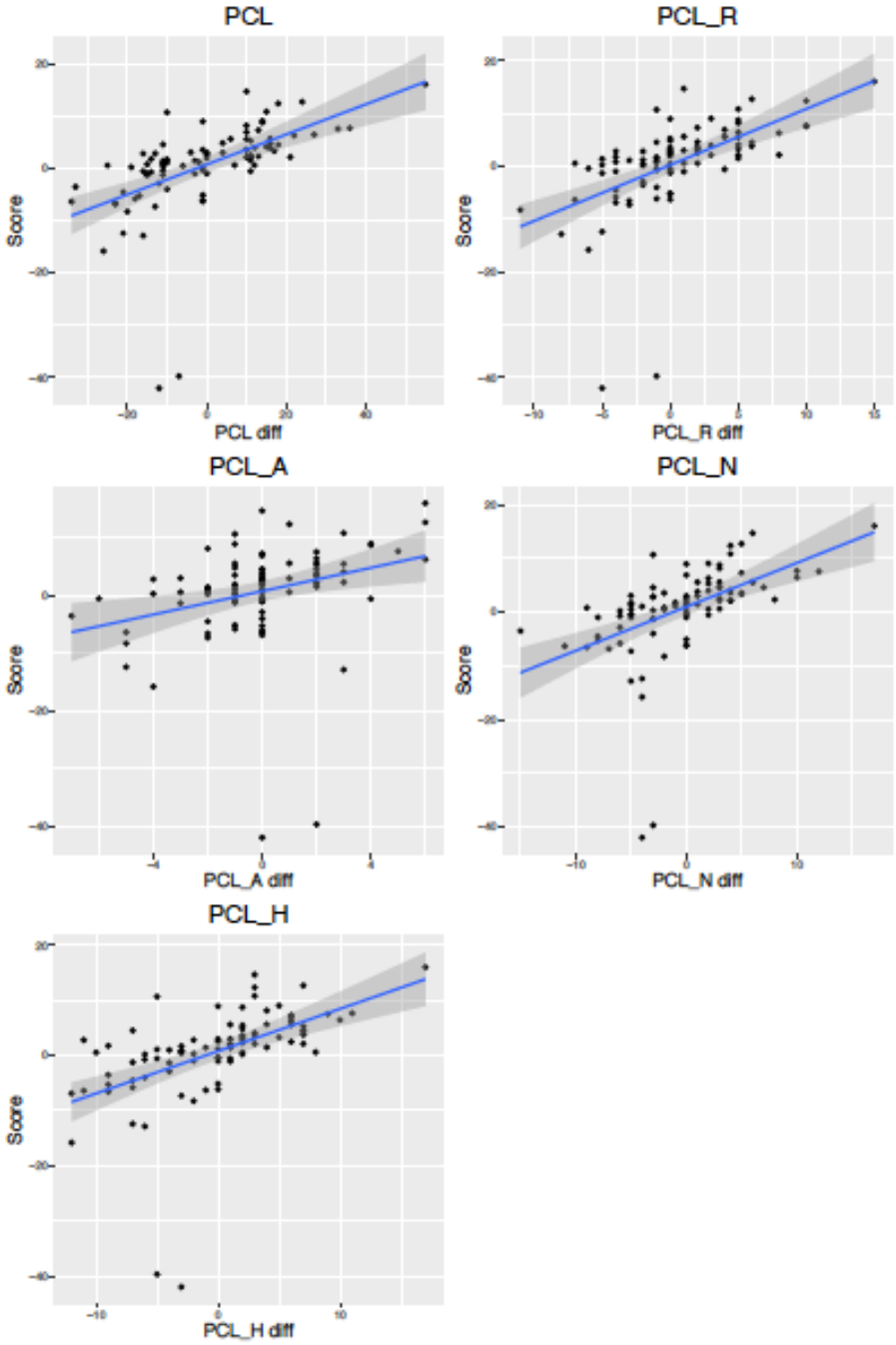
Significant GO categories at FDR < 0.05	KEGG pathways at p < 0.05
skin development intermediate filament detection of chemical stimulus involved in sensory perception intermediate filament cytoskeleton sensory perception of taste keratinization epidermis development sensory perception of mechanical stimulus sensory perception of smell photoreceptor outer segment photoreceptor cell cilium	Olfactory transduction Drug metabolism - cytochrome P450 Metabolism of xenobiotics by cytochrome P450 Bile secretion Neuroactive ligand-receptor interaction Cholesterol metabolism Pentose and glucuronate interconversions Staphylococcus aureus infection Retinol metabolism Estrogen signaling pathway Arrhythmogenic right ventricular cardiomyopathy

Aim 2. Evaluate whether the GES identified in Aim 1 is associated with change in each PTSD symptom dimension.

Method. Pearson correlation coefficients were computed between the predicted GES scores and changes in each PTSD symptom dimension on the same test set (n = 90) as metrics for performance evaluation.

Results. The GES achieved highest strength of associations with re-experiencing ($r = 0.554$, $p < 0.001$), followed by hyperarousal ($r = 0.520$, $p < 0.001$), numbing ($r = 0.503$, $p < 0.001$) and lowest with avoidance ($r = 0.302$, $p < 0.001$) as shown in Figure 6.

Figure 6. Pair plot of predicted GES vs changes in PTSD symptoms and each dimensions.



Aim 3. Test the directionality of prospective associations between the GES score and PTSD symptom severity.

Method. To test the directionality of the association between GES and PTSD symptom severity, we fitted the elastic net (37) algorithm on $log_2(FPKM + 1)$ using T1 as training set and T2 as test set, and vice versa. We considered several candidate feature sets, including (a) top 5000 genes ranked by the p-values from DESeq2 model (39) (DESeq2_top5000), (b) genes with $p < 0.05$ from DESeq2 model (DESeq2_pv0.05), (c) top 5000 genes ranked by the p-values from NBAMSeq model (29) (NBAMSeq_top5000), and (d) genes with $p < 0.05$ from NBAMSeq model (NBAMSeq_pv0.05). Pearson correlation coefficients were computed between the predicted scores and PTSD symptoms on the test set as metrics for performance evaluation. We also evaluated the predicted scores on each PTSD symptom dimension.

Results. The GES estimated using top 5000 genes from either DESeq2 or NBAMSeq models showed better performance, i.e., higher Pearson correlation coefficients compared to using genes with $p < 0.05$ from either model (Table 5). In general, the GES trained at base line (T1) achieved moderately lower correlation in predicting PTSD symptom severity at follow-up (T2) (for example $r = 0.384$, $p < 0.001$ using NBAMSeq_top5000) and the strength of correlation was comparable to the correlation between GES trained at T2 and PTSD symptom severity at T1 ($r = 0.378$, $p < 0.001$ using NBAMSeq_top5000), i.e., supporting a bidirectional prediction between GES and PTSD symptoms. Additionally, among the PTSD symptom dimensions, the GES achieved comparable strength of associations with re-experiencing, hyperarousal and numbing but lower for avoidance for models trained on T1 and tested on T2. For models trained on T2 and tested on T1, the strength of associations were comparable for re-experiencing and hyperarousal, but lower in avoidance and numbing.

Table 5. Pearson correlation coefficients between GES and PTSD symptoms and symptom dimensions.

Train on T1, test on T2					
	Total PCL	Re-experiencing	Avoidance	Numbing	Hyperarousal
DESeq2_top5000	0.392	0.344	0.225	0.399	0.374
DESeq2_pv0.05	0.331	0.304	0.168	0.344	0.308
NBAMSeq_top5000	0.384	0.338	0.229	0.395	0.359
NBAMSeq_pv0.05	0.346	0.307	0.289	0.360	0.324
Train on T2, test on T1					
	Total PCL	Re-experiencing	Avoidance	Numbing	Hyperarousal
DESeq2_top5000	0.369	0.364	0.319	0.288	0.346
DESeq2_pv0.05	0.314	0.336	0.294	0.236	0.271
NBAMSeq_top5000	0.378	0.369	0.311	0.302	0.351
NBAMSeq_pv0.05	0.297	0.321	0.257	0.224	0.263

Aim 4. Evaluate whether change in GES is associated with change in LRS.

Method. Lower respiratory symptoms (LRS) was determined from clinical assessments conducted by providers during the monitoring visit (40, 41). A composite based on four lower respiratory symptoms present in the past week was constructed: shortness of breath, chest tightness, wheezing, and regular cough. Symptom severity was rated on a scale from 0 ("none") to 4 ("almost a constant problem"). Since we did not have concurrent LRS at T2, we tested whether the GES trained on T1 was associated with LRS at T1. A linear regression model was

fitted using the predicted GES scores as outcome and LRS as predictor. To evaluate the effect of PTSD symptoms in moderating the gene expression-LRS relationship, a multivariable linear regression model was fitted using the predicted GES scores as outcome and LRS as predictor, adjusting for PTSD symptoms. All variables were standardized.

Results. The GES trained at T1 achieved moderate association with LRS ($r = 0.425$, $\beta = 0.538$, $p < 0.001$). After including PTSD symptoms in the model, the association became insignificant ($\beta = 0.009$, $p = 0.945$). The results indicated that PTSD symptoms fully accounted for the association between the GES and LRS.

Study A. Evaluate whether PTSD is associated with accelerated transcriptional aging.

Motivated by the results of GSEA analysis as described in Aim 1, we performed a computational study to evaluate whether PTSD is associated with accelerated transcriptional aging. Posttraumatic stress disorder (PTSD) is associated with shortened lifespan and health span, which suggests accelerated aging. Emerging evidence suggests that methylation age may be accelerated in PTSD. It is important to examine whether transcriptional age is also accelerated because transcriptome is highly dynamic, associated with age-related outcomes and may offer greater insight into the premature aging in PTSD.

Although both DNA methylation (DNAm) and gene expression have been found to be associated with PTSD and are hallmarks for understanding the aging process and associated diseases, no previous study has investigated transcriptional aging in PTSD. One possible explanation is the lack of availability of transcriptional age predictors. In addition, most existing human transcriptional age predictors were developed based on microarray data and are limited to a small number of tissues (42-46). RNA-Seq has emerged as the current state-of-the-art platform for transcriptional profiling. The only predictor constructed by using RNA-Seq data is the ensemble LDA predictor; however, this predictor was derived only from fibroblast data (47). Recognizing the gap in existing transcriptional aging research based on RNA-Seq data, our group has recently developed RNAAgeCalc, a multi-tissue transcriptional age calculator (48), by using the RNA-Seq data from the Genotype-Tissue Expression (GTEx) Program (49). Our calculator can perform both the across-tissue and tissue-specific transcriptional age prediction. We have further shown that RNAAgeCalc outperforms prior age-related gene expression signatures in predicting age of normal samples and offers complementary information to DNA methylation age in association analysis of mutation burden and mortality risk across different types of cancer (48).

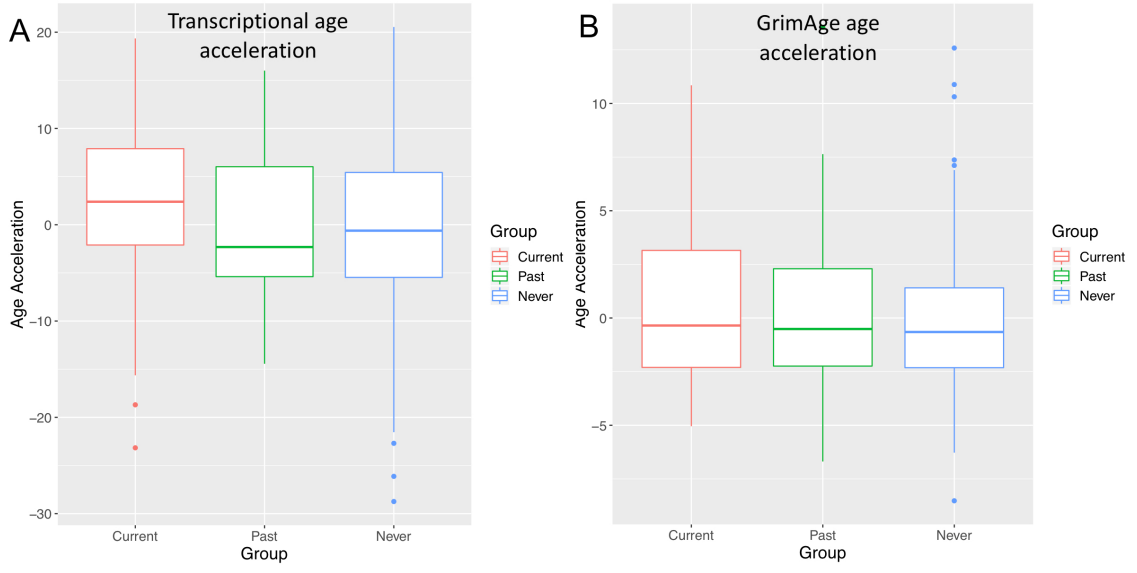
The objectives of Study A were twofold: (1) we performed the first investigation of the relationship between transcriptional aging and PTSD, and (2) we compared the different DNAm age calculators for PTSD and correlated these findings to transcriptional aging. To facilitate comparison with DNAm data, we used $n = 324$ (201 never had PTSD, 81 with current PTSD and 42 with past PTSD) whole blood RNA-Seq gene expression data profiled by using RNA-Seq and matching DNA methylation data from our previous studies of World Trade Center (WTC) responders (50, 51). We computed the DNAm and transcriptional age and evaluated the associations between PTSD and these biological age predictions.

Method. Epigenetic ages from DNAm data were computed with the online DNAm age calculator (http://dnamage.genetics.ucla.edu/), which implements several DNAm age estimators including Horvath (52), Hannum (53), PhenoAge (54) and GrimAge (55). On the other hand, transcriptional age was computed using the option in RNAAgeCalc which was trained on the 1,497 genes of the whole blood GTEx RNA-Seq data. The correlations between each biological age (i.e., the different DNAm age predictions and transcriptional age) and chronological age were assessed with Pearson correlation coefficients. Age acceleration was

defined as the residual by regressing biological age on chronological age. Positive age acceleration indicated a higher biological age than chronological age. A linear model was fitted by using age acceleration as the dependent variable and PTSD diagnosis (current versus never) as the independent variable, with adjustment for chronological age and race. Sensitivity analysis was conducted to compare the results from a model that was further adjusted for the proportions of CD8 and CD4 T cells, natural killer cells, B cells and monocytes. Analyses were repeated by substituting PTSD diagnosis with total PTSD symptom severity (total PCL) and each of the four dimensions (re-experiencing, avoidance, numbing and hyperarousal). All continuous variables were standardized in the linear model fit. Univariate associations between each cell type and age acceleration, as well as the associations between cell type proportions estimated on DNA methylation (Houseman et al. procedure (56)) versus RNA-Seq data (CIBERSORT software (16)) were assessed with Pearson correlation coefficients.

Results. The current PTSD group showed higher transcriptional age acceleration than the never PTSD group ($\beta = 0.354$, $p = 0.0077$) after adjustment for age and race. In contrast, transcriptional age acceleration was not significantly different between current PTSD and past PTSD ($\beta = 0.346$, $p = 0.075$) as well as between past PTSD and never PTSD ($\beta = 0.017$, $p = 0.918$), after adjustment for age and race (Figure 7A). Transcriptional age acceleration was also positively associated with the avoidance dimension ($\beta = 0.116$, $p = 0.037$) but was not significantly associated with total PCL, re-experiencing, hyperarousal dimensions ($0.05 < p < 0.1$) and numbing ($p = 0.183$). Among the four DNAm age predictions, only GrimAge age acceleration was significantly higher in the current PTSD group than the never PTSD group ($\beta = 0.335$, $p = 0.0097$) after adjustment for age and race. GrimAge age acceleration was not significantly different between current PTSD and past PTSD ($\beta = 0.190$, $p = 0.386$), and between past PTSD and never PTSD (0.132, $p = 0.410$), after adjustment for age and race (Figure 7B). GrimAge age acceleration was also positively associated with total PCL ($\beta = 0.146$, $p = 0.010$), avoidance ($\beta = 0.147$ $p = 0.0083$) and numbing dimensions ($\beta = 0.186$, $p = 0.012$), but was not significantly associated with re-experiencing and hyperarousal dimensions ($0.05 < p < 0.1$). Our results indicated that both epigenetic and transcriptional aging may provide biological insights into the mechanisms underpinning aging in PTSD. This study has been published (57).

Figure 7. A.Box plot comparing transcriptional age acceleration to PTSD diagnosis. B. Box plot comparing GrimAge age acceleration to PTSD diagnosis.



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Inclusions

Cumulative Inclusion Enrollment Table

View Burden Statement

PHS Inclusion Enrollment Report

This report format should NOT be used for collecting data from study participants.

OMB Number: 0925-0001 and 0925-0002
Expiration Date: 10/31/2018

***Study Title (must be unique):** Longitudinal genome-wide transcriptome study of PTSD symptom change in WTC responders

* Delayed Onset Study? ☐ Yes ☒ No

If study is not delayed onset, the following selections are required:

Enrollment Type ☐ Planned ☒ Cumulative (Actual)

Using an Existing Dataset or Resource ☒ Yes ☐ No

Enrollment Location ☒ Domestic ☐ Foreign

Clinical Trial ☐ Yes ☒ No **NIH-Defined Phase III Clinical Trial** ☐ Yes ☐ No

Comments:

Racial Categories	Ethnic Categories									
	Not Hispanic or Latino			Hispanic or Latino			Unknown/Not Reported Ethnicity			Total
	Female	Male	Unknown/ Not Reported	Female	Male	Unknown/ Not Reported	Female	Male	Unknown/ Not Reported	
American Indian/ Alaska Native	0	0	0	0	0	0	0	0	0	0
Asian	0	1	0	0	0	0	0	0	0	1
Native Hawaiian or Other Pacific Islander	0	0	0	0	0	0	0	0	0	0
Black or African American	0	6	0	0	0	0	0	1	0	7
White	11	167	0	0	6	0	0	0	0	184
More than One Race	0	1	0	0	9	0	0	76	0	86
Unknown or Not Reported	0	0	0	0	0	0	0	22	0	22
Total	11	175	0	0	15	0	0	99	0	300

Report 1 of 1

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Gender and minority study subjects

Both women and minorities were involved in the WTC-HP and thus were recruited into the baseline epigenetics study. Since this sample is drawn from the original sample of 2076 responders who banked their blood for future genetic studies, the involvement of women and minorities were based on these statistics accounting for oversampling for participants with significant PCL change. Please see the Enrollment Table for details.

Children

Since the sample was drawn from the original sample of 2076 responders who banked their blood for future genetic studies, there were no responders who were under 18 years of age in the biobank; and therefore, the study did not include children.

Materials available for other investigators

The subset of RNA-Seq data (n=226) was uploaded to the Gene Expression Omnibus (accession number GSE164877). Complete dataset will be uploaded to the same accession number upon publication of pending manuscripts.

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C. OVERALL PRODUCTS

C.1 PUBLICATIONS

Are there publications or manuscripts accepted for publication in a journal or other publication (e.g., book, one-time publication, monograph) during the reporting period resulting directly from this award?

Yes

Publications Reported for this Reporting Period

Public Access Compliance	Citation
N/A: Not NIH Funded	Uddin M, Ratanatharathorn A, Armstrong D, Kuan PF, Aiello AE, Bromet EJ, Galea S, Koenen KC, Luft B, Ressler KJ, Wildman DE, Nievergelt CM, Smith A. Epigenetic meta-analysis across three civilian cohorts identifies NRG1 and HGS as blood-based biomarkers for post-traumatic stress disorder. Epigenomics. 2018 December;10(12):1585-1601. PubMed PMID: 30456986; PubMed Central PMCID: PMC6331697; DOI: 10.2217/epi-2018-0049.
N/A: Not NIH Funded	Kuan PF, Yang X, Clouston S, Ren X, Kotov R, Waszczuk M, Singh PK, Glenn ST, Gomez EC, Wang J, Bromet E, Luft BJ. Cell type-specific gene expression patterns associated with posttraumatic stress disorder in World Trade Center responders. Translational psychiatry. 2019 January 15;9(1):1. PubMed PMID: 30664621; PubMed Central PMCID: PMC6341096; DOI: 10.1038/s41398-018-0355-8.
N/A: Not NIH Funded	Ren X, Kuan PF. methylGSA: a Bioconductor package and Shiny app for DNA methylation data length bias adjustment in gene set testing. Bioinformatics (Oxford, England). 2019 June 1;35(11):1958-1959. PubMed PMID: 30346483; DOI: 10.1093/bioinformatics/bty892.
N/A: Not NIH Funded	Ren X, Kuan PF. RNAAgeCalc: A multi-tissue transcriptional age calculator. PloS one. 2020;15(8):e0237006. PubMed PMID: 32750074; PubMed Central PMCID: PMC7402472; DOI: 10.1371/journal.pone.0237006.
N/A: Not NIH Funded	Ren X, Kuan PF. Negative binomial additive model for RNA-Seq data analysis. BMC bioinformatics. 2020 May 1;21(1):171. PubMed PMID: 32357831; PubMed Central PMCID: PMC7195715; DOI: 10.1186/s12859-020-3506-x.
N/A: Not NIH Funded	Kuan PF, Clouston S, Yang X, Kotov R, Bromet E, Luft BJ. Molecular linkage between post-traumatic stress disorder and cognitive impairment: a targeted proteomics study of World Trade Center responders. Translational psychiatry. 2020 August 4;10(1):269. PubMed PMID: 32753605; PubMed Central PMCID: PMC7403297; DOI: 10.1038/s41398-020-00958-4.
N/A: Not NIH Funded	Kuan PF, Clouston S, Yang X, Che C, Gandy S, Kotov R, Bromet E, Luft BJ. Single-cell transcriptomics analysis of mild cognitive impairment in World Trade Center disaster responders. Alzheimer's & dementia (Amsterdam, Netherlands). 2021;13(1):e12154. PubMed PMID: 33665344; PubMed Central PMCID: PMC7896635; DOI: 10.1002/dad2.12154.
N/A: Not NIH Funded	Kuan PF, Yang X, Ren X, Che C, Waszczuk M, Kotov R, Clouston S, Singh PK, Glenn ST, Gomez EC, Wang J, Bromet E, Luft BJ. Mapping the transcriptomics landscape of post-traumatic stress disorder symptom dimensions in World Trade Center responders. Translational psychiatry. 2021 May 24;11(1):310. PubMed PMID: 34031375; PubMed Central PMCID: PMC8144574; DOI: 10.1038/s41398-021-01431-6.
N/A: Not NIH Funded	Kuan PF, Ren X, Clouston S, Yang X, Jonas K, Kotov R, Bromet E, Luft BJ. PTSD is associated with accelerated transcriptional aging in World Trade Center responders. Translational psychiatry. 2021 May 24;11(1):311. PubMed PMID: 34031357; PubMed Central PMCID: PMC8144188; DOI: 10.1038/s41398-021-01437-0.

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

NOTHING TO REPORT

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Have inventions, patent applications and/or licenses resulted from the award during the reporting period? No

If yes, has this information been previously provided to the PHS or to the official responsible for patent matters at the grantee organization?

C.5 OTHER PRODUCTS AND RESOURCE SHARING

NOTHING TO REPORT

D. OVERALL PARTICIPANTS

D.1 WHAT INDIVIDUALS HAVE WORKED ON THE PROJECT?

Commons ID	S/K	Name	Degree(s)	Role	Cal	Aca	Sum	Foreign Org	Country	SS
PFKUAN	Y	Kuan, Pei-Fen	PHD	PD/PI	0.0	2.0	3.0			NA
EBROMET	Y	BROMET, EVELYN J	PHD,BA,MOTH	Co-Investigator	2.0	0.0	0.0			NA
BJLUFT	Y	LUFT, BENJAMIN J	MD,BA	Co-Investigator	1.0	0.0	0.0			NA
	Y	Waszczuk, Monika	PhD,BA,MOTH	Co-Investigator	2.0	0.0	0.0			NA
	N	Yang, Xiaohua		Technician	5.0	0.0	0.0			NA

Glossary of acronyms:
S/K - Senior/Key
DOB - Date of Birth
Cal - Person Months (Calendar)
Aca - Person Months (Academic)
Sum - Person Months (Summer)
Foreign Org - Foreign Organization Affiliation
SS - Supplement Support
RE - Reentry Supplement
DI - Diversity Supplement
OT - Other
NA - Not Applicable

D.2 PERSONNEL UPDATES

D.2.a Level of Effort

Not Applicable

D.2.b New Senior/Key Personnel

Not Applicable

D.2.c Changes in Other Support

Not Applicable

D.2.d New Other Significant Contributors

Not Applicable

D.2.e Multi-PI (MPI) Leadership Plan

Not Applicable

E. OVERALL IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES? Not Applicable
E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE? NOTHING TO REPORT
E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER? Not Applicable
E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)? NOTHING TO REPORT

G. OVERALL SPECIAL REPORTING REQUIREMENTS SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

NOTHING TO REPORT

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

G.4.a Does the project involve human subjects?

Not Applicable

G.4.b Inclusion Enrollment Data

File(s) uploaded:
inclusion-enrollment-report.pdf

G.4.c ClinicalTrials.gov

Does this project include one or more applicable clinical trials that must be registered in ClinicalTrials.gov under FDAAA?

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

NOT APPLICABLE

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Not Applicable

G.8 PROJECT/PERFORMANCE SITES

Not Applicable

G.9 FOREIGN COMPONENT No foreign component
G.10 ESTIMATED UNOBLIGATED BALANCE Not Applicable
G.11 PROGRAM INCOME Not Applicable
G.12 F&A COSTS Not Applicable

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I. OVERALL OUTCOMES

I.1 What were the outcomes of the award?

The study considerably advanced basic science, clarifying the role of gene expression in PTSD, with no previous studies in the gene expression field investigating longitudinal associations, individual PTSD dimensions, and physical symptom comorbidities. Other important contributions included the potential clinical implications for screening and treatment of PTSD. Taken together, by integrating psychiatric epidemiology and gene expression, this study offers a cutting-edge translational approach to inform clinical practice and improve the health of WTC responders.

Potential outcomes from the findings of this study include:
(a) Increased awareness of OSH issues
Study objectives and approaches were presented as a poster at the at the Society of the Biological Psychiatry 2018 Annual Meeting, New York, NY

(b) Basic/Etiologic only: Other researchers use knowledge as conceptual basis for additional basic or applied research
Our publications were cited by epigenetics researchers in psychiatric disorders, thus we anticipate that the findings and gene expression signature developed in this study can be tailored to other psychiatric disorders and trauma exposed cohorts.