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ORIGINAL RESEARCH

Potential Mitochondria-Related Key Genes in Post-Traumatic Stress Disorder Analyzed by Machine Learning Methods

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Purpose: Post-traumatic stress disorder (PTSD) is a debilitating psychiatric disorder triggered by exposure to traumatic events. Emerging evidence suggests that mitochondrial dysfunction may contribute to PTSD pathogenesis by disrupting cellular energy metabolism, increasing oxidative stress, and impairing neuroplasticity. This study investigates mitochondrial dysfunction-associated biomarkers, potentially opening new avenues for targeted therapeutic approaches.

Methods: Gene expression matrices from datasets GSE199841 and GSE81761 were derived from peripheral blood samples, used to identify differentially expressed genes (DEGs) between PTSD patients and healthy controls. Functional annotation and enrichment analysis were carried out using Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses. Next, least absolute shrinkage and selection operator (LASSO), support vector machine recursive feature elimination (SVM-RFE), and random forest were employed to screen and prioritize potential biomarkers, followed by validation through receiver operating characteristic (ROC) analysis and independent cohort from human verification. We also examined the expression of mitoDEGs in peripheral blood from PTSD-related mouse models by RT-qPCR. Given the close interplay between mitochondrial metabolism and immune function, we investigated the relationship between key MitoDEGs and immune infiltration proportions.

Results: DEGs in PTSD were enriched in mitochondrial pathways, including mitochondrial ribosome function and nitric oxide synthase regulation. Machine learning identified *UCP2, CISD1, NADK2* and *IDE* as key MitoDEGs. Then we assessed the diagnostic performance of four key genes through ROC curve analysis. We evaluated four key genes using ROC analysis, showing good diagnostic performance in the discovery cohort (AUC=0.871). Results were replicated in validation cohorts (GSE81761 AUC=0.745; GSE97356 AUC=0.638). These genes correlated with immune cell proportions (regulatory T cells, naïve B cells, CD4+/CD8+ T cells) and showed conserved dysregulation in murine blood, aligning with human data.

Conclusion: Mitochondrial-related genes *UCP2, CISD1, NADK2* and *IDE* may serve as cross-species diagnostic biomarkers for PTSD, with potential links to neuroimmune mechanisms.

Keywords: PTSD, mitochondria, immune infiltration, bioinformatics analysis, machine learning

Introduction

As a trauma-related psychiatric syndrome, post-traumatic stress disorder (PTSD) affects a proportion of individuals following exposure to potentially life-threatening events,[1] imposing a significant societal burden due to its frequent comorbidity with major depression, elevated suicide risk, and profound psychosocial impairments.[2] Current therapeutic strategies primarily involve pharmacotherapy such as selective serotonin reuptake inhibitors (SSRIs) and psychotherapy,[3] or their combination.[4] Recent clinical studies have demonstrated repetitive transcranial magnetic stimulation (rTMS) can ameliorate core PTSD symptoms within short-term treatment.[5] However, these interventions are constrained by prolonged treatment duration, high relapse rates, and limited therapeutic efficacy,[6] underscoring the critical need for early

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diagnostic biomarkers and preventive interventions in high-risk populations. While clinical diagnosis remains symptom-based, emerging research prioritizes the identification of molecular biomarkers that reflect PTSD pathophysiology.[7]

Mitochondrial dysfunction has recently emerged as a key pathophysiological mechanism in PTSD.[8] As central regulators of cellular energetics, steroidogenesis,[9,10] and inflammatory responses,[11] mitochondria influence multiple processes implicated in PTSD symptomatology, including abnormal fear conditioning, synaptic plasticity, and neuroendocrine signaling.[8] Notably, mitochondrial-immune crosstalk is increasingly recognized in psychiatric disorders, yet its specific contributions to PTSD pathogenesis—particularly through dysregulated immune-inflammatory pathways—remain poorly understood. MitoCarta3.0 is a curated database of mammalian mitochondrial protein-coding genes, essential for studying mitochondrial functions and disorders. Our work integrated MitoCarta3.0's comprehensive mitochondrial gene profiles with machine learning techniques to uncover PTSD-specific biomarkers. This approach uniquely identifies mitochondrial pathway interactions—rather than isolated gene effects—offering both predictive power and mechanistic insights that could guide future mitochondrial-targeted therapies for PTSD.

This knowledge gap is clinically significant, given that PTSD is now conceptualized as a disorder of neuro-immune-metabolic dysregulation.[12] Epidemiological studies reveal elevated rates of immune-mediated comorbidities in PTSD patients, likely driven by stress-induced perturbations in HPA axis function,[13] pro-inflammatory signaling, oxidative stress, and gut-brain axis interactions.[12] Critically, these immunological alterations may directly disrupt neurobiological processes central to PTSD, such as fear memory consolidation and extinction[14]—highlighting immunomodulation as a potential therapeutic target for trauma-related disorders.[14]

Transcriptomic profiling has become an indispensable tool for elucidating disease-associated molecular pathways.[15] The integration of machine learning algorithms further enhances analytical capacity for large-scale biological datasets,[16] enabling robust identification of disease-relevant genes and predictive biomarker discovery.[17] In the present study, we employed transcriptomic data from the GEO database (GSE199841, GSE81761) to investigate mitochondrial-immune interactions in PTSD progression. Through combined differential gene expression analysis and machine learning approaches, we identified mitochondrial-associated DEGs (MitoDEGs) with dual utility as diagnostic biomarkers and therapeutic targets.[18,19] Furthermore, we characterized immune cell infiltration patterns associated with key MitoDEGs, advancing understanding of immunometabolic mechanisms in PTSD pathogenesis.

Materials and Methods

Data Acquisition and Sample Collection

Gene expression datasets (accession numbers GSE199841 and GSE81761) were obtained from the NCBI GEO repository (http://www.ncbi.nlm.nih.gov/geo). Subsequent preprocessing and normalization were performed using R statistical software version 4.4.1 (https://www.r-project.org). GSE199841 contained RNA expression data from peripheral blood mononuclear cell (PBMC) generated by the GPL21185 platform (32 PTSD samples and 16 controls) from humans. And GSE81761 contained 39 PTSD and 27 controls from GPL570 platform. The raw data underwent background correction, inter-array normalization, and log2 transformation. Besides, samples from GSE97356 (81 PTSD/ 201 controls; GPL11154) was selected to validate the key genes.

Differential Expression Genes (DEGs) Analysis and Functional Enrichment Analysis

DEGs were identified using "limma" R package with thresholds of p.val < 0.05 and |log2FC| > 0.2. For multiple testing correction in differential expression analysis, we applied false discovery rate (FDR) adjustment, with results visualized through heatmaps (generated using pheatmap) and volcano plots (created with ggplot2).

Extraction of Mitochondria-related DEGs (MitoDEGs)

The 1136 mitochondrial genes from MitoCarta3.0 database (https://www.broadinstitute.org/mitocarta/mitocarta30) were intersected with DEGs to identify mitochondrial DEGs (mitoDEGs),[20] with results displayed in a Venn diagram.

Screening the Diagnostic Biomarkers

To briefly summarize, the R software package glmnet (https://cran.rproject.org/web/packages/glmnet/index.html) was employed to perform LASSO logistic regression analysis. The optimal penalty parameter was determined via 10-fold cross-validation to identify feature genes. Additionally, the R packages "e1071" (https://cran.r-project.org/web/packages/e1071) and "caret" were utilized to calculate the model with the minimum cross-validated error. Support vector machine recursive feature elimination was implemented through these packages to screen diagnostic biomarkers. Random forest screening was conducted using the "randomForest" R package (https://cran.r-project.org/web/packages/randomForest). Genes with common features across the three algorithms were selected by intersecting their results. Finally, receiver operating characteristic (ROC) curves were analyzed to evaluate the sensitivity and specificity of candidate genes for distinguishing normal individuals from PTSD patients.

Immune Infiltration Analysis

CIBERSORT was employed to calculate the immune cell content of each sample (https://cibersort.stanford.edu/). Subsequently, spearman's rank correlation analysis was implemented through the Cor.test function in R to assess monotonic relationships between gene expression levels and immune cell infiltration fractions.

Animals

Male C57BL/6 mice (10–12 weeks old) were group-housed (4/cage) under standard 12h light/dark cycles with ad libitum access to food and water. Following a 1-week acclimation period, all experimental procedures were conducted during the light phase under red-light illumination in accordance with protocols approved by the Animal Ethics Committee of Zhongnan Hospital of Wuhan University. This study was reviewed and approved by the Animal Ethics Committee of Zhongnan Hospital, Wuhan University (Approval No. ZN2023228).

Fear Conditioning and Tissue Collection

Two distinct contexts (A and B; AniLab Scientific Instruments Co., Ltd., China) were employed for behavioral assays as previously outlined.[21] Context A featured a metal grid floor (3.2 mm diameter, 8 mm spacing), while Context B was modified with a white plastic insert and LED lighting to reduce contextual overlap. Freezing behavior was recorded via ceiling-mounted cameras and analyzed using automated software (FreezeFrame). During conditioning (Context A), mice were exposed to a 120 s baseline period with lemon-scented aerosol (10% lemon extract, 10% ethanol), followed by three pairings of an auditory CS (80 dB, 16 kHz pure tone; 120 s duration) coterminating with a foot shock US (0.7 mA, 1 s; 2 min inter-trial intervals). Animals failing to exhibit ≥50% freezing during the final CS were excluded. Control mice underwent identical Context A exposure (14 min) without shocks. For testing, mice were reintroduced to Context B after a 2 min acclimation. Freezing was quantified across three CS presentations (120 s each; 120 s intervals). Memory retention was expressed as the percentage of freezing time. Peripheral blood samples were collected post-test for downstream analysis.

RNA Extraction and Quantitative RT-PCR

We manipulated the PBMC as described in previous studies.[22] PBMCs were homogenized in 500 μL PBS using a Dounce homogenizer, with 100 μL aliquots processed for RNA extraction using Trizol reagent (Invitrogen) per manufacturer's protocol. RNA quality was assessed by Qubit assay (Invitrogen). Reverse transcription of 500 ng total RNA was performed using the Prime Script Reverse Transcription Kit (Takara). qPCR analysis was conducted on a RotorGeneQ system (Qiagen) with SYBR Green Master Mix (Vazyme), using phosphoglycerate kinase 1 (PGK1) for normalization. Reactions were run in duplicate with 2 experimental replicates, and data analyzed via ΔΔCt method. The specific primer sequences utilized for PCR amplification are provided in Table 1.

Statistical Analysis

Results were expressed as mean ± standard error (SEM). Statistical differences between groups were determined by Student's *t*-test using GraphPad Prism 9 software (version 9), considering p-values <0.05 as significant. All figures in the

Table 1 qRT-PCR Primer Sequence List

Gene Symbol	Forward (3′–5′)	Reverse (3′–5′)
PGK1	ATGTCGCTTTCCAACAAGCTG	GCTCCATTGTCCAAGCAGAAT
UCP2	ATGGTTGGTTTCAAGGCCACA	CGGTATCCAGAGGGAAAGTGAT
CISD1	GCTGTGCGAGTTGAGTGGAT	TGGTGCGATTCTCTTTAGCGTA
NADK2	CACCGCTGCTCGAACTCGTAGCGGG	AAACCCCGCTACGAGTTCGAGCAGC
IDE	AATCCGGCCATCCAGAGAATA	GGGTCTGACAGTGAACCTATGT

manuscript were generated and finalized using Adobe Illustrator 2023 (v27.7) for graphical refinement and layout composition.

Results

Identification of DEGs in Control and PTSD Samples

The experimental workflow is summarized in Figure 1. Differential expression analysis of the GSE199841 dataset identified 1928 DEGs using stringent thresholds (adjusted p-value < 0.05, |log2FC| > 0.2), comprising 908 upregulated and 1020 downregulated transcripts (Figure 2A and B; Supplementary Table 1). Similarly, analysis of the GSE81761 dataset revealed 3357 DEGs (1356 upregulated; 2001 downregulated) under equivalent statistical criteria (Figure 2D and E; Supplementary Table 2). Hierarchical clustering of the 50 DEGs with maximal upregulation and 50 with maximal downregulation in each dataset generated distinct transcriptional profiles, as visualized through heatmap analysis (Figure 2C and F).

Potential Functions and Pathway of Differential Expression Genes

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses of DEGs from the GSE199841 and GSE81761 datasets revealed mitochondrial-centric functional signatures in PTSD pathogenesis. In GSE199841, GO

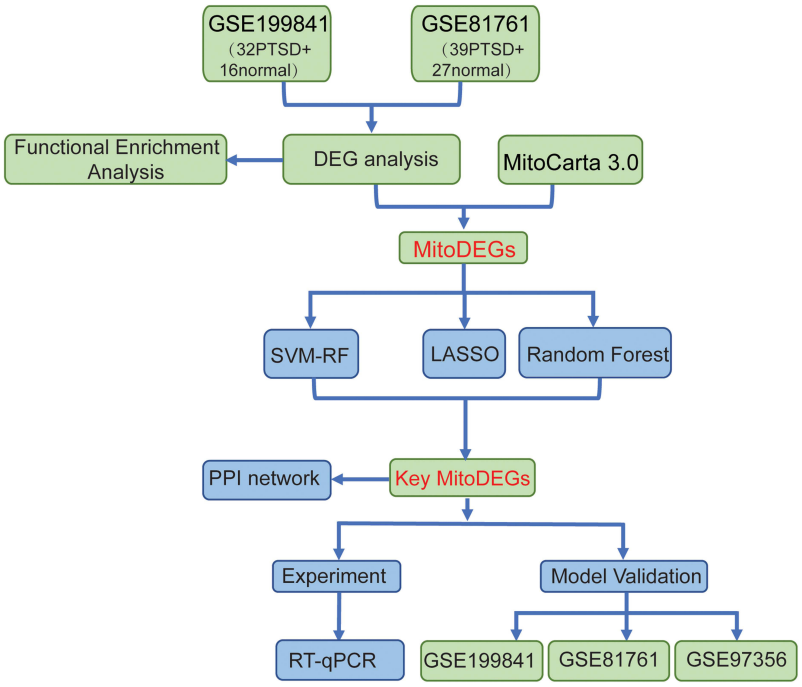

Figure 1 Schematic overview of the study design. Flow diagram illustrating the analytical workflow for identifying MitoDEGs in PTSD across two human transcriptomic datasets (GSE199841 and GSE81761). Key steps include differential expression analysis, functional enrichment, machine learning-based feature selection, and experimental validation in a murine PTSD-related model.

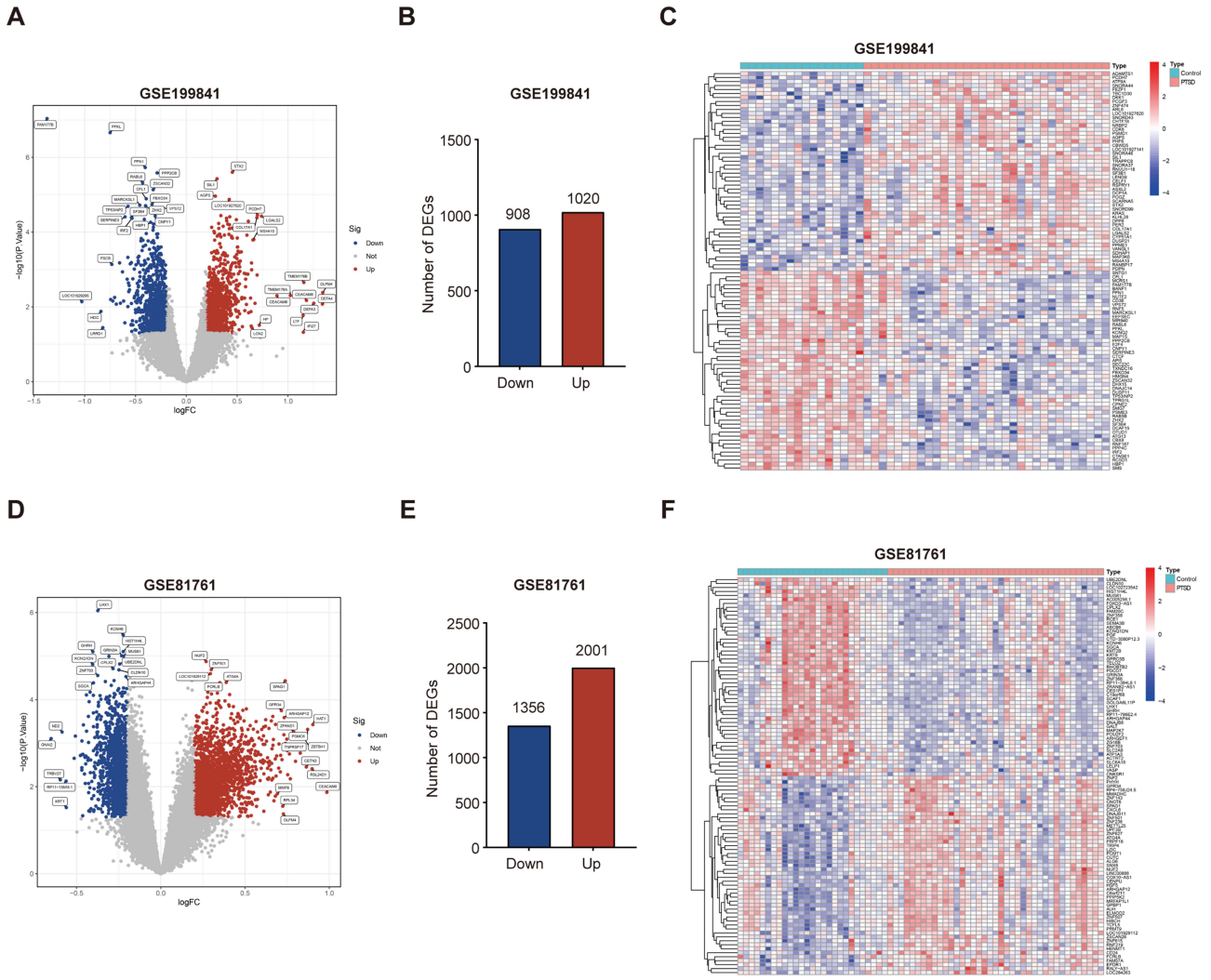

Figure 2 Identification of differentially expressed genes (DEGs) in PTSD cohorts. (**A**) Volcano plot depicting DEGs between PTSD and control groups in GSE199841. Red/blue points denote upregulated/downregulated genes. (**B**) Bar graph quantifying upregulated (red) and downregulated (blue) DEGs in GSE199841. (**C**) Hierarchical clustering heatmap of the top 50 DEGs (ranked by adjusted p-value) in GSE199841, demonstrating distinct expression patterns between groups. (**D**) Volcano plot depicting DEGs between PTSD and control groups in GSE81761. Red/blue points denote upregulated/downregulated genes. (**E**) Bar graph quantifying upregulated (red) and downregulated (blue) DEGs in GSE81761. (**F**) Hierarchical clustering heatmap of the top 50 DEGs (ranked by adjusted p-value) in GSE81761.

enrichment highlighted biological processes including upregulation of NOS enzymatic activity and microtubule motor activity, with cellular components including mitochondrial-associated structures (cell-substrate junctions, pseudopodia) and molecular functions like metalloendopeptidase activity (Figure 3A; Supplementary Table 3). Parallel analysis of GSE81761 further identified mitochondrial gene expression, inner membrane organization, and ribosomal complexes as key enriched terms (Figure 3B; Supplementary Table 4). KEGG pathway profiling delineated PTSD-related mechanisms spanning synaptic plasticity, neuroinflammatory signaling, and metabolic dysregulation (Figure 3C and D; Supplementary Tables 5 and 6). Multi-level integration of these findings underscores mitochondrial metabolism as a critical hub in PTSD pathology, linking energy homeostasis, neuroimmune crosstalk, and stress-responsive signaling networks.

Analysis of Screening of Mitochondria-related DEGs

Intersection analysis of MitoCarta3.0-derived mitochondrial genes with PTSD-associated DEGs from GSE199841 and GSE81761 identified seven upregulated (*SLC25A12, IDE, MRPL27, COX17, MRPS17, CISD1, NADK2*) and one downregulated (*UCP2*) consensus MitoDEGs (Figure 4A and B). Cross-dataset validation confirmed robust differential

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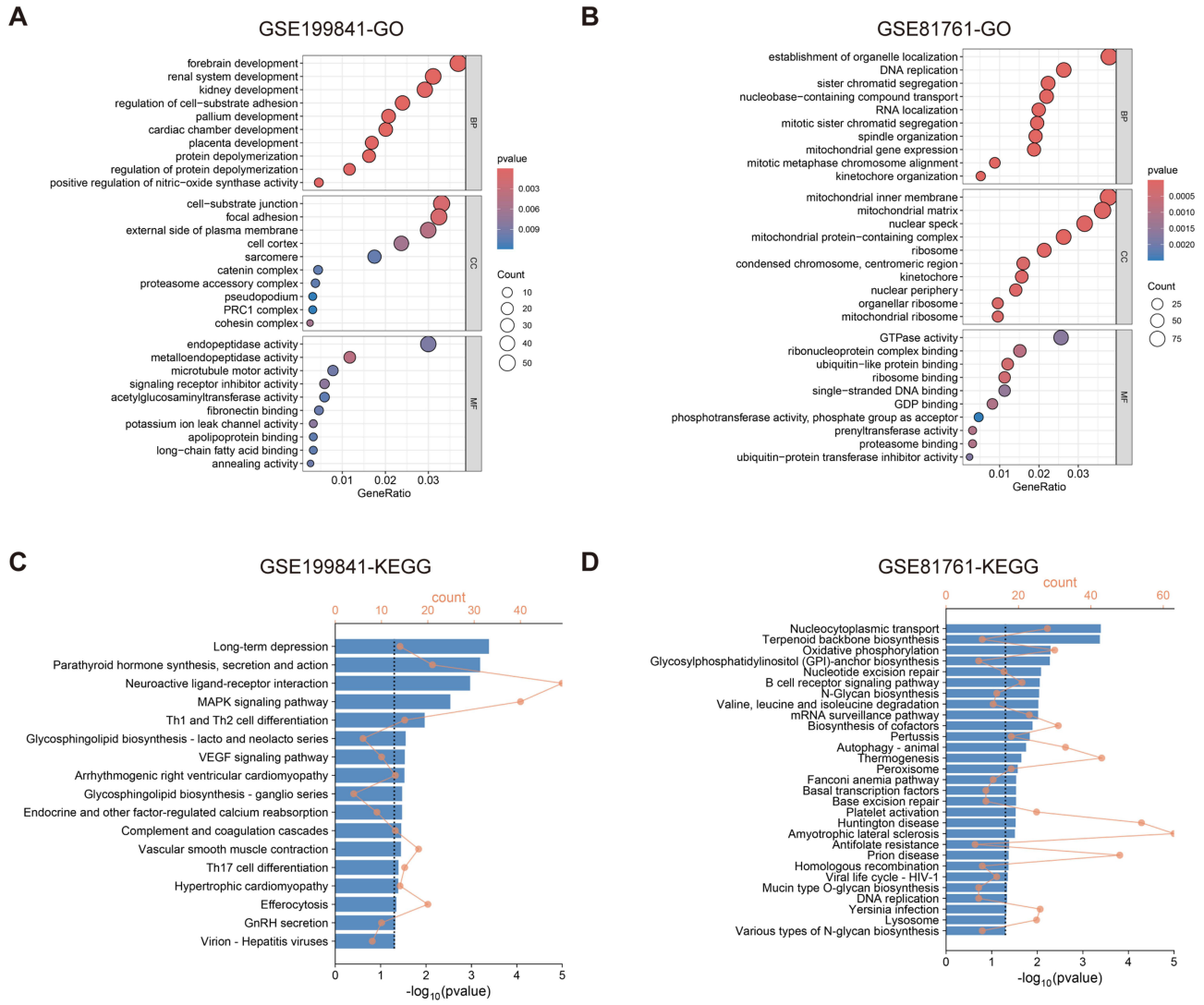

Figure 3 Functional enrichment analysis of PTSD-associated DEGs. GO terms (**A**) and KEGG pathways (**B**) enriched in DEGs from GSE199841. GO terms (**C**) and KEGG pathways (**D**) enriched in DEGs from GSE81761.
Abbreviations: BP, biological process; CC, cellular component; MF, molecular function; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes.

expression patterns across cohorts (Figure 4C and F; Supplementary Tables 7 and 8), with hierarchical clustering revealing distinct mitochondrial transcriptional signatures in PTSD. Functional enrichment profiling demonstrated MitoDEG involvement in bioenergetic pathways, including oxidative phosphorylation, mitochondrial inner membrane organization, and dicarboxylate transport (Figure 4G). KEGG pathway analysis further implicated these genes in ribosome biogenesis, thermogenesis, and neurodegenerative cascades, with oxidative phosphorylation (hsa00190) emerging as the central node (Figure 4H). This multi-omics convergence positions mitochondrial bioenergetic dysregulation as a hallmark of PTSD pathophysiology.

Machine Learning-Based Identification and Validation of Diagnostic Biomarkers

To refine mitochondrial-associated diagnostic signatures, we implemented a tripartite machine learning framework on the training cohort: 1) LASSO regression identified five feature genes through λ optimization (Figure 5A); 2) SVM-RFE algorithm selected six genes with maximal classification accuracy (Figure 5B); 3) Random Forest modeling prioritized five genes by Gini importance scoring (Figure 5C and D). Consensus analysis of these algorithmically derived candidates revealed four intersecting mitochondrial biomarkers—*UCP2, CISD1, NADK2*, and *IDE*—with robust cross-validation

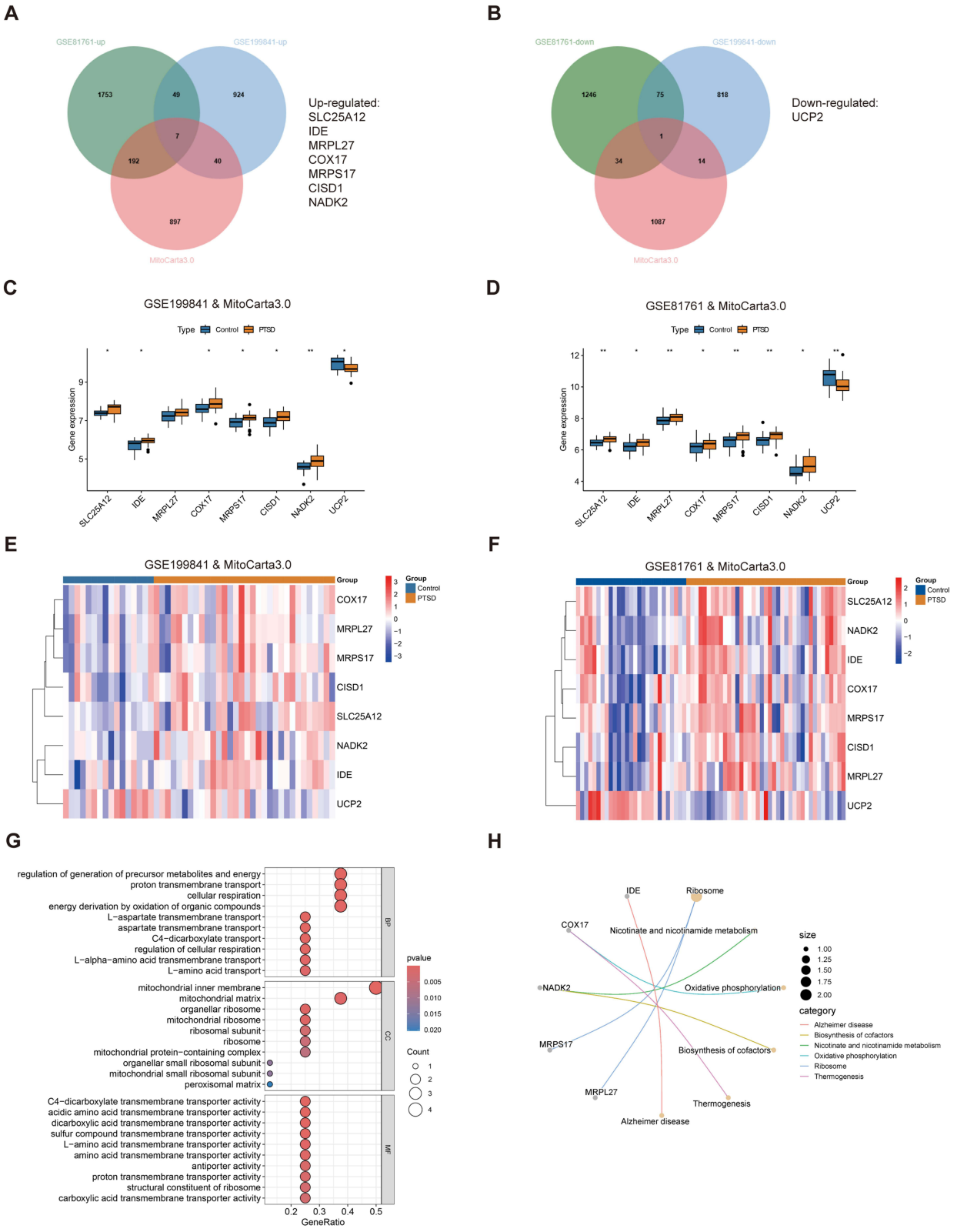

Figure 4 Mitochondrial DEG characterization and enrichment. Venn diagrams showing overlap between mitochondrial genes (MitoCarta3.0) and (**A**) upregulated or (**B**) downregulated DEGs. Normalized expression levels of eight consensus MitoDEGs in (**C**) GSE199841 and (**D**) GSE81761. Heatmaps of MitoDEG expression profiles in (**E**) GSE199841 and (**F**) GSE81761, clustered by Euclidean distance. (**G**) GO terms and (**H**) KEGG pathways enriched among MitoDEGs (*p < 0.05, **p < 0.01).

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performance (Figure 5E). PPI network analysis demonstrated functional coherence among these biomarkers, with *UCP2* and *IDE* serving as hub nodes connecting oxidative phosphorylation and neuroinflammatory modules (Figure 5F).

To evaluate the cross-dataset validity of mitochondrial-associated gene signatures for PTSD stratification, we assessed the diagnostic performance of four key mitochondrial dysfunction-related genes (UCP2, CISD1, NADK2, and IDE)

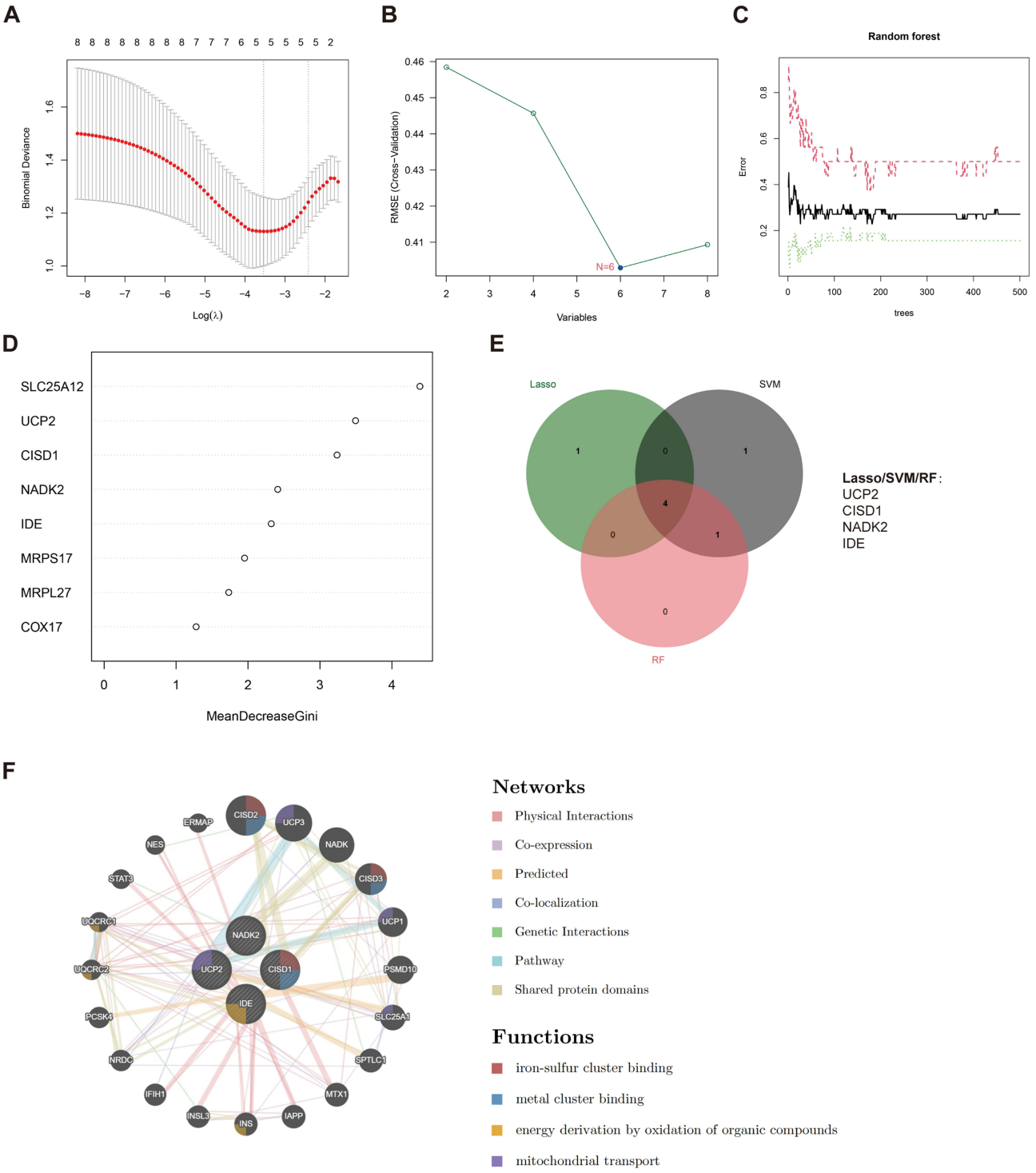

Figure 5 Machine learning-driven identification of diagnostic MitoDEGs. (**A**) Selection of the optimal parameter (λ) in the LASSO model. (**B**) SVM-RFE feature selection curve showing cross-validation error versus gene number. (**C**) RF error rate stabilization with increasing tree number. (**D**) Gini coefficient ranking of top five MitoDEGs. (**E**) Intersection of feature genes from LASSO, SVM-RFE, and RF. (**F**) PPI network of diagnostic genes (GeneMANIA), illustrating physical interactions (red), co-expression (blue), and pathway associations (green). **Abbreviations**: Lasso, least absolute shrinkage and selection operator; SVM, support vector machine; RF, random forest.

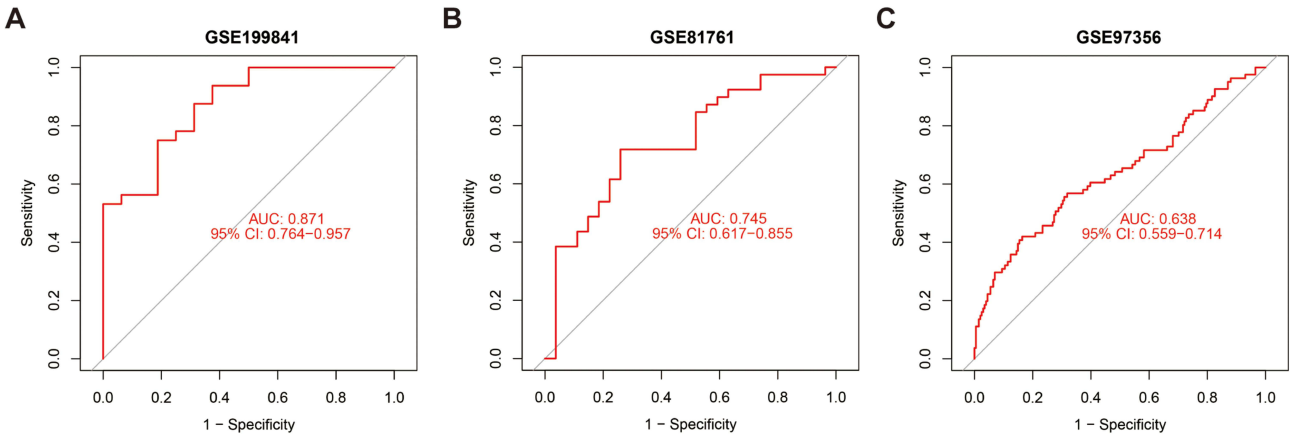

Figure 6 Diagnostic performance evaluation of key MitoDEGs. Receiver operating characteristic (ROC) curves for four mitochondrial biomarkers in (**A**) GSE199841, (**B**) GSE81761, and (**C**) independent validation cohort GSE97356. Area under the curve values quantify classification accuracy between PTSD and controls. 95% CI, 95% confidence interval.
Abbreviation: AUC, area under the curve.

through ROC curve analysis across multiple cohorts. In discovery cohorts, the gene panel demonstrated robust discriminative ability, with area under the curve (AUC) of 0.871 (95% CI: 0.764–0.957) in GSE199841 (Figure 6A). However, performance was attenuated in the independent validation cohort GSE81761 (AUC = 0.745, 95% CI: 0.617–0.855) (Figure 6B) and GSE97356 (AUC = 0.638, 95% CI: 0.559–0.714) (Figure 6C). The observed differential efficacy across cohorts may reflect variations in sample characteristics or underlying PTSD heterogeneity. Collective analysis of these multi-cohort AUC profiles supports the potential utility of mitochondrial gene signatures in PTSD stratification.

Immune Cell Infiltration Analysis

Immune infiltration analysis from GSE199841 revealed UCP2 showed significant positive correlations with regulatory T cells regulatory (Tregs) and naïve B cells naïve ($P<0.05$) among the four machine learning-derived feature genes, and was negatively associated with T cell gamma delta and neutrophils ($P<0.05$) (Figure 7A). *NADK2* was significantly correlated positively with T cell CD8 and T cell CD4 naïve ($P<0.05$), and showed a significant negative correlation with neutrophils ($P<0.05$) (Figure 7C). *IDE* was significantly correlated positively with T cell CD8 and T cell CD4 naïve ($P<0.05$), and was and was inversely associated with neutrophils and T cell gamma delta ($P<0.05$) (Figure 7D). However, *CISD1* was not associated with immune cell infiltration (Figure 7B).

Validation of Feature Genes by qRT-PCR in Rodent Model

A laboratory mouse model of PTSD was established using footshock conditioning[23] (Figure 8A). The results showed that the freeze percentage during the third CS was greater than 50% in all mice (Figure 8B), indicating successful model establishment. On the following day, during the fear memory recall test, the footshock-exposed mice exhibited a markedly increased freeze percentage compared to the contextual control group (Figure 8C). The expression of identified genes was experimentally confirmed in peripheral blood using qRT-PCR (Figure 8D–G). qRT-PCR results demonstrated that the feature genes *Cisd1, Nadk2, and Ide* were significantly upregulated in peripheral blood. Behavioral and qRT-PCR raw data were shown in Supplementary Table 9.

Discussion

PTSD has emerged as a critical global health challenge, exacerbated by the COVID-19 pandemic and protracted geopolitical conflicts that have precipitated widespread mental health crises. The clinical complexity of PTSD is amplified by its frequent comorbidities-including major depressive disorder, generalized anxiety, substance use disorder[24] and chronic insomnia-which collectively impair quality of life and synergistically escalate disease burden.

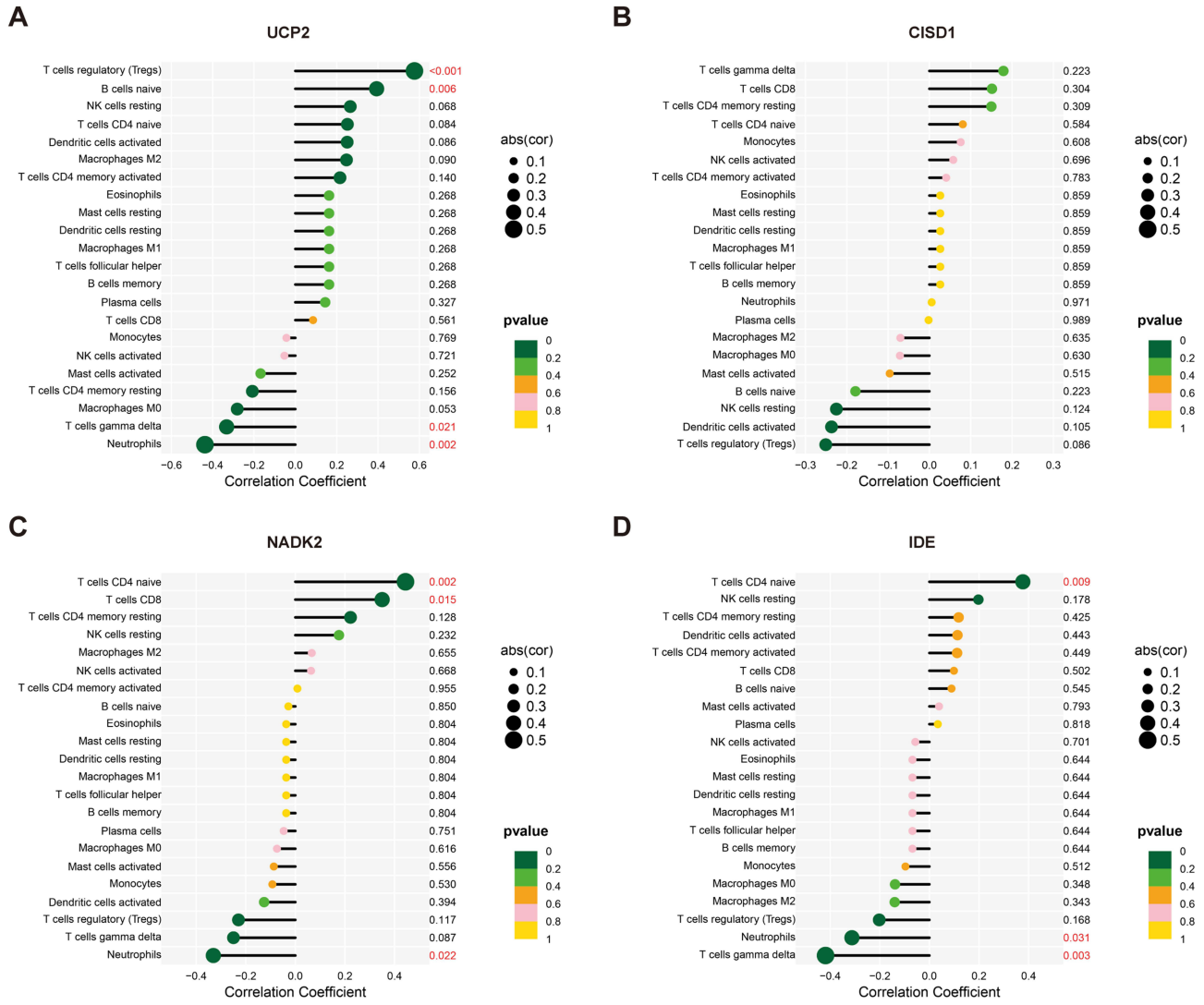

Figure 7 Immune infiltration correlates of mitochondrial biomarkers. Spearman correlation heatmaps between expression levels of (**A**) *UCP2*, (**B**) *CISD1*, (**C**) *NADK2*, and (**D**) *IDE* with immune cell proportions. A correlation coefficient greater than 0 indicates a positive correlation, while a coefficient less than 0 indicates a negative correlation. Results with $p < 0.05$ were considered statistically significant and were highlighted in red.

These intersecting pathologies not only hinder therapeutic efficacy but also necessitate multimodal intervention strategies, underscoring the urgent imperative to identify biomarkers for early diagnosis and personalized therapeutic targeting.

Using bioinformatics approaches, we identified DEGs in PTSD patient datasets, which showed significant involvement in mitochondrial function, immune response, and lipid metabolism pathways. These findings align with growing recognition of PTSD as a systemic disorder involving intertwined metabolic and immunological disturbances. Mitochondrial dysfunction can significantly impact immune responses by disrupting cellular energy metabolism,[25] triggering inflammatory signaling through mitochondrial DNA release,[26] and promoting oxidative stress-induced cytokine production,[27] with this mitochondria-immune interplay playing a particularly important role in PTSD pathogenesis. Epidemiological studies consistently show PTSD patients have higher incidence of immune-mediated comorbidities, including cardiovascular diseases and autoimmune disorders.[1] Clinical and preclinical evidence further demonstrates that PTSD is characterized by a proinflammatory state with elevated cytokines (eg, IL-6, TNF-α), metabolomic alterations, and mitochondrial metabolic abnormalities.[28] Our findings provide new insights into how mitochondrial metabolic alterations may interact with immune dysregulation in PTSD, highlighting several molecular pathways that warrant further investigation as possible therapeutic targets.

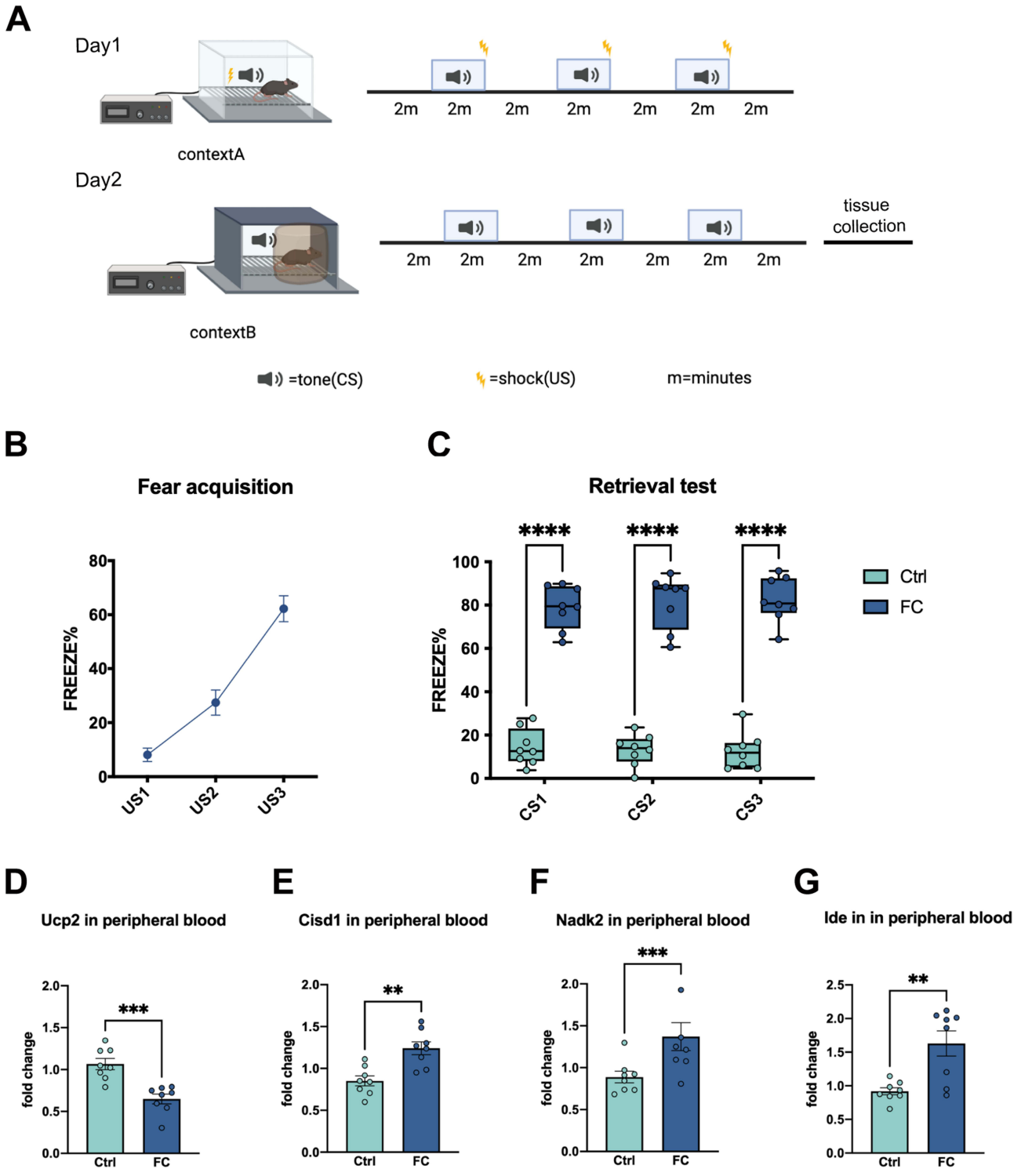

Figure 8 Experimental validation in footshock-exposed mice. (**A**) Schematic of the behavioral protocol. (**B**) FC group mice underwent conditioned fear training. (**C**) Freezing behavior quantification during fear recall. (**D–G**) Relative mRNA expression (qPCR, normalized to PGK1) of four MitoDEGs in peripheral blood. Data presented as mean ± SEM; $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$ (two-tailed *t*-test).

Abbreviations: Ctrl, contextual control; FC, fear acquisition; US, unconditioned stimulus; CS, conditioned stimulus.

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While research on PTSD's molecular basis has expanded, mitochondrial gene networks remain unexplored through systematic bioinformatics approaches in this disorder. To address this gap, our study represents the first application of MitoCarta 3.0-a comprehensive mitochondrial proteome database, to identify mitochondrial differentially expressed genes (MitoDEGs) associated with PTSD. Using this approach, we detected eight key MitoDEGs showing significant differential expression in PTSD cohorts. Subsequent comparative analysis of three machine learning algorithms refined these candidates to four feature MitoDEGs: *UCP2, CISD1, NADK2*, and *IDE*. To validate these findings, we employed a footshock-induced conditioned fear paradigm to establish a PTSD-related mouse model. Quantitative expression analysis revealed concordant dysregulation of *Ucp2, Cisd1, Nadk2*, and *Ide* in peripheral blood, aligning with our initial bioinformatics predictions. This multi-modal approach bridges computational discovery with experimental validation, highlighting mitochondrial pathways as potential contributors to PTSD pathophysiology.

Uncoupling protein 2 (UCP2), a mitochondrial transporter protein, disrupts the coupling between electron transport and ATP production by facilitating proton leakage across the inner mitochondrial membrane.[29] This activity modulates mitochondrial ATP production and regulates reactive oxygen species (ROS) generation, which serve as critical second messengers in cellular signaling.[30,31] Emerging evidence highlights UCP2's role in synaptic plasticity and mitochondrial dynamics, particularly its importance in neuronal bioenergetic adaptation.[29] Enhanced UCP2 expression correlates with increased neuronal activity and synaptic plasticity[30,31,] while chronic stress-induced synaptic deficits in cortical and limbic circuits—key regions for emotion and cognition—may underlie PTSD-related functional impairments.[32,33] UCP2 also modulates immune responses by influencing cellular metabolism and oxidative stress.[34] Specifically, UCP2 downregulates glycolysis, inhibits fatty acid synthesis, and reduces ROS generation, thereby regulating CD8+ T cell differentiation and survival.[35] Our data reveal a strong positive correlation between *UCP2* expression and T cell abundance. Notably, *Ucp2* expression showed marked downregulation in footshock-exposed mice relative to controls, a pattern similarly evident in peripheral blood samples. These findings suggest *UCP2*-associated mitochondrial metabolic dysregulation and immune dysfunction in PTSD, positioning it as a potential biomarker for the disorder.

CISD1, a mitochondrial membrane protein, is integral to mitochondrial redox regulation, calcium homeostasis, and antioxidant defense,[36] while also modulating apoptosis.[37] Pathological CISD1 accumulation in neurodegenerative diseases induces mitochondrial dysfunction by impairing mitophagy.[38] In lipopolysaccharide-induced acute lung injury (ALI) models, CISD1 upregulation exacerbates inflammation, whereas its inhibition via mitoNEET ligand-1 (NL-1) reduces apoptosis, pulmonary edema, and pro-inflammatory cytokines (TNF-α, IL-6 and IL-1β).[39] Our study detected significant *Cisd1* upregulation in PTSD mouse peripheral blood. Given mitoNEET's therapeutic efficacy in traumatic brain injury[40] and Parkinson's disease,[41] CISD1 represents a promising target for PTSD intervention.

NADK2 catalyzes NAD phosphorylation to generate NADP, a cofactor essential for cellular energy metabolism and redox homeostasis.[42] Elevated NADK2 expression increases intracellular NADP levels, disrupting redox equilibrium and promoting oxidative stress.[43] This metabolic dysregulation may impair antioxidant systems, exacerbating cellular damage —a mechanism potentially relevant to PTSD pathophysiology. Our findings demonstrate a significant elevation in *NADK2* expression levels within the peripheral blood of both PTSD patients and a murine footshock-exposed mice compared to healthy controls. This upregulation may reflect a compensatory response to trauma-induced stress, suggesting that pathological stimuli associated with PTSD could directly or indirectly modulate NADK2 expression. Notably, no published evidence currently establishes a mechanistic or clinical association between NADK2 and stress-related pathways or neuropsychiatric disorders, despite its recognized role in cellular redox homeostasis. The absence of prior investigations into this relationship underscores the novelty of our observations and highlights the critical need for further studies to elucidate the molecular mechanisms linking NADK2 to PTSD pathophysiology. Such research could advance our understanding of stress-related metabolic dysregulation and identify potential therapeutic targets for neuropsychiatric conditions.

The insulin-degrading enzyme (IDE), encoded by the *IDE* gene, is a multifunctional protease primarily responsible for insulin catabolism and the regulation of peptide hormone homeostasis.[44] Beyond its canonical metabolic roles, IDE exhibits amyloid beta (Aβ)-degrading activity, directly implicating it in Alzheimer's disease (AD) pathogenesis.[44] Notably, IDE co-deposits with Aβ plaques in AD-vulnerable cerebral regions, a pathological feature absent in non-diseased brains.[45] This observation aligns with higher concentrations of s-nitrosylated IDE detected in AD patients

relative to age-matched controls.[46] IDE's functional repertoire extends to mitochondrial compartments, where full-length IDE transcripts are translated into isoforms that interact with mitochondrial ribosomal proteins and contribute to the formation of respiratory chain complexes I and IV.[47] These mitochondrial isoforms may exert non-proteolytic chaperone-like activities, potentially supporting protein quality control and stress adaptation mechanisms.[47] Furthermore, emerging evidence positions IDE as an immunomodulatory factor, with studies demonstrating that IDE inhibition attenuates pro-inflammatory CD4+ T cell responses to insulin in diabetic models.[48] Concurrently, IDE exhibits heat shock protein-like properties that may influence central nervous system malignancy progression.[49] While extensive research has established IDE's involvement in neurodegenerative disorders such as AD and Parkinson's disease,[44–47] its potential role in PTSD remains underexplored. Our findings reveal upregulated *Ide* expression in peripheral blood, suggesting its candidacy as a novel therapeutic target.

To investigate potential biomarkers of PTSD-like phenotypes, this study employed a fear conditioning paradigm to delineate characteristic gene expression profiles in murine peripheral blood. As a fundamental form of associative learning, this paradigm ensures high experimental reproducibility and cross-study comparability through standardized footshock stimulation paired with quantifiable freezing behavior—methodological strengths that have been extensively validated in elucidating the etiological mechanisms, symptom maintenance principles, and therapeutic targets of PTSD and related anxiety disorders.[50] While this experimental model does not fully recapitulate the entire clinical spectrum of human PTSD, its well-characterized neural circuitry provides a neuroanatomically defined framework for mechanistic exploration at molecular and cellular levels. Our findings establish a translational foundation, with future plans to validate these gene signatures in PTSD models such as the single prolonged stress (SPS) paradigm.

While our machine learning model demonstrated strong predictive performance in the current cohort, several limitations regarding generalizability warrant discussion. The model's performance may be influenced by cohort-specific characteristics including demographic composition, clinical assessment protocols, and technical variations in data acquisition. Although we implemented rigorous cross-validation and regularization techniques to enhance robustness, the potential for overfitting to site-specific patterns remains a consideration. The biological pathways identified, while mechanistically plausible, may require validation across more diverse populations to establish their broader relevance. Future studies incorporating multi-center datasets and standardized protocols will be essential to assess the model's generalizability more comprehensively. Additionally, emerging approaches such as federated learning or data harmonization techniques could help address these challenges in subsequent research.

This study represents the first integrative analysis of mitochondrial genomics and immune microenvironment dynamics in PTSD using bioinformatics approaches. We identified UCP2, CISD1, NADK2, and IDE as key mitochondrial differentially expressed genes (MitoDEGs) with significant diagnostic and prognostic potential for PTSD. While animal experiments have successfully validated the expression patterns of these candidate genes, several critical gaps remain to be addressed in future research. Currently, validation has been restricted to peripheral blood samples, highlighting the need for expanded analyses across multiple tissue types, particularly in brain regions relevant to PTSD pathophysiology. Furthermore, while our findings in animal models are promising, their clinical relevance must be established through validation in human PTSD patient cohorts. Perhaps most importantly, the mechanistic relationships between these mitochondrial gene functions, immune cell dynamics, and PTSD development remain to be fully elucidated. Despite these limitations, our identification of these MitoDEGs in peripheral blood establishes a crucial foundation for translational research bridging molecular mechanisms with clinical applications. Future studies should particularly focus on investigating UCP2's role in fear circuitry regulation, NADK2's contribution to stress-induced redox imbalance, and IDE's potential involvement in PTSD-related neuroinflammation. These preliminary findings not only provide a strong rationale for subsequent mechanistic investigations but also underscore the importance of mitochondrial-immune interactions in understanding PTSD's complex etiology.

Conclusion

Through comprehensive bioinformatics analysis, we identified DEGs between healthy individuals and PTSD patients, with a focus on mitochondrial-associated genes. By intersecting MitoCarta3.0-derived mitochondrial genes with PTSD-associated genes from GSE199841 and GSE81761 datasets, we discovered eight candidate mitoDEGs (SLC25A12, IDE,

MRPL27, COX17, MRPS17, CISD1, NADK2, and UCP2). Subsequent validation using a tripartite machine learning framework (LASSO, SVM-RFE, and Random Forest) consistently identified four pivotal mitoDEGs—UCP2, CISD1, NADK2, and IDE—across all algorithm. Transcriptomic analysis demonstrated upregulated mRNA expression levels of *IDE, CISD1*, and *NADK2* in PTSD cohorts, whereas *UCP2* exhibited downregulation. Subsequent investigation revealed a significant association between key mitoDEGs and immune cell dynamics, along with PTSD-related changes in the immune micro-environment. Notably, these hub mitoDEGs displayed robust correlations with multiple immune cell populations, suggesting their potential role as co-regulatory molecules bridging mitochondrial metabolic processes and immune dysregulation in PTSD. To further validate these findings, we established a murine PTSD model via fear conditioning using a foot shock paradigm. Experimental quantification of the four mitoDEGs confirmed transcriptional changes consistent with bioinformatics predictions. Collectively, these results advance our understanding of PTSD pathogenesis by elucidating the interplay between mitochondrial metabolism and immune responses. This research offers new perspectives into the identification of diagnostic biomarkers and therapeutic strategies targeting mitochondrial-immune crosstalk in PTSD.

Data Sharing Statement

All analyzed datasets are publicly accessible via the GEO database (http://www.ncbi.nlm.nih.gov/geo) and MitoCarta3.0 (https://www.broadinstitute.org/mitocarta/mitocarta30). Further data are available from the corresponding authors upon request.

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Disclosure

The authors have no competing interests to disclose.

References

1. Hori H, Kim Y. Inflammation and post-traumatic stress disorder. *Psychiatry Clin Neurosci*. 2019;73(4):143–153. doi:10.1111/pcn.12820
2. Maercker A, Cloitre M, Bachem R, et al. Complex post-traumatic stress disorder. *Lancet*. 2022;400(10345):60–72. doi:10.1016/S0140-6736(22)00821-2
3. Fitzpatrick S, Saraiya T, Lopez-Castro T, Ruglass LM, Hien D. The impact of trauma characteristics on post-traumatic stress disorder and substance use disorder outcomes across integrated and substance use treatments. *J Subst Abuse Treat*. 2020;113:107976. doi:10.1016/j.jsat.2020.01.012
4. Thakur A, Choudhary D, Kumar B, Chaudhary A. A review on post-traumatic stress disorder (PTSD): symptoms, therapies and recent case studies. *Curr Mol Pharmacol*. 2022;15(3):502–516. doi:10.2174/1874467214666210525160944
5. Lin J, Xing Q, Zhang C, et al. Advances in repetitive transcranial magnetic stimulation for the treatment of post-traumatic Stress disorder. *Alpha Psychiatry*. 2024;25(4):440–448. doi:10.5152/alphapsychiatry.2024.241587
6. Parsons RG, Ressler KJ. Implications of memory modulation for post-traumatic stress and fear disorders. *Nat Neurosci*. 2013;16(2):146–153. doi:10.1038/nn.3296
7. Al Jowf GI, Ahmed ZT, Reijnders RA, de Nijs L, Eijssen LMT. To predict, prevent, and manage post-traumatic stress disorder (PTSD): a review of pathophysiology, treatment, and biomarkers. *Int J Mol Sci*. 2023;24(6). doi:10.3390/ijms24065238
8. Cheng A, Hou Y, Mattson MP. Mitochondria and neuroplasticity. *ASN Neuro*. 2010;2(5):e00045. doi:10.1042/AN20100019
9. Miller WL. Steroid hormone synthesis in mitochondria. *Mol Cell Endocrinol*. 2013;379(1–2):62–73. doi:10.1016/j.mce.2013.04.014
10. Smith SM, Vale WW. The role of the hypothalamic-pituitary-adrenal axis in neuroendocrine responses to stress. *Dialogues Clin Neurosci*. 2006;8(4):383–395. doi:10.31887/DCNS.2006.8.4/ssmith
11. Lopez-Armada MJ, Riveiro-Naveira RR, Vaamonde-Garcia C, Valcarcel-Ares MN. Mitochondrial dysfunction and the inflammatory response. *Mitochondrion*. 2013;13(2):106–118. doi:10.1016/j.mito.2013.01.003
12. Pivac N, Vuic B, Sagud M, et al. PTSD, immune system, and inflammation. *Adv Exp Med Biol*. 2023;1411:225–262. doi:10.1007/978-981-19-7376-5_11
13. Porgali Zayman E, Bay Karabulut A, Ozdemir S, Kartalci S. Oxytocin and vasopressin blood levels in people with post-traumatic stress disorder. *Alpha Psychiatry*. 2023;24(5):180–185. doi:10.5152/alphapsychiatry.2023.21628
14. Michopoulos V, Powers A, Gillespie CF, Ressler KJ, Jovanovic T. Inflammation in fear- and anxiety-based disorders: PTSD, GAD, and beyond. *Neuropsychopharmacology*. 2017;42(1):254–270. doi:10.1038/npp.2016.146
15. Wang Z, Gerstein M, Snyder M. RNA-Seq: a revolutionary tool for transcriptomics. *Nat Rev Genet*. 2009;10(1):57–63. doi:10.1038/nrg2484
16. Chang CH, Lin CH, Lane HY. Machine learning and novel biomarkers for the diagnosis of Alzheimer's disease. *Int J Mol Sci*. 2021;22(5). doi:10.3390/ijms22052761

17. Ngiam KY, Khor IW. Big data and machine learning algorithms for health-care delivery. *Lancet Oncol*. 2019;20(5):e262–e273. doi:10.1016/S1470-2045(19)30149-4
18. Vali M, Nezhad HM, Kovacs L, Gandomi AH. Machine learning algorithms for predicting PTSD: a systematic review and meta-analysis. *BMC Med Inform Decis Mak*. 2025;25(1):34. doi:10.1186/s12911-024-02754-2
19. Wang J, Ouyang H, Jiao R, et al. The application of machine learning techniques in posttraumatic stress disorder: a systematic review and meta-analysis. *NPJ Digit Med*. 2024;7(1):121. doi:10.1038/s41746-024-01117-5
20. Rath S, Sharma R, Gupta R, et al. MitoCarta3.0: an updated mitochondrial proteome now with sub-organelle localization and pathway annotations. *Nucleic Acids Res*. 2021;49(D1):D1541–D1547. doi:10.1093/nar/gkaa1011
21. Wei W, Zhao Q, Wang Z, et al. ADRAM is an experience-dependent long noncoding RNA that drives fear extinction through a direct interaction with the chaperone protein 14-3-3. *Cell Rep*. 2022;38(12):110546. doi:10.1016/j.celrep.2022.110546
22. Xie L, Zhang S, Huang L, et al. Single-cell RNA sequencing of peripheral blood reveals that monocytes with high cathepsin S expression aggravate cerebral ischemia-reperfusion injury. *Brain Behav Immun*. 2023;107:330–344. doi:10.1016/j.bbi.2022.11.001
23. Verbitsky A, Dopfel D, Zhang N. Rodent models of post-traumatic stress disorder: behavioral assessment. *Transl Psychiatry*. 2020;10(1):132. doi:10.1038/s41398-020-0806-x
24. Karabulut S, Genc H. A comparative study of women with substance use disorder with and without post-traumatic stress disorder: complex interaction between childhood trauma, empathy, personality traits, and substance use severity. *Alpha Psychiatry*. 2023;24(5):226–233. doi:10.5152/alphapsychiatry.2023.231195
25. Ye L, Jiang Y, Zhang M. Crosstalk between glucose metabolism, lactate production and immune response modulation. *Cytokine Growth Factor Rev*. 2022;68:81–92. doi:10.1016/j.cytogfr.2022.11.001
26. Newman LE, Shadel GS. Mitochondrial DNA Release in Innate Immune Signaling. *Annu Rev Biochem*. 2023;92:299–332. doi:10.1146/annurev-biochem-032620-104401
27. Qiao L, Zhang X, Pi S, et al. Dietary supplementation with biogenic selenium nanoparticles alleviate oxidative stress-induced intestinal barrier dysfunction. *NPJ Sci Food*. 2022;6(1):30. doi:10.1038/s41538-022-00145-3
28. Makowski L, Chaib M, Rathmell JC. Immunometabolism: from basic mechanisms to translation. *Immunol Rev*. 2020;295(1):5–14. doi:10.1111/imr.12858
29. Andrews ZB, Liu ZW, Walllingford N, et al. UCP2 mediates ghrelin's action on NPY/AgRP neurons by lowering free radicals. *Nature*. 2008;454(7206):846–851. doi:10.1038/nature07181
30. Dietrich MO, Antunes C, Geliang G, et al. Agrp neurons mediate Sirt1's action on the melanocortin system and energy balance: roles for Sirt1 in neuronal firing and synaptic plasticity. *J Neurosci*. 2010;30(35):11815–11825. doi:10.1523/JNEUROSCI.2234-10.2010
31. Dietrich MO, Andrews ZB, Horvath TL. Exercise-induced synaptogenesis in the hippocampus is dependent on UCP2-regulated mitochondrial adaptation. *J Neurosci*. 2008;28(42):10766–10771. doi:10.1523/JNEUROSCI.2744-08.2008
32. Duman RS, Aghajanian GK, Sanacora G, Krystal JH. Synaptic plasticity and depression: new insights from stress and rapid-acting antidepressants. *Nat Med*. 2016;22(3):238–249. doi:10.1038/nm.4050
33. Krystal JH, Abdallah CG, Averill LA, et al. Synaptic loss and the pathophysiology of PTSD: implications for ketamine as a prototype novel therapeutic. *Curr Psychiatry Rep*. 2017;19(10):74. doi:10.1007/s11920-017-0829-z
34. Jiang ZM, Yang QH, Zhu CQ. UCP2 in early diagnosis and prognosis of sepsis. *Eur Rev Med Pharmacol Sci*. 2017;21(3):549–553.
35. Chaudhuri L, Srivastava RK, Kos F, Shrikant PA. Uncoupling protein 2 regulates metabolic reprogramming and fate of antigen-stimulated CD8+ T cells. *Cancer Immunol Immunother*. 2016;65(7):869–874. doi:10.1007/s00262-016-1851-4
36. Zhong G, Li Y, Li L, et al. Mitochondrial miR-12294-5p regulated copper-induced mitochondrial oxidative stress and mitochondrial quality control imbalance by targeted inhibition of CISD1 in chicken livers. *J Hazard Mater*. 2023;458:131908. doi:10.1016/j.jhazmat.2023.131908
37. Gao J, Dong M, Tian W, et al. The role of CISD1 reduction in macrophages in promoting COPD development through M1 polarization and mitochondrial dysfunction. *Eur J Med Res*. 2024;29(1):541. doi:10.1186/s40001-024-02146-2
38. Martinez A, Sanchez-Martinez A, Pickering JT, et al. Mitochondrial CISD1/Cisd accumulation blocks mitophagy and genetic or pharmacological inhibition rescues neurodegenerative phenotypes in Pink1/parkin models. *Mol Neurodegener*. 2024;19(1):12. doi:10.1186/s13024-024-00701-3
39. Zhang X, Peng T, Li C, et al. Inhibition of CISD1 alleviates mitochondrial dysfunction and ferroptosis in mice with acute lung injury. *Int Immunopharmacol*. 2024;130:111685. doi:10.1016/j.intimp.2024.111685
40. Yonutas HM, Hubbard WB, Pandya JD, Vekaria HJ, Geldenhuys WJ, Sullivan PG. Bioenergetic restoration and neuroprotection after therapeutic targeting of mitoNEET: new mechanism of pioglitazone following traumatic brain injury. *Exp Neurol*. 2020;327:113243. doi:10.1016/j.expneurol.2020.113243
41. Geldenhuys WJ, Benkovic SA, Lin L, et al. MitoNEET (CISD1) knockout mice show signs of striatal mitochondrial dysfunction and a Parkinson's disease phenotype. *ACS Chem Neurosci*. 2017;8(12):2759–2765. doi:10.1021/acschemneuro.7b00287
42. Zhu J, Schworer S, Berisa M, et al. Mitochondrial NADP(H) generation is essential for proline biosynthesis. *Science*. 2021;372(6545):968–972. doi:10.1126/science.abd5491
43. Tran DH, Kesavan R, Rion H, et al. Mitochondrial NADP(+) is essential for proline biosynthesis during cell growth. *Nat Metab*. 2021;3(4):571–585. doi:10.1038/s42255-021-00374-y
44. Chen Z, Zhong C. Decoding Alzheimer's disease from perturbed cerebral glucose metabolism: implications for diagnostic and therapeutic strategies. *Prog Neurobiol*. 2013;108:21–43. doi:10.1016/j.pneurobio.2013.06.004
45. Dorfman VB, Pasquini L, Riudavets M, et al. Differential cerebral deposition of IDE and NEP in sporadic and familial Alzheimer's disease. *Neurobiol Aging*. 2010;31(10):1743–1757. doi:10.1016/j.neurobiolaging.2008.09.016
46. Akhtar MW, Sanz-Blasco S, Dolatabadi N, et al. Elevated glucose and oligomeric beta-amyloid disrupt synapses via a common pathway of aberrant protein S-nitrosylation. *Nat Commun*. 2016;7:10242. doi:10.1038/ncomms10242
47. Yilmaz A, Guerrera C, Waeckel-Enee E, Lipecka J, Bertocci B, van Endert P. Insulin-degrading enzyme interacts with mitochondrial ribosomes and respiratory chain proteins. *Biomolecules*. 2023;13(6). doi:10.3390/biom13060890
48. Bessard MA, Moser A, Waeckel-Enee E, et al. Insulin-degrading enzyme regulates insulin-directed cellular autoimmunity in murine type 1 diabetes. *Front Immunol*. 2024;15:1474453. doi:10.3389/fimmu.2024.1474453

49. Tundo GR, Sbardella D, Ciaccio C, et al. Insulin-degrading enzyme (IDE): a novel heat shock-like protein. *J Biol Chem*. 2013;288(4):2281–2289. doi:10.1074/jbc.M112.393108
50. Dunsmoor JE, Cisler JM, Fonzo GA, Creech SK, Nemeroff CB. Laboratory models of post-traumatic stress disorder: the elusive bridge to translation. *Neuron*. 2022;110(11):1754–1776. doi:10.1016/j.neuron.2022.03.001