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ORIGINAL RESEARCH

Role of LncRNA in Trauma Susceptibility and Resilience to Post-Traumatic Stress Disorder (PTSD): A Pilot Study in the African American Population

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Purpose: Childhood adversities are associated with the development of post-traumatic stress disorder (PTSD). However, not all individuals exposed to severe trauma develop psychopathology, underscoring the need for a better understanding of the molecular pathophysiology underlying vulnerability to PTSD. Evidence suggests that the peripheral olfactory system, which is accessible in the nose, regulates the structure and function of olfactory regions relevant to stress biology. Long non-coding RNAs (lncRNAs) control transcriptional regulation via epigenetic mechanisms, and since these elements are sensitive to environmental inputs, they may play a crucial role in diseases like PTSD, which are highly dependent on environmental experiences. This study aims to identify lncRNAs in the olfactory mucosa associated with vulnerability and resilience to PTSD in African American populations.

Patients and Methods: Thirty-eight adult residents in the Washington DC metropolitan region, aged 18–50 years were recruited based on a history of exposure to trauma during childhood. Participants were divided into three groups: those who experienced childhood trauma and developed PTSD, those with similar trauma but did not develop PTSD, and a control group who did not experience childhood trauma. Olfactory mucosa samples were collected through nasal brushings, and neurobehavioral assessments were conducted. RNA sequencing was performed to facilitate lncRNA-based analysis, exploring differentially expressed lncRNAs among the specified groups.

Results: Two lncRNAs, CYP1B1-AS1 and SLC7A11-AS1, known for their neuroprotective and anti-inflammatory functions, were significantly elevated in the PTSD group compared to the non-trauma-exposed controls. Ingenuity Pathway Analysis of the differentially expressed lncRNAs suggests their potential involvement in NFAT5-mediated inflammatory cascades, indicating a possible biological mechanism underlying PTSD vulnerability.

Conclusion: PTSD may be associated with epigenetic modifications of inflammatory and immunoregulatory pathways in the olfactory system. Further evaluation of the relationship between these differentially expressed lncRNAs and PTSD should be conducted with larger samples and more diverse cohorts.

Keywords: PTSD, lncRNA, CYP1B1-AS1, SLC7A11-AS1, NFAT5

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Introduction

Posttraumatic stress disorder (PTSD) is a psychiatric condition that develops in some individuals following exposure to psychologically traumatic events.[1] The prevalence of PTSD has been reported to reach approximately 40% among those who have experienced sexual assault and up to 30% among war veterans.[2–4] However, PTSD does not always develop in individuals exposed to severe psychological trauma or adversity, highlighting the importance of investigating the molecular mechanisms underlying vulnerability and resilience to PTSD in the nervous system. Our laboratory previously reported an association between olfactory bulb (OB) morphometry and PTSD vulnerability or resilience in adults exposed to childhood trauma.[1] The OB projects neural inputs to limbic structures, such as the amygdala, which links emotional processing to brain regions involved in learning, memory, and stress responses. Experimental ablation of the OB has been shown to induce cortical-hippocampal-amygdala dysfunction,[5–7] increase amygdaloid after-discharges and kindling effects in the medial amygdaloid nucleus,[8,9] and enhance the sensitization of acoustic startle reflex in response to acute or repeated stress stimuli.[10] These findings emphasize the significance of investigating the molecular regulation of the OB following stress exposure to better understand the pathophysiology of stress-related disorders, including PTSD. Although the OB is not realistically accessible for repeated sampling (biopsy) in humans, it is anatomically connected to, and structurally and functionally influenced by molecular events in the olfactory mucosa (OM) neuroepithelium in the nose. For instance, recent studies have shown that environmentally induced inflammatory changes in the olfactory-nasal epithelia in the nose reversibly influence OB volumes.[11] Therefore, the olfactory epithelia could be a useful platform for the investigation of epigenetic mechanisms by which stress and other environmental factors influence the development of PTSD.

Epigenetics, the study of molecular mechanisms through which environmental factors and behavior influence gene function, has emerged as a crucial field in understanding stress-related disorders. Preclinical studies suggest that epigenetic regulation provides a compelling mechanism by which stress affects brain structure and function.[12–14] Among epigenetic factors, non-coding RNAs (ncRNAs), including microRNAs (miRNAs) and long non-coding RNAs (lncRNAs), play critical roles in gene regulation and transgenerational epigenetic inheritance. lncRNAs, defined as RNA molecules longer than 200 nucleotides that are not translated into proteins,[15] have been implicated in several diseases, including cancer, neurodegenerative disorders, and cardiovascular diseases.[16,17] Mechanistic studies in animal models have also linked lncRNAs to the normal and abnormal development of sensory organs.[18–20] Recent evidence has shown that stress exposure affects the expression of specific lncRNAs in the hippocampus of mouse model of PTSD. For instance, a significant decrease in the expression of lncRNAs such as Gomafu, NONMMUT033604.2, and NONMMUT064397.2 has been observed in the hippocampus of a mouse model of PTSD.[21] Although, studies have indicated an interactive role of lncRNAs and environmental factors in the lifelong plasticity and development of olfactory structures, including the OM,[21–24] the potential role of childhood trauma-associated lncRNAs alterations in the olfactory system in the development of PTSD remains underexplored.

In this study, we leveraged the accessibility of the OM for repeated sampling in living subjects to investigate olfactory lncRNA mechanisms of PTSD resilience in individuals exposed to childhood trauma. Participants were categorized into three groups: those who experienced trauma and developed PTSD (T1P1), those who experienced trauma but did not develop PTSD (T1P0), and a control group with no trauma exposure or PTSD (T0P0). Nasal exfoliates were collected for lncRNA-based epigenomic transcriptomic analysis. This study identified differentially expressed lncRNAs in the OM as potential biomarkers of PTSD vulnerability and resilience, with a focus on the African American population.

Materials and Methods

Study Participants and Demographic Features

The study procedures were approved by the Howard University Institutional Review Board (HU-IRB) to ensure ethical research involving human participants, in accordance with the Declaration of Helsinki. Participants were recruited from the greater Washington, D.C., area. All participants provided written informed consent, as approved by the IRB, prior to engaging in any aspect of the study. They were asked to complete questionnaires covering their background, demographics, social, and medical histories.

Participants, within the age range of 18 to 50 years old, were recruited from the Washington, D.C., metro area. All participants had a history of severe childhood trauma exposures, except for controls who had no severe childhood trauma history, and were abstinent from substance use disorders for at least six months prior to recruitment. Exclusion criteria included a history of severe trauma exposures after the age of 16 years old, psychiatric disorders other than PTSD; a history of HIV; use of psychotropic medications or psychotherapy within the past six weeks; traumatic brain injury; intellectual disability, as determined by history or mental status examination; medical history of autoimmune disorders; and contraindications to MRI (eg, metal prostheses, aneurysmal clips, cardiac pacemakers, metal orthodontics, coronary artery bypass clips, or other implants). People with acute rhinosinusitis and acute infections in the past six weeks were also excluded from nasal exfoliates collections.

All procedures, including psychiatric measures and diagnostic interviews for PTSD, have been described previously by this team.[1] Briefly, all study participants were screened using PTSD Checklist for DSM-5 (PCL-5) and Life Events Checklist (LEC) for stressful events. The control group did not have Criterion A (traumatic exposure), hence did not meet the DSM-5 diagnostic criteria for PTSD on the Clinician-Administered PTSD Scale for DSM-5 (CAPS-5); and so, they were classified as non-trauma exposed healthy controls, ie trauma exposure negative and PTSD negative. The PTSD Checklist (PCL) for the Diagnostic and Statistical Manual of Mental Disorders fifth edition (DSM-5), ie, PCL-5[25] was used to assess PTSD symptoms. PCL-5 is a widely used self-report measure of PTSD symptom severity.[25,26] The CAPS-5,[27] a well-validated semi-structured interview for PTSD diagnosis,[27,28] was used to determine the severity and diagnostic status of PTSD by psychiatrists and physicians (EN and MH). In this study we enrolled study participants with or without exposure to childhood trauma (between the ages of 5 and 15 years) and excluded everyone who experienced Criterion A traumatic events after age 15 years. All study participants underwent a comprehensive psychiatric diagnostic interview, with the Diagnostic Interview for Genetic Studies (DIGS), 4th edition[29] performed by a psychiatrist (EN) and psychiatry research associate with more than 10 years of experience (MH), as described previously.[1] The DIGS was used to identify and exclude any participant with current or past diagnosis of any Major Depression, Anxiety Disorders, substance use disorders, and other major psychiatric conditions, except for PTSD. For substance use exclusionary purpose, we also conducted urine drug screen on all participants prior to baseline studies.

A total of 38 participants that met the eligibility criteria were enrolled for this study. Participants were categorized into three groups: a PTSD group, referred to as the childhood trauma-exposed-PTSD-positive (T1P1) group; a group of individuals exposed to childhood trauma who did not develop PTSD (Trauma- exposed PTSD -ve control: T1P0); and a control group who did not experience childhood trauma (non-trauma control: T0P0). The demographic data for each group are presented in Table 1.

Sample Collection

Olfactory mucosa samples were collected from each participant using FLOQBrushes (Copan Italia, Brescia, Italy) by otolaryngologists, as previously described.[30] Briefly, the samples were swabbed from both nasal cavities and placed into 15 mL tubes containing RNA preservation solution. The tubes were initially kept at room temperature and subsequently stored at −80°C until they were ready for RNA isolation.

RNA Isolation and Quality Control

Total RNA was isolated using the AllPrep Mini Kit (PureLink RNA Mini Kit, ThermoFisher Scientific, Cat# 12183018A) according to the manufacturer's protocol. RNA quantity and quality were assessed using a NanoDrop 800 spectrophotometer (NanoDrop Technologies, Wilmington, Delaware, USA). RNA integrity was evaluated through 1% formaldehyde denaturing gel electrophoresis. Only samples displaying clear ribosomal 28S and 18S RNA bands with a ratio >1.5:1 were selected for RNA sequencing (RNA-Seq).

RNA-Seq

Single-indexed libraries for RNA-seq were constructed from 200ng of total RNA using the TruSeq Stranded Total RNA Kit (Illumina, San Diego, CA, USA) according to the manufacturer's instructions. Briefly, coding RNA and multiple forms of non-coding ncRNAs were captured using bead-based cytoplasmic and mitochondrial rRNA depletion, cDNA

Table 1 Demographics of the Participants in Trauma Study

Characteristics	Diagnosis			*p* values[¥]
	Non-Trauma Control (T0P0) n (%) 23 (60.53)	Trauma-Exposed PTSD -ve Control (T1P0) n (%) 7 (18.42)	PTSD +ve (T1P1) n (%) 8 (21.05)	
Female	17 (73.91)	2 (28.57)	8 (100)	0.01
Education (>12 grade)	10 (43.48)	6 (85.71)	6 (75.00)	0.09
Race (African American)	17 (73.91)	6 (85.71)	8 (100)	0.4
Country of Origin (US)	20 (86.96)	7 (100)	7 (87.50)	0.23
Marital status (never married)	23 (100)	7 (100)	6 (75.00)	0.07
Employment status (unemployed)	17 (73.91)	4 (57.14)	4 (50.00)	0.16
Mean Age (SD)	22.52 (9.15)	24.42 (7.52)	34 (11.79)	0.02

Notes: T0P0: a control group who did not experience childhood trauma, referred as non-trauma control; T1P0: a childhood trauma-exposed people who did not develop PTSD, referred as Trauma- exposed PTSD -ve control and T1P1: childhood trauma-exposed-PTSD-positive group, referred as PTSD +ve. [¥] p values for all categorical variables were derived from Exact test, while P value for differences in mean age was derived from F-test with model degree of freedom of 2, and total degree of freedom of 37.

synthesis, adapter ligation, and PCR. The resulting sequencing libraries were assessed for quality using a BioAnalyzer 2100 with a DNA 1000 kit (Agilent, Santa Clara, CA) and quantified via fluorometry using Qubit 4.0 (Thermo Fisher, Waltham, MA). Libraries were pooled and sequenced on the NextSeq 550 system (Illumina, San Diego, CA, USA) using the High Output Kit v2.5 (150 cycles) with paired-end 75bp read mode to an average depth of 50M reads per sample.

RNA-Seq Data Analysis

Figure 1 summarizes the bioinformatic analysis workflow. Raw FASTQ files were uploaded to Partek Flow via its backend FTP server (http://www.partek.com/partek-flow/). Following pre-alignment quality control, alignment was performed using the STAR aligner (https://www.dnastar.com/workflows/rna-seq). Post-alignment assessment showed that 92.58% to 97.59% of reads were successfully aligned across all samples. These aligned reads were quantified using the Partek E/M annotation model to obtain transcript counts. Normalization of count data was performed using the Trimmed Mean of M-values (TMM) method, with TMM normalization factors incorporated into the statistical model used for testing differential gene expression.[31] TMM factors were calculated by selecting one sample as a reference and computing the normalization factor for each non-reference sample. Adjusting for age differences, differential expression analysis was conducted using DESeq2, a method designed for count data analysis that applies shrinkage estimation for dispersions and fold changes, improving the stability and interpretability of results.[32] DESeq2 enabled a more quantitative analysis by focusing on the strength of differential expression rather than its mere presence. Raw p-values were generated, and multiple testing corrections were applied using Benjamini-Hochberg adjusted p-values to control the False Discovery Rate (FDR). Fold changes were reported for each pairwise comparison.

Pathway Analysis

Pathway analysis of differentially expressed lncRNAs was conducted using QIAGEN IPA (version 42012434, Ingenuity Systems; Qiagen China Co., Ltd). The objective of the bioinformatics analysis was to predict canonical pathways, diseases and functions, regulatory effects, upstream regulators, and molecular networks.[33,34] IPA employs a network-generation algorithm to segment molecular network maps into multiple networks and assigns a score to each network. The scores are calculated based on a hypergeometric distribution, with the negative logarithm of the significance level derived using Fisher's exact test at the right tail. For canonical pathway analysis, disease, and function prediction, a −log (P-value) >2 was considered the threshold. Significant activation was defined by a Z-score >2, while significant inhibition was indicated by a Z-score <−2.[35]

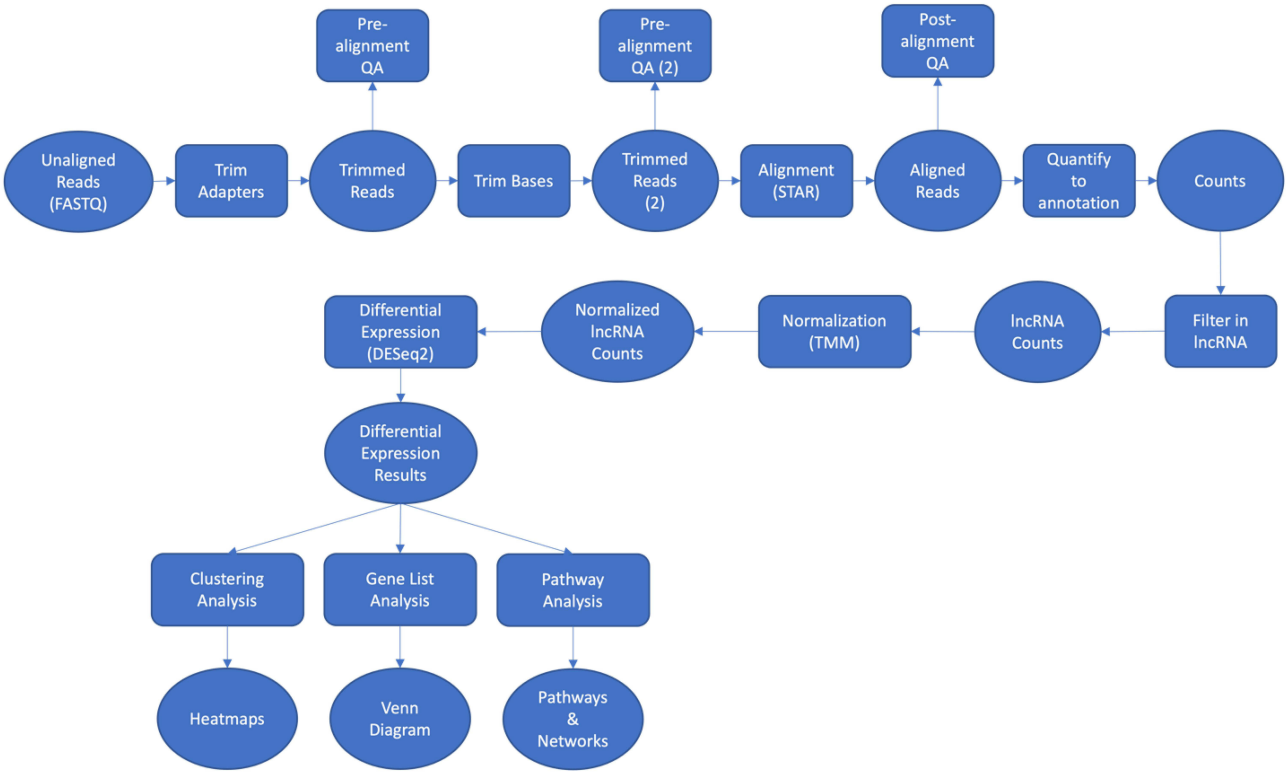

Figure 1 Flow chart for the LncRNA expression data analysis: It describes the entire pipeline of the RNASeq data analysis from the raw FASTQ data to the final results, which includes multiple quality assessments, alignment, normalization, differential expression, unsupervised clustering analysis, pathway analysis and etc. The unaligned reads were obtained from the instrument as FASTQ format, then the adapters of those reads are trimmed off, following quality assessment; the trimmed reads were further trimmed to remove unnecessary base, following another quality assessment; STAR algorithm then was used to align the cleaned reads to genome library; aligned reads were further quantified to counts of a variety of biological sources; those counts were filtered to obtain the list lncRNA counts only; the trimmed mean of M values (TMM) algorithm was used to normalized the lncRNA counts; DESeq2 algorithm then was used to do multiple pair-wise differential expression analyses (PlusVE vs Control + MinusVE, PlusVE vs Control, PlusVE vs MinusVE, MinusVE vs Control+PlusVE, MinusVE vs Control); the differential analysis results were further analyzed by other downstream methods, *ie* clustering, gene list and pathway analyses, and produced corresponding heatmaps, Venn diagram and pathway/network significance.

Results

Identification of Differentially Expressed LncRNA (DE-lncRNA)

The distribution of demographic characteristics of the 38 participants is presented in Table 1. The three groups were comparable in self-reported ethnicity, education, marital status, employment status, and country of birth, but they significantly differ in self-reported sex at birth and age. The first group is control group, who did not experience severe childhood trauma or qualified for PTSD (T0P0, non-trauma control), the second group is childhood trauma-exposed people who did not develop PTSD (T1P0, trauma- exposed PTSD -ve control), and the last group is PTSD group, also known as childhood trauma-exposed-PTSD-positive (T1P1, PTSD +ve) group. Notably, significant sex differences were observed across groups, with an over-representation of women in the PTSD +ve (T1P1) group.

DE-lncRNAs were identified based on fold change (>1.5, or <-1.5), significant p-value, and multiple testing (False Discovery Rate, FDR<0.05) q-value. The top ten DE-LncRNAs in the different groups are listed in Table 2 and Supplementary Tables 1 and 2. DE-lncRNAs were subjected to hierarchical clustering, as shown in Figure 2A, with red and green colors identifying the level of expression above and below the relative expression, respectively. Among the DE-lncRNAs, three Y-linked lncRNAs, LINC00278, TTTY14 and AC011297.1, were significantly downregulated in PTSD +ve (T1P1) vs non-trauma controls (T0P0) (Table 2), as well as in the PTSD +ve group (T1P1) compared to all controls ie trauma exposed PTSD -ve controls + non-trauma control (T1P0 + T0P0) (Supplementary Table 1). It was also observed that CYP1B1-AS1 and SLC7A11-AS1 were significantly expressed in PTSD +ve (T1P1) vs non-trauma controls (T0P0) (Table 2), but not in the contrast between PTSD +ve and all controls (Supplementary Table 1) or in the contrast between PTSD +ve vs trauma controls (trauma exposed PTSD -ve). Given the differential gender

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Table 2 Top 10 Significant LncRNA When Comparing PTSD+ve Vs Non-Trauma Control (ie, T1P1 Vs T0P0)

Gene ID	Gene name	P-Value (PTSD+ve vs Non-Trauma Control)	FDR q-Value (PTSD+ve vs Non-Trauma Control)	Fold Change (PTSD+ve vs Non-Trauma Control)
LINC00278	LINC00278‡	4.49E-58	5.27E-54	−1,000,000.0
TTTY14	TTTY14‡	3.25E-23	1.90E-19	−1,000,000.0
AC011297.1	AC011297.1‡	5.95E-15	2.32E-11	−502,289.2257
CYP1B1-AS1	CYP1B1-AS1	2.37E-6	0.0069	2.8472
SLC7A11-AS1	SLC7A11-AS1	4.84E-6	0.0113	3.3537
NCMAP-DT	NCMAP-DT	1.07E-4	0.2009	−4.4296
TTTY10	TTTY10‡	1.19E-4	0.2009	−5.2122
AC005041.4	AC005041.4	2.38E-4	0.3501	1.9623
AC005100.1	AC005100.1	5.30E-4	0.6914	−3.4665
AC005722.4	AC005722.4	6.03E-4	0.6949	2.2209

Note: ‡ Y chromosome-linked lncRNAs.

distributions between PTSD+ve group and control groups (Table 1), we examined the associations between the non-Y chromosome linked lncRNAs (ie, CYP1B1-AS1 and SLC7A11-AS1) in female-only samples (N=30) in the PTSD+ve (T1P1) vs non-trauma control (T0P0) (data not shown), and confirmed significant differential expressions between PTSD +ve (T1P1) vs non-trauma control (T0P0) for both CYP1B1-AS1 and SLC7A11-AS1. Figure 2B shows Venn diagrams of the upregulated and downregulated lncRNAs in the different study groups. Finally, exploratory regression analyses of normalized levels of the significantly expressed lncRNAs on messenger RNA (mRNA) data from all 38 participants identified large numbers of mRNAs that are significantly associated with and possibly altered by each lncRNA.

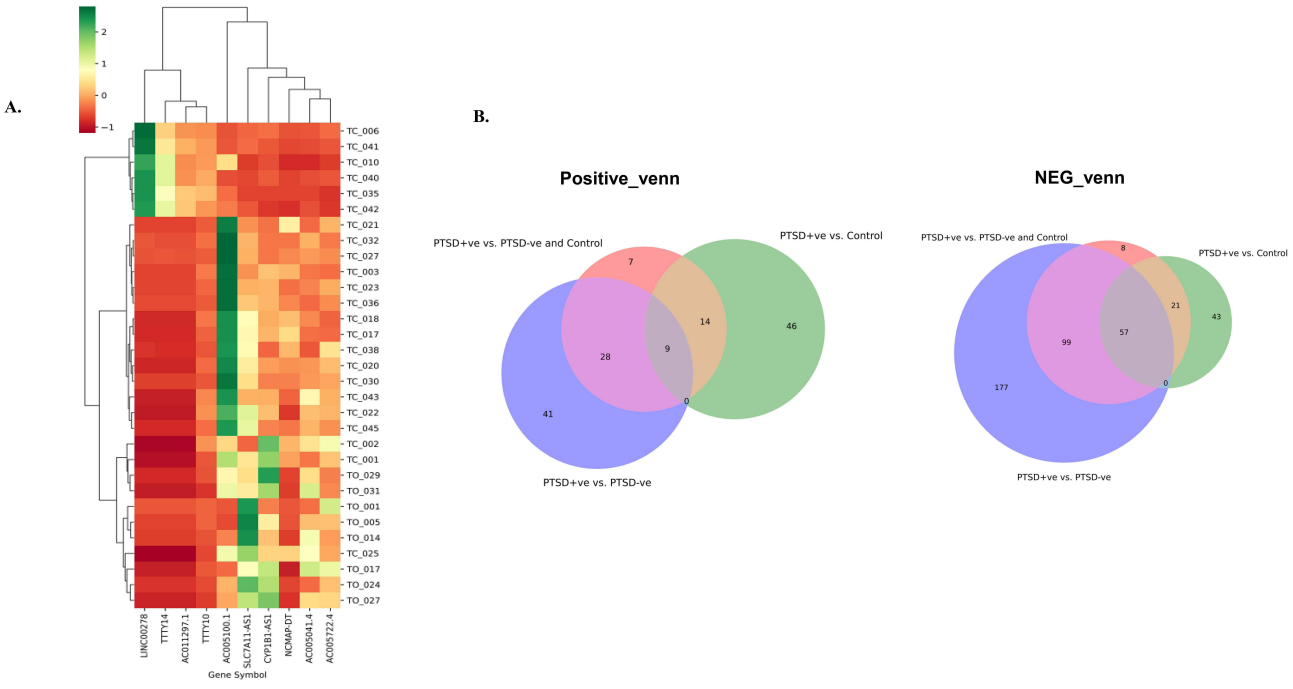

Figure 2 (**A**) displays a heatmap of significant lncRNAs across PTSD and control samples. We used a hierarchical bi-clustering algorithm to cluster both lncRNAs and samples, generating dendrograms in the process. TC and TO are the participants IDs for controls and PTSD, respectively. (**B**) presents two Venn diagrams depicting the significant lncRNAs identified in three comparisons for both positively and negatively regulated lncRNAs separately.

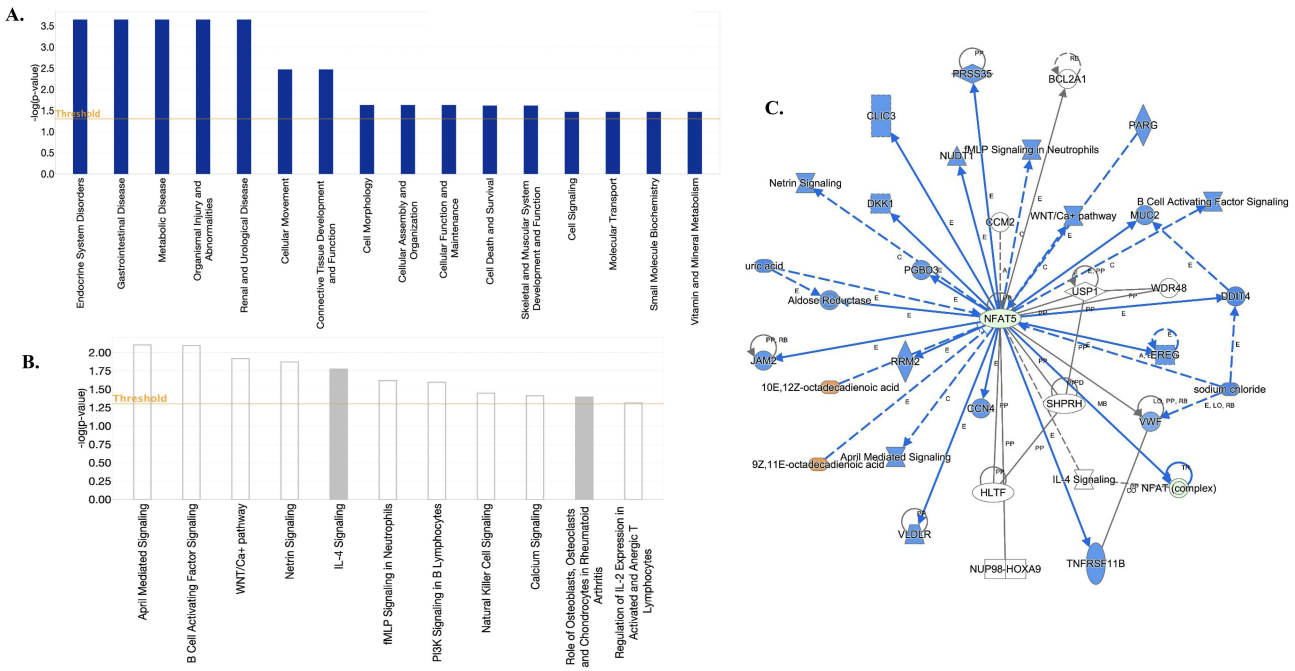

Figure 3 (**A**) illustrates the canonical pathways found to be significant based on the list of significant lncRNAs. (**B**) demonstrates the biological functions identified as significant based on the list of significant lncRNAs. White (Hollow) Bars: Represent pathways with a z-score of 0, indicating no predicted significant activation or inhibition, but the pathway is still enriched based on the input gene list. Gray Bars: Represent pathways where no activity pattern is available, meaning there is not sufficient directional information or data to predict activation or inhibition, even though the pathway is enriched. (**C**) reveals the regulatory network in which the given list of significant lncRNAs is involved.

Supplementary Tables 3–6 show the top mRNAs upregulated or downregulated by CYP1B1-AS1, SLC7A11-AS1, AC011297.1 and LIN00278, respectively. In Supplementary Table 3, it can be seen that the CYP1B1 mRNA is one of the top 20 mRNAa significantly associated with the expression of CYP1B1-AS1 lncRNA. Although we did not show all mRNAs significantly associated with each lncRNA, it should be noted that SLC7A11 was among the top 30 mRNAs associated with SLC7A11-AS1. Therefore, the results are consistent with lncRNA-associated genes selected for pathways analysis by the IPA.

Pathway and Network Analysis by DE-lncRNAs

IPA for canonical pathway analysis using differentially expressed (DE) lncRNAs revealed that biological pathways related to endocrine system disorders, gastrointestinal diseases, metabolic diseases, and organismal signaling pathways were the most significantly enriched when comparing PTSD +ve versus control samples (T1P1 vs T0P0) (Figure 3A). Sixteen pathways surpassed the threshold values.

Comprehensive biofunction pathway analysis suggested that several DE-lncRNAs in trauma-exposed PTSD+ve groups compared to controls were associated with various cellular functions (11 pathways surpassed the threshold value), such as B-cell activity factor signaling, the WNT/Ca+2 pathway, and natural killer cell signaling, among others (Figure 3B).

Finally, the network in which the given list of significant lncRNAs plays a regulatory role is shown in Figure 3C. IPA network analysis predicted activation of the NFAT5 pathway based on changes in DE-LncRNAs in the T1P1 vs T0P0 groups.

Discussion

In this cross-sectional study, we investigated the associations of differentially expressed long non-coding RNAs (DE-lncRNAs) among groups categorized by childhood trauma exposure and PTSD development. Previously, we reported a strong association between the olfactory bulb (OB) volume and PTSD, suggesting that the structure and function of the olfactory system may be linked to PTSD development. Several studies have demonstrated that epigenetic modifications,

including the expression patterns of lncRNAs, are associated with olfactory structure and function.[36–38] However, only a limited number of studies have explored the relationship between lncRNAs and PTSD development.[39] Moreover, previous research using multi-omics approaches has identified blood- and serum-based biomarkers for PTSD.[40,41] The uniqueness of our study lies in its focus on identifying lncRNAs in the olfactory mucosa, which is directly connected to the olfactory bulb and regulates structure and function of the limbic olfactory regions relevant to stress biology. This approach provides a novel perspective on PTSD-associated brain function, potentially revealing more significant risk factors for PTSD development.

Given the significant gender distribution differences between the PTSD+ve groups and controls, which could not technically be controlled by adjusting for sex -differences in our statistical models, we focus our discussions on the non-Y chromosome linked lncRNA findings with significant differential expressions. Two novel non-Y linked lncRNAs, CYP1B1-AS1 and SLC7A11-AS1, were found to be differentially expressed in trauma-exposed PTSD-positive individuals (T1P1) compared to non-trauma-exposed control subjects without PTSD (T0P0). Both CYP1B1-AS1 and SLC7A11-AS1 are known for their anti-inflammatory and neuroprotective roles.[42,43] This differential expression suggests that these lncRNAs are influenced by trauma exposure rather than PTSD itself. Association studies have linked CYP1B1-AS1 and its target gene CYP1B1 to 29 different types of cancer,[44] however, their specific role in PTSD remains unclear. SLC7A11-AS1, on the other hand, targets *SLC7A11*, a gene associated with scavenging reactive oxygen species (ROS) and has been implicated in the risk of Parkinson's disease.[20] Studies suggest that SLC7A11-AS1 acts as a tumor suppressor gene, with reduced expression linked to enhanced cell growth in gastric cancer.[45] Additionally, Yang Q et al demonstrated that SLC7A11-AS1 promotes chemoresistance in pancreatic cancer by stabilizing nuclear factor erythroid-2-related factor 2 (NRF2) to mitigate intracellular ROS.[46] Luo Y et al further reported that reduced SLC7A11-AS1 expression activates the p38MAPK-JNK signaling pathway, leading to increased expression of the cisplatin export gene *ATP7A* and the GSH biosynthesis gene *GCLM* in gastric cancer.[47] The p38MAPK-JNK pathway plays a significant role in stress and immune responses and is implicated in several neurological diseases.[48] Elevated levels of CYP1B1-AS1 and SLC7A11-AS1 in trauma-exposed individuals observed in our study may reflect a compensatory mechanism for the molecular changes associated with childhood trauma exposure. If validated in larger studies, these lncRNAs could serve as biomarkers of trauma exposure and potentially inform therapeutic strategies. Further, transcriptome regression analysis of mRNA data with the CYP1B1-AS1 and SLC7A11-AS1 expression shows a list of possibly altered mRNAs, which should be a focus of future molecular studies in PTSD.

IPA analysis of these DE-lncRNAs identified 16 canonical pathways and 11 biofunctional pathways that crossed the threshold value in the trauma-exposed PTSD group compared to controls, implicating several cellular functions. Network analysis revealed alterations in the NFAT5 network in trauma-exposed individuals relative to controls. NFAT5, a transcription factor, plays a crucial role in the development and activation of immune cells, particularly T cells and macrophages.[49] As a tonicity-responsive enhancer-binding protein, NFAT5 helps maintain cellular homeostasis in hypertonic and hyperosmotic environments. Loss of NFAT5 has been reported to alter the expression of over 3000 genes in the renal cortex and more than 5000 genes in the inner medulla, highlighting its role as a master regulator in renal tissues.[50] Emerging evidence also suggests that NFAT5 is critical in immune and inflammatory responses across diverse conditions.[49] For example, NFAT5 may activate the NLRP3 inflammasome to modulate inflammatory regulation in Parkinson's Disease (PD).[51] NFAT5 belongs to the Rel homology domain (RHD) protein family, which includes NF-κB factors and calcineurin-dependent NFAT1-4 (NFATc) proteins.[52,53] While the role of NFAT5 in PD pathogenesis is not well understood, NFATc proteins have been implicated in axonal outgrowth and neuronal responses, with their inhibition shown to mitigate PD development.[54,55] To further investigate the role of DE-lncRNAs such as CYP1B1-AS1, SLC7A11-AS1, and the downstream activation of NFAT5 in the olfactory mucosa of trauma-exposed and PTSD-positive individuals, future longitudinal studies with larger cohorts and translational validation are necessary. Additionally, larger sample sizes are required to determine the significance of NCMAP-DT, which is modestly downregulated in PTSD (Table 1). NCMAP-DT has been associated with height in young individuals, a characteristic potentially affected by childhood trauma.[56] While Y chromosome-linked genes (*LINC00278, TTTY14*, and *AC011297.1*) are potential confounders due to the disproportionate number of women in the PTSD group, including more men and women across all groups

in future studies will allow adjustments for sex and enable a clearer determination of their relevance as confounding factors.

Conclusion

Our study indicates the differential expression of LINC00278, TTTY14, AC011297.1, CYP1B1-AS1, SLC7A11-AS1, and downstream activation of NFAT5 in the olfactory mucosa of trauma-experienced and PTSD-positive African American individuals. We used for pathway analysis of differentially expressed lncRNAs in the three different groups to identify the specific canonical pathways, diseases, biological functions, and networks. These three lncRNAs, AC011297.1, CYP1B1-AS1, SLC7A11-AS1, and NFAT5, are linked to the inflammatory/immunoregulatory pathway and neurodevelopment in the olfactory mucosa, which are related to olfactory function. However, further studies are required to understand the relationship between DE-lncRNAs and mechanisms underlying PTSD development.

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Disclosure

The authors report no conflicts of interest in this work.

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