Gene expression profiling of individuals with bipolar disorder who died by suicide and those who died from causes other than suicide in the prefrontal cortex and cerebellum

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Abstract

Suicide is a major risk among individuals with bipolar disorder (BD), with up to 20% dying by suicide. However, the underlying genes and biological pathways remain poorly understood. RNA-Seq data from the prefrontal cortex (PFC) and cerebellum of Stanley Neuropathology Consortium collection were processed and analyzed to identify genes and biological pathways associated with BD as well as suicide in individuals with BD. In the PFC, 179 genes were differentially expressed in the BD patients who died from causes other than suicide (BD_NS) compared to controls, while 3 genes were differentially expressed in BD patients who died by suicide (BD_S) versus controls. Enrichment analysis identified 38 significantly enriched pathways, including inflammation and NF-κB signaling, in upregulated genes from BD_NS, but no pathways were enriched in BD_S. In the cerebellum, more genes were differentially expressed than in the PFC, with 2823 genes showing differential expression between BD_NS and controls and 26 genes between BD_S and controls. Various pathways, including synaptic vesicle cycle, endocannabinoid signaling, nicotine addiction, focal adhesion, and PI3K-Akt signaling, were enriched in the DEGs. This study suggests that biological pathways, including immune/inflammatory responses and synaptic signaling, may be associated with BD, while several genes related to cell signaling and protection against oxidative stress and DNA damage may play a role in suicide among BD patients.

Introduction

Bipolar disorder (BD) is a devastating and chronic psychiatric disorder characterized by extreme changes in mood, including manic episodes (or hypomania) and depression [1]. Moreover, BD is a common disorder with about 2.4% lifetime prevalence [2]. Mania episodes are defined by elevated mood, increased vitality, and recklessness, whereas depressive episodes are characterized by feelings of sadness, low energy and hopelessness [3]. These mood episodes can vary greatly in duration and intensity. This disorder can have a severe impact on brain functions including cognition and significantly lower an individual's daily functioning [4] [5].

Individuals with major psychiatric disorders, such as BD, depression, and schizophrenia, are significantly associated with an increased risk of suicide [6]. The rate of suicide attempts is between 20% and 60% among individuals with BD; moreover, up to 20% of BD patients die by suicide [7]. Several risk factors for suicide in BD patients include male gender, lack of social connections, unemployment, a history of suicide attempts, and depressive or mixed mood

episodes [8]. However, the genes and biological pathways involved in suicide in individuals with BD are not well understood.

Gene expression profiling using RNA sequencing (RNA-seq) has significantly contributed to the identification of genes and biological pathways associated with major psychiatric disorders and their comorbidities [9] [10]. Post-mortem brain samples have been utilized for these technologies. The gene expression profiling studies using brain samples from individuals with the disorders reveal gene expression alterations associated with these disorders and comorbidities, enabling researchers to explore the underlying mechanisms [11]. Transcriptome sequencing shows differential gene expression in the postmortem human dorsal striatum, emphasizing immune response and oxidative phosphorylation pathways [12], as well as genes related to immune/inflammatory processes and the postsynaptic membrane in the subgenual anterior cingulate cortex and amygdala in BD [13]. Moreover, genes associated with immune, inflammatory, and neurodevelopmental pathways are linked to suicide completion in the brains of individuals with mood disorders, including bipolar disorder [14].

Previous studies have primarily focused on depression-related suicides, and research on suicide in individuals with BD has been limited. In this study, we conducted RNA-seq data analysis from two brain regions, the PFC and cerebellum, of individuals with BD and unaffected controls. Gene expression profiles from BS_NS was compared with those from controls and BD_S was also compared with those from the controls. Comparative analysis of gene expression was performed among three groups, BD_NS, BD_S and controls, to identify genes and pathways associated with BD as well as suicide in individuals with BD.

Methods

RNA-seq data

The RNA-Seq data were generated from the PFC and cerebellum of 28 individuals as part of the Stanley Neuropathology Consortium (NPC) (Torrey et al., 2000) (Table 1).

The samples were collected from three groups: 5 BD_NS individuals, 8 BD_S individuals, and 15 control individuals in the PFC, and 6 BD_NS individuals, 7 BD_S individuals, and 15 control individuals in the cerebellum. The data were created through international collaborative efforts and deposited in the Stanley Neuropathology Consortium Integrative Database (http://sncid.stanleyresearch.org) [15]. Raw FASTQ files from the SNCID database were downloaded and analyzed.

Preprocessing and Quality Control of RNA-Seq Data

Raw paired-end sequencing data were processed with Trimmomatic (v0.39) to remove adapter sequences and low-quality bases, using the TruSeq3-PE adapter file and applying quality filters

for leading/trailing bases, sliding window trimming, and a minimum read length of 90 bases. Quality control (QC) of the trimmed sequencing reads was then performed using FastQC v0.12.1 to assess various quality metrics.

Reads Mapping and Gene Expression Quantification

RNA-seq reads were mapped to the human GRCh38 reference genome using HISAT2 (v2.2.1) for gene model annotations. Paired-end read orientation was set using the --fr option to indicate forward-reverse orientation, and the alignment results were stored as SAM files in the specified output directory. The alignment process was performed by iterating through paired R1 and R2 FASTQ files, with no more than two mismatches allowed during alignment. The aligned read count data were processed by converting SAM files to sorted BAM files using samtools (1.19.2) and then quantifying gene expression with featureCounts (Version 2.0.3), utilizing the Homo_sapiens.GRCh38.95.gtf annotation file.

Differential Gene Expression Analysis with Surrogate Variable Adjustment

Raw read counts were imported into R for differential expression analysis using the edgeR and SVA packages. Paired count data were processed, and counts per million (CPM) were calculated, followed by data normalization. Genes that have a CPM<1 in 50% or more of the samples were filtered out. Surrogate variables were identified using the SVA package. These surrogate variables were incorporated into the differential expression model to ensure accurate identification of biologically relevant gene expression changes to create a generalized linear model framework. Genes with an FDR < 0.05 were considered statistically significant.

Functional Enrichment Analysis

Differentially expressed genes (DEGs) were filtered based on logFC and FDR thresholds. Upregulated genes were identified as DEGs with logFC > 0 (indicating higher expression in the disorder group compared to the control group) and FDR < 0.05, while downregulated genes were identified as DEGs with logFC < 0 and FDR < 0.05. Enrichment analysis was performed using the gprofiler2 R package. KEGG pathways were identified for both upregulated and downregulated genes and pathways with FDR <0.05 were considered statistically significant.

Results

RNA-seq data used for this study

Publicly available RNA-seq data from the brains of 13 individuals with BD and 15 controls were downloaded and used for this study. Among the 13 individuals with BD, there were 5 BD_NS and 8 BD_S from the PFC, and 6 BD_NS and 7 BD_S from the cerebellum. The data were

created through international collaborative efforts and deposited in the Stanley Neuropathology Consortium Integrative Database (http://sncid.stanleyresearch.org) [15].

Table 1. Demographic and clinical variables for the samples in the RNA-seq data

Variables	PFC			Cerebellum		
	BD_NS (n=5)	BD_S (n=8)	CTL (n=15)	BD_NS (n=6)	BD_S (n=7)	CTL (n=15)
Age	48.2±10.59	39.5±12.27	48.07±10.66	50.33±9.88	36.42±8.67	48.07±10.3
Sex (M/F)	3/2	5/3	9/6	3/3	5/2	9/6
Brain pH	6.1±0.19	6.26±0.23	6.27±0.24	6.17±0.21	6.23±0.21	6.27±0.23
PMI	33.8±18.02	31.5±17.15	23.73±9.95	38.17±17.66	27.43±12.7	23.73±9.61
RIN	7.61±0.91	7.94±1.30	8.28±0.86	7.47±1.02	8.06±0.85	8.58±0.39

Values are mean ± S.D. BD_NS, bipolar disorder patients who died from causes other than suicide; BD_S, bipolar disorder patients who died by suicide; CTL, normal control; PMI, post-mortem interval; RIN, RNA integrity number

Differentially expressed genes in the PFC

The analysis compared gene expression profiles in the PFC between BD_NS vs unaffected controls as well as BD_S vs controls. A total 179 and 3 genes were differentially expressed in the brain region between BD_NS vs controls and between BD_S vs controls respectively (Figure 1). One gene is differentially expressed in both comparisons (Figure 1).

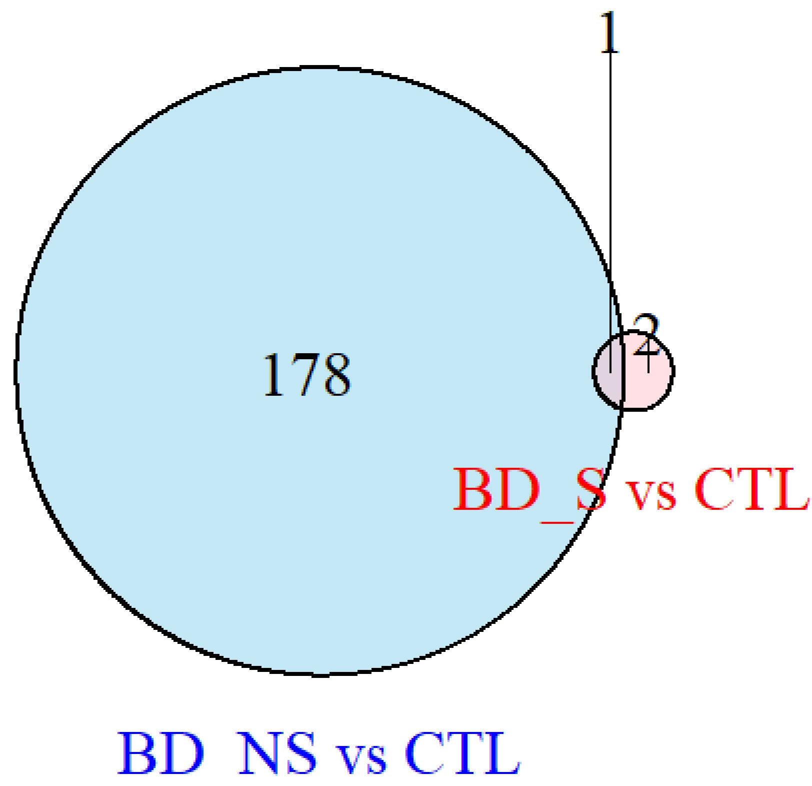
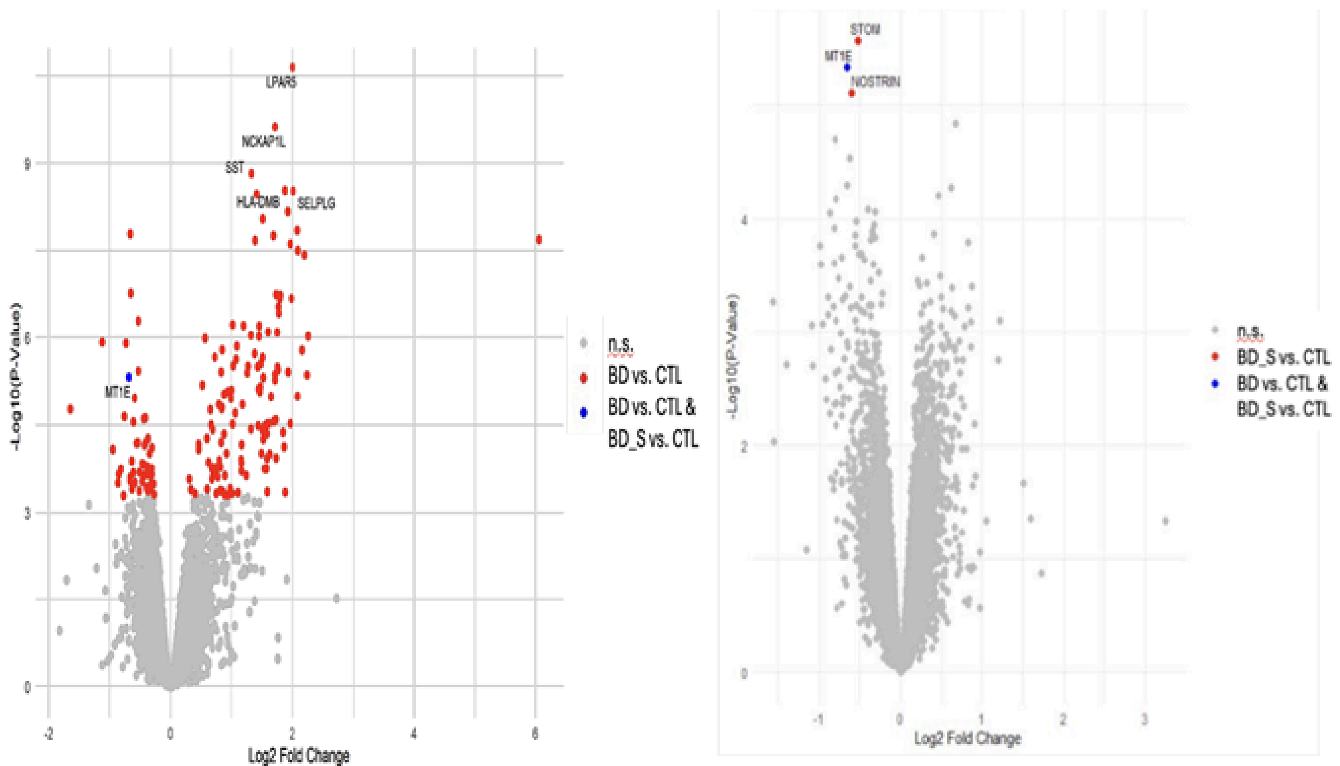


Figure 1. Venn diagram of differentially expressed genes in the PFC between BD_NS (bipolar disorder who died from causes other than suicide) vs controls and BD_S (bipolar disorder patients who died by suicide) vs controls.

126 genes were up-regulated and 53 genes were down-regulated in the PFC of BD_NS, respectively (Figure 2 A). Two genes, *STOM* (stomatin) and *NOSTRIN* (nitric oxide synthase trafficking), were specifically down-regulated in the PFC of BD_S and one gene, *MT1E* (metallothionein 1E), was down-regulated in both BD_NS and BD_S, as compared to controls (Figure 2 B).



A **B**

Figure 2. Volcano plot of the DEGs in the PFC between BD_NS vs controls (A), and BD_S vs control (B). DEGs with an FDR less than 0.05 are shown in red, commonly significantly DEGs are shown in blue, and non-significant genes are shown in gray. The five most significantly differentially expressed genes and the commonly significantly differentially expressed genes were labeled with gene symbols.

Enrichment analysis was performed to identify biological pathways associated with the DEGs. 38 KEGG pathways were significantly enriched in the up-regulated genes in the PFC of BD_NS, as compared to controls, which includes infectious diseases, complementation system, inflammation and NF-kB signaling pathway (Figure 3). There were no significantly enriched KEGG pathways in the DEG between BD_S and controls.

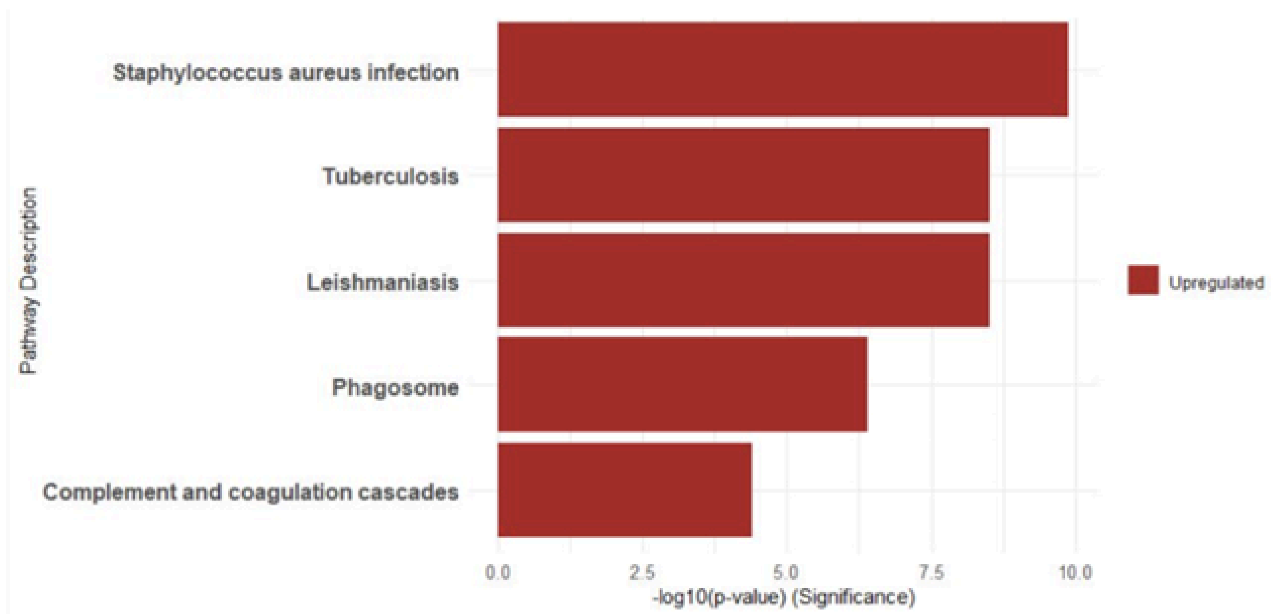


Figure 3. Five most significant KEGG pathways enriched in the up-regulated genes in the PFC of BD_NS compared to controls.

Differentially expressed genes in the cerebellum

More genes were differentially expressed in the cerebellum than the PFC. A total of 2823 and 26 genes were differentially expressed in the cerebellum between BD_NS vs controls and between BD_S vs controls respectively (Figure 4). Eight genes were differentially expressed in both comparisons (Figure 4).

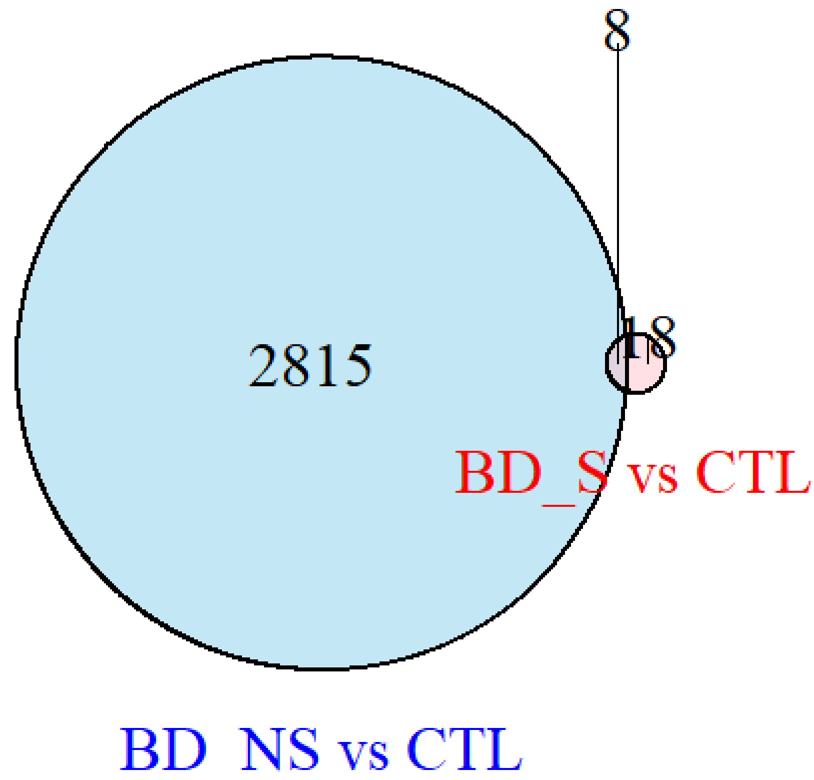


Figure 4. Venn diagram of differentially expressed genes in the cerebellum between BD_NS vs controls and BD_S vs controls.

1897 genes were up-regulated and 926 genes were down-regulated in the cerebellum of BD_NS, respectively (Figure 5 A). 20 genes were up-regulated and 6 genes were down-regulated in the PFC of BD_S, respectively (Figure 5 B). Among the DEGs, 8 genes were commonly up-regulated in both comparisons (Figure 5).

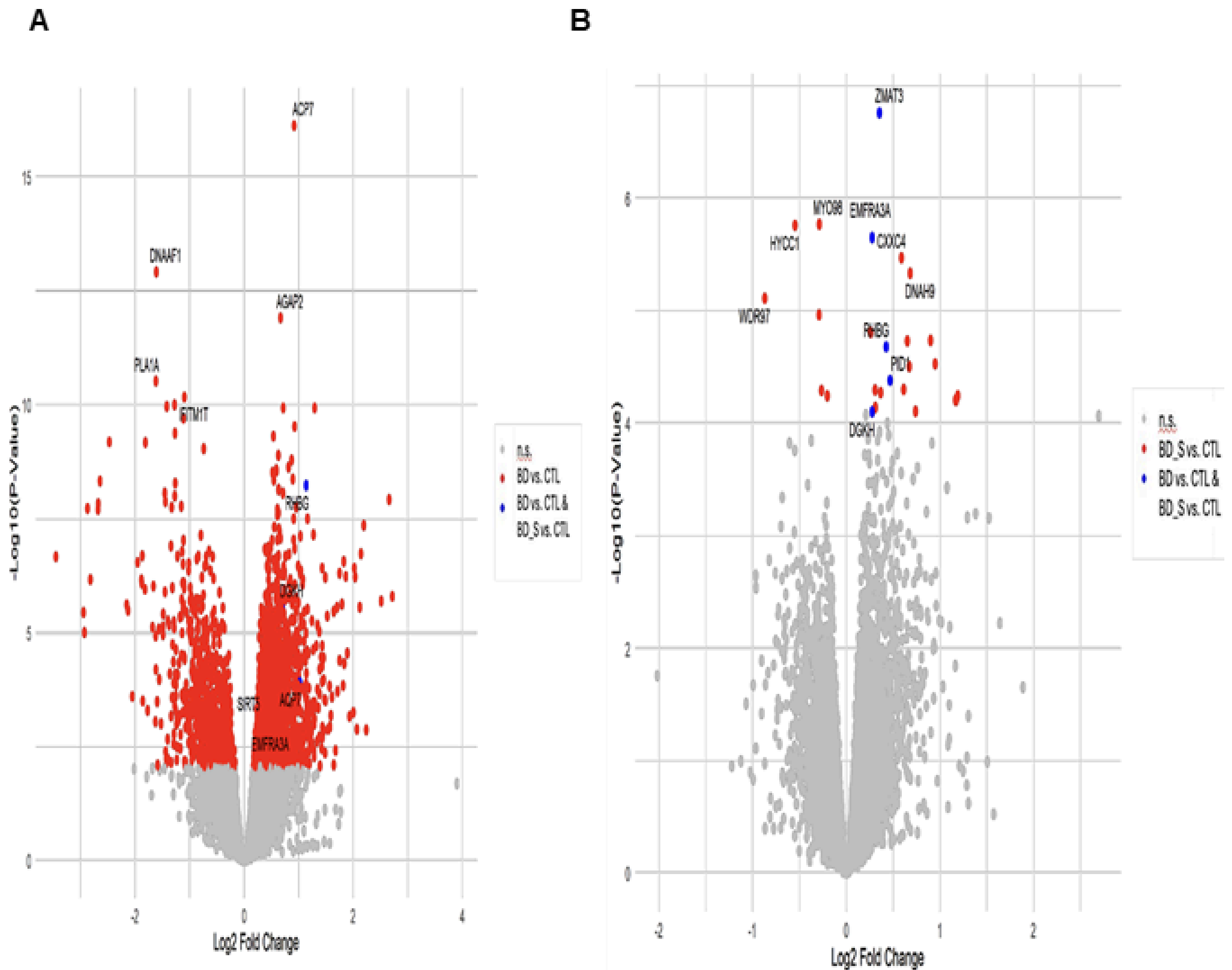


Figure 5. Volcano plot of the DEGs in the cerebellum between BD_NS vs controls (A), and BD_S vs control (B). DEGs with an FDR less than 0.05 are shown in red, commonly significantly DEGs are shown in blue, and non-significant genes are shown in gray. The five most significantly differentially expressed genes and the commonly significantly differentially expressed genes were labeled with gene symbols.

34 KEGG pathways including synaptic vesicle cycle, endocannabinoid signaling, and nicotine addiction were significantly enriched in the up-regulated genes and 12 pathways including focal adhesion and PI3K-Akt signaling were significantly enriched in the down-regulated genes in the cerebellum of BD, as compared to controls (Figure 6).

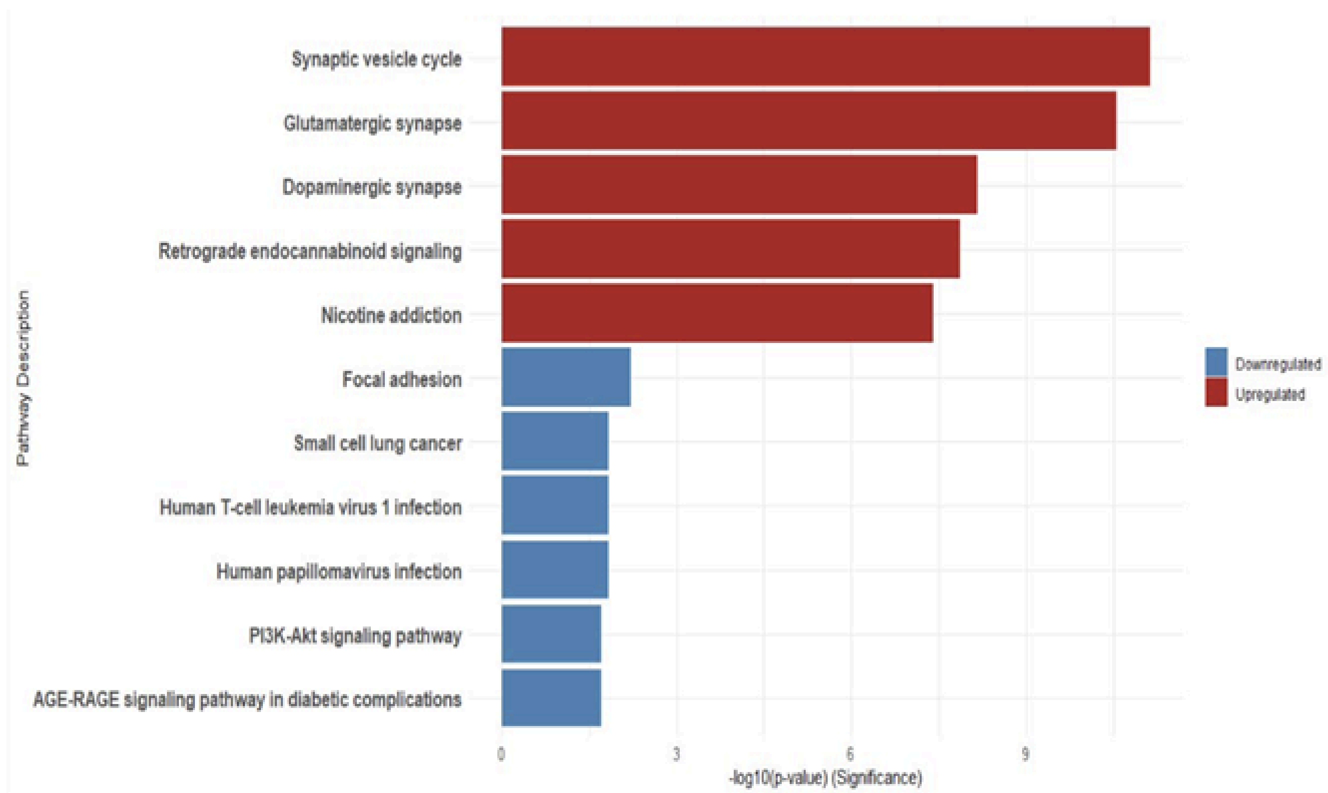


Figure 6. Five most significant KEGG pathways enriched in the differentially expressed genes in the cerebellum of BD_NS as compared to controls.

Discussion

Differential expression of genes related to immune/inflammation responses, oxidative phosphorylation and postsynaptic membrane have been reported in the various brain regions of individuals with BD [11] [12]. Consistent with the results, genes related to infection, immune/inflammation responses and NF-κB (nuclear factor kappa B) pathway were up-regulated in the PFC of individuals with BD who died from causes other than suicide in this study. The NF-κB plays a crucial role in regulation of immune functions, inflammatory responses and immune cell regulation [16]. The results from this study, along with previous findings, suggest that the upregulation of genes associated with immune/inflammation responses, including those in the NF-κB pathway, may contribute to the pathophysiology of BD.

In a previous study using human post-mortem brain tissue, several genes such as *HTR2A (*5-hydroxytryptamine receptor 2A*)*, *MT1E* (metallothionein 1E) and *MT1F* (metallothionein 1F) associated with suicide have been reported [17]. *MT1E* is a functional isoform of metallothioneins, which are small cysteine-rich proteins that play roles in protecting

against oxidative stress and DNA damage. However, its roles in psychiatric disorders and comorbidities remain unknown [18]. In this study, *MT1E* was down-regulated in the PFC of BD_NS and BD_S, as compared to controls, suggesting that *MT1E* may be associated with the pathophysiology of BD and may contribute to risk factor for suicide of the individuals with the disorder.

Another transcriptomics study shows differential expression of genes including *WDR97* (WD repeat domain 97), *STOM* (Stomatin), *MT1E*, and *NOSTRIN* (Nitric oxide synthase trafficking receptor) in the dorsomedial prefrontal cortex in suicide victims as compared to controls [19]. Consistent with the results, *STOM* and *NOSTRIN* were down-regulated and *WDR97* was down-regulated in the PFC and cerebellum in BP_S respectively. *STOM* is involved in regulating ion channels and membrane trafficking, *NOSTRIN* modulates nitric oxide signaling and endothelial cell function, and *WDR97* plays a role in the regulation of protein transport and cell signaling pathways. These results suggest that dysregulation of genes such as *STOM*, N*OSTRIN*, *WDR97*, and *MT1E* in the brains may contribute to the pathophysiology of suicide of individuals with BD by affecting neuronal function, stress responses, and cell membrane integrity.

This transcriptomics study has several limitations because it used a small sample size. One of the most important limitations is the reduced statistical power due to the small sample size, which increases the risk of Type II errors and may make it difficult to identify subtle differences in gene expression between the disorder group and the control group [20]. Moreover, although this study performed surrogate variable analysis to control for hidden confounding factors, the small sample size could still pose challenges in identifying and controlling for confounders [21]. To address these issues, future research should replicate these findings in larger and independent samples. Additionally, statistical methods such as Bayesian approaches or bootstrapping should be utilized with small sample sizes to obtain more reliable results. Nevertheless, this study may provide novel insights into the molecular mechanisms that underlie BD and the risk of suicide in individuals with the disorder by highlighting specific genes.

Note

The complete list of DEGs and KEGG pathways can be downloaded from the following website: https://lydialibbykim.wixsite.com/transcriptomics-to-t/research

The code for data preprocessing and analysis is provided upon request.

Abbreviations:

BD, bipolar disorder

BD_NS, bipolar disorder patients who died from causes other than suicide

BD_S, bipolar disorder patients who died by suicide

CTL, normal control

PFC, prefrontal cortex

PMI, post-mortem interval

RIN, RNA integrity number

RNA-seq, RNA sequencing

CPM, count per million

FDR, false discovery rate

DEGs, differentially expressed genes

LogFC, log fold change

KEGG, Kyoto Encyclopedia of Genes and genomes

g:Profiler, Gene Ontology Profiler

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