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Identification of Key Genes and Pathways Associated with Post-Traumatic Stress Disorder

A research submitted in partial fulfillment of the requirements for the degree of Master in Bioinformatics

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Abstract

Post-Traumatic Stress Disorder (PTSD) is a complex multifactorial mental health condition characterized by a range of symptoms, including intrusive thoughts, nightmares, hypervigilance, and emotional distress, significantly affecting an individual's quality of life. While PTSD is relatively common, with a prevalence ranging from 6% to 10%, it varies depending on the population and specific traumatic events experienced. However, the exact pathogenesis of PTSD remains unclear, and accurate diagnosis can be challenging due to the possibility of inaccuracies in reporting symptoms. Furthermore, preventive therapies for PTSD development are limited. This study aimed to bridge these gaps by conducting an integrative bioinformatics analysis that explores the molecular mechanisms, identifies diagnostic markers, and discovers therapeutic targets for PTSD. The analysis utilized mRNA microarray data from the GEO database, specifically the GSE860 dataset including only full PTSD patients and full healthy controls. Through this analysis, 418 differentially expressed genes (DEGs) were identified. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses were performed to gain insights into the biological processes and significant pathways associated with PTSD. Subsequently, a protein-protein interaction (PPI) network was constructed, leading to the identification of 14 hub genes and three key modules. Notably, all 14 hub genes, including NUP153, NUP50, NUP160, UBE2N, SP100, BIRC3, SMC4, YWHAZ, RB1, SUMO1, CDKN1B, CASP3, MAP2K4, and NBN, were found to be underexpressed in PTSD patients. The in-silico validation of these hub genes was performed using expression profiles and reference datasets, in addition to the Comparative Toxicogenomics Database (CTD). Functional enrichment analysis revealed the significant roles of the key genes in various biological processes, including cytokine signaling in the immune system, cell cycle regulation, and apoptosis-related processes such as DNA damage response and oxidative damage response. Moreover,

44 microRNAs (miRNAs) and 13 key transcription factors (TFs) targeting these hub genes were identified. Furthermore, computational drug repurposing analysis yielded 25 potential therapeutic compounds. Five of the findings, namely MAP2K4, NUP153, NFKB, hsa-mir-212-3p, and hsa-mir-199b-3p, were validated as PTSD biomarkers using the PTSD Biomarker Database (PTSDDB). This comprehensive bioinformatics analysis offers valuable insights into the mechanism of PTSD, potential biomarkers for clinical treatment, and the discovery of drug targets. The findings contribute to our understanding of PTSD and hold promise for further advancements in diagnosis and therapeutic interventions.

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List of Abbreviations

APA: American Psychiatric Association

BP: Biological Processes

CAPS: The Clinician-Administered PTSD Scale

CBT: Cognitive Behavioral Therapy

CC: Cellular Components

CNS: Central Nervous System

CPT: Cognitive Processing Therapy

CTD: The Comparative Toxicogenomics Database

DAVID: The Database for Annotation, Visualization and Integrated Discovery

DEGs: Differentially Expressed Genes

DGIdb: The Drug-Gene Interaction Database

DSM: The Diagnostic and Statistical Manual of Mental Disorders

EMDR: Eye Movement Desensitization and Reprocessing

ER: Emergency Room

ER Stress: Endoplasmic Reticulum Stress

FC: Fold Change

FDA: The US Food and Drug Administration

FDR: False Discovery Rate

GEO: Gene Expression Omnibus Database

GO: Gene Ontology

HPA Axis: Hypothalamic-Pituitary-Adrenal Axis

IES: Impact of event scale

KEGG: Kyoto Encyclopedia of Genes and Genomes Pathway

MCODE: Molecular Complex Detection

MF: Molecular Functions

miRNAs: microRNAs

M1 PTSD: Clinical diagnosis 1 month after the trauma

M4 PTSD: Clinical diagnosis 4 month after the trauma

PCL: The PTSD Checklist

PE: Prolonged Exposure

PMBCs: Peripheral Mononuclear Blood Cells

PPI: Protein-Protein Interaction

PTSD: Post-Traumatic Stress Disorder

PTSDDB: PTSD Biomarker Database

SNRIs: Serotonin-Norepinephrine Reuptake Inhibitors

SSRIs: Selective Serotonin Reuptake Inhibitors

STRING: Search Tool for Retrieval of Interacting Genes/Proteins

TFs: Transcription Factors

TRRUST: The Transcriptional Regulatory Relationships Unraveled by Sentence Based Text Mining database

UMAP: Uniform Manifold Approximation and Projection Plot

UPR: Unfolded Protein Response

WebGestalt: The Web-based Gene Set Analysis Toolkit

Introduction

Post-Traumatic Stress Disorder (PTSD) is a chronic and multifactorial psychological disorder that can occur in individuals who have experienced or witnessed a traumatic event with a prevalence that is estimated to be around 6-10% in the general population, and as high as 35% among individuals with a history of significant trauma (Núñez-Rios et al., 2022). According to American Psychiatric Association (APA), PTSD symptoms can be categorized into four main symptom clusters: intrusive re-experiencing of the traumatic event, avoidance of trauma-related cues, negative alterations in mood and cognition, and hyperarousal symptoms (American Psychiatric Association, 2017). Intrusive re-experiencing involves distressing memories, flashbacks, or nightmares related to the traumatic event. Avoidance behaviors may manifest as efforts to avoid any reminder of the trauma including thoughts, feelings, or situations. Negative alterations in mood and cognition can lead to persistent negative beliefs, distorted thoughts, emotional numbness, and loss of interest in previously enjoyed activities. Hyperarousal symptoms include irritability, difficulty sleeping, hypervigilance, and exaggerated startle responses. These symptoms can significantly impair social relationships, work performance, and overall quality of life (Brewin et al., 2017).

PTSD stands out from other mental disorders due to its distinctive requirement of exposure to a traumatic event as a prerequisite for its development. These events can vary widely and may include experiences such as war, natural disasters, physical or sexual assault, or serious accidents. The severity and nature of the trauma can have a significant impact on the development and course of PTSD symptoms. Notably, individuals who have experienced prolonged exposure to combat situations or survived life-threatening earthquakes may be particularly vulnerable to developing PTSD (Bryant et al., 2009; Norris et al., 1999).

The diagnosis of PTSD requires a thorough assessment of an individual's symptoms, history, and background. The Diagnostic and Statistical Manual of Mental Disorders (DSM-5), published by APA, states that individuals must have been exposed to a traumatic event and exhibit specific symptoms that last for at least one month and significantly impact their functioning and well-being. Evaluating symptoms at multiple time points, such as one month and four months, helps ensure that the symptoms are persistent and not temporary reactions to the trauma (American Psychiatric Association, 2017). The assessment typically includes a detailed assessment of the individual's trauma history, current symptoms, and their impact on daily functioning. Clinicians may use validated assessment tools, such as the Clinician-Administered PTSD Scale (CAPS) or the PTSD Checklist for DSM-5 (PCL-5), to aid in the diagnostic process (Blevins et al., 2015).

While 60-90% of people experience at least one traumatic event in their lifetime, not all of them develop PTSD, highlighting the concept of resilience (Benjet et al., 2016; Broekman et al., 2007). The variability in individuals' response to trauma reveals the involvement of underlying biological mechanisms that contribute to the risk of developing PTSD (Wolf et al., 2018).

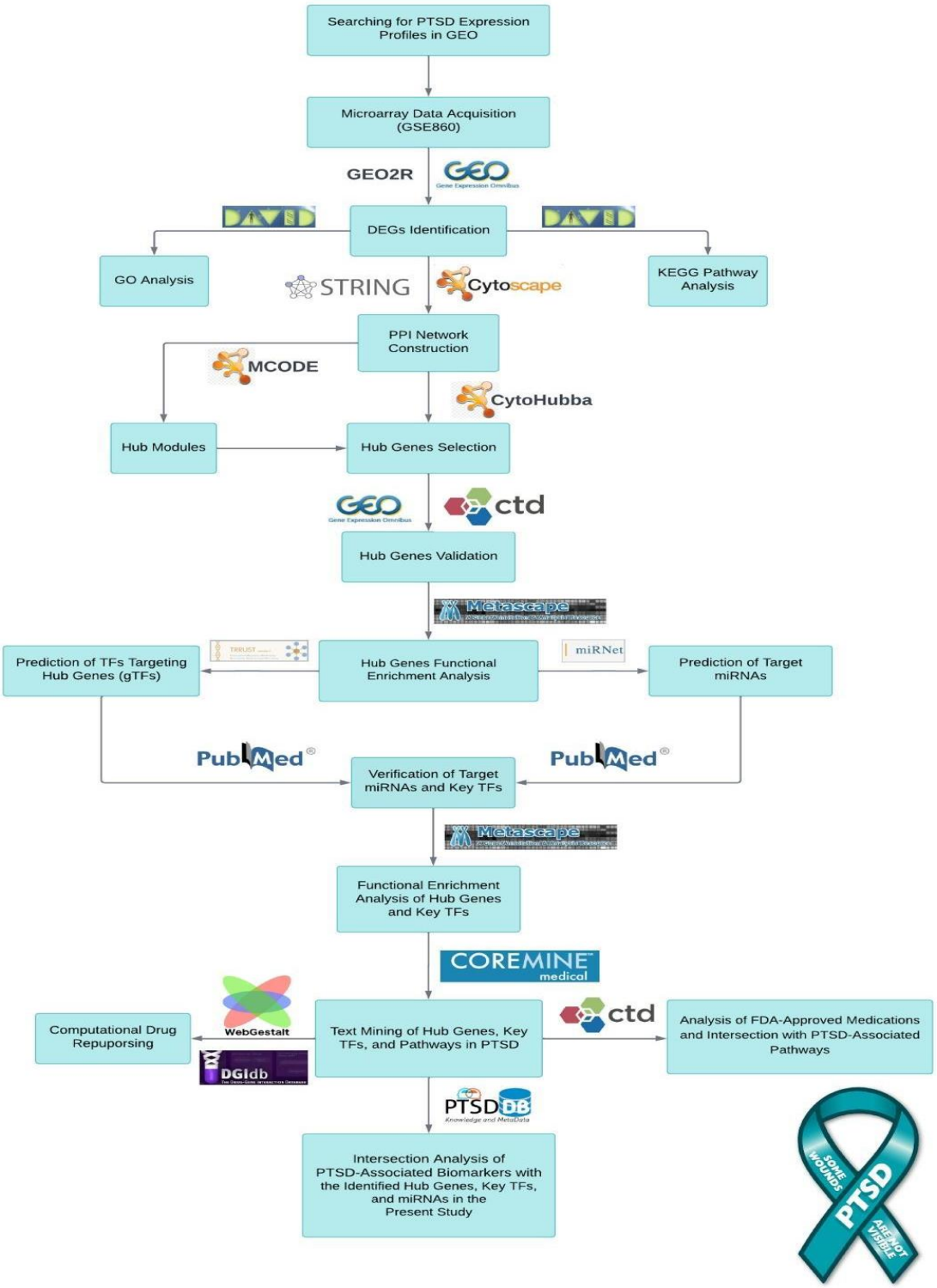
Numerous studies have been conducted to better understand the causes, risk factors, and effective treatment approaches for PTSD. Research suggests that the development of PTSD is influenced by a complex interplay of genetic and environmental factors. Research has shown that genetic factors contribute to individual differences in vulnerability to PTSD, with certain genes involved in stress response and fear regulation playing a significant role (Almli et al., 2014; Binder et al., 2008). Variations in genes related to stress response systems, such as the hypothalamic-pituitary-adrenal axis and neurotransmitter pathways involved in fear and anxiety, may directly influence an individual's susceptibility to PTSD (Logue et al., 2015; Nievergelt et al., 2018). Heritability estimates for PTSD range from approximately 30% to 40%, suggesting a significant genetic component in its development (Smoller et al., 2019).
However, it is important to note that genetic factors alone do not determine the occurrence of PTSD. Additionally, environmental factors, including early life experiences, trauma severity, and social support, also contribute to its development. As well as, epigenetic mechanisms, such as DNA methylation and histone modifications, may play a role in how PTSD affects physiological responses by altering gene expression (Mehta et al., 2013; Yehuda et al., 2016).

The management of PTSD requires a comprehensive approach that includes both pharmacological and psychotherapeutic interventions. Currently, selective serotonin reuptake inhibitors (SSRIs) and serotonin-norepinephrine reuptake inhibitors (SNRIs), are commonly used medications for PTSD, targeting symptoms such as anxiety, depression, and sleep disturbances. In addition, psychological treatments such as cognitive-behavioral therapy (CBT) and eye movement desensitization and reprocessing (EMDR) have shown efficacy in reducing PTSD symptoms and enhancing overall psychological well-being (Stein et al., 2006).

Current research indicates that the development of PTSD is linked to the autonomic nervous system, hypothalamic-pituitary-adrenal axis, neural circuits, and immune system. Despite advancements, the precise pathogenesis of PTSD remains incompletely understood. Therefore, there is a growing need to delve deeper into identifying the causal factors, molecular mechanisms, and pathways involved in PTSD
Fortunately, advancements in high-throughput technology, such as microarrays and next-generation sequencing, along with the application of bioinformatics analysis, have greatly facilitated the evaluation of genomic, transcriptomic, epigenomic, and proteomic

alterations in both central and peripheral tissues of individuals with PTSD, as well as animal models of the disorder and have enabled the identification of numerous genes and pathways that are associated with the development and progression of PTSD (Bian et al., 2019; Núñez-Rios et al., 2022).

In this study, an integrative bioinformatics approach was employed to analyze mRNA microarray data from PTSD patients sourced from the GEO database. The primary objectives were to explore hub genes, elucidate molecular mechanisms, identify potential diagnostic biomarkers, and uncover therapeutic targets for PTSD. The analysis successfully identified DEGs, with a particular focus on 14 hub genes. To gain insights into the GO and KEGG pathway enrichment analyses were conducted. Additionally, the study predicted and verified the regulatory network involving miRNAs and key TFs associated with PTSD and their influence on the hub genes. Computational drug repurposing analysis was also performed to investigate potential therapeutic compounds. Furthermore, the study examined PTSD biomarkers using the PTSDDB to enhance understanding in this area. Overall, this bioinformatics study provides valuable insights into the pathogenesis of PTSD, potential biomarkers for clinical treatment, and drug target discovery, with implications for diagnosis and therapies.



The Concise Diagram of Research Flowchart

Materials and Methods

1. The Subjects:

This study involved individuals who experienced a traumatic event and were immediately admitted to the emergency room (ER). These individuals were assessed for acute or chronic PTSD according to the DSM IV1 diagnostic criteria at two subsequent follow-up points, one month and four months later. Additionally, individuals who did not meet any DSM IV1 diagnostic criteria during these two time points were also included in the study.Therefore, the samples in this study are subdivided into two groups:

1- Subjects with full consistent PTSD at month 1 and month 4.
2- Subjects that hadn't met any PTSD criterion at any time.

The exclusion criteria of this study was subjects that had head injury, burn injury or serious physical injury, had current or lifetime history of alcohol or illicit drugs abuse, had past or present psychiatric diagnoses other than depressive or anxiety disorders, or had medical or neurological illness that could confound the assessments (Segman et al., 2005).

A clear representation of the data pertaining to the subjects was provided **(Table_1)**, including:

M1_PTSD: Clinical diagnosis 1 month after the trauma (Y: full-blown acute PTSD, N: No diagnostic criteria for PTSD).
M4_PTSD: Clinical diagnosis 4 month after the trauma (Y: full-blown chronic PTSD, N: No diagnostic criteria for PTSD).
M4 IES_Intrusion, M4 IES_Avoidance, M4 IES_Arousal: Impact of event scale for the mentioned symptoms 4 months after the trauma.
M4 IES_Total: Total score for impact of event scale 4 months after the trauma.

Table_1: *Subjects Characteristics*

Subject #	M1 PTSD	M4 PTSD	Gender	Age	M4 IES_ intrusion	M4 IES_ avoidance	M4 IES_ arousal	M4 IES_ total	Comorbid Psychiatric Diagnoses	Trauma Severity	PTSD Status
1P	Y	Y	F	56	33	17	24	74		20	Full consis-tent PTSD at 1 and 4 months
2P	Y	Y	M	36	15	14	15	44		15	
3P	Y	Y	F	21.5	15	18	21	54		13	
4P	Y	Y	M	21	35	34	27	96		22	
5P	Y	Y	M	43	19	20	23	62		12	
6P	Y	Y	M	21	35	18	33	86	Depression/ OCD/Body Dysmorphic Disorder	26	
7P	Y	Y	F	25	29	19	17	65	Depression	15	
8P	Y	Y	F	46	35	16	35	86		28	
14C	N	N	M	31	1	1	3	5		19	No PTSD criter-ion met at any time
15C	N	N	F	22	0	0	1	1		9	
16C	N	N	M	25	3	0	4	7		23	
17C	N	N	F	20	3	5	5	13		10	
18C	N	N	M	23	0	0	0	0		10	
19C	N	N	F	24	2	11	0	13		9	

2. Microarray Data Acquisition:

GEO stands for **G**ene **E**xpression **O**mnibus (https://www.ncbi.nlm.nih.gov/geo/) is an international public genomics database for high-throughput gene expression data, chips, microarrays and RNA methylation profiling managed by the National Center of Biotechnology Information (NCBI) (Barrett et al., 2013).
The dataset used in this research, namely GSE860, consists of normalized mRNA microarray expression profiling data obtained from peripheral mononuclear blood cells (PMBC) of 33 individuals diagnosed with post-traumatic stress disorder (PTSD). The data was sourced from the GEO database.
However, it is important to note that only individuals who met the criteria for full PTSD diagnosis at both the 1-month and 4-month follow-up assessments, as well as individuals who did not receive a PTSD diagnosis at any time point, were included in this study. Consequently, the study sample size consists of 8 PTSD subjects (11 PMBC samples: 6 ER and 5 M4) and 6 control subjects (9 PMBC samples: 5 ER and 4 M4).

3. Identification of Differentially Expressed Genes:

DEGs were determined using **GEO2R** (https://www.ncbi.nlm.nih.gov/geo/geo2r/) an online analytical tool available at GEO that uses the GEOquery and limma R packages from the Bioconductor project.
The GEOquery R package is utilized to extract GEO data and convert it into R data structures, allowing for further analysis using other R packages. Among these packages, limma (Linear Models for Microarray Analysis) has gained popularity as a powerful statistical tool for identifying genes that are differentially expressed. (Barrett et al., 2013)
The analysis was made based on the following parameters; multiple testing correction is the Benjamini and Hochberg False Discovery Rate (FDR), Significance level cut-off $P < 0.05$, and $|\text{Log2 Fold Change (FC)}| > 0.2$.

4. Functional Annotation of DEGs by GO and KEGG Pathway Enrichment Analysis:

Functional annotation of genes that exhibit differential expression is an essential and crucial step in analyzing microarray data. The **D**atabase for **A**nnotation, **V**isualization and **I**ntegrated **D**iscovery **DAVID** (https://david.ncifcrf.gov/home.jsp) was used to functionally categorize the significant DEGs, as the functionality provided by DAVID accelerates the analysis of genome-scale datasets by facilitating the transition from data collection to biological meaning (Dennis et al., 2003).
Gene Ontology (GO) covers three main aspects of biology: Biological Process (BP), Cellular Component (CC), and Molecular Function (MF), while Kyoto Encyclopedia of Genes and Genomes (KEGG) is used to understand the relevant signaling pathways.

In order to obtain GO and KEGG enrichment analysis of the significant DEGs, DAVID was used with P-value>0.05.

5. PPI Network Construction & Analysis:

STRING Search **T**ool for **R**etrieval of **I**nteracting **G**enes/Proteins (https://string-db.org/) is a comprehensive database that provides predicted functional associations between proteins used to explore protein interactions, pathways, and functional relationships (von Mering et al., 2003).

The PPI network of the identified DEGs was built by considering only interactions with a minimum required interaction score of 0.9, indicating the highest level of confidence in the interactions included in the network.

6. PPI Network Module Analysis & Hub Genes Selection:

The analysis results of the PPI network were loaded into **Cytoscape** software (version 3.9.1) for visual adjustment. Cytoscape, an open-source software project, facilitates the integration of biomolecular interaction networks with high-throughput expression data and other molecular states, providing a unified conceptual framework (Shannon et al., 2003).

The Cytoscape plugin **CytoHubba** was used to calculate the connectivity scores and identify the intersections among the first 30 genes. Since a single algorithm sometimes produces false positives, a four-fold algorithm was adopted, combining two local-based algorithms (MCC and Degree) and two global-based algorithms (Stress and Radiality).
To identify the central hub genes, a Venn diagram was generated using Venny 2.1, (https://bioinfogp.cnb.csic.es/tools/venny/), overlapping the first 30 genes obtained from the four aforementioned methods.

Then, the **M**olecular **Co**mplex **De**tection (**MCODE**) plug-in within Cytoscape, which enables the clustering of a given network based on topology to discover densely connected regions, was applied for further analysis.

7. Validation of Hub Genes:

Due to the absence of a dedicated gene expression database for PTSD, it was necessary to employ multiple databases and methodologies to validate the identified hub genes.
First, **CTD**, **C**omparative **T**oxicogenomics **D**atabase (https://ctdbase.org/) a robust database that provides manually curated information about chemical–gene/protein interactions, chemical–disease and gene–disease relationships was used to confirm the association between the hub genes and PTSD (Davis et al., 2021).

Subsequently, **GEO Profiles** (https://www.ncbi.nlm.nih.gov/geoprofiles) was used to search for expression profiles related to chronic stress. A dataset with the title “Chronic stress effect on peripheral blood monocytes” GDS3383 was queried.

Finally, I referred to PTSD Blood Transcriptome Mega-Analysis which incorporates five independent PTSD blood transcriptome studies covering seven types of traumas in 229 PTSD and 311 comparison individuals (Breen et al., 2018).

8. Hub Genes Expression Profiles:

After intersection of four CytoHubba algorithms, extracting the top 3 most significant clusters, then finally validate the results in-silico by querying CTD and retrieving expression profiles of chronic stress patients from GEO Profile database and referring to five independent PTSD blood transcriptome studies, the final set of key genes could be identified.
The Hub genes expression profiles were generated by using **ExpressAnalyst** (https://www.expressanalyst.ca/) ; a unified platform for gene expression analysis.

9. Hub Genes Functional Enrichment Analysis:

At first, the **STRING** database was used to visualize the PPI of the hub genes. The first analysis was performed by **Metascape** (http://metascape.org/), a gene function annotation tool used to apply bioinformatics methods to batch analysis of genes and proteins. It was used to conduct the functional and pathway enrichment analysis of the selected hub genes (Y. Zhou et al., 2019).

The **DAVID** database was used to generate a functional annotation clustering of the 14 hub genes based on the associated biological processes and pathways. It categorized genes based on their functional annotations which facilitate the identification of biological themes and enriched functional categories which provide a valuable feature for the analysis and interpretation of hub genes.

10. Prediction of Target miRNAs:

The prediction of miRNAs targeting the hub genes was performed using **miRNet** (https://www.mirnet.ca/), an open-source online platform created to help understanding microRNA (miRNA) functions by integrating users' data with existing knowledge through network-based visual analytics (Chang et al., 2020).

To verify the differential expression of miRNAs in PTSD and healthy patients, a list of miRNAs previously validated in two studies was extracted from Pubmed (Martin et al., 2017; Snijders et al., 2019). This step aimed to assess the expression patterns of these miRNAs in the context of PTSD.

11. Prediction of Target Transcription Factors (TFs):

To enhance our understanding of the hub genes,further investigation was conducted to explore the molecular mechanisms that regulate their expression.
The **T**ranscriptional **R**egulatory **R**elationships **U**nraveled by **S**entence Based **T**ext Mining **(TRRUST)** database (https://www.grnpedia.org/trrust/) -a manually curated database of human transcriptional regulatory networks- was used through **miRNET** to predict the TFs that regulate the identified hub genes (H. Han et al., 2015).
To validate the differential expression of the identified TFs in individuals with PTSD compared to healthy individuals, I conducted an extensive review of multiple studies, in addition to the findings of this study (Breen et al., 2015, 2019; Seah et al., 2022).
Finally, a functional and pathway enrichment analysis was performed for a total of 26 genes including hub genes and key TFs by **Metascape**.

12. Text Mining Analysis of Hub Genes, Key TFs, and Pathways in PTSD:

Text mining was conducted using **Coremine Medical** (https://coremine.com/medical/), the world's most advanced medical information retrieval platform, to investigate the association between hub genes, key TFs, and the main enriched pathways in the context of PTSD.

13. Analysis of FDA-Approved Medications and Intersections with PTSD-Associated Pathways:

According to the US Food and Drug Administration FDA (https://www.accessdata.fda.gov/), Sertraline and Paroxetine Hydrochloride are the only two medications that are FDA-approved in PTSD.
CTD (https://ctdbase.org/) was used to study the chemical-gene and chemical-pathway interactions of these medications in purpose of evaluating and comparing the results with our findings.

14. Computational Drug Repurposing for PTSD Treatment:

The goal of computational drug repurposing is to predict enriched drug target genes and explore potential therapeutic options for hub genes and key TFs. This approach enabled the identification of potential therapeutic options and the exploration of gene-drug interactions for further analysis and consideration.

WebGestalt (https://www.webgestalt.org/) , also known as the **WEB**-based **GE**ne **S**e**T** **A**na**L**ysis **T**oolkit, is a popular tool used for gene set enrichment analysis. It enables users to gain biological insights from their genes of interest by employing three main

methods: Over Representation Analysis (ORA), Gene Set Enrichment Analysis (GSEA), and Network Topology-based Analysis (NTA) (Liao et al., 2019).
The analysis involved utilizing a recommended functional database, **DrugBank**, in conjunction with **WebGestalt**.

A second drug repurposing analysis was performed utilizing the **D**rug-**G**ene **I**nteraction **D**ata**b**ase **DGIdb** (https://www.dgidb.org/) to identify druggable gene targets with the aim of exploring their potential as therapeutic options for pharmacological treatments.
DGIdb analyzes and examines interactions between drugs and genes. It assigns an interaction score to each gene-drug pair. This scoring system aids in identifying and prioritizing gene-drug interactions for further investigation and analysis (Freshour et al., 2021).

15. Intersection analysis of PTSD-associated Biomarkers with the Identified Hub Genes, Key TFs, and miRNAs in the Present Study through PTSD Biomarker Database:

The PTSD Biomarker Database **PTSDDB** (https://ptsd.scai.fraunhofer.de/) is a comprehensive database that presents an overview of physiological markers investigated as potential biomarkers in the current PTSD research. Its purpose is to facilitate quick exploration and comparison of findings for researchers. The database currently includes more than 900 biomarkers along with relevant information from 109 original articles published between 1997 and 2017. The curated content is accompanied by a web application featuring interactive visualizations, allowing researchers to examine biomarker knowledge in PTSD. These visualizations encompass clinical study metadata, biomarker findings, experimental methods, and more. By compiling results from biomarker studies, the database offers insights into the level of evidence supporting individual biomarkers and functional categories. This resource represents the initial effort to gather and organize biomarker data and metadata in the field of PTSD, providing a comprehensive database that can support future analysis and research in this area (Domingo-Fernández et al., 2019).

Initially, PTSDDB was used to extract the 10 most frequently observed biomarkers in PTSD. Furthermore, PTSDDB provides an examination of PTSD biomarkers in different biofluids separately. Consequently, I specifically retrieved PTSD biomarkers associated with PMBCs since my study focuses on mRNA microarray expression profiling data from PMBC samples.
As a final step, an intersection analysis was conducted between the identified hub genes, key TFs, and miRNAs associated with PTSD in my study, and the content available in PTSDDB.

Results

1. Identification of Differentially Expressed Genes (DEGs):

The analysis was conducted using specific parameters, including the Benjamini and Hochberg False Discovery Rate (FDR) for multiple testing correction, a significance level cut-off of $P < 0.05$, and a threshold of $|\text{Log2 Fold Change (FC)}| > 0.2$. As a result, a total of 418 genes were identified as differentially expressed genes (DEGs), with 372 genes being upregulated and 46 genes being downregulated in the control group compared to the PTSD group.

A volcano plot was generated to visualize DEGs by displaying statistical significance (-log10 P value) versus magnitude of change (log2 fold change) where highlighted genes are significantly differentially expressed at a default adjusted p-value cutoff of 0.05 (red = upregulated, blue = downregulated) **(Figure_1)**.

In addition to the volcano plot, a Uniform Manifold Approximation and Projection (UMAP) can be generated through **GEO2R** in order to visualize how samples are related to each other, where the number of nearest neighbors used in the calculation is 9 **(Figure_2)**.

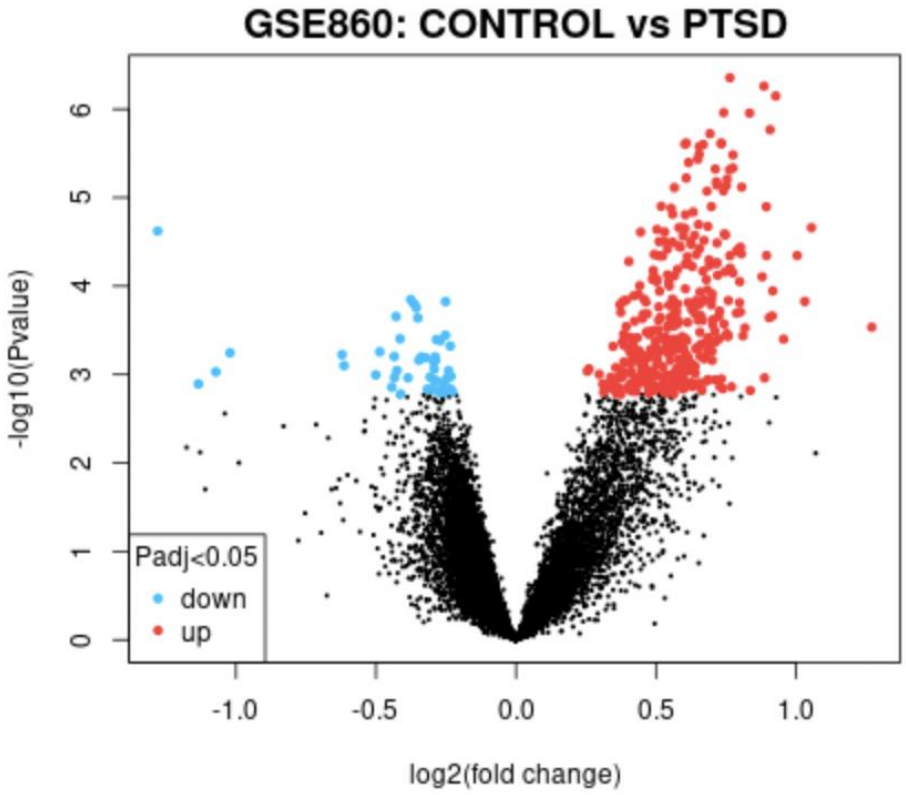


Figure_1: ***Volcano Plot of DEGs.***

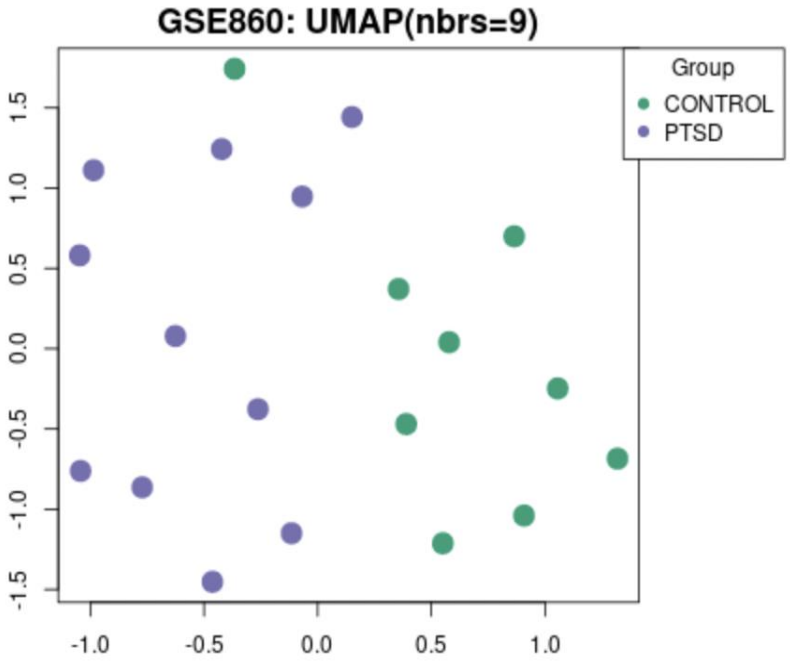


Figure_2: *UMAP Plot.*

2. Functional Annotation of DEGs by GO and KEGG Pathway Enrichment Analysis:

DAVID was employed to conduct GO and KEGG enrichment analysis of the significant DEGs with a significance threshold of P-value<0.05 **(Table_2)**.

The analysis showed that these DEGs were mainly involved in many biologic processes associated with mRNA maturation and Transcription including; RNA splicing, mRNA processing, mRNA splicing via spliceosome, DNA-templated transcription, positive regulation of transcription by RNA polymerase II, negative regulation of transcription by RNA polymerase II, negative regulation of DNA-templated transcription, regulation of transcription by RNA polymerase II. In addition, several biologic processes related to cell cycle regulation were highlighted including DNA damage response, cell division, regulation of cell cycle, and apoptotic process.

The screened DEGs were mainly located in nucleus, nucleoplasm, cytosol, cytoplasm, intracellular membrane-bounded organelle, nucleolus, endoplasmic reticulum, and Golgi apparatus.

With regards to molecular functions, the DEGs were enriched in binding of several molecular compounds and enzymes including; protein, RNA, DNA, ATP, cadherin, chromatin, zinc ion, protein kinase, and identical protein.

Finally, the analysis of KEGG pathways indicated that the pathways associated with DEGs were Platinum drug resistance, spliceosome, nucleocytoplasmic transport, apoptosis, cell cycle, TNF signaling pathway, Ubiquitin mediated proteolysis, transcriptional misregulation in cancer, and viral carcinogenesis.

Table_2: ***Gene Ontology and KEGG Pathways Analysis of DEGs Associated with PTSD.***

Category	Term	Gene Count	P-Value
Biological Process			
GOTERM_BP_DIRECT	RNA splicing	18	1.82E-07
GOTERM_BP_DIRECT	mRNA processing	15	6.19E-05
GOTERM_BP_DIRECT	cellular response to DNA damage stimulus	15	6.83E-04
GOTERM_BP_DIRECT	mRNA splicing, via spliceosome	12	1.26E-03
GOTERM_BP_DIRECT	chromatin remodeling	11	2.01E-03
GOTERM_BP_DIRECT	cell division	17	2.03E-03
GOTERM_BP_DIRECT	transcription, DNA-templated	12	2.65E-03
GOTERM_BP_DIRECT	protein transport	18	3.28E-03
GOTERM_BP_DIRECT	positive regulation of transcription from RNA polymerase II promoter	36	5.34E-03
GOTERM_BP_DIRECT	regulation of cell cycle	14	7.19E-03
GOTERM_BP_DIRECT	apoptotic process	21	8.86E-03
GOTERM_BP_DIRECT	negative regulation of transcription from RNA polymerase II promoter	29	1.27E-02
GOTERM_BP_DIRECT	negative regulation of transcription, DNA-templated	20	1.35E-02
GOTERM_BP_DIRECT	regulation of transcription from RNA polymerase II promoter	44	2.41E-02
Cell Component			
GOTERM_CC_DIRECT	nucleus	183	4.67E-18
GOTERM_CC_DIRECT	nucleoplasm	139	1.03E-17
GOTERM_CC_DIRECT	cytosol	166	4.97E-15
GOTERM_CC_DIRECT	cytoplasm	132	7.25E-05
GOTERM_CC_DIRECT	intracellular membrane-bounded organelle	32	1.16E-03
GOTERM_CC_DIRECT	nucleolus	41	1.45E-03
GOTERM_CC_DIRECT	endoplasmic reticulum	30	2.88E-02
GOTERM_CC_DIRECT	Golgi apparatus	30	2.99E-02
Molecular Function			

GOTERM_MF_DIRECT	protein binding	304	1.85E-14
GOTERM_MF_DIRECT	RNA binding	57	4.26E-07
GOTERM_MF_DIRECT	DNA binding	45	2.50E-04
GOTERM_MF_DIRECT	ATP binding	49	4.61E-04
GOTERM_MF_DIRECT	ATPase activity	17	1.28E-03
GOTERM_MF_DIRECT	cadherin binding	15	3.01E-03
GOTERM_MF_DIRECT	chromatin binding	19	3.94E-03
GOTERM_MF_DIRECT	zinc ion binding	27	1.61E-02
GOTERM_MF_DIRECT	protein kinase binding	18	1.85E-02
GOTERM_MF_DIRECT	identical protein binding	44	3.93E-02
KEGG Pathways			
KEGG_PATHWAY	Platinum drug resistance	9	1.69E-04
KEGG_PATHWAY	Spliceosome	11	1.31E-03
KEGG_PATHWAY	Nucleocytoplasmic transport	9	2.33E-03
KEGG_PATHWAY	Apoptosis	10	2.74E-03
KEGG_PATHWAY	Apoptosis - multiple species	5	4.79E-03
KEGG_PATHWAY	Cell cycle	9	6.28E-03
KEGG_PATHWAY	TNF signaling pathway	8	1.08E-02
KEGG_PATHWAY	Ubiquitin mediated proteolysis	9	1.20E-02
KEGG_PATHWAY	Transcriptional misregulation in cancer	10	2.45E-02
KEGG_PATHWAY	Viral carcinogenesis	10	3.34E-02

3. PPI Network Construction:

With defining the minimum required interaction score as the highest confidence 0.9, a gene network that contains; 382 nodes, 145 edges, 0.759 as average node degree, 0.238 as average local clustering coefficient, and 0.000448 as PPI enrichment P-values was built by **STRING**.

The expected number of edges is 109. However, the constructed network has more interactions than expected which means that the screened proteins have more interactions among themselves than what would be expected for a random set of proteins of the same size and degree distribution drawn from the genome. This indicated that the proteins are at least partially biologically connected as a group **(Figure_3).**

The functional enrichment analysis of the PPI network showed that the highlighted pathways are apoptosis, spliceosome, and Platinum drug resistance.

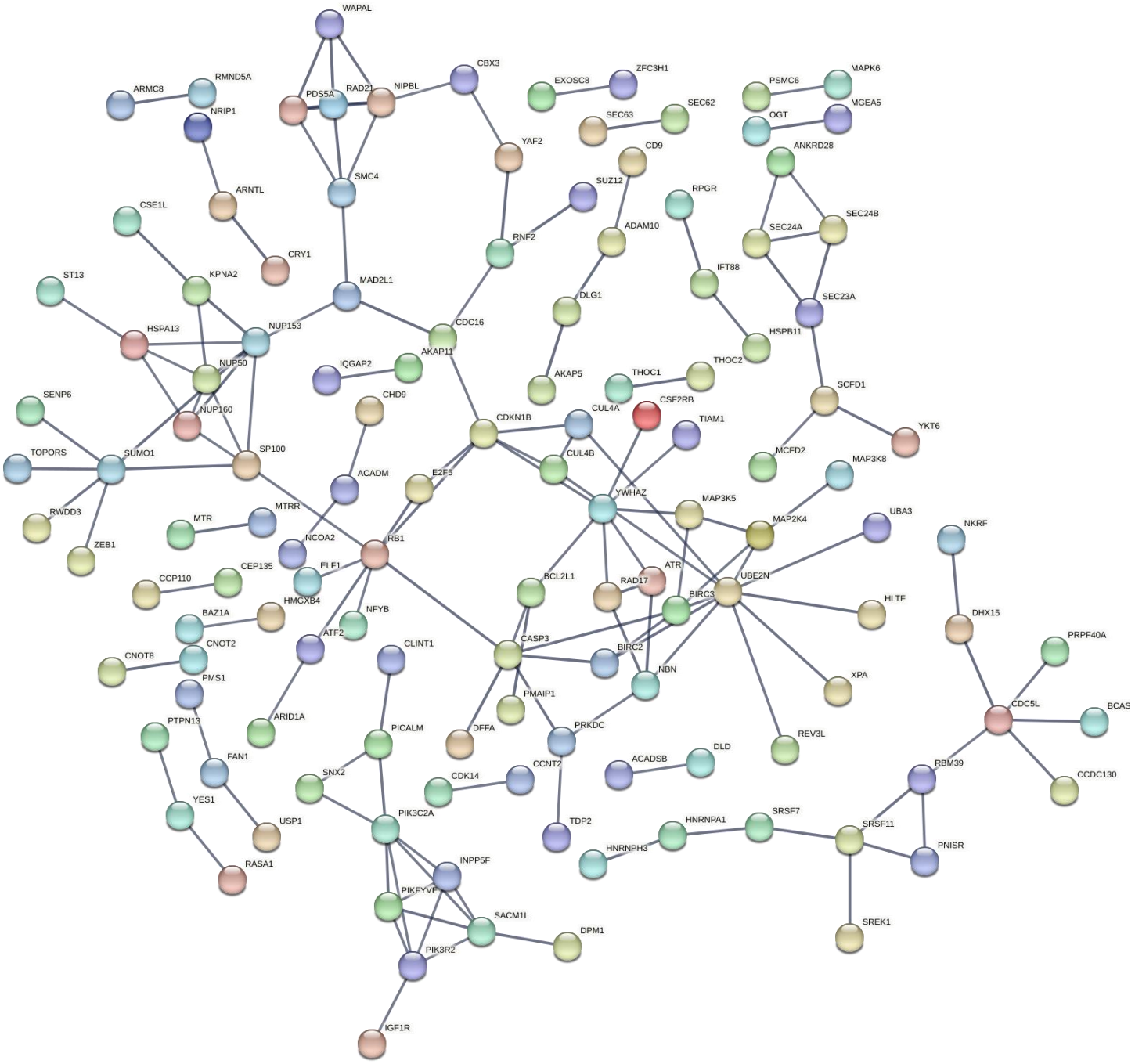


Figure_3: ***The DEGs Protein-Protein Interaction (PPI) Network by STRING Database.***

4. PPI Network Module Analysis & Hub Genes Selection:

The **CytoHubba** plugin was employed to calculate connectivity scores and determine the common genes among the top 30 genes with a combination of four algorithms (MCC, Degree, Stress, and Radiality) **(Table_3) (Figure_4)**.

All 4 methods identified 14 common central hub genes among the top 30 hub genes: NUP153, NUP50, NUP160, UBE2N, SP100, BIRC3, SMC4, YWHAZ, RB1, SUMO1, CDKN1B, CASP3, MAP2K4, NBN. On the other hand, 7 genes (PIK3C2A, PIK3R2, SACM1L, PIKFYVE, INPP5F, WAPAL, CDC5L, SRSF11) were identified by 3 of the 4 algorithms, and 8 genes (PIK3C2A, PIK3R2, SACM1L, PIKFYVE, INPP5F, WAPAL, CDC5L, SRSF11) were identified by 2 of the 4 algorithms **(Figure_5)**.

A total of 3 significant modules were defined from the PPI network using the plugin **MCODE** with a criterion of selection as follows: degree cut-off = 2, node score cut-off = 0.2, k-score = 2, and Max depth = 100 **(Table_4) (Figure_6)**.

To ensure the presence of the 14 defined hub genes in the most significant modules, an overlap analysis was conducted. The intersection revealed five genes: NUP153, NUP50, NUP160, SP100, and SMC4 **(Figure_7)**.

Table_3: ***The Top 30 Hub Genes Rank in CytoHubba by 4 Different Methods.***

Local-Based Methods		Global-Based Methods	
MCC	**Degree**	**Stress**	**Rdiality**
PIK3C2A	UBE2N	RB1	CDKN1B
PIK3R2	NUP153	CDKN1B	RB1
SACM1L	YWHAZ	SP100	CDC16
PIKFYVE	RB1	UBE2N	SP100
INPP5F	SUMO1	CDC16	CASP3
NUP153	PIK3C2A	CASP3	YWHAZ
NUP50	CDKN1B	MAD2L1	E2F5
NIPBL	CASP3	NUP153	CUL4A
RAD21	SP100	YWHAZ	CUL4B
PDS5A	PIK3R2	SMC4	MAD2L1
NUP160	NUP50	CUL4A	NUP153
UBE2N	SACM1L	CUL4B	BIRC3
SP100	NIPBL	SUMO1	BCL2L1
BIRC3	BIRC3	BIRC3	SUMO1
SMC4	CDC5L	NUP50	UBE2N
HSPA13	SRSF11	HSPA13	NUP50
YWHAZ	RAD21	RNF2	BIRC2
RB1	PDS5A	BIRC2	NUP160
SUMO1	SMC4	KPNA2	ATF2
WAPAL	PIKFYVE	MAP2K4	NFYB
CDKN1B	INPP5F	PRKDC	ELF1
CASP3	NUP160	NIPBL	PRKDC
MAP2K4	HSPA13	NUP160	MAP3K5
CDC5L	MAP2K4	MAP3K5	RNF2
SRSF11	NBN	BCL2L1	RAD17
CUL4A	SEC23A	NBN	ATR
CUL4B	WAPAL	ATF2	NBN
BIRC2	SCFD1	RAD21	MAP2K4
RAD17	KPNA2	PDS5A	DFFA
NBN	PICALM	YAF2	SMC4

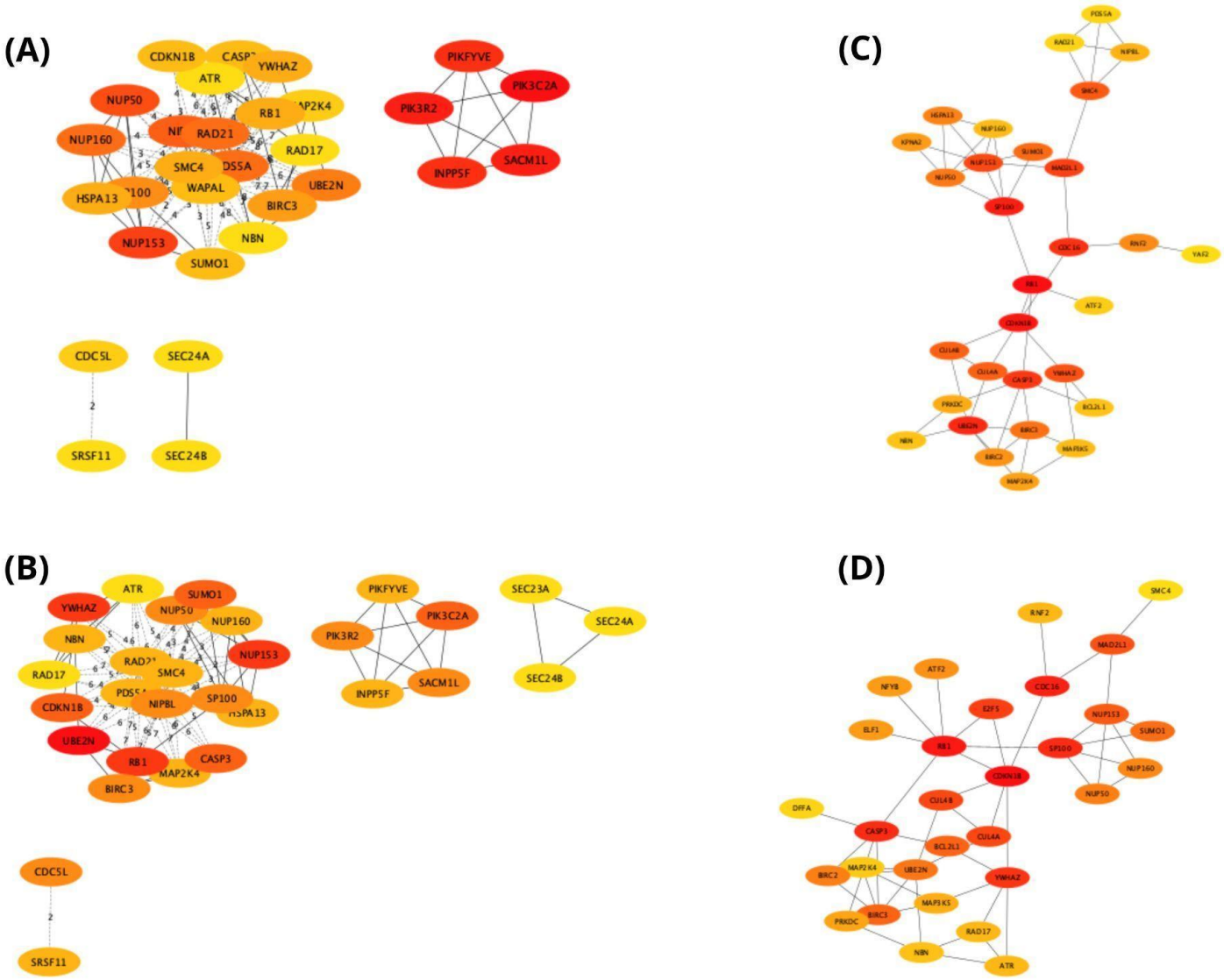


Figure_4: ***Hub Gene Networks Identified from the PPI Network Using (A) MCC Algorithm; (B) Degree Algorithm; (C) Stress Algorithm; and (D) Radiality Algorithm of the Cytoscape Plug-in CytoHubba.***

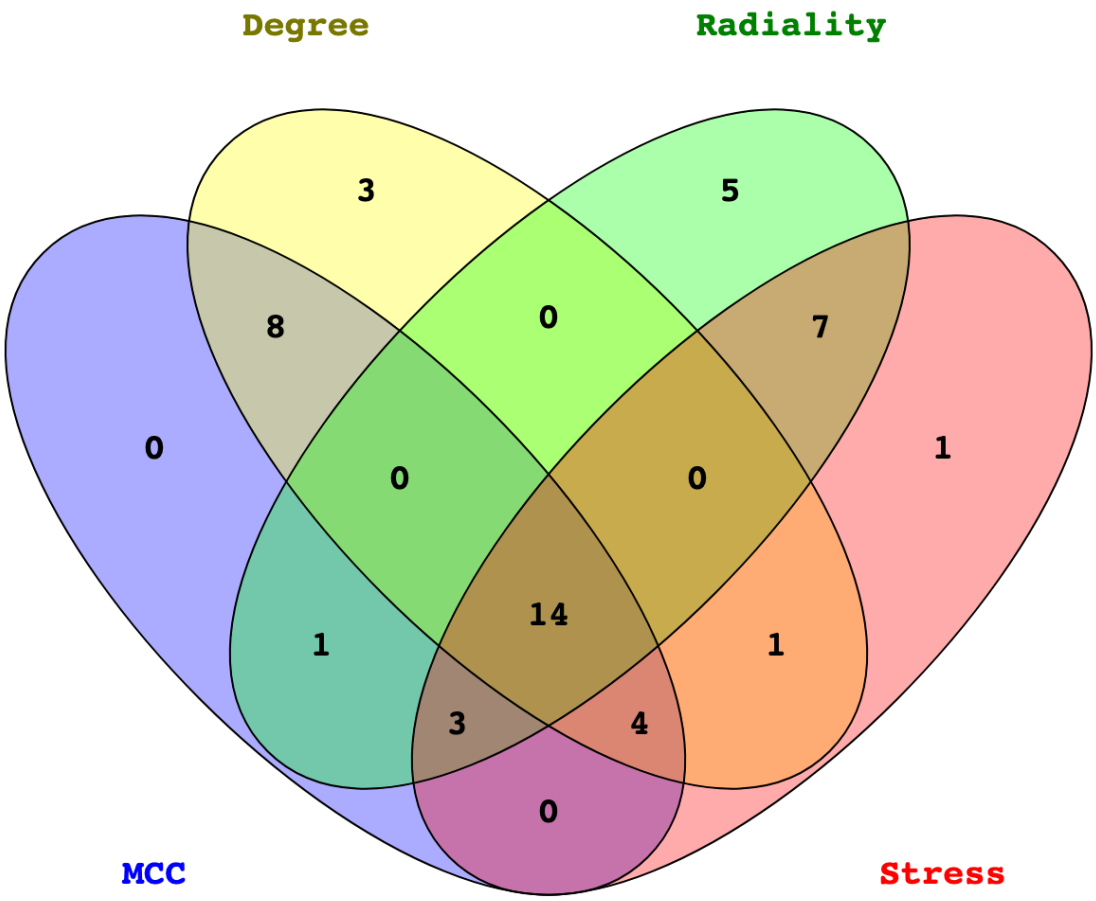


Figure_5: ***The Venn Diagram of the Top 30 Genes in 4 Classification Methods of CytoHubba.***

Table_4: ***The Top 3 Clusters Identified by MCODE***

Cluster #	Score	Nodes	Edges	Genes
Cluster 1	5	5	10	SACM1L, PIK3R2, INPP5F, PIKFYVE, PIK3C2A
Cluster 2	4.5	5	9	HSPA13, NUP50, NUP160, NUP153, SP100
Cluster 3	4.5	5	9	NIPBL, SMC4, WAPAL, PDS5A, RAD21

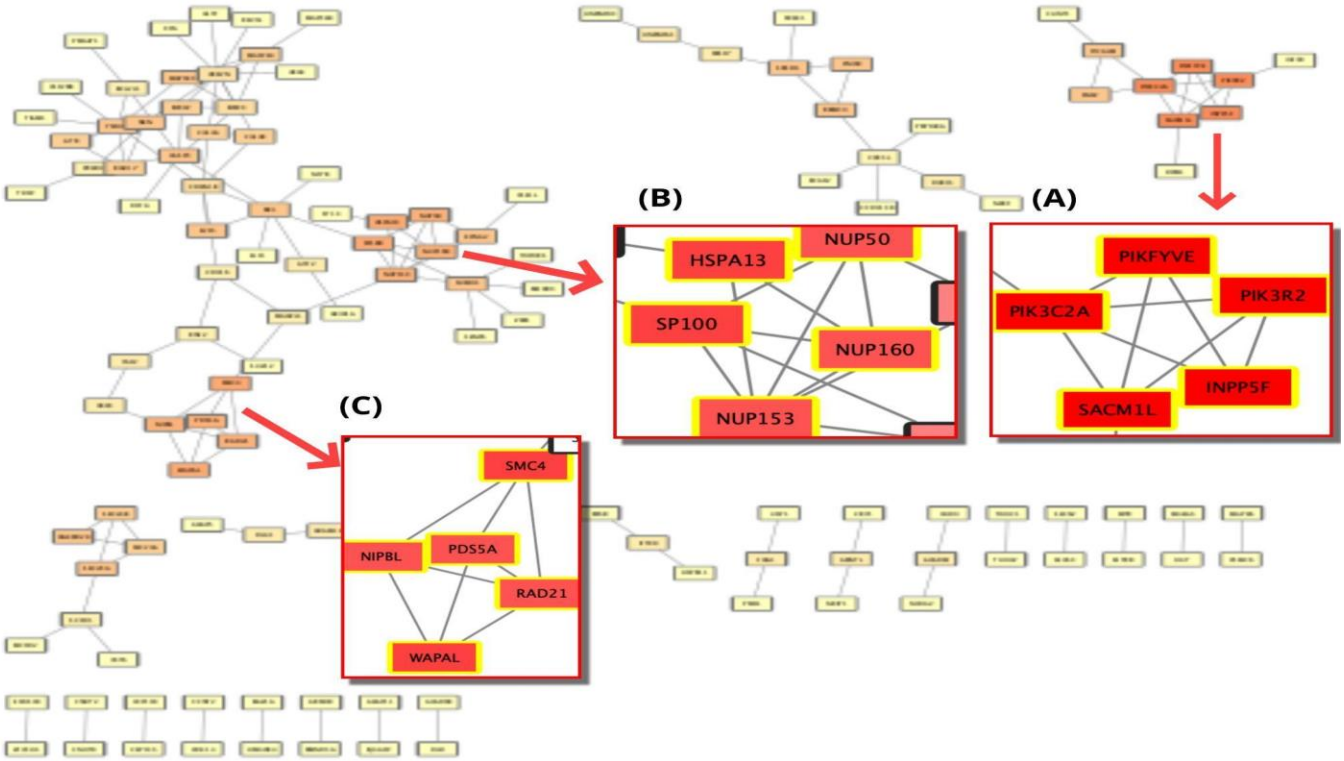


Figure_6: ***The Three Most Important Modules Generated by MCODE; (A) Cluster 1, (B) Cluster 2, (C) Cluster 3.***

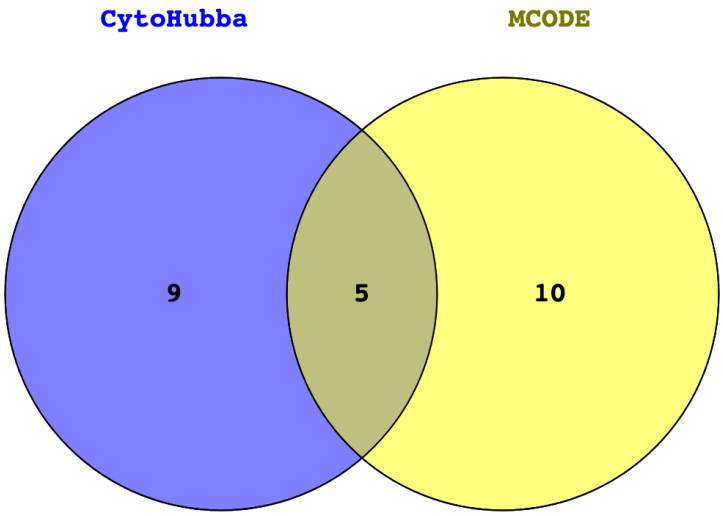


Figure_7: ***Intersection of the Hub Genes Identified through CytoHubba and MCODE Top 3 Clusters Genes.***

5. Validation of Hub Genes:

The **CTD** intersection analysis between hub genes and PTSD showed that all 14 hub genes exhibit inferred association with PTSD **(Table_5) (Figure_8)**.

The dataset GDS3383 titled "Chronic Stress Effect on Peripheral Blood Monocytes" in **GEO Profiles** was queried, and the expression of five hub genes (NUP50, NUP160, CASP3, SMC4, and MAP2K4) was validated. These genes were found to be overexpressed in normal patients compared to chronic stress patients **(Figure_9 (A)-(E))**.

Analysis of PTSD Blood Transcriptome Mega-Analysis which incorporates five independent PTSD blood transcriptome studies revealed that six out of the 14 identified hub genes exhibited differential expression; NUP153, SP100.YWHAZ, MAP2K4, CDKN1B, and NBN **(Table_6)**.

Table_5: ***Hub Genes with Inferred Association to PTSD***

Gene	Inference Score
CASP3	52.44
CDKN1B	10.04
RB1	6.97
NUP50	6.86
SMC4	6.16
YWHAZ	6.11
NUP160	2.43
SP100	2.37
NUP153	2.33
UBE2N	2.3
SUMO1	2.24
NBN	2.23
MAP2K4	2.12
BIRC3	1.74

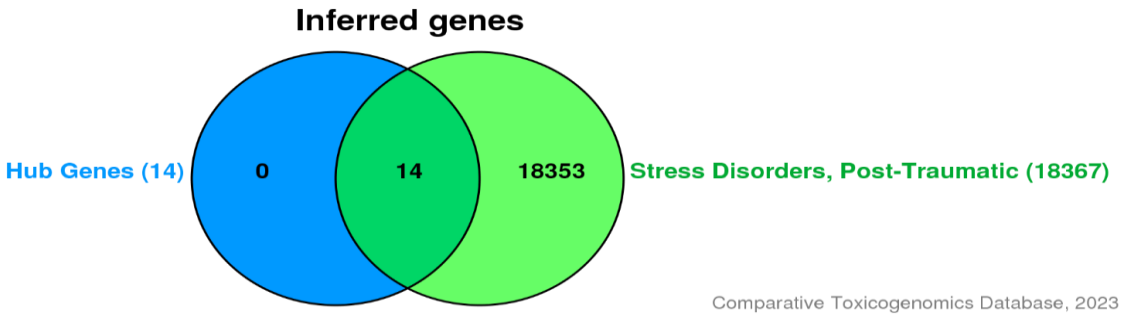
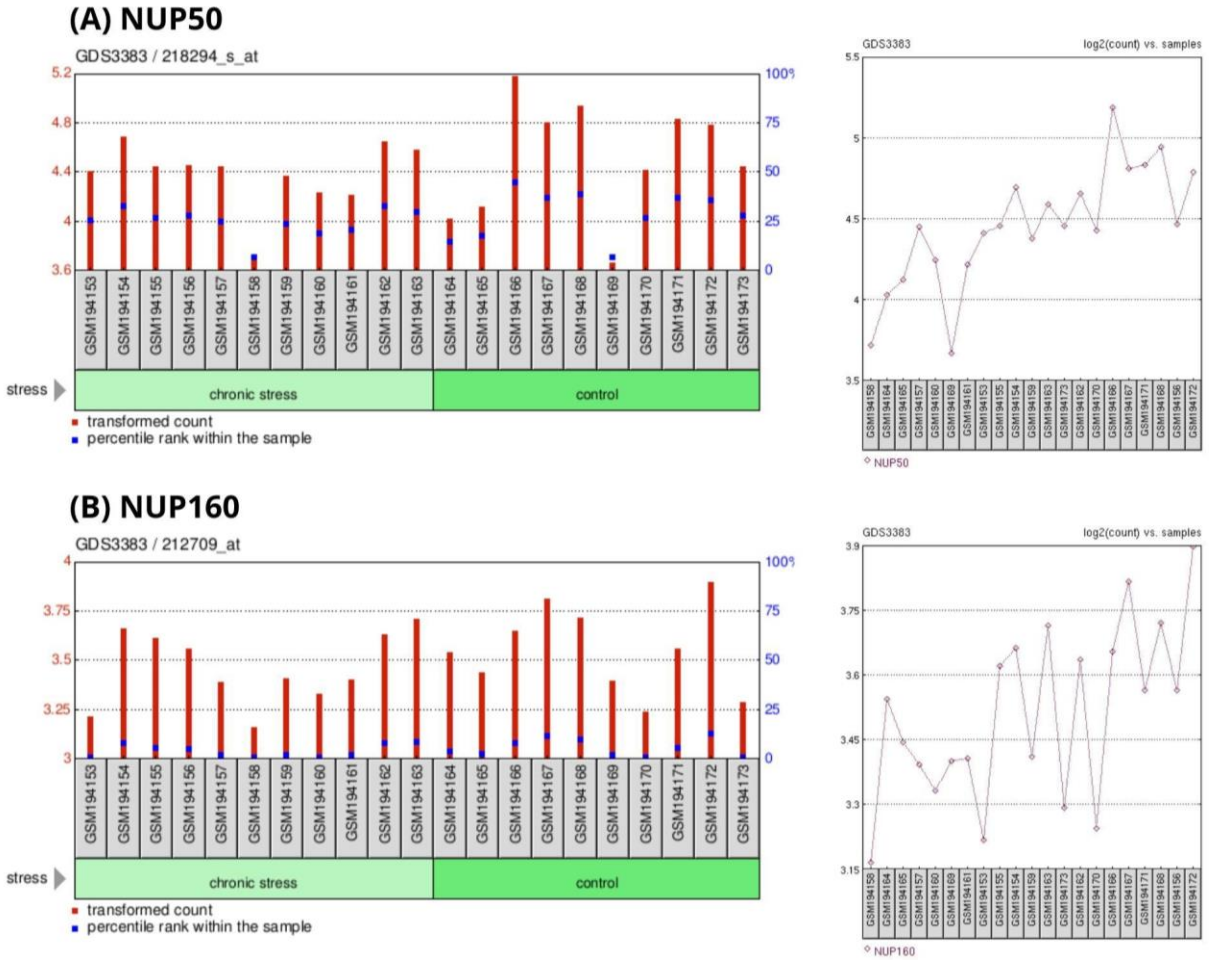


Figure_8: ***The Venn Diagram of Hub Genes and PTSD-Inferred Genes Generated by CTD.***



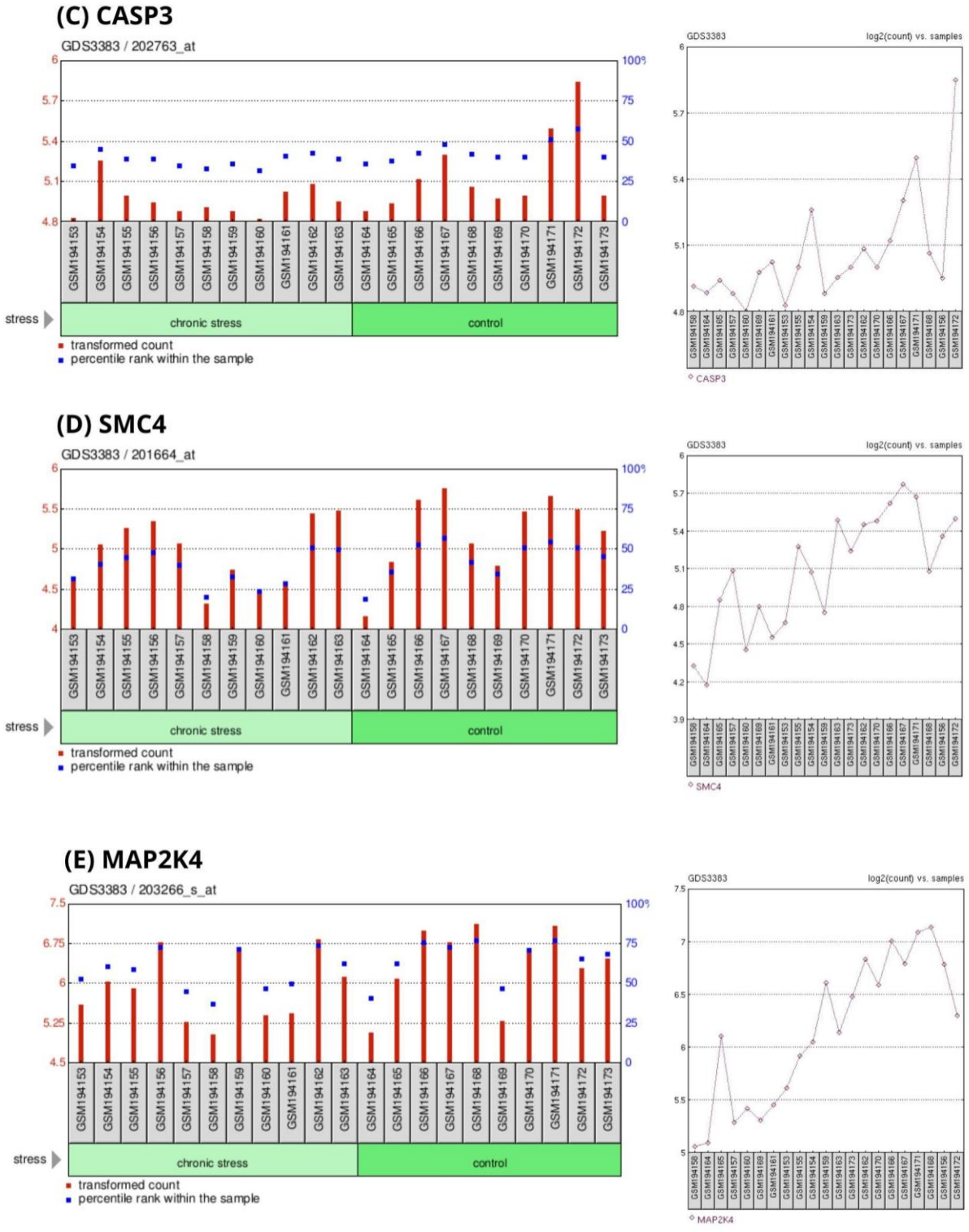


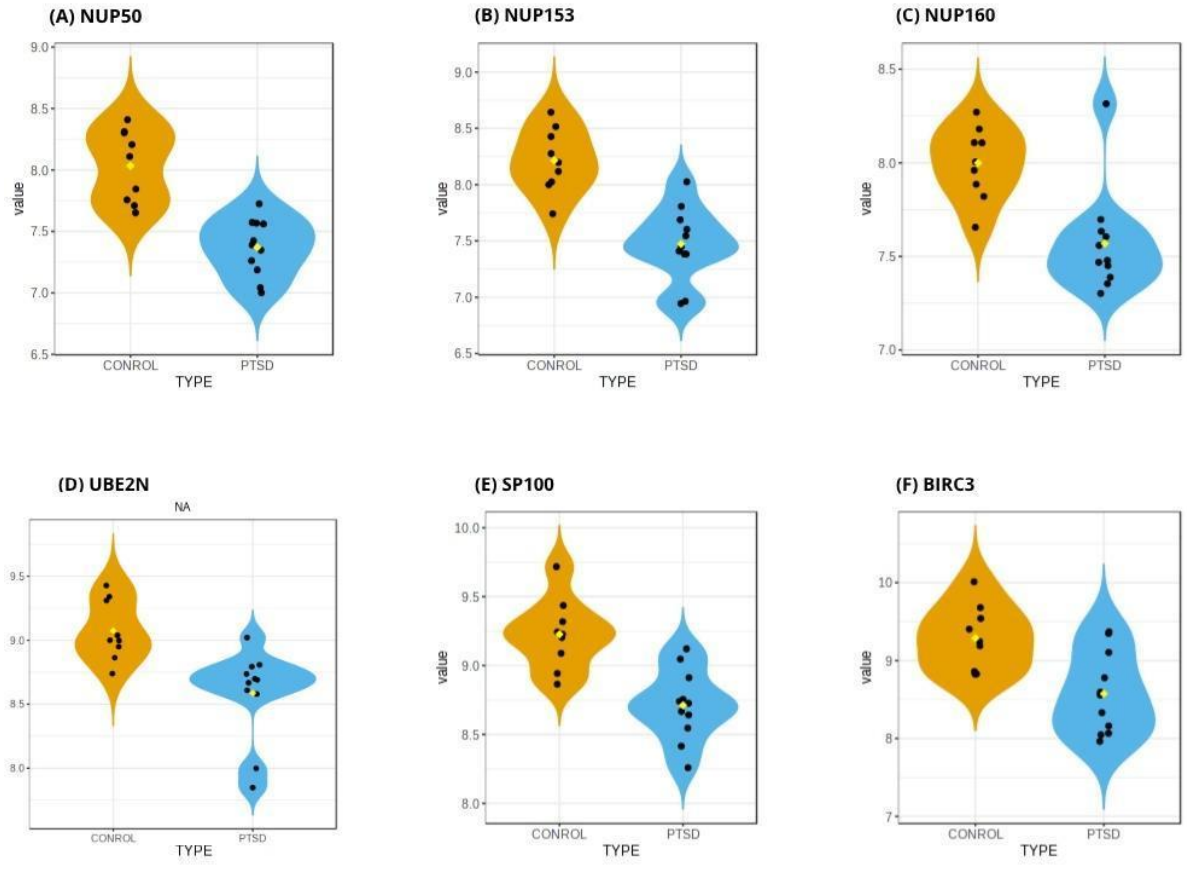
Figure_9: ***The Expression Profiles of 5 Hub Genes (A-E: NUP50, NUP160, CASP3, SMC4, and MAP2K4) Extracted from GEO Profile (GDS3383).***

Table_6: Differentially Expressed Genes (P<0.05) in Independent PTSD Blood Transcriptome Studies.

Gene	Log Fold-change	P-Value	Study
NUP153	-0.3	0.017	Segman (emergency room trauma)
SP100	-0.1	0.03368	Breen (combat trauma)
YWHAZ	0.0944	0.0457	Tylee (combat trauma)
CDKN1B	0.23	0.021	Segman (emergency room trauma)
MAP2K4	-0.21	0.0058	Mehta (interpersonal traumas)
NBN	0.12	0.0107	Neylan (assault trauma)

6. Hub Genes Expression Profiles:

The expression profiles of the 14 key genes were visualized using violin plots, with the control group represented by the orange violin and the PTSD group represented by the blue violin **(Figure_10 (A)-(M)) (Table_7)**.



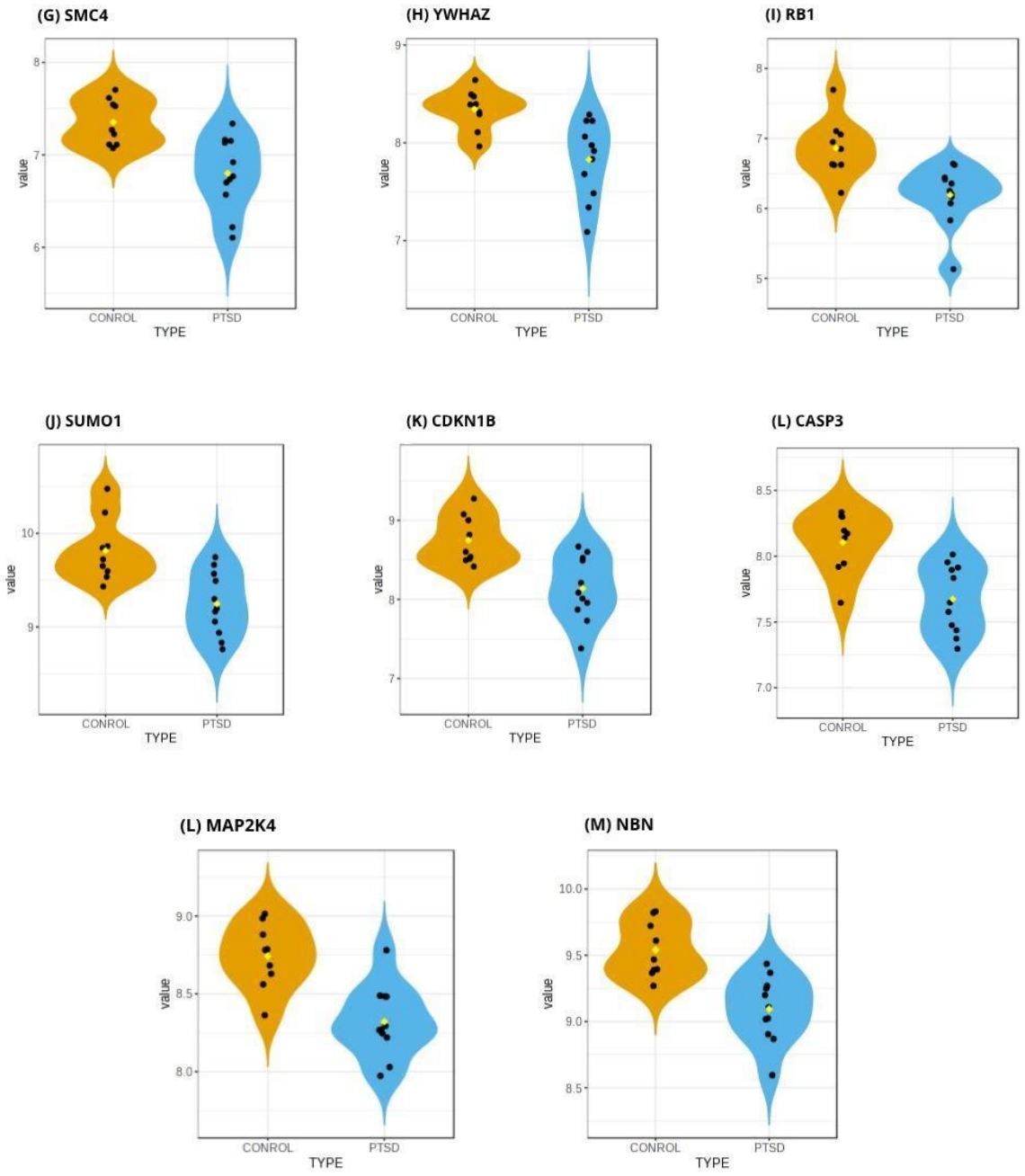


Figure_10: ***Expression Profiles of the 14 Hub Genes Generated by Express Analyst; The Orange Violin Represents the Control Group While the Blue Violin Represents the PTSD Group.***

Table_7: ***The Hub Genes Associated with PTSD***

Gene Symbol	Gene Title	Log2 (fold change)	-Log10 (Pvalue)
NUP50	Nucleoporin 50	0.683	5.073
NUP153	Nucleoporin 153	0.754	5.209
NUP160	Nucleoporin 160	0.452	3.634
UBE2N	Ubiquitin Conjugating Enzyme E2 N	0.486	2.956
SP100	SP100 Nuclear Antigen	0.513	3.941
BIRC3	Baculoviral IAP Repeat Containing 3	0.71	2.94
SMC4	Structural Maintenance of Chromosomes 4	0.578	3.29
YWHAZ	Tyrosine 3-Monooxygenase/Tryptophan 5-Monooxygenase Activation Protein Zeta	0.525	3.085
RB1	RB Transcriptional Corepressor 1	0.693	3.312
SUMO1	Small Ubiquitin-Like Modifier 1	0.556	3.256
CDKN1B	Cyclin Dependent Kinase Inhibitor 1B	0.608	3.05
CASP3	Caspase 3	0.456	3.213
MAP2K4	Mitogen-Activated Protein Kinase Kinase 4	0.423	3.602
NBN	Nibrin	0.431	3.244

7. Hub Genes Functional Enrichment Analysis:

The **STRING** database was used to visualize the PPI of the hub genes with setting the minimum required interaction score as the medium confidence 0.4 **(Figure_11_A)** and k-mean clustering was also applied dividing the genes into 3 clusters **(Figure_11_B)**.

The functional enrichment analysis by **Metascape** showed that the key genes mainly contribute to Cytokine signaling in the immune system (CASP3, CDKN1B, NUP50, YWHAZ, NUP160, SP100, NUP153, UBE2N, SUMO1,MAP2K4, BIRC3), Toll Like Receptor 3 (TLR3) Cascade (CDKN1B, UBE2N, MAP2K4, BIRC3), cell cycle (CASP3, CDKN1B, RB1, NUP50, SMC4,YWHAZ, NUP160, NUP153, UBE2N, SUMO1, NBN, MAP2K4), and numerous apoptosis-related processes including oxidative damage response (CASP3, CDKN1B, YWHAZ, SP100, UBE2N, MAP2K4, BIRC3) and DNA damage response (CASP3, CDKN1B, RB1, SMC4, YWHAZ, SP100, SUMO1, NBN, MAP2K4, BIRC3) **(Figure_12) (Figure_13).**

While the functional annotation Clustering by **DAVID** revealed that the key genes are associated with regulation of cell cycle, apoptosis, viral carcinogenesis, and several viral

infections such as Hepatitis B, Hepatitis C, Human Papilloma Virus and Epstein-Barr infections.

The figure displays the annotation cluster, accompanied by an enrichment score of 2.4 where the positive corresponding is denoted by the green squares, whereas the negative corresponding is represented by the dark ones **(Figure_14)**.

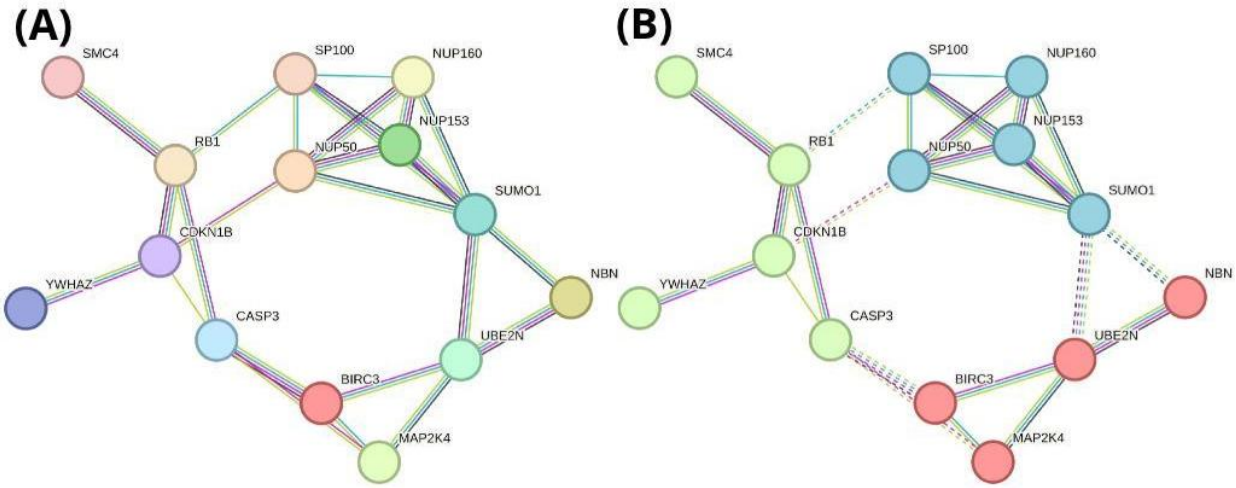


Figure_11: ***The 14 Key Genes Associated with PTSD Network Generated by STRING.***

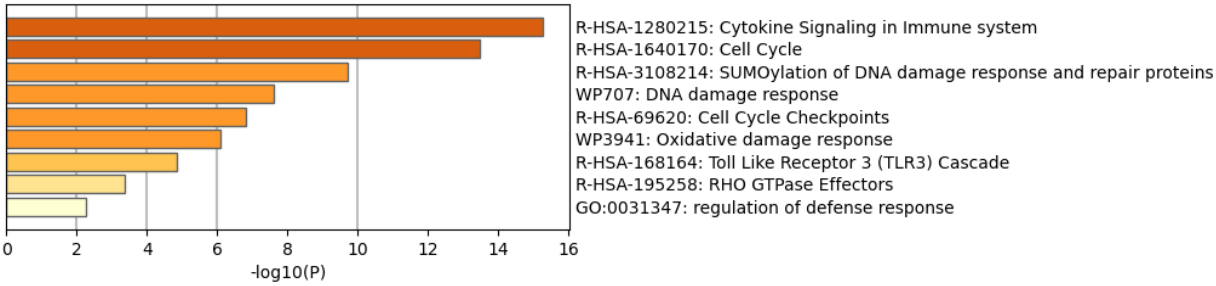


Figure_12: ***Bar Graph of Enriched Pathways and Biological Processes of the 14 Hub Genes Identified by CytoHubba, Colored by P-values.***

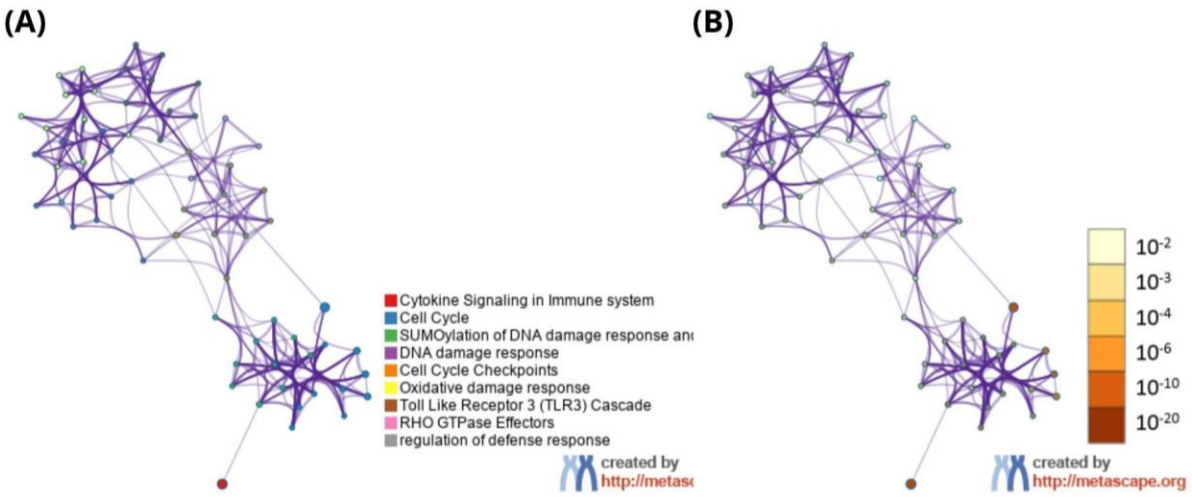


Figure_13: ***Network of Enriched Pathways and Biological Processes of the 14 Hub Genes: (A) Colored by Cluster ID, Where Nodes That Share the Same Cluster ID Are Typically Close To Each Other; (B) Colored by p-value, Where Terms Containing More Genes Tend to Have a More Significant P-Value.***

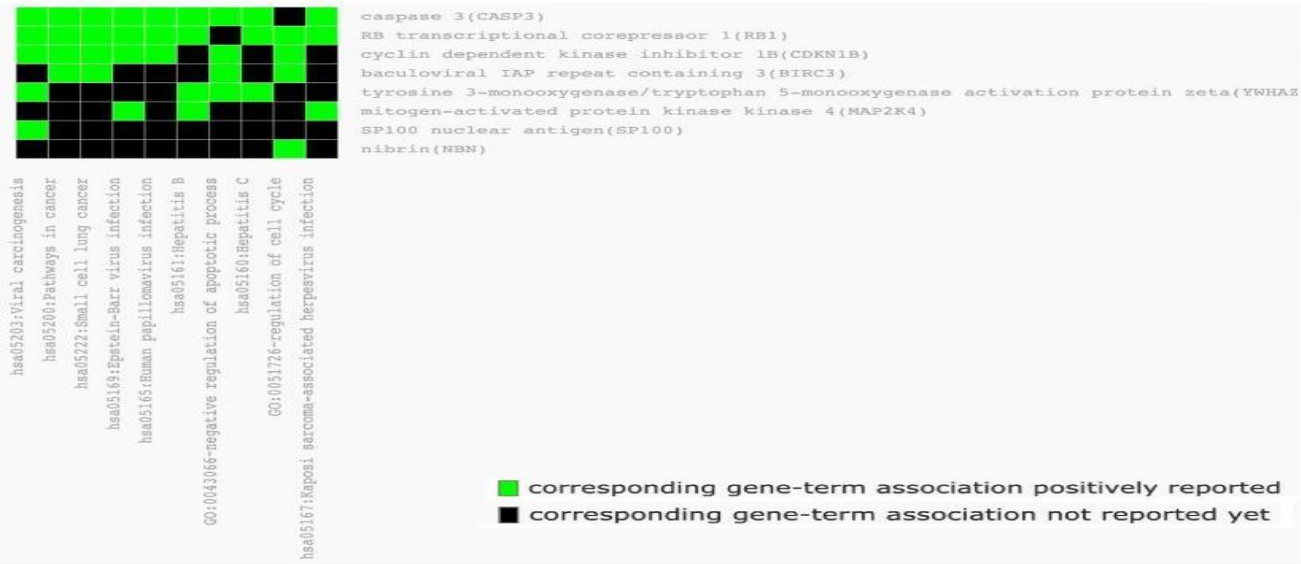


Figure_14: ***2D View Functional Annotation Clustering Heatmap of Hub Genes Associated with PTSD Generated by DAVID.***

8. Prediction of Target miRNA:

The hub genes were uploaded into **miRNet** and a total of 865 miRNAs were obtained with 1613 edges **(Figure_15)**.

The overlap between the 865 miRNAs from miRNet and the 82 miRNAs from **PubMed** revealed that 44 miRNAs identified in this study have previously been confirmed to exhibit differential expression in individuals with PTSD. Out of these, 23 miRNAS were found to be downregulated, while 21 were upregulated in PTSD patients **(Table_8)**.

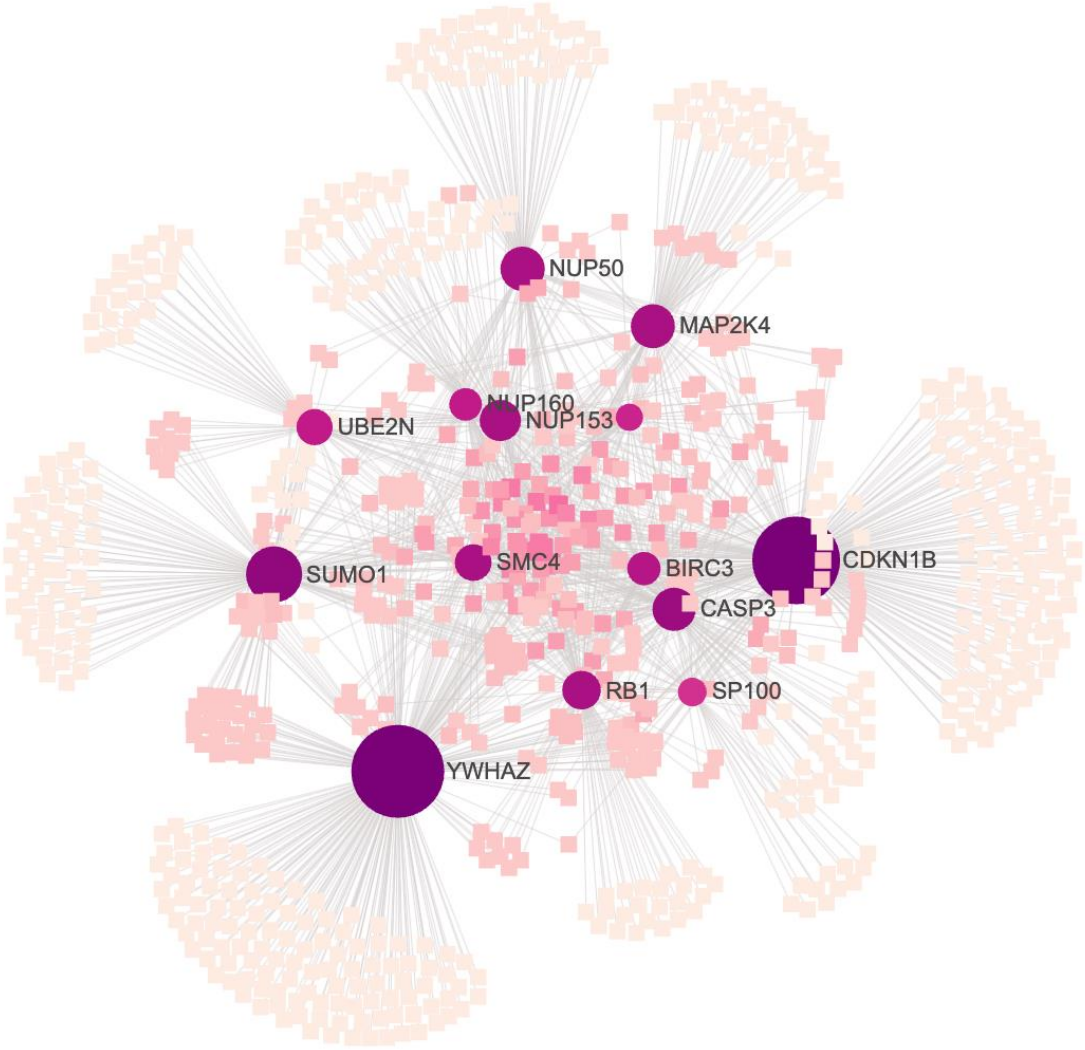


Figure_15: ***miRNA-Hub Gene Regulatory Network Extracted from miRNet; Purple Circles Represent Hub Genes while PinkSquares Represent miRNAs.***

Table_8: ***The 44 Differentially Expressed miRNAs in PTSD Patients.***

Downregulated miRNAs			Upregulated miRNAs		
miRNAs	**Target Hub Genes**	**Log2 FC**	**miRNAs**	**Target Hub Genes**	**Log2 FC**
hsa-mir-146a-5p	BIRC3/NUP160/NUP160/NUP153	-2.04	hsa-mir-23a-3p	NUP153/MAP2K4/NUP50/RB1/CASP3	0.15
hsa-mir-708-5p	YWHAZ	-1.27	hsa-mir-151a-3p	BIRC3	0.15
hsa-mir-490-5p	YWHAZ	-1.27	hsa-mir-145-3p	SMC4	0.22
hsa-mir-628-5p	BIRC3/NUP153	-1.27	hsa-mir-130a-3p	BIRC3/NUP160NUP160/CDKN1B/SMC4/NUP153/RB1	0.22
hsa-mir-641	BIRC3	-1.27	hsa-mir-20a-5p	RB1/YWHAZ/BIRC3/CDKN1B/SMC4	0.39
hsa-mir-675-5p	RB1	-1.27	hsa-mir-302a-5p	NUP50/CASP3	0.41
hsa-mir-504-5p	SUMO1	-1.27	hsa-mir-212-3p	RB1/NUP50/NUP160/NUP160/SP100/CDKN1B	0.41
hsa-mir-455-5p	CDKN1B	-0.82	hsa-mir-19a-3p	NUP50/CDKN1B/CASP3	0.43
hsa-mir-335-5p	RB1/YWHAZ	-0.75	hsa-mir-127-3p	MAP2K4	0.8
hsa-mir-340-5p	NUP50/NBN	-0.75	hsa-mir-20b-5p	RB1/YWHAZ/CDKN1B	0.8
hsa-mir-210-3p	BIRC3/NUP160/NUP160/NBN/SMC4/RB1/UBE2N	-0.74	hsa-mir-199a-3p	YWHAZ	0.8
hsa-mir-31-5p	CDKN1B	-0.57	hsa-mir-181c-5p	NUP50/NBN/NUP153	0.8
hsa-mir-199b-3p	YWHAZ	-0.51	hsa-mir-143-3p	NUP153	0.8
hsa-mir-18a-3p	CDKN1B/SMC4	-0.51	hsa-mir-132-3p	NBN/RB1/NUP50	0.8
hsa-mir-148a-5p	CDKN1B	-0.51	hsa-mir-3200-3p	CASP3	0.91
hsa-mir-335-3p	CDKN1B/YWHAZ/SP100/CASP3	-0.45	hsa-mir-148a-3p	CDKN1B/NUP153/RB1	1.25
hsa-mir-3175	CDKN1B/SUMO1/YWHAZ	-0.38	hsa-mir-128-3p	CASP3/UBE2N/YWHAZ/BIRC3/MAP2K4/NBN/SMC4	1.55
hsa-mir-214-3p	BIRC3/SP100	-0.36	hsa-mir-222-3p	CDKN1B/UBE2N/BIRC3/CASP3	2.06
hsa-mir-34a-5p	BIRC3/CASP3/CDKN1B/NUP160/NUP160/NUP50/NBN/SMC4/NUP153/YWHAZ/UBE2N	-0.24	hsa-mir-101-3p	BIRC3/NUP160/NUP160/NUP50/SMC4/CASP3/YWHAZ/UBE2N	2.1202
hsa-mir-221-5p	CDKN1B/NUP50/YWHAZ	-0.21	hsa-mir-138-5p	CASP3/SP100/SUMO1	2.29
hsa-mir-183-5p	NBN	-0.21	hsa-mir-146b-5p	NUP153	2.29
hsa-mir-15b-3p	NUP50	−1.5629			
hsa-mir-125b-5p	NBN/CDKN1B	−1.6985			

9. Prediction of Target Transcription Factors (TFs):

Using **TRRUST** through **miRNet**, a total of 49 transcription factors (gTFs) targeting 8 of the hub genes were extracted, and multiple networks depicting their relationships were generated. However, the biggest network with 43 TFs and 5 hub genes was visualized **(Figure_16)**.

The verification results determine that 10 TFs were differentially expressed, and these TFs target 6 of the Hub genes (CASP3, BIRC3, SUMO1, CDKN1B, RB1, SP100). Among these TFs, NFKB, TP53, RELA, HDAC1, STAT5A, and FOXO1 were found to be downregulated, whereas PML, IRF1, SP1, and E2F1 were upregulated **(Figure_17)**.
Besides the previously validated 10 TFs, three more TFs were identified and found to be undepressed in PTSD patients in GSE860 dataset: DEK, NFYB, RB1 with 0.396, 0.501, 0.693 log(fc) respectively **(Figure_18)**.
In total, 13 key TFs which target 6 were identified on the basis of TRRUST and then verified by GSE860 dataset and 3 previous studies **(Table_9)**.

The analysis showed that key TFs participate in main pathways associated with PTSD hub genes including: Cytokine Signaling in Immune system (NFKB1, TP53, RELA, STAT5A, PML, IRF9, FOXO1), cell cycle (TP53, HDAC1, E2F1, RB1), Cellular responses to stress (NFKB1, TP53, RELA, SP1, E2F1, NFYB, RB1), and Toll Like Receptor 3 (TLR3) Cascade (NFKB1, TP53, RELA) **(Figure_19)**.

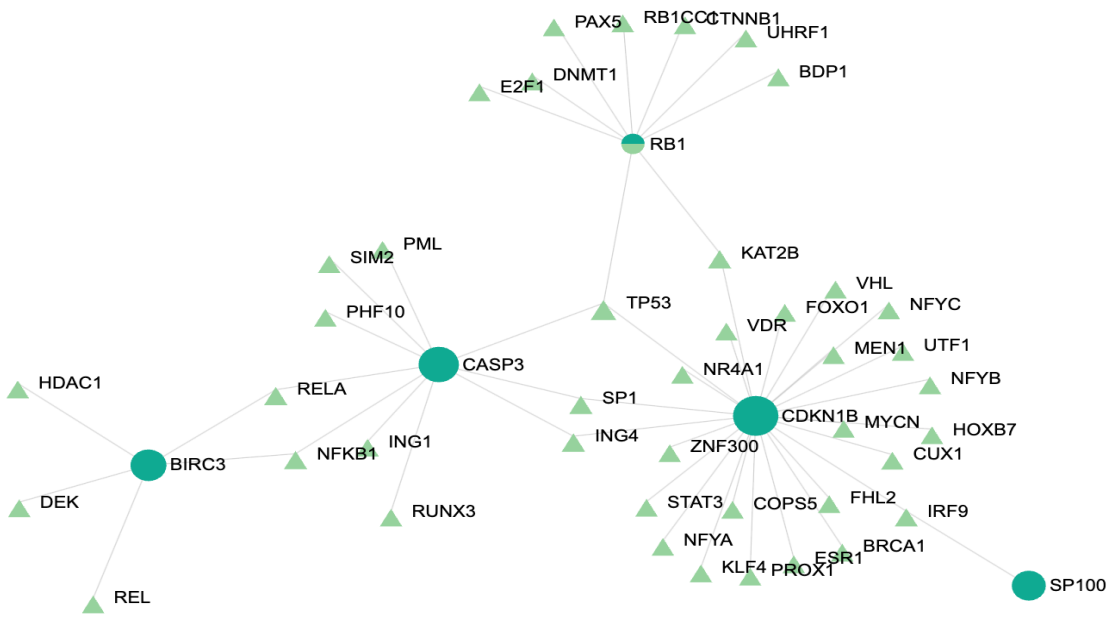


Figure_16: ***The Network of 5 Hub Genes and the TFs Targeting Hub Genes gTF: The dark Green Circles Represents Hub Genes, while the Light Green Triangles Represents gTFs***

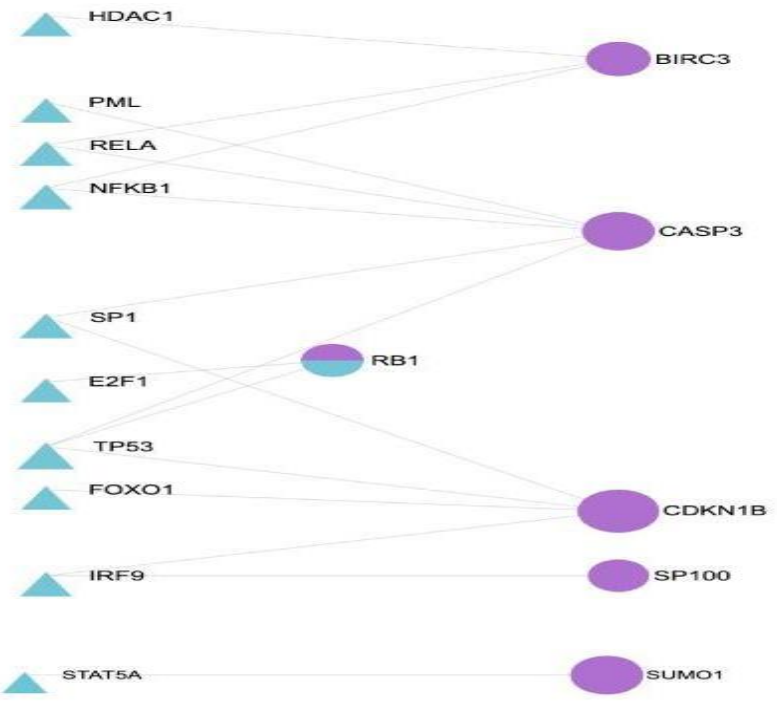


Figure_17: ***The Network of Verified TFs with Targeted Hub Genes by Previous Studies; The Purple Circle Represents Hub Genes and The Blue Triangles Represent TFs.***

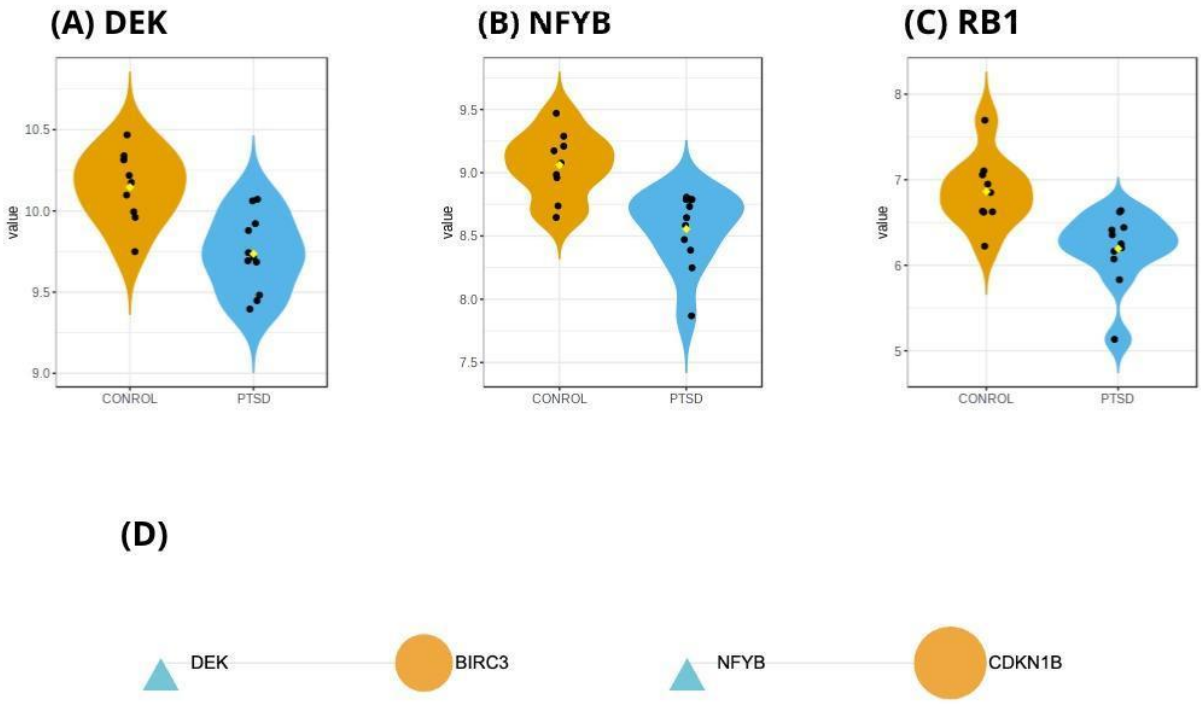


Figure_18: ***The Expression Levels (P < 0.05) of Verified TFs in GSE860: (A) DEK, (B) NFYB, (C) RB1, (D) TFs Regulatory Network.***

Table_9: ***Key Transcriptional Factors of Hub Genes.***

	Key TFs	Description	Genes
1	NFKB1	Nuclear Factor Kappa B Subunit 1	BIRC3/CASP3
2	TP53	Tumor Protein p53	CASP3/CDKN1B/RB1
3	RELA	RELA proto-oncogene, NF-kB subunit	BIRC3/CASP3
4	HDAC1	Histone Deacetylase 1	BIRC3
5	STAT5A	Signal Transducer and Activator of Transcription 5A	SUMO1
6	PML	PML Nuclear Body Scaffold	CASP3
7	IRF9	Interferon Regulatory Factor 9	CDKN1B/SP100
8	FOXO1	Forkhead Box O1	CDKN1B
9	SP1	Sp1 Transcription Factor	CASP3/CDKN1B

10	E2F1	E2F Transcription Factor 1	RB1
11	DEK	DEK Proto-Oncogene	BIRC3
12	NFYB	Nuclear Transcription Factor Y Subunit Beta	CDKN1B
13	RB1	RB Transcriptional Corepressor 1	RB1

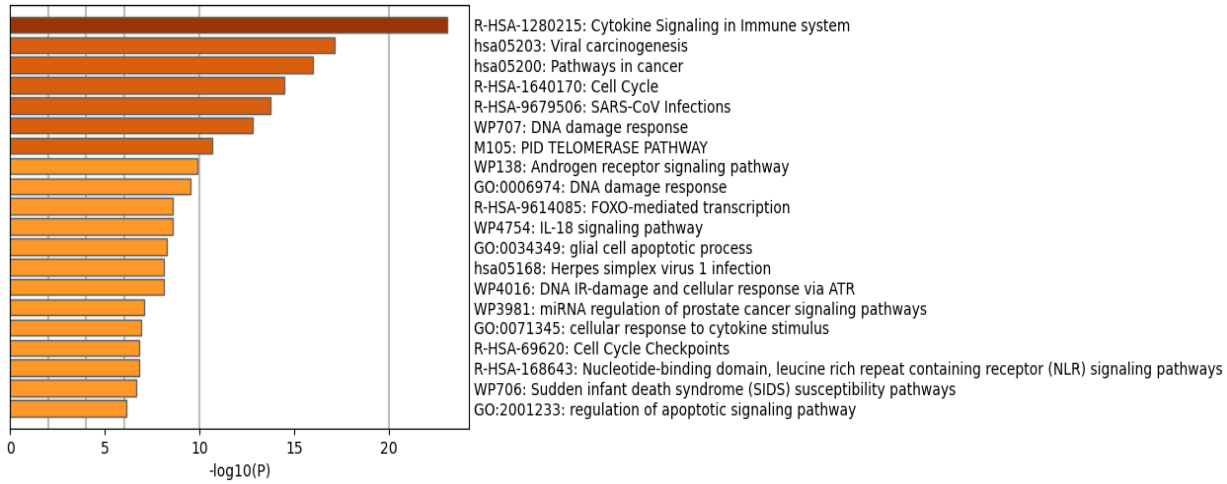


Figure_19: ***Bar Graph of Enriched Pathways and Biological Processes of The Hub Genes and Key TFs Colored by P-values.***

10. Text Mining Analysis of Hub Genes, Key TFs, and Pathways in PTSD:

Coremine Medical was utilized through two steps to investigate the relationship between hub genes, key TFs, and the main enriched pathways associated with PTSD.

In the first step, the search terms "post-traumatic stress disorder," "immune response", "apoptosis", "cell cycle", and "hub gene symbols" were used. The analysis revealed that PTSD is directly correlated with three hub genes: CASP3, YWHAZ, and RB1. Furthermore, a total of 13 hub genes were found to be associated with the three pathways,except NUP160 was specifically linked to the apoptotic process and cell cycle (**Figure_20)**.

While in the second step, a new analysis was conducted using the search terms "post-traumatic stress disorder," "immune response," "apoptotic process," "cell cycle," and "gene symbols of key TFs." All of the key TFs examined were found to be related to the three pathways, except for DEK, which was specifically associated with the apoptotic process and cell cycle. On the other hand, PTSD was linked directly to six key TFs: NFKB1, HDAC1, STAT5A, TP53, RB1, and RELA **(Figure_21)**.

By performing these analyses, valuable insights were obtained regarding the relationships among hub genes, TFs, and the pathways involved in PTSD. This information contributes to a deeper understanding of the molecular mechanisms underlying PTSD and provides potential targets for further research and therapeutic interventions.

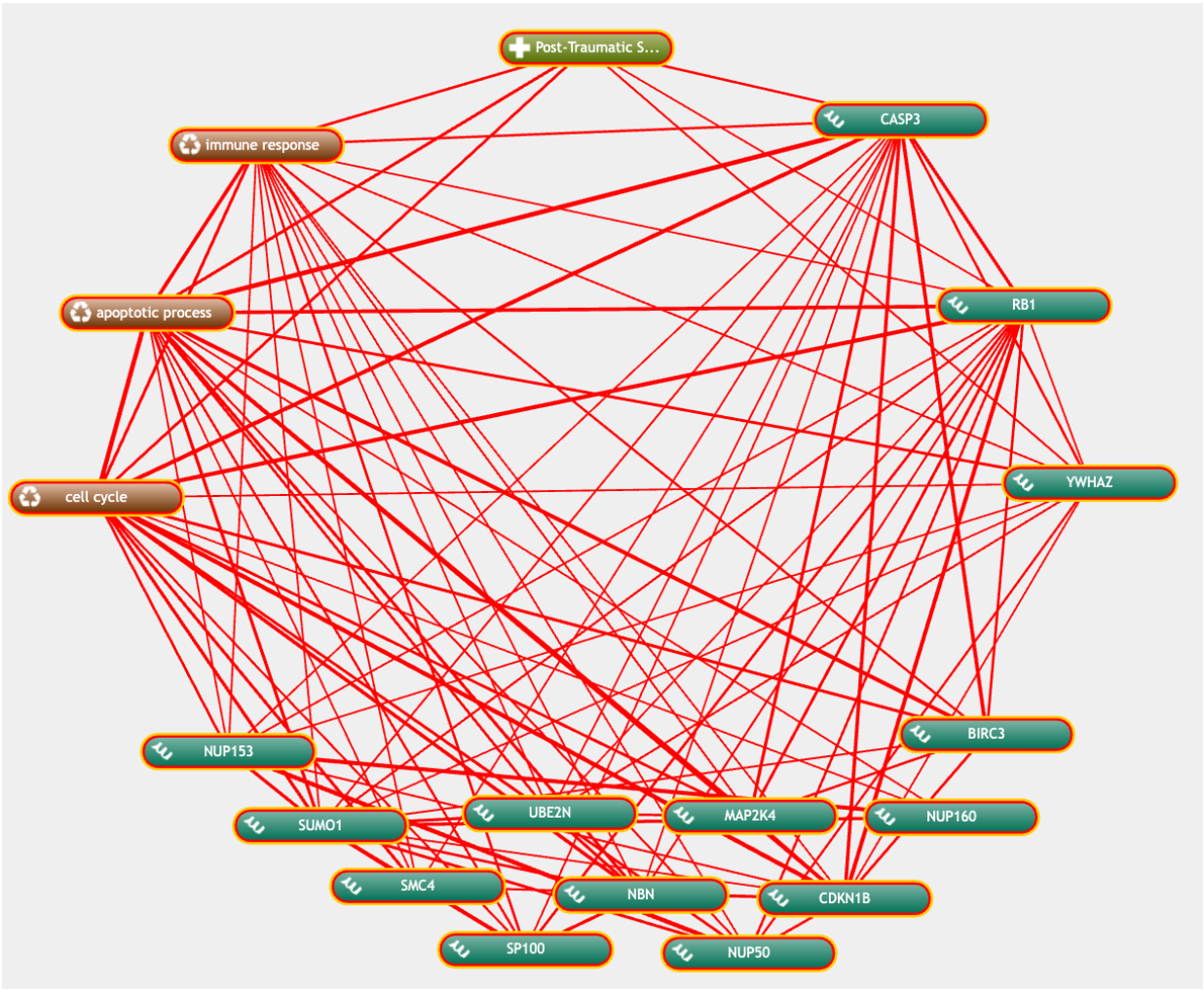


Figure_20: ***Linear Association among Hub Genes and The Main Enriched Pathways in PTSD Using Coremine Medical, The Thicker the Line, the Closer the Connection.***

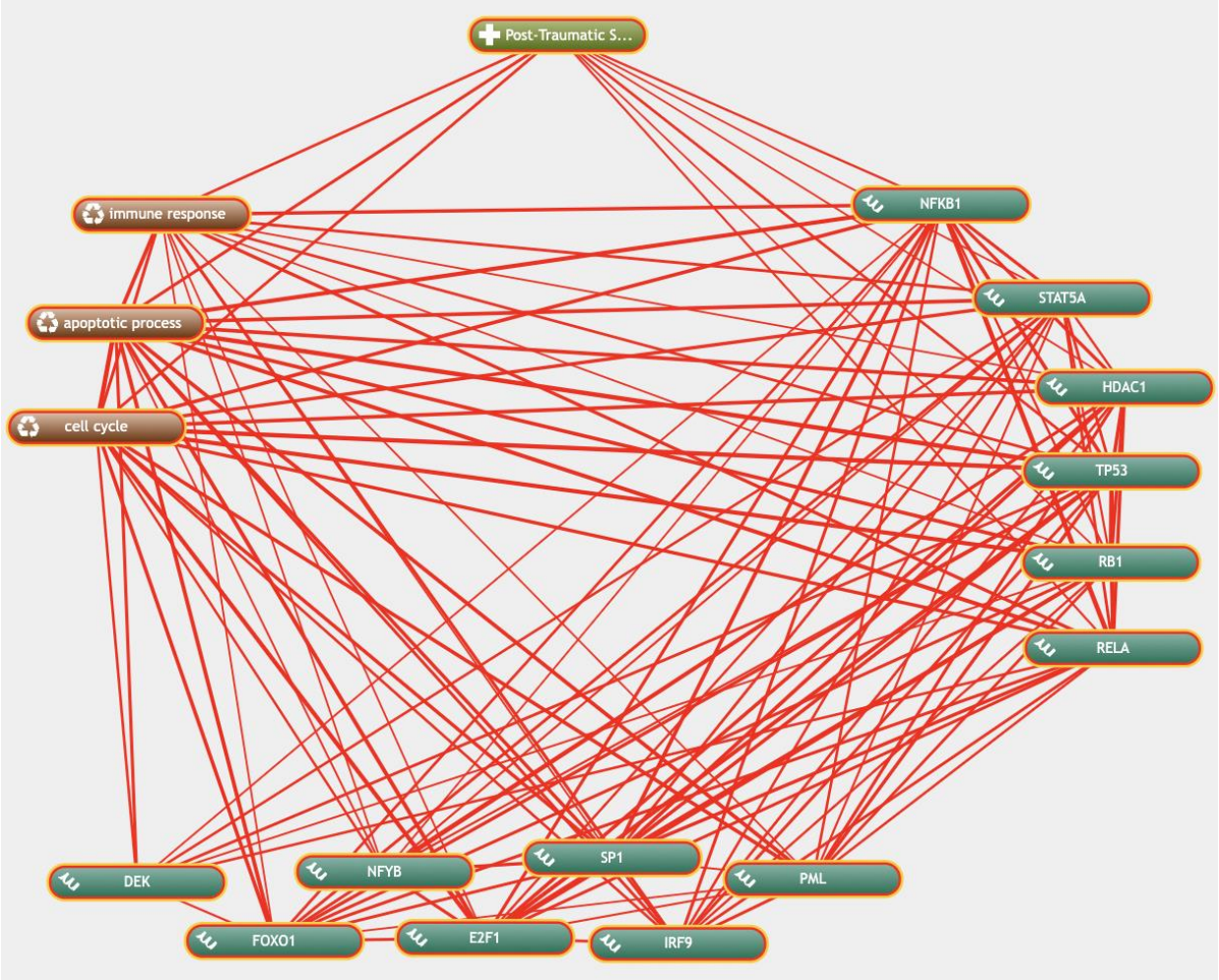


Figure_21: ***Linear Association among Key TFs and The Main Enriched Pathways in PTSD Using Coremine Medical, The Thicker the Line, the Closer the Connection.***

11. Analysis of FDA-Approved Medications and Intersections with PTSD-Associated Pathways:

Regarding chemical-gene interaction, **CTD** analysis showed that Sertraline is associated with 127 genes while Paroxetine interacts with 50 genes. The intersection of these genes with Hub genes and key TFs associated with PTSD showed that Sertraline and Paroxetine increase activity of CASP3 protein while only Sertraline results in increased expression of NFKB1 mRNA **(Figure_22)**.

On the other hand, the chemical-pathway interaction analysis clarified that Sertraline and Paroxetine are associated with 24 enriched pathways related to PTSD Hub Genes **(Table_10)**.

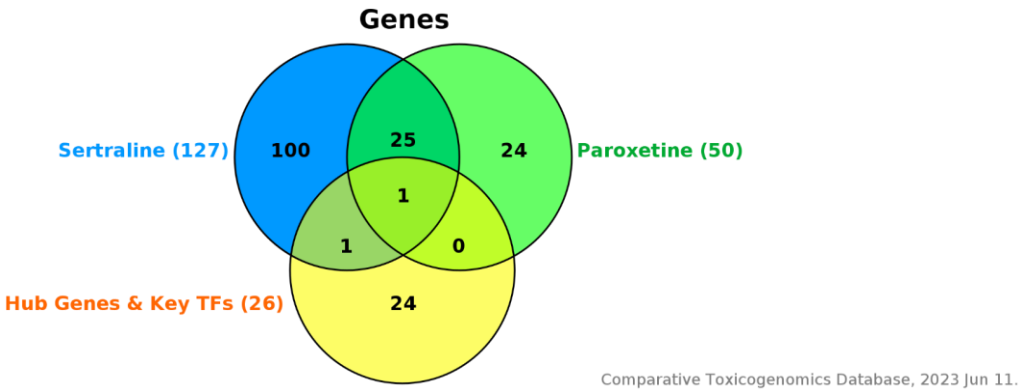


Figure_22: ***Venn Diagram of Hub genes and Key TFs associated with PTSD, Sertraline-associated genes, and Paroxetine-associated genes.***

Table_10: ***The Mutual Enriched Pathways of Hub Genes, Sertraline, and Paroxetine.***

Pathway ID	Enriched Pathways	Sertraline P-Value	Paroxetine P-Value
REACT:R-HSA-1280215	Cytokine Signaling in Immune system	4.71E-23	1.18E-12
KEGG:hsa04110	Cell Cycle	9.23E-07	-
REACT:R-HSA-2262752	Cellular responses to stress	5.99E-14	9.31E-06
KEGG:hsa05203	Viral carcinogenesis	5.91E-12	2.70E-06
KEGG:hsa05222	Small cell lung cancer	9.14E-08	-
KEGG:hsa05161	Hepatitis B	7.37E-22	3.54E-12
REACT:R-HSA-2559583	Cellular Senescence	2.50E-09	-
KEGG:hsa05169	Epstein-Barr virus infection	6.14E-18	-
REACT:R-HSA-3371453	Regulation of HSF1-mediated heat shock response	3.04E-08	-
KEGG:hsa04210	Apoptosis	1.58E-29	1.64E-10
REACT:R-HSA-3371556	Cellular response to heat stress	1.29E-07	-
REACT:R-HSA-168164	Toll Like Receptor 3 (TLR3) Cascade	8.64E-21	8.06E-08
KEGG:hsa04668	TNF signaling pathway	7.15E-26	3.41E-13

KEGG:hsa05014	Amyotrophic lateral sclerosis	2.28E-07	-
KEGG:hsa04350	TGF-beta signaling pathway	1.20E-12	-
REACT:R-HSA-166016	Toll Like Receptor 4 (TLR4) Cascade	2.96E-19	2.69E-07
KEGG:hsa05160	Hepatitis C	2.03E-12	6.69E-09
REACT:R-HSA-449147	Signaling by Interleukins	8.50E-26	1.17E-14
KEGG:hsa05200	Pathways in cancer	7.78E-15	4.30E-13
REACT:R-HSA-5357801	Programmed Cell Death	4.02E-07	-
KEGG:hsa05132	Salmonella infection	2.96E-09	2.43E-06

12. Computational Drug Repurposing for PTSD Treatment:

The first analysis involved utilizing **DrugBank** as a functional database in conjunction with **WebGestalt**. After submitting 27 genes including 14 Hub genes (NUP50. NUP153, NUP160, UBE2N, SP100, BIRC3, SMC4, YWHAZ, RB1, SUMO1, CDKN1B, CASP3, MAP2K4, NBN) and 12 key TFs (NFKB1, TP53, RELA, HDAC1, STAT5A, PML, IRF9, FOXO1, SP1, E2F1, DEK, NFYB), considering the significance level of P value < 0.05, and Over Representation Analysis (ORA) as the method of Interest, WebGestalt gene-drug analysis predicted ten existing drugs **(Figure_23) (Table_11)**.

The second analysis was performed by **DGIdb** through several steps.
Initially, hub genes and key TFs were categorized to indicate their druggability potential; 11 genes came out to be druggable genomes while 12 genes are clinically actionable **(Table_12)**.
Then, a drug repurposing analysis was conducted for the 26 genes (hub genes and key TFs) identified in PTSD. DGIbd analyzed drug-gene interactions and assigned an interaction score for each pair of gene-drug pairs. After applying a query score threshold of 5, the filtration results stated that 5 genes CASP3, NFKB1, TP53, RELA, HDAC1 are the final druggable target with 32 potential compounds/drugs **(Table_13)**.
After manual study and analysis, a total of 15 final potential drugs were listed based on the enriched pathways associated with the genes including ; Cytokine Signaling in Immune system (CAPS3, NFKB1, TP53, RELA), cell cycle (HDAC1, TP53), Toll Like Receptor 3 (TLR3) Cascade (NFKB1, RELA, TP53), and Cellular Responses to Stress (NKB1, RELA, TP53) **(Table_13)**.

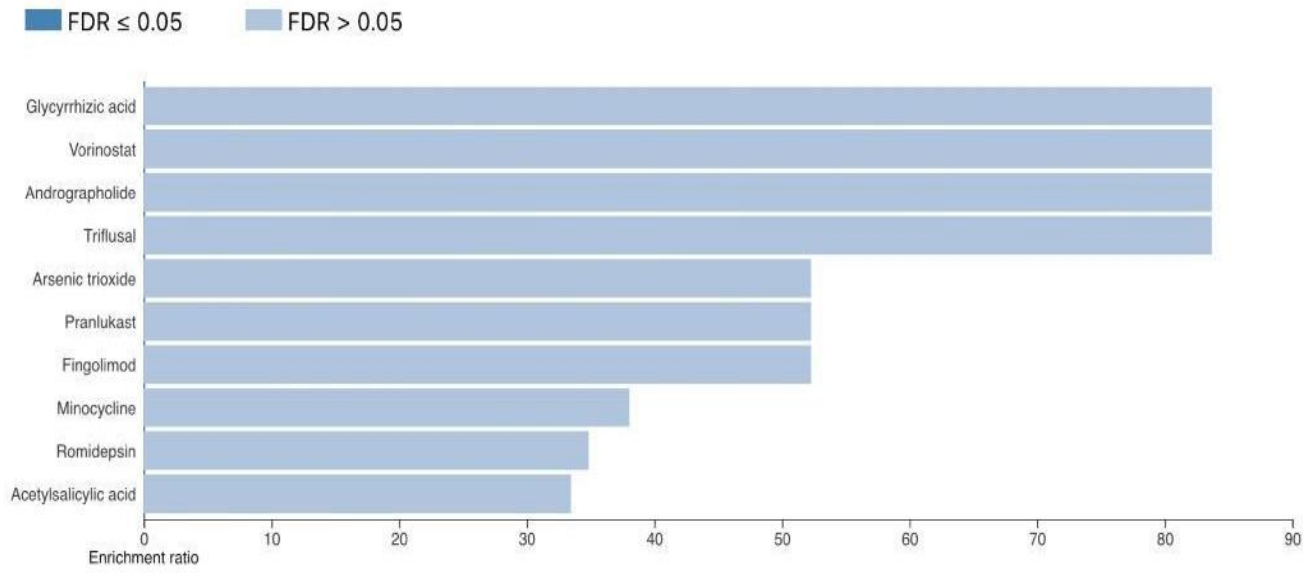


Figure_23: ***Bar Chart of Enriched Drugs and Potential Therapeutic Options for Hub Genes and Key TFs Associated with PTSD: A DrugBank-based Analysis Using WebGestalt.***

Table_11: ***Table of Enriched Drugs and Potential Therapeutic Options for Hub Genes and Key TFs Associated with PTSD: A DrugBank-based Analysis Using WebGestalt.***

Gene Set	Description	Size	Expect	Ratio	P Value	↑ FDR
DB13751	Glycyrrhizic acid	10	0.023899	83.686	0.00021838	0.25616
DB01169	Arsenic trioxide	16	0.038238	52.304	0.00057837	0.33921
DB00945	Acetylsalicylic acid	25	0.059747	33.474	0.0014311	0.55958
DB02546	Vorinostat	5	0.011949	83.686	0.011901	1
DB05767	Andrographolide	5	0.011949	83.686	0.011901	1
DB08814	Triflusal	5	0.011949	83.686	0.011901	1
DB01411	Pranlukast	8	0.019119	52.304	0.018982	1
DB08868	Fingolimod	8	0.019119	52.304	0.018982	1
DB01017	Minocycline	11	0.026289	38.039	0.026021	1
DB06176	Romidepsin	12	0.028679	34.869	0.028357	1

Table_12: *Categorization of Hub Genes and Key TFs Generated by DGIdb.*

Results Grouped by Categories

Druggable Gene Category	Matching Gene Count	Matching Gene(s)
CLINICALLY ACTIONABLE	12	BIRC3, RB1, CDKN1B, MAP2K4, NBN, TP53, RELA, HDAC1, STAT5A, PML, FOXO1, DEK
DRUGGABLE GENOME	11	NUP153, UBE2N, BIRC3, RB1, CASP3, MAP2K4, NFKB1, TP53, RELA, HDAC1, STAT5A
KINASE	10	BIRC3, RB1, CDKN1B, MAP2K4, NBN, NFKB1, TP53, RELA, STAT5A, IRF9
TRANSCRIPTION FACTOR	10	RB1, NFKB1, RELA, STAT5A, PML, IRF9, FOXO1, SP1, E2F1, NFYB
TUMOR SUPPRESSOR	5	BIRC3, RB1, CDKN1B, NBN, TP53
DRUG RESISTANCE	4	RB1, CDKN1B, TP53, PML
ENZYME	3	UBE2N, BIRC3, SUMO1
TRANSCRIPTION FACTOR COMPLEX	3	RB1, TP53, RELA
DNA REPAIR	1	SUMO1
PROTEASE	1	CASP3
SERINE THREONINE KINASE	1	MAP2K4
TRANSCRIPTION FACTOR BINDING	1	SUMO1
TRANSPORTER	1	TP53
TYROSINE KINASE	1	MAP2K4

Table_13: ***Table of Potential Drugs Targeting CASP3, NFKB1, TP53, RELA, and HDAC1 (QueryScore>=5).***

Search Term: "CASP3" ► CASP3

Drug	Interaction Type & Directionality	Sources	PMIDs	Query Score	Interaction Score
1,4-DICHLOROBENZENE	n/a	DTC	16699520	8.61	3.75
CHEMBL560532	n/a	DTC	19631549	8.61	3.75
PHATHALIMIDE	n/a	DTC	18296491	8.61	3.75
KOBOPHENOL A	n/a	DTC	17300930	8.61	3.75
TELEOCIDIN B	n/a	DTC	21696964	8.61	3.75

Search Term: "NFKB1" ► NFKB1

Drug	Interaction Type & Directionality	Sources	PMIDs	Query Score	Interaction Score
DEHYDROXYMETHYLEPOXYQUINOMICIN	n/a	DTC	18729348	8.61	0.69
ANDROGRAPHOLIDE	n/a	DTC TdgClinicalTrial	25615020	8.61	0.69
DIOSMETIN	n/a	DTC	25190486	5.74	0.46
KAEMPFERIDE	n/a	DTC	25190486	5.74	0.46
CHRYSOERIOL	n/a	DTC	25190486	5.74	0.46
TAMARIXETIN	n/a	DTC	25190486	5.74	0.46
ISORHAMNETIN	n/a	DTC	25190486	5.74	0.46
SORBINIL	n/a	DTC	24819954	5.74	0.46
QUERCETAGETIN	n/a	DTC	25190486	5.74	0.46

Search Term: "TP53" ► TP53

Drug	Interaction Type & Directionality	Sources	PMIDs	Query Score	Interaction Score
APR-246	n/a	JAX-CKB TTD	22965953 21239882 21415220 26086967 27179033	30.13	1.15
SAR-405838	n/a	JAX-CKB TTD	25145672 27550099 27765850	21.52	0.82
NUTLIN-3	n/a	DTC JAX-CKB	25964101 21058726 26494859 26298654 24268795 19928922 26883273	19.37	0.74
CHEMBL1241268	n/a	DTC	16862141	8.61	0.33
AVASTIN	n/a	JAX-CKB	21399866	8.61	0.33
ATRACTYLENOLIDE I	n/a	DTC	22365756	8.61	0.33
4-ISOTHIOUREIDOBUTYRONITRILE	n/a	JAX-CKB TTD	*None found*	8.61	0.33
CHEMBL1801219	n/a	DTC	20605096	8.61	0.33
YONDELIS	n/a	NCI	16888811	8.61	0.33
CAMPTOSAR	n/a	JAX-CKB	18618574	8.61	0.33
PHA-680632	n/a	JAX-CKB	18026196	8.61	0.33
RO-5045337	n/a	JAX-CKB CIViC	28459177	6.46	0.25

Search Term: "RELA" ► RELA

Drug	Interaction Type & Directionality	Sources	PMIDs	Query Score	Interaction Score
MULBERROFURAN H	n/a	DTC	24901948	8.61	2.42
CYNAROPICRIN	n/a	DTC	23232059	8.61	2.42
DEHYDROXYMETHYLEPOXYQUINOMICIN	n/a	DTC	18729348	8.61	1.21
BROMOPYROGALLOL RED	n/a	DTC	16759101	8.61	2.42
PYROCATECHOL VIOLET	n/a	DTC	16759101	8.61	2.42
ISORHAMNETIN	n/a	DTC	25190466	5.74	0.81
DIOSMETIN	n/a	DTC	25190466	5.74	0.81
CHRYSOERIOL	n/a	DTC	25190466	5.74	0.81
ANDROGRAPHOLIDE	n/a	DTC	22026410	5.74	0.81
QUERCETAGETIN	n/a	DTC	25190466	5.74	0.81
TAMARIXETIN	n/a	DTC	25190466	5.74	0.81
KAEMPFERIDE	n/a	DTC	25190466	5.74	0.81
SORBINIL	n/a	DTC	24819954	5.74	0.81

Table_14: ***Table of Final Potential Drugs for PTSD Generated by DGIdb.***

Drug	Gene	Query Score	Interaction Score
PHATHALIMIDE	CASP3	8.61	3.75
KOBOPHENOL A	CASP3	8.61	3.75
DIOSMETIN	NFKB1/RELA	5.74	0.46
KAEMPFERIDE	NFKB1/RELA	5.74	0.46
CHRYSOERIOL	NFKB1/RELA	5.74	0.46
DHMEQ	NFKB1	8.61	0.69
	RELA	8.61	1.21
TAMARIXETIN	NFKB1	5.74	0.46
	RELA	5.74	0.81
ISORHAMNETIN	NFKB1	5.74	0.46
	RELA	5.74	0.81
SORBINIL	NFKB1	5.74	0.46
	RELA	5.74	0.81
QUERCETAGETIN	NFKB1	5.74	0.46
	RELA	5.74	0.81
ANDROGRAPHOLIDE	NFKB1	8.61	0.69
	RELA	5.74	0.81
ATRACTYLENOLIDE I	TP53	8.61	0.33
MULBERROFURAN H	RELA	8.61	2.42
VORINOSTAT	HDAC1	5.61	0.57
TUBASTATIN A	HDAC2	8.61	2.63

I. Potential Drugs for PTSD predicted by WebGestalt-DrugBank:

1. **Glycyrrhizic acid** (Antagonist of CASP3 and Translocation Inhibitor of NFKB1):

An Anti-inflammatory, anti-ulcer, anti-allergic, antioxidant, anti-tumor, anti-diabetic and hepatoprotective.

Glycyrrhizin treatment in rats with PTSD showed significant improvements in anxiety and fear memory, as well as restoration of disrupted circadian rhythm in serotonin and Tryptophan Hydroxylase 2 (TPH2) (Lai et al., 2020).

2. **Arsenic trioxide** (Associated with HDAC1 and PML):

A chemotherapeutic agent used in the treatment of refractory or relapsed acute promyelocytic leukemia in patients with prior retinoid and anthracycline chemotherapy.

3. **Acetylsalicylic Acid** (TP53 Inducer and NFKB1 Inhibitor):

A salicylate used to treat pain, fever, inflammation, migraines, and reducing the risk of major adverse cardiovascular events. Aspirin use, particularly current, long-term, and low-dose, is associated with a decreased risk of depression, anxiety, and stress-related disorders following cancer diagnosis (Hu et al., 2020).

4. **Vorinostat** (HDAC Inhibitor):

a histone deacetylase (HDAC) inhibitor that induces cell apoptosis. it is used to treat cutaneous manifestations in patients with cutaneous T-cell lymphoma (CTCL) who have not responded to other therapies, and it has demonstrated effectiveness as an inhibitor of human papillomavirus (HPV)-18 DNA amplification (Banerjee et al., 2018).

A new approach for treating anxiety and fear-related disorders such as PTSD involves combining cognitive behavioral therapy (CBT) with cognitive enhancers. This strategy aims to target and enhance the learning mechanisms underlying CBT, particularly extinction-based exposure therapy, in order to overcome learning deficits and promote long-term fear inhibition for relapse prevention. Recent preclinical studies have shown that increasing histone acetylation, either through genetic or pharmacological inhibition of histone deacetylases (HDACs), or by targeting histone acetyltransferases (HATs), can enhance fear extinction and generate long-lasting extinction memory. These effects are mediated by molecular mechanisms and pathways involving brain-derived neurotrophic factor (BDNF) and N-methyl-D-aspartate (NMDA) receptor signaling.This presents a clear recommendation for a new therapeutic approach in treating PTSD by enhancing the process of fear extinction (Matsumoto et al., 2013; Whittle & Singewald, 2014).

5. **Andrographolide** (NFKB1 Inhibitor):

A botanic extract from the herb Andrographis Paniculata, exhibits anti-inflammatory, anticancer, and hepatoprotective activities. It specifically inhibits the activation of NFKB1 through covalent modification of a cysteine residue on p50 in endothelial cells (Xia et al., 2004; Zhai et al., 2014).

Recent research investigated the potential antidepressant effects of andrographolide in mice exposed to chronic unpredictable mild stress (CUMS) (Geng et al., 2019).

Another study examined the antidepressant effects andrographolide in a zebrafish model of chronic unpredictable stress (CUS). The administration of andrographolide as a single treatment in the CUS zebrafish resulted in increased locomotion activity and decreased cortisol levels, indicating an antidepressant-like effect of the compound (Aldurrah et al., 2023).

6. **Triflusal** (NFKB1 Antagonist):

A medication related to acetylsalicylic acid with antithrombotic effects used in the treatment of thromboembolic diseases.Triflusal has shown potential neuroprotective effects by inhibiting inflammatory pathways in rat models with permanent middle cerebral artery occlusion (Alvarez-Sabín et al., 2009).

7. **Pranlukast** (NFKB1 Inhibitor):

A leukotriene receptor antagonist for the treatment of allergic rhinitis and asthma symptoms.

8. **Fingolimod** (HDAC1 Inhibitor):

A sphingosine 1-phosphate receptor modulator used to treat patients with the relapsing-remitting form of multiple sclerosis (MS) and studied to manage lung complications of COVID-19.Fingolimod exhibits a protective effect on hippocampal neurons against stress-induced damage and mitigates depressive symptoms through the inhibition of neuroinflammation (Guo et al., 2020).

9. **Minocycline** (CASP3 Negative Modulator):

A tetracycline analog used to treat a wide variety of infections in the body. Minocycline has shown promising results in both preclinical and human studies for the treatment of PTSD. In animal models, it was found to alleviate PTSD-like behaviors, reduce pro-inflammatory cytokine levels, and inhibit NF-κB in the hippocampus. Additionally, in a 12-week human study, adjunctive minocycline treatment resulted in a reduction in PTSD symptoms and improvements in depression symptoms (Gerst et al., 2021; W. Wang et al., 2018).

10. **Romidepsin** (HDAC1 Antagonist Inhibitor):

It is used to treat cutaneous T-cell lymphoma.

II. Potential Drugs for PTSD predicted by DGIdb:

1. **Phtalamide:** a precursor of Thalidomide and other drugs that belong to Immunomodulatory Imide Drugs (IMiDs) (Y. J. Jung et al., 2021). Thalidomide is an anti-TNF agent that has neuroprotective effects, apparently due to its anti-apoptotic, anti-oxidant, and anti-inflammatory effects (Palencia et al., 2015).
   A recent study focused on using Thalidomide as a Potential Treatment for Comorbid Pain in PTSD since chronic pain is one of the most frequent comorbidities for patients with PTSD and higher levels of the proinflammatory cytokine tumor necrosis factor α (TNF-α) have been detected in these patients (Illouz, 2022).

2. **Kobophenol A:** a natural oligomeric stilbenoid isolated from Caragana genus and is a tetramer of resveratrol (Gangadevi et al., 2021). Kobophenol A has been recognized for its ability to inhibit the activity of acetylcholinesterase; an enzyme involved in the breakdown of acetylcholine. This property of kobophenol A is significant in the context of neuroprotection, making it a potential candidate for the treatment of neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease (Lee et al., 2007).

3. **Diosmetin:** a naturally occurring O-methylated flavone found in citrus fruits, is the aglycone component of diosmin, a flavonoid glycoside. Pharmacologically, diosmetin has been shown to possess a range of beneficial effects, including anticancer, antimicrobial, antioxidant, estrogenic, and anti-inflammatory activities (Patel et al., 2013).

4. **Kaempferide:** an O-methylated flavonol that is a derivative of kaempferol. It can be found in Kaempferia galanga (aromatic ginger). Kaempferol has been found to significantly decrease the level of HMGB1 and inhibit the TLR4/MyD88 inflammatory pathway at both the transcriptional and translational levels. Additionally, numerous studies have provided evidence of the antioxidant and anti-inflammatory properties of kaempferol (Hussein et al., 2018; Mahat et al., 2010).
   Kaempferol exhibits neuroprotective effects, particularly in several neurodegenerative diseases like Parkinson's disease, Alzheimer's disease, and Huntington's disease. Its beneficial effects contribute to the protection and preservation of neuronal health in neuroinflammation conditions by inhibiting NKB1 (Yu et al., 2013).

5. **Chrysoeriol:** is a 30-O-methoxy flavone, which means that it is a chemically derived product of luteolin found in a variety of plant species (Mishra et al., 2003). Chrysoeriol ameliorates TLR4-mediated inflammatory responses by inhibiting

NFKB1 (Yoon & Park, 2021). In vivo pharmacokinetic studies have demonstrated the excellent stability of this compound, indicating its promising potential for the prevention or treatment of various diseases. These include cancer, diabetes, inflammation, osteoporosis, Parkinson's disease, and cardiovascular diseases (Aboulaghras et al., 2022).

6. **DHMEQ:** Dehydroxymethylepoxyquinomicin is a potent, selective and irreversible NFKB1 inhibitor that distresses its nuclear translocation and shows anti-inflammatory and anticancer activity (Watanabe et al., 2005).

7. **Tamarixetin:** a natural flavonoid derivative of quercetin, with anti-oxidative and anti-inflammatory effects. It protects against cardiac hypertrophy (Fan et al., 2019). Tamarixetin also has been shown to decrease the release of different inflammatory cytokines by dendritic cells. Additionally, it enhances the production of the anti-inflammatory cytokine interleukin (IL)-10 and specifically boosts the population of immune cells that secrete IL-10 (Park et al., 2018).

8. **Isorhamnetin:** A monomethoxyflavone derivative of Quercetin that is found in many plant species. Many in-vivo studies suggested the anxiolytic effect of Quercetin in many anxiety disorders including PTSD (Wróbel-Biedrawa et al., 2022).
   A study investigating the anxiolytic properties of Quercetin found that its anxiolytic effect is mediated through GABAA-ρ receptors. Importantly, the administration of quercetin did not induce any hypoactivity or myorelaxation, suggesting that it could be developed as an effective anxiolytic drug without any associated side effects (J. Jung & Lee, 2014).

9. **Sorbinil:** an Aldose reductase inhibitor (ARI) that plays therapeutic role in treating diabetes and diabetic complications, decreases AR activity and inhibits polyol pathway which has been linked to the development of secondary diabetic complications (Huang et al., 2019). A study suggested that inhibition of aldose reductase, which prevents NFB1 activation, may be a useful approach for treating vascular inflammation caused by diabetes (Huang et al., 2019).

10. **Quercetagetin:** Quercetagetin is a flavonol, a type of flavonoid and a derivative of Quercetin. Quercetagetin has antioxidant and antiviral effects.In a cohort primarily consisting of male veterans, individuals diagnosed with PTSD had a nearly twofold increased risk of developing dementia compared to those without PTSD. A history of PTSD may exhibit neurocognitive deficits and increased tau deposition in the brain many years after their traumatic experiences (Mohamed et al., 2019; Yaffe et al., 2010). Quercetagetin has been shown to effectively inhibit tau accumulation and reverse neuroinflammation and cognitive deficits in P301S-

Tau transgenic mice by prevention of Tau phosphorylation in the hippocampus and cortex and inhibition the of NFKB1 activation (Zhong et al., 2023).

11. **Andrographolide:** It was among the compounds predicted by WebGestalt too, its antidepressant-like effect in PTSD has been explained in **page 54**.

12. **Atractylenolide I:** a major active component of the Rhizoma of Atractylodes Macrocephala Koidz. Atractylenolide I exhibits a diverse array of pharmacological activities that include anti-inflammatory properties, anti-tumor effects by promoting tumor antigen presentation of both human and mouse colorectal cancer (CRC) cells and thereby enhances the cytotoxic response of CD8+ T cells (H. Xu et al., 2021).
    On the other hand, Atractylenolide I (AT-I) demonstrates antidepressant-like effects in a mouse model of depression induced by chronic unpredictable mild stress (CUMS). The molecular mechanism underlying these effects involves the inhibition of NLRP3 inflammasome activation, leading to a reduction in the production of interleukin-1β (IL-1β). This suggests that the antidepressant properties of AT-I may be attributed to its ability to modulate the inflammatory response mediated by the NLRP3 inflammasome and IL-1β (Gao et al., 2018).

13. **Mulberrofuran:** a 2-arylbenzofuran derivative from the cultivated mulberry tree (Nomura et al., 1982).
    PTSD is a significant risk factor for cognitive decline and increased deposition of Amyloid β (Aβ) plaques, which resembles the pathological hallmark of Alzheimer's disease (Mohamed et al., 2018). It has been predicted that mulberry fruit, due to its properties, can potentially prevent the formation of Amyloid β plaques through the inhibition of phospho-extracellular signal-regulated protein kinases 1 and 2 (p-ERK1/2) or the inhibition of β-site amyloid precursor protein cleaving enzyme 1 (BACE1) in in-vitro models. This suggests that mulberry fruit possesses antioxidant properties and has the potential to improve cognitive function, enhance the learning process, reduce memory impairment, alleviate Parkinson's disease-like behaviors, and exhibit anxiolytic, anti-depressant, and anti-ischemic activities (Tam et al., 2021).

14. **Vorinostat:** It was among the compounds predicted by WebGestalt too, and its participation in PTSD management as a novel approach by enhancing the process of fear extinction has been previously elucidated in **page 54**.

15. **Tubastatin A:** a selective inhibitor of HDAC6 has been found to exhibit a neuroprotective effect and has shown therapeutic potential in animal models of cognitive and neurodegenerative disorders (Butler et al., 2010).
    In addition, it has shown therapeutic effectiveness in animal models of cognitive

and neurodegenerative disorders by decreasing the overall level of Tau protein (Selenica et al., 2014). The findings of a study aimed to assess the neuroactive properties of Vorinostat and Tubastatin A in both in vitro and in vivo suggested that the newly developed HDAC inhibitors hold promise as potential therapeutics for anxiety, depression, and other related psychiatric disorders (Reddy et al., 2019).

13. Intersection analysis of PTSD-associated Biomarkers with the Identified Hub Genes, Key TFs, and miRNAs in the Present Study through PTSD Biomarker Database:

At first, the 10 most frequently observed biomarkers in PTSD were extracted from **PTSDDB** and they were represented in a bar chart, which provided a clear visualization of their relative occurrence across the reported studies. The top ten biomarkers identified were BDNF, Cortisol, DHEA-S, FKBP5, NR3C1, IL6, CRP, CRH, Serotonin, and NPY **(Figure_24)**.

In addition, PTSD biomarkers that are linked to PBMCs were retrieved including FKBP5, NR3C1, PBMC Ratio, NR3C1-α, miR-125a-5p, miR-296, B-Lymphocytes, CD56brightCD16- NK cells, and Monocyte number **(Figure_25)**.

Finally, the intersection analysis of **PTSDDB** content and the present study results revealed that MAP2K4, NUP153, NFKB, hsa-mir-212-3p, and hsa-mir-199b-3p are among the biomarkers present in the database. In terms of their observed effect, MAP2K4, NUP153, and NFKB showed increased upregulation in PTSD patients compared to control cases, while hsa-mir-212-3p and hsa-mir-199b-3p exhibited downregulation and a decrease in change. (**Figure_26)**.

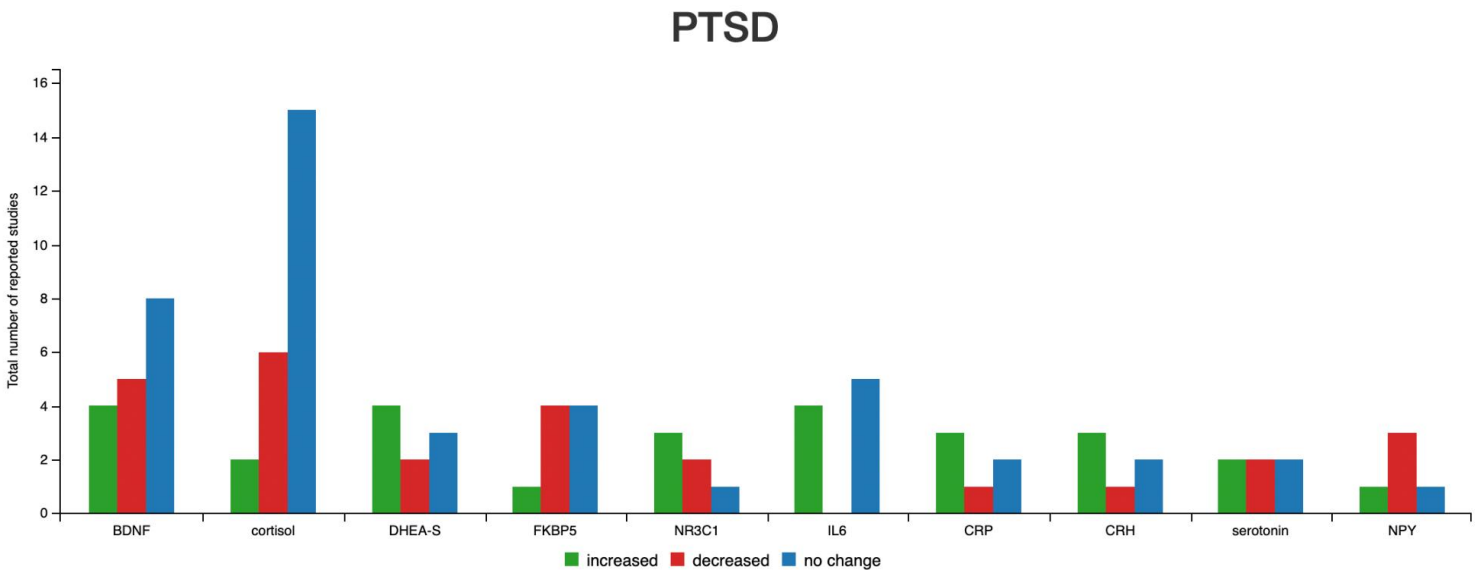


Figure_24: ***Bar Chart of the Relative Changes for the Most Common Biomarkers Collected in PTSD Extracted from PTSDDB.***

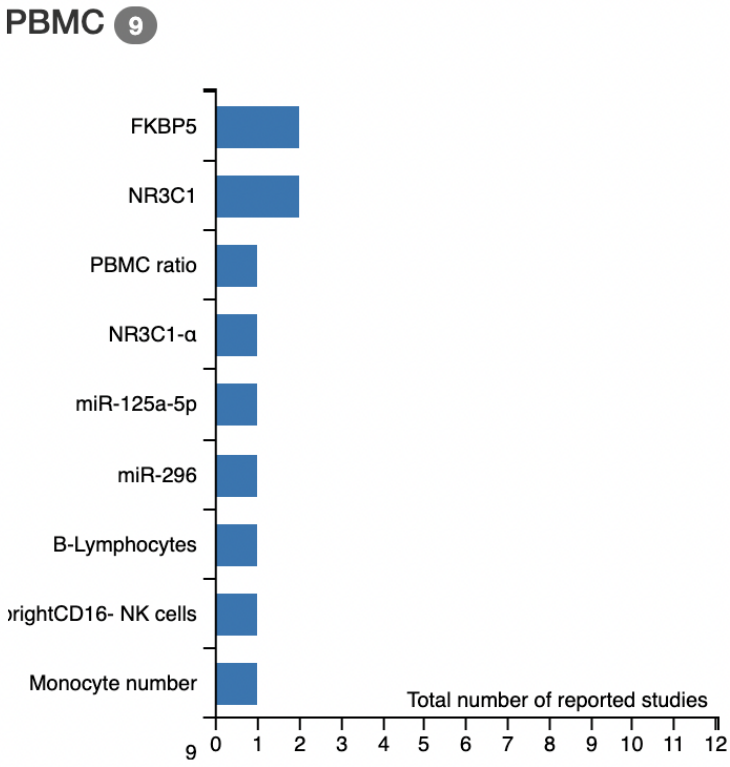


Figure_25: ***Chart of PTSD Markers in PBMC with the Number of Studies in which Each Biomarker Was Measured by PTSDDB.***

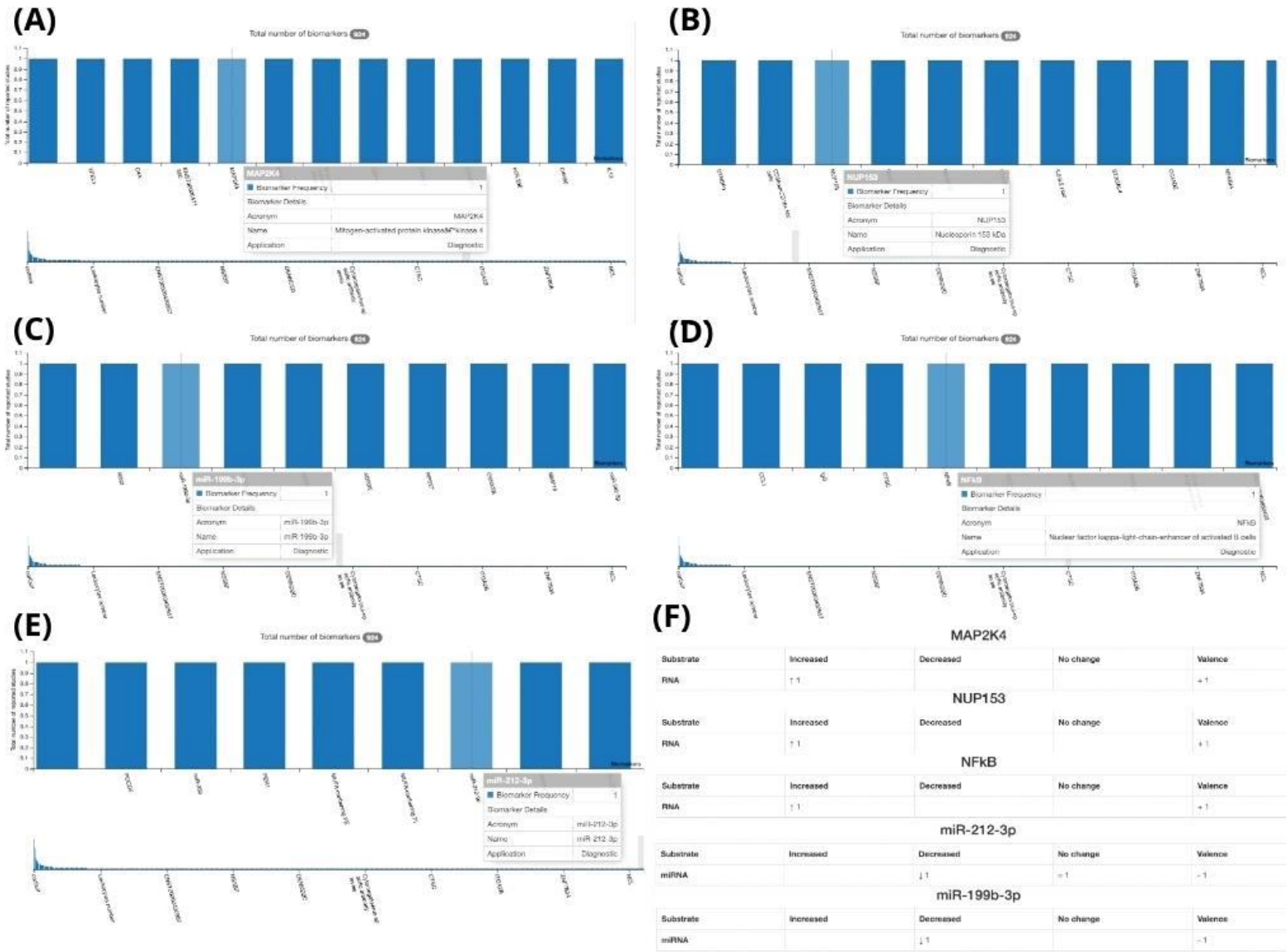


Figure_26: ***Intersection Analysis of PTSD-Associated Biomarkers: Hub Genes, Key TFs, and miRNAs in Comparison with PTSDDB: (A) MAP2K4, (B) NUP153, (C) miR-199b-3p, (D) NFKB, (E) miR-212-3p, (F) Their Observed Change.***

Discussion

PTSD is a severe stress and anxiety-related disorder that occurs in response to particular traumatic events or experiences.
Gaining insights into the gene pathways linked to PTSD and their interactions with the fear and stress circuitry is crucial for understanding the mechanisms underlying the risk and resilience factors associated with this complex disorder. Such understanding paves the way for the development of targeted therapeutic interventions aimed at preventing the onset of PTSD or reducing its severity. (S. B. Banerjee et al., 2017).
The analysis of gene expression in PTSD poses certain difficulties, primarily because the molecular events underlying this disorder are believed to occur within the central nervous system (CNS). Unfortunately, the availability of postmortem brain tissue from individuals affected by PTSD lags behind that of other CNS disorders such as Parkinson's and Alzheimer's Disease. Nevertheless, accumulating evidence suggests that PTSD is associated with systemic immune and metabolic disruptions resulting from stress-induced alterations in the hypothalamic-pituitary-adrenal (HPA) axis. Despite these challenges, comprehensive analyses of gene expression in peripheral blood are performed to shed light on the underlying neurobiological mechanisms of PTSD and its co-occurring conditions (Guardado et al., 2016; Michopoulos et al., 2016).
Therefore, in this present study, the primary objective was to identify significant contributors and potential molecular mechanisms associated with PTSD and investigate novel targets in PTSD pharmacotherapy through bioinformatics analysis.

Initially, a normalized RNA-seq dataset of PTSD patients was retrieved from the GEO database. The analysis started by employing GEO2R which revealed that out of the total gene pool, 417 genes exhibited differential expression patterns in response to PTSD. Among these genes, 372 were up-regulated, indicating increased expression, while 46 were down-regulated, suggesting decreased expression, when comparing the PTSD group to the control group.
The DEGs were subjected to functional annotation and KEGG pathway enrichment analysis using the DAVID database. The annotation analysis involved categorizing the genes into three groups based on Gene Ontology (GO), which includes Biological Processes (BP), Cell Component (CC), and Molecular Function (MF).
The results of the analysis revealed that the majority of DEGs are mainly abandoned in the nucleus and cytoplasm, participating in many biological processes related to mRNA maturation and transcription, additionally, the genes were found to have functional roles in binding processes of many biomolecules, particularly proteins and RNAs.

Furthermore, the DEGs were involved in a variety of essential pathways in cell cycle, cancer, apoptosis, inflammation, and immune response including TNF-signaling pathway.

These initial findings went along with several previous studies that suggested the strong link between PTSD and immune response alteration including high production of pro-inflammatory cytokines and TNF-a in PBMCs in PTSD patients compared to healthy individuals (Gola et al., 2013).
On the other hand, a reduced level of apoptotic markers in the serum of subjects with PTSD was detected (Mkrtchian et al., 2013) and abnormal apoptosis in specific brain regions of animals undergoing single prolonged stress as a model for PTSD (F. Han et al., 2013a; Jia et al., 2018; Li et al., 2013).These findings suggest that dysfunction in apoptosis may contribute to the observed inflammation pattern commonly seen in individuals with PTSD (Mkrtchian et al., 2013).

The DEGs were analyzed using the STRING database to assess their interaction relationships where interactions with a confidence score greater than 0.9 were considered significant. The constructed network exhibited a higher number of interactions than expected which indicates that these proteins are biologically connected as a group, at least to some extent.
Cytoscape was employed to visually explore the interactive networks, with a cut-off criterion of a confidence score greater than 0.4 to determine the significance of the interactions. While CytoHubba was used to calculate the connectivity score of the genes based on a four-fold algorithm. By intersecting the results, a total of 14 genes were identified as common among the four algorithms, with 5 of these genes (NUP153, NUP50, NUP160, SP100, and SMC4) appearing in the three most significant modules identified by MCODE.
The 14 candidate hub genes were cross-referenced with data from the Comparative Toxicogenomics Database (CTD) and examined in relation to previous studies (Breen et al., 2018; Miller et al., 2008). As a result, 10 out of the 14 genes demonstrated differential expression in patients with PTSD and chronic stress, indicating their potential involvement in the disorder. While all the 14 genes show inferred association to PTSD according to CTD, CASP3 and CDKN1B had the highest inferred scores.
Both CASP3 and CDKN1B are involved in the regulation of apoptosis, but they operate through different mechanisms. CDKN1B acts as a negative regulator of the cell cycle by inhibiting cyclin-dependent kinases, thereby leading to cell cycle arrest. Dysregulation of CDKN1B and the consequent activation of cyclin-dependent kinases have been implicated in neuronal apoptosis observed in neurodegenerative diseases (Castedo et al., 2002).
On the other hand, CASP3 functions as a key effector caspase that plays a central role in the execution phase of apoptosis. It is involved in various cellular processes, including neurogenesis and synaptic activity in the central nervous system. Abnormal regulation of CASP3 has been associated with the pathogenesis of chronic neurodegenerative diseases (D'Amelio et al., 2010, 2012; Louneva et al., 2008). Therefore, the low

expression of CASP3 in PTSD may disrupt apoptotic processes, leading to impaired cell death or aberrant cell survival in response to stressors.

The previous step confirmed that final identified hub genes in the current study are NUP150, NUP153, NUP160, UBE2N, SP100, BIRC3, SMC4, YWHAZ, RB1, SUMO1, CDKN1B, CASP3, MAP2K4, and NBN, and all hub genes were downregulated in PTSD. The hub genes play significant roles in many biological processes starting from protein hemostasis, immune system dysregulation, inflammatory response, cellular stress response, signal transduction, cell cycle regulation, and apoptosis.
The functional analysis of the hub genes that was performed using Metascape confirmed this contribution by revealing that 11 of the hub genes (all hub genes except RB1, SMC4, NBN) participate in Cytokine signaling in the immune system while 10 genes contributed in cell cycle regulation.
Immune system has been associated with PTSD through several studies and these studies were either Focusing on exploring the possibility of an immune-related or inflammatory cause for PTSD (Cohen et al., 2011; Pervanidou et al., 2007), or suggesting that PTSD contributes to inflammation (Solomon et al., 2017; Toft et al., 2018).While others propose a bidirectional relationship between PTSD and inflammation (Sumner et al., 2018).
An important example is BIRC3, which is a cell signaling regulator involved in innate immune responses specifically in regulation of canonical and non-canonical signaling to the inflammatory transcription factor nuclear factor-κB (NF-κB).When NF-κB is activated, it not only triggers the expression of inflammatory cytokines, chemokines, and enzymes associated with inflammation, but also stimulates the expression of numerous anti-apoptotic genes (Berthelet & Dubrez, 2013; Estornes & Bertrand, 2015).
On the other hand, several hub genes are linked to neuronal function and survival. For example, YWHAZ has been associated with the regulation of neuronal activity and synaptic plasticity. Recent research indicates that a lack of YWHAZ can result in a disruption of the normal functioning of dopamine and serotonin in Zebrafish (Antón-Galindo et al., 2022; Wan et al., 2023).

A prediction analysis of miRNAs and transcription factors that target the identified hub genes was needed to fully understand the regulatory network associated with PTSD, including both genetic and epigenetic factors.
Epigenetics encompasses processes influenced by environmental factors that induce long-lasting but reversible changes in gene expression. Environmental influences play a significant role in PTSD, leading to epigenetic modifications that alter gene expression. miRNAs, as key epigenetic regulators, bind to complementary sequences on mRNA, particularly in the 3' UTR region, to suppress translation. This comprehensive approach provides insights into the complex mechanisms underlying PTSD (Bartel, 2004).

Numerous studies have shown that miRNAs play a crucial role in regulating immune system genes, highlighting the tight control exerted by miRNAs on the immune system (Bam et al., 2016; J. Zhou et al., 2014).
Using miRNet, 44 miRNAs were identified as targeting hub genes and differentially expressed in PTSD patients. Among them, hsa-mir-222-3p is notably overexpressed in PTSD patients. This overexpression leads to the downregulation of its target hub genes (CDKN1B, CASP3, BIRC3, and UBE2), resulting in the dysregulation of the biological functions and mechanisms associated with these genes, as mentioned previously (Snijders et al., 2019).
In addition to studying epigenetic factors that affect the regulation of hub genes, it was crucial to explore and identify the TFs of the hub genes since they are the direct regulators and might represent potential therapeutic targets for PTSD.
Thirteen key TFs were identified and confirmed to exhibit differential expression in PTSD patients through the use of TRRUST, miRnet, and comprehensive review of previous studies. NFKB, an essential transcription factor identified in our study, plays a critical role in immune response regulation and it represents a promising therapeutic target for many disorders, including PTSD (Kumar et al., 2004).
Moreover, text-mining analysis was conducted using Coremine Medical to gather additional evidence supporting the association between Hub genes, key TFs, and pathways relevant to PTSD.

One of the main objectives of this study was to gain new insights into the management of PTSD. To achieve this, an analysis of currently FDA-approved medications was performed. Then it was crucial to predict potential drugs that could be repurposed for PTSD treatment using computational drug repurposing methods.
Using CTD, Chemical-gene and chemical-pathway analyses of Sertraline and Paroxetine with hub genes,key TFs, and PTSD pathways revealed their potential to increase CASP3 activity, while Sertraline specifically increases NFKB1 expression. In addition to their association with many pathways involved in cell cycle regulation and apoptosis.
It is well-established that individuals with PTSD have smaller hippocampal volumes compared to those without the disorder. Interestingly, recent studies have highlighted a connection between PTSD and endoplasmic reticulum (ER) stress, specifically the apoptosis of neuronal cells in the hippocampus, which is a key brain region involved in memory and emotional regulation (F. Han et al., 2013b; Mahendra et al., 2017).
Sertraline impacts the brain by increasing oxidative stress, impairing spatial memory, and reducing the expression of the GRP78-Sig1R complex in the hippocampus. This complex is important for unfolded protein response (UPR) signaling and promoting cell survival during stress. Consequently, the use of sertraline may exacerbate ER stress by enhancing oxidative stress, potentially contributing to the increased risk of suicidality associated with SSRIs in PTSD patients (Hayashi, 2015; Mahendra & Putra, 2018).

These findings highlighted the need to distinguish PTSD pharmacotherapy from medications used for other psychological disorders like major depression disorder. This emphasizes the importance of identifying novel drugs specifically targeted for PTSD treatment which was performed in the current study by computational drug repurposing.

The first drug repurposing analysis of hub genes and key TFs was conducted using WebGestalt through DrugBank as a functional database aiming to predict gene-drug interactions.
Ten potential compounds were identified by WebGestalt. Among these compounds, Glycyrrhizic acid, Vorinostat, and Minocycline have undergone preclinical studies to assess their efficacy in managing PTSD, suggesting their potential as therapeutic options for the condition (Fujita et al., 2012; Gerst et al., 2021; Lai et al., 2020).
Additionally, Aspirin and Andrographolide have demonstrated antidepressant and anxiolytic activity (Aldurrah et al., 2023; Hu et al., 2020). While Triflusal and Fingolimod have shown potential neuroprotective effects (Alvarez-Sabín et al., 2009; Guo et al., 2020).

Another drug repurposing analysis was conducted using DGIdb, taking into account the enrichment pathways associated with the genes under consideration.
DGIdb was utilized at multiple stages of the analysis. Initially, it was employed to predict 11 druggable genes. To refine the selection, a querying score threshold of 5 was set, followed by manual filtration and analysis. As a result, 15 potential compounds were identified based on the enriched pathways associated with the genes, including Cytokine Signaling in the Immune System (CASP3, NFKB1, TP53, RELA), Cell Cycle (HDAC1, TP53), Toll-Like Receptor 3 (TLR3) Cascade (NFKB1, RELA, TP53), and Cellular Responses to Stress (NFKB1, RELA, TP53).
The identified drugs were classified into two categories based on their origin: synthetic compounds and botanical active ingredients. Interestingly, two of the previously mentioned compounds, Andrographolide and Vorinostat, were found to intersect with the results of this analysis.
Regarding synthetic compounds, Phtalamide -a precursor of Thalidomide- was identified. Further studies should evaluate its potential pharmacology effect in PTSD management since Thalidomide has already been shown to effectively reduce the proinflammatory cytokine TNF-α, indicating its potential as a treatment for comorbid pain in individuals with PTSD (Illouz, 2022).
Furthermore, Tubastatin A, another inhibitor of HDAC (histone deacetylase), has shown to inhibit the accumulation of Tau protein and maintain its neuroprotective properties. These results, along with the effectiveness of Vorinostat in treating PTSD, highlight the potential of newly developed HDAC inhibitors as promising therapeutic options for anxiety, depression, and other psychiatric disorders (Reddy et al., 2019).

Among the numerous botanical active ingredients, Kaempferide, Tamarexetin, Isorhamnetin, and Quercetagetin have been identified as having potential direct effects on PTSD treatment or neuroprotective properties. These findings highlight Ginkgo Biloba as a promising treatment option for PTSD, as it contains these compounds or their derivatives. Furthermore, Ginkgo extract reduces mitochondrial free radical generation associated with PTSD symptoms and demonstrates potential anxiolytic and mild antidepressant effects by inhibiting monoamine oxidase A and B in the brain (Aliev et al., 2020; Singh et al., 2017).
Interestingly, a significant number of the predicted botanical active compounds are found in Ginkgo Biloba, Mulberry, and Glycyrrhiza Glabra. These plants are widely recognized as adaptogenic plants due to their remarkable ability to restore and enhance bodily functions while fortifying systems that are vulnerable to the effects of stress (Nomura et al., 1982; Singh et al., 2017).

Currently, the management of PTSD involves both pharmaceutical treatment with medications like Sertraline and Paroxetine, as well as psychological treatment options recommended by the American Psychological Association (APA). These psychological treatments include Prolonged Exposure (PE), Cognitive Processing Therapy (CPT), and trauma-focused Cognitive Behavioral Therapy (CBT). (Watkins et al., 2018).
However, exploring alternative approaches for treating PTSD may hold significant potential. Six non-pharmacological and non-psychological interventions for PTSD were identified as effective including acupuncture, neurofeedback, aikokeishikankyoto (a herbal preparation, somatic experiencing, transcranial magnetic stimulation, and yoga as effective interventions (Bisson et al., 2020).
Another noteworthy approach is music therapy which offers a promising and more accessible alternative treatment option for individuals with PTSD. Theoretical and empirical evidence indicates that individuals who have experienced trauma and suffer from PTSD may benefit from music therapy since It can improve functioning, foster resilience, and help individuals recover from trauma (Landis-Shack et al., 2017). Additionally, previous studies have shown its effectiveness in treating trauma-exposed individuals with PTSD (Dokter, 1998; Heidenreich, 2005; MacIntosh, 2003).
Despite the evidence from brain imaging studies showing that listening to music can impact brain structure and function, the specific molecular mechanisms underlying these effects remained unknown. However, a pioneer study recently investigated the impact of music on the transcriptome, shedding light on these mechanisms (Kanduri et al., 2015).
Music therapy has been found to have an impact on epigenetic mechanisms, influencing gene expression in a therapeutic manner.Recent studies have shown that music can modulate miRNA expression and impact behavior, indicating a connection between music and biochemical changes.Furthermore, there is growing evidence of the epigenetic foundation of music therapy, with studies highlighting its benefits in various

populations, from preterm infants to individuals with Alzheimer's disease (Mastnak, 2023).
Two studies conducted an analysis of the impact of listening to music and practicing music on the expression of miRNAs. The findings revealed that the miRNAs that exhibited differential expression are involved in regulating critical neurological molecular and biological functions (P. Nair et al., 2021; P. S. Nair et al., 2019).
Interestingly, the results from these studies revealed that music has an impact on the expression of three miRNAs, which have already been identified and confirmed as differentially expressed in individuals with PTSD: hsa-mir-23a-3p, hsa-mir-132-3p, and hsa-mir-222-3p.
This finding establishes a connection between PTSD and music therapy at the epigenetic level, highlighting the potential of music therapy as an adjunctive treatment in managing PTSD.

As a final step in my research, I used PTSD Biomarker database to intersect my findings with its content and it came out that MAP2K4, NUP153, NFKB, hsa-mir-212-3p, and hsa-mir-199b-3p are identified as biomarkers.
The valuable information derived from PTSDDB includes the identification of significant biomarkers in various body fluids. This discovery has brought attention to the potential of utilizing routine low-cost blood tests, in combination with neuropsychological evaluation, for diagnosing PTSD. Clinical interviews and self-report measures have proven to be challenging in accurately diagnosing PTSD, as patients may under-report or over-report symptoms due to factors such as stigma or compensation-seeking. Therefore, the exploration of biomarkers through blood tests offers a promising avenue for more objective and reliable PTSD diagnosis, reducing the reliance on subjective self-reporting methods (M. Xu et al., 2023).
PTSDDB identified several blood biomarkers, including PBMC Ratio (along with separate biomarkers for Monocyte and Leukocyte Count), CRP, Glucose, Cortisol, TG, and HDL.These tests are readily accessible, affordable, and widely available in all countries, including those with economic challenges. This accessibility facilitates the diagnosis and evaluation of PTSD, making it less challenging especially in low-income countries.
A recent study could define five key biomarkers that differentiate individuals with PTSD from the healthy control group: glucose, CRP, HbA1c, WBC, and alkaline phosphatase. Glucose and HbA1c levels were higher in the PTSD group, indicating impaired glucose metabolism that may lead to pre-diabetes and diabetes. Elevated CRP and WBC levels suggest an inflammatory state in individuals with PTSD which goes along with my findings in the current study. While alkaline phosphatase levels were lower in the PTSD group, contrary to expectations. Lower alkaline phosphatase levels are rare and may be associated with mineral deficiencies such as zinc and magnesium that are linked to chronic stress and depression (J. Wang et al., 2018; M. Xu et al., 2023).

Ultimately, it is essential to address the issue of PTSD in Syria, a country that has endured a prolonged decade of conflict characterized by various traumatic events, including witnessing physical aggression, violence, clashes, explosions, injured individuals, loss of loved ones and then dealing with the aftermath of a devastating earthquake. Extensive research indicates that experiencing multiple traumatic events significantly heightens the risk of severe and long-lasting PTSD symptoms compared to experiencing a single traumatic event (Breslau et al., 1999; Gerber et al., 2018; Suliman et al., 2009).

A population-based survey found that experiencing four or more traumatic events is associated with greater functional impairment, earlier onset of PTSD, longer symptom duration, higher comorbidity, and other psychological dysfunctions (Karam et al., 2014). Nevertheless, concerning managing PTSD from multiple traumas, a recent study suggests a focused cognitive treatment approach (Kube et al., 2023).

Syrian children and adolescents have faced traumatic events and daily hardships that greatly impact their psychological well-being. Limited access to mental health care services, language barriers, denial, misunderstanding, and stigma surrounding mental illness, may have delayed or even hindered their recovery
(Hassan et al., 2016; Tabur et al., 2019).

Determining the prevalence of PTSD in Syria is a complex task due to the challenges in diagnosis and the diverse Syrian population segments, including residents, expatriates, and refugees, each with varying levels of traumatic experiences. However, a meta-analysis of 26 studies involving 11,400 Syrian children and adolescents revealed a PTSD prevalence of 36% among those exposed to the 2011 Syrian war. This estimate is higher than rates observed in studies of children and adolescents exposed to other traumatic events or conflicts in different regions (Kanan & Leão, 2022).

Conclusion

In conclusion, this research employed an integrative bioinformatics approach to analyze a microarray dataset of PTSD patients. The analysis identified 418 DEGs and constructed PPI networks, leading to the identification of 14 hub genes using a four-fold algorithm method. These hub genes were found to be involved in key pathways associated with PTSD, such as cytokine signaling in the immune system, cell cycle regulation, and apoptosis-related processes. Additionally, the study predicted 44 miRNAs and 13 key TFs that were confirmed to regulate the majority of the hub genes associated with PTSD. Separate networks were constructed for the interactions between miRNAs and hub genes, as well as between key TFs and hub genes. Furthermore, computational drug repurposing led to the identification of 25 potential medications. Finally, five of the findings identified in this study, including two hub genes, one key TF, and two miRNAs, were validated as PTSD biomarkers.

This study holds important implications for advancing our understanding of the molecular foundations of PTSD and identifying potential targets for therapeutic interventions. By elucidating the molecular mechanisms and identifying biomarkers, this research contributes to the development of improved diagnostic tools and personalized treatment approaches. As a final point, it is important to emphasize that the findings of this bioinformatics research need to be further validated and experimented in wet lab settings. Conducting further wet lab experiments is essential to confirm the reliability and accuracy of the results incorporating and will provide a more comprehensive understanding of the pathogenesis of PTSD and ensure the robustness of the identified biomarkers and therapeutic targets.

Research Limitations

Despite the valuable insights gained from this study, it is important to acknowledge certain limitations and challenges in PTSD research. Firstly, the availability of large and well-characterized datasets specifically focused on PTSD is limited, which resulted in a relatively small dataset for analysis. This may restrict the generalizability of the findings to a broader population of individuals with PTSD.

Additionally, the scarcity of gene expression databases specifically dedicated to mental disorders, including an absolute lack of a specialized database for PTSD, posed challenges in the verification of hub genes.

Furthermore, the absence of experimental validation represents a limitation in fully confirming the identified hub genes, biomarkers, and therapeutic targets. The verification of at least the hub genes using RT-qPCR would have been highly beneficial. Therefore, future research should prioritize experimental validation to enhance the robustness and reliability of the study's outcomes.

References

1. Aboulaghras, S., Sahib, N., Bakrim, S., Benali, T., Charfi, S., Guaouguaou, F.-E., Omari, N. E., Gallo, M., Montesano, D., Zengin, G., Taghzouti, K., & Bouyahya, A. (2022). Health Benefits and Pharmacological Aspects of Chrysoeriol. *Pharmaceuticals (Basel, Switzerland)*, *15*(8), 973.
2. Aldurrah, Z., Mohd Kauli, F. S., Abdul Rahim, N., Zainal, Z., Afzan, A., Al Zarzour, R. H., Muhamad Salhimi, S., Che Zain, M. S., & Zakaria, F. (2023). Antidepressant evaluation of Andrographis paniculata Nees extract and andrographolide in chronic unpredictable stress zebrafish model. *Comparative Biochemistry and Physiology. Toxicology & Pharmacology: CBP*, *271*, 109678.
3. Aliev, G., Beeraka, N. M., Nikolenko, V. N., Svistunov, A. A., Rozhnova, T., Kostyuk, S., Cherkesov, I., Gavryushova, L. V., Chekhonatsky, A. A., Mikhaleva, L. M., Somasundaram, S. G., Avila-Rodriguez, M. F., & Kirkland, C. E. (2020). Neurophysiology and Psychopathology Underlying PTSD and Recent Insights into the PTSD Therapies—A Comprehensive Review. *Journal of Clinical Medicine*, *9*(9), 2951.
4. Almli, L. M., Fani, N., Smith, A. K., & Ressler, K. J. (2014). Genetic approaches to understanding post-traumatic stress disorder. *The International Journal of Neuropsychopharmacology*, *17*(2), 355–370.
5. Alvarez-Sabín, J., Penalba, A., Quintana, M., Krupinski, J., García-Bonilla, L., & Montaner, J. (2009). Triflusal and Aspirin Have Different Effects on Inflammatory Biomarkers Measured in Patients with Acute Ischemic Stroke. *Cerebrovascular Diseases*, *28*(4), 371–377.
6. American Psychiatric Association. (2017). Practice guideline for the treatment of patients with posttraumatic stress disorder. American Journal of Psychiatry, 174(9), 1-78.
7. Antón-Galindo, E., Dalla Vecchia, E., Orlandi, J. G., Castro, G., Gualda, E. J., Young, A. M. J., Guasch-Piqueras, M., Arenas, C., Herrera-Úbeda, C., Garcia-Fernàndez, J.,

Aguado, F., Loza-Alvarez, P., Cormand, B., Norton, W. H. J., & Fernàndez-Castillo, N. (2022). Deficiency of the ywhaz gene, involved in neurodevelopmental disorders, alters brain activity and behaviour in zebrafish. *Molecular Psychiatry*, *27*(9), 3739–3748.

8. Bam, M., Yang, X., Zhou, J., Ginsberg, J. P., Leyden, Q., Nagarkatti, P. S., & Nagarkatti, M. (2016). Evidence for Epigenetic Regulation of Pro-inflammatory Cytokines, Interleukin-12 and Interferon Gamma, in Peripheral Blood Mononuclear Cells from PTSD patients. *Journal of Neuroimmune Pharmacology : The Official Journal of the Society on NeuroImmune Pharmacology*, *11*(1), 168–181.
9. Banerjee, N. S., Moore, D. W., Broker, T. R., & Chow, L. T. (2018). Vorinostat, a pan-HDAC inhibitor, abrogates productive HPV-18 DNA amplification. *Proceedings of the National Academy of Sciences of the United States of America*, *115*(47), E11138–E11147.
10. Banerjee, S. B., Morrison, F. G., & Ressler, K. J. (2017). Genetic approaches for the study of PTSD: Advances and challenges. *Neuroscience Letters*, *649*, 139–146.
11. Barrett, T., Wilhite, S. E., Ledoux, P., Evangelista, C., Kim, I. F., Tomashevsky, M., Marshall, K. A., Phillippy, K. H., Sherman, P. M., Holko, M., Yefanov, A., Lee, H., Zhang, N., Robertson, C. L., Serova, N., Davis, S., & Soboleva, A. (2013). NCBI GEO: Archive for functional genomics data sets--update. *Nucleic Acids Research*, *41*(Database issue), D991-995.
12. Bartel, D. P. (2004). MicroRNAs: Genomics, biogenesis, mechanism, and function. *Cell*, *116*(2), 281–297.
13. Benjet, C., Bromet, E., Karam, E. G., Kessler, R. C., McLaughlin, K. A., Ruscio, A. M., Shahly, V., Stein, D. J., Petukhova, M., Hill, E., Alonso, J., Atwoli, L., Bunting, B., Bruffaerts, R., Caldas-de-Almeida, J. M., de Girolamo, G., Florescu, S., Gureje, O., Huang, Y., … Koenen, K. C. (2016). The epidemiology of traumatic event exposure worldwide: Results from the World Mental Health Survey Consortium. *Psychological*

Medicine, *46*(2), 327–343.

14. Berthelet, J., & Dubrez, L. (2013). Regulation of Apoptosis by Inhibitors of Apoptosis (IAPs). *Cells*, *2*(1), 163–187.
15. Bian, Y., Yang, L., Zhao, M., Li, Z., Xu, Y., Zhou, G., Li, W., & Zeng, L. (2019). Identification of Key Genes and Pathways in Post-traumatic Stress Disorder Using Microarray Analysis. *Frontiers in Psychology*, *10*.
16. Binder, E. B., Bradley, R. G., Liu, W., Epstein, M. P., Deveau, T. C., Mercer, K. B., Tang, Y., Gillespie, C. F., Heim, C. M., Nemeroff, C. B., Schwartz, A. C., Cubells, J. F., & Ressler, K. J. (2008). Association of FKBP5 polymorphisms and childhood abuse with risk of posttraumatic stress disorder symptoms in adults. *JAMA*, *299*(11), 1291–1305.
17. Bisson, J. I., Van Gelderen, M., Roberts, N. P., & Lewis, C. (2020). Non-pharmacological and non-psychological approaches to the treatment of PTSD: Results of a systematic review and meta-analyses. *European Journal of Psychotraumatology*, *11*(1), 1795361.
18. Blevins, C. A., Weathers, F. W., Davis, M. T., Witte, T. K., & Domino, J. L. (2015). The Posttraumatic Stress Disorder Checklist for DSM-5 (PCL-5): Development and Initial Psychometric Evaluation. *Journal of Traumatic Stress*, *28*(6), 489–498.
19. Breen, M. S., Bierer, L. M., Daskalakis, N. P., Bader, H. N., Makotkine, I., Chattopadhyay, M., Xu, C., Buxbaum Grice, A., Tocheva, A. S., Flory, J. D., Buxbaum, J. D., Meaney, M. J., Brennand, K., & Yehuda, R. (2019). Differential transcriptional response following glucocorticoid activation in cultured blood immune cells: A novel approach to PTSD biomarker development. *Translational Psychiatry*, *9*, 201.
20. Breen, M. S., Maihofer, A. X., Glatt, S. J., Tylee, D. S., Chandler, S. D., Tsuang, M. T., Risbrough, V. B., Baker, D. G., O'Connor, D. T., Nievergelt, C. M., & Woelk, C. H. (2015). Gene Networks Specific for Innate Immunity Define Post-Traumatic Stress Disorder. *Molecular Psychiatry*, *20*(12), 1538–1545.
21. Breen, M. S., Tylee, D. S., Maihofer, A. X., Neylan, T. C., Mehta, D., Binder, E. B.,

Chandler, S. D., Hess, J. L., Kremen, W. S., Risbrough, V. B., Woelk, C. H., Baker, D. G., Nievergelt, C. M., Tsuang, M. T., Buxbaum, J. D., & Glatt, S. J. (2018). PTSD Blood Transcriptome Mega-Analysis: Shared Inflammatory Pathways across Biological Sex and Modes of Trauma. *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology*, *43*(3), 469–481.

22. Breslau, N., Chilcoat, H. D., Kessler, R. C., & Davis, G. C. (1999). Previous exposure to trauma and PTSD effects of subsequent trauma: Results from the Detroit Area Survey of Trauma. *The American Journal of Psychiatry*, *156*(6), 902–907.
23. Brewin, C. R., Cloitre, M., Hyland, P., Shevlin, M., Maercker, A., Bryant, R. A., Humayun, A., Jones, L. M., Kagee, A., Rousseau, C., Somasundaram, D., Suzuki, Y., Wessely, S., van Ommeren, M., & Reed, G. M. (2017). A review of current evidence regarding the ICD-11 proposals for diagnosing PTSD and complex PTSD. *Clinical Psychology Review*, *58*, 1–15.
24. Broekman, B. F. P., Olff, M., & Boer, F. (2007). The genetic background to PTSD. *Neuroscience and Biobehavioral Reviews*, *31*(3), 348–362.
25. Bryant, R. A., Creamer, M., O'Donnell, M., Silove, D., & McFarlane, A. C. (2009). A Study of the Protective Function of Acute Morphine Administration on Subsequent Posttraumatic Stress Disorder. *Biological Psychiatry*, *65*(5), 438–440.
26. Butler, K. V., Kalin, J., Brochier, C., Vistoli, G., Langley, B., & Kozikowski, A. P. (2010). Rational design and simple chemistry yield a superior, neuroprotective HDAC6 inhibitor, tubastatin A. *Journal of the American Chemical Society*, *132*(31), 10842–10846.
27. Castedo, M., Perfettini, J.-L., Roumier, T., & Kroemer, G. (2002). Cyclin-dependent kinase-1: Linking apoptosis to cell cycle and mitotic catastrophe. *Cell Death and Differentiation*, *9*(12), 1287–1293.
28. Chang, L., Zhou, G., Soufan, O., & Xia, J. (2020). miRNet 2.0: Network-based visual analytics for miRNA functional analysis and systems biology. *Nucleic Acids Research*,

48(W1), W244–W251.

29. Cohen, M., Meir, T., Klein, E., Volpin, G., Assaf, M., & Pollack, S. (2011). Cytokine levels as potential biomarkers for predicting the development of posttraumatic stress symptoms in casualties of accidents. *International Journal of Psychiatry in Medicine*, *42*(2), 117–131.
30. D'Amelio, M., Cavallucci, V., & Cecconi, F. (2010). Neuronal caspase-3 signaling: Not only cell death. *Cell Death and Differentiation*, *17*(7), 1104–1114.
31. D'Amelio, M., Sheng, M., & Cecconi, F. (2012). Caspase-3 in the central nervous system: Beyond apoptosis. *Trends in Neurosciences*, *35*(11), 700–709.
32. Davis, A. P., Grondin, C. J., Johnson, R. J., Sciaky, D., Wiegers, J., Wiegers, T. C., & Mattingly, C. J. (2021). Comparative Toxicogenomics Database (CTD): Update 2021. *Nucleic Acids Research*, *49*(D1), D1138–D1143.
33. Dennis, G., Sherman, B. T., Hosack, D. A., Yang, J., Gao, W., Lane, H. C., & Lempicki, R. A. (2003). DAVID: Database for Annotation, Visualization, and Integrated Discovery. *Genome Biology*, *4*(5), P3.
34. Dokter, D. (1998). *Arts Therapists, Refugees, and Migrants: Reaching Across Borders*. Jessica Kingsley Publishers.
35. Domingo-Fernández, D., Provost, A., Kodamullil, A. T., Marín-Llaó, J., Lasseter, H., Diaz, K., Daskalakis, N. P., Lancashire, L., Hofmann-Apitius, M., & Haas, M. (2019). PTSD Biomarker Database: Deep dive metadatabase for PTSD biomarkers, visualizations and analysis tools. *Database: The Journal of Biological Databases and Curation*, *2019*, baz081.
36. Estornes, Y., & Bertrand, M. J. M. (2015). IAPs, regulators of innate immunity and inflammation. *Seminars in Cell & Developmental Biology*, *39*, 106–114.
37. Fan, C., Li, Y., Yang, H., Cui, Y., Wang, H., Zhou, H., Zhang, J., Du, B., Zhai, Q., Wu, D., Chen, X., & Guo, H. (2019). Tamarixetin protects against cardiac hypertrophy via

inhibiting NFAT and AKT pathway. *Journal of Molecular Histology*, *50*(4), 343–354.

38. Freshour, S. L., Kiwala, S., Cotto, K. C., Coffman, A. C., McMichael, J. F., Song, J. J., Griffith, M., Griffith, O. L., & Wagner, A. H. (2021). Integration of the Drug-Gene Interaction Database (DGIdb 4.0) with open crowdsource efforts. *Nucleic Acids Research*, *49*(D1), D1144–D1151.
39. Fujita, Y., Yamamoto, S., & Morinobu, S. (2012). [Novel therapeutic approach for the treatment of post-traumatic stress disorder (PTSD): Facilitating fear extinction]. *Nihon Shinkei Seishin Yakurigaku Zasshi = Japanese Journal of Psychopharmacology*, *32*(4), 195–201.
40. Gangadevi, S., Badavath, V. N., Thakur, A., Yin, N., De Jonghe, S., Acevedo, O., Jochmans, D., Leyssen, P., Wang, K., Neyts, J., Yujie, T., & Blum, G. (2021). Kobophenol A Inhibits Binding of Host ACE2 Receptor with Spike RBD Domain of SARS-CoV-2, a Lead Compound for Blocking COVID-19. *The Journal of Physical Chemistry Letters*, *12*(7), 1793–1802.
41. Gao, H., Zhu, X., Xi, Y., Li, Q., Shen, Z., & Yang, Y. (2018). Anti-depressant-like effect of atractylenolide I in a mouse model of depression induced by chronic unpredictable mild stress. *Experimental and Therapeutic Medicine*, *15*(2), 1574–1579.
42. Geng, J., Liu, J., Yuan, X., Liu, W., & Guo, W. (2019). Andrographolide triggers autophagy-mediated inflammation inhibition and attenuates chronic unpredictable mild stress (CUMS)-induced depressive-like behavior in mice. *Toxicology and Applied Pharmacology*, *379*, 114688.
43. Gerber, M. M., Frankfurt, S. B., Contractor, A. A., Oudshoorn, K., Dranger, P., & Brown, L. A. (2018). Influence of Multiple Traumatic Event Types on Mental Health Outcomes: Does Count Matter? *Journal of Psychopathology and Behavioral Assessment*, *40*(4), 645–654.
44. Gerst, A., Murthy, T., Elson, A., Driscoll, D., Bittner, M. J., & Ramaswamy, S. (2021).

Adjunctive Minocycline for Treatment of Posttraumatic Stress Disorder. *WMJ: Official Publication of the State Medical Society of Wisconsin*, *120*(4), 321–324.

45. Gola, H., Engler, H., Sommershof, A., Adenauer, H., Kolassa, S., Schedlowski, M., Groettrup, M., Elbert, T., & Kolassa, I.-T. (2013). Posttraumatic stress disorder is associated with an enhanced spontaneous production of pro-inflammatory cytokines by peripheral blood mononuclear cells. *BMC Psychiatry*, *13*, 40.
46. Guardado, P., Olivera, A., Rusch, H. L., Roy, M., Martin, C., Lejbman, N., Lee, H., & Gill, J. M. (2016). Altered gene expression of the innate immune, neuroendocrine, and nuclear factor-kappa B (NF-κB) systems is associated with posttraumatic stress disorder in military personnel. *Journal of Anxiety Disorders*, *38*, 9–20.
47. Guo, Y., Gan, X., Zhou, H., Zhou, H., Pu, S., Long, X., Ren, C., Feng, T., & Tang, H. (2020). Fingolimod suppressed the chronic unpredictable mild stress-induced depressive-like behaviors via affecting microglial and NLRP3 inflammasome activation. *Life Sciences*, *263*, 118582.
48. Han, F., Yan, S., & Shi, Y. (2013a). Single-prolonged stress induces endoplasmic reticulum-dependent apoptosis in the hippocampus in a rat model of post-traumatic stress disorder. *PloS One*, *8*(7), e69340.
49. Han, F., Yan, S., & Shi, Y. (2013b). Single-Prolonged Stress Induces Endoplasmic Reticulum—Dependent Apoptosis in the Hippocampus in a Rat Model of Post-Traumatic Stress Disorder. *PLOS ONE*, *8*(7), e69340.
50. Han, H., Shim, H., Shin, D., Shim, J. E., Ko, Y., Shin, J., Kim, H., Cho, A., Kim, E., Lee, T., Kim, H., Kim, K., Yang, S., Bae, D., Yun, A., Kim, S., Kim, C. Y., Cho, H. J., Kang, B., … Lee, I. (2015). TRRUST: A reference database of human transcriptional regulatory interactions. *Scientific Reports*, *5*, 11432.
51. Hassan, G., Ventevogel, P., Jefee-Bahloul, H., Barkil-Oteo, A., & Kirmayer, L. J. (2016). Mental health and psychosocial wellbeing of Syrians affected by armed conflict.

Epidemiology and Psychiatric Sciences, *25*(2), 129–141.

52. Hayashi, T. (2015). Conversion of psychological stress into cellular stress response: Roles of the sigma-1 receptor in the process. *Psychiatry and Clinical Neurosciences*, *69*(4), 179–191.
53. Heidenreich, V. (2005). *Music therapy in war-effected areas*. *3*(2).
54. Hu, K., Sjölander, A., Lu, D., Walker, A. K., Sloan, E. K., Fall, K., Valdimarsdóttir, U., Hall, P., Smedby, K. E., & Fang, F. (2020). Aspirin and other non-steroidal anti-inflammatory drugs and depression, anxiety, and stress-related disorders following a cancer diagnosis: A nationwide register-based cohort study. *BMC Medicine*, *18*(1), 238.
55. Huang, Q., Liu, Q., & Ouyang, D. (2019). Sorbinil, an Aldose Reductase Inhibitor, in Fighting Against Diabetic Complications. *Medicinal Chemistry (Shariqah (United Arab Emirates))*, *15*(1), 3–7.
56. Hussein, R. M., Mohamed, W. R., & Omar, H. A. (2018). A neuroprotective role of kaempferol against chlorpyrifos-induced oxidative stress and memory deficits in rats via GSK3β-Nrf2 signaling pathway. *Pesticide Biochemistry and Physiology*, *152*, 29–37.
57. Illouz, H. M. C. (2022). Anti-TNF-α Therapy as a Potential Treatment for Comorbid Pain in Post-traumatic Stress Disorder. *Douleur et Analgésie*, *35*(1), Article 1.
58. Jia, Y., Han, Y., Wang, X., & Han, F. (2018). Role of apoptosis in the Post-traumatic stress disorder model-single prolonged stressed rats. *Psychoneuroendocrinology*, *95*, 97–105.
59. Jung, J., & Lee, S. (2014). Anxiolytic Effects of Quercetin: Involvement of GABAergic System. *Journal of Life Science*, *24*.
60. Jung, Y. J., Tweedie, D., Scerba, M. T., Kim, D. S., Palmas, M. F., Pisanu, A., Carta, A. R., & Greig, N. H. (2021). Repurposing Immunomodulatory Imide Drugs (IMiDs) in Neuropsychiatric and Neurodegenerative Disorders. *Frontiers in Neuroscience*, *15*, 656921.

61. Kanan, J., & Leão, T. (2022). Post-traumatic stress disorder in youth exposed to the Syrian conflict: A systematic review and meta-analysis of prevalence and determinants. *Journal of Health Psychology*, 13591053221123140.
62. Kanduri, C., Raijas, P., Ahvenainen, M., Philips, A. K., Ukkola-Vuoti, L., Lähdesmäki, H., & Järvelä, I. (2015). The effect of listening to music on human transcriptome. *PeerJ*, *3*, e830.
63. Karam, E. G., Friedman, M. J., Hill, E. D., Kessler, R. C., McLaughlin, K. A., Petukhova, M., Sampson, L., Shahly, V., Angermeyer, M. C., Bromet, E. J., de Girolamo, G., de Graaf, R., Demyttenaere, K., Ferry, F., Florescu, S. E., Haro, J. M., He, Y., Karam, A. N., Kawakami, N., … Koenen, K. C. (2014). Cumulative traumas and risk thresholds: 12-month PTSD in the World Mental Health (WMH) surveys. *Depression and Anxiety*, *31*(2), 130–142.
64. Kube, T., Elssner, A. C., & Herzog, P. (2023). *The relationship between multiple traumatic events and the severity of posttraumatic stress disorder symptoms – evidence for a cognitive link*.
65. Kumar, A., Takada, Y., Boriek, A. M., & Aggarwal, B. B. (2004). Nuclear factor-kappaB: Its role in health and disease. *Journal of Molecular Medicine (Berlin, Germany)*, *82*(7), 434–448.
66. Lai, S., Shi, L., Jiang, Z., & Lin, Z. (2020). Glycyrrhizin treatment ameliorates post-traumatic stress disorder-like behaviours and restores circadian oscillation of intracranial serotonin. *Clinical and Experimental Pharmacology & Physiology*, *47*(1), 95–101.
67. Landis-Shack, N., Heinz, A. J., & Bonn-Miller, M. O. (2017). Music Therapy for Posttraumatic Stress in Adults: A Theoretical Review. *Psychomusicology*, *27*(4), 334–342.
68. Lee, S. R., Kwak, J. H., Kim, H. J., & Pyo, S. (2007). Neuroprotective effects of kobophenol A against the withdrawal of tropic support, nitrosative stress, and

mitochondrial damage in SH-SY5Y neuroblastoma cells. *Bioorganic & Medicinal Chemistry Letters*, *17*(7), 1879–1882.

69. Li, Y., Han, F., & Shi, Y. (2013). Increased neuronal apoptosis in medial prefrontal cortex is accompanied with changes of Bcl-2 and Bax in a rat model of post-traumatic stress disorder. *Journal of Molecular Neuroscience: MN*, *51*(1), 127–137.
70. Liao, Y., Wang, J., Jaehnig, E. J., Shi, Z., & Zhang, B. (2019). WebGestalt 2019: Gene set analysis toolkit with revamped UIs and APIs. *Nucleic Acids Research*, *47*(W1), W199–W205.
71. Logue, M. W., Amstadter, A. B., Baker, D. G., Duncan, L., Koenen, K. C., Liberzon, I., Miller, M. W., Morey, R. A., Nievergelt, C. M., Ressler, K. J., Smith, A. K., Smoller, J. W., Stein, M. B., Sumner, J. A., & Uddin, M. (2015). The Psychiatric Genomics Consortium Posttraumatic Stress Disorder Workgroup: Posttraumatic Stress Disorder Enters the Age of Large-Scale Genomic Collaboration. *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology*, *40*(10), 2287–2297.
72. Louneva, N., Cohen, J. W., Han, L.-Y., Talbot, K., Wilson, R. S., Bennett, D. A., Trojanowski, J. Q., & Arnold, S. E. (2008). Caspase-3 is enriched in postsynaptic densities and increased in Alzheimer's disease. *The American Journal of Pathology*, *173*(5), 1488–1495.
73. MacIntosh, H. B. (2003). Sounds of healing: Music in group work with survivors of sexual abuse. *The Arts in Psychotherapy*, *30*(1), 17–23.
74. Mahat, M. Y. A., Kulkarni, N. M., Vishwakarma, S. L., Khan, F. R., Thippeswamy, B. S., Hebballi, V., Adhyapak, A. A., Benade, V. S., Ashfaque, S. M., Tubachi, S., & Patil, B. M. (2010). Modulation of the cyclooxygenase pathway via inhibition of nitric oxide production contributes to the anti-inflammatory activity of kaempferol. *European Journal of Pharmacology*, *642*(1–3), 169–176.
75. Mahendra, A. N., Pramartha, I. N. T., Darmayanti, N. L. S., & Dewi, N. W. S. (2017).

Pharmaco-ethological and Hippocampal CA1 region neurohistological study of Sertraline effects on Single-prolonged Stress-induced Rodent model of PTSD. *Bali Medical Journal*, *6*(3), Article 3.

76. Mahendra, A. N., & Putra, I. N. A. J. (2018). Hippocampal Endoplasmic Reticulum Stress: Novel Target in PTSD Pharmacotherapy? *Biomedical and Pharmacology Journal*, *11*(3), 1269–1274.
77. Martin, C. G., Kim, H., Yun, S., Livingston, W., Fetta, J., Mysliwiec, V., Baxter, T., & Gill, J. M. (2017). Circulating miRNA associated with posttraumatic stress disorder in a cohort of military combat veterans. *Psychiatry Research*, *251*, 261–265.
78. Mastnak. (2023). *Music Therapy: Scientific Perspectives and Clinical Prospects*.
79. Matsumoto, Y., Morinobu, S., Yamamoto, S., Matsumoto, T., Takei, S., Fujita, Y., & Yamawaki, S. (2013). Vorinostat ameliorates impaired fear extinction possibly via the hippocampal NMDA-CaMKII pathway in an animal model of posttraumatic stress disorder. *Psychopharmacology*, *229*(1), 51–62.
80. Mehta, D., Klengel, T., Conneely, K. N., Smith, A. K., Altmann, A., Pace, T. W., Rex-Haffner, M., Loeschner, A., Gonik, M., Mercer, K. B., Bradley, B., Müller-Myhsok, B., Ressler, K. J., & Binder, E. B. (2013). Childhood maltreatment is associated with distinct genomic and epigenetic profiles in posttraumatic stress disorder. *Proceedings of the National Academy of Sciences of the United States of America*, *110*(20), 8302–8307.
81. Michopoulos, V., Vester, A., & Neigh, G. (2016). Posttraumatic Stress Disorder: A Metabolic Disorder in Disguise? *Experimental Neurology*, *284*(Pt B), 220–229.
82. Miller, G. E., Chen, E., Sze, J., Marin, T., Arevalo, J. M. G., Doll, R., Ma, R., & Cole, S. W. (2008). A functional genomic fingerprint of chronic stress in humans: Blunted glucocorticoid and increased NF-kappaB signaling. *Biological Psychiatry*, *64*(4), 266–272.
83. Mishra, B., Priyadarsini, K. I., Kumar, M. S., Unnikrishnan, M. K., & Mohan, H. (2003).

Effect of O-glycosilation on the antioxidant activity and free radical reactions of a plant flavonoid, chrysoeriol. *Bioorganic & Medicinal Chemistry*, *11*(13), 2677–2685.

84. Mkrtchian, G. M., Boiadzhian, A. S., Avetian, D. G., & Sukiasian, S. G. (2013). [The involvement of abnormal apoptosis in the disturbance of synaptic plasticity in posttraumatic stress disorder]. *Zhurnal Nevrologii I Psikhiatrii Imeni S.S. Korsakova*, *113*(1), 26–29.
85. Mohamed, A. Z., Cumming, P., Götz, J., Nasrallah, F., & Department of Defense Alzheimer's Disease Neuroimaging Initiative. (2019). Tauopathy in veterans with long-term posttraumatic stress disorder and traumatic brain injury. *European Journal of Nuclear Medicine and Molecular Imaging*, *46*(5), 1139–1151.
86. Mohamed, A. Z., Cumming, P., Srour, H., Gunasena, T., Uchida, A., Haller, C. N., Nasrallah, F., & Department of Defense Alzheimer's Disease Neuroimaging Initiative. (2018). Amyloid pathology fingerprint differentiates post-traumatic stress disorder and traumatic brain injury. *NeuroImage. Clinical*, *19*, 716–726.
87. Nair, P., Raijas, P., Ahvenainen, M., Philips, A. K., Ukkola-Vuoti, L., & Järvelä, I. (2021). Music-listening regulates human microRNA expression. *Epigenetics*, *16*(5), 554–566.
88. Nair, P. S., Kuusi, T., Ahvenainen, M., Philips, A. K., & Järvelä, I. (2019). Music-performance regulates microRNAs in professional musicians. *PeerJ*, *7*, e6660.
89. Nievergelt, C. M., Ashley-Koch, A. E., Dalvie, S., Hauser, M. A., Morey, R. A., Smith, A. K., & Uddin, M. (2018). Genomic Approaches to Posttraumatic Stress Disorder: The Psychiatric Genomic Consortium Initiative. *Biological Psychiatry*, *83*(10), 831–839.
90. Nomura, T., Fukai, T., Matsumoto, J., & Ohmori, T. (1982). Constituents of the cultivated mulberry tree. *Planta Medica*, *46*(1), 28–32.
91. Norris, F. H., Perilla, J. L., Riad, J. K., Kaniasty, K., & Lavizzo, E. A. (1999). Stability and change in stress, resources, and psychological distress following natural disaster: Findings from hurricane Andrew. *Anxiety, Stress, & Coping*, *12*(4), 363–396.

92. Núñez-Rios, D. L., Martínez-Magaña, J. J., Nagamatsu, S. T., Andrade-Brito, D. E., Forero, D. A., Orozco-Castaño, C. A., & Montalvo-Ortiz, J. L. (2022). Central and Peripheral Immune Dysregulation in Posttraumatic Stress Disorder: Convergent Multi-Omics Evidence. *Biomedicines*, *10*(5), Article 5.
93. Palencia, G., Medrano, J. Á. N.-, Ortiz-Plata, A., Farfán, D. J., Sotelo, J., Sánchez, A., & Trejo-Solís, C. (2015). Anti-apoptotic, anti-oxidant, and anti-inflammatory effects of thalidomide on cerebral ischemia/reperfusion injury in rats. *Journal of the Neurological Sciences*, *351*(1), 78–87.
94. Park, H. J., Lee, S. J., Cho, J., Gharbi, A., Han, H. D., Kang, T. H., Kim, Y., Lee, Y., Park, W. S., Jung, I. D., & Park, Y.-M. (2018). Tamarixetin Exhibits Anti-inflammatory Activity and Prevents Bacterial Sepsis by Increasing IL-10 Production. *Journal of Natural Products*, *81*(6), 1435–1443.
95. Patel, K., Gadewar, M., Tahilyani, V., & Patel, D. K. (2013). A review on pharmacological and analytical aspects of diosmetin: A concise report. *Chinese Journal of Integrative Medicine*, *19*(10), 792–800.
96. Pervanidou, P., Kolaitis, G., Charitaki, S., Margeli, A., Ferentinos, S., Bakoula, C., Lazaropoulou, C., Papassotiriou, I., Tsiantis, J., & Chrousos, G. P. (2007). Elevated morning serum interleukin (IL)-6 or evening salivary cortisol concentrations predict posttraumatic stress disorder in children and adolescents six months after a motor vehicle accident. *Psychoneuroendocrinology*, *32*(8), 991–999.
97. Reddy, R. G., Surineni, G., Bhattacharya, D., Marvadi, S. K., Sagar, A., Kalle, A. M., Kumar, A., Kantevari, S., & Chakravarty, S. (2019). Crafting Carbazole-Based Vorinostat and Tubastatin-A-like Histone Deacetylase (HDAC) Inhibitors with Potent in Vitro and in Vivo Neuroactive Functions. *ACS Omega*, *4*(17), 17279–17294.
98. Seah, C., Breen, M. S., Rusielewicz, T., Bader, H. N., Xu, C., Hunter, C. J., McCarthy, B., Deans, P. J. M., Chattopadhyay, M., Goldberg, J., Desarnaud, F., Makotkine, I., Flory,

J. D., Bierer, L. M., Staniskyte, M., Team, N. G. S. C. A., Noggle, S. A., Huckins, L. M., Paull, D., … Yehuda, R. (2022). Modeling gene × environment interactions in PTSD using human neurons reveals diagnosis-specific glucocorticoid-induced gene expression. *Nature Neuroscience*, *25*(11), 1434.

99. Segman, R. H., Shefi, N., Goltser-Dubner, T., Friedman, N., Kaminski, N., & Shalev, A. Y. (2005). Peripheral blood mononuclear cell gene expression profiles identify emergent post-traumatic stress disorder among trauma survivors. *Molecular Psychiatry*, *10*(5), 500–513, 425.

100. Selenica, M.-L., Benner, L., Housley, S. B., Manchec, B., Lee, D. C., Nash, K. R., Kalin, J., Bergman, J. A., Kozikowski, A., Gordon, M. N., & Morgan, D. (2014). Histone deacetylase 6 inhibition improves memory and reduces total tau levels in a mouse model of tau deposition. *Alzheimer's Research & Therapy*, *6*(1), 12.

101. Shannon, P., Markiel, A., Ozier, O., Baliga, N. S., Wang, J. T., Ramage, D., Amin, N., Schwikowski, B., & Ideker, T. (2003). Cytoscape: A Software Environment for Integrated Models of Biomolecular Interaction Networks. *Genome Research*, *13*(11), 2498–2504.

102. Singh, S. K., Barreto, G. E., Aliev, G., & Echeverria, V. (2017). Ginkgo biloba as an Alternative Medicine in the Treatment of Anxiety in Dementia and other Psychiatric Disorders. *Current Drug Metabolism*, *18*(2), 112–119.

103. Smoller, J. W., Andreassen, O. A., Edenberg, H. J., Faraone, S. V., Glatt, S. J., & Kendler, K. S. (2019). Psychiatric genetics and the structure of psychopathology. *Molecular Psychiatry*, *24*(3), 409–420.

104. Snijders, C., Krauskopf, J., Pishva, E., Eijssen, L., Machiels, B., Kleinjans, J., Kenis, G., van den Hove, D., Kim, M. O., Boks, M. P. M., Vinkers, C. H., Vermetten, E., Geuze, E., Rutten, B. P. F., & de Nijs, L. (2019). Circulating Serum MicroRNAs as Potential Diagnostic Biomarkers of Posttraumatic Stress Disorder: A Pilot Study.

Frontiers in Genetics, *10*.

105. Solomon, Z., Levin, Y., Assayag, E. B., Furman, O., Shenhar-Tsarfaty, S., Berliner, S., & Ohry, A. (2017). The Implication of Combat Stress and PTSD Trajectories in Metabolic Syndrome and Elevated C-Reactive Protein Levels: A Longitudinal Study. *The Journal of Clinical Psychiatry*, *78*(9), e1180–e1186.

106. Stein, D. J., Ipser, J. C., Seedat, S., Sager, C., & Amos, T. (2006). Pharmacotherapy for post traumatic stress disorder (PTSD). *The Cochrane Database of Systematic Reviews*, *2006*(1), CD002795.

107. Suliman, S., Mkabile, S. G., Fincham, D. S., Ahmed, R., Stein, D. J., & Seedat, S. (2009). Cumulative effect of multiple trauma on symptoms of posttraumatic stress disorder, anxiety, and depression in adolescents. *Comprehensive Psychiatry*, *50*(2), 121–127.

108. Sumner, J. A., Chen, Q., Roberts, A. L., Winning, A., Rimm, E. B., Gilsanz, P., Glymour, M. M., Tworoger, S. S., Koenen, K. C., & Kubzansky, L. D. (2018). Posttraumatic stress disorder onset and inflammatory and endothelial function biomarkers in women. *Brain, Behavior, and Immunity*, *69*, 203–209.

109. Tabur, S., Tufan, A. E., Çeri, V., & Semerci, B. (2019). Syrian Civil War's effects on Turkish school children: Prevalence and predictors of psychopathology. *Psychiatry and Clinical Psychopharmacology*, *29*(4), 811–816.

110. Tam, D. N. H., Nam, N. H., Elhady, M. T., Tran, L., Hassan, O. G., Sadik, M., Tien, P. T. M., Elshafei, G. A., & Huy, N. T. (2021). Effects of Mulberry on The Central Nervous System: A Literature Review. *Current Neuropharmacology*, *19*(2), 193–219.

111. Toft, H., Bramness, J. G., Lien, L., Abebe, D. S., Wampold, B. E., Tilden, T., Hestad, K., & Neupane, S. P. (2018). PTSD patients show increasing cytokine levels during treatment despite reduced psychological distress. *Neuropsychiatric Disease and Treatment*, *14*, 2367–2378.

112. von Mering, C., Huynen, M., Jaeggi, D., Schmidt, S., Bork, P., & Snel, B. (2003). STRING: A database of predicted functional associations between proteins. *Nucleic Acids Research*, *31*(1), 258–261.

113. Wan, R.-P., Liu, Z.-G., Huang, X.-F., Kwan, P., Li, Y.-P., Qu, X.-C., Ye, X.-G., Chen, F.-Y., Zhang, D.-W., He, M.-F., Wang, J., Mao, Y.-L., & Qiao, J.-D. (2023). YWHAZ variation causes intellectual disability and global developmental delay with brain malformation. *Human Molecular Genetics*, *32*(3), 462–472.

114. Wang, J., Um, P., Dickerman, B. A., & Liu, J. (2018). Zinc, Magnesium, Selenium and Depression: A Review of the Evidence, Potential Mechanisms and Implications. *Nutrients*, *10*(5), 584.

115. Wang, W., Wang, R., Xu, J., Qin, X., Jiang, H., Khalid, A., Liu, D., Pan, F., Ho, C. S. H., & Ho, R. C. M. (2018). Minocycline Attenuates Stress-Induced Behavioral Changes via Its Anti-inflammatory Effects in an Animal Model of Post-traumatic Stress Disorder. *Frontiers in Psychiatry*, *9*, 558.

116. Watanabe, M., Ohsugi, T., Shoda, M., Ishida, T., Aizawa, S., Maruyama-Nagai, M., Utsunomiya, A., Koga, S., Yamada, Y., Kamihira, S., Okayama, A., Kikuchi, H., Uozumi, K., Yamaguchi, K., Higashihara, M., Umezawa, K., Watanabe, T., & Horie, R. (2005). Dual targeting of transformed and untransformed HTLV-1-infected T cells by DHMEQ, a potent and selective inhibitor of NF-kappaB, as a strategy for chemoprevention and therapy of adult T-cell leukemia. *Blood*, *106*(7), 2462–2471.

117. Watkins, L. E., Sprang, K. R., & Rothbaum, B. O. (2018). Treating PTSD: A Review of Evidence-Based Psychotherapy Interventions. *Frontiers in Behavioral Neuroscience*,

118. Whittle, N., & Singewald, N. (2014). HDAC inhibitors as cognitive enhancers in fear, anxiety and trauma therapy: Where do we stand? *Biochemical Society Transactions*, *42*(2), 569–581.

119. Wolf, E. J., Miller, M. W., Sullivan, D. R., Amstadter, A. B., Mitchell, K. S., Goldberg, J., & Magruder, K. M. (2018). A classical twin study of PTSD symptoms and resilience: Evidence for a single spectrum of vulnerability to traumatic stress. *Depression and Anxiety*, *35*(2), 132–139.

120. Wróbel-Biedrawa, D., Grabowska, K., Galanty, A., Sobolewska, D., & Podolak, I. (2022). A Flavonoid on the Brain: Quercetin as a Potential Therapeutic Agent in Central Nervous System Disorders. *Life (Basel, Switzerland)*, *12*(4), 591.

121. Xia, Y.-F., Ye, B.-Q., Li, Y.-D., Wang, J.-G., He, X.-J., Lin, X., Yao, X., Ma, D., Slungaard, A., Hebbel, R. P., Key, N. S., & Geng, J.-G. (2004). Andrographolide attenuates inflammation by inhibition of NF-kappa B activation through covalent modification of reduced cysteine 62 of p50. *Journal of Immunology (Baltimore, Md.: 1950)*, *173*(6), 4207–4217.

122. Xu, H., Van der Jeught, K., Zhou, Z., Zhang, L., Yu, T., Sun, Y., Li, Y., Wan, C., So, K. M., Liu, D., Frieden, M., Fang, Y., Mosley, A. L., He, X., Zhang, X., Sandusky, G. E., Liu, Y., Meroueh, S. O., Zhang, C., … Lu, X. (2021). Atractylenolide I enhances responsiveness to immune checkpoint blockade therapy by activating tumor antigen presentation. *The Journal of Clinical Investigation*, *131*(10), e146832, 146832.

123. Xu, M., Lin, Z., Siegel, C. E., Laska, E. M., Abu-Amara, D., Genfi, A., Newman, J., Jeffers, M. K., Blessing, E. M., Flanagan, S. R., Fossati, S., Etkin, A., & Marmar, C. R. (2023). Screening for PTSD and TBI in Veterans using Routine Clinical Laboratory Blood Tests. *Translational Psychiatry*, *13*(1), 64.

124. Yaffe, K., Vittinghoff, E., Lindquist, K., Barnes, D., Covinsky, K. E., Neylan, T., Kluse, M., & Marmar, C. (2010). Posttraumatic stress disorder and risk of dementia among US veterans. *Archives of General Psychiatry*, *67*(6), 608–613.

125. Yehuda, R., Daskalakis, N. P., Bierer, L. M., Bader, H. N., Klengel, T., Holsboer, F., & Binder, E. B. (2016). Holocaust Exposure Induced Intergenerational Effects on

FKBP5 Methylation. *Biological Psychiatry*, *80*(5), 372–380.

126. Yoon, H.-S., & Park, C. M. (2021). Chrysoeriol ameliorates COX-2 expression through NF-κB, AP-1 and MAPK regulation via the TLR4/MyD88 signaling pathway in LPS-stimulated murine macrophages. *Experimental and Therapeutic Medicine*, *22*(1), 718.

127. Yu, L., Chen, C., Wang, L.-F., Kuang, X., Liu, K., Zhang, H., & Du, J.-R. (2013). Neuroprotective effect of kaempferol glycosides against brain injury and neuroinflammation by inhibiting the activation of NF-κB and STAT3 in transient focal stroke. *PloS One*, *8*(2), e55839.

128. Zhai, Z. J., Li, H. W., Liu, G. W., Qu, X. H., Tian, B., Yan, W., Lin, Z., Tang, T. T., Qin, A., & Dai, K. R. (2014). Andrographolide suppresses RANKL-induced osteoclastogenesis in vitro and prevents inflammatory bone loss in vivo. *British Journal of Pharmacology*, *171*(3), 663–675.

129. Zhong, S., Ye, J., Deng, Y., Zhang, M., Zou, M., Yao, X., & Xiao, S. (2023). Quercetagitrin Inhibits Tau Accumulation and Reverses Neuroinflammation and Cognitive Deficits in P301S-Tau Transgenic Mice. *Molecules (Basel, Switzerland)*, *28*(9), 3964.

130. Zhou, J., Nagarkatti, P., Zhong, Y., Ginsberg, J. P., Singh, N. P., Zhang, J., & Nagarkatti, M. (2014). Dysregulation in microRNA Expression Is Associated with Alterations in Immune Functions in Combat Veterans with Post-Traumatic Stress Disorder. *PLoS ONE*, *9*(4), e94075.

131. Zhou, Y., Zhou, B., Pache, L., Chang, M., Khodabakhshi, A. H., Tanaseichuk, O., Benner, C., & Chanda, S. K. (2019). Metascape provides a biologist-oriented resource for the analysis of systems-level datasets. *Nature Communications*, *10*(1), 1523.